

Helium Virus S and Y—Two New Viruses from Commercially Grown Helium Hybrids

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This work is a portion of a thesis submitted by the senior author to the University of Hamburg in partial fulfillment of the requirements for the doctoral degree. In that thesis the names Helium virus B and A are used instead of Helium virus S and Y, respectively.

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ABSTRACT

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A carlavirus and a potyvirus were isolated from commercially grown *Helium amarum* hybrids showing typical symptoms of virus infection. The carlavirus, for which the name Helium virus S (HelVS) is proposed, has slightly curved particles with a normal length of 640 nm and a sedimentation coefficient of 160 S. Its UV-absorption spectrum has a maximum at 255 nm and a minimum at 238 nm. The ratio of A_{max}/A_{min} is approximately 1.2. In sap from infected *Chenopodium quinoa* HelVS has a thermal inactivation point of 60 to 65 C and an infectivity dilution endpoint of 10^{-4} to 10^{-5} . In the cytoplasm of infected cells typical aggregates of virus particles were arranged in parallel and accumula-

tions of endoplasmic reticulum together with ribosomes and scattered virus particles were found. Serologically, HelVS is related to carnation latent, chrysanthemum B, and potato M viruses. The molecular weight of its protein subunit is 31,000. The potyvirus, for which the name Helium virus Y (HelVY) is proposed, has flexuous particles with a normal length of 721 nm. In sap from *C. quinoa* it has a thermal inactivation point of 55 to 60 C and an infectivity dilution endpoint of 10^{-4} . Helium virus Y induces typical pinwheels, bundles, and laminate inclusions. Serologically, it shows some relationship with bean yellow mosaic, lettuce mosaic, and potato Y viruses.

Additional key words: serological relationships, cellular inclusions, particle properties.

Inspection of commercially grown Helium hybrids revealed many plants which showed typical symptoms of virus infection. Reports of virus infections in plants of the genus *Helium* are scarce. Rush and Gooding (9) found that *Helium amarum* plants in North Carolina are naturally infected with tobacco ringspot virus. In the present paper we describe two elongated viruses from cultivated Helium hybrids—one belonging to the carlavirus group, the other one belonging to the potyvirus group. The names Helium virus S (HelVS) and Helium virus Y (HelVY) are proposed for these viruses which apparently are not identical to any of the well characterized viruses.

MATERIALS AND METHODS

Sap from the leaves of Helium hybrids was rubbed on carborundum-dusted leaves of indicator plants. Helium virus S was purified by homogenizing 100 g of leaves of infected *Chenopodium quinoa* Willd. in 150 ml 0.5 M sodium borate-HCl buffer pH 7.5 containing 0.001% EDTA and 0.1% thioglycolic acid. A supernatant was obtained by low-speed centrifugation and 8.5% butanol was added to it dropwise under continuous stirring. The final virus sediment after three cycles of low- and high-

speed centrifugation was resuspended in 0.02 M borate buffer pH 7.5. Further purification was achieved by centrifugation on a linear 10-40% sucrose density gradient buffered in 0.2 M borate, pH 7.5.

Helium virus Y was purified by homogenizing 100 g of infected leaves of *C. quinoa* in 50 ml of chloroform and 200 ml of 0.5 M borate buffer pH 7.5 containing 0.01% EDTA and 0.1% thioglycolic acid. To the aqueous phase obtained after low-speed centrifugation 8.5% butanol was added dropwise under continuous stirring in an icebath. The final virus sediment obtained after two cycles of low- and high-speed centrifugation was resuspended in 0.02 M borate buffer pH 7.5.

Protein molecular weights (5, 7), sedimentation behavior (8), UV-absorption spectrum (8), and electron microscopical properties (6) were studied as described previously. Serological tests—if not stated otherwise—were done with the slide precipitin test (1).

RESULTS

Virus symptoms on Helium hybrids.—Typical virus symptoms on Helium hybrids were flower color break with the appearance of yellow stripes in red flowers (Fig. 1), distortion and reduction in number of marginal florets (Fig. 1), chlorotic and necrotic spots or ringspots on leaves (Fig. 2), leaf distortion, leaf mosaic, growth reduction of leaves and flower heads, and stunting of the whole plants owing to the development of shortened

internodes. On the whole, symptoms were quite variable in different cultivars.

