A Closterovirus from a Stem-Pitting-Diseased Grapevine

Maurizio Conti, Robert G. Milne, Enrico Luisoni, and Guido Boccardo

Laboratorio di Fitovirologia applicata del CNR, Via O. Vigliani 104, 10135 Turin, Italy.

We thank M. Bar-Joseph, A. A. Brunt, M. F. Clark, D. A. Govier, B. D. Harrison, and A. F. Murant for antisera; M. F. Clark for apple stem grooving virus; L. Giunchedi for healthy cuttings of cultivar St. George grapevine; and K. Yora for sending us his paper before publication.

We thank M. d'Aquilio, A. Casetta, R. Lenzi, and V. Masenga for skilled technical assistance.

Accepted for publication 24 October 1979.

ABSTRACT

CONTI, M., R. G. MILNE, E. LUISONI, and G. BOCCARDO. 1980. A closterovirus from a stem-pitting-diseased grapevine. Phytopathology 70:394-399.

A closterovirus was isolated in *Nicotiana clevelandii* following mechanical inoculation from a grapevine with stem-pitting symptoms. The virus was easily mechanically transmissible thereafter between *N. clevelandii* plants, in which it caused vein clearing and stunting. Leaf crinkle symptoms appeared in *N. megalosiphon* but the virus was not transmitted to 22 other herbaceous plant species. In *N. clevelandii* sap, the virus lost infectivity when heated to 50 C for 10 min, diluted with water to 10^{-5}, or stored for 6 days at 20 C or for 15 days at 5 C. The virus was not seed transmitted in *N. clevelandii*, and was not transmitted by *Myzus persicae* or *Macrosiphum euphorbiae*. It was purified best by extraction in 0.05 M tris-HCl buffer, pH 7.8, containing 0.01 M $MgSO_4$, clarification with bentonite, precipitation with polyethylene glycol, and rate-zonal density gradient centrifugation in Cs_2SO_4. In uranyl acetate negative stain, the very flexous particles had a diameter of 11–12 nm, a clearly visible pitch of 3.6–4.0 nm, and a modal length near 800 nm. The modal length was similar in neutral sodium phosphotungstate. The particle structure was typical of closteroviruses. Three antisera (bleedings from one rabbit) gave titers up to 1/64 in slide precipitin tests, and 1/512 in immunoelectron microscopic decoration tests. The virus was not serologically related to apple chlorotic leafspot, beet yellows, carnation yellow fleck, heracleum latent, lilac chlorotic leafspot, apple stem grooving, or potato T viruses.

Additional key words: immunoelectron microscopy, purification, serology.

Grapevine stem-pitting (GSP) disease, first described as "legno riccio" in Italy (8,13) has been reported in several European countries, Israel, California, and South Africa (4,14). Affected plants show stunting, abnormal bark rugosity, and stem pitting; spring bud burst is delayed; and there is an imbalance between rootstock and scion growth.

The GSP pathogen is graft-transmissible, but the etiology of the disease is obscure. Procaryotes as causative agents seem to be excluded (1,14), but tomato ringspot virus (22) and grape fanleaf virus (GFV) (10,14,15) sometimes have been found associated with the disease, which may even be due to synergism between two or more agents. Present knowledge on the disease is discussed in (1).

We have attempted to recover viruses present in grapevines with GSP by both tissue insertion and sap inoculation to herbaceous hosts. Tissue insertion was always unsuccessful, but of 47 plants checked by sap inoculation, four yielded GFV and one gave a virus with flexuous filamentous particles characteristic of the closterovirus group. This paper describes some properties of this virus, referred to provisionally as GSP-associated virus (GSP-AV).

MATERIALS AND METHODS

Apple stem grooving virus (ASGV). This virus was inoculated to leaves of *Chenopodium quinoa* Willd., and the resulting local lesions were used as source of virus for comparisons with GSP-AV. The virus was purified by method A (below).

Source and isolation of GSP-AV. The following grapevine tissues were tested: (i), young apical leaves from field plants in spring; (ii), opening buds on glasshouse-grown cuttings raised in sterilized perlite (Perles, V. I. C. Italiana SpA., Viale Maino 3, Milan) and watered with sterile water; (iii), young roots of the above cuttings; (iv), grape skins; and (v), cortex plus underlying cambium from the stem of a field grown plant in autumn.

The tissues were ground with a pestle and mortar in an equal volume (w/v) of 2.5% nicotine in 0.1 M phosphate buffer, pH 7.2 (NP buffer). The extract was further diluted with three volumes of phosphate buffer before mechanical inoculation. In addition, tissues (ii) and (iv) above were extracted in two volumes of 0.02 M HEPES buffer (Calbiochem, San Diego, CA 92112) pH 8, mixed with 5% polyvinyl pyrrolidone (6), and tissue (v) was extracted in 0.1 M Na_2SO_3 brought to pH 8.1 with KH_2PO_4 (11). Extracts were inoculated to *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Gomphrena globosa* L., *Nicotiana clevelandii* Gray, and *N. glutinosa* L.

Host range. Following inoculation from grapevine, GSP-AV was recovered only in *N. clevelandii*. Twenty-three other herbaceous test plants were inoculated with GSP-AV isolated from this host and suspended in 0.01 M phosphate buffer, pH 7. Plants were later checked by back-inoculation to *N. clevelandii* and by electron microscopy.

