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Preliminary description of a potyvirus from Vallota speciosa

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Abstract

A potyvirus, isolated from *Vallota speciosa*, is tentatively designated *Vallota* mosaic virus (VMV).

VMV was easily transmissible in sap and could be transferred by *Myzus persicae* in a non-persistent manner. Infection was symptomless in *Nicotiana clevelandii* and *Spinacia oleracea*, whereas *Hyoscyamus niger*, *chenopodium quinoa*, *C. amaranticolor*, *Tetragonia expansa* and *Gomphrena globosa* reacted with local lesions.

Dilution end-point was 10^4–10^5, thermal inactivation at c. 60 °C and ageing in vitro 4–8 days.

With the electron microscope elongate particles with a normal length of about 750 nm could be detected in crude sap.

In ultrathin sections virus particles were observed in the cytoplasm, dispersed as well as aggregated. Pinwheel and laminate aggregated inclusions were present in sectioned leaf material. The inclusions observed in negatively stained crude sap preparations exhibited fine linear striations with a periodicity of 5.3 nm.

Additional keywords: host range, inclusions, pinwheels, virus transmission.

Introduction

Vallota speciosa (Amaryllidaceae) is a minor ornamental crop in the Netherlands. Commercial expansion of this crop is hindered by the occurrence of a bulb rot caused by *Fusarium* sp. and virus-like symptoms in leaves and flowers. The present study was undertaken in order to elucidate the possible virus origin of the flower colour breaking and the mosaic symptoms in the leaves.

Materials and methods

Determination of *virus transmission with sap*, *host-range tests and determination of persistence of infectivity in expressed sap* were in the conventional ways, with carborundum 500 mesh as an abrasive. Plants were grown and kept in an insect-proof glasshouse at 18–20 °C.

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Virus isolates. The virus was isolated from *Vallota speciosa* plants, collected in the Netherlands in April 1978. They showed a mosaic on the leaves and colour breaking in the flowers.

In all experiments a virus isolate was used that was carried through three single lesion transfers on *Chenopodium quinoa*, followed by two successive transfers from local lesions in this host by *Myzus persicae*.

The host range was determined by inoculation, using sap from leaves of *C. quinoa*, showing chlorotic local lesions.

Back inoculation was made on *C. quinoa*, *C. amaranticolor*, and sometimes on *Tetragonia expansa* and *Hyoscyamus niger*.

C. quinoa was used as source and test plant in most of the experiments on physical properties.

Electron microscopy was applied to inoculated leaves of test plants, using a Philips EM 300. Preparations of crude sap were observed after negative staining with 2% aqueous phosphotungstic acid, pH 6.5. Tissues of *Vallota* leaves with mosaic symptoms, of inoculated *C. quinoa*, *C. amaranticolor*, *T. expansa* and *H. niger*, showing chlorotic local lesions, and of latently infected *Nicotiana clevelandii*, were fixed in 6% glutaraldehyde in 0.1 M phosphate buffer for 2 h, followed by 90 min in chilled 2% osmium tetroxide. After dehydration the tissues were embedded in a 3:2:1 mixture of Epon 812, methylnadic anhydride and dodecenyl succinic anhydride. Sections were cut with glass knives and post-stained with uranyl acetate or lead citrate.

Results

Host range and symptoms. The virus was readily transmissible with sap from diseased *Vallota* leaves (Fig. 1., above) to test plants.

Host range studies were carried out in serial tests, as the one presented in Fig. 2. The results were determined by visual observation. Latent infections were checked by back inoculation onto indicator plants and by direct electron microscopy.

None of the plants tested reacted with systemic symptoms. Local lesions were produced on the inoculated leaves of: *C. quinoa*, *C. amaranticolor*, *T. expansa*, *Gomphrena globosa* and *H. niger*, but *N. clevelandii* and *Spinacia oleracea* remained symptomless.

