Neth. J. Pl. Path. 84 (1978) 227–237

# Shallot latent virus, a new carlavirus

L. BOS, H. HUTTINGA and D. Z. MAAT[1]

Research Institute for Plant Protection (IPO), Wageningen

Accepted 12 May 1978

**Abstract**

A new carlavirus, apparently omnipresent in shallot (*Allium ascalonicum*) without causing symptoms, is described as shallot latent virus. It has also been detected in naturally infected onion (*A. cepa*) and leek (*A. porrum*).

The virus was easily transmissible in sap and could be transferred with *Myzus ascalonicus*. Infection after inoculation was symptomless in onion, leek, *A. fistulosum* and *A. jailae*. *Chenopodium album*, *C. amaranticolor* and *C. quinoa* reacted with local lesions. *A. neapolitanum* and *A. schoenoprasum* and 20 other plant species were immune.

Dilution end-point was $10^4$–$10^5$, thermal inactivation at ca. 80 °C and ageing in vitro 8–11 days. Purification by molecular sieving on Sephadex G-200 followed by equilibrium density-gradient centrifuging in CsCl was successful. Sedimentation coefficient was 147.5 S, buoyant density 1.313 g/cm$^3$ and molecular mass of protein subunits 23 200 dalton. With the antiserum (titre 1024) distant serological relationships to some carlaviruses were determined. No inclusion bodies were observed with the light microscope. With the electron microscope a high concentration of straight or slightly curved particles with a normal length of 650 to 652 nm could easily be detected in crude sap and in purified preparations.

## Introduction

Shallot *(Allium ascalonicum)* is propagated vegetatively and thus likely to harbour viruses. It is widely known as an important host of onion yellow dwarf virus (OYDV, Bos, 1976). Soon after initiation of studies on leek yellow stripe virus (LYSV) in leek *(A. porrum)* and its relationships to OYDV (Bos et al., 1978) a symptomless virus was detected in shallot. It occurred in high concentrations, was more rigid in particle form than OYDV and obviously belonged to the carlavirus group (Bos, 1972).

In the course of investigations on *Allium* viruses in the Netherlands the virus was soon found to be omnipresent in shallot. We have therefore studied it in some detail. The present paper describes shallot latent virus (SLV) as an apparently new virus.

## Materials and methods

*Virus isolates.* The virus was isolated a number of times without showing appreciable variation from plants of 'Noordhollandse Strogele', the main shallot cultivar grown in the Netherlands. It was also detected several times electron microscopically, among others in plants of the Dutch cultivar 'Ouddorpse Bruine'. Most research was on an isolate from an unidentified shallot cultivar in a private garden.

[1] The last two authors are responsible for purification and serology, respectively.

*Maintenance and propagation* was usually in leek cv. Goliath (or Winterreuzen). *Virus assay* was on *Chenopodium amaranticolor* and *C. quinoa* or with the electron microscope.

*Virus transmission in 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 18–22 °C.

*Insect-transmission tests* were with virus-free aphid cultures provided by the Entomology Department (IPO). Aphids were first starved for 2 h and then acquisition fed for $^1/_2$ h on leaves of leek plants infected after sap inoculation. They were then transferred to healthy leek seedlings (5 per pot), 10 aphids per plant and after $^1/_2$ h of inoculation feeding transferred to another series of leek seedlings, where they were left overnight, and then killed with an insecticide. Three months after test feeding, the test plants were examined for infection.

*Virus purification* was from *A. porrum* ('Goliath') as described for LYSV (Huttinga, 1975) and for OYDV (Bos et al., 1978) using molecular sieving on Sephadex G-200 and extended by equilibrium density-gradient centrifuging in CsCl when for antiserum production. Biophysical properties were also determined as for OYDV.

*Serology.* For antiserum production two intravenous injections were given to a rabbit with a one-week interval. Two weeks after the second injection, an emulsion of equal volumes of virus and Freund's incomplete adjuvant was injected intramuscularly. Per injection the virus obtained from 100–200 g of leaf material was administered. Bleeding was started two weeks later.

Antiserum titers and serological relationships were determined in the microprecipitin test, using purified virus preparations. To determine reactions with normal plant antigens, clarified extracts or concentrated preparations of virus-free plants were used. Dilutions were made with 0.1 M tris, adjusted to pH 8 with citric acid and containing 0.05% $NaN_3$. In one test with *Hippeastrum*, tris-HCl, pH 9, was used.

Antisera to poplar mosaic virus, lily symptomless virus, and carnation latent virus were from the Bulb Research Centre, Lisse, the Netherlands, and to *Narcissus* latent virus from the Bulb Research Centre and from Dr A. A. Brunt, Littlehampton, England. The other antisera used were from own stock.

*Light and electron microscopy* were as described by Bos et al. (1978) for LYSV and OYDV.