Young healthy rooted cuttings of grapevine cultivar St. George were inoculated with either GSP-AV alone, GFV alone, or with both. The inocula were prepared from leaves of *N. clevelandii* homogenized in 0.01 M phosphate buffer, pH 7. Control cuttings were inoculated with healthy plant extracts. There were 18 cuttings in each group. All cuttings were checked by back-inoculation to *N. clevelandii* using NP buffer.

Properties in vitro. The thermal inactivation point and longevity in vitro were determined in crude sap. The dilution end point was determined by dilution in distilled water. *N. clevelandii* was used as both virus donor and indicator.

Seed and aphid transmissibility. Seeds from infected *N. clevelandii* were sown in sterilized soil shortly after harvest. The seedlings were checked for symptoms, and tested 3–4 wk after emergence by sap inoculation to *N. clevelandii*, and by electron microscopy.

GSP-AV was tested for aphid transmission in the nonpersistent (acquisition, 60 sec; inoculation, 12 hr), semipersistent (acquisition, 16 hr; inoculation, 24 hr), and persistent modes (acquisition, 24 hr; incubation, 24 and 48 hr; inoculation, 24 hr) with both *Myzus persicae* Sulzer and *Macrosiphum euphorbiae* Thomas. Three to five virus-exposed aphids were used per plant; for each aphid

0031-949X/80/05039406/$03.00/0

species 35, 45, and 30 plants were used for the nonpersistent, semipersistent, and persistent tests, respectively.

Purification. GSP-AV was purified from freshly harvested leaves of systemically infected *N. clevelandii* inoculated 3 wk previously. About 50 g of leaves were used in each experiment. Two methods were tested.

Method A. Leaves were ground in the cold with three volumes (w/v) of 0.05 M tris-HCl buffer, pH 7.8, containing 0.01 M $MgSO_4$ (TM buffer). The homogenate was filtered through nylon cloth and centrifuged in the Sorvall GSA rotor at 4,000 rpm for 10 min, then at 8,000 rpm for an additional 10 min (2). The supernatant was clarified by adding 3.5 ml of bentonite suspension (17) to every 100 ml, followed by centrifugation at 10,000 rpm for 10 min in the GSA rotor. Five grams of polyethylene glycol, molecular weight 6,000 (PEG-6000) and 0.5 ml 5 M NaCl were added to each 100 ml of supernatant, and the mixture was placed on ice for 2–3 hr then centrifuged at 10,000 rpm for 15 min in the GSA rotor. The pellets were taken up in 10 ml of TM buffer, and centrifuged at 10,000 rpm for 10 min in the Sorvall SS-34 rotor. The supernatant was layered onto a preformed 25–40% linear gradient of Cs_2SO_4 in 0.01 M phosphate buffer, pH 7.5, and centrifuged at 37,000 rpm for 150 min in a Beckman SW41 rotor. The lowest band, found by electron microscopy to consist of the virus particles, was withdrawn; the Cs_2SO_4 was removed either by overnight dialysis against 0.05 M phosphate buffer, pH 7.5, or by dilution followed by pelleting at 40,000 rpm for 60 min in a Beckman rotor 40, and resuspension in the same buffer.

Method B. Leaves were homogenized with three volumes of 0.5 M phosphate buffer, pH 7.5, containing 0.005 M EDTA, 0.01 M DIECA, and 0.02 M Na_2SO_3. The homogenate was filtered through nylon cloth, shaken for 10 min with an equal volume of chloroform, and centrifuged at low speed. The virus was precipitated from the aqueous phase with PEG-6000 and the pellets were resuspended in the extraction buffer without the additives. The suspension was centrifuged at 10,000 rpm for 10 min in the SS-34 rotor, and the supernatant was centrifuged into a Cs_2SO_4 density gradient as in method A.

Electron microscopy. Grapevine extracts, and crude and purified virus preparations from *N. clevelandii* were touched to Formvar-backed carbon films on 38μm (400-mesh) grids. These were rinsed with water, negatively stained with 2% aqueous uranyl acetate (UA) or 2% neutral aqueous sodium phosphotungstate (PTA), and examined in a Philips EM 300 electron microscope calibrated with a diffraction grating replica and also with tobacco mosaic virus particles. Negatives taken at approximately ×20,000 were projected at a magnification of ×8 and the images were traced on paper. The tracings were measured with a map measurer passed over each tracing three times. The structures of GSP-AV and ASGV particles were compared by mixing the partially purified viruses and negatively staining the mixture in UA.

Serology. A rabbit was injected intramuscularly with virus purified by method B and emulsified with an equal volume of Freund's complete adjuvant. The rabbit was bled after 22 days (serum I) and then received two more injections, both with incomplete adjuvant, the first intramuscularly and the second in the footpads, 67 and 80 days after the first injection. Bleedings were made 6 days (serum II) and 15 days (serum III) after the last injection. The homologous titers of these sera were determined by the slide precipitin test and by immunoelectron microscopy (IEM) using the decoration test (18,19). Agar gel double diffusion, with 0.7% Difco Noble agar in phosphate buffered saline containing 0.02% sodium azide, was used to determine the titers against normal plant constituents, and to distinguish the reactions due to intact and disaggregated virus.

