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Characterization of a carlavirus in elderberry (Sambucus spp.)

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Abstract

A carlavirus was isolated from *Sambucus racemosa* and *S. nigra* in the Netherlands. The virus was sap-transmissible and capable of infecting 14 out of 58 plant species and cultivars tested, causing symptoms in five of them. It was also transmitted by *Myzus persicae* at a low rate. Dilution end-point was 10^{-3}–10^{-4}, thermal inactivation at 70–75°C and ageing in vitro 2–4 days. The virus had a sedimentation coefficient of 155 S and molecular weight of capsid protein subunits of 31 000 dalton. The average buoyant density of the four isolates used was 1.315 g/cm^3. The virus particles had an average normal length of 678 nm and a width of approximately 12 nm. In ultrathin sections of leaf tissue of *S. racemosa* 'Plumosa Aurea' bundles of virus particles were observed in the cytoplasm. Close serological relationship was found to a virus isolated from elderberry in Britain and a distant relationship to carnation latent virus. In its reaction on host plants and its persistence in crude sap it also resembled the former virus, originally code-named elderberry virus A. We propose the name elderberry carlavirus for it.

Additional keywords: elderberry virus A, carnation latent virus, cherry leaf roll virus, elderberry carlavirus.

Introduction

European red elder (*Sambucus racemosa* L.), a native of mountain forests in Europe, is grown as an ornamental shrub in the Netherlands, and especially its cultivar Plumosa Aurea is valued for its bright-yellow, graceful foliage. Virus-like symptoms were observed on *S. racemosa* at Eelde and Haren on *S. racemosa* 'Plumosa Aurea' at Wageningen, and on *S. nigra* L. at Wageningen. *Sambucus racemosa* at Eelde showed chlorotic, bright-yellow or orange ringflecks and oak-leaf pattern, sometimes surrounded by a brown, purple-brown or even blackish border (Fig. 1 and 2), whereas its cultivar Plumosa Aurea exhibited chlorotic (ring)flecks on the leaves of some branches only (Fig. 3). *Sambucus racemosa* at Haren displayed bright-yellow ringflecks, whereas plants of the same species at Rhenen had shown similar symptoms in previous years but were symptomless at the moment of virus isolation. Symptoms on *S. nigra* consisted of chlorotic (ring)flecks and some oak-leaf pattern on the leaves (Fig. 4).

Dip preparations for the electron microscope of symptom-showing leaves of both *S. racemosa* and its cultivar Plumosa Aurea showed the presence of filamentous virus-like particles. In a preliminary experiment, a few test plants were inoculated with crude sap from symptom-showing leaves of 'Plumosa Aurea'. Only *Gomphrena globosa* developed symptoms (necrotic local lesions, Fig. 5).

Fig. 1. Leaves of *Sambucus racemosa* with bright-yellow ringspots and some oak-leaf pattern.

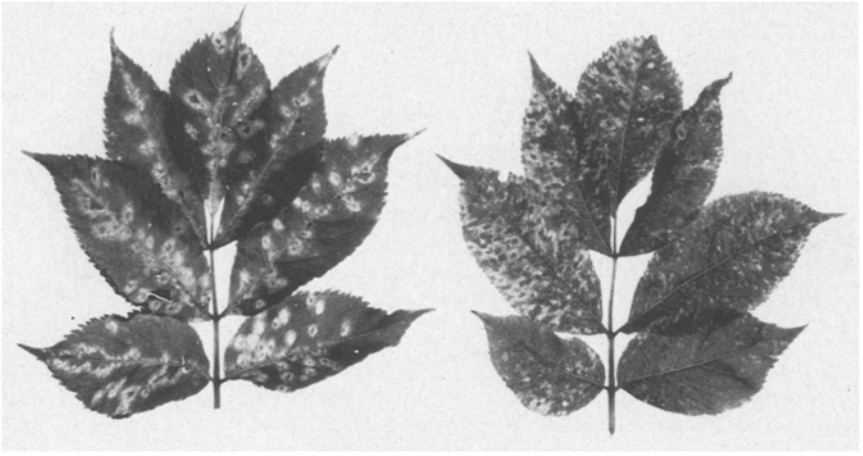


Fig. 1. Bladeren van Sambucus racemosa met heldergele kringvlekken en wat eikebladpatroon.

Sambucus racemosa has been reported as a natural host for cherry leaf roll virus (CLRV) (Grbelja, 1972; Schimanski and Schmelzer, 1972; Schmelzer, 1964, 1965, 1966) and for a virus with flexuous particles, code-named elderberry virus A (Jones, 1970; 1972).

From other *Sambucus* species and cultivars a filamentous virus has been recorded too, such as from cultivars of *S. canadensis* L. (Jones, 1972; Uyemoto and Gilmer, 1971; Uyemoto et al., 1971), from *S. nigra* (Brčák, 1964; Brčák and Polák, 1966) and from non-specified *Sambucus* spp. in S. E. England (Hollings, 1972).

Fig. 2. Leaves of *Sambucus racemosa* with deep-yellow ringspots and oak-leaf pattern surrounded by a purple-brown border.

Fig. 2. Bladeren van Sambucus racemosa met donkergele kringvlekken en eikebladpatroon omgeven door een purperbruine rand.

Fig. 3. Leaf of *Sambucus racemosa* 'Plumosa Aurea' with chlorotic (ring)spots.

Fig. 3. Blad van Sambucus racemosa 'Plumosa Aurea' with chlorotic (ring)spots.

Fig. 4. Leaf of *Sambucus nigra* with chlorotic (ring)spots and some oak-leaf pattern.