The symptoms in susceptible plants were as follows:

1. *Chenopodium quinoa*. Chlorotic local lesions, 1–1.5 mm diameter, developed on the inoculated leaves, 6–7 days after inoculation (Fig. 3, left). Occasionally the lesions had a white necrotic centre.
2. *Chenopodium amaranticolor*. Chlorotic local lesions appeared on the inoculated leaves 6–7 days after inoculation (Fig. 3, right). When the inoculated leaves began to turn yellow, the chlorotic spots became light brown, with a purple edge.
3. *Tetragonia expansa*. Local green ringspots developed on the inoculated lower leaves, when they began to turn yellow. On the inoculated upper leaves chlorotic lesions were formed, 6–8 mm diameter, 12–14 days after inoculation. Green spots or ringspots sometimes occurred on the inoculated lower leaves 8–10 days after inoculation.
4. *Gomphrena globosa*. Red-edged local lesions with light brown necrotic centre were formed on the inoculated leaves 12–15 days after inoculation.

Fig. 1. Symptoms of VMV in leaves of *Vallota speciosa* (above) and local lesions of this virus in *Hyoscyamus niger* (under) 17 days after inoculation.

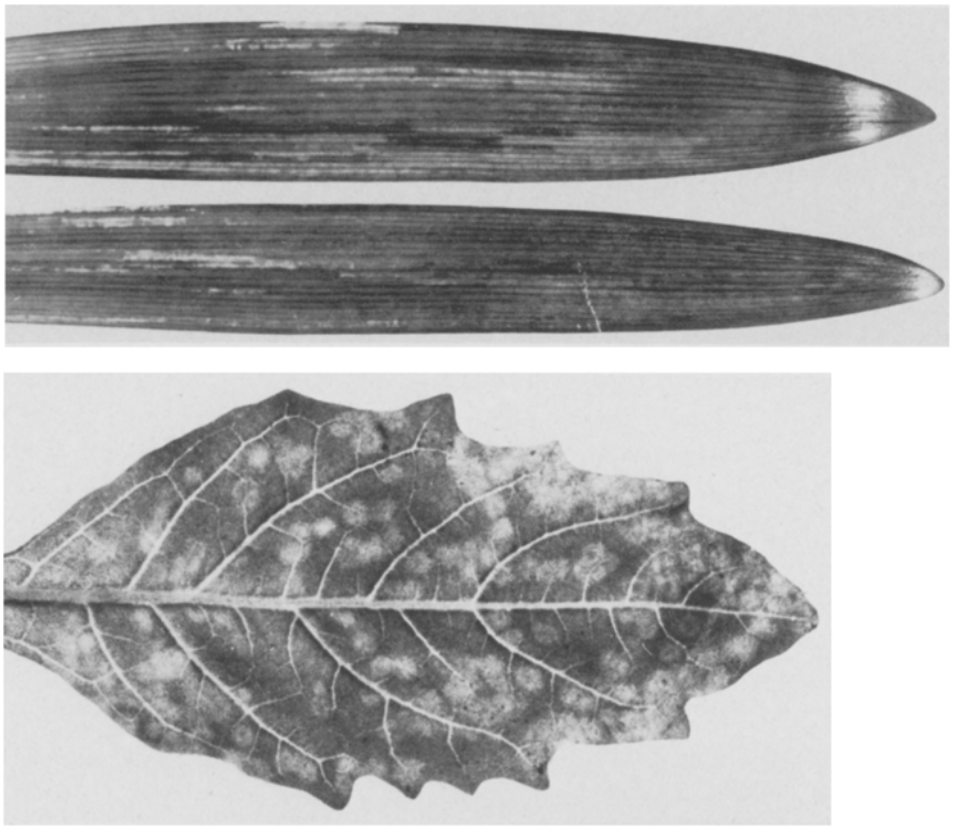

Fig. 1. Symptomen van VMV in bladeren van Vallota speciosa (boven) en lokale lesies van dit virus in Hyoscyamus niger (onder) 17 dagen na inoculatie.

5. *Hyoscyamus niger*. Light chlorotic local lesions, approximately 3 mm diameter, appeared on the inoculated leaves, 7–10 days after inoculation (Fig. 1, under). The lesions later extended to form large spots with a diameter of 10 mm or more.
6. *Nicotiana clevelandii* and *Spinacia oleracea*. Inoculation led to latent local infection. In *S. oleracea* occasionally very vague chlorotic lesions were observed in the inoculated leaves, around 15 days after inoculation.
7. *Freesia spec.* Leaves showed mosaic symptoms, 80 days after inoculation (Fig. 4).

