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Hippeastrum mosaic virus and another filamentous virus in Eucharis grandiflora

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Abstract

Two viruses were found in mosaic-diseased plants of *Eucharis grandiflora* in a glasshouse of the laboratory. One virus with a normal particle length of 733 nm caused local lesions on *Hyoscyamus niger* and mosaic symptoms in leaves of healthy-looking *Eucharis* and *Hippeastrum* plants. On the basis of its host range, physical properties and serology it was identified as *Hippeastrum* mosaic virus, a member of the potyvirus group. This was confirmed by the presence of spherical nuclear inclusions and pinwheels in different tissues of diseased *Eucharis* plants.

The second virus with a normal particle length of 598 nm was present in both healthy-looking and mosaic-diseased *Eucharis* plants, and it inconsistently induced local lesions on *Gomphrena globosa*. According to its morphology and its reaction on *Gomphrena*, it might be identical or related to *Hippeastrum* latent virus.

Crystal-like inclusions were observed in the cytoplasm of cells of both healthy-looking and mosaic-showing *Eucharis* leaves. As no virus-free seedlings of *Eucharis* were available, the virus nature of these inclusions could not be established.

Additional keywords: Hippeastrum latent virus, pinwheels.

Introduction

Some plants of *Eucharis grandiflora* Planch. (Amaryllidaceae) grown in a glasshouse of the laboratory for many years showed severe mosaic symptoms in the leaves (lamina and stalk) (Fig. 1) while others looked healthy.

Dip preparations for the electron microscope prepared from *Eucharis* leaves with mosaic showed the presence of virus-like particles of two different lengths: one of c. 750 nm, the other of c. 600 nm. The former length prevailed on the latter in the preparations.

The disease could readily be transmitted from mosaic-showing *Eucharis* plants to healthy-looking ones by sap inoculation. As symptoms first appeared in newly-developed leaves only, the time at which mosaic became visible depended on the development of the growing point at the time of inoculation. It varied from 14 days to a couple of months. The symptoms did not always last; sometimes they disappeared in due course of time (after 10–15 months) and even the newly-developed leaves looked healthy.

In 1923 Whetzel reported a mosaic disease of *E. grandiflora* in Bermuda.

Two viruses are known to occur in *Eucharis* plants, viz. a strain of tobacco ringspot

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Fig. 1. Leaf of *Eucharis grandiflora* with mosaic symptoms.

Fig. 1. Blad van Eucharis grandiflora met mozaïeksymptomen.

virus (Kahn et al., 1962) and a filamentous virus, c. 740 nm long, called *Eucharis* mosaic virus (Brandes, 1964).

The aim of this investigation was to identify the incitant(s) of the disease.

Materials and methods

Mosaic-showing plants of *E. grandiflora* and healthy-looking ones were collected from the Laboratory of Virology and from the Laboratory of Horticultural Research, Wageningen. They were maintained in a glasshouse of the former laboratory at temperatures ranging from 20 to 30 °C. No *Eucharis* plants were raised from seed as we could not obtain the latter. For comparison, we also used *Hippeastrum* plants infected with *Hippeastrum* mosaic virus (HMV) and healthy-looking *Hippeastrum* seedlings, both raised in our glasshouse.

Host range tests. Plants of 42 species and cultivars were inoculated manually after dusting the leaves with 600-mesh carborundum, with sap obtained by grinding the *Eucharis* leaves in either 1% K_2HPO_4 or in water. After about three weeks return inoculations to indicator plants (*Hyoscyamus niger* 'Pallidus' and *Gomphrena globosa*) were always made from inoculated and from uninoculated leaves. Host range tests were performed in November, December, February and March. Besides in sap transmission tests, *H. niger* and *G. globosa* were also used as assay plants in experiments on persistence of infectivity in crude sap and on purification.

Inhibitors of infectivity. The possible presence of inhibitors of infectivity in sap from *Eucharis* leaves was examined by mixing sap from healthy-looking or mosaic-showing *Eucharis* plants 1 : 1 with a purified tobacco mosaic virus (TMV) suspension of 10 µg/ml. The mixture was assayed on 10 leaf halves of *Nicotiana glutinosa*. The same TMV suspension diluted 1 : 1 with distilled water served as a control.

Transmission experiments with Myzus persicae were performed with apterous aphids which were starved for 30 min and then allowed to feed on mosaic-showing plants of

Eucharis (10 aphids per plant) for 10 min. Thereafter, they were transferred to healthy-looking plants of *Eucharis*.

Virus purification was from mosaic-showing leaves of *Eucharis*, intermediate in size. Due to the high content of mucilage in *Eucharis* leaves virus purification was very difficult. The mucilage easily sedimented in high-speed centrifugation. The molecular sieving method adopted by Huttinga (1975) for the purification of onion yellow dwarf virus from leek did not consistently yield satisfactory results in our case. After trying out a number of different purification procedures in which we tried to get rid of the mucilage by varying the amount of leaf material, the buffers, centrifugal forces and time of centrifugation, and by using a sugar cushion during high-speed centrifugation, a detergent (Triton X-100) or polyethylene glycol 6000 (PEG), we eventually had the best results with the following purification procedure. Leaf material (20 g) of *Eucharis* was homogenised in a Waring blendor with 250 ml of 0.15 *M* phosphate buffer (pH 6.75), 0.1% thioglycolic acid, 1 *M* urea, 10 ml of chloroform, 10 ml of carbon tetrachloride, and 5 ml of diethyl ether. The homogenate was centrifuged at 15000 *g* for 10 min and PEG with NaCl was added to the supernatant to a final concentration of 2.5% and 0.2 *M*, respectively. After stirring at 4 °C for 15 min and allowing to stand for 1 h the mixture was centrifuged at 6000 *g* for 10 min, yielding a pellet (A) and supernatant (B). The pellet was resuspended in 50 ml of 0.15 *M* phosphate buffer (pH 6.75), stirred gently at 4 °C for 30 min and centrifuged at 7500 *g* for 10 min. The resulting supernatant and the supernatant B were centrifuged at 54000 *g* for 2.5 h. The pellets formed (A′ and B′, respectively) were resuspended in 2.85 ml of 0.15 *M* phosphate buffer (pH 6.75), stirred gently at 4 °C for 1 h, and centrifuged at 7500 *g* for 10 min. The resulting supernatants (A″ and B″, respectively) were then mixed with 0.75 ml of distilled water and 5.7 ml of CsCl solution (0.535 g/ml). Three ml of the respective mixtures were added to each of the centrifuge tubes (Beckman SW 50.1), overlaid with paraffin oil and centrifuged at 114000 *g* for 17.5 h. Virus zones were dialysed against 0.15 *M* phosphate buffer (pH 6.75) to remove the CsCl.

Extinction coefficient. As the prevailing c. 750 nm particle probably belongs to the potyviruses the specific extinction coefficient of another member of this group, viz. tobacco etch virus with $E\ 260\ {}^{0.1\%}_{1\ cm} = 2.4$ (Gibbs and Harrison, 1971), was used to estimate the virus concentration.

Antisera were produced in rabbits, injected intravenously with 0.56 mg and 1.16 mg of purified fractions A″ and B″, respectively. Thereafter, the rabbits were injected intramuscularly on the 5th, 12th, and 19th day with 0.56, 0.46 and 0.8 mg, respectively, of virus from fraction A″, and with 0.56, 1.3 and 1.2 mg, respectively, of virus from fraction B″. A week after the last injection the titre of the antisera was determined. As the titres were very low another intramuscular injection of the two virus fractions (0.90 mg for fraction A″ and 1.14 mg for fraction B″) was given. Five days after the last injection blood was collected. Microprecipitin methods according to Van Slogteren (1954) and Noordam (1973) were used for serological tests. For these tests purified virus preparations were dialysed against a solution of 0.85% sodium chloride in 0.01 *M* Tris buffer (pH 7.6) containing 0.01% sodium azide. Antisera (unabsorbed and absorbed with healthy plant material) to narcissus yellow stripe virus,

HMV, *Ornithogalum thyrsoides* virus, hyacinth mosaic virus, broken tulip virus, *Scilla* virus and *Galtonia* virus were kindly provided by Ir D. H. M. van Slogteren, Bulb Research Centre, Lisse, The Netherlands, and antisera to potato virus Y^N, bean common mosaic virus, onion yellow dwarf virus, freesia mosaic virus, *Alstroemeria* mosaic virus, bean yellow mosaic virus and leek yellow stripe virus were from Ing. D. Z. Maat, Research Institute for Plant Protection, Wageningen.