The IEM decoration test was used to check GSP-AV against antisera to potato virus T (PVT) (from B. D. Harrison), heracleum latent virus (from A. F. Murant), apple chlorotic leafspot virus (ACLV) and ASGV (from M. F. Clark), carnation yellow fleck virus (from M. Bar-Joseph), lilac chlorotic leafspot virus (LCLV) (from A. A. Brunt), and beet yellows virus (from D. A. Govier). The test also was used to show whether ASGV reacted with the GSP-AV antisera. For literature on the above viruses, see (3,5,9,16).

RESULTS

GSP-AV was isolated only on one occasion, when *N. clevelandii* plants were inoculated with young roots of glasshouse-grown cuttings from a plant of cultivar Pigato grape growing near Savona in northern Italy. The cuttings had been taken in winter 1977, kept at 4 C, and tested in April 1978. Further attempts to recover the virus from the same or other plants by all methods listed have so far been unsuccessful.

The symptoms of GSP-AV in *N. clevelandii,* appearing 3–4 wk after inoculation, were vein clearing on the young leaves followed by general vein clearing and rosetting (Fig. 1). In subsequent passages in *N. clevelandii*, the incubation period shortened to about 2 wk, and in winter symptoms became more severe. Leaf crinkle symptoms appeared in *N. megalosiphon* Huerck & Muell.

Host range. Besides *N. clevelandii* and *N. megalosiphon,* no other species was infected. Those tested were: *Amaranthus*

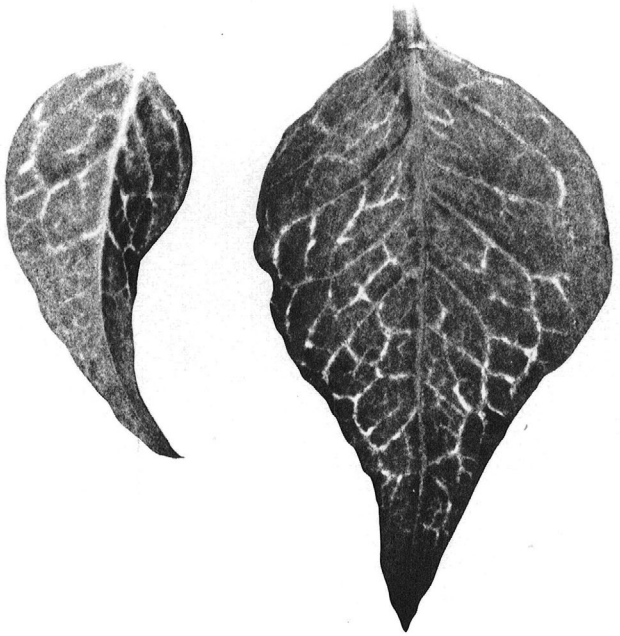

Fig. 1. Vein clearing on leaves of *Nicotiana clevelandii* systemically infected with grapevine stem pitting-associated virus.

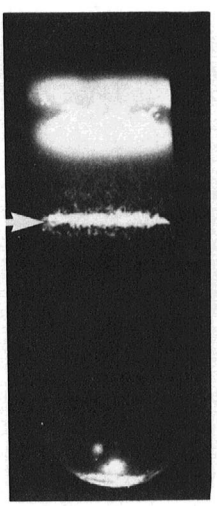

Fig. 2. Band of purified grapevine stem pitting-associated virus (arrowed) after rate zonal centrifugation in a preformed density gradient of Cs_2SO_4.

caudatus L., *A. hybridus* L., *Capsicum annuum* L., *Chenopodium album* L., *C. amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Cucumis sativus* L., *Datura stramonium* L., *Gomphrena globosa* L., *Lycopersicum esculentum* Mill., *Nicotiana alata* Link & Otto, *N. debneyi* Domin., *N. glauca* R. Grah., *N. glutinosa* L., *N. rustica* L., *N. tabacum* L. 'White Burley', *Ocimum basilicum* L., *Phaseolus vulgaris* L. 'Saxa', *Solanum melongena* L., *Tetragonia expansa* Murr., *Catharanthus roseus* (L.) G. Don (formerly *Vinca rosea* L.), and *Vigna unguiculata* (L.) Walp. Back inoculation to grapevine has also so far not proved successful. Neither GSP-AV nor GFV has been recovered from cuttings inoculated up to 9 mo previously.

Properties in vitro. The virus lost infectivity when heated for 10 min at 50 C but not 45 C, diluted to 10^{-5} but not 10^{-4}, or stored at 20 C for 6 but not 4 days, or at 5 C for 15 but not 12 days.

Seed and aphid transmissibility. No virus infection was detected in over 200 seedlings from 12 infected *N. clevelandii* mother plants. These plants were either self-pollinated or pollinated by other infected plants in the same group, which was maintained in isolation.

The virus was not transmitted by *Myzus persicae* or *Macrosiphum euphorbiae* in any of our tests.

Purification. Both purification methods tested were successful but method A was slightly better, judging by infectivity, serology, electron microscopy, and the appearance of the virus-containing band after density gradient centrifugation. This band (arrowed in Fig. 2) consisted of purified virus. Attempts to use sucrose instead of Cs_2SO_4 for the gradient failed as no clear virus-containing zone resulted. The virus was destroyed by high concentrations of CsCl. As noted by Smookler and Loebenstein (21), high-speed mechanical blending of the starting material resulted in severe particle breakage. A sample of purified virus is shown in Fig. 3.