No infection was obtained by mechanical inoculation of the following 24 species in 8 families. Chenopodiaceae: *Beta vulgaris*; Compositae: *Zinnia elegans*; Cruciferae: *Brassica oleracea, Raphanus sativus*; Cucurbitaceae: *Cucumis sativus, Cucurbita hybrida, C. maxima, C. moschata, C. pepo*; Gramineae: *Zea mays*; Leguminosae: *Glycine max, Phaseolus vulgaris, Pisum sativum, Trifolium incarnatum, Vicia faba, Vigna sesquipedalis*; Pedaliaceae: *Sesamum indicum*; Solanaceae: *Datura stramonium, Lycopersicum esculentum, Nicotiana glutinosa, N. rustica, N. tabacum* (Samsun, Samsun NN, White Burley), *Petunia hybrida, Physalis floridana*.

Fig. 2. Serial testing of VMV from *Vallota speciosa* on a host range of 6 test plant species. L = local lesion; l = local latent infection; (pw) = pinwheels; × = not tested for pinwheels.

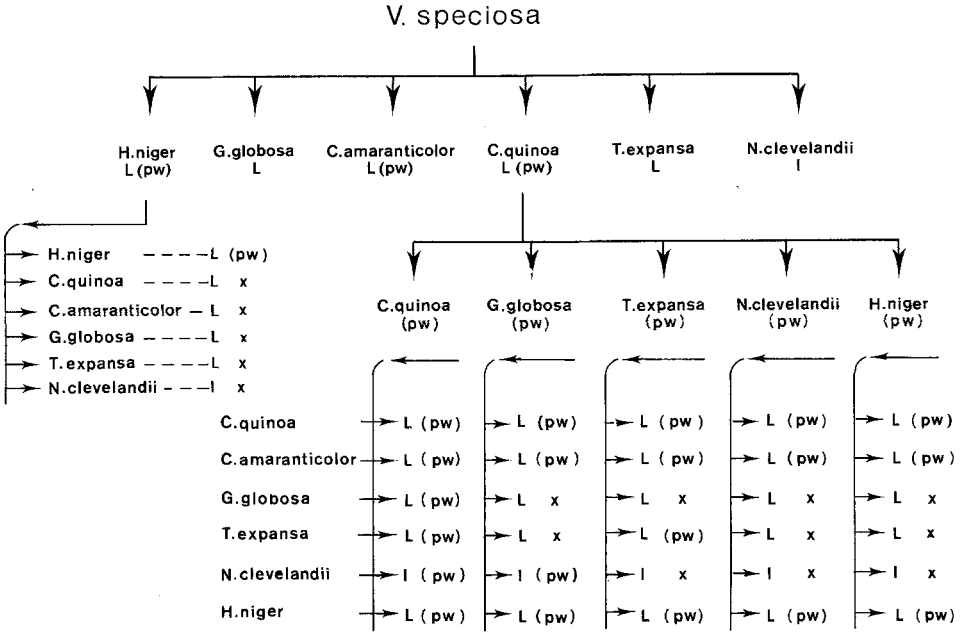

Fig. 2. Schema van inoculaties van VMV van Vallota speciosa op een waardplantenreeks van 6 soorten. L = lokale lesies; l = lokale latente besmetting; (pw) = 'pinwheels'; × = niet onderzocht op voorkomen van 'pinwheels'.

Fig. 3. Local lesions of VMV in inoculated leaves of *Chenopodium quinoa* (left) and *C. amaranticolor* (right) 14 days after inoculation.