Light microscopy. For light microscopic observations, epidermal strips from leaves of diseased and healthy-looking *Eucharis* plants were prepared according to the methods described by Bos and Rubio-Huertos (1969) and Christie and Edwardson (1977).

Electron microscopy was performed in crude sap and in purified preparations after negative staining with 2% potassium phosphotungstate (pH 5.6). 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, TMV from 'White Burley' tobacco was used as an internal size standard (Bos, 1975). Specimens for studying viral inclusions were prepared as described by Hitchborn and Hills (1965) and stained with 2% phosphotungstate (pH 5.6). For in situ studies 1 mm^2 pieces of leaf lamina (green and yellow areas), petioles, immature anthers and ovules of mosaic-showing *Eucharis* plants were fixed with Karnovsky fixative in 0.07 *M* cacodylate buffer at room temperature for 1 h and at a vacuum of 50 Torr for 30 min, washed six times for 10 min each in 0.1 *M* cacodylate buffer with 0.1% $CaCl_2$, and post-fixed in a solution of 1% osmium tetroxide in 0.1 *M* cacodylate buffer with 0.1% $CaCl_2$ at room temperature for 1 h. The material was then washed twice in 0.1 *M* cacodylate buffer with 0.1% $CaCl_2$ for five min each, and once in veronal acetate buffer (pH 5.1) for five min. The second post-fixation was done by using cold uranyl acetate (2%) in veronal acetate buffer at room temperature for 1 h. The plant material was then washed twice in veronal acetate buffer (pH 5.1) for five min each, and once in doubly distilled water for five min. The fixed tissue pieces were then dehydrated in a graded series of ethanol solutions, infiltrated with methacrylate, and embedded. Ultrathin sections were made with and LKB ultratome III, using a glass knife. The grids with the sections were placed in a 2% uranyl acetate solution and later in lead citrate with 0.001 *M* NaOH for 10 min. The sections were examined with Siemens Elmiskop I and 101 electron microscopes.

Results

Sap transmission. Diluted sap from mosaic-showing and healthy-looking *Eucharis* leaves was inoculated to plants of *H. niger* and *G. globosa*. Small chlorotic local lesions, often with a light-brown necrotic centre, developed on *Hyoscyamus* leaves 6–10 days after inoculation (Fig. 2), and red-bordered necrotic lesions sometimes appeared on *Gomphrena* leaves 14–21 days after inoculation of sap from diseased leaves (Fig. 3). Sap from healthy-looking *Eucharis* plants inconsistently induced the above-mentioned local lesions on *Gomphrena* only.

Out of the 42 plant species and cultivars inoculated with diluted sap from mosaic-showing *Eucharis* plants five showed symptoms. Besides *Hyoscyamus* and *Gomphrena*, *Chenopodium hybridum* and *C. murale* produced some (faintly chlorotic) local lesions

Fig. 2. Leaf of *Hyoscyamus niger* with local, chlorotic lesions after inoculation with sap from leaves of a mosaic-showing *Eucharis* plant.

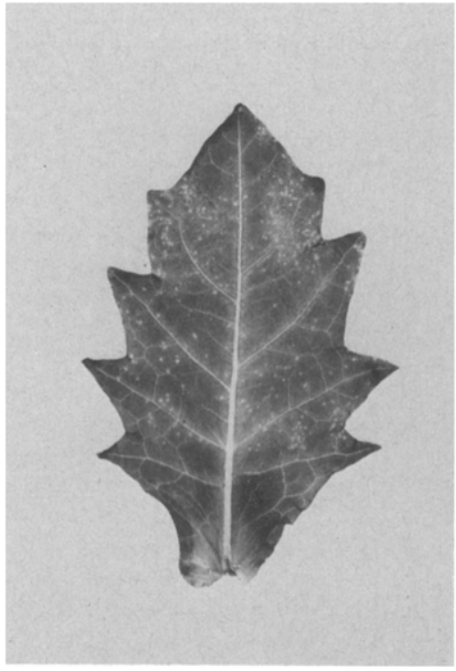

Fig. 2. Blad van Hyoscyamus niger met lokale, chlorotische lesies na inoculatie met sap van een mozaïekzieke Eucharis.

Fig. 3. Leaf of *Gomphrena globosa* with local, necrotic lesions after inoculation with sap from leaves of a mosaic-showing *Eucharis* plant.

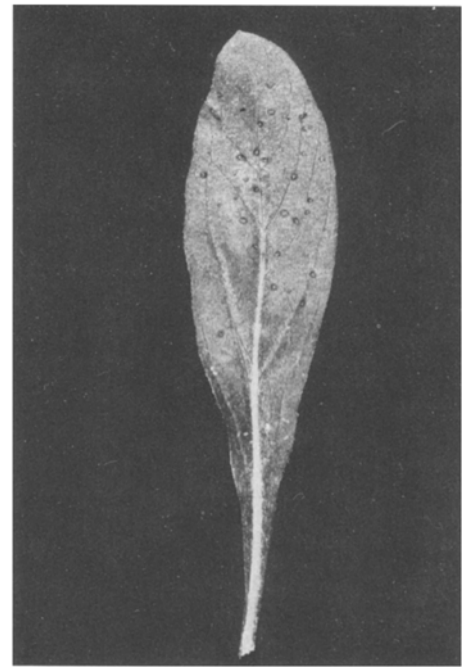

Fig. 3. Blad van Gomphrena globosa met lokale, necrotische lesies na inoculatie met sap van een mozaïekzieke Eucharis plant.

about three weeks after inoculation. However, return inoculations from these *Chenopodium* species to *Hyoscyamus*, *Gomphrena*, *C. hybridum* and *C. murale* did not yield any symptoms. *Hippeastrum* seedlings were systemically infected and showed bright mosaic symptoms. The following species and cultivars were not infected: *Amaranthus caudatus*, *Beta vulgaris* 'Groeningia' *Brassica pekinensis*, *Calceolaria hybrida*, *Capsicum annuum*, *Celosia argentea*, *Chenopodium amaranticolor*, *C. foetidum*, *C. quinoa*, *Chlorophytum spec.*, *Chrysanthemum segetum*, *Cichorium endivia*, *Cineraria hybrida*, *Citrullus vulgaris*, *Coleus* spec., *Cucumis sativus* 'Lange gele tros', *Datura stramonium*, *Emilia sagittata*, *Gynura aurantiaca*, *Lycopersicon esculentum* 'Moneymaker', *Nicotiana clevelandii*, *N. glutinosa*, *N. megalosiphon*, *N. rustica*, *N. tabacum* 'Samsun NN', *N. tabacum* 'White Burley', *Petunia hybrida*, *Phaseolus vulgaris* 'Noordhollandse bruine', *Phlox drummondii*, *Pisum sativum* 'Koroza', *Portulaca oleracea*, *Solanum melongena* 'Radja RZ', *Tetragonia expansa*, *Trifolium incarnatum*, *Vicia faba* 'Driemaal wit', *Vigna unguiculata* 'Blackeye' and *Zinnia elegans*.

Experiments on the possible presence of an inhibitor of infectivity showed that sap from *Eucharis* plants reduced the infectivity of TMV by 40–54%. Therefore, the same plant species and cultivars (minus *Hippeastrum*) were inoculated with sap from infected *Hyoscyamus* leaves exhibiting a large number of local lesions. Only *Hyoscyamus* reacted and none of the other test plants got infected.