Isolation of two different viruses.—Sap from diseased *Helenium* sp. plants contained elongated virus particles. Some of these virus particles appeared slightly curved, others were more flexible. Ultrathin sections revealed the presence of pinwheels.

Sap from *Helenium* was rubbed on 69 indicator plants in 20 different plant families. Symptoms developed only on *C. quinoa*, *C. amaranticolor* Coste & Reyn., *C. album* L., and *Gomphrena globosa* L. In *G. globosa* local lesions only developed which were first chlorotic and later turned necrotic. In these lesions only the flexible particles were detected. Plants of the three *Chenopodium* spp. developed systemic symptoms and in the first experiments both types of particles were found. In later experiments we used highly diluted sap from *Helenium* sp. for inoculations and two viruses could be selected which in subsequent transfers consistently caused different types of symptoms on *C. quinoa*. One virus had slightly curved particles and was named HelVS (Fig. 3), the other one had flexuous particles and was named

HelVY (Fig. 4).

The following plants showed no symptoms after inoculation with HelVS and HelVY in sap from infected *C. quinoa* and *Helenium* sp. plants and no virus could be transmitted back from these plants to *C. quinoa*: *Ageratum conyzoides* L., *Amaranthus caudatus* L., *A. retroflexus* L., *Antirrhinum majus* L., *Apium graveolens* L., *Arachis hypogea* L., *Atriplex hortensis* L., *Beta vulgaris* L., *Brassica oleracea* L., *B. rapa* L., *Calendula officinalis* L., *Caltha palustris* L., *Campanula rapunculoides* L., *Capsicum annum* L., *Cheiranthus cheiri* L., *Chrysanthemum segetum* L., *Citrullus vulgaris* Schard., *Conium maculatum* L., *Cucumis sativus* L., *Cucurbita maxima* Duch., *C. pepo* L., *Dactylis glomerata* L., *Dahlia pinnata* Car., *Datura metel* L., *Daucus carota* L., *Dianthus armeria* L., *D. barbatus* L., *Glycine max* (L.) Merr., *Helianthus annuus* L., *Hordeum sativum* Jess., *Impatiens balsamina* L., *I. glandulifera* Royle, *Latuca sativa* L., *Lobelia cardinalis* L., *L. fulgens* Willd., *L. siphilitica* L., *Lupinus albus* L., *Lycopersicon pimpinellifolium* Mill., *Matthiola incana* R. Br., *Nicotiana clevelandii* Gray, *N. debneyi* Domin, *N.*



Fig. 1-2. Symptoms on *Helenium* hybrids infected with *Helenium virus S* and *Helenium virus Y*. 1) Color break and distortion of marginal florets in flower heads. 2) Spotting and mosaic on leaves.

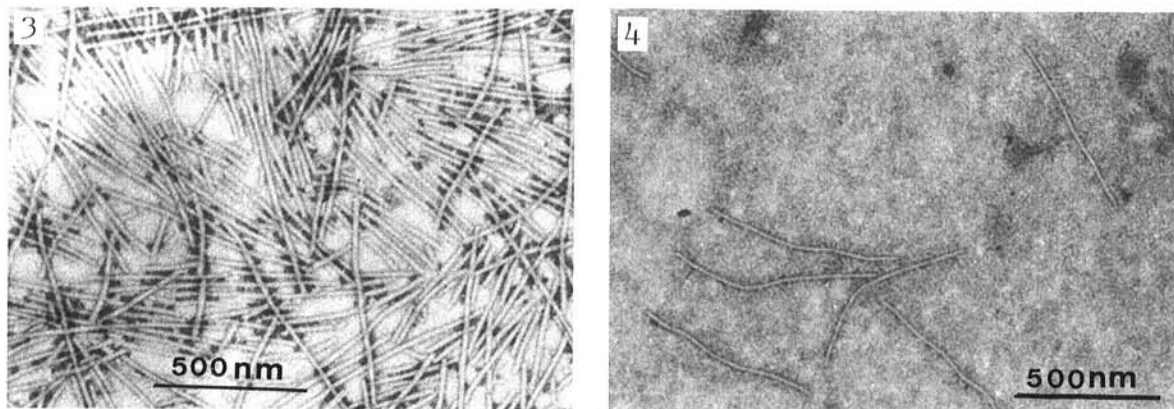


Fig. 3-4. Electron micrographs of *Helenium virus S* (HelVS) and *Helenium virus Y* (HelVY) negatively stained with sodium phosphotungstate. 3) Purified preparation of HelVS. 4) Particles of HelVY in crude sap of infected *Gomphrena globosa*.

