Purification and Characterization of Strawberry Crinkle Virus

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ABSTRACT

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Strawberry crinkle virus (SCV), a cytoplasmic rhabdovirus, has proven difficult to purify from strawberry leaves. By testing alternative hosts and different procedures, we have succeeded in purifying SCV from leaves of *Physalis floridana* using a celite filtration method. Two classes of particles differing in sedimentation rate could be distinguished by sucrose density gradient centrifugation. Electron microscopic examination of these purified preparations revealed the presence of bacilliform particles and an apparent size difference between the two classes. Polyacrylamide gel electrophoresis of sodium dodecyl sulfate-disrupted virions showed that SCV contains three major structural proteins with molecular weights estimated to be 78, 47, and 25 kDa. The 78-kDa protein bound lectins, identifying it as the viral glycoprotein. The SCV protein profile resembles that of lettuce necrotic yellows virus (LNYV) but differs appreciably from sonchus yellow net virus (SYNV). SCV has no detectable serological relatedness to LNYV or SYNV. These results suggest that SCV is distinct from other previously characterized rhabdoviruses.

The crinkle disease of strawberry (*Fragaria* spp.) was described first by Zeller and Vaughan (31), and cytoplasmic rhabdoviruslike particles were found in diseased plants by Richardson et al (24). Strawberry crinkle is one of the more important components of the viral diseases affecting strawberries worldwide because it reduces both the yield and quality of the fruit. The causal agent, strawberry crinkle virus (SCV), often appears in combination with other strawberry viruses where it contributes an additive effect to disease severity (13). Very little is known about strains of SCV, its epidemiology, movements in nature, or natural reservoirs.

SCV can be transmitted to strawberry by grafting and by the aphids *Chaetosiphon fragaefolii* (Cockerell) and *C. jacobi* (Hille Ris Lambers) (13). To provide certified SCV-free planting stock, viral infection is diagnosed routinely by grafting and/or aphid transmission to indicator plants (4,13). This approach has not proved practical for field testing because it is time consuming and can be complicated by the presence of other interfering viruses. Although these problems could be circumvented through the use of diagnostic antisera and nucleic acid probes (5), development of these tools has not been possible because of the inability to purify the virus. There have been two major impediments to purification of SCV. The first obstacle was the inability to transmit the virus by mechanical inoculation. Transmission by grafting is time consuming and labor intensive, and transmission via fed aphids results in a low frequency of infection. Second, until recently, strawberry was the only identified host, and because of a high concentration of phenolic compounds, it is a very difficult host from which to purify viruses. A search for other aphid species suitable for transmission now has resulted in a broader range of hosts (27). These investigations have shown that the polyphagous pink and green potato aphid *Macrosiphum euphorbiae* (Thomas), after injection with SCV and an appropriate incubation period, is able to transmit the virus to solanaceous plants in the genera *Nicotiana* (27) and *Physalis* (25). Once in these alternative host plants, SCV can be propagated by mechanical sap inoculation, which provides an opportunity to investigate purification of the virus in hosts other than strawberry.

This paper describes the purification of SCV from infected leaves of *Physalis floridana* Rybd., the identification of the viral glycoprotein by lectin binding, and the generation of an antibody to the glycoprotein. We also have investigated some of the physicochemical properties of SCV and have compared its structural proteins with those of two other plant rhabdoviruses.

MATERIALS AND METHODS

Virus propagation. SCV was maintained in *P. floridana* grown under greenhouse conditions. The virus was transferred approximately every 2–3 wk to uninfected seedlings with three to four true leaves. Inoculum was prepared by grinding symptomatic leaves in 5 v of cold 0.5% sodium sulfite and 1% celite. The homogenate was rubbed onto the leaves of previously darkened host plants.

SCV purification. Symptomatic leaves were harvested 15–23 days postinoculation, stored at 4 C, and extracted within 3 days using minor, but important, modifications of the procedure described by Jackson and Christie (18) for sonchus yellow net virus (SYNV). The adapted procedure involved blending 40 g of tissue in approximately 3 v of extraction buffer (100 mM Tris-HCl, pH 8.2, 10 mM magnesium acetate, 40 mM sodium sulfite, 1 mM manganese chloride). The homogenate was squeezed through four layers of cheesecloth and centrifuged for 10 min at 5,000 rpm in a Sorvall SS34 rotor (Dupont Instruments, Wilmington, DE) to remove insoluble material. The supernatant was immediately layered on a step gradient formed from 8 ml of 300 mg/ml of sucrose and 5 ml of 600 mg/ml of sucrose in maintenance buffer (extraction buffer at pH 7.4). After centrifugation for 1 hr in an SW28 rotor (Beckman Instruments, Inc., Fullerton, CA) at 25,000 rpm at 4 C, the dark green band at the 300/600 mg/ml interface was collected with a syringe and bent needle, mixed once in a glass tissue homogenizer, and diluted