Electron microscopy. GSP-AV was easily detected in the crude sap of *N. clevelandii* but was not detected in that of grapevine. The particles (Fig. 3) were very flexuous, with typical closterovirus form and structure, and a diameter of 11–12 nm. The modal lengths of particles (Fig. 4) in both crude and purified preparations, negatively stained in UA or PTA, were all close to 800 nm, within the calibration error of about 4%. In UA, the particles had a pitch of about 3.7 nm (range 3.6–4.0).

Fig. 5 shows representative particles from an artificial mixture of

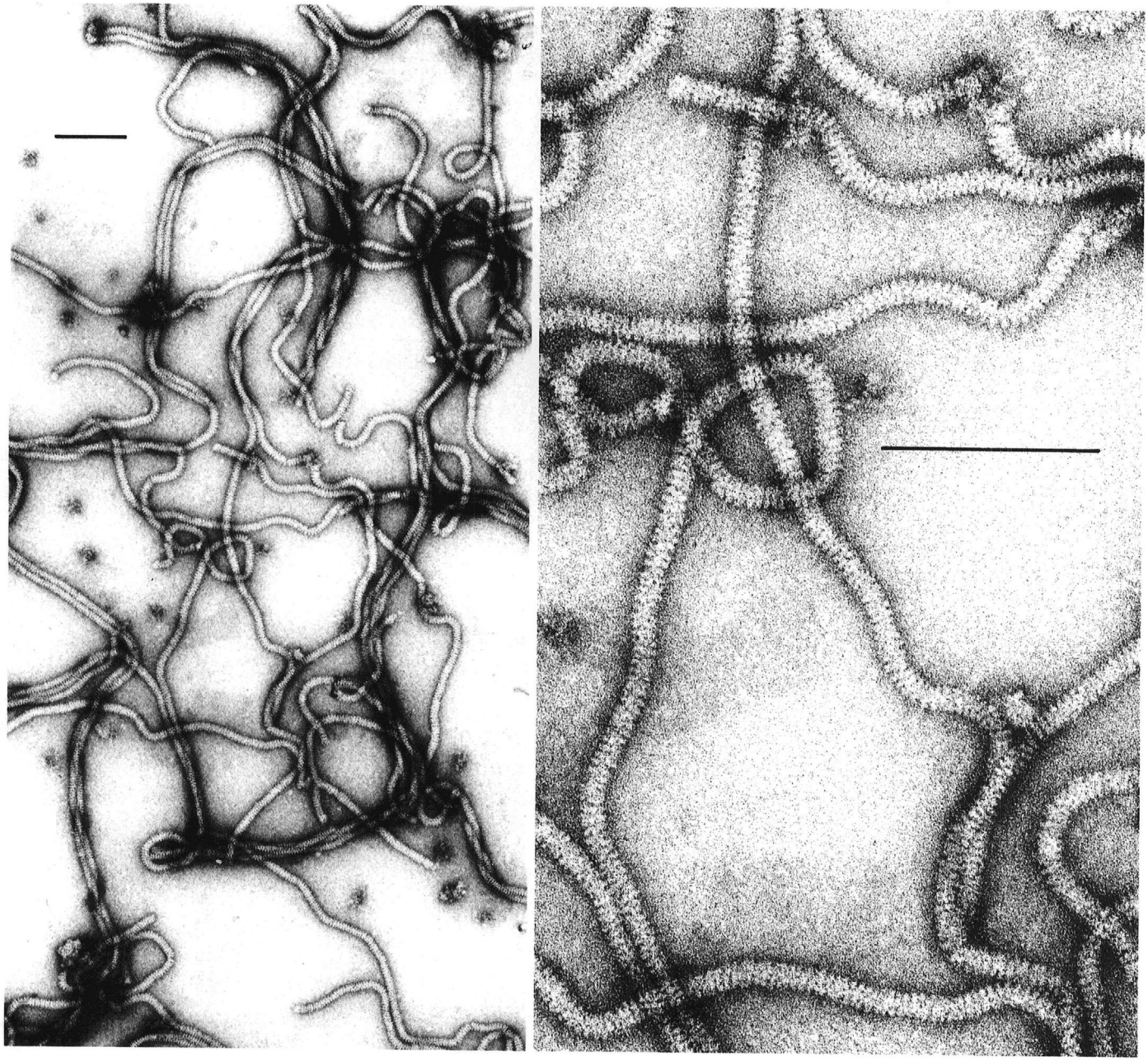

Fig. 3. A sample of purified grapevine stem pitting-associated virus at two magnifications. The bars represent 100 nm. Uranyl acetate negative stain.

