Properties of a Virus Isolated from Golden Elderberry, Sambucus nigra aurea

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ABSTRACT

A virus has been consistently isolated from Golden Elderberry, *Sambucus nigra aurea*. Two other golden forms, *S. canadensis aurea* and *S. plumosa aurea*, were found to be free from sap-transmissible viruses. The Golden Elderberry virus (GEV) was capable of infecting 44 out of 55 herbaceous hosts tested, and was seed-transmitted in *Chenopodium amaranticolor*, *Nicotiana clevelandii*, *N. megalosiphon*, *N. tabacum*, and *Phaseolus vulgaris*. Twenty per cent of the sap-inoculated *Prunus avium* and *P. persica* seedlings, and 66% of the similarly inoculated *Sambucus* seedlings, became infected. The virus was spherical, and had a diameter of ca. 30 nm. On a sucrose gradient, it separated into two opalescent bands with sedimentation coefficients of 114 S and 132 S. Both bands were infective and serologically identical. A GEV-antiserum with a titer of 1:640 was used to establish that GEV is unrelated to 14 other spherical viruses, six of which had previously been isolated from elderberry. Serological relationships between GEV and some strains of cherry leaf roll virus may exist. The virus seems to be responsible for the rings and arcs observed in *S. nigra aurea*, but not for the golden leaf coloration. Phytopathology 61:1222-1229.

Golden Elderberry, *Sambucus nigra aurea* L., is an ornamental shrub valued for its unusual coloration, winter hardiness, ease of propagation, and adaptability to many environmental conditions. It derives its common name from the bright-yellow foliage which is especially prominent during June and July. In *S. nigra aurea*, this coloration generally appears in the form of diffuse rings, arcs, and oak leaf patterns, which stand out because of their different shade of yellow or green (Fig. 1-B). Two other ornamental elderberries, *S. canadensis aurea* L. and *S. plumosa-aurea* Weezelenburg (*S. serratifolia aurea* Barbier), also display golden leaf coloration but lack modifying patterns (Fig. 1-A).

While conducting a survey for viruses which may pose a threat to the stone fruit industry, we found that commercially available *S. nigra aurea* is generally virus-infected. Since elderberries can carry viruses which may cause serious losses in stone fruit cultivars (6, 14, 20), studies were initiated on the identity of the virus present, and on its economic importance. The identification of the virus in *S. nigra aurea*, its potential threat to the stone fruit industry, and its relationship to the golden leaf coloration were made the subject of the study reported here. Preliminary accounts have been published (8, 10).

Virus infection and viruslike diseases in *Sambucus* have been reported from various species and cultivars. Martin (17) reported a mosaic of *S. canadensis* from Pennsylvania and Florida, and Wilkinson (22) found a similar disease to be caused by tobacco ringspot virus. Tomato ringspot virus (6, 21) and tobacco ringspot virus (R. M. Gilmer, *personal communication*) have been detected in native *Sambucus* in New York. A virus thus far unidentified occurs in the elderberry cultivar, Adams No. 2 (*unpublished data*). The only other report from North America refers to the symptomless occurrence of Pierce's disease (5). In Europe, a number of other viruses have been detected in elderberry. The most extensive work is by Schmelzer (19, 20), who detected cherry leafroll virus in *S. nigra* and in native *S. racemosa* L. in Germany and Poland. He also detected tomato black ring virus in *S. nigra* with yellow net symptoms. Arabis mosaic virus has been isolated from elderberry in Britain (3, 11). Other viruses isolated from elderberry are strawberry latent ringspot (14) and tobacco mosaic virus (18). Some workers have reported viruslike diseases from elderberry, but do not specify the virus or viruses involved (1, 2, 7, 13, 15). Apparently no previous work has been conducted with the Golden Elderberry virus (GEV), the subject of our study.

MATERIALS AND METHODS.—*Sambucus* cultivars were obtained through the retail and wholesale nursery trade. As far as could be ascertained, the stock had been grown in Canada and in the USA. Verbal reports that the *S. nigra aurea* originally had been imported from Europe could be neither confirmed nor refuted. All source- and test plants were grown in sterilized soil, in an insect-proof greenhouse kept at 20-23 C, unless otherwise mentioned. Additional light was supplied during winter months to provide a day-length of 16 hr.

