Neth. J. Pl. Path. 81(1975) 226–236

Tentative description of Hippeastrum latent virus in Hippeastrum hybridum plants and differentiation from Hippeastrum mosaic virus

J. E. BRÖLMAN-HUPKES

Phytopathological Laboratory 'Willie Commelin Scholten', Baarn, the Netherlands

Abstract

In mosaic-diseased plants of *Hippeastrum hybridum* two viruses were found. One virus with a normal length of 706 nm caused local lesions on *Hyoscyamus niger* test plants and mosaic symptoms in the leaves of *H. hybridum*. This virus was identified with the *Hippeastrum* mosaic virus (HMV) (*/*: */*: E/E : S/*) and had a dilution end point between 10^{-3} and 10^{-4}, a thermal inactivation point between 55–60 °C and a longevity at room temperature of 28–32 hours. The second virus had a normal length between 584 and 611 nm depending on the method used. It caused local lesions on *Gomphrena globosa* and *Chenopodium quinoa* leaves, and after inoculation of *H. hybridum* was found to be present without showing symptoms. It was readily purified from inoculated leaf tissue of *C. quinoa* and *Nicotiana clevelandii* by differential centrifugation and of *H. hybridum* by density-gradient centrifugation. Purified virus had an absorption minimum at 242 nm, a maximum at 262 nm and a 260/280 absorption ratio of 1.19. The dilution end point was between 10^{-3} and 10^{-4}, the thermal inactivation point between 70 and 80 °C and the longevity in vitro at room temperature 28–32 hours. Although no direct comparisons have been made with other members of the potexvirus group, the virus seems to be a new one now named *Hippeastrum* latent virus. Both viruses were not seed-borne.

Introduction

Several viruses have been reported from *Hippeastrum hybridum* plants (Amaryllidaceae) viz. tomato spotted wilt virus (Smith, 1935), sunflower mosaic virus (Smith, 1957), cucumber mosaic virus (Kahn and Smith, 1963), *Hippeastrum* mosaic virus (Brants and Van den Heuvel, 1965), a virus designated as '*Hippeastrum* streak' (Van Velzen, 1967) and tobacco mosaic virus (De Leeuw, 1972a).

Inconsistent results were published on the characterisation of the *Hippeastrum* mosaic virus (HMV). Brants and Van den Heuvel (1965) indicated *Gomphrena globosa* as an assay host, but Brants et al. (1970) stated that it did not react with all investigated extracts from mosaic-diseased *H. hybridum* plants, while extracts from symptomless *H. hybridum* plants even induced lesions on *G. globosa* plants. The length of the virus particles was reported to be 643 $\pm$ 24 nm. De Leeuw (1972b) described *Hyoscyamus niger* as a useful local lesion host for a mosaic virus in *H. hybridum* and suggested that this virus might be identical to HMV, although no local lesions were induced on *G. globosa*. Brunt (1973) stated that both H. niger and G. globosa are diagnostic species for HMV. However, he also mentioned that *G. globosa* is much less susceptible than other local lesion hosts. He reported the sizes of the HMV to be 750 $\times$ 12 nm, while he also mentioned the presence of the virus of 650 $\times$ 13 nm, referring to Brants et al. (1970).

We have now tried to isolate and characterize the agent causing the local lesions on *H. niger* and *G. globosa* plants after inoculation from *H. hybridum* plants.

Materials and methods

Plant material and inoculation. Hippeastrum hybridum plants, showing mosaic symptoms and plants without leaf symptoms but still able to induce reactions on some test plants served as virus sources.

The mosaic of the *H. hybridum* plants consisted of dark and light green spots on the lamina and stalk; the dark areas were usually sharply defined. Seeds from mosaic-diseased *H. hybridum* plants were harvested to test seed transmission.

Symptoms in *H. hybridum* 'Scarlet globe' consisted of a faint mosaic pattern on the upper side of the red-coloured leaves.

The test plants used were: *Capsicum annuum*, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Cucumis sativus*, *Datura stramonium*, *Gomphrena globosa*, *H. hybridum* seedlings, *Hyoscyamus niger* 'Pallides', *Lycopersicon esculentum*, *Nicotiana clevelandii*, *N. tabacum* 'Samsun' and *N. tabacum* 'Xanthi'. They were grown in a glasshouse at 20–23 °C using supplementary artificial light in winter to obtain a 16-hour photoperiod. The plants were grown in a rich soil mixture, mostly in large pots with a diameter of 12 cm or more, and were inoculated when 4–8 leaves were present, depending on the plant species. Test plants dusted with 500-mesh carborundum were inoculated with undiluted, fresh sap from squeezed leaves.

Virus purification. Sucrose density gradient columns were prepared by layering 4 ml, 7 ml 7 ml and 7 ml of buffer solution (0.001 M KCl, 0.005 M K_2HPO_4, 0.0005 M

Fig. 1. Leaf of *Hyoscyamus niger* with chlorotic local lesions, 7 days after inoculation with the *Hippeastrum mosaic* virus.

