Neth. J. Plant Path. 71 (1965): 145–151

INVESTIGATION OF *HIPPEASTRUM* MOSAIC VIRUS IN *HIPPEASTRUM HYBRIDUM*[1]

Onderzoek over het Hippeastrum-*mozaïek-virus in* Hippeastrum hybridum

D. HENDRINA BRANTS and J. VAN DEN HEUVEL

Phytopathological Laboratory "Willie Commelin Scholten", Baarn

An attempt was made to identify a mosaic disease in *Hippeastrum hybridum*. Infectious virus material could be demonstrated in roots, leaves, stem, perianth, stamen and pistil. Inclusion bodies were found in the epidermis of leaves, stem, spatha leaves and in the perianth. Virus concentration in a young stage was high but decreased by aging of the perianth. Efforts to transmit the virus by aphids failed. However, the virus was transmitted by seed in a few cases. Plants of 30 species reacted negatively upon inoculation with the virus. *Hippeastrum hybridum*, *Gomphrena globosa*, *Chlorophytum* spec. and *Lycopersicum esculentum* could be infected experimentally. On account of the host range and presence of inclusion bodies the mosaic symptoms in *Hippeastrum* are not caused by tomato spotted wilt virus or Cucumber mosaic virus. Results suggest that the virus under investigation is the *Hippeastrum* mosaic virus. Dr. M. K. CORBETT, Wageningen, succeeded in purifying the virus by density gradient centrifugation. Whe preparation contained flexuous rod particles. Plants of *Datura stramonium*, *Nicotiana glutinosa* and *N. tabacum* 'Samsun' could be infected. Within two weeks after inoculation with purified virus solution these plants showed systemic symptoms.

INTRODUCTION

In a nursery at Hoorn, the Netherlands, many *Hippeastrum* plants (Amaryllidaceae) showed mosaic in the leaves. The symptoms suggested that a virus might be the causal agent.

There has been some confusion in the literature as to the genus name of *Hippeastrum* plants. The problem has been discussed by MOORE (1963). By international agreement in 1954 the genus name *Amaryllis* is the correct name for only the South-African species *Amaryllis belladonna* and the name *Hippeastrum* should be used for American species and hybrids.

Since 1922 many authors (a.o. DICKSON, 1922; KUNKEL, 1924) described viruslike symptoms of *Hippeastrum* or *Amaryllis* under the name of *Hippeastrum* mosaic virus or *Amaryllis* mosaic virus without identifying the virus in question. At least three viruses are now known to cause mosaic symptoms in leaves of *Hippeastrum* and *Amaryllis*: 1. Tomato spotted wilt virus, 2. Cucumber mosaic virus, 3. *Hippeastrum* mosaic virus.

Hippeastrum plants infected by Tomato spotted wilt virus (TSWV) show many yellowish spots and red necrotic lesions on the leaves which finally turn yellow and die. Growth and blossoming of the plant is not influenced by the virus. Transmission of TSWV by sap is possible but according to NOORDAM (1943) and SMITH (1957) it is not seed transmitted.

It was reported (ANONYMUS, 1958) that plants of *Hippeastrum* could be infected by Cucumber mosaic virus (CMV). STOUFFER (1963) confirmed this finding. KAHN & SCOTT (1964) isolated CMV from *Hippeastrum* plants and

Accepted for publication 8 July, 1965.

based their identification on symptoms, thermal inactivation point, dilution end point, longevity in vitro, serology and transmission. The virus is transmissable by sap as well as by the aphid *Myzus persicae*. Symptoms are characterized by scattered light and dark green spots or a stripe mosaic. The virus does not seem to be seed transmitted.