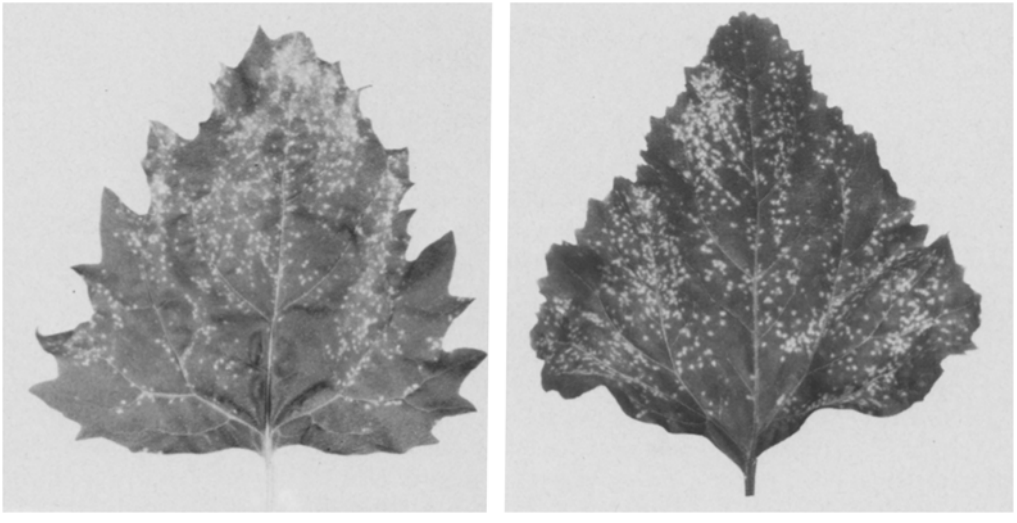

Fig. 3. Lokale lesies van VMV in geïnoculeerde bladeren van Chenopodium quinoa (links) en C. amaranticolor (rechts) 14 dagen na inoculatie.

Fig. 4. Mosaic symptoms in *Freesia spec.*, 80 days after inoculation with sap of VMV-infected *Chenopodium quinoa.*

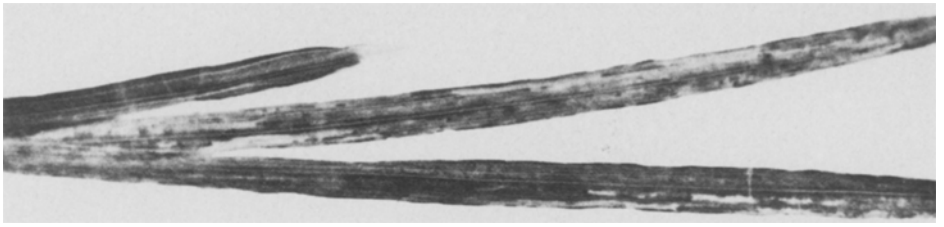

Fig. 4. Mozaïeksymptomen in Freesia spec., 80 dagen na inoculatie met sap van Chenopodium quinoa, geïnfecteerd met VMV.

Cross and back inoculations were made with sap expressed from each plant of the infected *C. quinoa*, *G. globosa*, *T. expansa*, *H. niger* and *N. clevelandii*, onto *C. quinoa*, *C. amaranticolor*, *G. globosa*, *T. expansa*, *H. niger* and *N. clevelandii*. The results of these inoculations are presented in Fig. 2. Sap from all infected plants, used as inoculation sources, infected *H. niger*, *C. quinoa*, *C. amaranticolor*, *T. expansa*, *G. globosa*, and *N. clevelandii*, which developed symptoms on the inoculated leaves as mentioned above.

Symptoms in Vallota

Approximately one year after inoculation, *Vallota* seedlings showed mosaic symptoms (Fig. 5), similar to those observed in the plants from which the VMV isolate originated.

Aphid transmission. Transmission was achieved with *Myzus persicae*, in a non-persistent manner. The effect of aphid-transmission tests was checked by back inoculation onto test plants and by electron microscopy.

Persistence of virus infectivity in expressed sap. Dilution end-point in crude sap of inoculated *C. quinoa* was between 10^4 and 10^5. Thermal inactivation was between 60 and 65 °C. The ageing end-point was between 4 and 8 days.

Fig. 5. Mosaic symptoms in seedlings of *Vallota speciosa*, 12 months after inoculation with sap of VMV-infected *Chenopodium quinoa.*

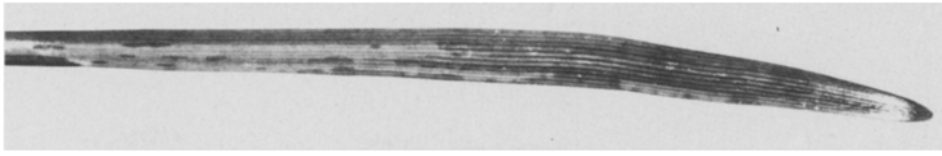

Fig. 5. Mozaïeksymptomen in Vallota speciosa zaailingen, 12 maanden na inoculatie met sap van Chenopodium quinoa, geïnfecteerd met VMV.