glutinosa L., *N. megalosiphon* Heurck & Muell., *N. rustica* L., *N. tabacum* L. 'Samsun' and 'Xanthi', *Ocimum basilicum* L., *Pastinaca sativa* L., *Petunia hybrida* Vilm., *Phaseolus vulgaris* L., *Plantago lanceolata* L., *Pisum sativum* L., *Solanum melongena* L., *Spinacia oleracea* L., *Tetragonia expansa* Murr., *Trifolium hybridum* L., *T. incarnatum* L., *Tropaeolum majus* L., *Verbena encelioides* Benth. & Hook., *Vicia faba* L., *Vigna sinensis* Erdl., *Viola tricolor* L., *Zea mays* L., *Zinnia elegans* Jacq.

Electron microscopy, host reactions, and physical properties of Helenium virus S.—A population of 334 particles of HelVS from sap of *C. quinoa* negatively stained with phosphotungstate had a normal length of 640 nm. The virus infects *C. quinoa*, *C. amaranticolor*, and *C. album*, but not *Gomphrena globosa*. On the infected *Chenopodium* spp. chlorotic local lesions are formed which are later followed by systemic symptoms. In *C. quinoa* the intercostal areas of systemically infected leaves turn yellow and only narrow bands along the veins remain green. The leaves are somewhat smaller than those from healthy plants, but on the whole the growth of the plants is not markedly affected. Normal seeds are formed.

The thermal inactivation point of HelVS is between 60 and 65 C and its infectivity dilution endpoint in sap from infected *C. quinoa* is 10^{-4} to 10^{-5} .

In cells of *C. quinoa* infected with HelVS no pinwheel inclusions were present. However, in cells from infected *C. quinoa* and Helenium hybrids cytoplasmic inclusions were found which contained conspicuous accumulations of endoplasmic reticulum together with ribosomes, scattered virus particles, and aggregates of virus particles in parallel arrangement (Fig. 5). Similar accumulations of endoplasmic reticulum have been observed with several carlaviruses, including Wisconsin pea streak, red clover vein mosaic, and cole latent viruses [for a review, see (2)].

Properties of purified preparations of Helenium virus S.—Purified preparations of HelVS (Fig. 3) have a sedimentation coefficient of $s_{20,w} = 160$ S. The UV-absorption spectrum—not corrected for light scattering—shows a maximum at 255–256 and a minimum at 238–239 nm. The ratio of A_{max}/A_{min} is about 1.2 and the ratio A_{280}/A_{260} is about 0.61. In SDS-polyacrylamide electrophoresis a single protein band is formed for which a molecular weight of about 31,000 daltons was calculated. This value was independent of the polyacrylamide concentration which ranged from 1–10%. The protein thus showed a normal behavior in SDS-polyacrylamide electrophoresis.

Serological properties of Helenium virus S.—Helenium virus S is a good immunogen. Antisera with titers of 1:4,096 were readily obtained. In agar gel double diffusion tests these antisera reacted with pyrolydine degraded virus in crude sap from *Helenium* sp.

Purified preparations of HelVS reacted in the slide precipitin test with antisera to several carlaviruses; i.e., carnation latent (8,000/100), chrysanthemum virus B (1,024/64), and potato virus M (4,000/100)—the reciprocals of the homologous and the heterologous titer with HelVS are given in parentheses. Weak reactions with titers below 1:10 were obtained with one antiserum each to lily symptomless and potato virus S. No reactions were observed with antisera to poplar mosaic virus and to several potyviruses; i.e., bean yellow, lettuce mosaic, and

potato virus Y.

A conjugate of alkaline phosphatase and antibodies to chrysanthemum virus B could be used for the detection of low concentrations of purified HelVS in enzyme-linked immunosorbent assays (ELISA) (6).