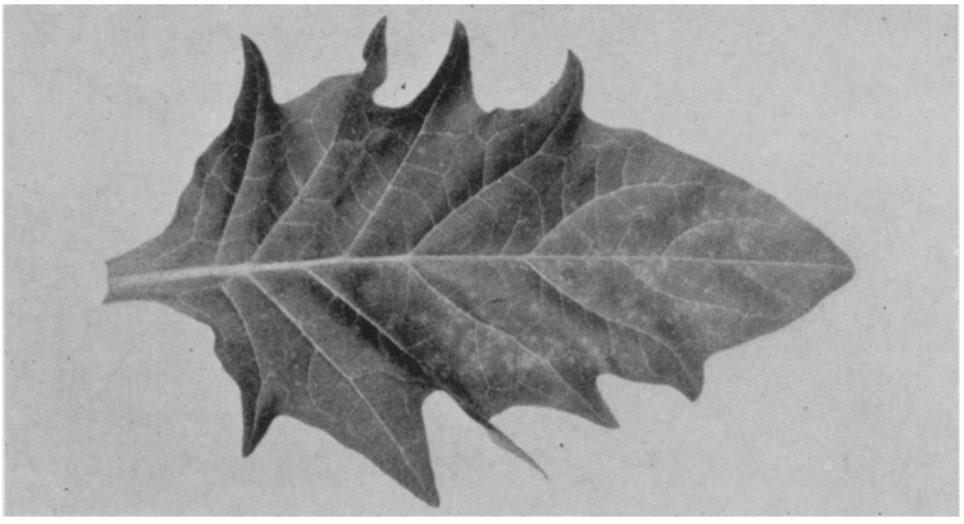

Fig. 1. Blad van Hyoscyamus niger, met chlorotische lokale lesies 7 dagen na inoculatie met Hippeastrum mozaïekvirus.

KH_2PO_4, pH 7.5) containing 40, 30, 20, 10% w/v sucrose in phosphate buffer, respectively. Expressed plant sap was centrifuged for 10 min at 3000 rev/min and 2 ml was layered on top of the gradient. After centrifugation in a swinging-bucket rotor of a Spinco L_2 ultracentrifuge for 1.5 h at 5°C at 24,500 rev/min the columns were fractionated in a LKB fraction collector and the fractions tested for infectivity. The viruses were also purified by differential centrifugation using relatively low centrifugal forces and a high pH buffer (Huttinga, 1973), sometimes followed by sucrose density-gradient centrifugation.

Particle length. The particle length was studied from electron micrographs obtained with a Philips EM 300. Purified preparations or dip-preparations were shadow-cast with platinum/carbon at an angle of 20°. If present, sugars were removed by the method of Webb (1973). The preparations were photographed at × 5500; measurements were made at × 38,500 using dividers and a nonius (De Leeuw, 1975). Particles were selected at random for measurement on condition that they were intact with both ends clearly discernible. They were measured in length classes of 10 nm. The normal length was determined according to Brandes (1964).

UV spectrum. The UV spectrum was determined in an Optica Spectrophotometer model 10.

Results

Seed transmission. In order to investigate whether seedlings of *H. hybridum* may contain the viruses sap from more than 100 seedlings from plants known to be virus-infected, was tested separately on several plant species and some were examined by electron microscopy. No reaction could be observed and no virus particle was found. Hence, seed transmission was not detected.

Host range and symptoms. All inoculations were made with sap from mosaic-diseased *H. hybridum* plants. Leaves of *H. niger* always showed chlorotic local lesions about 7 days after inoculation (Fig. 1). In *G. globosa* chlorotic local lesions sometimes occur-

Fig. 2. Leaf of *Gomphrena globosa*, with red-bordered local lesions 16 days after inoculation with the *Hippeastrum* latent virus.

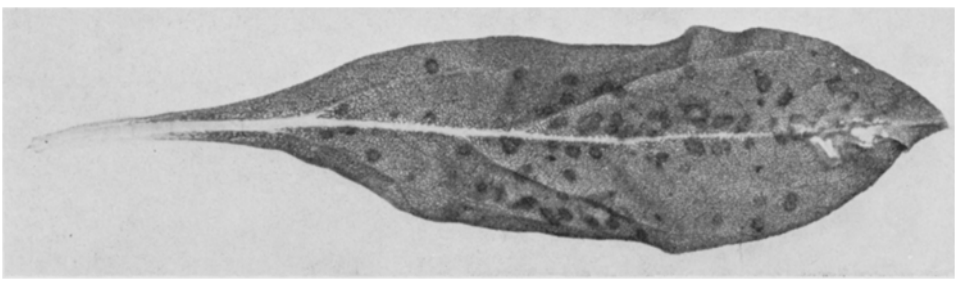

Fig. 2. Blad van Gomphrena globosa, met roodgerande lokale lesies 16 dagen na inoculatie met het latente Hippeastrum virus.