All mechanical virus transmission attempts were made by macerating approximately one part of plant tissue in five parts of 0.5% nicotine (alkaloid), and by applying the tissue suspension to Carborundum-dusted, fully expanded cotyledons or leaves. Isolations from *Sambucus* were made between March and June, whereas host range studies, including inoculations of *Sambucus and Prunus* seedlings, were conducted throughout the year.

Several virus purification procedures were tested. The method finally selected was as follows: inoculated tobacco leaves were homogenized in 0.5 M citrate buffer, pH 6.5, and chloroform at the rate of 1:1:1 (w/v). After centrifugation at 9,000 rpm (7,200 *g*) for 10 min, the aqueous phase was centrifuged at 28,000 rpm (68,000 *g*) for 90 min in a Spinco No. 30 rotor. The resulting pellets were each dissolved in 0.5 ml of

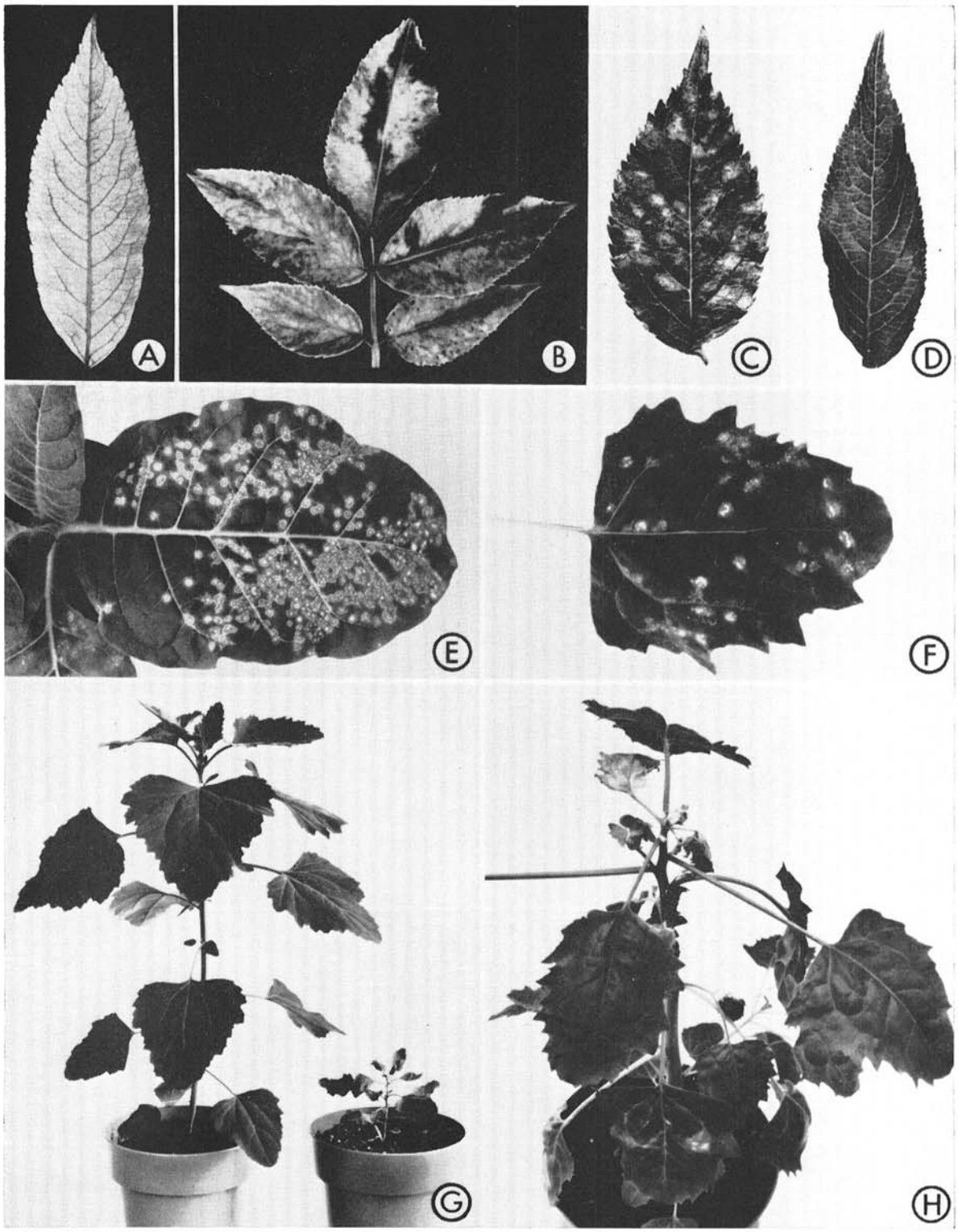

Fig. 1. Symptoms induced by Golden Elderberry virus (GEV) on various hosts. **A)** *Sambucus canadensis aurea,* uniformly yellow and free from GEV. **B)** GEV-infected *S. nigra aurea* with green rings and patterns on yellow background. **C)** GEV-infected *S. nigra* seedling with light green rings on normally green background. **D)** Healthy *S. nigra* seedling, normally green. **E)** Concentric rings on inoculated leaf of tobacco. **F)** Seminecrotic lesions on inoculated leaf of *Chenopodium quinoa.* **G)** Six-week-old plants of *C. amaranticolor* from healthy seed (left) and from seed infected with GEV (right). **H)** Epinasty and tip death of systemically infected plant of *Chenopodium quinoa.*

0.01 M Tris [tris (hydroxymethyl) amino methane]-HCl buffer, pH 7.3, and the pooled suspension was centrifuged at 10,000 rpm (9,000 *g*) for 10 min. The supernatant fluid was again centrifuged at 36,000 rpm (88,000 *g*) for 60 min in a Spinco No. 40 rotor. The resulting pellets were dissolved in Tris buffer. This suspension was layered onto 10-40% sucrose gradients in Tris buffer, and the gradients were centrifuged at 23,000 rpm (56,000 *g*) for 120 min in a SW 25.1 rotor. The resulting two opalescent bands were removed separately with a hypodermic syringe through the side of the tube. Infectivity of the bands was determined by inoculation of tobacco.

Sedimentation coefficients were determined for both gradients bands on a Spinco Model-E analytical centrifuge. Centrifuge runs were done in 0.1 M sodium chloride, 0.01 M Tris at 20 C, and the values were determined by the graphical method developed by Markham (16).