Fig. 6. VMV in crude sap, *Chenopodium quinoa*, stained with 2% phosphotungstic acid, pH 6.5.

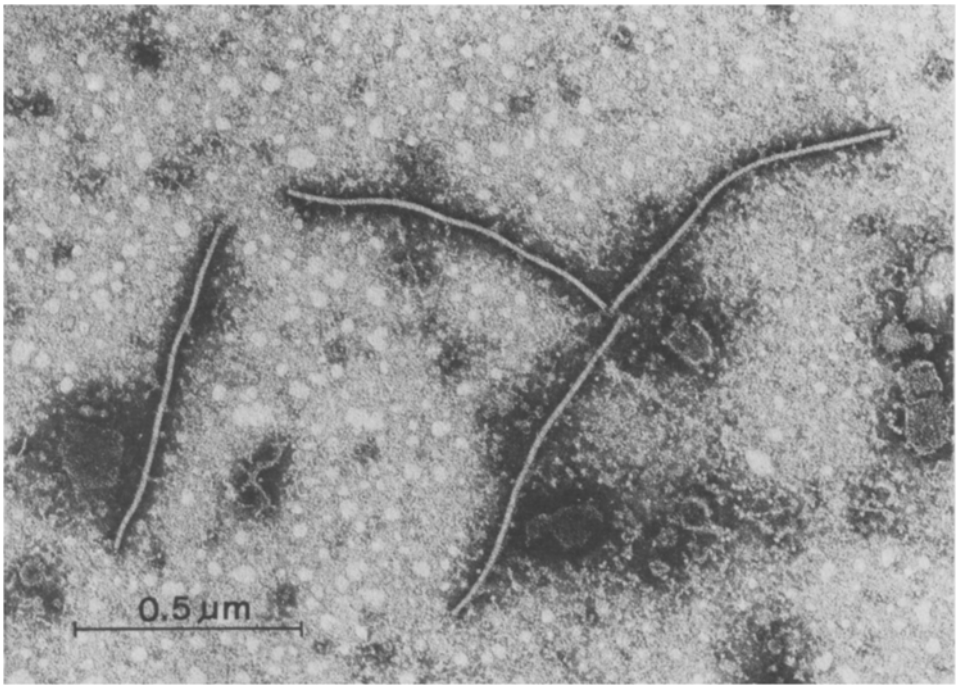

Fig. 6. VMV in ruw sap van Chenopodium quinoa, in 2% fosforwolfraamzuur, pH 6,5.

Fig. 7. Particle length distribution of VMS.

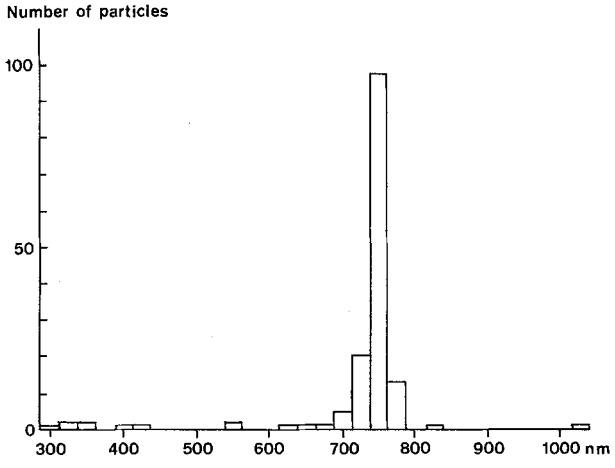

Fig. 7. Verdeling van deeltjeslengten van VMV.

Fig. 8. Striated, laminate inclusions of VMV in crude sap of *Vallota speciosa*, stained with 2% phosphotungstic acid, pH 6.5.