Fig. 3. Leaf of *Chenopodium quinoa* with chlorotic local lesions, sometimes with a necrotic centre, 21 days after inoculation with the *Hippeastrum* latent virus.

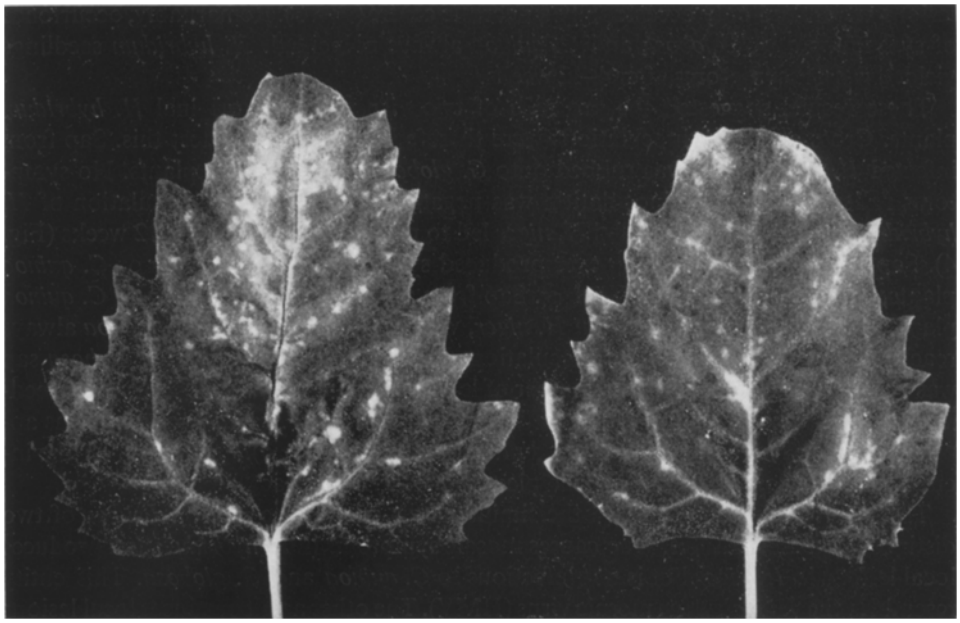

Fig. 3. Blad van Chenopodium quinoa met chlorotische lokale lesies, soms met een necrotisch centrum 21 dagen na inoculatie met het latente Hippeastrum virus.

Fig. 4. *Hippeastrum hybridum* leaves. Top: Leaf with the *Hippeastrum* mosiac virus; middle: leaf with the *Hippeastrum* latent virus; bottom: virus-free leaf.

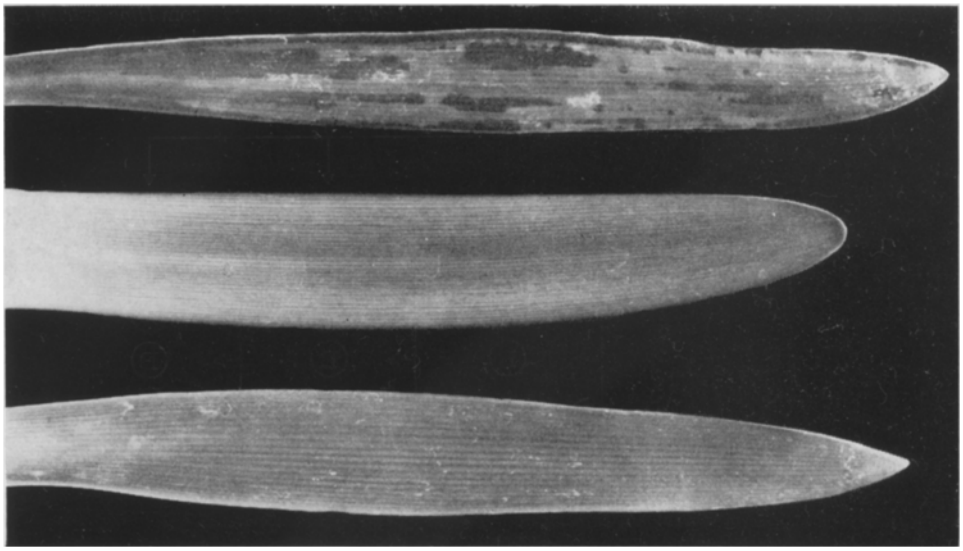

Fig. 4. Bladeren van Hippeastrum hybridum. Boven: blad met het Hippeastrum mozaïekvirus; midden: blad met het latente Hippeastrum virus; onder: blad zonder virus.

red after 9–11 days. Three days later these became necrotic and showed a red border (Fig. 2), *C. quinoa* leaves always reacted after about 16–20 days with chlorotic local lesions which had only occasionally a necrotic centre (Fig. 3). Remarkably, occurrence of symptoms on *G. globosa* and *C. quinoa* always coincided. *H. hybridum* seedlings yielded mosaic symptoms after 4–12 weeks.