These results suggested the presence of two viruses in mosaic-showing *Eucharis* plants. To test this, on the one hand sap from infected *Hyoscyamus* leaves was inoculated onto *Gomphrena*, *Hyoscyamus* and healthy-looking *Eucharis* plants. After 6–10 days local lesions appeared on *Hyoscyamus* and after 2–15 weeks mosaic symptoms on *Eucharis*. No symptoms developed on *Gomphrena* and return inoculation to *Gomphrena* and *Hyoscyamus* was also negative. On the other hand sap from infected *Gomphrena* leaves was inoculated onto *Gomphrena*, *Hyoscyamus* and healthy-looking *Eucharis* plants. Only a few lesions appeared on *Gomphrena* after about 18 days. The other plant species did not react at all and return inoculation to *Gomphrena* and *Hyoscyamus* was negative.

These results confirmed the presence of two different viruses in mosaic-showing *Eucharis* plants. One caused local lesions in *Hyoscyamus* but was not infectious to *Gomphrena*, the other produced local lesions in *Gomphrena* but not in *Hyoscyamus*. Off and on the latter could also be demonstrated in healthy-looking *Eucharis* plants.

As the symptoms on *Hyoscyamus* and *Hippeastrum* very much resembled those caused by HMV, sap from *Hippeastrum* plants infected with this virus was inoculated onto *Gomphrena*, *Hyoscyamus* and healthy-looking *Eucharis* plants. Within seven days *Hyoscyamus* produced local lesions and *Eucharis* showed mosaic symptoms within 3–15 weeks; both the symptoms were of the same type as those caused by one of the viruses from mosaic-showing *Eucharis* plants. In some of these experiments *Gomphrena* produced a few local lesions similar to those caused by inoculation with sap from *Eucharis* plants, but in others these plants failed to react. In all sap transmission experiments *Gomphrena* proved to be a very unreliable assay plant, as sometimes (especially in spring and summer) it reacted with the formation of aspecific local lesions

Table 1. Effect of different concentrations of polyethylene glycol 6000 (PEG) added to clarified sap from mosaic-showing *Eucharis* leaves, on the infectivity of pellet and supernatant formed after low speed centrifugation.

% PEG (w/v) added	Number of local lesions[1] on			
	Hyoscyamus		*Gomphrena*	
	pellet	supernatant	pellet	supernatant
0	0	437	0	31
0.5	10	400	0	20
1.0	32	253	1	7
1.5	16	722	0	21
2.0	102	360	49	23
2.5	42	646	143	2
3.0	417	105	107	2
3.5	593	26	206	0
4.0	201	104	109	0
4.5	641	63	26	0

[1] Average number of local lesions per leaf.

Tabel 1. Effect van verschillende concentraties polyethyleenglycol 6000 (PEG) toegevoegd aan helder gemaakt sap uit mozaïek-zieke Eucharis-bladeren, op de infectiositeit van pellet en bovenstaande vloeistof gevormd na centrifugering bij laag toerental.

only, a phenomenon already described by Francki (1967). *Hyoscyamus* also showed tremendous differences in susceptibility and sometimes it produced irregularly-shaped, necrotic lesions only or no local lesions at all (in winter and early spring) upon inoculation with sap from mosaic-showing *Eucharis* plants or with purified virus preparations.

Aphid transmission. The mosaic disease was transmitted by *Myzus persicae* to one out of six, and to three out of six healthy-looking *Eucharis* plants in two experiments, respectively.

Properties in crude sap. Sap from mosaic-showing *Eucharis* plants was diluted five times because of the phytotoxicity of undiluted sap. This sap was infective after further dilution to 10^{-2}–10^{-3} when assayed in *Gomphrena* and also to 10^{-2}–10^{-3} when *Hyoscyamus* was used as assay plant. The thermal inactivation point was 70–80 °C in *Gomphrena* and 60–70 °C in *Hyoscyamus*. At 22 °C infectivity was retained for less than one day when assayed in *Gomphrena* and for 2–3 days in *Hyoscyamus*.

Virus purification. It was found that after addition of PEG to the first supernatant followed by low-speed centrifugation the resuspended pellet contained more virus infective to *Gomphrena* along with most of the mucilage, whereas the supernatant was more infective to *Hyoscyamus*. Therefore, different concentrations of PEG were used with a view to completely separate the two infectious entities. Table 1 shows that at 2.5% PEG most of the material infective to *Hyoscyamus* was in the supernatant, whereas the resuspended pellet was mostly infective to *Gomphrena*. At high concentrations of PEG (3.5–4.5%) the resulting pellet was hard and difficult to resuspend so that the results might be less reliable. Therefore, a PEG concentration of 2.5% was chosen in order to achieve separation of the two viruses present. However, the final fractions A″ and B″ invariably produced local lesions on both *Gomphrena* and *Hyoscyamus*, although A″ mostly on *Gomphrena* and B″ mostly on *Hyoscyamus*. When A″ or B″ were subjected to CsCl-gradient centrifugation off and on two light scattering

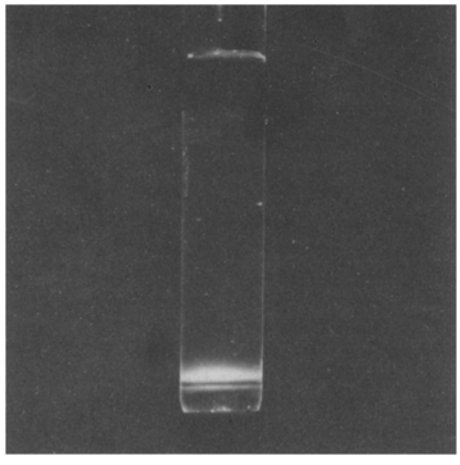

Fig. 4. Two light-scattering bands formed after subjecting fraction B″ of a purified preparation of virus from mosaic-showing *Eucharis* plants to CsCl-density centrifugation.

Fig. 4. Twee licht-verstrooiende bandjes die optraden nadat fractie B″ van een gezuiverd viruspreparaat uit mozaïek-zieke Eucharis-planten onderworpen was aan dichtheidsgradiëntcentrifugering in CsCl.

and ultraviolet radiation absorbing bands appeared, very close to each other (Fig. 4) which we were unable to separate. In addition to these two bands fraction A″ very often yielded a third band, consisting of mucilage. This band was never constant in the gradient; it occupied a position above or below the virus band(s) or at times formed a pellet. The ultraviolet absorption curve of the fractions A″ and B″ showed fraction B″ to be rather pure, with an absorption maximum at 262 nm and a minimum at 242 nm, in contrast to fraction A″ which failed to produce any peak. Because of these results and those from electron microscopical examinations (see chapter electron microscopy) the concentration of the viruses was only estimated in the B″ fraction and amounted to 22.5–77.6 mg virus per 1000 g leaf material.

Light microscopy. The staining method of Christie and Edwardson (1977) yielded better results than that of Bos and Rubio-Huertos (1969) due to the differential staining of inclusions and cell organelles with the former method. Epidermal cells of leaves of diseased and healthy-looking *Eucharis* plants showed the presence of large, needle-shaped, crystal-like inclusions sometimes extending over 3–4 cells. This type of inclusions was also observed in the parenchyma cells of petioles and at times they seemed to originate in bundles from the vascular tissue. In addition to these inclusions rectangular, hexagonal and 'cigar-shaped' crystal-like inclusions were also observed. They were stained slightly yellowish green with the O–G combination of Christie and Edwardson (1977), but not with Azur B or phloxin-ethylene blue stains.

The nuclei of epidermal cells from healthy-looking plants showed the presence of at least two nucleoli.