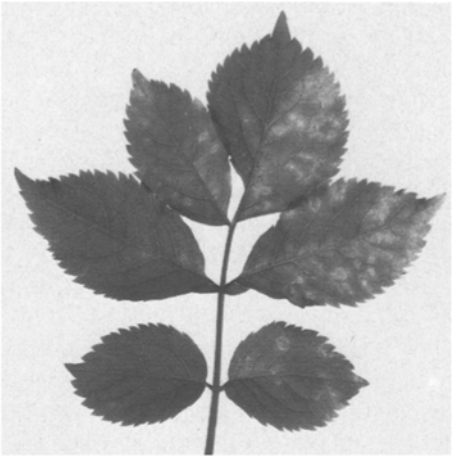


Fig. 4. Blad van Sambucus nigra met chlorotische (kring)vlekken en wat eikebladpatroon.

Fig. 5. Leaf of *Gomphrena globosa* with necrotic local lesions after inoculation with sap from diseased *Sambucus racemosa* 'Plumosa Aurea'.

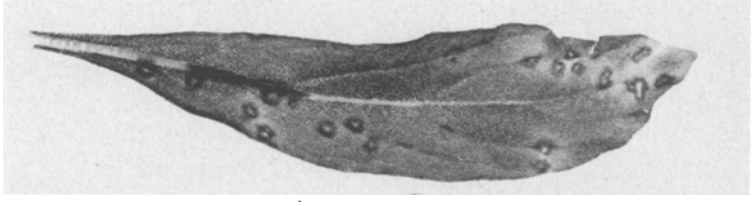


Fig. 5. Blad van Gomphrena globosa met necrotische lokale lesies na inoculatie met sap van zieke Sambucus racemosa 'Plumosa Aurea'.

Viruses in woody plants may constitute a permanent hazard to (other) cultivated plants in the neighbourhood. It is known, for instance, that elderberries can carry viruses which may cause serious losses in fruit culture (Gilmer and Kelts, 1968; Lister, 1964; Schmelzer, 1966).

The aim of the present study was, therefore, to further characterize the filamentous virus in *S. racemosa* and to investigate the detectability of the virus in infected bushes, also with a view to indexing of commercial stocks.

Materials and methods

Virus isolates. The virus was isolated from bushes of *S. racemosa* growing in the experimental garden of the Plant Protection Service (PD) at Eelde (isolate RE), in the municipal nursery at Haren (isolate RH), at the edge of a hill, called the Grebbeberg, at Rhenen (isolate RR), from *S. racemosa* 'Plumosa Aurea' bushes growing in the experimental garden of the PD at Wageningen (isolate RPA) and from a bush of *S. nigra*

growing in the demonstration garden of the Laboratory of Virology (isolate N). Most research was on RPA which was repeatedly isolated from the infected *Sambucus* bushes during the growing season.

Maintenance of the virus was in *G. globosa*, showing local lesions in 5–8 days after inoculation.

Infectivity assay was on *G. globosa*.

Virus transmission in sap, host range tests and determination of persistence of infectivity in crude sap were performed in the conventional ways, with carborundum 600 mesh as an abrasive and water or the extraction buffers used in the purification procedures (with or without the addition of 2% (w/v) polyvinyl pyrrolidone (PVP) and 0.1% Na_2SO_3) as diluants).

All test and assay plants were grown in sterilized soil in the glasshouse kept at 20–25°C.

In host range tests, plants of 58 species and cultivars were inoculated with dilute sap from ground *G. globosa* leaves showing local lesions. After three to four weeks return inoculations to *G. globosa* were made from inoculated and from non-inoculated leaves of the test plants. In case of seedlings of *S. pubens* Michx. (syn. *S. racemosa* var. pubens Wats.) return inoculations were from non-inoculated leaves only, about eight months after inoculation. The *S. pubens* seedlings were about seven months old when they were inoculated.

As it is known that pruning may have a beneficial effect on development of visible symptoms in virus-infected woody plants (Fischer, 1955) all the inoculated *S. pubens* seedlings were pruned drastically in April 1979. Schmelzer (1966) obtained symptoms on *Sambucus* plants only after inoculation of young, not yet fully expanded leaves in the first week of May. Therefore, we reinoculated six of those seedlings which had failed to become infected, on their young flush on May 8, 1979.

For comparison of possible symptoms eight *S. pubens* seedlings, about nine months of age, were inoculated with CLRV obtained during purification of the filamentous virus from one sample of *S. racemosa* leaves. Another batch of eight seedlings was inoculated with sap containing both the filamentous virus and CLRV. All these seedlings were pruned and four of each batch reinoculated at the same time as those reinoculated with the filamentous virus alone.

Aphid transmission experiments were with virus-free cultures of *Aphis fabae* Scop. and *Myzus persicae* (Sulz.) Apterous aphids were starved for 30–60 min and then given an acquisition access period of 10–45 min either on cotyledons of infected plants of *Cyamopsis tetragonoloba* or on young shoots with not yet fully developed leaves of infected 'Plumosa Aurea' (with *A. fabae* only). Thereafter they were transferred to the cotyledons of healthy plants of *C. tetragonoloba* (10 aphids per plant) for inoculation feeding of about 90 min. From these plants the aphids were transferred to another series of healthy plants of the same species and killed with an insecticide 24 h later. Infection of the *C. tetragonoloba* plants was checked by return inoculation to *G. globosa* about a fortnight after the inoculation feeding.

Dodder (*Cuscuta subinclusa*) was maintained on healthy plants of *Beta vulgaris*. Shoots of dodder were established on infected plants of *C. tetragonoloba* and trained onto healthy plants of the latter species. Infection of the *C. tetragonoloba* plants was checked by return inoculation onto *G. globosa* about three weeks after the shoots of dodder on the infected plants had established on healthy plants.

Virus purification. In earlier experiments the virus was purified from leaves of infected *Sambucus* bushes by the method described by Van Oosten (1972), whereas in all the later experiments the purification method used by Veerisetty and Brakke (1978) for legume carlaviruses was adopted.

Extinction coefficient. The optical density of purified virus preparations was measured in a Zeiss spectrophotometer or in a Gilford 2400-2 self-recording spectrophotometer.

Absorbance values were not corrected for light-scattering.

Virus concentrations were estimated using an arbitrary extinction coefficient $E260^{0.1\,\%}_{1\,cm}$ of 3.0 (Veerisetty and Brakke, 1978).