Electron microscopy was confined to examination of material in the light-scattering zones sucked from sucrose gradient tubes. Sucrose was removed by dialysis overnight against a large volume of 0.1 M ammonium acetate, pH 6.5. Portions to be examined by the negative staining technique were mixed with an equal volume of 2% uranyl acetate and examined in a Phillips EM 200 microscope.

Antiserum was prepared by giving one rabbit intramuscular injections of 0.5 mg of the bottom component in adjuvant in monthly intervals. Serological relationships were determined by the Ouchterlony gel diffusion method in 0.8% agar dissolved in 0.05 phosphate buffer, pH 7.0, plus 0.05% sodium azide to prevent bacterial growth. The antiserum was tested against 14 spherical viruses.

RESULTS.—*Isolation from Sambucus.*—Repeated attempts to obtain virus from *S. canadensis aurea, S. plumosa aurea,* and *S. nigra aurea* were successful only with *S. nigra aurea.* Nine bushes of this cultivar, which had been obtained from five different commercial sources, consistently yielded virus isolates similar in their characteristics and in their symptom expression on tobacco, *Gomphrena globosa* L. and *Chenopodium quinoa* Willd. The range of variation among these five isolates was about that which could be obtained by comparing single lesion lines selected from individual isolates. Therefore, subsequent work was carried out with one isolate only.

Host range.—In the herbaceous host range studies, at least four plants of each species were inoculated at two different times of year. Inoculum consisted of heavily infected tobacco leaves macerated in 0.5% nicotine. Inoculated herbaceous hosts were rated infected when definite virus symptoms appeared within 14 days, or when back-inoculations to *Nicotiana tabacum* L. or *C. quinoa* Willd. induced typical GEV symptoms.

The virus was capable of infecting 44 of 55 herbaceous hosts tested. In addition to the differential and common hosts listed in Table 1, the following became infected: *Ammi majus* L.; *Browallia speciosa* Hook.; *B. viscosa* HBK; *Crotalaria spectabilis* Roth; *Cucurbita pepo* L. (2 cultivars); *Emilia flammea* Cass. (no systemic invasion); *Helianthus annuus* L. (no systemic invasion); *Solanum sisymbriifolium* Lam.; *Torenia fournieri* Lind.; *Vigna sinensis* L.; *Vinca rosea* L.; and *Zinnia elegans* Jacq. In simultaneous tests, the following species were not infected: *Aster* sp.;

TABLE 1. Symptoms induced by Golden Elderberry virus on some differential and common herbaceous hosts