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to 100 ml with maintenance buffer. After the addition of 2 g of celite analytical filter aid (Manville Corp. & Subsidiaries, Denver, CO), the mixture was filtered under vacuum through a 10-g celite pad in an 11-cm Büchner funnel. Just as the last of the viral solution passed through the pad, it was rinsed with an additional 100 ml of maintenance buffer. This filtration step through celite was especially critical for SCV recovery because suboptimal amounts of celite resulted in appreciable contamination with chloroplast materials, whereas greater than optimal amounts drastically reduced viral recovery. Under optimal filtration conditions, a colorless filtrate was recovered. However, the filtration procedure was somewhat variable and occasionally produced green filtrates. In such cases, most of the green material could be removed while still retaining virus by immediately passing the filtrate through an additional 0.5- to 2-g celite pad in a 7-cm Büchner funnel. Virus particles subsequently were pelleted from the filtrate by centrifugation at 25,000 rpm for 1 hr at 4 C in a Beckman 50.2 Ti rotor (Beckman Instruments). The pellets were usually relatively clear to pale green, jellylike in consistency, and easily resuspended in maintenance buffer. When more than one batch of tissue was extracted, all resuspended pellets were pooled in a final volume of 1 to 2 ml. The virus suspension was layered over gradients formed from layers of 5, 10, 10, and 10 ml of 50, 100, 200, and 300 mg of sucrose/ml of maintenance buffer, which were allowed to diffuse overnight at 4 C. The gradients were centrifuged at 25,000 rpm for 20 min at 4 C in a Beckman SW28 rotor and fractionated, and the absorbance at 254 nm was monitored. Virus particles were pelleted from the peak fractions by centrifugation at 30,000 rpm for 30 min in a Beckman 50.2 Ti rotor, resuspended in 50–100 μl of maintenance buffer, and stored frozen at −20 C.

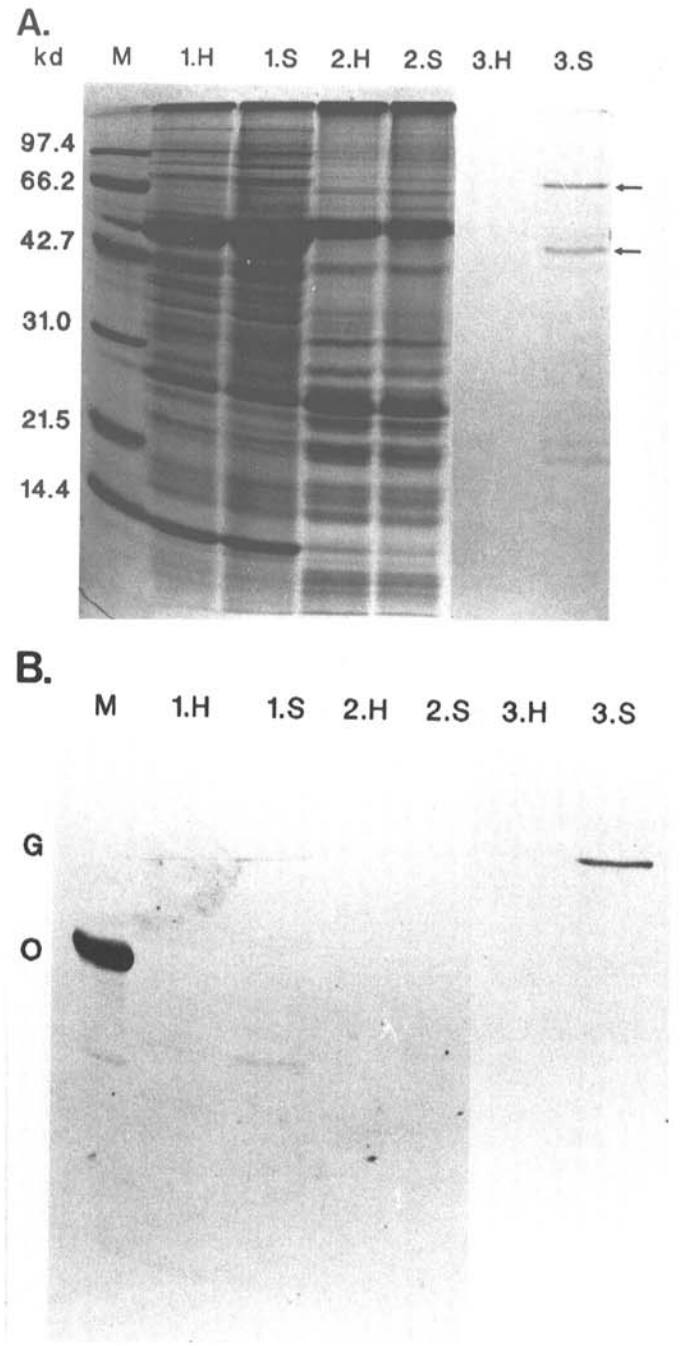
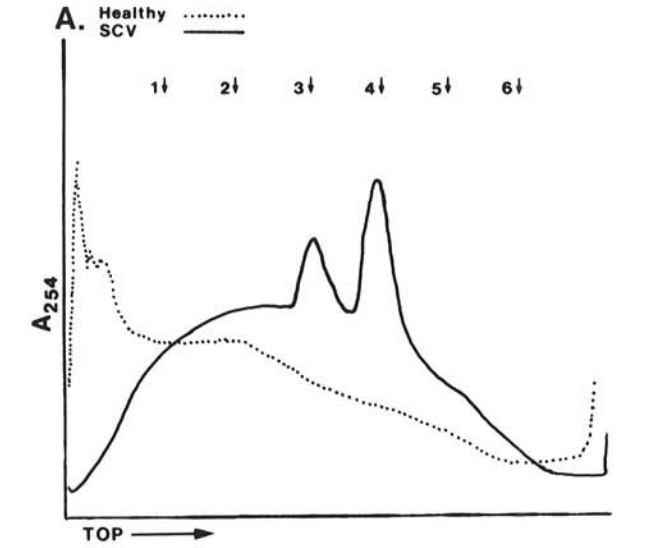