The most striking feature observed in the epidermal cells of leaves of mosaic-showing plants was the shape of the nucleus which was not oval, but egg-shaped and sometimes divided into a number of lobes. Many nuclei had club-shaped appendages on one side. The nucleoli were greatly enlarged and usually had a ring-like appearance with a lighter centre. These ring-shaped nucleoli were observed only in specimens stained with Azur B and phloxin-methylene blue, but not with the O–G combination. Besides the abnormal nucleoli large inclusions were observed in some of the nuclei. These inclusions were either distinct and regular, or they had an irregular shape with a wavy margin. With the O–G combination they were stained yellowish brown and it was difficult to distinguish them from the rest of the nucleus. However, with Azur B they were clearly distinguishable, because they were not stained at all in contrast to the purple- or violet-stained nuclear material. Besides inclusions in the nucleus, cytoplasmic inclusions were also found in the epidermal cells. They did not stain well with Azur B and phloxin-methylene blue, but staining with the O–G combination revealed the presence of large masses of irregularly shaped inclusions consisting of cylindrical plates.

No inclusions other than the above-mentioned crystal-like ones were found in cells of healthy-looking plants.

Electron microscopy. In the electron microscope fractions A″ and B″ revealed a large number of flexuous particles (Fig. 5A and B, respectively). The frequency of the lengths of the particles from these two fractions is represented in Fig. 6A and B, respectively. The results show that fraction A″ comprised mostly of particles with a normal length of 598 nm in addition to particles with a normal length of 740 nm, while fraction B″

Fig. 5. Electron micrographs of purified preparations of virus from mosaic-showing *Eucharis* plants after CsCl-density centrifugation. Bar represents 300 nm. A. Fraction A″; B. Fraction B″.

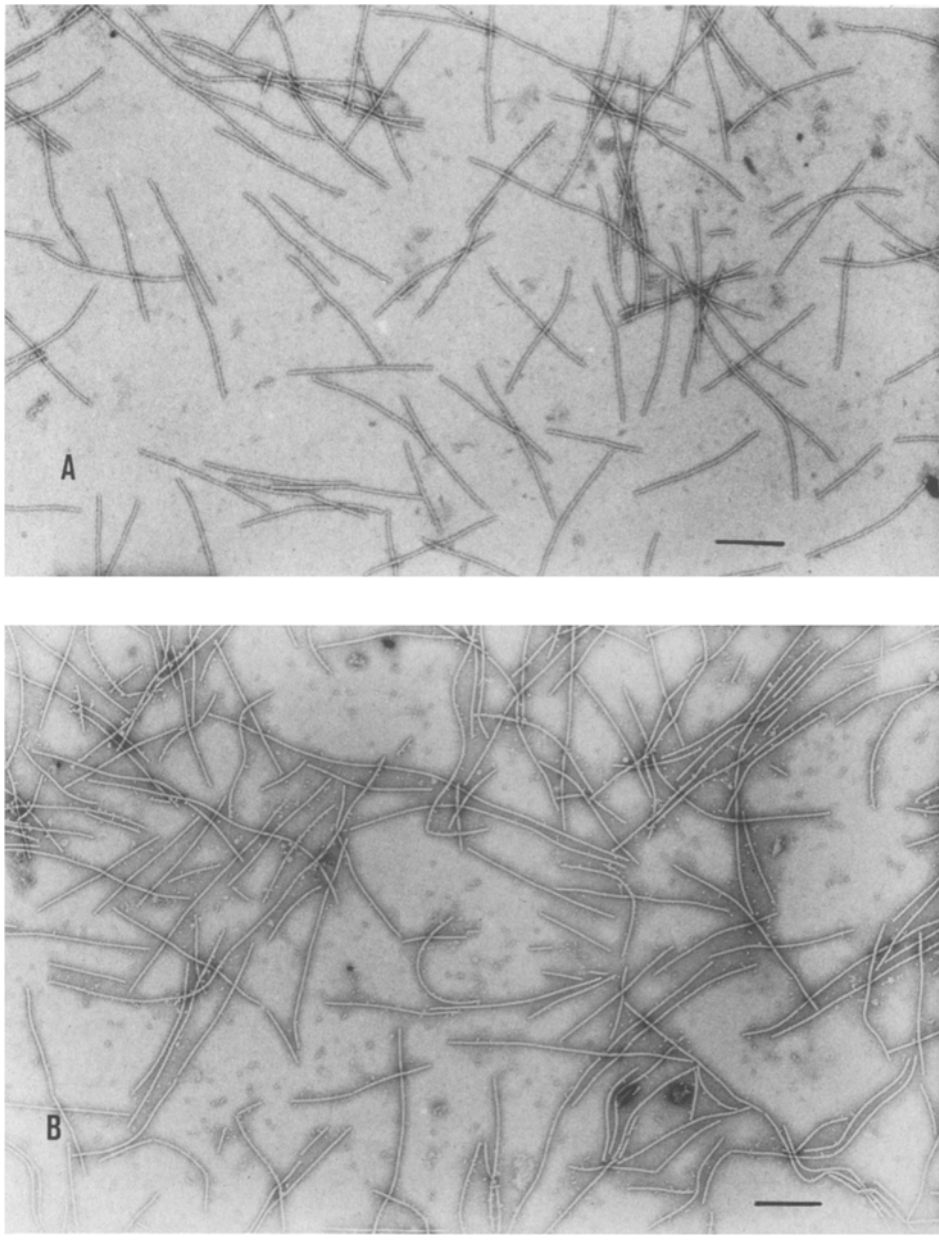

Fig. 5. Elektronenmicroscopische opnamen van een gezuiverd viruspreparaat uit mozaïek-zieke Eucharis-planten na dichtheidsgradiëntcentrifugering in CsCl. De vergrotingsstreep geeft 300 nm weer. A. Fractie A″; B. Fractie B″.

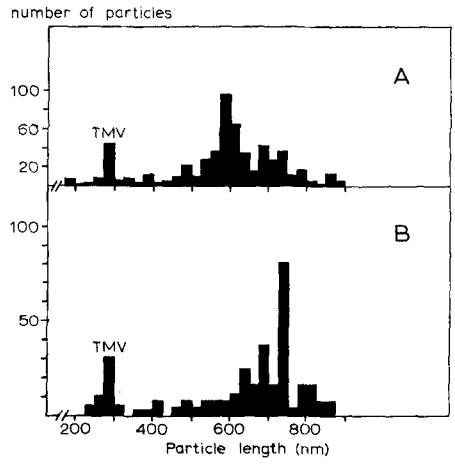

Fig. 6. Histogram of length of particles from purified preparation of virus from mosaic-showing *Eucharis* plants after CsCl-density centrifugation. Tobacco mosaic virus was added as an internal magnification standard. A. Particles from fraction A″; B. Particles from fraction B″.

Fig. 6. Lengteverdeling van deeltjes in een gezuiverd viruspreparaat uit mozaïek-zieke Eucharisplanten na dichtheidsgradiëntcentrifugering in CsCl. Tabaksmozaïekvirus was toegevoegd als interne vergrotingsstandaard. A. Deeltjes uit fractie A″; B. Deeltjes uit fractie B″.

Fig. 7. Ultrathin section of a mesophyll cell of a diseased *Eucharis* leaf with pinwheels (P). V = vacuole; R = ribosomes; CW = cell wall. The arrow points to a slightly curved plate of a pinwheel. Bar represents 200 nm.