## Results

*Incidence.* The virus was readily transmissible in sap and isolated with test plants or demonstrated to be present by electron microscopy all 17 times when material from various parts of the Netherlands was tested. In one of these instances when individual plants were randomly taken from 'Noordhollandse Strogele' origins obtained via the General Netherlands Inspection Service of Vegetable and Flower Seeds, all contained the virus. Symptoms were never observed except in two cases when the virus occurred in complex with OYDV.

The virus was once isolated together with OYDV from a naturally infected onion plant 'Rijnsburger' and once from five leek plants growing near shallot plants at a trial field to study possible natural spread of OYDV from shallot to leek (Bos et al., 1978).

Fig. 1. Local lesions of shallot latent virus in *Chenopodium amaranticolor* 18 days after inoculation.

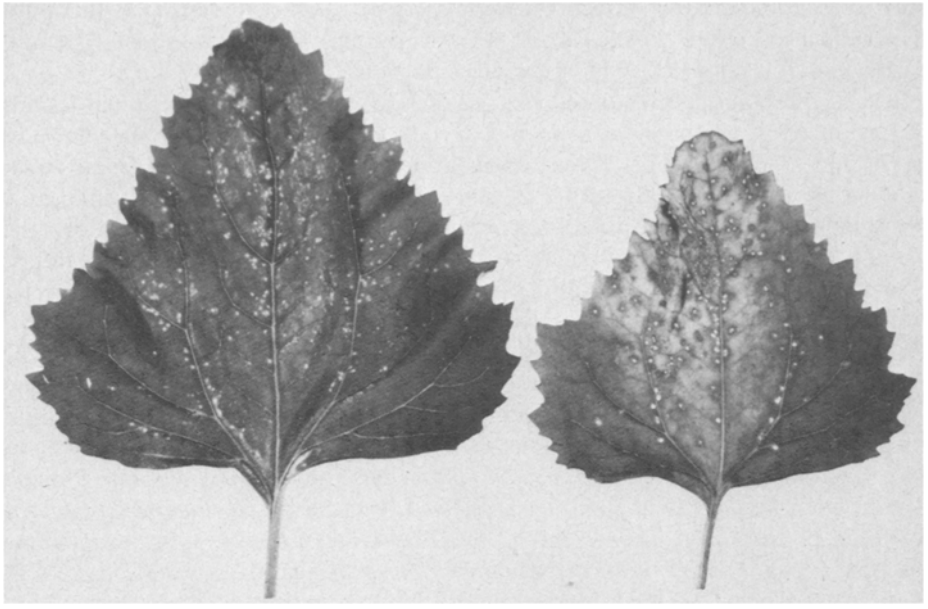

*Fig. 1. Lokale lesies van het latente sjalottevirus in Chenopodium amaranticolor 18 dagen na inoculatie.*

Fig. 2. Local lesions of shallot latent virus in *Chenopodium quinoa* 14 (left) and 18 days (right) after inoculation.

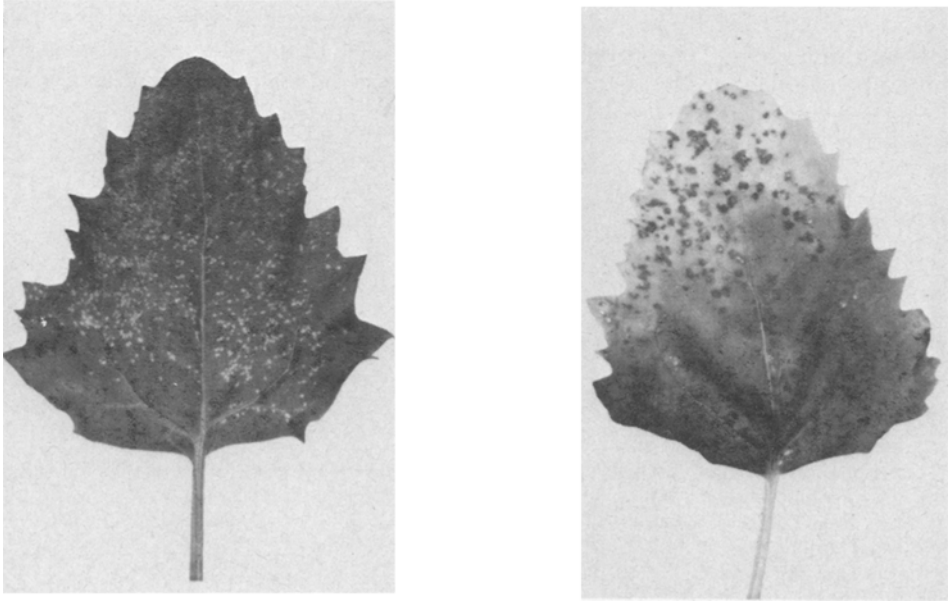

*Fig. 2. Lokale lesies van het latente sjalotte virus in Chenopodium quinoa 14 dagen (links) en 18 dagen (rechts) na inoculatie.*

*Host range and symptoms.* Of each species or cultivar at least two and often more plants were tested. Latent infections were checked by back inoculation (bi) onto *Chenopodium* species, by direct electron microscopy (em), or by back inoculation onto leek and electron microscopy in that species (lem).