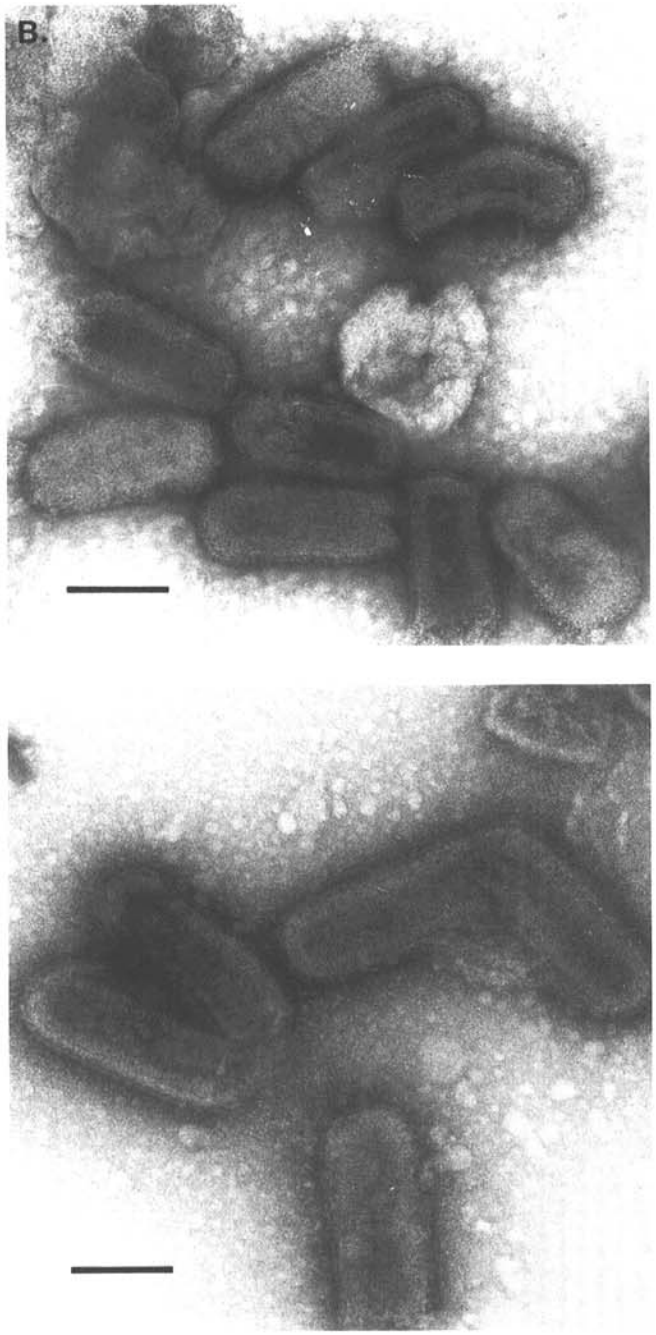
Fig. 1. Purification of strawberry crinkle virus (SCV). Samples from healthy (H) and SCV-infected (S) plants of *Physalis floridana*, recovered at different stages of purification, were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. **A,** The gel was stained with Coomassie Brilliant Blue. Arrows indicate the 78- and 47-kDa proteins. **B,** The proteins were transferred to nitrocellulose and incubated with wheat germ agglutinin to monitor increase in the viral glycoprotein during purification. The G indicates the putative SCV glycoprotein. The O indicates ovalbumin, a glycosylated protein in the molecular-weight standards that serves as a control for the binding of the lectin. Lane M, protein molecular-weight standards of Bio-Rad Laboratories, Richmond, CA. The molecular weights in kilodaltons are indicated on the left. Lane 1, protein samples after homogenization of tissue and low-speed centrifugation. Lane 2, protein samples from the dark green interface formed after centrifugation through the 30 and 60% sucrose step gradient. Lane 3, protein sample after filtration through celite and concentration by centrifugation.