Many authors have mentioned the occurrence of inclusion bodies in epidermal cells of *Hippeastrum* leaves showing mosaic symptoms (KUNKEL, 1922; KLINKOWSKI, 1958). Inclusions are not detectable before mosaic symptoms have appeared in the leaves. Cell inclusions are never found in *Hippeastrum* plants infected with TSWV or CMV. Inclusion bodies seem to be caused by *Hippeastrum* mosaic virus (HMV). Infected plants show a mottling in the leaves and sometimes light as well as dark green stripes (KAHN, 1960). BRIERLEY (1948) stated that HMV is not transmitted by insects nor by seed but according to TRAUB (1958) insects or mites are vectors. SMITH (1933) reported seed transmission of a mosaic inducing virus in *Hippeastrum*. CEVAT (1964) suggested that HMV is transmitted by sucking insects and possibly by mites. He reported seed transmission of the virus. Electron microscopic investigation by JOHNSON (1951) revealed that the particles of HMV are long flexuous rods. The exact length, however, was not reported. PROCENKO & PROCENKO (1964) described a mosaic in *Hippeastrum* leaves characterized by narrow lightgreen stripes which later sometimes necrotize. Under low temperatures the mosaic was masked. Long, narrow, slightly curved or bent threadlike particles of 500–550 mμ were found in the leaves. This disease is probably identical with the one described by BRIERLEY (1948) and JOHNSON (1951).

SMITH (1957) reports *Hippeastrum* to be a host plant for sunflower mosaic virus.

The aim of our investigation was to identify the virus in *Hippeastrum* found in the nursery at Hoorn and to obtain a useful test plant.

MATERIALS AND METHODS

Plants of *Hippeastrum hybridum* showing mosaic symptoms served as a virus source. Sap was pressed out of the leaves or flowers from infected plants by squeezing and in most cases diluted 1:1 with 0.2% Na_2SO_3-solution or water. Leaves of test plants were rubbed with the sap and 500-mesh Carborundum. Control plants were treated with sap from healthy *Hippeastrum*. *Hippeastrum* and many other plant species grown in the greenhouse were used as test material.

Inclusion bodies were examined microscopically after staining. For this purpose epidermal strips of *Hippeastrum* leaves or flowers were fixed in 8% formaldehyde during 15 minutes, stained with 0.5% trypan-blue solution for 30 minutes and rinsed with water. Preparations were kept in water or alcohol-glycerine-water mixture (1:1:1).

In efforts to transmit the virus by *Myzus persicae* 10 aphids were placed on each of a *Hippeastrum* leaf showing mosaic symptoms. The aphids were removed at different times – up to three days – after the beginning of the experiment and placed on healthy *Hippeastrum* seedlings. Four months later none of the seedlings showed virus symptoms.

Host range

Affected *Hippeastrum* plants exhibit mosaic symptoms on both sides of the leaves, characterized by a mixture of irregularly shaped dark and light green spots on the lamina. Particularly the dark areas are sharply defined.

Sometimes many small spots are united to stripes and bordered by veins (Fig. 1). Mosaic symptoms may occur also on the flower stem as vague light- and dark coloured spots. In a few cases the petals of old flowers show light mosaic symptoms. Growth and blossoming of the plants is not influenced by the virus.

To determine the host range of the virus many plant species were inoculated. Efforts to infect the following 30 species with virus containing sap proved negative: *Begonia semperflorens*, *Calceolaria hybrida*, *Chenopodium amaranticolor*, *Chrysanthemum segetum* and *C. sinense*, *Cichorium endivia*, *Cineraria hybrida*, *Citrullus vulgaris*, *Coleus* spec., *Datura stramonium*, *Emilia sagittata*, *Galanthus nivalis*, *Hyacinthus orientalis*, *Impatiens balsamina* and *I. holstii*, *Lactuca sativa*, *Narcissus* spec., *Nicotiana glutinosa*, *N. tabacum* var. 'Samsun' and 'Xanthi nc'., *Petunia hybrida*, *Pisum sativum*, *Tetragonia expansa*, *Tropaeolum majus*, *Tulipa hybrida*, *Vicia faba* and *Zinnia elegans*.