None of the plant species tested reacted with systemic symptoms. Local lesions were produced in all three *Chenopodium* species tested. *C. album*, *C. amaranticolor* (Fig. 1) and *C. quinoa* (Fig. 2) reacted in about 10 days with many small vague local lesions, which soon thereafter became more clear without further enlarging. They sometimes showed up as soon as 6 days after inoculation and often had the appearance of pinpoint dry lesions. In yellowing old leaves they sometimes developed into distinct green rings surrounding dry tissue. In all three *Chenopodium* species characteristic virus particles could be detected with the electron microscope.

Latent systemic infection following inoculation was obtained in *Allium cepa* 'Noordhollandse Bloedrode' (bi), 'Noordhollandse Strogele' (bi), 'Witte Lissabon' (bi) and 'Zilverui' (bi), *A. fistulosum* (em), *A. jailae* (em) and *A. porrum* 'Brabantse Winter' (bi), 'Goliath' (bi), 'Olifantsreuzen' (bi), and 'Winterreuzen' (bi).

No infection was obtained in *A. neapolitanum* (em) and *A. schoenoprasum* (bi) and the same held for all other plant species tested, viz. *Chrysanthemum* sp. (bi, lem), *Crotalaria* sp. (bi), *Cichorium endivia* (bi), three cultivars of *Freesia* hybr. (tested three times, bi), *Gomphrena globosa* (bi), *Lilium formosanum* (em), *Lycopersicon esculentum* (bi), *Nicotiana clevelandii* (bi), *N. glutinosa* (bi), *N. tabacum* 'White Burley' (bi), *Petunia* hybr. (bi), *Phaseolus vulgaris* 'Bataaf' (bi), *Pisum sativum* 'Koroza' and 'Rondo' (bi), *Tetragonia expansa* (bi, lem), *Tulipa* sp. (lem), *Vicia faba* 'Kompakta' (not back tested), *Vigna sinensis* 'Early Ramshorn' (bi) and *Zea mays* (bi, em).

During 1973 a series of plants of *Hyacinthus* No. 673 and of *Narcissus* 'Mount Hood' and 'Talma' were inoculated with our shallot virus by Ir C. J. Asjes at the Bulb Research Centre, Lisse. The bulbs were harvested and replanted at Wageningen. The next spring no symptoms were observed in any of the 29, 77 and 28 plants, respectively. Of two hyacinth plants, however, tested with the electron microscope, one contained

Table 1. Results of insect transmission test.

| Period of testing \ Aphid species | | *Aphis fabae* | | *Myzus ascalonicus* | | *Myzus persicae* | |
|---|---|---|---|---|---|---|---|
| | | em[1] | bi[3] | em | bi | em | bi |
| first ½ h | pot 1 | 0/5[2] | −[4] | 3/5 | ++[6] | 0/5 | − |
| | pot 2 | 0/5 | − | 1/5 | + | 0/5 | − |
| subsequent | pot 1 | nt[7] | − | nt | − | nt | − |
| period overnight | pot 2 | nt | +[5] | nt | ++ | nt | − |

[1] Testing of individual plants by electron microscopy three months after inoculation feeding.
[2] Number of plants infected out of plants tested.
[3] Testing of pooled samples of 5 plants per pot on *C. amaranticolor* and *C. quinoa*, 2 plants each, three months after inoculation feeding.
[4] − = no local lesions in indicator plants.
[5] + = few local lesions.
[6] ++ = many local lesions.
[7] nt = not tested.

*Tabel 1. Resultaten van de insektenoverbrengingsproef.*

thread-like virus particles but upon back inoculation no local lesions were obtained in the two *Chenopodium* assay species and no infection was obtained in leek. The same situation was observed with one of three 'Talma' plants. Two 'Mount Hood' plants tested did not contain visible virus particles.

*Insect-transmission tests.* Details of the insect-transmission tests, the aphid species used and the way of testing the plants used for test feeding, as well as the results obtained are recorded in Table 1. Transmission was achieved with *Myzus ascalonicus* and possibly with *Aphis fabae*.

*Persistence of virus infectivity in expressed sap.* Dilution end-point in sap from artificially infected leek plants was between $10^4$ and $10^5$ although at dilution $10^3$ already most infectivity was lost. Thermal inactivation was slightly over 80 °C, with very few local lesions still obtained at that temperature and numerous lesions at 75 °C. Most infectivity was lost after one day of storage in vitro, with a little infectivity left during the next week until the aging end-point between 8 and 11 days.