Host	Symptoms: Inoculated leaves	Symptoms: Systemically infected parts	Result of back-index
Antirrhinum majus L.	None	None	+
Atriplex hortensis L.	None	None	+
Beta vulgaris L. (3 cultivars) and var. *cicla* L.	None, or faint rings	None	+
Chenopodium amaranticolor Coste & Reyn.	White rings	Mottle, slight epinasty	+
C. quinoa Willd.	Semi-necrotic lesions	Mottle, severe epinasty, tip death	+
Commelina coelestis Willd.	None	None	—
Cucumis sativus L.	Green lesions	Mottle	+
Cucurbita maxima Duchesne	Occasional green lesions	None	+
Cyamopsis tetragonoloba (L.) Taub.	Black lesions	Necrotic spots	+
Gomphrena globosa L.	Red lesions: none in winter	None	+
Lycopersicon esculentum Mill.	None	None	—
Momordica balsaminea L.	None	None	—
Nicotiana tabacum L. (7 cultivars)	White concentric rings	White line patterns	+
N. clevelandii Gray	White circles	Numerous white spots	+
N. megalosiphon Heurch & Mueller	None	Mottle	+
Ocimium basilicum L.	None	None	—
Petunia hybrida Vilm.	None	None	+
Phaseolus vulgaris L. (6 cultivars)	Necrotic lesions	Necrotic streaks	+
Phlox drummondi Hook.	None	None	+
Plantago sp.	None	None	+
Raphanus sativus L.	None	None	—
Sesbania exaltata (Raf.) Cory	Faint rings	None	+
Spinacea oleracea L.	None, or faint rings	None	+

Citrullus vulgaris Schrad.; *Datura tatula* L.; *Fragaria vesca* L.; *Pulmonaria officinalis* L.; and *Tithonia speciosa* Hook.

Infection occurred in most of the hosts which were used by Schmelzer (19, 20) and others (12, 14) to distinguish between other elderberry viruses. The most distinctive symptoms appeared in members of the *Chenopodiaceae, Leguminosae,* and in species and cultivars of *Nicotiana.* Tobacco and *C. quinoa* developed discrete symptoms, which made them suitable as index plants for the detection of symptomless infections in other hosts. Of the two, *C. quinoa* was more susceptible to low concentrations of the virus, whereas tobacco was more suitable for quantitative work. On tobacco, the virus induced clear-white or sometimes brownish necrotic rings and lesions on the inoculated leaves (Fig. 1-E). On *C. quinoa,* a few necrotic or chlorotic rings usually appeared on the inoculated leaves, followed by severe systemic mottle and epinasty (Fig. 1-F, H). The results of the two tests carried out on each group of four host plants indicated only small seasonal differences in symptom expression, except in the case of lima bean and *G. globosa.* Once, lima bean reacted with red lesions on the inoculated leaves, but in a second test it did not become infected. Occasionally, *G. globosa* did not develop red lesions during the winter months, but it always became infected.

In the woody host range studies, seedlings of three *Sambucus* species, *Prunus avium* L., *P. persica* Sieb. & Zucc., and *P. tomentosa* Thunb., in the four-eight leaf stage were sap-inoculated with GEV in the greenhouse, planted outdoors in the spring following inoculation, and back-indexed in early summer. For comparative purposes, two similar groups of *P. avium* seedlings were simultaneously sap-inoculated with necrotic ringspot and prune dwarf virus, respectively.

Sap inoculation of *Sambucus* seedlings was successful when the plants had not developed beyond the six-leaf stage. In one test, 20 *S. nigra* seedlings remained uninfected when inoculated in the eight-leaf stage. Successful inoculation of *Prunus* seedlings with GEV was less common (Table 2), as only ca. 23% of those inoculated eventually became infected. This percentage was considerably higher than that obtained with the two groups of *P. avium* seedlings simultaneously inoculated with cherry necrotic ringspot and prune dwarf viruses. On the *Sambucus* seedlings, no symptoms were observed on the inoculated leaves, but vein clearing and ring patterns developed on the third and fourth leaf pair (Fig. 1-C). Subsequently, symptoms became more diffused but were still discernible under greenhouse conditions. In those *Prunus* seedlings which became infected with GEV, virus was detected in the inoculated leaves by indexing 1 week after inoculation; after another 5-8 weeks it was detected in the noninoculated upper leaves. In *P. tomentosa* and *P. persica,* a mild mottle appeared ca. 2 months after the inoculation; the infected *P. avium* seedlings remained symptomless.