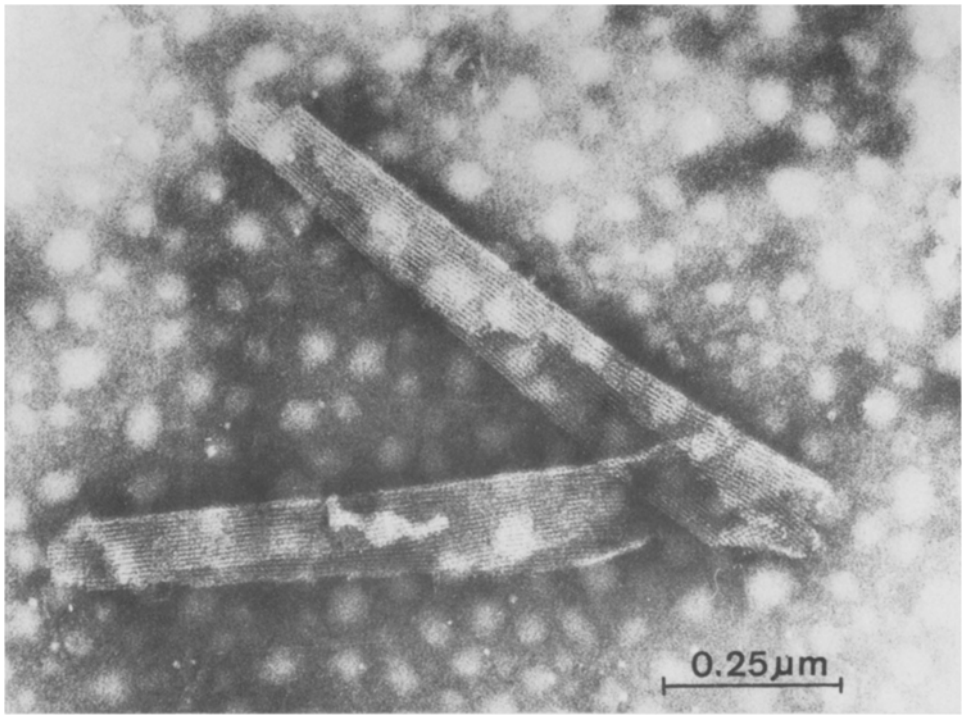

Fig. 8. Gestreepte, plaatvormige insluitsels van VMV in ruw sap van Vallota speciosa, in 2% fosforwolfraamzuur, pH 6,5.

Virus particles. Elongate particles were observed in crude-sap preparations from diseased *Vallota* plants, as well as from *C. quinoa*, *C. amaranticolor*, *G. globosa*, *T. expansa*, and *H. niger*, infected with the virus (Fig. 6). After measuring 147 particles in a crude-sap preparation from *C. quinoa* a normal length of approximately 750 nm was calculated (Fig. 7) and a width of about 13 nm.

Inclusions. Fragments of inclusions were observed in crude sap preparations from diseased *Vallota*, from *C. quinoa*, *C. amaranticolor*, *T. expansa*, *G. globosa* and *H. niger*, showing chlorotic local lesions, as well as from latently infected *N. clevelandii*. The plates of the inclusions showed a pattern of fine linear striations with a periodicity of approximately 5.3 nm (Fig. 8).

Ultrastructure of infected plants. In ultrathin sections of mesophyll cells of diseased *Vallota* inclusions were observed in the cytoplasm. They were laminate aggregates, the plates of which were straight or slightly curved.

In the cytoplasm of cells in or near local lesions on inoculated leaves of *C. quinoa*, *C. amaranticolor*, *T. expansa*, and *H. niger* no laminate aggregates, but pinwheels were present (Fig. 9).

Virus particles were dispersed or clustered in the cytoplasm.

Fig. 9. Pinwheel inclusions in mesophyll cells of *Chenopodium quinoa* (A) *Tetragonia expansa* (B) and *Hyoscyamus niger* (C), infected with VMV.