These results suggested the presence of two viruses in the original *H. hybridum* plants. Cross and back inoculations were therefore carried out to test this. Sap from infected *H. niger* leaves was rubbed onto *G. globosa* and *C. quinoa* plants. No symptoms followed and back inoculation was negative also. However, inoculation of *H. hybridum* seedlings from infected *H. niger* led to mosaic symptoms in 4–12 weeks (Fig. 4). Sap from the latter plants never produced symptoms on *G. globosa* or *C. quinoa* plants, but was infectious to *H. niger*. Sap from virusinfected *G. globosa* or *C. quinoa* plants never induced symptoms in *H. niger*, whereas *G. globosa* and *C. quinoa* always reacted with local lesions after inoculation. However, when infected *G. globosa* sap was rubbed onto *G. globosa* the number of lesions decreased. When *H. hybridum* seedlings were inoculated with sap from infected *G. globosa* or *C. quinoa* no symptoms appeared but th virus could be recovered after 4–12 weeks (Fig. 4). During winter the incubation time was usually longer and plants often failed to react.

These results indeed indicated the simultaneous occurrence in *H. hybridum* of two distinct infectious entities. One causes mosaic symptoms in *H. hybridum* and produces local lesions in *H. niger* but is not infectious to *C. quinoa* and *G. globosa*. This entity resembles the *Hippeastrum* mosaic virus (HMV). The other agent produces local lesions on *G. globosa* and *C. quinoa* and can be present in *H. hybridum* plants without producing symptoms and therefore might be named a *Hippeastrum* latent virus (HLV). It does not infect *H. niger*. Various cross inoculations have confirmed this hypothesis. Fig. 5 represents the results.

Fig. 5. Serial testing of *Hippeastrum* mosaic virus and *Hippeastrum* latent virus from *Hippeastrum hybridum* plants on 4 test plant species. The infecting virus is indicated after the species name.

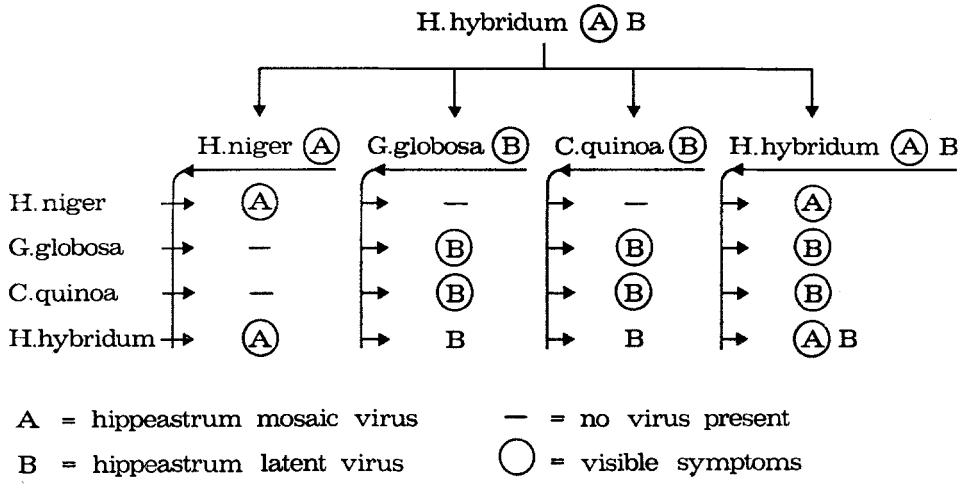

Fig. 5. Doortoetsing van Hippeastrum-mozaïekvirus en latente Hippeastrum-virus uit Hippeastrum hybridum op 4 toetsplantsoorten. Achter de naam van de soort wordt het infecterende virus vermeld.

Both viruses produced very vague symptoms in leaves of *N. clevelandii* sometimes followed by a systemic vein-clearing after about 19 days. The non-inoculated leaves were found to contain one or two viruses, dependent on the inoculum used for *N. clevelandii*, even when the symptoms were scarcely visible.

The HLV produced also chlorotic local lesions on *D. stramonium* after 15 days and on *C. murale* and *C. amaranticolor* after about 20 days. The lesions on the latter turned red after 28 days.

No symptoms were recorded on *C. annuum*, *C. sativus*, *L. esculentum*, *N. tabacum* 'Samsun' and *N. tabacum* 'Xanthi', neither was any virus recovered from these species.

When sap from *H. hybridum* 'Scarlet globe' was used on the same test plants, the reactions were identical with these obtained from HLV, with the exception that *C. quinoa* additionally reacted with a systemic mosaic after 17 days.

Virus purification. Crude sap from *H. hybridum* leaves, containing only HLV, was centrifuged in sucrose density-gradient columns. A very vague light-scattering zone not occurring in the control was found at 2–2.5 cm beneath the meniscus. Fractionation of the column and testing of the fractions revealed only that zone to contain virus, only infectious to hosts of HLV. With sap from infected *G. globosa* or *C. quinoa* plants the same results were obtained.