Electrophoretic analysis of SCV structural proteins. Aliquots of SCV preparations were disrupted in Laemmli sample buffer (62.5 mM Tris, pH 6.8, 2% sodium dodecyl sulfate [SDS], 10% glycerol, 5% β-mercaptoethanol). Molecular-weight standards were purchased from Bio-Rad Laboratories, Richmond, CA, and included phosphorylase B (97.4 kDa), bovine serum albumin (BSA) (66.2 kDa), ovalbumin (42.7 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa). The proteins were electrophoresed in 12.5% SDS-polyacrylamide gels (20) and were either stained with Coomassie Brilliant Blue or transferred to nitrocellulose (28) for analysis with lectins or antibodies. The use of lectins for detection of glycosylated proteins was essentially as described by Adam et al (2). Biotinylated wheat germ agglutinin (WGA), concanavalin A (conA), agglutinin of *Pisum sativum* L. (PSA), and agglutinin of *Lycopersicon esculentum* Miller (LEA) (Sigma Chemical Co., St. Louis, MO) were used at a concentration of 1 μg/ml, and streptavidin, conjugated with alkaline phosphatase (Bethesda Research Laboratories, Gaithersburg, MD), was used at 0.05 μg/ml. The alkaline phosphatase reactions used the substrates 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt and nitro-blue tetrazolium chloride as described by Bio-Rad Laboratories.

Serological analysis. To reduce the level of contamination by host plant proteins, the SCV glycoprotein band was excised from Coomassie Brilliant Blue-stained polyacrylamide gels, equilibrated in phosphate-buffered saline (PBS) (10 mM sodium phosphate buffer, pH 7.4, 140 mM NaCl, 3.0 mM KCl) for 20 min, and stored at −20 C. Gel slices containing approximately 2 μg of protein were homogenized with 200 μl of incomplete Freund's adjuvant (Sigma Chemical Co.) and injected subcutaneously into female Swiss Webster mice at weekly intervals. Serum samples obtained from the tails of the mice were assayed for the presence of SCV antibodies by Western immunoblotting as described below. When the antibody titer was greater than 1/1,600, the mice were injected intraperitoneally with approximately 10^7 T-180 mouse sarcoma cells (American Type Culture Collection #TIB 66) in 1 ml of PBS to induce the production of ascites fluid (8,15). The ascites fluid was harvested and clarified by centrifugation, and aliquots were stored at −20 C. The production of the antisera to lettuce necrotic yellows virus (LNYV) and SYNV was described in McLean et al (21) and Jackson and Christie (18).

The immunoblots were performed as follows. After protein transfer, the nitrocellulose was blocked with 3% newborn calf serum, 1% BSA, and 0.05% Tween 20 in PBS overnight at 4 C





or 2 hr at room temperature. The primary antibodies were diluted in blocking buffer plus 5 units/ml of heparin (6) and incubated overnight at 4 C or 2 hr at room temperature. After three washes in PBS at room temperature for 15 min each, the nitrocellulose was incubated with goat antimouse or goat antirabbit antibody conjugated with horseradish peroxidase (Bio-Rad Laboratories), which also was diluted in blocking buffer with heparin. After four washes with PBS, the color reaction was performed with the substrate 4-chloro-1-naphthol as recommended by Bio-Rad Laboratories.

Electron microscopy. Copper grids (300 mesh), coated with Formvar and evaporated carbon, were floated on virus samples for 1 min. They then were moved to 4% glutaraldehyde for 1 min, washed twice with H_2O, and floated on 2.5% uranyl acetate, pH 3.2, for 1 min. Excess liquid was removed with filter paper, and the dried grids were examined in a Phillips EM 200 electron microscope (Phillips Electronic Instruments, Inc., Mahwah, NJ) operating at 60 kV.

RESULTS AND DISCUSSION

Purification of SCV. In initial experiments to purify SCV, we tested several variables, each of which proved to be critical for efficient recovery of the virus. These variables included host plants from which virus was purified and purification strategies. We began by testing modifications of the purification procedure developed for SYNV (18) because the protocol has been applied successfully to purification of several other plant rhabdoviruses, including maize mosaic virus (9), sowthistle yellow vein virus (26), and potato yellow dwarf virus (PYDV) (10; A. O. Jackson, *unpublished observations*). Polyacrylamide gel electrophoresis (PAGE) and specific binding of a lectin, WGA, to the viral glycoprotein to demonstrate enrichment of SCV were of particular value in monitoring the early stages of development of a purification protocol (Fig. 1). Electron microscopy, or inoculation of host plants, lacked the sensitivity and speed needed to estimate the efficiency of successive steps in purification of the virus. Samples from infected tissue at later steps of purification contained two bands (78 and 47 kDa) of approximately equal intensity when examined by PAGE, which were not present in comparable samples from uninfected *P. floridana* (Fig. 1A). After the proteins were transferred to nitrocellulose, the 78-kDa protein, but not the 47-kDa protein, specifically bound WGA, suggesting that the 78-kDa protein is glycosylated (Fig. 1B). The binding of the lectin to different samples shows the enrichment of the glycoprotein (G) relative to other plant proteins. Other plant rhabdovirus G and nucleocapsid (N) proteins have relative concentrations and sizes similar to the 78- and 47-kDa polypeptides present in the SCV preparation. This suggests that the 78- and 47-kDa proteins are the SCV G and N proteins, respectively.