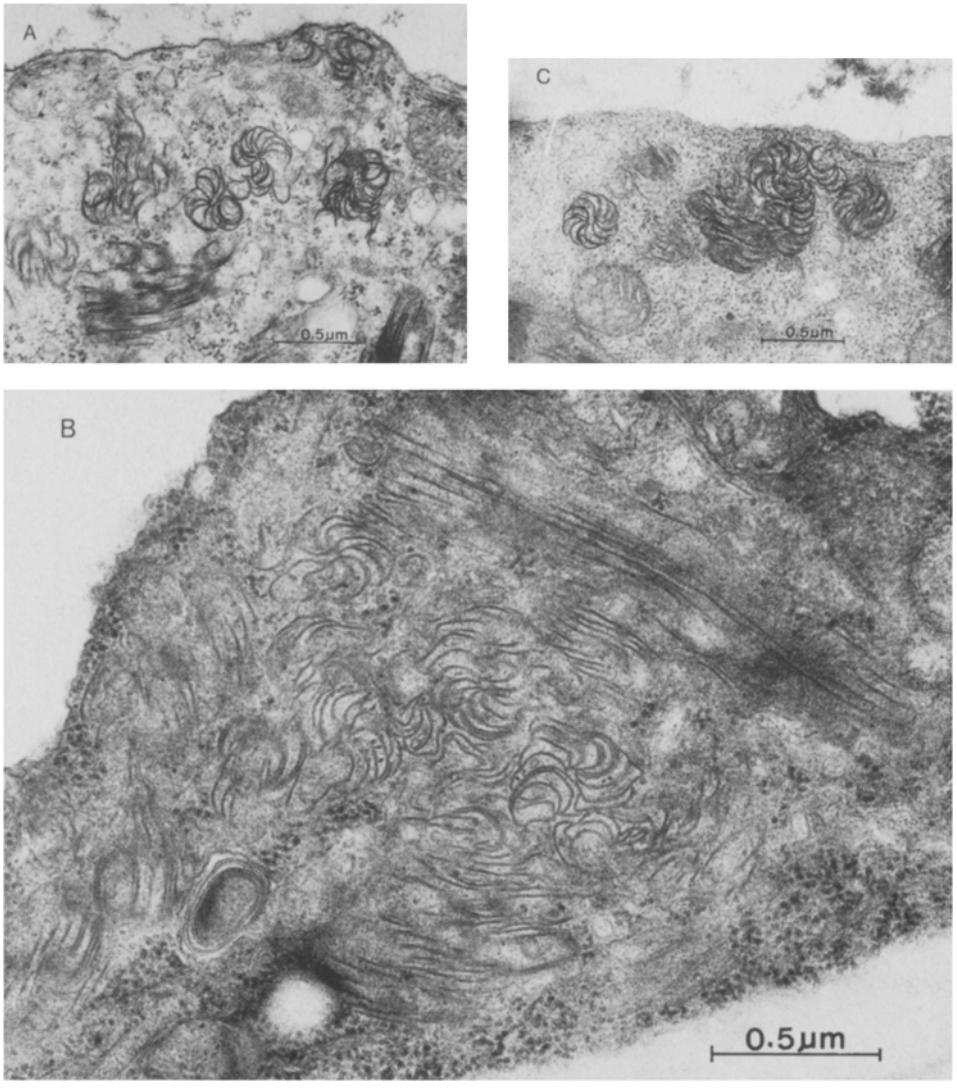

Fig. 9. 'Pinwheel' insluitsels in mesofylcellen van Chenopodium quinoa (A), Tetragonia expansa (B) en Hyoscyamus niger (C), geïnfecteerd met VMV.

Discussion

The virus isolated from *Vallota speciosa* belongs to the potyvirus group in its particle size and morphology, physical behaviour in plant sap, aphid transmissibility, ease of detection by electron microscopy, and pinwheel inclusions. It is characterized by local lesions on *H. niger* as well as *C. quinoa*, *C. amaranticolor*, *T. expansa*, *G. globosa*, but is latent in *N. clevelandii* and *S. oleracea*.

Several potyviruses have been reported from amaryllidaceous plants. Our *Vallota* virus is distinguished from *Hippeastrum* mosaic virus in that it infects both *C. quinoa* and *H. niger*, whereas *Hippeastrum* mosaic virus infects *H. niger*, but not *C. quinoa* (De Leeuw, 1972; Brölman-Hupkes, 1975). Regarding narcissus yellow stripe virus only *Tetragonia expansa* has been recorded as a herbaceous indicator plant (Brunt, 1971). The potyvirus from *Alstroemeria* was infectious to *C. quinoa* and *H. niger*, but without clear symptoms (Maat, 1978). Jonquil mild mosaic virus (Brunt, 1977) induced symptomless systemic infection in *N. clevelandii*, whereas our virus remained local. The 750 nm 'Soleil d'Or' virus is not known to infect herbaceous test plants (Brunt, 1970). Neither are there herbaceous indicator plants known of the 750 nm narcissus white streak virus (Brunt, 1969).