In addition to *Hippeastrum hybridum* three plant species have been experimentally infected: *Gomphrena globosa*, *Chlorophytum* spec. and *Lycopersicum esculentum*. When *G. globosa* is inoculated with virus-containing sap, local lesions are formed after 7–10 days. The lesions resemble those produced by potato virus X on *Gomphrena* but they appear later. *Chlorophytum* and tomato did not exhibit symptoms after infection but sap of both affected plants produced local lesions on *G. globosa*. Attempts to infect healthy *Hippeastrum* seedlings with sap of affected plants of the three species failed. The host range of this virus differs markedly from that reported for TSWV. The latter included *Chenopodium amaranticolor*, *Cucumis sativus* var. 'Gele tros' and 'Improved Telegraph', *Datura stramonium*, *N. glutinosa*, *N. tabacum* 'Samsun' and *Petunia hybrida*. All these plants reacted negatively upon inoculation with sap from *Hippeastrum*. *Gomphrena globosa* proved to be a local lesion host for the virus and was therefore used in further experiments, in spite of the fact that it was not possible to infect *Hippeastrum* with sap from *Gomphrena*.

Localization of virus in Hippeastrum

To determine in which parts of *Hippeastrum* plants the virus is present, sap expressed from roots and different flower parts was tested on *Gomphrena globosa* (Table 1).

The virus was present in all plant parts except the spatha leaves. The concentration of virus, as measured by local lesions on plants of *Gomphrena*, varied greatly with different plant parts. The bloom of a flower lasts nine days at most. The virus concentration during this time was examined (Table 1b). From the number of local lesions induced on the inoculated leaves of *G. globosa* it is evident that the virus concentration in the perianth is high in very young leaves and decreases with aging of the perianth. The cause of this decrease is unknown.

TABLE 1. Number of local lesions on eight leaves of *G. globosa* inoculated with sap from different plant parts of *Hippeastrum* showing mosaic.
Het aantal lesies op acht bladeren van G. globosa, *geïnoculeerd met sap, afkomstig van verschillende plantedelen van* Hippeastrum *met mozaïek.*

a) Plant part	Number of days after opening of the flower	Number of lesions	b) Plant part	Number of days after opening of the flower	Number of lesions
Roots/*Wortels*		15	Perianth/ *Bloemdek*	0	526
Stem/*Stengel*		79	,,	1	177
Spatha leaves/ *Bloemschede*		0	,,	2	214
Pedicellus/ *Bloemsteeltje*		322	,,	3	95
Stamen/ *Meeldraden*	6	0	,,	4	94
,,	7	15	,,	5	70
,,	8	13	,,	6	48
,,	9	131	,,	7	21
Pistil/*Stamper*	6	2	,,	8	27
,,	8	14	,,	9	19
Plantedeel	*Aantal dagen na het opengaan van de bloem*	*Aantal lesies*	*Plantedeel*	*Aantal dagen na het opengaan van de bloem*	*Aantal lesies*

Cell inclusions

Epidermal strips of *Hippeastrum hybridum* were stained after fixation as described. In strips from leaves showing mosaic symptoms inclusions were visible. They differed from the nuclei by less intensive colouration and vague contours. The variation in size, number, shape and localisation of the inclusion bodies per cell was great. In many cases one or two inclusions approximately the size of the nucleus occurred in one cell (Fig. 2). Sometimes many of the epidermal cells did not contain inclusions. They were never found in healthy leaves. The inclusions resemble much the ones described by KUNKEL (1924) and HOLMES (1928). It was not reported in the literature whether other plant parts besides leaves may contain inclusions. Therefore various flowerparts were examined microscopically. Inclusion bodies were found in the stem, in spatha leaves at those places where the colour was lost and in the perianth depending on the flowering stage. Flowers three to four days old did not contain inclusions in the perianth but flowers eight days of age did (Fig. 3). Sometimes flowers of six days old showed inclusion bodies. The relationship between the presence of inclusion bodies and the reduction in the amount of available infectious virus material in the flowers is of interest. The first five till six days of flowering, when the virus concentration was high, inclusions were not demonstrable in the perianth (Table 1 b). After this period inclusions were detectable and the virus concentration was greatly diminished.

Transmission

All efforts to transmit the virus by *Myzus persicae* proved negative. It is

possible, however, that other insects are able to transmit the virus. To determine whether the virus can be transmitted by seed, flowers of affected *Hippeastrum* plants were pollinated with pollen from diseased plants. As a control healthy flowers were pollinated with pollen originating from healthy flowers. The resulting seeds were sown and the seedlings examined. During several months the plants did not show virus symptoms but sap expressed from leaves and tested on *G. globosa* caused local lesions in two cases. After a year these plants showed symptoms. The percentage of seed transmission is not yet determined but seems to be rather low.