Sap from non-lesion areas of inoculated *G. globosa* leaves was also centrifuged in a sucrose-gradient and the columns were subsequently fractionated. No visible zone was formed and no infectivity recovered, this in contrast to the results obtained when lesion-areas were used. Crude sap from HMV-infected *H. niger* leaves centrifuged in a

Fig. 6. Electron micrograph of the *Hippeastrum* latent virus; bar represent 1000 nm.

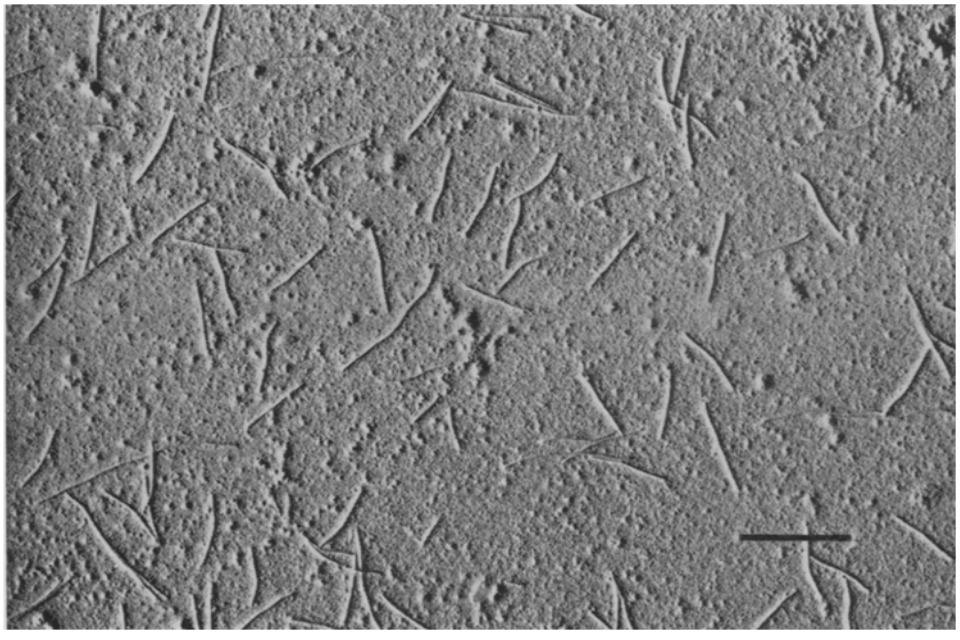

Fig. 6. Elektronenmicroscopische foto van het latente Hippeastrum-virus; staaf geeft 1000 nm aan.

sucrose density-gradient also yielded light scattering zones at 2–2.5 cm beneath the meniscus, which appeared to be infectious to *H. niger* plants and *H. hybridum* seedlings only.

Fig. 7. Length distribution curves of particles of the *Hippeastrum* latent virus.
A. Particles obtained from leaf dip preparation of *Gomphrena globosa, Chenopodium quinoa* and *Nicotiana clevelandii* leaves. Normal length: 586 nm.
B. Particles obtained by density-gradient centrifugation of sap from *Hippeastrum hybridum* leaves. Normal length: 611 nm.
C. Particles obtained after purification by differential centrifugation from sap of *Chenopodium quinoa* and *Nicotiana clevelandii* leaves. Normal length: 585 nm.