Sedimentation coefficients were determined on a Spinco Model-E analytical centrifuge. Centrifuge runs were done with a purified virus suspension in citrate/phosphate buffer of pH 9.0 at 20°C, and the values were determined by the graphical method developed by Markham (1960).

Buoyant density was determined on partially purified virus suspension (i.e. the purified preparation before rate-zonal and equilibrium-gradient centrifugation), containing 0.24 mg/ml virus. About 200 µl of this virus suspension was layered on a solution of 0.419 g CsCl/ml in citrate/phosphate buffer. Centrifugation was at 149000 *g* for 18–20 h in a Beckman SW 50.1 rotor. The gradient was fractionated in samples of 10 drops each and the solute concentration in each sample determined refractometrically. To establish the position of the virus in the gradient, each sample was examined electron microscopically. Densities were calculated by using the relation $\rho^{25°} = 10.2402\eta_D^{25°} - 12.6483$ for densities between 1.00 and 1.38 and $\rho^{25°} = 10.8601\eta_D^{25°} - 13.4974$ for densities higher than 1.37 (Bruner and Vinograd, 1965).

Polyacrylamide gel electrophoresis. The molecular weight of capsid protein of isolate N was estimated on 7.5% polyacrylamide cylindrical gels according to the method of Weber and Osborne (1969) only once. Purified virus suspension was boiled in a buffer (pH 6.8) containing 0.125 M ethylenediamine-tetraacetate, 1% sodium dodecyl sulphate (SDS), 0.01% crystal violet, 0.1% dithiothreitol and 30% (w/v) sucrose.

The following markers were used: phosphorylase, ovalbumin, concanavalin A, bovine serum albumin (BSA), RNase A and cytochrome C.

Electron microscopy was performed in crude sap and in (partially) purified virus preparations after staining with 2% potassium phosphotungstate (pH 6.5). The mixture was mounted on carbon-reinforced formvar-coated copper grids and examined with a Siemens Elmiskop I electron microscope. For length measurements of the virus particles either tobacco mosaic virus (TMV) from *Nicotiana tabacum* 'White Burley' was used as an internal size standard (Bos, 1975) or fixed catalase crystals with a lattice spacing of 8.6 ± 0.22 nm (Wrigley, 1968). Particle measurements were made from negatives with a binocular microscope at × 12.5 using a micrometer eyepiece. Of each isolate at least 300 virus particles were measured.

Specimens for in situ studies were prepared as described by Jayasinghe and Dijkstra (1979).

Serology. For antiserum production a rabbit was injected intravenously with 4.8 mg purified virus isolate RPA. After 18 days a second injection was given intramuscularly with 2.4 mg virus emulsified with Freund's incomplete adjuvant. As after a fortnight the titre was found too low, another intravenous injection was given with 3.4 mg purified virus (isolate RE). Nineteen days later the rabbit was bled again.

The microprecipitin test according to Van Slogteren (1954) and the microplate method of enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977) were used for serological tests.

Microprecipitin test. Dilutions of purified virus preparations were made with 0.01 M Tris-HCl, pH 7.0. Readings were made after 18 h at room temperature.

ELISA test. In this test antiserum with a titre of 128 was used. Purification of the antiserum γ-globulin and preparation of the conjugate were according to Ellens et al. (1978). The microplates were coated at 37°C for 3–4 h. Incubation with the virus samples was at 6°C overnight, and with the dilute enzyme-antiserum conjugate at 37°C for 3–4 h. Thereafter, the microplates were filled with enzyme substrate and the colour of the reaction product was assessed by eye or measured in a photometer at 450 nm after about 2 h. Between two reaction stages the microplates were rinsed thoroughly with tap water. The enzyme and substrate used were horseradish peroxidase and 5-aminosalicylic acid, respectively. Dilution series of purified virus suspension and sap of herbaceous plants were made with PBS containing 0.05% Tween 20 (Clark and Adams, 1977) and 1% PVP. Sap of *Sambucus* leaves and twigs (prepared by crushing the woody parts in a mortar with liquid nitrogen and thawing the powder thus obtained) was diluted in PBS-Tween with 2% PVP and 0.2% BSA. Optimum concentrations of antiserum γ-globulin and enzyme conjugates were determined in a chequerboard titration.

Antisera to a number of carlaviruses, viz. carnation latent virus, chrysanthemum virus B, *Lonicera* virus S, *Nerine* latent virus, poplar mosaic virus, potato virus M, potato virus S, red clover vein mosaic virus-RK 31, shallot latent virus and Wisconsin pea streak virus were kindly supplied by Ing. D. Z. Maat, Research Institute for Plant Protection, Wageningen. From Dr A. A. Brunt, Glasshouse Crops Research Institute, Littlehampton, England, we received antiserum to a carlavirus isolated from elderberry in Britain.

Results

Isolation from Sambucus and sap transmission. As all the isolates used were similar in their symptom expression on *G. globosa* (Fig. 5) and in their appearance in the electron microscope, it was taken for granted that they were isolates of the same virus.

Transmission of the virus from *S. racemosa* to *G. globosa* by sap was more difficult than from *S. nigra* to this indicator plant; in most of the former transmission experiments only 2–3 local lesions appeared on one *G. globosa* plant, in constrast to more than 100 in the latter experiments.

There was no difference in infectivity between inoculum prepared from *S. racemosa* with water or with buffer.

Host range. Of each species or cultivar three plants (12 in case of *S. pubens* seedlings) were inoculated with water-diluted sap from *G. globosa* leaves showing more than 40 local lesions per leaf. Out of the 58 plant species and cultivars used five showed symptoms (Table 1). On *Chenopodium amaranticolor* and *Ch. quinoa* irregular, greyish-white necrotic local lesions with a chlorotic halo (Fig. 6) were formed only when the inoculations were performed in late summer; throughout the rest of the year the plants were symptomless carriers. *Crotalaria juncea* showed systemic symptoms consisting of

Table 1. Results of host range tests.