Physical properties

Crude juice pressed out of the leaves from *Hippeastrum* plants showing symptoms was used to determine the dilution end point of the virus. Ten leaves of two *G. globosa* plants were inoculated with one dilution. A hundredfold dilution produced 27 lesions while no lesions were formed with sap diluted 10^{-3}. The thermal inactivation point was found to be 70 °C. Tests on longevity in vitro were made with untreated crude juice from *Hippeastrum* stored at 20 °C. Samples were removed at intervals and tested for infectivity on *G. globosa.* Juice stored for 1 and 24 hours produced 558 and 5 local lesions on 10 leaves, respectively. Shorter intervals over a narrower range were used in a confirmatory run. The first three hours there was hardly any loss in infectivity. Then a rapid decrease of infectivity could be observed and at 7 hours after preparation of the juice the infectivity was about 10% of that at 0 hours. After 30 hrs the infectivity was practically lost.

Purification

Efforts to purify the virus from crude juice by salting out with $(NH_4)_2\ SO_4$ failed. It was not possible to resolve the virus from the precipitate.

Dr. M. K. CORBETT from the University of Florida, Gainesville, U.S.A., an exchange professor in Plant Virology in Wageningen, purified the virus for us by density gradient centrifugation. Examination of the preparation in the electron microscope revealed that the preparation from *Hippeastrum* contained flexuous rod particles. This work will be published elsewhere.

Dr. CORBETT provided us with a partially purified preparation of the virus with which further experiments could be carried out. When plants of *Datura stramonium, N. glutinosa, N. tabacum* 'Samsun' and *Lycopersicum esculentum* were inoculated with the purified HMV solution all plants were infected systemically within two weeks. It is remarkable that in our earlier experiments with virus-containing *Hippeastrum* juice all plants except the last named reacted negatively. The concentration of the virus in *N. glutinosa* reached a high level. In this plant species no cell inclusions could be demonstrated. Efforts to inoculate *Allium cepa* and *Narcissus pseudonarcissus* with purified virus solution proved negative. Further work will be accomplished with purified virus solution.

DISCUSSION

The virus under investigation occurring in *Hippeastrum hybridum* seems to be the *Hippeastrum* mosaic virus described by JOHNSON (1951) and PROCENKO

& PROCENKO (1964). It is not TSWV nor CMV because the host ranges of these viruses differ. Moreover, cell inclusions are never found in plants infected with TSWV or CMV.

It is quite possible that a virus inhibitor occurs in *Hippeastrum*. If so this could explain why *Datura stramonium*, *N. tabacum* 'Samsun' and *N. glutinosa* were not infected with virus-containing sap originating from *Hippeastrum* but became systemically infected with juice from *N. glutinosa*.

The reverse relation between infectious virus material and inclusion bodies in the flowers is still unexplained. Possibly virus material is accumulated in inclusion bodies and cannot serve as inoculum. The decrease of the virus concentration with aging of the perianth might be due to an inhibitor. There is perhaps less inhibitor in young flowers than in old ones. Further work on the identification of the virus is in progress.

ACKNOWLEDGMENTS

The authors are greatly indebted to Dr. M. K. CORBETT for the purification of the virus and correction of the English text. Miss I. ZOUTEWELLE took the photographs.

SAMENVATTING

In een kwekerij te Hoorn werden planten van *Hippeastrum hybridum* aangetroffen, die mozaïekverschijnselen vertoonden. De oorzaak hiervan werd nagegaan. Infectieus virusmateriaal kon worden aangetoond in wortels, bladeren, bloemstengel, bloemdekbladen, meeldraden en stijl. Celinsluitsels kwamen voor in de epidermis van bladeren en stengel en in de bloemschede. De aanwezigheid van insluitsels in het bloemdek was afhankelijk van de ouderdom van de bloem. Er schijnt een omgekeerd evenredige relatie te bestaan tussen de virusconcentratie en het aantal insluitsels in bloemen.