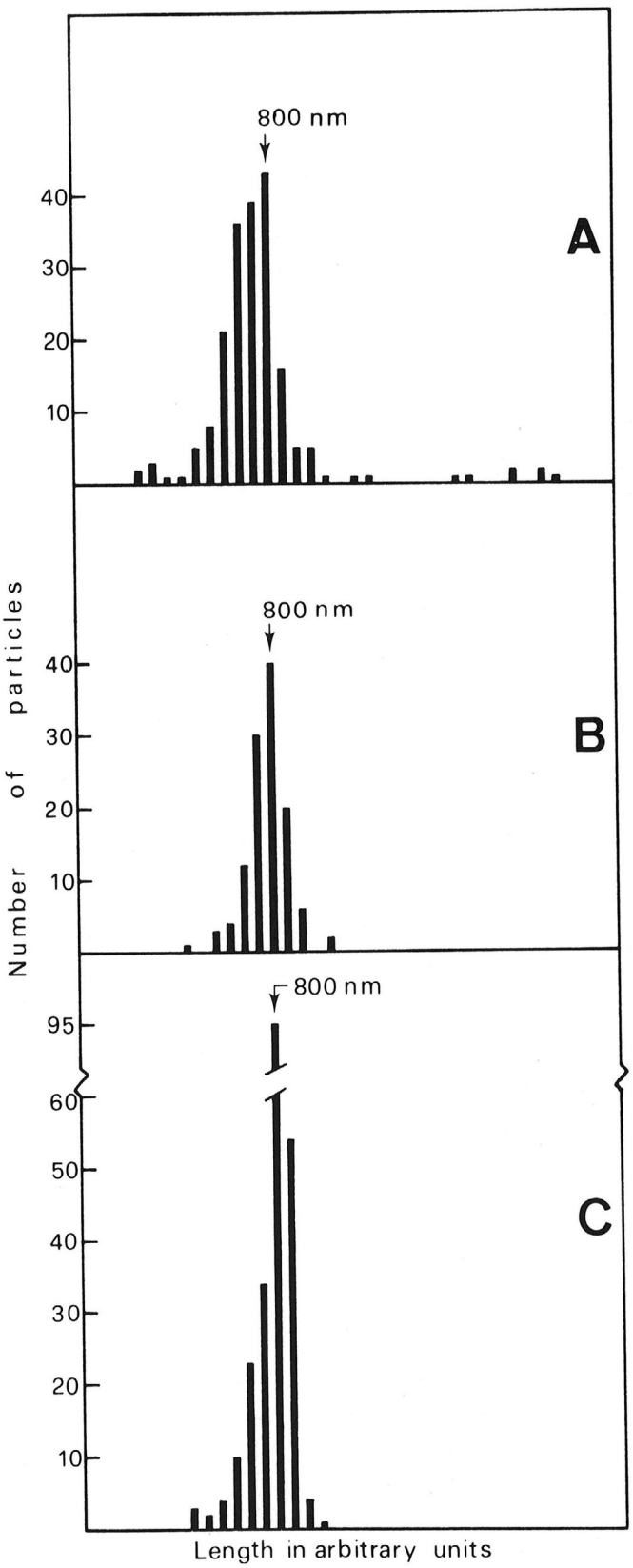

Fig. 4. Length histograms of grapevine stem pitting-associated virus particles. **A,** combined lengths of 98 particles from a crude preparation and 97 particles from a purified preparation, both in uranyl acetate; **B,** lengths of 118 particles from a crude preparation in sodium phosphotungstate (PTA); and **C,** lengths of 230 particles from a purified preparation, in PTA. The modal lengths of all preparations were near 800 nm and the class interval was 30 nm.