Test plant	Infection of test plant	Test plant	Infection of test plant
Amaranthus caudatus	l, s	*Petunia hybrida*	–
Arachis hypogaea 'Valencia'	l	*Phaseolus vulgaris* 'Amanda'	–
Beta vulgaris 'Groeningia'	l	*Phaseolus vulgaris* 'Dubbele Witte'	–
Capsicum annuum 'Westlandse Zoete'	–	*Phaseolus vulgaris* 'Noordhollandse Bruine'	–
Celosia argentea	l	*Phaseolus vulgaris* 'Pinto'	–
Chenopodium album	–	*Phaseolus vulgaris* 'Prelude'	–
Chenopodium amaranticolor	L	*Phaseolus vulgaris* 'Processor'	l
Chenopodium foetidum	–	*Phaseolus vulgaris* 'Saxa'	–
Chenopodium hybridum	–	*Phaseolus vulgaris* 'The Prince'	–
Chenopodium quinoa	L	*Phlox drummondii*	–
Crotalaria juncea	l, S	*Physalis floridana*	–
Cucumis sativus 'Lange Gele Tros'	–	*Pisum sativum* 'Koroza'	–
Cyamopsis tetragonoloba	l, S	*Sambucus pubens*	–
Datura metel	–	*Solanum dulcamara*	–
Datura stramonium	–	*Solanum luteum*	–
Dianthus barbatus	–	*Solanum melongena* 'Lange Violette'	–
Dianthus caryophyllus	–	*Solanum nigrum*	–
Gomphrena globosa	L	*Solanum rostratum*	–
Lycopersicon esculentum 'Moneymaker'	–	*Solanum tuberosum* 'Eigenheimer'	–
Melilotus alba	–	*Sorghum halepense*	–
Nicotiana benthamiana	–	*Trifolium hybridum*	–
Nicotiana clevelandii	–	*Trifolium incarnatum*	–
Nicotiana debneyi	–	*Trifolium pratense*	–
Nicotiana glutinosa	–	*Trifolium repens*	–
Nicotiana megalosiphon	–	*Vicia faba* 'Driemaal Wit'	l?, s
Nicotiana rustica	–	*Vigna unguiculata* 'Blackeye'	s
Nicotiana tabacum 'Samsun NN'	–	*Vigna unguiculata* 'Early Red'	l
Nicotiana tabacum 'White Burley'	–	*Vinca rosea* 'Delicata'	–
Nicotiana tabacum 'Xanthi-nc'	–	*Zinnia elegans* 'Persian Carpet'	–

l, s = latent local (l) or systemic (s) infection as established by return inoculation onto *Gomphrena globosa*; – = no infection; L = local symptoms; S = systemic symptoms; l? = no return inoculation from inoculated leaves.

Tabel 1. Resultaten van het waardplantonderzoek.

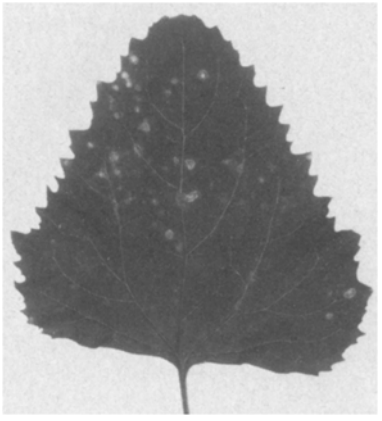
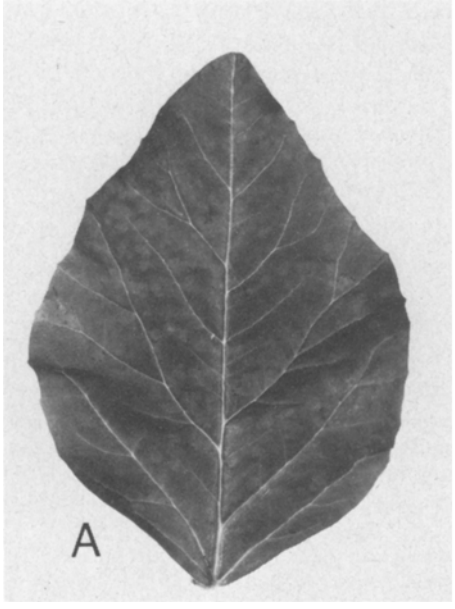


Fig. 6. Leaf of *Chenopodium amaranticolor* with necrotic local lesions after inoculation with elderberry carlavirus.

Fig. 6. Blad van Chenopodium amaranticolor met necrotische lokale lesies na inoculatie met het carlavirus van vlier.

chlorotic mottling, brownish-black necrosis of the veins and epinasty of the leaves more than three weeks after inoculation. *Cyamopsis tetragonoloba* showed local chlorotic (ring)spots on the first leaf above the cotyledons (Fig. 7A), a systemic faint chlorotic mottle and mosaic on the second leaf (Fig. 7B), and sometimes also on the third undivided leaf and on the first trifoliolate leaf. The cotyledons were infected

Fig. 7. Leaves of *Cyamopsis tetragonoloba* with local chlorotic (ring)spots on the first undivided leaf above the cotyledons (A) and with a faint systemic chlorotic mottle and mosaic on the second undivided leaf (B) after inoculation with elderberry carlavirus.