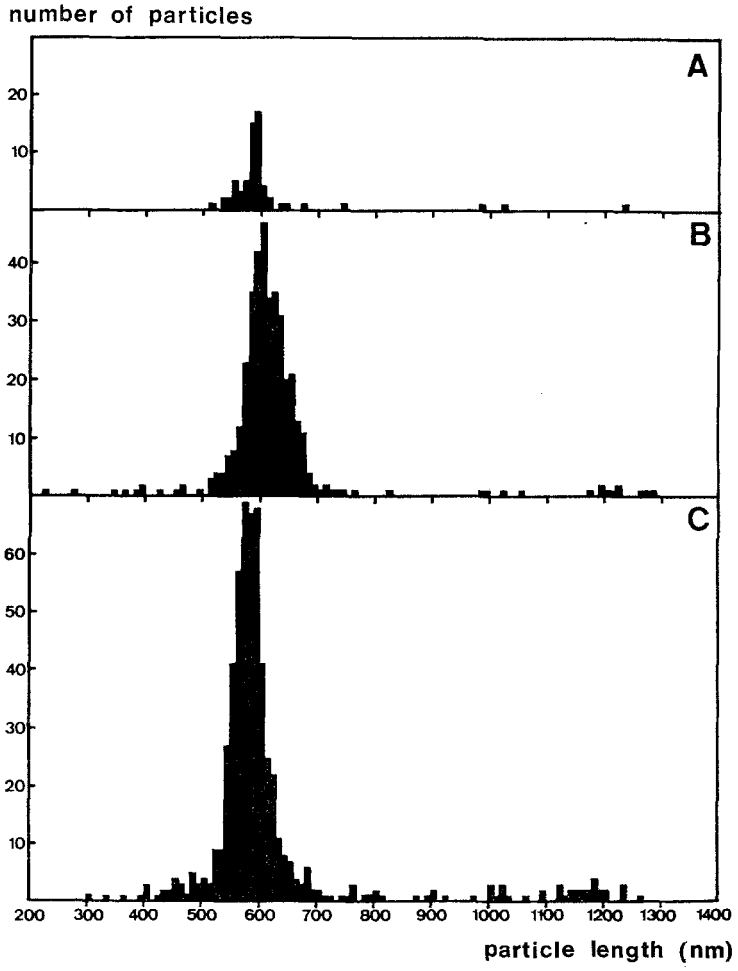

Fig. 7. Lengteverdeling van deeltjes van het latente Hippeastrum-virus.
A. Deeltjes verkregen uit dooppreparaten van Gomphrena globosa, Chenopodium quinoa en Nicotiana clevelandii. Normaallengte 586 nm.
B. Deeltjes verkregen via dichtheids-gradiëntcentrifugering van bladsap van Hippeastrum hybridum. Normaallengte 611 nm.
C. Deeltjes verkregen na zuivering door middel van differentieel centrifugeren van bladsap van Chenopodium quinoa en Nicotiana clevelandii. Normaallengte: 585 nm.

Differential centrifugation was very suitable to purify HLV. Leaves of *N. clevelandii* and of *C. quinoa* were good virus sources 22–26 days and 18–26 days after inoculation, respectively. The purified preparations obtained were very infectious and contained a high concentration of virus particles. Inoculated leaves of *G. globosa* were less suitable, as the purified preparations, although rather infectious, appeared to be not clear when examined with the electron microscope. Purification of HMV from *H. niger* leaves by differential centrifugation was unsuccessful.

Particle length. The light scattering zones obtained from *H. niger* plants infected with HMV, contained flexuous rods of 660–790 nm. The normal length was 706 nm. In dip-preparations of plants infected with this virus particle length was similar.

Dip-preparations from HLV-infected *H. quinoa*, *G. globosa* and *N. clevelandii* leaves also showed flexuous filaments (Fig. 6). Of the 58 particles measured 92% had a length of 520–690 nm with a normal length of 586 nm (Fig. 7A). The infectious fractions from densitygradient centrifugation of *H. hybridum* leaves containing HLV also contained flexuous threads. Of the 390 particles measured 90% were 520–690 nm long, with a normal length of 611 nm (Fig. 7B). When HLV was purified from *N. clevelandii* or *C. quinoa* by differential centrifugation, 83% of the particles measured had a length between 520–690 nm. Virus particles from *N. clevelandii* had a normal length of 584 nm and those from *C. quinoa* 585 nm (Fig. 7C). The normal length, calculated from all HLV-preparations was 596 nm.

Purified virus preparations and dip preparations from *H. hybridum* 'Scarlet globe' contained flexuous rods. Of the 350 particles measured from electron micrographs 56% had a length of 525–650 nm. The normal length appeared to be 581 nm.

Physical properties. Crude sap from leaves of *H. hybridum* containing HMV and HLV, or crude sap from plants containing either HMV or HLV, were used to determine the dilution end point, the longevity in vitro at room temperature and the thermal inactivation point. Both viruses had dilution end points between 10^{-3} and 10^{-4} and a longevity in vitro between 28 and 32 h. However, the thermal inactivation point was different for both viruses; HMV was inactivated between 55 and 60°C, HLV between 70 and 80°C. The absorption spectrum of HLV was determined after purification of the virus using density-gradient centrifugation. The maximum absorption was reached at 262 nm and the minimum absorption at 242 nm; the maximum/minimum absorption ratio was 1.08. The A 260/280 ratio was 1.19.

Discussion

The data presented here show that two viruses were present in the *H. hybridum* plants studied. As these two viruses clearly differ in host range and particle morphology from those of tomato spotted wilt virus, sunflower mosaic virus, cucumber mosaic virus, *Hippeastrum* streak virus and tobacco mosaic virus, also reported from *H. hybridum*, it is evident that the so-called HMV and HLV are in fact distinct (see also Brunt, 1973).