Although the purification protocol yielded reproducible preparations of SCV from *P. floridana* that were free of appreciable host contamination, we were unable to recover virus from infected *Nicotiana edwardsonii* (Christie and Hall) or *N. glutinosa* L., both of which had striking mosaic symptoms. Similar host specificity for optimum recovery has been demonstrated previously for SYNV and PYDV. SYNV is readily recovered from *N. edwardsonii* and *N. benthamiana* Domin but not from *N. glutinosa* L. or *N. rustica* L., and PYDV can be recovered in high yields from *N. rustica* but not from *N. glutinosa*, despite the fact that equally severe symptoms are induced on all these

◀ **Fig. 2.** Centrifugation of strawberry crinkle virus (SCV) through sucrose density gradients. Extracts from uninoculated plants of *Physalis floridana* and plants inoculated with SCV were concentrated after filtration through celite and centrifuged through 5 to 30% sucrose gradients. **A,** The absorbance at 254 nm of the gradients was monitored, and fractions 1 through 6, indicated by the arrows, were collected. **B,** Fractions 3 (top micrograph) and 4 (bottom micrograph), corresponding to the upper and lower peaks, respectively, were examined by electron microscopy. Bars = 100 nm.

plants (A. O. Jackson, *unpublished observations*). These observations thus agree with previous studies showing that the choice of host is a critical consideration when developing protocols for purification of rhabdoviruses (19).

During development of purification procedures, we also tested calcium phosphate gel chromatography, which is routinely used for purification of LNYV from *N. glutinosa* (12). However, we were unable to recover SCV from *N. glutinosa* or *P. floridana* using this method. Similar difficulties also were observed when the calcium phosphate method was tested for purification of SYNV (18; Dietzgen and Francki, *unpublished observations*). These observations emphasize that no single procedure is suitable for purification of all plant rhabdoviruses and that many different variables may need to be tested when devising purification protocols for uncharacterized plant rhabdoviruses (11,19).

Physicochemical properties of SCV. Preparations of SCV were analyzed by centrifugation through 5 to 30% sucrose density gradients. The virus resolved into two sharp bands that sedimented about halfway into the sucrose gradients (Fig. 2A). Electron microscopic examination revealed that both peaks contained typical bacilliform rhabdovirus particles (Fig. 2B), but many of the particles were swollen or broken, making it difficult to obtain accurate measurements of the sizes. However, based on a limited number of measurements, there does appear to be a size difference between the two sedimenting components. Forty-five particles from the more slowly sedimenting peak were measured and averaged 74 × 163 nm (Fig. 2B, upper micrograph). The more rapidly sedimenting peak appeared to contain two classes of particles averaging 87 × 207 nm (38 particles) and 88 × 383 nm (13 particles) (Fig. 2B, lower micrograph). Samples from both peaks were infectious on *P. floridana*, which developed similar symptoms irrespective of the peak used as inoculum.

To determine the protein composition of the particles, six samples from the gradient, indicated by arrows in Fig. 2A, were denatured with SDS and analyzed by PAGE (Fig. 3). Samples 3 and 4, corresponding to the peaks in Fig. 2A, had identical banding patterns consisting of three major bands of approximately 78, 47, and 25 kDa (Fig. 3A). The 78-kDa protein from both fractions bound WGA (Fig. 3B). These three proteins are the most concentrated in the peak fractions and are not present in the corresponding fractions from uninfected plants, suggesting that they are viral structural proteins.

The reason for two different sized particles is unclear. However, different sedimenting classes of particles also have been observed in preparations of PYDV (1) and SYNV (16) and have been speculated to be defective interfering particles. We currently are performing more detailed studies to investigate whether this is also the case with SCV.

When the structural proteins of SCV were compared with other plant rhabdoviruses, the protein profile of SCV more closely resembled that of LNYV than SYNV (Fig. 4). Based on physicochemical properties (7), the LNYV proteins have been designated the envelope glycoprotein (G), the nucleocapsid protein (N), and the matrix protein (M) to correspond with the nomenclature proposed for rhabdovirus proteins (30). Because the PAGE patterns of the SCV proteins resemble those of LNYV, we have used corresponding designations for the SCV structural proteins. As is common with rhabdoviruses, the structural proteins of SCV and LNYV, two viruses that undergo morphogenesis in the cytoplasm, differed in size and staining patterns from the four major proteins of SYNV, which is a nuclear-associated rhabdovirus (18). The four SYNV proteins have been designated G, N, M1, and M2, respectively (17).

As an additional comparison, the proteins of SCV, LNYV, and SYNV were examined for their ability to bind lectins. The viruses were similar in that their putative G proteins all bind WGA (Fig. 5A), conA (Fig. 5B), LEA (Fig. 5C), and PSA (Fig. 5D). WGA and LEA will bind to N-acetyl-β-D-glucosamine