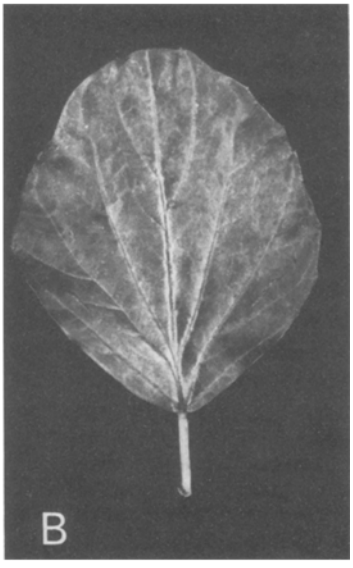


Fig. 7. Bladeren van Cyamopsis tetragonoloba met lokale chlorotische (kring) vlekken op het eerste ongedeelde blad boven de cotylen (A) en met een zwakke, systemische, chlorotische vlekkerigheid en mozaïek op het tweede ongedeelde blad (B) na inoculatie met het carlavirus van vlier.

symptomlessly. However, infection of and symptoms on the leaves above the inoculated cotyledons only occurred when besides the cotyledons the first undivided leaf was inoculated too; if inoculation was restricted to the cotyledons the virus remained localized. Usually, symptom-showing *C. tetragonoloba* plants showed recovery about four weeks after inoculation.

In nine test plant species and cultivars only latent infections were obtained (Table 1).

About eight months after inoculation with the filamentous virus, 2 out of 12 *S. pubens* seedlings were shown to be infected (Table 5). Neither the *S. pubens* seedlings inoculated with CLRV nor those with a mixture of the filamentous virus and CLRV were infected at that time. About nine days after reinoculation on the young leaves with CLRV one seedling showed (concentric) chlorotic ringspots and some line pattern on two leaves. A couple of days later one more plant from this batch displayed similar symptoms, whereas all four seedlings reinoculated with the mixture of the two viruses exhibited more severe symptoms consisting of concentric ringspots, chlorotic flecks, mottling and mosaic. In the latter case both the viruses could be recovered from the four plants. None of the six seedlings reinoculated with the filamentous virus alone showed any symptoms, although the virus was recovered from three of these plants.

Aphid transmission. Myzus persicae transmitted the virus to two out of six, to none out of five, and to one out of five seedlings of *C. tetragonoloba* in three experiments, respectively. With *A. fabae* no virus transmission was obtained. None of the *C. tetragonoloba* plants of the second series to which the aphids were transferred after inoculation feeding on the first series became infected.

Dodder transmission. No virus transmission was obtained from systemically infected plants of *C. tetragonoloba* to healthy plants of the same species by dodder.

Persistence of infectivity in crude sap. Crude sap from infected *G. globosa* leaves was infective after dilution with water to 10^{-3} but not 10^{-4}. The thermal inactivation point was 70–75 °C, and at 20 °C infectivity was retained for two days but not for four days.

Virus purification. Virus purification according to Van Oosten (1972) yielded rather pure infective preparations, but the virus particles showed much end-to-end aggregation. In the first purification experiment the purified virus (isolate RE) suspension obtained evoked not only the normal symptoms on *G. globosa*, but also one local necrotic lesion on a plant of *N. tabacum* 'Samsun NN'. Inoculum prepared from this lesion and applied to a small number of test plant species and cultivars yielded symptoms on most of these plants, except on *G. globosa*. The causative agent was identified as CLRV, a member of the nepovirus group. In later purification experiments (with RE and other isolates) there was no indication that besides the filamentous virus CLRV was present too.

Because of the above-mentioned end-to-end aggregation of the virus particles we tried the method described by Veerisetty and Brakke (1978). The suspension obtained after adding polyethylene glycol 6000 (PEG) to the clarified sap and dissolving the resulting PEG-precipitate was still green. We could eliminate the green-coloured material by high-speed centrifugation in 20% sucrose. The pellet thus formed was dark-brown and jelly-like. Against the walls of the centrifuge tubes and on top of the

Fig. 8. Electron micrograph of a purified preparation of elderberry carlavirus. Bar represents 500 nm.

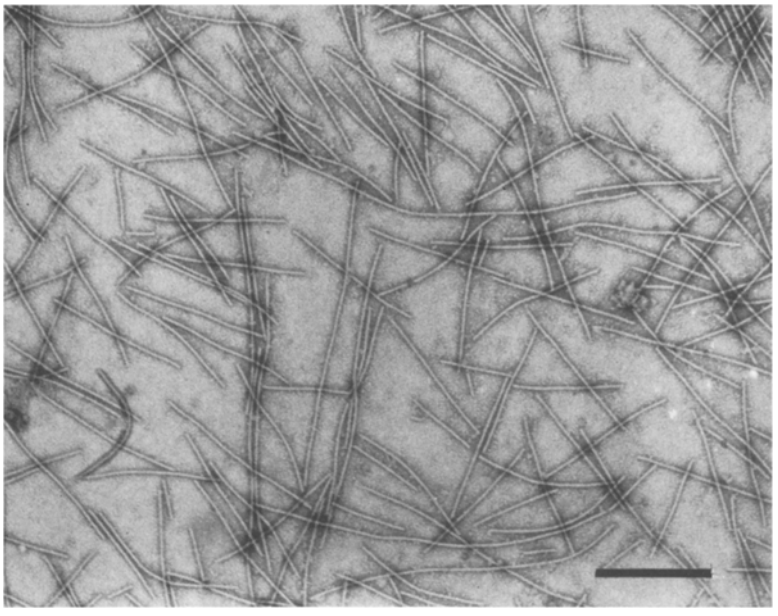


Fig. 8. Elektronenmicroscopische opname van een gezuiverd preparaat van het carlavirus van vlier. De vergrotingsstreep geeft 500 nm weer.

supernatants, obtained both after precipitation with PEG and after high-speed centrifugation on a sugar cushion, a bright-yellow, fatty substance was observed. This substance which was insoluble in ethanol, acetone or chloroform may have originated from the waxy cuticle of the *Sambucus* leaves.

The pellet formed after passing the virus suspension through a 30% sucrose cushion was usually light-brown and gelatinous. After resuspending of the pellet and low-speed centrifugation a slightly coloured supernatant was obtained and most of the brown colour was in the pellet. The infectivity of this supernatant, henceforth called 'partially purified virus' was high and there was very little aggregation of the virus particles (Fig. 8).

Purified virus preparations, obtained after rate-zonal and equilibrium-gradient centrifugation, had an absorption maximum at 260 nm and minimum at 248 nm. Without correction for light scattering the A_{260}/A_{280} absorption ratio varied from 1.14 to 1.25 (average 1.17). The yield was 2–3 mg virus per 100 g leaf material.