In the literature there has been much uncertainty about the exact identification of HMV, because of unknown contamination with HLV. Brants and Van den Heuvel (1965) introduced the name HMV for the virus which induces lesions on *G. globosa*. This name has been adopted by Iwaki (1967) and Nowicki and Derrich (1974). Brunt

(1973) stated also that *G. globosa*, *C. quinoa* and *C. murale* are host plants for HMV. However, the present results indicate that these plant species are differential hosts of HLV. *H. niger* always produced chlorotic local lesions when inoculated from mosaic-diseased *H. hybridum* plants. It was also possible to provoke mosaic symptoms in *H. hybridum* seedlings by inoculating them with sap from virus-infected *H. niger* plants. Therefore *H. niger* can be used as assay and differential host for HMV (De Leeuw, 1972b). It is worth noting that during November and December the susceptibility of this species was less than during the rest of the year, but this phenomenon is common (Hollings, 1966). The fact that the number of lesions of HLV on *G. globosa* leaves decreased in serial inoculations within this species suggest the presence of inhibitors in *G. globosa*. These might also be responsible for the lack of symptoms on *H. niger* leaves after passage of the original complex from *H. hybridum* (see Fig. 5) to *G. globosa*. However, purified HLV dit not induce symptoms on *H. niger* leaves and this shows the species to be a non-suscept to HLV.

The method of Huttinga (1973) has proved to be very successful for purification of HLV from *C. quinoa* and *N. clevelandii* plants, and at high pH and moderate centrifugal forces the fragmentation and aggregation of filamentous viruses is indeed kept at a minimum. Why his method failed for purification of HMV from *H. niger* plants is not known. With sucrose density-gradient centrifugation good results were obtained with both viruses.

The host plant has no apparent influence on the length of HLV as it did not differ when purified either from *N. clevelandii* or *C. quinoa*. Neither did length differ between purifications, although Brandes and Bercks (1965) state that the host plant influences the length and that the length of the particles is variable after purification. The difference in lengths of virus particles obtained from *H. hybridum* plants by density-gradient centrifugation and from test plants by differential centrifugation is remarkable. However, the circumstances during purification and preparation should be carefully considered (Govier and Woods, 1971; Hampton et al., 1974). This fact as well as the technique used to measure particle-length (De Leeuw, 1975) may be the reason why Brunt (1973) and Brants et al. (1970) found a virus length of 619–667 nm, while the mean normal length in this investigation was found to be 596 nm. This would also explain the 44 nm difference between the length of HMV found by Brunt (1973) and the length of HMV measured by the present author.

A nucleic acid content of 10% or more might be expected (Paul, 1959) with the A 260/280 ratio for the HLV. Nevertheless great caution is necessary when drawing such conclusions (Bar-Joseph and Hull, 1974).

It is likely that HLV belongs to the potex virus group, but extensive comparisons with other members of this group are needed before it can be concluded that HLV is distinct.

Acknowledgment

The author is very much indebted to Mrs A. L. Nelis-Blomberg for competent technical assistance and to Mr H. J. Miller for correcting the English text.

Samenvatting

Voorlopige beschrijving van het latente Hippeastrum virus in Hippeastrum hybridum, en onderscheiding van het Hippeastrum mozaïekvirus

In dit onderzoek is gebruik gemaakt van planten van *Hippeastrum hybridum* met mozaïeksymptomen in de bladeren. *H. hybridum*, *Gomphrena globosa*, *Chenopodium quinoa* en *Hyoscyamus niger* werden op de bladeren geïnoculeerd met sap van deze mozaïekzieke planten van *H. hybridum*. *H. hybridum* gaf na 4–12 weken mozaïeksymptomen te zien. *H. niger* reageerde altijd met chlorotische lokale lesies na ca. 7 dagen, terwijl *G. globosa* en *C. quinoa* slechts in enkele gevallen reageerden met respektievelijk roodgerande lokale lesies na 14 dagen en chlorotische lesies na 16–20 dagen. Teruginoculatie van *H. niger* op bladeren van zaailingen van *H. hybridum* gaf na 4–12 weken mozaïeksymptomen te zien; teruginoculatie van *G. globosa* en *C. quinoa* gaf geen symptomen op zaailingen van *H. hybridum*, terwijl 4–12 weken na inoculatie de betrokken bladeren het virus wel bleken te bevatten. Het lukte niet om virus over te brengen van *H. niger* naar *G. globosa* en *C. quinoa* en omgekeerd (Fig. 1). Hieruit kan geconcludeerd worden dat het virus dat mozaïeksymptomen op *H. hybridum* en lokale lesies of *H. niger* geeft het *Hippeastrum*-mozaïekvirus (HMV) is (De Leeuw, 1972b). Het virus dat op *G. globosa* en *C. quinoa* lesies geeft is een virus dat symptoomloos voorkomt in *H. hybridum*. Dit laatste wordt nu het latente *Hippeastrum* virus (HLV) genoemd. Verdere waardplanten van het HLV zijn: *Datura stramonium*, *Chenopodium murale*, *C. amaranticolor* en *Nicotiana clevelandii*. Alleen deze laatste behoort ook tot de waardplantenreeks van het HMV.

Het HMV is gezuiverd via dichtheids-gradiëntcentrifugering uit geïnoculeerde bladeren van *H. niger* en bleek een normaallengte te hebben van ca. 706 nm. Het verdunningseindpunt lag tussen 10^{-3} en 10^{-4}, de inactiveringstemperatuur tussen 55 en 60 °C en de houdbaarheid in vitro bij kamertemperatuur bedroeg 28–32 uur.