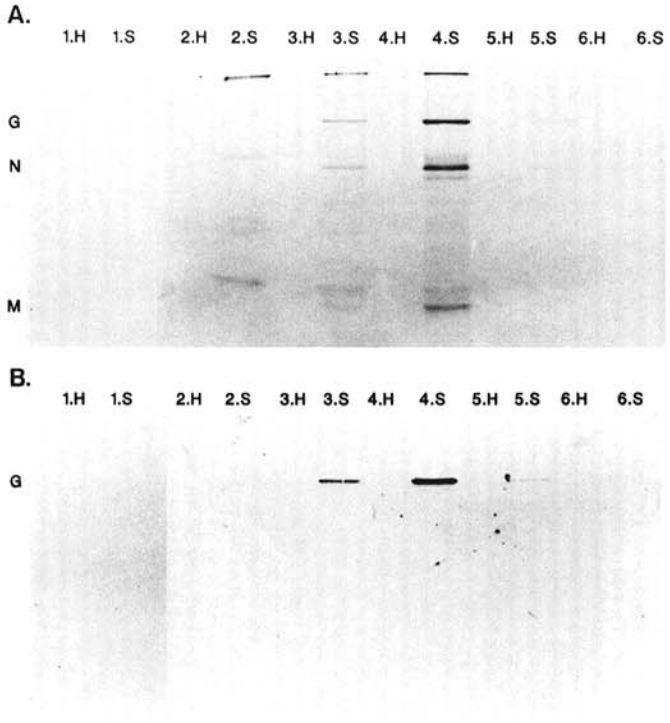


Fig. 3. Gel analysis of fractions from sucrose gradients. Samples from the gradients as indicated in Figure 2A were denatured and electrophoresed through 12.5% polyacrylamide gels. (H = healthy plants; S = plants infected with strawberry crinkle virus [SCV].) The material at the top of the lanes is protein that collected at the interface between the stacking and resolving gels. **A,** The gel was stained with Coomassie Brilliant Blue. **B,** The proteins were transferred to nitrocellulose and reacted with wheat germ agglutinin. G = SCV glycoprotein; N = nucleocapsid protein; M = membrane protein.

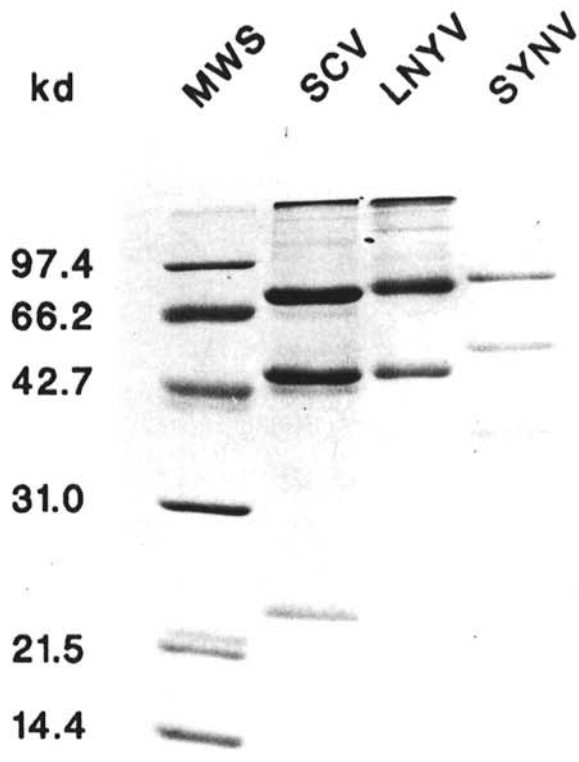


Fig. 4. Comparison of rhabdovirus structural proteins. Disrupted virions from strawberry crinkle virus (SCV), lettuce necrotic yellows virus (LNYV), and sonchus yellow net virus (SYNV) were electrophoresed through polyacrylamide gels and stained with Coomassie Brilliant Blue.

oligomers (3,22,23); PSA recognizes mannose residues (29); and conA recognizes both mannose and glucose, as well as a number of other sugars (14). The specific binding of these lectins to the viral glycoprotein suggests that at least the sugars n-acetyl-β-D-glucosamine and mannose are present. Lectins have been used previously to demonstrate the presence of carbohydrate on LNYV (7), as well as PYDV and eggplant mottled dwarf virus (2).

Although the glycoproteins of SCV, LNYV, and SYNV, isolated from solanaceous host plants in the genera *Physalis* and *Nicotiana*, contain similar carbohydrate moieties, they are serologically distinct. Ascites fluid antibodies raised against gel-purified SCV G failed to cross react with either LNYV or SYNV G (Fig. 6). Antisera raised against purified LNYV and SYNV also did not cross react with the other rhabdoviruses, thus illustrating that these viruses do not have a close serological relationship.

Preliminary experiments using the SCV-G ascites antibodies to detect the viral protein in unconcentrated extracts from *P. floridana* and strawberry by Western analysis were unsuccessful, using conditions where SYNV can be detected (data not shown). This may reflect a lower concentration of SCV in infected tissue compared with SYNV and hence a lower yield obtained during purification of SCV. We are beginning to clone SCV RNA with the objective of providing cDNA probes suitable for virus detection. The recombinant clones should enable us to extend diagnostic capabilities and carry out important ecological and epidemiological studies of the virus in the plant hosts and aphid vectors.