Sedimentation coefficient. In the analytical ultracentrifuge the virus sedimented as a single component. The average sedimentation of the virus at a concentration of 2.5 mg/ml in citrate/phosphate buffer (0.0018 M trisodium citrate, 0.0165 M disodium phosphate) at 20°C was 155 S.

Buoyant density. The buoyant densities of the isolates RPA, RE, RR and N were 1.314, 1.316, 1.315 and 1.314, respectively.

Capsid protein. Electrophoresis of virus coat protein in polyacrylamide gels containing SDS revealed one component with an estimated molecular weight of 31 000 dalton.

Electron microscopy. The number of virus particles in crude sap preparations of *Sambucus* material was small. Table 2 presents the normal lengths of the particles in purified virus suspensions. The average normal length was 678 nm and the width was approximately 12 nm.

In ultrathin sections of leaf tissues of *S. racemosa* 'Plumosa Aurea' large numbers of filamentous virus particles could be observed in the cytoplasm of most of the cells (Fig. 9). The virus particles were arranged in bundles not surrounded by membrane-like structures.

Fig. 9. Ultrathin section of leaf tissue of *Sambucus racemosa* 'Plumosa Aurea' infected with elderberry carlavirus. CW = cell wall; M = mitochondrion; V = bundles of virus particles; VA = vacuole. Bar represents 500 nm.

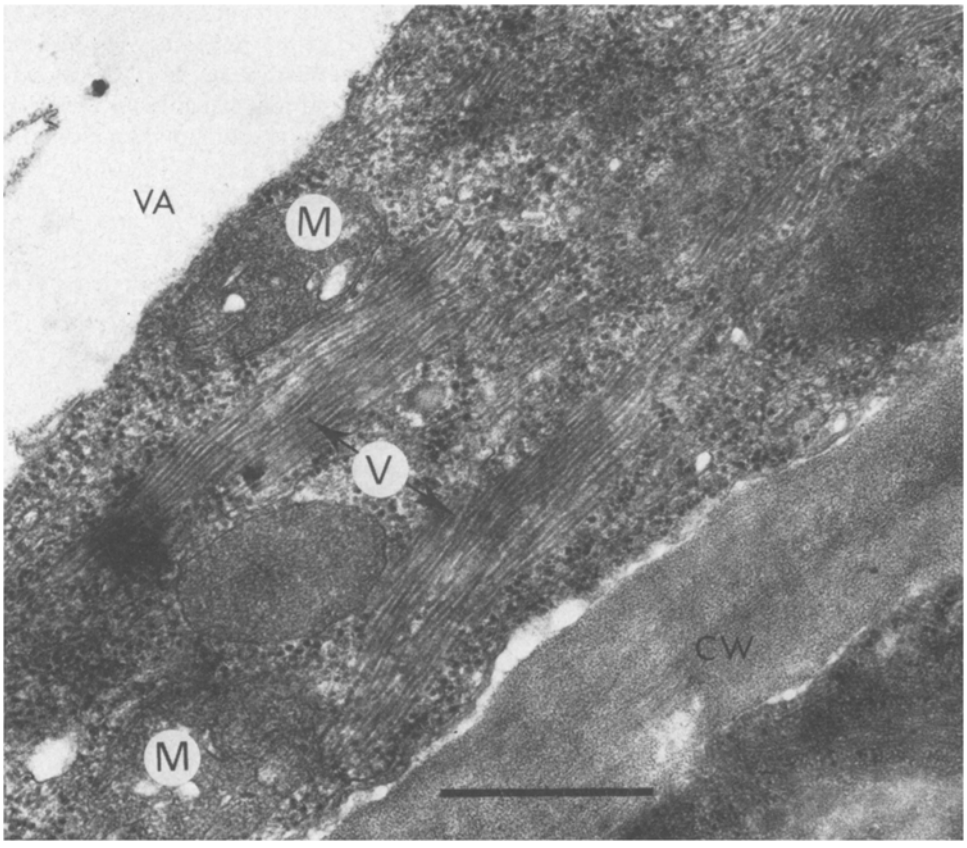


Fig. 9. Ultradunne coupe van bladweefsel van Sambucus racemosa 'Plumosa Aurea', geïnfecteerd met het carlavirus van vlier. CW = celwand; M = mitochondrium; V = bundels van virusdeeltjes; VA = vacuole. De vergrotingsstreep geeft 500 nm weer.

Table 2. Normal lengths of the virus particles in purified preparations from infected *Sambucus* leaves.

Virus isolate	Normal length (nm)	Internal standard used
RPA	675	tobacco mosaic virus
RPA	684	catalase
N	670	catalase
RE	682	catalase

Tabel 2. Normale lengtes van de virusdeeltjes in gezuiverde preparaten uit geïnfecteerde Sambucus bladeren.

Serology

Microprecipitin test. The antiserum to the purified virus had a homologous titre of 256 with isolate RE and of at least 512 with isolate N. The results of tests performed to determine serological relationships of the virus from *Sambucus* to carlaviruses, are given in Table 3. The results show that the virus from our *Sambucus* spp. is closely related to a virus isolated from elderberry in Britain, and to a much lesser extent to carnation latent virus. The titres of the other antisera in the heterologous reactions did not exceed those to normal plant antigens.

Table 3. Results of microprecipitin tests to determine the relationship of the virus from *Sambucus* spp. (isolates RE and N) to carlaviruses.

Antisera	Antigens		Homologous titres (according to the donor)
	Isolate RE	Isolate N	
Carnation latent virus	32	32	1024
Chrysanthemum virus B	1	2	256
Elderberry virus from Britain	512	NT	4096
Lonicera virus S	–	–	1024
Nerine latent virus	4	4	256
Poplar mosaic virus	2	4	1024
Potato virus M	2	2	1024
Potato virus S	4	4	1024
Red clover vein mosaic virus	2	2	256
Shallot latent virus	2	2	1024
Wisconsin pea streak virus	–	–	1024

– = No reaction; NT = not tested.