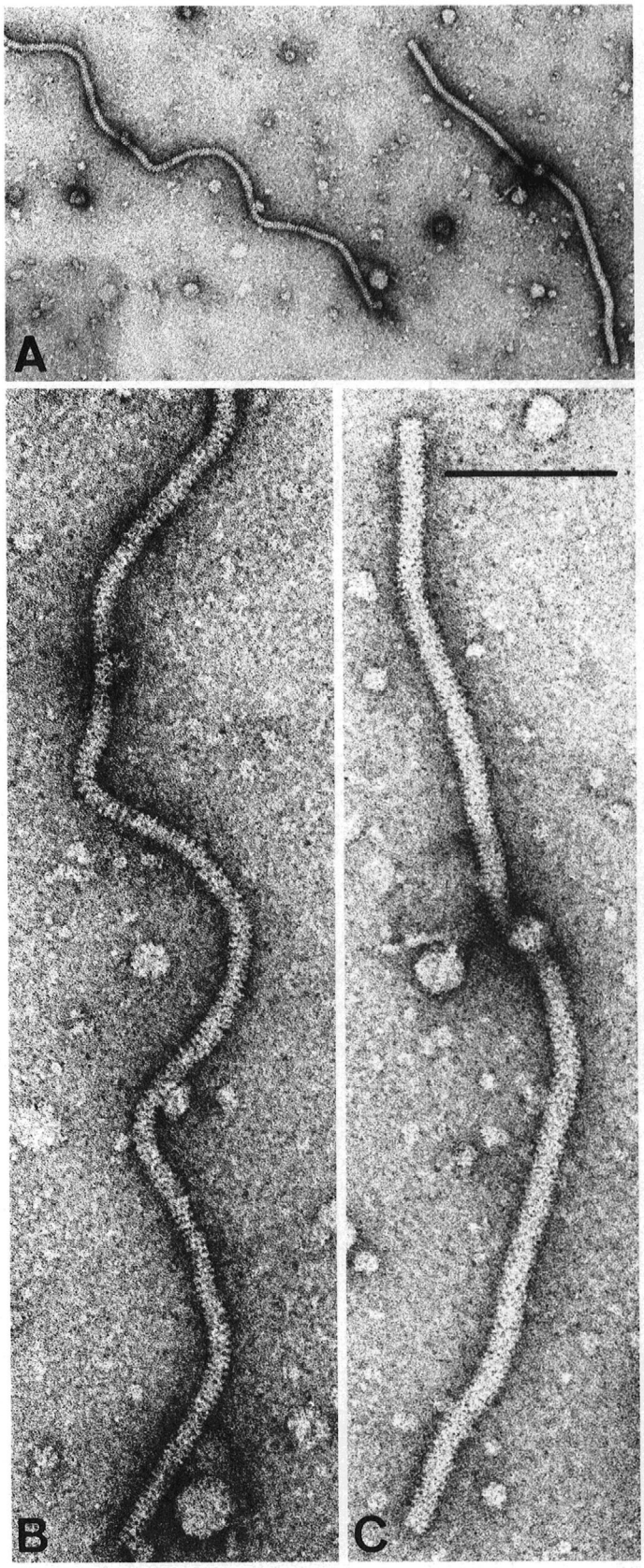

Fig. 5. Particles of grapevine stem pitting-associated virus (GSP-AV) and apple stem-grooving virus (ASGV) from an artificial mixture co-imaged in uranyl acetate. **A,** two particles on the same negative; **B** and **C,** the same particles equally enlarged, those of GSP-AV and ASGV, respectively. The GSP-AV particle is more than full length, due to end-to-end aggregation. The bar represents 100 nm.

partially purified GSP-AV and ASGV. There is a notable difference in structure. Specifically, the ratio of particle diameters (ASGV/GSP-AV) was about 1.2, and that of the pitches of the primary helices was about 0.87. GSP-AV particles were much more flexible than those of ASGV.

Serology. The sera I, II, and III reacted with GSP-AV preparations in slide precipitin tests up to 1/16, 1/32, and 1/64, respectively. In the IEM decoration test they had titers of 1/128, 1/256, and 1/512, respectively, against intact virus particles. In agar gel diffusion tests the sera reacted with normal plant constituents only when undiluted. All three sera, when absorbed with healthy sap, reacted in gel diffusion tests with virus preparations forming a band near the antigen well. Sera II and III gave, in addition, one or two bands near the antiserum well (Fig. 6). The different bands were cut out, crushed in phosphate-buffered saline, negatively stained, and examined in the electron microscope. The band formed near the antigen well was seen to consist of clumped, approximately full-length virus particles (Fig. 7); the other bands contained clumps of very fine material, probably disassembled coat protein.

The IEM decoration test was used to assess whether GSP-AV was serologically related to the antisera or viruses listed under negative, although the homologous reactions were strongly positive in those cases we were able to check. An example of the decoration test is seen in Fig. 8.

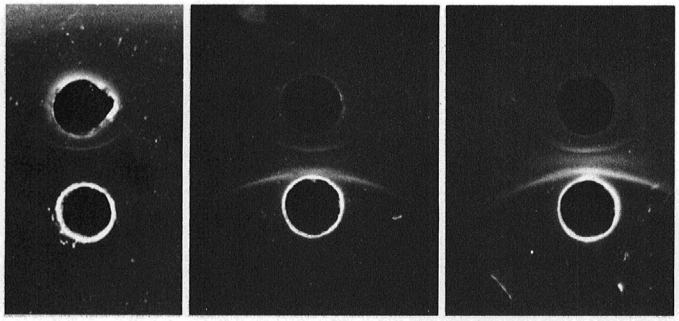

Fig. 6. Agar gel double diffusion reactions of purified grapevine stem pitting-associated virus (upper wells) with homologous antisera I, II, and III in the lower left, center, and right wells, respectively. The bands near the antigen wells are formed from undegraded virus (see Fig. 7). The other bands are formed from more rapidly diffusing viral degradation products.

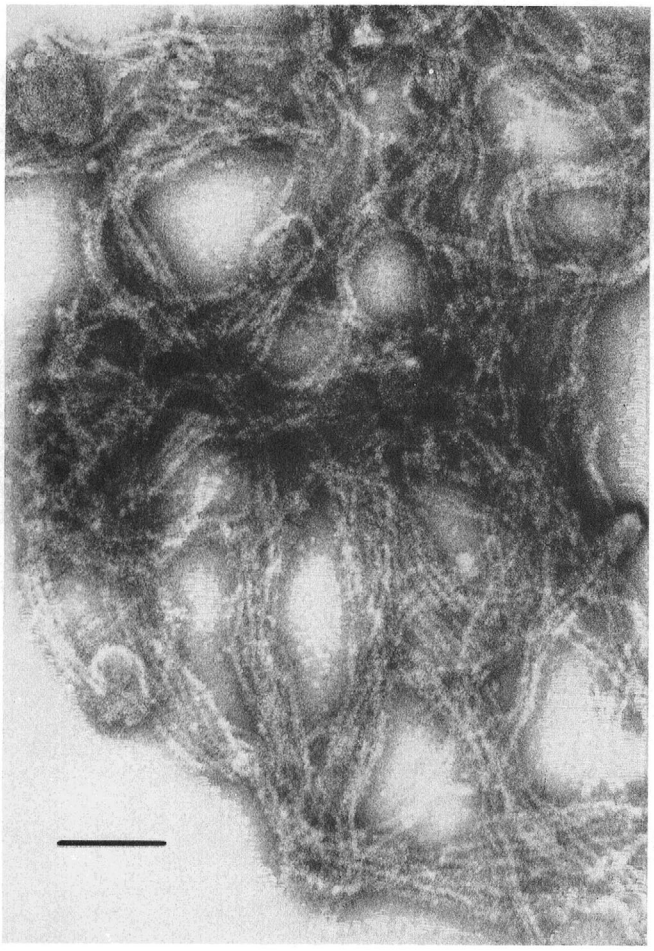

Fig. 7. Undegraded grapevine stem pitting-associated virus particles clumped by antibodies, forming the bands near the antigen wells in Fig. 6, negatively stained in uranyl acetate. The bar represents 200 nm.