In a separate test, 12 *Prunus* species and cultivars were inoculated by insertion of two buds from GEV-infected *P. avium* seedlings in May 1969. As bud take was poor, two more buds from infected *P. avium* and *P. tomentosa* were inserted into the same trees in April 1970.

TABLE 2. Results of mechanical inoculations of *Sambucus* and *Prunus* seedlings with Golden Elderberry virus (GEV), nd of *Prunus* seedlings with necrotic ringspot virus (NRSV) and prune dwarf virus (PDV)

Seedlings[a] of	Inoculum	No. plants: Inoculated	No. plants: With symptoms	No. plants: Infected, according to back-index
Sambucus nigra aurea 4 leaf stage	GEV	20	17	17
S. nigra, 4 leaf stage	GEV	20	18	18
S. nigra, 4-6 leaf stage	GEV	10	8	8
S. nigra, 8 leaf stage	GEV	20	0	0
S. glauca	GEV	10	6	9
S. melanocarpa	GEV	10	8	8
Prunus avium	GEV	40	1	5
P. persica	GEV	20	7	7
P. tomentosa	GEV	10	6	6
P. avium	NRSV	40	2	2
P. avium	PDV	40	0	1

[a] Unless specifically mentioned, seedlings were in the four- to six-leaf stage.

The 12 species and cultivars displayed no symptoms and no virus could be recovered by back-indexing during 1970.

Seed and pollen transmission.—Virus occurrence in seeds from systemically infected *Nicotiana clevelandii* Gray, *N. megalosiphon* Heurch & Mueller, and *N. tabacum* was detected by grinding 5 lots of surface-sterilized seeds and indexing on tobacco. Four of these yielded virus (Table 3) which was identical to the original inoculum. The tested seed of *N. tabacum* 'Bashi Bagli' had been stored for 2 years at room temperature; the virus may have lost its infectivity during this period, or it may not be seed-transmitted in this cultivar. The exact percentage of seed transmission in tobacco was not determined. Seed-borne infection in *N. tabacum* 'Haronova' seedlings was similarly investigated by indexing on tobacco. All 64 seedlings tested were free of virus. Seed transmission in *C. amaranticolor* was checked by visual examination of 15-day-old plants raised in sterile medium from seed obtained from two infected, individual mother plants. Preliminary testing had shown that visual inspection was sufficient, as all infected plants developed severe stunting (Fig. 1-G). Seed transmission was detected in 39% of the seedlings from one mother plant and in 87% of the seedlings from the other. Seed transmission in *Phaseolus vulgaris* L. was tested by indexing 27 seedlings from two infected mother plants. Eight of the seedlings were found to be infected.

No virus was recovered from a lot of 100 seeds

TABLE 3. Results of seed transmission test of various hosts infected with Golden Elderberry virus

Source	No. tested	Virus present
Nicotiana megalosiphon	Lot of 100 seeds	Yes[a]
N. clevelandii		
Plant A	Lot of 100 seeds	Yes[a]
Plant B	Lot of 100 seeds	Yes[a]
N. tabacum 'Bashi Bagli'	Lot of 100 seeds	No[a]
N. tabacum 'Haronova'	64 Seedlings	No[a]
N. tabacum 'White Burley'	Lot of 25 seeds	Yes[a]
Sambucus nigra aurea	Lot of 100 seeds	No[a]
Chenopodium amaranticolor		
Plant A	16 Seedlings	14 Seedlings[b]
Plant B	137 Seedlings	52 Seedlings[b]
Phaseolus vulgaris		
Plant A	12 Seedlings	5 Seedlings[a]
Plant B	15 Seedlings	3 Seedlings[a]

[a] Indexed on tobacco.
[b] Examined visually.

from the original, GEV-infected *S. nigra aurea* bush.

Presence of virus in pollen of *N. clevelandii* and *N. megalosiphon* was investigated in one experiment in which 35 individual pollen grains and four batches of 50 pollen grains were ground in ca. 25 times their volume of 0.1% nicotine. The suspension was applied to 1-mm^2 areas of F2C1 tobacco leaves by a method originally developed for single-cell virus assay (9). No virus was detected in any of the pollen tested.