Tabel 3. Resultaten van microprecipitatietoetsen ter bepaling van de verwantschap van het virus uit Sambucus spp. met carlavirussen.

ELISA test. In chequerboard titration 30 and 15 µg/ml γ-globulin proved to be optimum for coating of the plates. However, as there were only minor differences in reaction between the different concentrations used all the ELISA plates were coated with 10 µg/ml γ-globulin. An enzyme conjugate dilution of 1:500 proved to be best. When the above concentrations of γ-globulin and enzyme conjugate were used, a concentration of 17 ng/ml virus just gave rise to a visible reaction.

Table 4. Results of ELISA with 1:100 diluted crude sap of healthy and infected *Sambucus* shrubs, of healthy and infected test plants, and with purified virus suspensions diluted 1:100 with buffer or plant sap.

Antigen	Extinction value at 450 nm
Sambucus racemosa seedlings (H)	0.17
Sambucus racemosa from Eelde[1]	2.07
Sambucus racemosa from Haren[1]	2.35
Sambucus racemosa from Rhenen[1]	1.02
Sambucus racemosa 'Plumosa Aurea'	1.67
Sambucus racemosa 'Plumosa Aurea'[1, 2]	1.54
Sambucus nigra[1]	1.90
Sambucus nigra[2, 3]	0.99
Sambucus nigra[2, 4]	0.25
Sambucus nigra[2, 5]	0.26
Chenopodium amaranticolor (H)	0.12
Chenopodium amaranticolor (NI)	0.15
Chenopodium amaranticolor (I)	0.88
Crotalaria juncea (NI)	0.11
Crotalaria juncea (I)	1.93
Cyamopsis tetragonoloba (H)	0.09
Cyamopsis tetragonoloba (I)	0.98
Gomphrena globosa (H)	0.14
Gomphrena globosa (NI)	0.11
Gomphrena globosa (I)	0.32
Purified suspension[6]	1.48
Purified suspension[7]	1.57
Purified suspension[8]	1.79

[1] Crude sap of infected leaves.
[2] Plant tissue homogenized with liquid nitrogen.
[3] Crude sap of young twigs of infected shrubs.
[4] Crude sap of old twigs of infected shrubs.
[5] Crude sap of buds of infected shrubs.
[6] Virus suspension diluted in PBS-Tween with 2% PVP and 0.2% BSA.
[7] Virus suspension diluted in 1:100 diluted crude sap of leaves of healthy *C. tetragonoloba.*
[8] Virus suspension diluted in 1:100 diluted crude sap of healthy *S. racemosa* leaves.
(H) = crude sap of healthy leaves; (NI) = crude sap of non-inoculated leaves of inoculated plants; (I) = crude sap of inoculated leaves.

Tabel 4. Resultaten van ELISA met 1:100 verdund ruw sap van gezonde en geïnfecteerde Sambucus struiken, gezonde en geïnfecteerde toetsplanten en met gezuiverde virussuspensies 1:100 verdund met buffer of plantesap.

Table 5. Results of ELISA and bio-assay performed on 12 seedlings of *Sambucus pubens* about 3.5 and 8 months after inoculation, respectively.

No. of seedling	ELISA[1] (extinction value at 450 nm)	Bio-assay[2] (number of local lesions)[3]
1	0.21	0
2	0.24	0
3	0.29	6
4	0.20	0
5	0.21	0
6	0.28	15
7	0.20	0
8	0.21	0
9	0.25	0
10	0.19	0
11	0.19	0
12	0.19	0

[1] 1:100 diluted crude sap of non-inoculated leaves was assayed.
[2] Water-diluted crude sap of a mixture of non-inoculated young and old leaves was assayed on young *Gomphrena globosa* plants.
[3] Total number of local lesions on two *G. globosa* plants.

Tabel 5. Resultaten van toetsingen uitgevoerd op 12 Sambucus pubens zaailingen met behulp van ELISA en een indicatorplant, respectievelijk 3,5 en 8 maanden na inoculatie.

The results of ELISA, obtained with the antiserum prepared to the purified virus from *Sambucus* are summarized in Table 4. Extinction values ranged from 1.02 to 2.35 for infected *Sambucus* leaves. With sap from healthy plants extinction values ranged from 0.09 (sap from *C. tetragonoloba*) to 0.17 (sap from *Sambucus* leaves).

Leaf samples of the 12 *S. pubens* seedlings were tested with ELISA about $3\frac{1}{2}$ months after inoculation. Table 5 shows that the extinction values were very low and that there was not much difference between them; only seedling nos. 3 and 6 gave a slightly higher value. The same two seedlings reacted positively in a bio-assay performed about eight months after inoculation (Table 5).

Discussion

The filamentous virus in *Sambucus* spp. is a typical representative of the carlavirus group in its latency in most of its hosts, narrow host range, high concentration and persistence of infectivity in crude sap, single capsid protein, buoyant density, particle size and morphology, and distant serological relationship to other carlaviruses (Fenner, 1976).

From the results of serological tests, its reaction on *Ch. quinoa* and *G. globosa*, persistence of infectivity in crude sap and particle size, our filamentous virus in *Sambucus* resembles a virus isolated from *S. canadensis*, *S. nigra* and *S. racemosa laciniata-aurea* in Britain and code-named elderberry virus A (Jones, 1970, 1972).

Hollings (1972) also isolated a virus with filamentous particles (c. 650 × 13 nm) from elderberry in S.E. England. The latter was serologically distantly related to carnation latent and chrysanthemum B viruses.

In the literature virtually nothing is reported on symptoms in *Sambucus* spp. to be attributed to a filamentous virus. Uyemoto and Gilmer (1971) mentioned that *S. canadensis* plants from which an unidentified virus belonging to the potato virus S group was isolated, showed vein clearing and faint diffuse mottle on their leaves. After inoculation of this virus to healthy *S. canadensis* plants Uyemoto et al. (1971) obtained symptomlessly infected plants only.