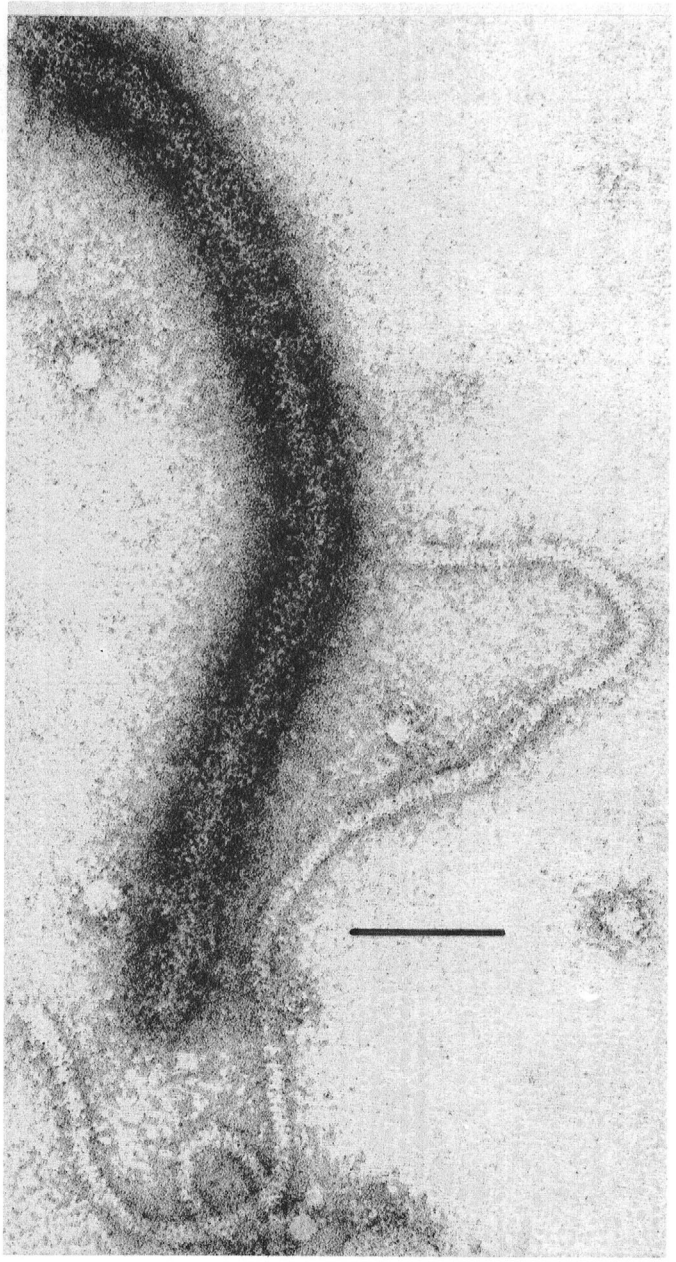

Fig. 8. Example of the immunoelectron microscopic decoration test. Apple stem-grooving virus (ASGV) and grapevine stem pitting-associated virus (GSP-AV) particles were mixed, placed on the electron microscope grid, incubated with undiluted ASGV antiserum, and negatively stained in uranyl acetate. The bar represents 100 nm. The ASGV particle is heavily decorated, but the GSP-AV particle has not reacted.

DISCUSSION

The morphology and fine structure of the particles, the symptoms in *N. clevelandii* and *N. megalosiphon*, the restricted host range, and the properties in vitro only partly characterize GSP-AV, but do indicate that it is a closterovirus (9).

If we exclude the atypical LCLV, ASGV, and PVT, all currently recognized closteroviruses are aphid-transmitted except ACLV and GSP-AV. If GSP-AV is a grapevine virus, then both this and ACLV would come from vegetatively propagated woody hosts, and vegetative propagation could have encouraged the loss of aphid transmissibility.

We were interested to see whether ASGV, superficially similar morphologically to GSP-AV, resembled it in structural detail when co-imaged in UA. In fact, it appeared rather different, confirming the suggestion (20) that ASGV and the serologically related PVT are not typical closteroviruses.

Serologically, GSP-AV is not related to any of the likely viruses tested, although citrus tristeza virus antisera have not yet been tried, and a relationship with other strains of, for example, ACLV (7) is not yet excluded.

The agar gel double diffusion test, rather unsuccessful with many elongated viruses, worked with GSP-AV and its antisera, the band forming near the antigen well consisting of clumped virus particles. Chairez and Lister (7) considered that ACLV did not readily diffuse in agar unless degraded, and that it became degraded in the presence of NaCl. However, we observe from their Fig. 1, and from Fig. 9 of Lister and Hadidi (17) that the ACLV band near the antigen well occurred in the same form and position as the corresponding band of GSP-AV intact particles in our experiments. Therefore, we suggest that ACLV, like GSP-AV, can diffuse in agar when intact, perhaps due to particle flexibility, and that, in physiological saline and in the absence of Mg^{++}, a significant proportion of the particles remains undegraded. Electron microscopy of the material in precipitin bands should be performed, and can be decisive in identifying the antigen involved.