Pogingen om het virus over te brengen door bladluizen mislukten. In enkele gevallen had zaadoverdracht plaats.

Dertig plantesoorten reageerden negatief op een inoculatie met virushoudend sap. *Hippeastrum hybridum*, *Gomphrena globosa*, *Chlorophytum* spec. en *Lycopersicum esculentum* konden wel worden geïnfecteerd. Gezien de waardplantenreeks en het voorkomen van celinsluitsels kunnen de mozaïeksymptomen in *Hippeastrum* niet toegeschreven worden aan „Tomato spotted wilt"-virus of het komkommer-mozaïek-virus, maar is het waarschijnlijk dat zij worden veroorzaakt door het *Hippeastrum*-mozaïek-virus, reeds beschreven door BRIERLEY (1948), JOHNSON (1951) en PROCENKO & PROCENKO (1964).

Dr. M. K. CORBETT, Wageningen, slaagde erin het virus te zuiveren door middel van „density gradient"-centrifugering. *Datura stramonium*, *N. glutinosa* en *N. tabacum* 'Samsun' werden systemisch ziek binnen twee weken na inoculatie met de gezuiverde virus-oplossing.

REFERENCES

ANONYMUS, – 1958. Versl. Meded. plziektenk. Dienst Wageningen 132 (Jaarboek 1958): 43.
BRIERLEY, P., – 1948. Diseases of Amaryllidaceae, excluding those of Allium and Narcissus. Herbertia, La Jolla 15: 113–119.
CEVAT, H. N., – 1964. Virusziekten bij Hippeastrum. Vakbl. Bloemist. 19: 716.

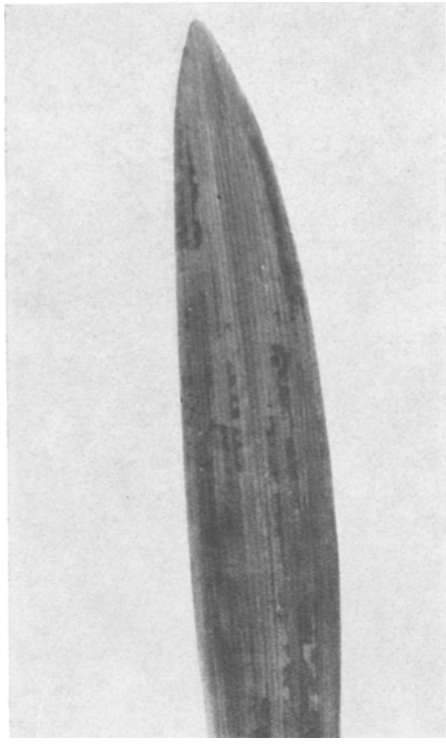

FIG. 1. *Hippeastrum* leaf showing mosaic symptoms.
Hippeastrum-*blad met mozaïek-symptomen.*

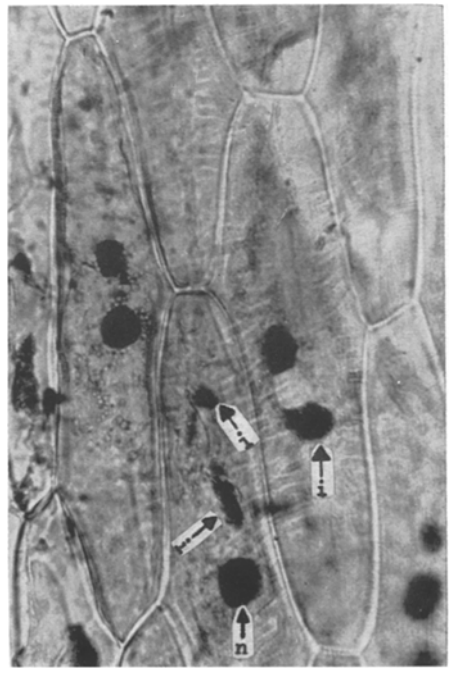

FIG. 2. Epidermal cells of a diseased *Hippeastrum* leaf.
Epidermiscellen van een ziek Hippeastrum-*blad.*