*Purification.* Sephadex molecular sieving chromatography gave highly purified preparations, free of mucilage but containing many particles characteristic of the carlavirus group with much end-to-end aggregation, most particles being present as dimers (Fig. 5). With purified material characteristic local lesions could be reproduced in *C. amaranticolor* and *C. quinoa*, whereas infection in leek was symptomless.

The sedimentation coefficient at infinite dilution in 0.1 M tris-HCl, pH 9, at 20 °C was 147.5 S. The buoyant density at 25 °C in CsCl was 1.313 g/cm$^3$ and the molecular mass of the coat protein subunit was 23 200 dalton.

*Serology.* The antiserum had titers of 1024 to the homologous virus, of 1 to a clarified extract from virus-free onions, and of 4 and 16 to concentrated preparations of virus-free *Nerine bowdenii* and *Hippeastrum* sp., respectively.

Antisera to *Nerine* latent virus, potato viruses S and M, red clover vein-mosaic virus, pea streak virus, chrysanthemum virus B, poplar mosaic virus, lily symptomless virus, *Narcissus* latent virus (Brunt, 1976), and carnation latent virus, with titers between 256 and 4000 to the homologous viruses and between 0 and 16 to normal plant antigens, did not react with the shallot virus, or their titers to this virus did not exceed those to normal plant antigens. Only two antisera clearly reacted with the shallot virus: an antiserum to an unidentified carlavirus from *Lonicera* sp. (F. A. van der Meer, unpublished) with titers of 1024, 64, and 1 to the homologous virus, to the shallot virus, and to normal plant antigens, respectively, and an antiserum to *Narcissus* latent virus (Bulb Research Centre) with titers of 640, 16 and 1 for homologous virus, shallot virus, and normal plant antigens, respectively.

*Light microscopy.* Neither inclusion bodies characteristic of OYDV (Bos, 1976; Bos et al., 1978) or of LYSV (Bos et al., 1978) nor other cytological abnormalities could be detected after staining and viewing by light microscopy in epidermal strips of infected leek plants 'Olifantsreuzen' or of shallot plants.

*Electron microscopy.* In crude sap preparations the virus particles could be readily

Fig. 3. Electron micrograph of chop preparation in PTA from leek artificially infected by the shallot latent virus. Note contamination by the longer and more flexuous particles of leek yellow stripe virus (arrows). Bar represents 1000 nm.

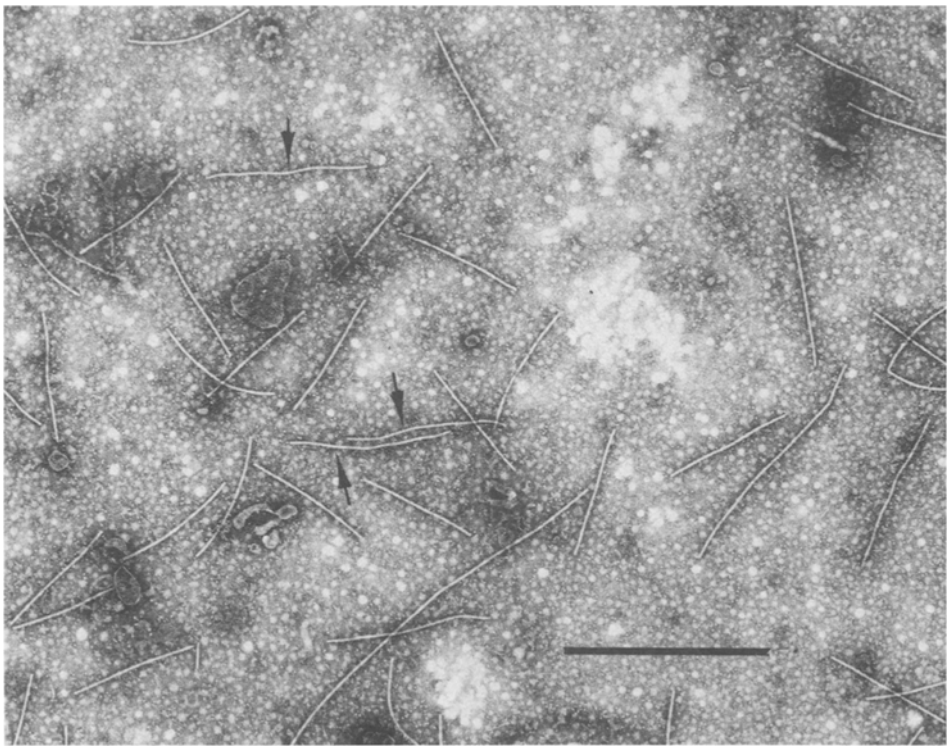

*Fig. 3. Elektronenfoto van hakselpreparaat in PTA van prei na kunstmatige infectie met het latente sjalottevirus. Let op de verontreiniging met de langere en flexibele deeltjes van het preigeelstreepvirus (pijltjes). Vergrotingsstaaf geeft 1000 nm weer.*

detected. They usually occurred in great numbers and were mostly spread evenly throughout the preparation (Fig. 3). End-to-end aggregation frequently occurred and subsequent breakage often led to deviating particle lengths. Individual particles were more or less straight or slightly curved and this was more pronounced after aggregation. In their morphology, distribution and thickness they could mostly be readily distinguished from particles of OYDV or LYSV, when occurring in mixed infections (Fig. 3). Occasionally groups of particles were observed at one end attached to membranous structures (Fig. 4).