Nature of yellow leaf factor.—In an effort to detect a suspected genetic "yellow" factor, or an interaction between a genetic predisposing factor and GEV, 39 seedlings were grown from seed obtained from a solitary *S. nigra aurea* bush and were indexed for GEV. Twenty of these were then sap-inoculated in the four-leaf stage with GEV, as were 20 comparable seedlings of *S. nigra*. All seedlings were planted outdoors in the following spring. Simultaneously, two bushes of *S. canadensis aurea* were inoculated by insertion of buds from GEV-infected *S. nigra* seedlings. The 39 seedlings from *S. nigra aurea* were similar to normal *S. nigra* seedlings, developed no yellow leaf coloration at any time, and contained no seed-transmitted virus. Those 19 seedlings which had not been inoculated continued to grow normally after being planted outdoors, whereas 17 of the 20 inoculated *S. nigra aurea* seedlings showed GEV-induced rings and mottle (Fig. 1-C). Symptoms on the *Sambucus* species were similar. The two bud-inoculated *S. canadensis aurea* plants developed similar rings and arcs, and thus displayed the full "golden" syndrome of *S. nigra aurea* (Fig. 1-B).

Biophysical properties.—Thermal inactivation point (10-min exposure), longevity (20 C), and dilution end point were determined in crude sap by the usual methods, using tobacco as a source and indicator host. The results indicated a thermal inactivation point of ca. 53 to 55 C; longevity of 5 to 7 days; and a dilution end point between 1:1,000 and 1:10,000.

Serology.—Antiserum prepared against GEV was tested by R. Bercks (B), H. Diaz (D), A. F. Murant (M), and the authors (Au) against 14 spherical viruses, among which were the six that had been isolated by other workers from elderberry. The viruses tested were: Andean potato latent (B); Arabis mosaic (B, Au); belladonna mottle (B); carnation ringspot (B, Au); cherry leafroll (Cropley's type strain) (Au); grape fanleaf (B); peach rosette mosaic (grape and peach strain) (D); pelargonium leaf curl (petunia asteroid mosaic strain) (B); raspberry ringspot (B, Au); strawberry latent ringspot (Au); tobacco ringspot (B, Au); tomato black ring (type, and beet ringspot strain) (B, M, Au); tomato ringspot (Au); and turnip yellow mosaic (B). Reciprocal tests were conducted with GEV and our own antisera against Arabis mosaic (Au); cherry leafroll (Cropley's type strain) (Au); and strawberry latent (Au). Healthy plant protein and GEV controls were included to ensure that positive results were due to antiserum-virus reaction, and not to reactions between healthy plant components and corresponding antibodies.

The titer for the homologous reaction between GEV and GEV antiserum was 1:160 after the first bleeding, and 1:640 after the second and subsequent bleedings. All serological tests with GEV antiserum and heterologous spherical viruses, and with GEV and the heterologous antisera, were negative. The GEV antiserum gave a slight positive reaction with healthy plant protein at a titer of 1:1 (M), but not at a titer of 1:10 (Au).

Purification and electron microscopy.—The purification procedure that we employed consistently yielded two virus peaks when concentrated solutions were examined in the Schlieren optics of the analytical ultracentrifuge (Fig. 2-A). This material, when layered on a sucrose gradient, yielded two opalescent bands upon centrifugation (Fig. 2-B). The bands on the gradient tube corresponded in intensity and position to the peaks observed in the analytical ultracentrifuge. Material comprising the two bands had sedimentation coefficients ($S_{20,w}$) at infinite dilution of 114 S for the upper band and 132 S for the lower band. Upon dilution to a concentration of A_{260} of 0.01 for inoculation to tobacco, different preparations induced from 50 to 150 local lesions/leaf.

Under the electron microscope, material from the two opalescent bands revealed polyhedral particles 30 nm in diam (Fig. 2-C). The hexagonal shape was better defined in the material from the lower band than in that from the upper band.

Ultraviolet absorption.—Both components of GEV demonstrated a typical nucleoprotein spectrum with minimum absorption at 240 nm and maximum absorption at 260 nm. There was more variation between different preparations than there was between top and bottom components of the individual preparations. Of six separate preparations of top and bottom components that were examined, the absorption spectra of