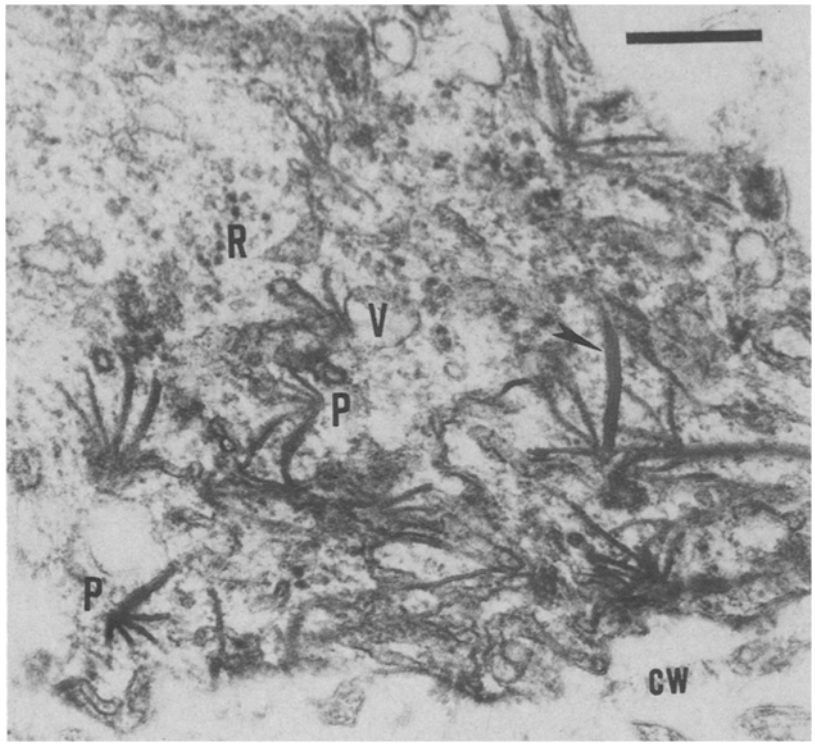

Fig. 7. Ultradunne coupe van een mesofylcel uit een ziek Eucharis-blad met schoepenradvormige insluitsels (P). V = vacuole; R = ribosomen; CW = celwand. De pijl wijst naar een enigszins gebogen plaat van een insluitsel. De vergrotingsstreep geeft 200 nm weer.

Fig. 8. Ultrathin section of a stoma cell and an adjacent epidermis cell of a diseased *Eucharis* leaf with pinwheels (P) and lamellated plates (arrows). G = deformed thylakoid system; S = starch; V = vacuole; M = mitochondrion; CW = cell wall. Bar represents 500 nm.

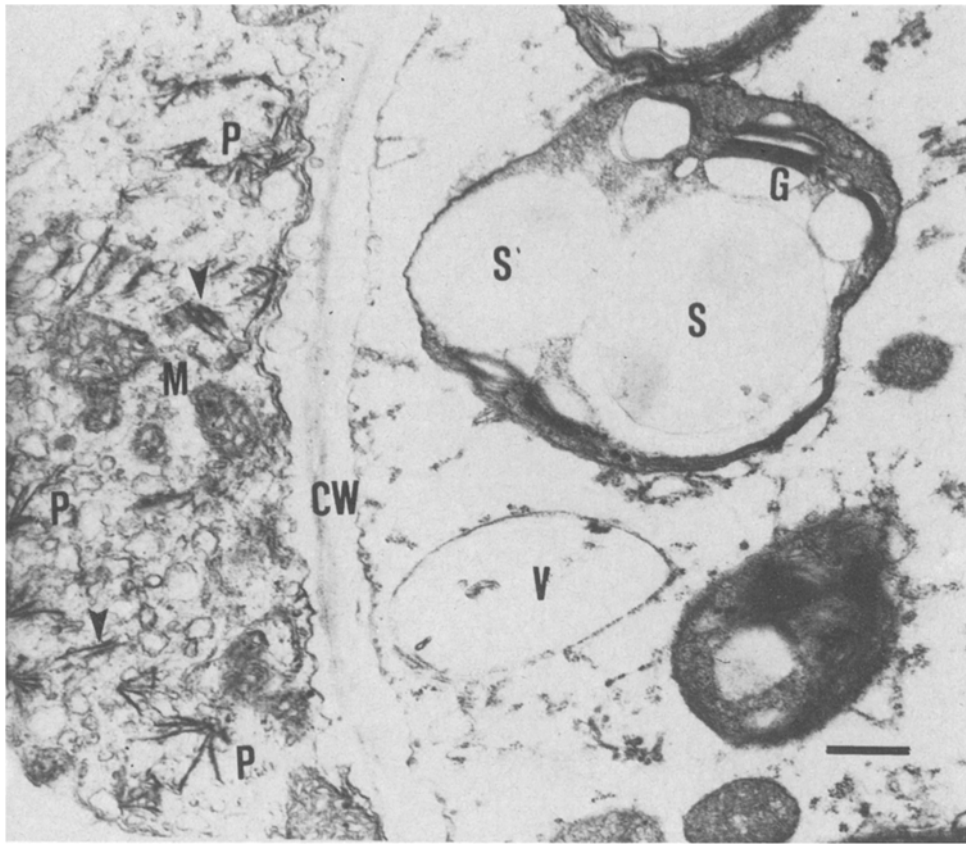

Fig. 8. Ultradunne coupe van een sluitcel van een huidmondje en een daarnaast gelegen epidermiscel van een ziek Eucharis-blad met schoepenradvormige insluitsels (P) en uit lamellen opgebouwde platen (pijlen). G = misvormd thylakoïd systeem; S = zetmeel; V = vacuole; M = mitochondrium; CW = celwand. De vergrotingsstreep geeft 500 nm weer.

comprised mostly of particles with a normal length of 733 nm in addition to a few particles of about 600 nm.

The number of virus particles in crude sap preparations of mosaic-showing *Eucharis* plants was so small that it was impossible to calculate their normal length; the specimens showed particles ranging in length from 520–800 nm.

In sections of mesophyll cells from the yellow area of a mosaic-showing leaf inclusions characteristic of potyviruses were found in the cytoplasm. In some areas thick and thin plates converged on central points to form pinwheels. These plates were usually straight but slightly curved ones also occurred (Fig. 7). In some cases, plates of varying thickness were arranged into bundles. Usually, where these bundles occurred pinwheels were also present. The latter were also observed in the epidermal cells (Fig. 8), the integument cells of the ovule (Fig. 9), the sieve elements of the petiole (Fig. 10),

Fig. 9. Ultrathin section of an ovule of a diseased *Eucharis* plant with pinwheels (P) and lamellated plates (arrows) in the integument cells. CW = cell wall. Bar represents 400 nm.

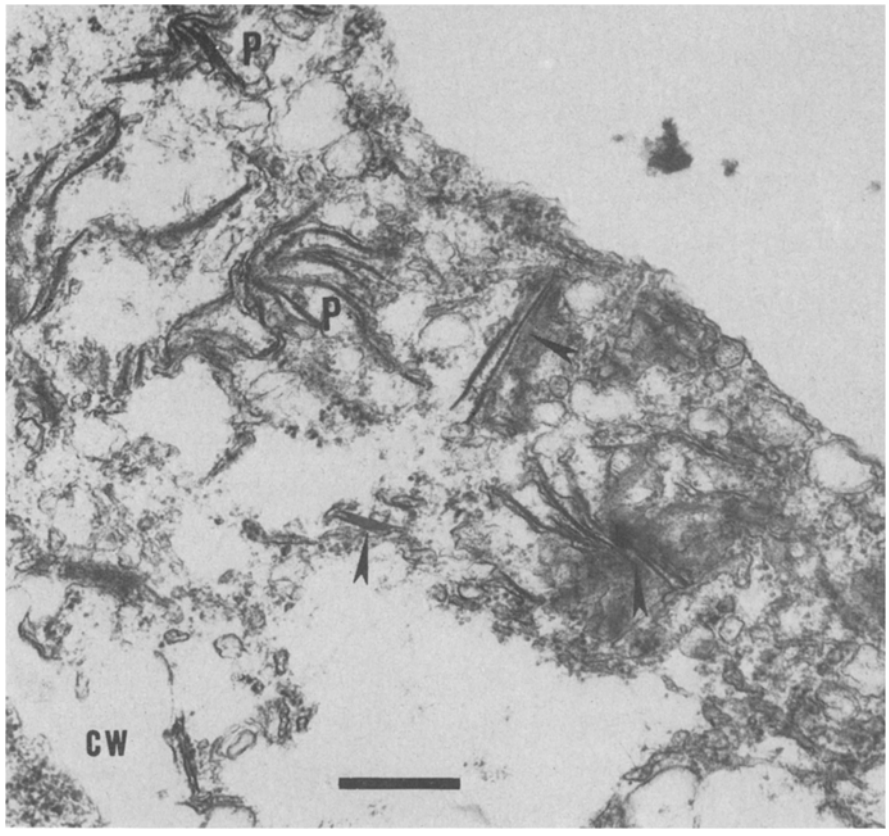

Fig. 9. Ultradunne coupe van een zaadknop van een zieke Eucharis-plant met schoepenradvormige insluitsels (P) en uit lamellen opgebouwde platen (pijlen). CW = celwand. De vergrotingsstreep geeft 400 nm weer.

and possibly in the pollen (Fig. 11) of mosaic-diseased *Eucharis* plants. The pinwheels in the integument cells comprised of plates which were more curved than those in the mesophyll cells. In the sieve elements the plates had a banded appearance by alternating dark and light areas. The pinwheels in the sieve elements seemed to be always associated with fibrillar structures (Fig. 10). The latter structures could only be observed in the sieve elements and not in mesophyll or integument cells. In the mosaic-showing leaf lamina pinwheels were only observed in the yellow areas and not in the green areas.