From the potyviruses infecting iridaceous plants freesia mosaic virus is not known to infect other plants than freesia (Van Koot et al., 1954). Gladiolus is susceptible to bean yellow mosaic virus (Bos et al., 1974), but our *Vallota* virus is not infectious to bean, pea, garden bean and *Trifolium incarnatum*. Iris mosaic virus is not infectious to *G. globosa* nor *S. oleracea* (Brunt, 1968). Iris severe mosaic virus does not infect *T. expansa* (Brunt, 1973). The bearded iris mosaic virus, described by Barnett et al. (1971) and also belonging to the potyvirus group, does not infect *C. amaranticolor*, *C. quinoa* and *T. expansa*. A mosaic of *Tigridia pavonia* is caused by turnip mosaic virus (Brunt, 1976); this *Tigridia* virus caused local and systemic symptoms in *Petunia hybrida*, which was not infected by our *Vallota* virus.

From the potyviruses infecting Liliaceae, leek yellow stripe virus has an incubation time of about three weeks in *C. amaranticolor* and *C. quinoa*, is latent in *G. globosa* and is not infectious to *N. clevelandii*, whereas onion yellow dwarf virus does not infect *C. amaranticolor* and *C. quinoa* (Bos et al., 1978). Spargelvirus 1 is infectious to *Beta vulgaris* (Hein, 1969). The potyvirus from *Gloriosa rothschildiana* does not infect *C. quinoa* nor *G. globosa* (Koenig and Lesemann, 1974). The only known hosts of tulip breaking virus are tulip and lily (Brunt, 1967).

The *Vallota* virus did not react with a mixed antiserum the components of which had been prepared against *Hippeastrum* mosaic, narcissus yellow stripe, bean yellow mosaic, iris mosaic, iris severe mosaic, and turnip mosaic viruses (Inouye, unpublished results).

For these reasons our *Vallota* virus is considered to be new and the tentative name *Vallota* mosaic virus is proposed.

Vallota mosaic virus proved to be infectious to *Vallota* seedlings, causing mosaic symptoms in the leaves. Due to the fact that the seedlings were too young to flower, colour breaking of the flowers was not yet observed. Because we assume that the virus is responsible not only for leaf, but also for flower symptoms, elimination of VMV from *Vallota* planting material seems desirable. Since freesia was found susceptible, diseased *Vallota* crops may also be a potential danger of freesia plants.

Acknowledgments

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Samenvatting

Voorlopige beschrijving van een potyvirus uit Vallota speciosa

Een potyvirus, geïsoleerd uit *Vallota speciosa*, wordt beschreven onder de voorlopige naam *Vallota*-mozaïekvirus (*VMV*).

VMV was gemakkelijk mechanisch overdraagbaar en kon door *Myzus persicae* op non-persistente wijze worden overgebracht. Infectie was symptoomloos in *Nicotiana clevelandii* en *Spinacia oleracea; Hyocyamus niger*, *Chenopodium quinoa*, *C. amaranticolor*, *Tetragonia expansa* en *Gomphrena globosa* reageerden met lokale vlekken.

Het verdunningseindpunt was 10^4–10^5, het thermale-inactiveringspunt ca. 60 °C en de houdbaarheid in vitro was 4–8 dagen.

Met de elektronenmicroscoop werden in ruw sap draadvormige deeltjes waargenomen van ca. 750 nm. In ultradunne coupes werden in het cytoplasma verspreide, zowel als geaggregeerde virusdeeltjes aangetroffen.

In bladweefsel kwamen bovendien 'Pinwheels' en plaatvormige insluitsels voor. De insluitsels in negatief-gekleurde preparaten van ruw sap vertoonden een fijne streping met een periodiciteit van 5,3 nm.

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