The problem of whether GSP-AV is a grapevine virus is not resolved. GSP-AV is unlikely to be seedborne in *N. clevelandii*, or to be a glasshouse contaminant because it is not aphid-transmitted, at least by *M. persicae* and *M. euphorbiae*, and as we have no similar virus in the laboratory. We regularly raise *N. clevelandii* plants in large numbers but have never observed "spontaneous" GSP-AV-like symptoms or found a closterovirus associated with them. The virus was, therefore, probably transmitted from the tested grapevine. There is a recent report (K. Yora, *personal communication*) that a clostero-like virus has been seen in grapevines.

However, three difficulties remain concerning GSP-AV and the etiology of the stem-pitting disease. First, GSP-AV so far has been isolated only once, but lack of consistent isolation is a problem common to many viruses of the grapevine. Second, we have not demonstrated reinfection of the grapevine with GSP-AV; this also is a common situation as there is only one report of a virus isolated from grapevine being returned to it by mechanical inoculation. This report (12) describes the production of atypical symptoms in about 4% of young grapevine seedlings inoculated with tobacco ringspot virus. Third, if GSP-AV should be confirmed as a grapevine virus, the question would arise whether it contributes to or causes the stem-pitting disease.

Authors' addendum, 19 March 1980:

(i) GSP-AV and its antiserum have now been tested against tristeza virus antiserum and antigen, kindly sent by D. E. Purcifull, Gainesville, FL. All cross reactions in SDS-agar and in immunoelectron microscopy were negative except for a very slight positive decoration of tristeza virus by undiluted GSP-AV antiserum, possibly nonspecific.

(ii) GFV has now been isolated from three of 20 St. George grapevine plants inoculated with this virus alone 14 mo previously. No virus has yet been recovered from the plants inoculated with GSP-AV alone or with GSP-AV and GFV together.

LITERATURE CITED

1. ANONYMOUS. 1979. Il legno riccio della vite in Italia. Inf. Fitopatol. 29:3-18.
2. BAR-JOSEPH, M., and R. HULL. 1974. Purification and partial characterization of sugarbeet yellows virus. Virology 62:552-562.
3. BEM, F., and A. F. MURANT. 1979. Host range, purification and serological properties of heracleum latent virus. Ann. Appl. Biol. 92:243-256.
4. BOVEY, R., and J. J. BRUGGER. 1973. Stem pitting of grapevine in Switzerland. Riv. Patol. Veg. 9:37-42.
5. BRUNT, A. A. 1978. The occurrence, hosts and properties of lilac chlorotic leafspot virus, a newly recognized virus from *Syringa vulgaris*. Ann. Appl. Biol. 88:383-391.
6. CASPER, R. 1976. Natural occurrence of cucumber mosaic virus with plum pox virus (Sharka) and prunus necrotic ringspot virus in plum. Acta Hortic. 67:327-328.
7. CHAIREZ, R., and R. M. LISTER. 1973. Soluble antigens associated with infection with apple chlorotic leafspot virus. Virology 54:506-514.
8. CICCARONE, A. 1961. La degenerazione infettiva ed altre alterazioni della vite. Ital. Agric. 98:900-921.
9. FENNER, F. 1976. Classification and nomenclature of viruses. Second Rep., Int. Comm. Taxonomy of Viruses. Intervirology 7:1-116.
10. GARAU, R., U. PROTA, and O. SERVAZZI. 1973. Indagini su alterazioni del tipo "legno riccio" della vite in Sardegna. Ann. Fac. Agric., Univ. Sassari 21:321-323.
11. GARSNEY, S. M., D. GONSALVES, and D. E. PURCIFULL. 1977. Mechanical transmission of citrus tristeza virus. Phytopathology 67:965-968.
12. GILMER, R. M., J. K. UYEMOTO, and L. J. KELTS. 1970. A new grapevine disease induced by tobacco ringspot virus. Phytopathology 60:619-627.
13. GRANITI, A., and A. CICCARONE. 1961. Osservazioni su alterazioni virosiche e virus-simili della vite in Puglia. Notiz. Mal. Piante 55:99-102.
14. GRANITI, A., and G. P. MARTELLI. 1970. Legno riccio. Pages 243-245 in: N. W. Frazier, ed. Virus Diseases of Small Fruits and Grapevines. University of California, Div. Agric. Sci., Berkeley.
15. HEWITT, W. B. 1968. Viruses and virus diseases of the grapevine. Rev. Appl. Mycol. 47:433-455.
16. LISTER, R. M. 1970. Apple stem grooving virus. No. 31 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England.
17. LISTER, R. M., and A. F. HADIDI. 1971. Some properties of apple chlorotic leaf spot virus and their relations to purification problems. Virology 45:240-251.
18. MILNE, R. G., and D. E. LESEMANN. 1978. An immunoelectron microscopic investigation of oat sterile dwarf and related viruses. Virology 90:299-304.
19. MILNE, R. G., and E. LUISONI. 1977. Rapid immune electron microscopy of virus preparations. Pages 265-281 in: Methods in Virology, Vol. VI. Academic Press, New York.
20. SALAZAR, L. F., and B. D. HARRISON. 1978. Host range, purification and properties of potato virus T. Ann. Appl. Biol. 89:223-235.
21. SMOOKLER, M., and G. LOEBENSTEIN. 1974. Carnation yellow fleck virus. Phytopathology 64:979-984.
22. UYEMOTO, J. K. 1974. An association of phloem necrosis and stem pitting symptoms in grapevine infected with tomato ringspot virus. Phytopathology 64:588.