LITERATURE CITED

1. Adam, G., Gaedigk, K., and Mundry, K. W. 1983. Alterations of a plant rhabdovirus during successive mechanical transfers. Z. Pflanzenkrankh. Pflanzenschutz 90:28-35.
2. Adam, G., Heegard, P., Bog-Hansen, T. C., and Mundry, K.-W. 1987. Lectins as probes for the assay of rhabdovirus infections in plants. J. Virol. Methods 17:263-275.
3. Allen, A. K., Neuberger, A., and Sharon, N. 1973. The purification, composition and specificity of wheat germ agglutinin. Biochem. J. 131:155-162.
4. Converse, R. H. 1987. Detection and elimination of virus and viruslike diseases in strawberry. Pages 2-10 in: Virus Diseases of Small Fruits. R. H. Converse, ed. Agric. Handb. 631 (U.S. Dep. Agric. Sci. Educ. Adm.).
5. Converse, R., Adams, A. N., Barbara, D. J., Clark, M. F., Caspar, R., Hepp, R. F., Martin, R. R., Morris, T. J., Spiegel, S., and Yoshikawa, N. 1988. Laboratory detection of viruses and mycoplasmalike organisms in strawberry. Plant Dis. 72:744-749.
6. Dietzgen, R. G., and Francki, R. I. B. 1987. Nonspecific binding of immunoglobulins to coat proteins of certain plant viruses in immunoblots and indirect ELISA. J. Virol. Methods 15:159-164.
7. Dietzgen, R. G., and Francki, R. I. B. 1988. Analysis of lettuce necrotic yellows virus structural proteins with monoclonal antibodies and concanavalin A. Virology 166:486-494.
8. Dunham, L. J., and Stewart, H. L. 1953. A survey of transplantable and transmissible animal tumors. J. Natl. Cancer Inst. (U.S.) 13:1299-1377.
9. Falk, B. W., and Tsai, J. H. 1983. Physicochemical characterization of maize mosaic virus. Phytopathology 73:1536-1539.

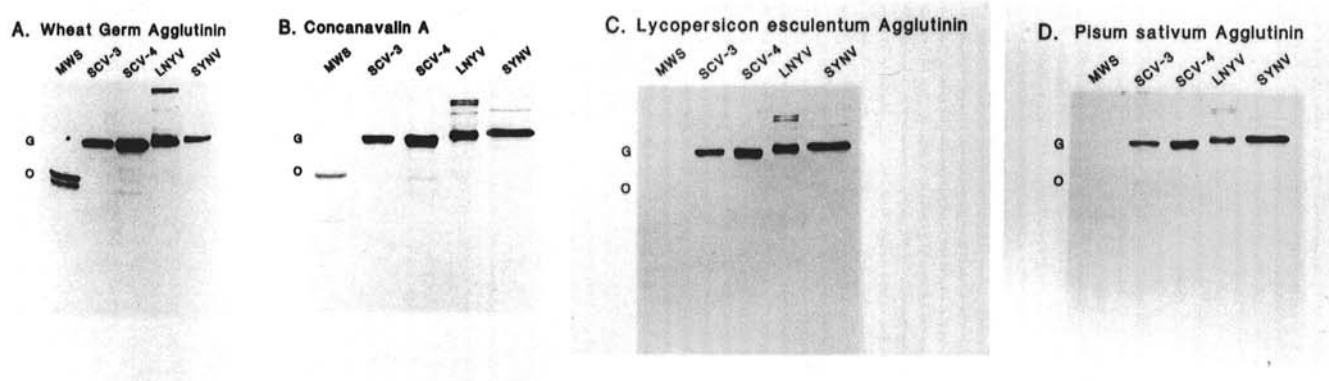


Fig. 5. Lectin analysis of rhabdovirus glycoproteins. Strawberry crinkle virus (SCV) from the gradient fractions 3 and 4, lettuce necrotic yellows virus (LNYV), and sonchus yellow net virus (SYNV) were disrupted, electrophoresed through polyacrylamide gels, transferred to nitrocellulose, and reacted with lectins. The proteins were reacted with: **A,** wheat germ agglutinin; **B,** concanavalin A; **C,** agglutinin of *Lycopersicon esculentum*; and **D,** agglutinin of *Pisum sativum*. G = glycoprotein; O = ovalbumin in the molecular-weight standards.