Purified preparations contained many of the above particles, also with much end-to-end aggregation. The preparation of Fig. 5 contained many dimers.

After measuring 169 particles in a crude-sap preparation from shallot together with 73 particles of TMV a normal length of 651 nm was calculated. When the same procedure was applied to 163 particles of a purified preparation together with 92 TMV particles, this length was 652 nm, and to 67 particles of another purified preparation together with 59 particles of TMV it was 650 nm.

Fig. 4. Electron micrograph of the shallot latent virus in crude sap in PTA of shallot with numerous particles attached with one end to membranous structures. Bar represents 1000 nm.

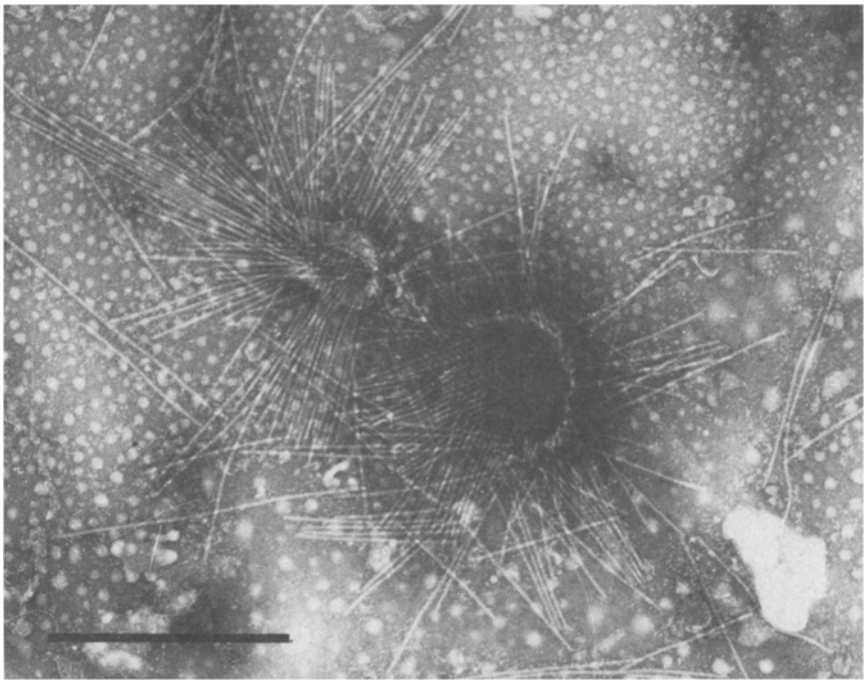

*Fig. 4. Elektronenfoto van het latente sjalottevirus in ruw sjalottesap in PTA met talrijke deeltjes gehecht aan membraanstructuren. Vergrotingsstaaf geeft 1000 nm weer.*

## Discussion

The shallot virus typically represents the carlavirus group in its particle size and morphology, high concentration and physical behaviour in plant sap, ease of detection by electron microscopy, weak serological relationship to some other carlaviruses, narrow host range, and latency in most of its hosts (see Fenner, 1976; descriptions in C.M.I./A.B.B. Descriptions of Plant Viruses). Its sedimentation coefficient (147.5 S) and buoyant density (1.313 g/cm$^3$) more resemble those of potyviruses.

The antiserum produced had a reasonable titer to its homologous virus (1024) but in some experiments also rather strongly reacted with normal plant antigens (titer up to 16 to *Hippeastrum* extracts). This indicates that the virus preparations used for antiserum preparation were not very pure. Unfortunately, virus-free shallots were not available. Clear relationships were demonstrated with two antisera: one to the unidentified *Lonicera*-virus, and one of the two *Narcissus* mosaic virus antisera, but they were distant. Though relationships to some other viruses tested are not excluded by the serological tests performed, they will be very distant. Thus the shallot virus is distinct from all viruses of which antisera were tested.

The virus apparently differs from all carlaviruses described sofar, including those of ornamental Liliiflorae (Liliaceae and some related families). It did not infect three

Fig. 5. Electron micrograph of purified preparation of shallot latent virus in PTA. Note the high percentage of particles being present as dimers. Bar represents 1000 nm.