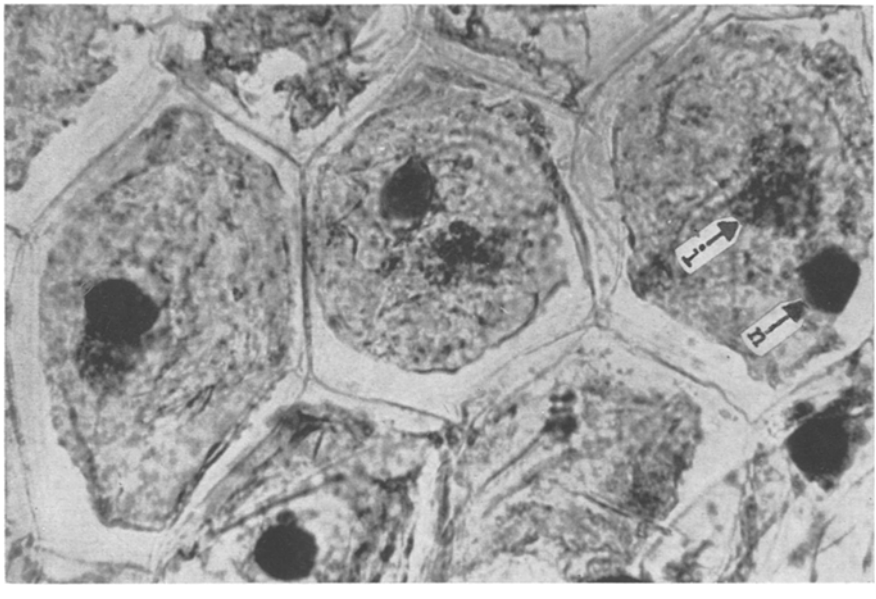

FIG. 3. Epidermal cells of the perianth of a flower eight days old with inclusions.
Epidermiscellen van een bloemdekblad van een acht dagen oude bloem met insluitsels.
i = inclusion / *insluitsel*
n = nucleus / *kern*

DICKSON, B. T., – 1922. Studies concerning mosaic diseases. Techn. Bull. MacDonald Coll. McGill Univ. No. 2.
HOLMES, F. O., – 1928. Cytological study of the intracellular body characteristic of Hippeastrum mosaic. Bot. Gaz. 86: 50–58.
JOHNSON, J., – 1951. Virus particles in various plant species and tissues. Phytopathology 41: 78–93.
KAHN, R. P., – 1960. The present status of the Amaryllis mosaic disease in the United States. Louisiana Soc. hortic. Res. Bull. no. 5: 24–30.
KAHN, R. P. & H. A. SCOTT, – 1964. Serological relationship of cumcumber mosaic virus and certain virus isolates that incite Amaryllis mosaic symptoms. Phytopathology 54: 360–362.
KLINKOWSKI, M., – 1958. Pflanzliche Virologie II. Berlin.
KUNKEL, L. O., – 1922. Amoeboid bodies associated with Hippeastrum mosaic. Science 55: 73.
KUNKEL, L. O., – 1924. Further studies on the intracellular bodies associated with certain mosaic diseases. Bull. Exp. Sta. Hawaiian Sug. Pl. Ass. Bot., Series 3: 108–114.
MOORE, H. E., – 1963. Amaryllis and Hippeastrum. Baileya 11: 15–16.
NOORDAM, D., – 1943. Over het voorkomen van spotted wilt in Nederland. Tijdschr. PlZiekt. 49: 117–119.
PROCENKO, A. E. & E. P. PROCENKO, – 1964. In: Plant Virology, Proc. 5th Conf. Czechosl. Pl. Virologists, Prague 1962: 242.
SMITH, K. M., – 1933. In: Recent Advances in the study of Plant viruses, London. Churchill Ltd.
SMITH, K. M., – 1957. A textbook of Plant Virus Diseases, London. 2nd ed.
STOUFFER, R. F., – 1963. A mosaic disease of hybrid Amaryllis caused by cucumber mosaic virus. Fla. State. Hort. Soc. Proc. 76: 462–466.
TRAUB, H. P., – 1958. The Amaryllis Manual. New York. Amer. Amaryllis Soc.