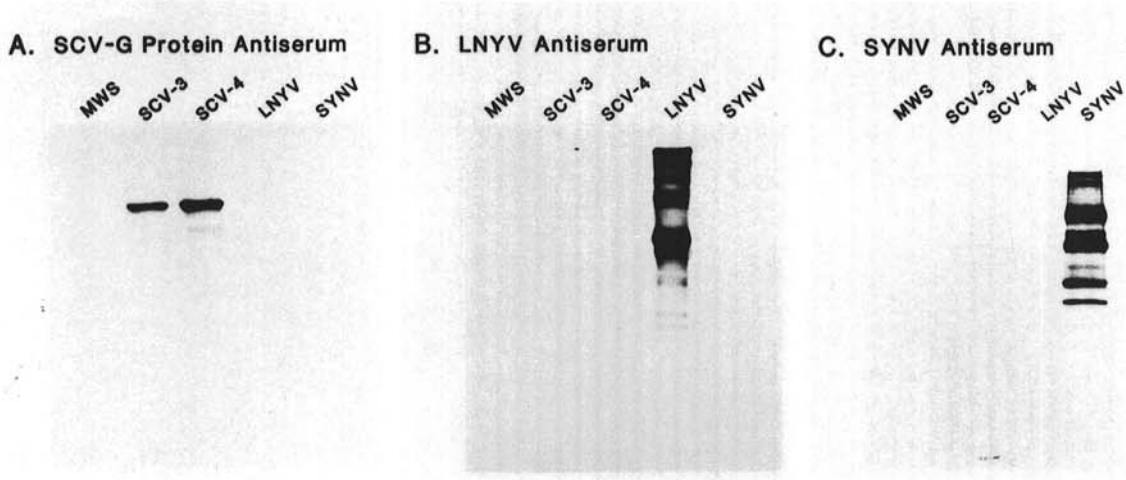


Fig. 6. Serological analysis of strawberry crinkle virus (SCV). Samples from SCV gradient fractions 3 and 4, lettuce necrotic yellows virus (LNYV), and sonchus yellow net virus (SYNV) were disrupted, electrophoresed through polyacrylamide gels, transferred to nitrocellulose, and reacted with the various antibodies. **A,** Reaction of the glycoproteins with a 1/500 dilution of mouse ascites fluid raised against SCV glycoprotein. **B,** Rabbit serum against LNYV was used at a 1/5,000 dilution. **C,** Rabbit serum against SYNV was used at a 1/400 dilution.

10. Falk, B. W., Weathers, L. G., and Greer, F. C. 1981. Identification of potato yellow dwarf virus occurring naturally in California. Plant Dis. 65:81-83.
11. Francki, R. I. B. 1973. Plant rhabdoviruses. Pages 257-345 in: Advances in Virus Research. M. A. Lauffer, F. B. Bang, K. Maramorosch, and K. M. Smith, eds. Academic Press, New York.
12. Francki, R. I. B., Randles, J. W., and Dietzgen, R. G. 1989. Lettuce necrotic yellows virus. No. 343 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England.
13. Frazier, N. W., Sylvester, E. S., and Richardson, J. 1987. Strawberry crinkle. Pages 20-25 in: Virus Diseases of Small Fruits. R. H. Converse, ed. Agric. Handb. 631 (U.S. Dep. Agric. Sci. Educ. Adm.).
14. Goldstein, I. J., Hollerman, C. E., and Smith, E. E. 1965. Protein-carbohydrate interaction. II. Inhibition studies on the interaction of concanavalin A with polysaccharides. Biochemistry 4:876-883.
15. Harlow, E., and Lane, D. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
16. Ismail, I. D., and Milner, J. J. 1988. Isolation of defective interfering particles of sonchus yellow net virus from chronically infected plants. J. Gen. Virol. 69:999-1006.
17. Jackson, A. O. 1978. Partial characterization of the structural proteins of sonchus yellow net virus. Virology 87:172-181.
18. Jackson, A. O., and Christie, S. R. 1977. Purification and some physicochemical properties of sonchus yellow net virus. Virology 77:344-355.
19. Jackson, A. O., Francki, R. I. B., and Zuidema, D. 1987. Biology, structure, and replication of plant rhabdoviruses. Pages 427-508 in: The Rhabdoviruses. R. R. Wagner, ed. Plenum Publishing Corp., New York.
20. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680-685.
21. McLean, G. D., Wolanski, B. S., and Francki, R. I. B. 1971. Serological analysis of lettuce necrotic yellows virus preparations by immunodiffusion. Virology 43:480-487.
22. Nachbar, M. S., Oppenheim, J. D., and Thomas, J. O. 1980. Lectins in the U.S. diet: Isolation and characterization of a lectin from the tomato (*Lycopersicon esculentum*). J. Biol. Chem. 255:2056-2061.
23. Nagata, Y., and Burger, M. M. 1974. Wheat germ agglutinin: Molecular characteristics and specificity for sugar binding. J. Biol. Chem. 249:3112-3116.
24. Richardson, J., Frazier, N. W., and Sylvester, E. 1972. Rhabdovirus-like particles associated with strawberry crinkle virus. Phytopathology 62:491-492.
25. Richardson, J., and Sylvester, E. S. 1988. Successful juice inoculation of the aphid-vectored strawberry crinkle virus. Calif. Agric. 42:6-7.
26. Stenger, D. C., Richardson, J., Sylvester, E. S., Jackson, A. O., and Morris, T. J. 1988. Analysis of sowthistle yellow vein virus-specific RNAs in infected hosts. Phytopathology 78:1473-1477.
27. Sylvester, E. S., Richardson, J., and Stenger, D. C. 1987. Use of injected *Macrosiphum euphorbiae* aphids as surrogate vectors for transfer of strawberry crinkle virus to *Nicotiana* species. Plant Dis. 71:972-975.
28. Towbin, H., Staehlin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. 76:4350-4354.
29. Trowbridge, I. S. 1974. Isolation and chemical characterization of a mitogenic lectin from *Pisum sativum*. J. Biol. Chem. 249:6002-6012.
30. Wagner, R. R., Prevec, L., Brown, F., Summers, D. F., Sokol, F., and MacLeod, R. 1972. Classification of rhabdovirus proteins: A proposal. J. Virol. 10:1228-1230.
31. Zeller, S. M., and Vaughan, E. K. 1932. Crinkle disease of strawberry. Phytopathology 22:709-713.