Het HLV kon gemakkelijk gezuiverd worden door differentieel centrifugeren uit geïnoculeerde bladeren van *C. quinoa* en *N. clevelandii* en door middel van dichtheidsgradiëntcentrifugeren uit *H. hybridum*. De virusdeeltjes waren flexibele draden met een normaallengte tussen de 584 en 611 nm, afhankelijk van de manier waarop werd gezuiverd (Fig. 7). Gezuiverd virus had een absorptieminimum bij 242 nm, een maximum bij 262 nm en een 260/280 nm verhouding van 1.19. Het verdunningseindpunt lag tussen 10^{-3} en 10^{-4}, de inactiveringstemperatuur tussen 70 en 80 °C en de houdbaarheid in vitro bij kamertemperatuur bedroeg 28–32 uur. Hoewel dit virus niet direkt vergeleken is met andere leden uit de potexvirusgroep schijnt het een nieuw virus te zijn: het latente *Hippeastrum*-virus.

Beide virussen gingen niet over met zaad.

References

Bar-Joseph, M. & Hull, R., 1974. Purification and partial characterisation of sugar beet yellows virus. Virology 62: 552–562.

Brandes, J., 1964. Identifizierung von gestreckten pflanzenpathogenen Viren auf morphologischer Grundlage. Mitt. biol. BundAnst. Ld-u. Forstw. 110; 1–130.

Brandes, J. & Bercks, R., 1965. Gross morphology and serology as a basis for classification of elongated plant viruses. Adv. Virus Res. 11: 1–24.

Brants, D. H., Fokkema, N. J. & Bode, R. de, 1970. Further identification of *Hippeastrum* mosaic virus. Neth. J. Pl. Path. 76: 171–173.
Brants, D. H. & Heuvel, J. van den, 1965. Investigation of *Hippeastrum* mosaic virus in *Hippeastrum hybridum*. Neth. J. Pl. Path. 78: 107–109.
Brunt, A. A., 1973. Hippeastrum mosaic virus. C.M.I./A.A.B. Descriptions of plant viruses 117: 4 pp.
Govier, D. A. & Woods, R. D., 1971. Changes induced by magnesium ions in the morphology of some plant viruses with filamentous particles. J. gen. Virol. 13: 127–132.
Hampton, R. O., Knesek, J. E. & Mink, G. I., 1974. Particle-length variability of the pea seedborne mosaic virus. Phytopathology 64: 1158–1358–1363.
Hollings, M., 1966. Local lesion and other test plants for the identification and culture of viruses. In: Beemster, A. B. R. and Dijkstra, J. (Eds), Viruses of plants. North-Holland Publishing Company, Amsterdam.
Huttinga, H., 1973. Properties of viruses of the potyvirus group. 1. A simple method to purify bean yellow mosaic virus, pea mosaic virus lettuce mosaic virus and potato virus Y. Neth. J. Pl. Path. 79: 125–129.
Iwaki, M., 1967. Viruses causing mosaic diseases of *Amaryllis* in Japan. Ann. phytopath. Soc. Japan 33, 237–243.
Kahn, R. P. & Smith, F. F., 1963. Transmission of a virus inciting *Amaryllis* mosaic symptoms. Pl. Life 19, 133–145.
Leeuw, G. T. N. de, 1972a. Tobacco mosaic virus in *Hippeastrum hybridum*. Neth. J. Pl. Path. 78: 69–71.
Leeuw, G. T. N. de, 1972b. *Hyoscyamus niger*, a useful local lesion host for a mosaic virus in *Hippeastrum*. Neth. J. Pl. Path. 78: 107–109.
Leeuw, G. T. N. de, 1975. An easy and precise method to measure the length of flexuous virus particles from electron micrographs. Phytopath. Z. 82: 347–351.
Nowicki, M. E. & Derrick, K. S., 1974. Mosaic disease in Leopoldii *Amaryllis* hybrids. Pl. Life 30: 103–108. The Amaryllis year book.
Paul, H. L., 1959. Die Bestimmung des Nucleinsäuregehaltes pflanzlicher Viren mit Hilfe einer spektrophotometrischen Methode. Z. Naturforschg. 14b: 427–432.
Smith, K. M., 1935. Some diseases of ornamental plants caused by the virus of tomato spotted wilt. Jl R. hort. Soc. 60: 304–310.
Smith, K. M., 1957. A textbook of plant viruses. Churchill, J. and A. Ltd. (Eds) London, 2nd ed.
Velsen, R. J. van, 1967. *Hippeastrum* streak, a virus disease of *Hippeastrum vittatum* in Papua and New Guinea. Papua New Guin. Agric. J. 19: 13–16.
Webb, M. J. W., 1973. A method for the rapid removal of sugars and salts from virus preparations on electron microscope grids. J. Microsc. (London) 98(1): 109–111.

Address

Phytopathologisch Laboratorium 'Willie Commelin Scholten', Javalaan 20, Baarn, the Netherlands.