No pinwheels could be found in tissues of healthy-looking *Eucharis* plants.

The ring-like nucleolus and inclusions within the nucleus of epidermal cells observed with the light microscope could also be seen in ultrathin sections of this tissue (Fig. 12). The inclusions were more or less hexagonal in shape, did not show any periodicity, and were usually larger than the nucleolus. The nucleus itself was enlarged and deviated from the normal oval shape.

Although no pinwheels could be observed in the green area of a mosaic-showing leaf,

Fig. 10. Ultrathin section of a petiole of a diseased *Eucharis* plant with banded pinwheels (P) in the sieve elements and a large number of fibrillar structures (F) in the cytoplasm. M = mitochondrion. Bar represents 200 nm.

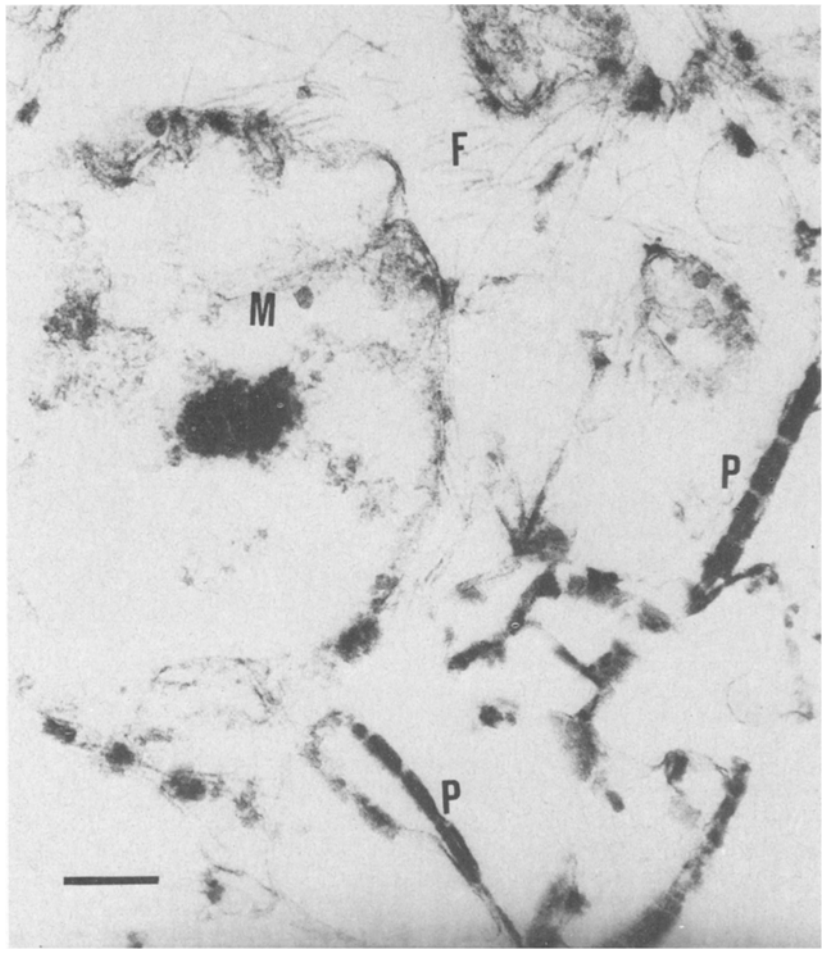

Fig. 10. Ultradunne coupe van een bladsteel van een zieke Eucharis-plant met gestreepte schoepenradvormige insluitsels (P) in de zeefelementen en een groot aantal fibrillaire structuren (F) in het cytoplasma. M = mitochondrium. De vergrotingsstreep geeft 200 nm weer.

many abnormalities could be seen, such as a very large number of ribosomes distributed singly, in aggregates or in chains forming polyribosomes, a large number of vacuoles of different sizes, and darkly stained membranes in the cytoplasm. The vacuoles were filled with fibrous material. Some chloroplasts had long appendages originating from one end and there was a considerable accumulation of starch grains by which the thylakoid system was completely disrupted.

The above-mentioned abnormalities were observed in both the green and yellow areas of mosaic-showing leaves, but not in cells of a healthy-looking plant, except for a few darkly stained ribosome clusters forming polyribosomes.

Fig. 11. Ultrathin section of an anther of a diseased *Eucharis* plant with pinwheel-like structures (arrow) in the vacuole of a pollen grain. Inset shows the structures at a higher magnification. Bar represents 200 nm.

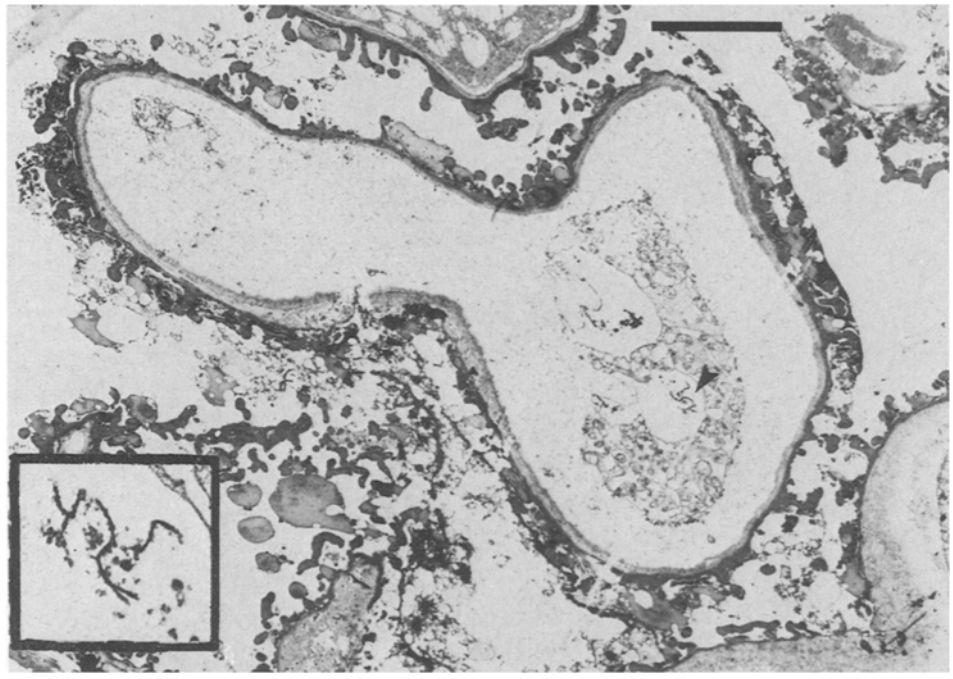

Fig. 11. Ultradunne coupe van een helmknop van een zieke Eucharis-plant met op schoepenradvormige insluitsels gelijkende structuren (pijl) in de vacuole van een pollenkorrel. Inzet toont de structuren bij grotere vergroting. De vergrotingsstreep geeft 200 nm weer.