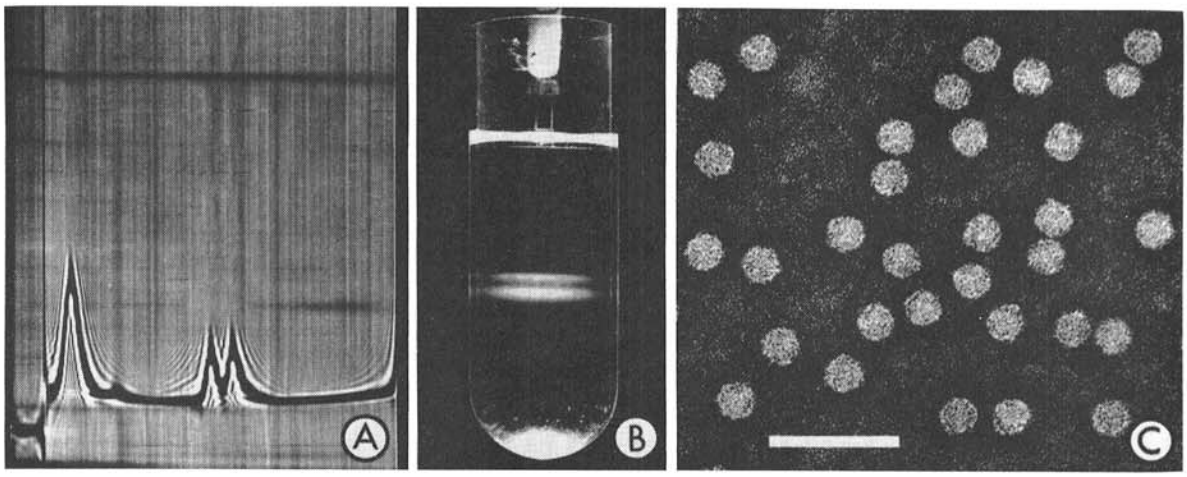

Fig. 2. **A)** Analytical ultracentrifuge photograph of partially purified Golden Elderberry virus showing relative proportion of the two virus component (center). Peak at left represents host constituents. Photograph taken with Schlieren optics 15 min after reaching speed of 35,600 rpm. **B)** Density gradient tube showing the two zones that result from layering material similar to that shown in Fig. 1-A and centrifuging 120 min at 23,000 rpm. **C)** Electron micrograph of particles sucked from the bottom zone of the density gradient, dialyzed, and stained with uranyl acetate. Bar represents 100 nm.

the top and bottom components were essentially identical. The 260:280 ratio of these preparations, corrected for light-scattering, varied from 1.69 to 1.86 (average 1.77) for the top component, and from 1.70 to 1.86 (average 1.78) for the bottom component.

Infectivity of purified virus.—Partially purified solutions, prior to centrifugation on a sucrose gradient, were highly infective. Material comprising the two light-scattering zones on a sucrose gradient, corresponding to the 114 S and 132 S peaks in the analytical ultracentrifuge, were removed separately and tested for infectivity. The particles comprising both zones were infective. The level of infectivity was high: upon dilution to a concentration of A_{260} of 0.001, different preparations induced up to 80 local lesions/inoculated tobacco leaf in replicated tests. Solutions from the top and bottom zone, adjusted to the same concentration based upon ultraviolet absorption at 260 nm, were approximately equal in their infectivity.

Although partially purified virus solutions containing 114 and 132 S particles may be separated into two apparently distinct zones on a sucrose gradient, it is recognized that even with the most exacting extraction technique, separation of particles comprising the two zones is incomplete. If only one type of particle was infective, low infectivity associated with particles from the second zone could be attributed to contamination resulting from incomplete separation of the zones. Our results suggested that both types of particles were infective, but we investigated the possibility that only one type was infective, and that this type was contaminating the other zone. The upper and lower zones were extracted from a sucrose gradient, dialyzed against 0.01 M Tris buffer to remove the sucrose, pelleted in a Spinco No. 40 rotor, resuspended, and subjected to a second sucrose gradient centrifugation. When these tubes were examined under a beam of light, each showed a major zone and a faint second zone, indicating that the initial separation was in fact incomplete. The tube of top zone material exhibited a major zone corresponding in position to top zone material and a faint zone corresponding in position to the bottom zone material. Conversely, the tube of bottom zone material exhibited a major light-scattering zone and a minor zone above it. Each of the major zones was extracted, adjusted to the same A_{260} concentration, and tested for infectivity. Again, there was a high level of infectivity associated with the particles from each zone. In a replicated test where six half-leaves were inoculated with comparable solutions of top and bottom zone particles, the average number of local lesions per half-leaf was 56 for top zone material and 87 for bottom zone material.