Besides a carlavirus-like virus a large number of other viruses have been isolated from *Sambucus* spp. In *S. racemosa* CLRV was detected (Schmelzer, 1964, 1965, 1966; Grbelja, 1972).

From *S. nigra Arabis* mosaic virus (AMV) (Cadman, 1960; Harrison and Winslow, 1961; Brčak and Polák, 1966), CLRV (Schmelzer, 1965; Štefanac, 1969; Jones and Murant, 1971; Grbelja, 1972; Jones, 1972, 1973; Horvath et al., 1974), strawberry latent ringspot virus (SLRV) (Lister, 1964) and tomato black ring virus (TBRV) (Schmelzer, 1964, 1966; Jones, 1972) were isolated, whereas its cultivar Aurea was shown to be infected by golden elderberry virus (GEV), a CLRV-related virus (Hansen and Stace-Smith, 1971) and by tobacco ringspot virus (TobRV) (Hansen, 1967).

Sambucus canadensis was shown to be a host for CLRV (Jones and Murant, 1971; Jones, 1972), cucumber mosaic virus (Uyemoto and Gilmer, 1971; Uyemoto et al., 1971), elderberry latent virus, an isometric virus (Jones, 1972), TMV (Müller, 1967), tobacco necrosis virus (TNV) (Uyemoto et al., 1971), TobRV (Wilkinson, 1952; Uyemoto and Gilmer, 1971; Uyemoto et al., 1971), TBRV (Jones, 1972), and tomato ringspot virus (TomRV) (Uyemoto, 1970; Uyemoto and Gilmer, 1971; Uyemoto et al., 1971).

From *S. ebulus* Mamula and Miličić (1975) isolated CLRV.

However, only in a few instances symptoms observed on diseased *Sambucus* plants were reported to be correlated with the virus isolated from them. Grbelja (1972) and Schmelzer (1965, 1966) proved that the vein clearing, chlorotic spots, rings and line pattern in *S. racemosa* were caused by CLRV. Similar symptoms in *S. nigra* were shown to be evoked by either CLRV (Grbelja, 1972; Jones, 1973), or TBRV (Schmelzer, 1966), or AMV (Harrison and Winslow, 1961; Brčak, 1964), and those in *S. nigra* 'Aurea' by GEV (Hansen and Stace-Smith, 1971) and a TobRV-like virus (Hansen, 1967). Tobacco ringspot virus was shown to be responsible for ringspotting, vein clearing and dark-green mottle in *S. canadensis*, whereas TNV was proved to be the killing agent of apical buds and leaves of this *Sambucus* species (Uyemoto et al., 1971).

In our experiments *S. pubens* plants inoculated with the filamentous virus remained symptomless, whereas those inoculated with CLRV or with a mixture of CLRV and the filamentous virus exhibited concentric chlorotic rings, (ring) spots, line pattern and mosaic.

However, most of the *Sambucus* bushes from which we had isolated the filamentous virus originally, had shown severe symptoms, ranging from bright-yellow ringspotting to oak leaf patterns. It seems, therefore, unlikely that those symptoms were caused by the filamentous virus alone. The possibility cannot be excluded that besides the filamentous virus either CLRV, or AMV or TBRV or a TobRV-like virus was present in the bushes too. In fact, in one of our purification experiments the virus suspension ob-

tained from leaves of *S. racemosa* from Eelde proved to contain CLRV as well, albeit in a very low concentration. Such a low concentration of CLRV might also have been the cause of the negative results of ELISA tests performed on all the original *Sambucus* material used (results not given in this paper). As we have not conducted an extensive survey for AMV, TBRV and TobRV in this material it is still feasible that one of these viruses was present too. The results of bio-assay, however, did not give us a clue into this direction.

Although the filamentous virus is most likely identical to elderberry virus A we would like to propose the name elderberry carlavirus (ECV) for our virus because of firstly the very incomplete characterization of elderberry virus A so that no proper comparison can be made, and secondly the fact that the name of the latter virus is only a code-name. Elderberry carlavirus and CLRV seem to act synergistically, as symptoms on doubly infected *S. pubens* seedlings were much more severe than those on singly infected ones.

With ELISA tests ECV in *Sambucus* can be detected, even in low concentrations.

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Samenvatting

Karakterisering van een carlavirus in vlier (Sambucus spp.)

Een carlavirus werd geïsoleerd uit *Sambucus racemosa* en *S. nigra* in Nederland. Het virus kon met sap worden overgebracht en was in staat 14 van de 58 getoetste plantesoorten en -cultivars te infecteren waarbij op vijf van deze symptomen verschenen. Ook met *Myzus persicae* vond overdracht plaats, zij het in beperkte mate. De verdunningsgrens was 10^{-3}–10^{-4}, de inactiveringstemperatuur 70–75°C en de houdbaarheid in vitro 2–4 dagen. Het virus had een sedimentatiecoëfficiënt van 155 S en het molecuulgewicht van de structuurelementen van het capside-eiwit bedroeg 31000 dalton. De deeltjes van de vier gebruikte isolaten hadden een gemiddelde zweefdichtheid van 1,315 g/cm^3. De gemiddelde normale lengte van de virusdeeltjes bedroeg 678 nm bij een breedte van ongeveer 12 nm. In ultradunne coupes van bladweefsel van *S. racemosa* 'Plumosa Aurea' werden bundels draadvormige virusdeeltjes waargenomen in het cytoplasma. Het virus vertoonde een zeer sterke serologische verwantschap met een virus uit vlier geïsoleerd in Groot-Brittannië en een geringe verwantschap met het anjer-latenvirus. In zijn reactie op waardplanten en zijn eigenschappen in ruw sap vertoonde het ook veel gelijkenis met eerstgenoemd virus, in de literatuur vermeld onder de code-naam 'elderberry virus A'. We stellen voor de naam carlavirus van vlier aan dit virus te geven.

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