Electron microscopy, host reactions, and physical properties of Helenium virus Y.—A population of 393 particles of HelVY from sap of *C. quinoa* negatively stained with phosphotungstate had a normal length of 721 nm.

On *G. globosa*, *C. quinoa*, *C. amaranticolor*, and *C. album* HelVY causes chlorotic local lesions. The infection later becomes systemic only in the three *Chenopodium* spp. In *C. quinoa* chlorotic and later necrotic spots develop on the first leaves which are formed after infection. The leaves are severely distorted. About 2 wk after inoculation the growth of the main shoot ceases. Some axillary shoots with strongly distorted leaves may develop. Flowers and some seed are produced only under high light intensity.

With sap from infected *C. quinoa* the infectivity dilution endpoint of HelVY is 10^{-4} , the thermal inactivation point is between 55 and 60 C.

Infected cells of *Helenium* sp. and *Chenopodium* sp. plants contain typical pinwheels, bundles, and laminate inclusions (Fig. 6 and 7). Based on morphology HelVY belongs to the subdivision II of the potyvirus group (3).

Purification and serological properties of Helenium virus Y.—Owing to the low concentration of HelVY in plant sap, its strong tendency to aggregate and its almost lethal effect on *Chenopodium* spp. only small amounts of partially purified HelVY were obtained which were not sufficient to produce an antiserum or to study the sedimentation behavior, the UV-absorption spectrum, and the protein molecular weight.

Serological properties were studied with virus in crude sap from infected *C. quinoa* and antisera to several potyviruses. Either no reactions at all or weak reactions up to serum dilutions of 1:8 at the most were observed with antisera to Bidens mottle, celery mosaic, plum pox, potato virus A, and tulip breaking viruses. Antisera to PVY and lettuce mosaic virus reacted at dilutions up to 1:16; the homologous titers of these antisera were 1:1,024 and 1:256, respectively. A reaction up to a dilution of 1:100 was observed with an antiserum to a bean isolate of bean yellow mosaic virus; the homologous titer of this antiserum was 1:2,000. No reactions were observed with antiserum to HelVS and to other carlaviruses including carnation latent virus, potato virus M, and chrysanthemum virus B.

DISCUSSION

Particle morphology, serological properties, and cytological observations clearly indicate that HelVS is a typical carlavirus and HelVY a typical potyvirus. The sedimentation coefficient of 160 S, the protein molecular weight of about 31,000 daltons, and the UV-absorption spectrum of HelVS further support its classification as a carlavirus. The only other well characterized carlavirus which naturally infects a member of the family Compositae is chrysanthemum virus B (4). Serologically HelVS is related to, but also is clearly different from chrysanthemum virus B. The two viruses also have very