The needle-shaped, crystal-like inclusions found in light microscopic investigations were also observed in ultrathin sections of vascular bundles of petioles both in diseased and in healthy-looking plants. These inclusions had an approximate periodicity of 13.8 Å.

Serology. The antisera to virus fractions A″ and B″ had titres of 32 and 512, respectively, in the first blood sample. After the booster injection there was no increase in the titre of the antiserum to fraction A″, but the titre of the antiserum to B″ increased to 1024. The titres were determined in the microprecipitin test according to Noordam (1973). No further serological tests were performed with fraction A″ because of the large amount of impurities present in this fraction and the low titre of its antiserum. The results of microprecipitin tests according to Van Slogteren (1954) and Noordam (1973) performed to determine serological relationships of this virus in fraction B″ and potyviruses, are given in Table 2. The results show that the virus in fraction B″ is rather closely related to HMV and narcissus yellow stripe virus. With Noordam's microprecipitin test serological reactions were obtained with all the antisera used except with bean yellow mosaic virus antiserum. As the homologous titre of antiserum to fraction B″ was 1024 with Noordam's method and 256 with Van Slogteren's one it seemed that the former was about four times more sensitive than the latter and possibly included

Fig. 12. Ultrathin section of an epidermal cell of a diseased *Eucharis* leaf with a ring-shaped nucleolus (No) and inclusions (NI) within the nucleus (N). ER = endoplasmic reticulum; M = mitochondrion; V = vacuole. Bar represents 1000 nm.

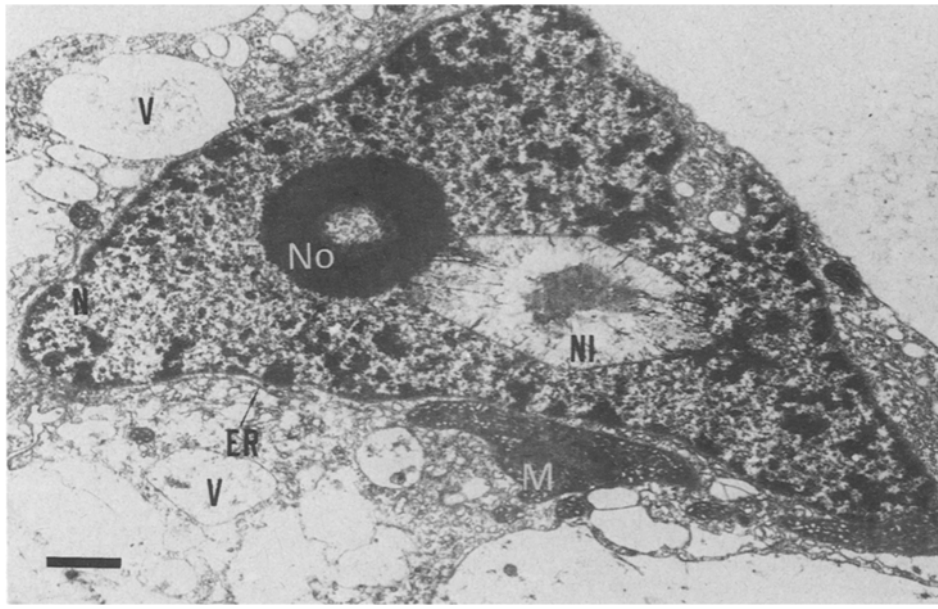

Fig. 12. Ultradunne coupe van een epidermiscel van een ziek Eucharis-blad met een ringvormige nucleolus (No) en insluitsels (NI) in de nucleus (N). ER = endoplasmatisch reticulum; M = mitochondrium; V = vacuole. De vergrotingsstreep geeft 1000 nm weer.

aspecific reactions, as can be judged from the results with unabsorbed antisera. Therefore, when we assume that titres of 16 and less with absorbed antisera in Noordam's tests were due to aspecific reactions, the results obtained with both types of microprecipitin tests were comparable.

Discussion

Much similarity in host plant reactions exists between the viruses from mosaic-showing *Eucharis* plants and those from mosaic-diseased plants of *Hippeastrum* (Brölman-Hupkes, 1975). The local lesions on *Hyoscyamus* appeared the same as those caused by HMV, whereas the reaction on *Gomphrena* was reminiscent of that caused by *Hippeastrum* latent virus (Brölman-Hupkes, 1975) which might be identical to *Nerine* latent virus (Maat et al., 1978). Moreover, healthy-looking *Hippeastrum* seedlings inoculated with sap from mosaic-showing *Eucharis* plants showed symptoms similar to those caused by HMV, and HMV from mosaic-diseased *Hippeastrum* induced mosaic symptoms in healthy-looking *Eucharis* plants indistinguishable from those in the original diseased ones.

As sap from infected *Hyoscyamus* leaves inoculated onto healthy-looking *Eucharis* plants and *Gomphrena* caused mosaic symptoms in the former and no symptoms in the latter, it is clear that in mosaic-diseased *Eucharis* plants two viruses may occur: one

Table 2. Results of microprecipitin tests according to Van Slogteren and Noordam performed with purified virus fraction B″ from mosaic-diseased *Eucharis* leaves, its homologous antiserum and antisera to a number of potyviruses.

Antisera	Titres of antisera to virus fraction B″		Homologous titres (according to the donors)
	Van Slogteren	Noordam	
Alstroemeria mosaic virus	–[1]	16	256
Bean common mosaic virus	–	1	256
Bean yellow mosaic virus	–	–	1024
Broken tulip virus[2]	–	16	640
Broken tulip virus[3]	–	160	1280
Eucharis virus fraction B″	256	1024	
Freesia mosaic virus	–	8	2048
Galtonia virus[2]	–	2	160
Galtonia virus[3]	–	16	320
Hippeastrum mosaic virus[2]	64	128	160
Hippeastrum mosaic virus[3]	160	160	320
Hyacinth mosaic virus[2]	–	16	640
Hyacinth mosaic virus[3]	–	160	1280
Leek yellow stripe virus	–	8	512
Narcissus yellow stripe virus[2]	80	80	160
Narcissus yellow stripe virus[3]	40	160	320
Onion yellow dwarf virus	–	16	2048
Ornithogalum virus[2]	–	20	160
Potato virus Y^N	–	8	512
Scilla virus[2]	–	8	320
Scilla virus[3]	–	40	not known

[1] no reaction; [2] absorbed serum; [3] unabsorbed serum.

Tabel 2. Resultaten van micro-precipitatieproeven volgens Van Slogteren en Noordam, uitgevoerd met gezuiverde virusfractie B″ uit mozaïek-zieke bladeren van Eucharis, het homologe antiserum en antisera tegen een aantal potyvirussen.

possibly causing the mosaic symptoms, the other symptomless.

Electron microscopy revealed the presence of two different filamentous particles with lengths of c. 740 nm and c. 600 nm, respectively. With purified preparations containing a majority of c. 740 nm particles (fraction B″) *Hyoscyamus* reacted much more than *Gomphrena*, whereas in case of fraction A″ it was the reverse. It is likely that the virus with the c. 740 nm particle is identical to HMV and possibly to *Eucharis* mosaic virus (Brandes, 1964) although no proper comparison with the latter virus could be made due to its incomplete characterization. The virus from fraction B″ and HMV were also found to be closely related serologically. Other characteristics of one of the viruses in mosaic-showing *Eucharis* also point to the potyviruses (aphid transmission in the non-persistent manner, the presence of pinwheels, and spherical nuclear inclusions). Similar nuclear inclusions have been reported by Christie and Edwardson (1977) in celery infected with celery mosaic virus. On the basis of the structure of the pinwheels (laminated aggregates attached to the central portion of the cylindrical inclusions) the virus with the c. 740 nm particle can be classed into subdivision II of potyviruses (Edwardson, 1974).