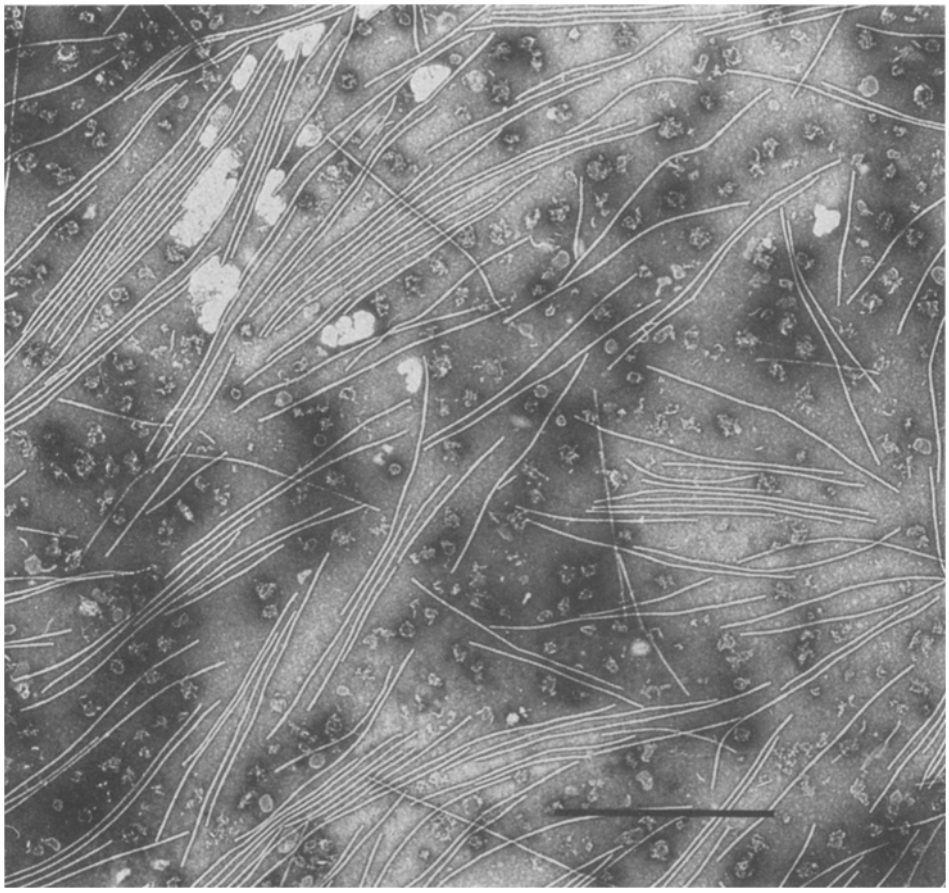

*Fig. 5. Elektronenfoto van gezuiverd preparaat van het latente sjalottevirus in PTA. Let op het hoge percentage deeltjes dat als dimeer aanwezig is. Vergrotingsstaaf geeft 1000 nm weer.*

freesia cultivars, so it differs from freesia mosaic virus. Not only could no serological relationship be detected with lily symptomless virus, but the shallot virus did not infect *Lilium formosanum* or *Tulipa* sp. The virus reacted with one of the two antisera to narcissus latent virus, but it did not infect two *Narcissus* cultivars, or *Nicotiana clevelandii* and *Tetragonia expansa*, hosts of the narcissus virus (Brunt, 1976). It was not related serologically to *Nerine* latent virus recently described by Maat et al. (1978). Even among liliiflorous plant species there appears much specialization of carlaviruses and the shallot virus is now described as a new carlavirus named *shallot latent virus*.

Hollings (1968) briefly reported on a shallot yellows virus readily transmissible to *C. quinoa*, *Tetragonia expansa* and onion (in which it induced only a mild mosaic). Infected shallot and onion plants contained intracellular inclusions detectable by light microscopy, as well as 620–640 nm particles. Symptoms recorded in shallot and

onion and the intracellular inclusions have undoubtedly been due to OYDV (see also Bos, 1976; Bos et al., 1978) often present in shallot. Hollings' electron microscope observations as well as the symptoms obtained in *C. quinoa* almost certainly indicate contamination of his material with shallot latent virus.

Since 1960 (Messiaen and Arnoux, 1960) in France a mosaic disease of garlic *(Allium sativum)* is being studied on and off. The virus was considered to be different from OYDV (Messiaen and Marrou, 1965). It was present in most autumn garlics and in all spring garlics in France. The virus was later (Quiot et al., 1975) claimed to be 640 nm long and able to induce a severe mosaic in garlic and 'Jersey' shallots and weak symptoms in onion and leek, but to cause symptomless infection in grey shallots. The virus was found to often occur in mixed infections with 770 nm long OYDV. Cadilhac et al. (1976) reported the occurrence of an OYDV-like virus and of a virus resembling garlic mosaic virus in diseased 'Jersey' shallots. Most of their information was by electron microscopy of ultrathin sections and they stressed the importance of purification and serology for exact identification.