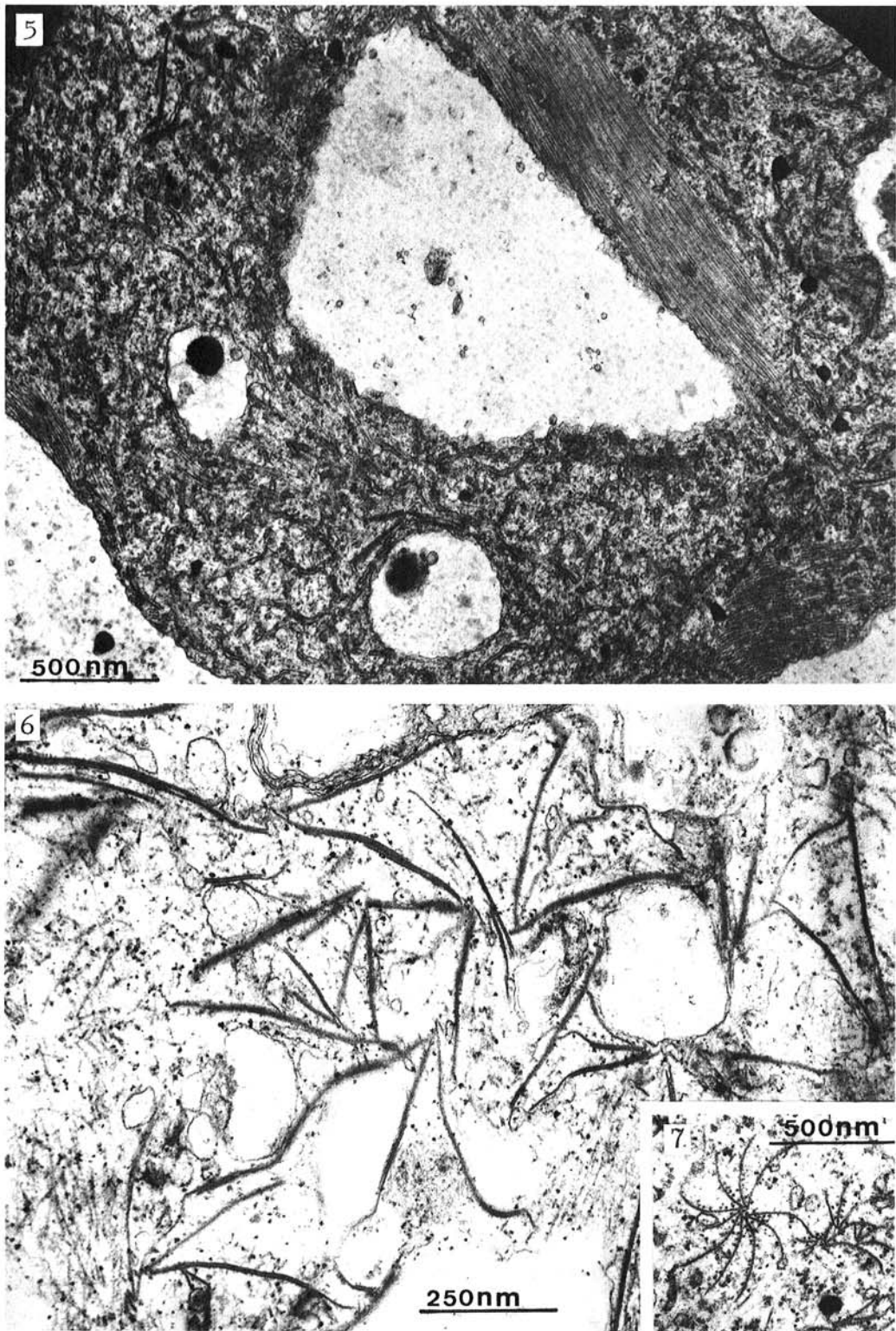


Fig. 5-7. Ultrastructural changes caused in infected cells by Helenium virus S (HelVS) and Helenium virus Y (HelVY). 5) Ultrathin section of leaf cell from HelVS-infected *Helenium* sp. leaf. Cytoplasmic inclusion with accumulation of endoplasmic reticulum containing ribosomes, scattered virus particles and aggregates of virus particles arranged in parallel. 6) Ultrathin section of leaf cell from HelVY-infected *Chenopodium quinoa* with laminated aggregates. 7) Ultrathin section of leaf cell of HelVY-infected *Helenium* with pinwheel inclusion.

different host ranges, *Nicotiana clevelandii* is systemically infected by chrysanthemum virus B, but not by HelVS. *Chenopodium* spp. are systemically infected by HelVS, but not by chrysanthemum virus B. So far no common host is known for the two viruses.

According to Edwardson (3) the following well characterized potyviruses can infect members in the family Compositae: beet mosaic, bidens mottle, bean yellow mosaic, lettuce mosaic, pea necrosis, plum pox, potato virus Y, tobacco etch, turnip mosaic, and watermelon mosaic II. Helenium virus Y is differentiated from all these viruses by differences in the host range. It also is differentiated from plum pox, potato virus Y, turnip mosaic, and watermelon mosaic virus II by the induction of a different type of cylindrical inclusion (3). In addition, lack or relative weakness of serological relationship differentiate HelVY from bean yellow mosaic, bidens mottle, plum pox, lettuce mosaic, and potato virus Y viruses.

Thus HelVS and HelVY are clearly differentiated from all other well characterized carla- and potyviruses. The names HelVS and HelVY are proposed to give reference to their group membership. So far we cannot relate any particular symptom seen in the different *Helenium* sp. hybrids with the presence of a particular virus because no virus free plants were available for such studies.

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