Much less can be said about the virus with the c. 600 nm particle. Its erratic presence in healthy-looking and mosaic-diseased *Eucharis* plants could not always be established as *Gomphrena* inconsistently reacted with local lesions and the virus concentration was very low. It never induced local lesions on *C. quinoa* in contrast to *Hippeastrum* latent virus (Brölman-Hupkes, 1975). The normal length of its particle was 598 nm which falls within the range of 584–611 nm reported for *Hippeastrum* latent virus (Brölman-Hupkes, 1975), but differs from the length reported by Maat et al. (1978) for *Nerine* latent virus (664 nm), a member of the carlavirus group.

As no virus-free seedlings of *Eucharis* were available it is not certain whether the crystal-like inclusions observed in the cytoplasm of both the diseased and healthy-looking *Eucharis* plants are due to virus infection or that they form part of the healthy cell.

Samenvatting

Hippeastrum-mozaïekvirus en een ander draadvormig virus in Eucharis grandiflora

Sommige *Eucharis grandiflora*-planten, die in de kas van het laboratorium gedurende een aantal jaren waren gekweekt, vertoonden een hevig mozaïek in de bladeren (Fig. 1), terwijl andere er gezond uitzagen. De ziekte kon gemakkelijk mechanisch worden overgebracht van mozaïek-zieke naar gezond-uitziende *Eucharis* planten. Daar de eerste symptomen zich uitsluitend op de pas ontwikkelde bladeren manifesteerden, hing het tijdstip waarop het mozaïek zichtbaar werd af van de ontwikkeling van het vegetatiepunt en varieerde van 14 dagen tot een aantal maanden na inoculatie. Verdund bladsap van mozaïek-zieke en gezond-uitziende *Eucharis* planten werd geïnoculeerd op bladeren van *Hyoscyamus niger* en *Gomphrena globosa*. Op *Hyoscyamus* ontwikkelden zich 6–10 dagen na inoculatie kleine, chlorotische lokale lesies, die vaak een lichtbruin necrotisch centrum hadden (Fig. 2) en soms verschenen op *Gomphrena* rood-omrande necrotische lokale lesies (Fig. 3) 14–21 dagen na inoculatie met sap van een zieke plant. Sap van een gezond-uitziende plant gaf alleen de eerder genoemde lokale lesies op *Gomphrena*. Gezond-uitziende zaailingen van *Hippeastrum*, geïnoculeerd met sap van zieke *Eucharis*, vertoonden een hevig mozaïek. Sap van *Gomphrena* bladeren met lokale lesies gaf geen symptomen op *Hyoscyamus* en gezond-uitziende *Eucharis*, terwijl sap van *Hyoscyamus* bladeren met lokale lesies geen symptomen op *Gomphrena* teweegbracht, maar wel mozaïek op gezond-uitziende *Eucharis*. Deze resultaten wezen dus op de aanwezigheid van twee virussen in mozaïek-zieke *Eucharis*. De symptomen op *Hyoscyamus* en *Hippeastrum* leken zeer sterk op die welke door *Hippeastrum*-mozaïekvirus (HMV) worden veroorzaakt. De mozaïekziekte werd op non-persistente wijze door *Myzus persicae* overgebracht op gezond-uitziende *Eucharis*.

Zuivering van de virussen was zeer moeilijk door het hoge gehalte aan slijmstoffen in bladeren van *Eucharis*-planten. Wanneer 2,5% polyethyleenglycol 6000 werd toegevoegd aan het helder gemaakte sap en dit mengsel werd gecentrifugeerd bij laag toerental, dan was de bovenstaande vloeistof (B) meer infectieus voor *Hyoscyamus* dan voor *Gomphrena*, terwijl de opgeloste pellet (A), waarin zich het meeste slijmachtige materiaal bevond, meer reactie gaf op *Gomphrena* dan op *Hyoscyamus* (Tabel 1). Van de uiteindelijke zuiveringsfracties A″ en B″ gaf A″ de meeste reactie op *Gomphrena*,

terwijl B″ hoofdzakelijk *Hyoscyamus* infecteerde. Werden beide fracties tenslotte nog onderworpen aan dichtheidsgradiëntcentrifugering in CsCl dan ontstonden vaak twee bandjes (Fig. 4), die zeer dicht bij elkaar lagen en niet konden worden gescheiden. De fracties A″ en B″ bevatten veel virusdeeltjes (Fig. 5A en B). Fractie A″ bestond uit deeltjes met een normale lengte van 598 nm, terwijl fractie B″ hoofdzakelijk deeltjes van 733 nm bevatte (Fig. 6A en B). Het virus uit fractie B″ was serologisch nauw verwant aan het HMV (Tabel 2).

Op grond van deze resultaten kon geconcludeerd worden, dat één van beide virussen in mozaïek-zieke *Eucharis* identiek is aan het HMV, een lid van de potyvirusgroep. Ultradunne coupes van het mesofyl van zieke *Eucharis*-planten vertoonden schoepenradvormige insluitsels, die bestonden uit platen (Fig. 7). Ze werden ook aangetroffen in de epidermiscellen (Fig. 8), integumentcellen van de zaadknop (Fig. 9), zeefelementen van de bladsteel (Fig. 10) en misschien in pollenkorrels (Fig. 11) van mozaïek-zieke *Eucharis*. Ook werden insluitsels in de nucleus gevonden, alsmede ringvormige nucleoli (Fig. 12). In bladeren van zowel mozaïek-zieke als in gezond-uitziende *Eucharis* werden tevens naaldvormige, rechthoekige, hexagonale en sigaarvormige, kristalachtige insluitsels aangetoond. Daar we niet de beschikking hadden over virusvrije zaailingen van *Eucharis* is het niet zeker of genoemde kristalachtige insluitsels een gevolg zijn van virusinfectie of dat zij bestanddelen zijn van de gezonde cel.

Het virus dat lokale lesies gaf op *Gomphrena* kon door zijn ongeregelde optreden en zijn lage concentratie in *Eucharis* niet nader worden gekarakteriseerd. Wat betreft zijn reactie op *Gomphrena* en zijn deeltjeslengte (598 nm) doet het denken aan het latente *Hippeastrum*-virus.

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Book review

International Virology IV. Abstracts of the Fourth International Congress for Virology held at The Hague, the Netherlands August 30–September 6, 1978. Centre for Agricultural Publishing and Documentation (Pudoc), Wageningen, the Netherlands, 1978. 674 pp. Price Dfl 85.

From 30 August to 6 September 1978, 1700 virologists from 55 countries gathered in The Hague to discuss developments in fundamental and applied research on viruses. About 200 of the participants were plant virologists.

The six mornings were devoted to plenary sessions on subjects of general interest. In four of these sessions, a paper on plant viruses was given. These four papers reviewed the use of resistance genes in crops, properties of viruses favouring their survival in different plant communities, the assembly of tobacco mosaic virus, and the genome structure and regulation of gene expression in plant viruses.

For the afternoon sessions, participants split up to attend workshops or to visit posters sessions on a great variety of special topics. Of the 1100 papers and posters, about 130 were on plant viruses and viroids. Almost half were accommodated in workshops and poster sessions exclusively devoted to plant viruses. These sessions covered multiplication of plant viruses in protoplasts, symptomatology and pathogenesis in virus infected plants, viroids, potyviruses, and the organization of seed and serum banks. All other presentations on plant viruses were placed in sessions dealing also with viruses of vertebrates and invertebrates. Such sessions included those on virus structure and assembly, replication of small RNA viruses, early stages of virus-cell interactions, ecology of vector-borne viruses, viruses in their arthropod vectors, and evolution of viruses.

The tendency to give papers on plant viruses in sessions dealing with general virological topics is indicative of the growing notion that plant viruses are not a separate group of pathogens but share many properties with viruses of other host groups.

Abstracts of all papers and posters are published in the present book, arranged in the sequence of the sessions. The abstracts were directly reproduced by a photographic process. For those who attended the congress, the book was rather heavy to carry around but that inconvenience is compensated by the wealth of recent information contained. Unfortunately the accessibility of the information is limited by the lack of a subject index. But this is probably an inevitable feature of books produced so rapidly. An index of contributors is inserted on the last pages.

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