In Czechoslovakia Havránek (1973) reported garlic mosaic virus to occur in most origins of garlic and in most plants tested, but onion, shallot and leek were immune to the virus. However, local lesions were produced in *C. amaranticolor* and *C. quinoa*. Brčák (1975) also reported the detection of garlic mosaic virus-like particles of 640 nm long in garlic and a number of wild *Allium* spp. from Russia. With one garlic isolate tested local lesions were produced on *C. quinoa* in 10–11 days. We have not been able to obtain reliably virus-free garlic plants for inoculation with our shallot virus.

Since none of the ca. 640 nm isolates reported from other countries have yet been properly identified by their intrinsic properties other than particle size, it is hard to judge their mutual relationships and their possible identity with our shallot latent virus. Our paper presents the first detailed description (including serology) of a carlavirus from *Allium* spp. The possible existence of a separate garlic mosaic virus should not be excluded in view of the rather narrow host ranges of carlaviruses.

In crude sap preparations viewed in the electron microscope the virus particles behaved like other carlaviruses in usually being present in high concentrations often with much end-to-end but never side-by-side aggregation. Virus particles occurring in large quantities with their ends attached to membrane remnants have earlier been reported and depicted by Bos and Rubio-Huertos (1972) for pea streak virus and by Bos et al. (1972) for a pea latent strain of red clover vein-mosaic virus. Such complexes have also been described for a carlavirus in shallot by Cadilhac et al. (1976).

The shallot latent virus may indeed be omnipresent in shallots because of vegetative propagation. All shallot plants we have tested so far contained the virus and usually did so in high concentration. The same may hold for all shallot plants grown in Northern France (Messiaen, personal communication, October 17th, 1975). Although carlaviruses may not all be readily transmitted by aphids, our tentative test has proved such transmission by *Myzus ascalonicus* and possibly by *Aphis fabae*. Acquisition was in short probes. Likely, transfer was in similar short probes characteristic of non-persistent transmission. 'Retention' of virus until after the first $^1/_2$ h inoculation 'feeding' period might have been due to delayed probing as is especially suggested by absence of transmission by *A. fabae* during the first $^1/_2$ h. Spread by insects is also suggested by the detection of the virus in leek grown from

seed and planted near shallot on a shallot trial field (Bos et al., 1978). Hence, shallot cultures may readily become infected without this being noticed.

Since no virus-free shallot plants seem to be available it is impossible to judge the effect of the virus on shallot. The virus may very well reduce yield considerably, as has for example been detected for a carlavirus (potato virus S) in potato. Infected plots were compared with plots of potato plants grown from tubers from plants serologically detected free from the virus and were found to yield 12% less on average in four cultivars tested (Scholz, 1962). Freeing shallots from virus by meristem tip culture might therefore pay, unless reinfection by aphids rapidly annuls its effect as was demonstrated for garlic mosaic virus by Marrou et al. (1972).


**Samenvatting**

*Latent sjalottevirus, een nieuw carlavirus*

Bij onderzoek over virussen van *Allium*-soorten zijn we in 1971 gestoten op een blijkbaar in alle sjalotteplanten *(Allium ascalonicum)* voorkomend symptoomloos virus. Het virus werd ook éénmaal in ui en éénmaal in prei aangetroffen.

Het virus kon gemakkelijk worden overgebracht met sap en gaf latente infecties in ui, prei, *A. fistulosum* en *A. jailae*. Het veroorzaakte spoedig na inoculatie talrijke lokale lesies in *Chenopodium album*, *C. amaranticolor* (Fig. 1) en *C. quinoa* (Fig. 2). Onvatbaar bleken *A. neapolitanum* en *A. schoenoprasum* en een 20-tal andere plantesoorten. De bladluis *Myzus ascalonicus* kon het virus in korte zuigtijden overbrengen (Tabel 1).

Het verdunningseindpunt in uitgeperst plantesap lag tussen $10^4$ en $10^5$, het thermale inactiveringspunt bij ongeveer 80 °C, en de houdbaarheid in vitro bedroeg 8–11 dagen.

Door moleculair zeven op een kolom van Sephadex G-200 gevolgd door evenwichtszonecentrifugering in een CsCl-dichtheidsgradiënt was het virus goed vanuit prei te zuiveren (Fig. 5). De sedimentatiecoëfficiënt bedroeg 147,5 S, het soortelijk gewicht 1,313 g/cm$^3$ en de moleculaire massa van het manteleiwit 23 200 dalton.

Het tegen gezuiverd virus bereide antiserum had een titer van 1024 en het virus bleek een verre serologische verwantschap te vertonen met enkele carlavirussen.

Er werden met de lichtmicroscoop geen celinsluitsels waargenomen. In ruw sap van sjalot en prei (Fig. 3 en 4) en in gezuiverde suspensies (Fig. 5) zijn grote aantallen stugge, rechte of zwak gebogen virusdeeltjes waarneembaar. Na meting bleken deze deeltjes gemiddeld 650 à 652 nm lang te zijn.