Discussion.—The host range and symptom expression of GEV were similar to, but not identical with, those of several other spherical viruses. The similarity between GEV and cherry leafroll virus (CLRV) was especially close. Symptoms were similar to those found by Cropley (4) for CLRV; host range data differed slightly from those found by Kegler et al. (12) for a cherry isolate and by Schmelzer (20) for an elderberry isolate. *Solanum sisymbriifolium* was susceptible in our tests, and *Commelina coelestis, Ocimium basilicum,* and *Raphanus sativus* did not become infected. Kegler et al. (12) and Schmelzer (20) had found the opposite reactions. The host range data enabled us to narrow the range of viruses to which GEV might be related, but they were not considered specific enough for definite identification.

Most of the biophysical properties of GEV were identical to those of a large number of polyhedral viruses, and were therefore of no assistance in the identification of the virus.

Production of a relatively high-titered antiserum allowed further identification tests. Since no reaction was found between GEV antiserum and 14 other vi-

ruses, and since corresponding reciprocal tests were also negative, GEV apparently is unrelated to any of them.

A. T. Jones & A. F. Murant (*personal communication*) recently found positive serological reactions between GEV and antisera to a Dutch cherry isolate and an English rhubarb isolate of CLRV; the titers were 1:128 and 1:64, respectively. This contrasts with our own tests, in which no serological relationship was found in reciprocal tests between GEV and Cropley's type strain of CLRV. The discrepancy between these results may be due to varying titers, or to differences in the specificity of the antisera. [Results from our most recent tests (*unpublished data*), in which a microscope agar gel diffusion method was used, confirm that a serological relationship exists between GEV and CLRV.] Until all these isolates are compared in one laboratory under uniform conditions, judgment should be reserved on whether GEV is closely related to CLRV or not. The designation "golden elderberry virus" should therefore be considered temporary.

The sedimentation coefficients of GEV do not match those of any spherical virus for which S-values have been published, and GEV is therefore considered to be unrelated to any of these. Unfortunately, S-values for CLRV have not yet been determined.

All purified preparations of GEV exhibited two peaks in the analytical ultracentrifuge, and these separated into two light-scattering zones upon sucrose gradient centrifugation. Initially, this appeared to be another instance of a multicomponent spherical virus. However, infectivity tests of the separate components demonstrated that GEV was unusual in that both components were approximately equal in their infectivity. This suggested that the top component was not deficient in RNA, as is the case with other multicomponent viruses. The ultraviolet absorption spectra of the two components also suggested that the RNA content was similar. Since there was a consistent difference of ca. 16% between the S-values of the two components, the most plausible explanation is of a deficiency of protein coat material in the upper component. Observations of particle morphology in the electron microscope indicate a less stable top component, which may be related to the suspected deficiency in protein.

The successful and relatively easy sap inoculation of cherry, peach, and *P. tomentosa* seedlings shows that these hosts are susceptible, and that GEV constitutes a potential threat to the stone fruit industry. Since bud transmission from the infected seedlings to *Prunus* cultivars failed to induce systemic infection during the 1st year, it is too early to determine the seriousness of this threat.

Although seed transmission occurred in *Chenopodium, Nicotiana,* and *Phaseolus,* there is no indication of the mode of field transmission of this virus. The wide experimental host range suggests that natural infection with GEV may not be restricted to one species only; on the other hand, it is surprising that a virus which can be isolated and handled easily was not discovered earlier, providing it was indeed present in many other hosts in North America.

It had been originally suspected (8) that GEV alone might be capable of inducing both the ring pattern and the golden leaf coloration, because GEV-inoculated *S. nigra* seedlings and vegetatively propagated *S. nigra aurea* plants displayed identical symptoms under greenhouse conditions. Under the field conditions of the present study, however, *S. nigra aurea* did develop the typical yellow foliage, whereas the inoculated *S. nigra* and *S. nigra aurea* seedlings remained green. This reaction indicated that the yellow leaf coloration was induced by a factor other than GEV. This explanation was made even more likely when it could be shown that the analogous *aurea* forms of *S. canadensis* and *S. plumosa* were virus-free, and that the modifying patterns, which are typical of *S. nigra aurea* only, can be introduced into *S. canadensis aurea* by inoculation with GEV. The lack of coloration in the 39 self seedlings of *S. nigra aurea* indicates that the color factor arose as a vegetative mutation, although it does not completely exclude the possibility of a gene-dependent mechanism.

On the basis of these data, it seems likely that the yellow coloration of *S. nigra aurea* is a cytoplasmatic mutation, while GEV is responsible for the modifying rings and patterns.

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