Het virus blijkt in veel opzichten karakteristiek voor de aardappelvirus-S-groep (carlavirusgroep), maar in waardplantenreeks en serologie van alle tot dusver in deze groep beschreven virussen te verschillen. Het wordt nu gepresenteerd als een nieuw virus met de naam *latent sjalottevirus* ('shallot latent virus'). Het lijkt enigszins op het nog zeer onvolledig gekarakteriseerde knoflook mozaïekvirus ('garlic mosaic virus').



**Acknowledgments**

Thanks are due to Miss M. P. Schor for performing most of the biological tests, to

Mr N. Huijberts for further technical assistance and to Ir C. J. Asjes (Lisse) for providing test plants of hyacinth, narcissus and tulip and for inoculating these with our virus.

## References

Bos, L., 1972. Ernstige uitbreiding van uiegeelstreepvirus in prei. Gewasbescherming 3: 81–87.

Bos, L., 1976. Onion yellow dwarf virus. C.M.I./A.A.B. Descr. Pl. Viruses No. 158: 4 pp.

Bos, L., Huijberts, N., Huttinga, H. & Maat, D. Z., 1978. Leek yellow stripe virus and its relationships to onion yellow dwarf virus; characterization, ecology and possible control. Neth. J. Pl. Path. 84: 185–204.

Bos, L., Maat, D. Z. & Markov, M., 1972. A biologically highly deviating strain of red clover vein mosaic virus, usually latent in pea (*Pisum sativum*), and its differentiation from pea streak virus. Neth. J. Pl. Path. 78: 125–152.

Bos, L. & Rubio-Huertos, M., 1972. Light and electron microscopy of pea streak virus in crude sap and tissues of pea (*Pisum sativum*). Neth. J. Pl. Path. 78: 247–257.

Brčák, J., 1975. Garlic mosaic virus particles and virus infections of some wild *Allium* species. Ochrana Rostl. 11: 237–242.

Brunt, A. A., 1976. Narcissus latent virus. C.M.I./A.A.B. Descr. Pl. Viruses No. 170: 4 pp.

Cadilhac, B., Quiot, J. B., Marrou, J. & Leroux, J. P., 1976. Mise en évidence au microscope électronique de deux virus différents infectant l'ail (*Allium sativum* L.) et l'échalotte (*Allium cepa* L. var. *ascalonicum*). Annls Phytopath. 8: 65–72.

Fenner, F., 1976. Classification and nomenclature of viruses. Second Report of the International Committee on Taxonomy of Viruses. Karger, Basel, München, Paris, London, New York, Sydney: 116 pp.

Havránek, P., 1973. Occurrence of viruses in the genus *Allium* and virus-free clones of common garlic (*Allium sativum*). Proc. 7th Conf. Czechosl. Pl. Virol. High Tatras 1971. Publ. House Slov. Acad. Sci. Bratislava: 133–136.

Hollings, M., 1968. Virology. A. Rep. Glassh. Crops Res. Inst. 1967: 108.

Huttinga, H., 1975. Purification by molecular sieving of a leek virus related to onion yellow dwarf virus. Neth. J. Pl. Path. 81: 81–83.

Maat, D. Z., Huttinga, H. & Hakkaart, F. A., 1978. *Nerine* latent virus: some properties and serological detectability in *Nerine bowdenii*. Neth. J. Pl. Path. 84: 47–59.

Marrou, J., Leclant, F. & Leroux, J. P., 1972. Epidémiologie du virus de la mosaïque de l'ail. Actas 3. Congr. Un. fitopat. medit. Oeiras, 22–28 Oct. 1972: 53–55.

Messiaen, C. M. & Arnoux, M., 1960. Une maladie de l'ail probablement due a un virus, son influence sur le rendement. Etudes Virol. appl. Inst. nat. Rech. agron.: 29–30.

Messiaen, C. M. & Marrou J., 1965. Sélection sanitaire de l'ail: deux solutions possibles au problème de la mosaïque de l'ail: plantes sensibles saines, ou plantes virosées tolérantes. C.r. 18 J. Phytiatr. Phytopharm. Tr. circum-médit. Marseille: 204–206.

Quiot, J. B., Marrou, J., Leroux, J. P., Leclant, L. & Messiaen, C. M., 1975. Viroses des *Allium*, sélection sanitaire des semences d'ail. Annls Phytopath. 7: 234–235.

Scholz, M., 1962. Die Bedeutung des S-Virus für den Kartoffelbau und Probleme der S-Virussanierung. NachrBl. dt. PflSchD. (Berlin) 16: 174–179.

## Address

Research Institute for Plant Protection (IPO), P.O. Box 42, 6700 AA Wageningen, the Netherlands