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Nerine latent virus: some properties and serological detectability in Nerine bowdenii

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Abstract

Nerine latent virus (NeLV), first found in *Nerine bowdenii*, may occur also in the other *Nerine* species investigated so far: *N. sarniensis*, *N. flexuosa* 'Alba', and *N.* 'Mansellii'.

Chenopodium amaranticolor, *C. quinoa*, and *Gomphrena globosa* sometimes reacted with local lesions after mechanical inoculation with NeLV. *Nicotiana clevelandii* and *Hippeastrum* were symptomless hosts. In this respect NeLV resembled the incompletely described *Hippeastrum* latent virus (HLV).

Serologically NeLV was closely related to HLV and to carnation latent virus (CaLV), but differed from the latter in host plant reactions. A more distant relationship was observed with some other carlaviruses, whereas NeLV also reacted with an antiserum to potato virus X.

Depending on the lot, NeLV could be detected rather reliably with the micro-precipitin test in *N. bowdenii* 'Van Roon', but less well in '63'. Better results were obtained with the microplate method of enzyme-linked immunosorbent assay (ELISA).

The average particle length was 664 nm, the sedimentation coefficient 155 S and the buoyant density 1.298 g/cm^3.

NeLV can be considered as a member of the carlavirus group. On basis of priority HLV may be considered as NeLV.

Introduction

The cultivation of various species of the bulbous plant genus *Nerine* for cut flower production is rapidly expanding in the Netherlands. The leaves, flower stalks and flowers may show disease symptoms, presumably caused by virus. Therefore, earlier, a research program was started to detect and identify the viruses occurring in *Nerine* and to study their effects on this ornamental plant.

Hakkaart (1972) reported the occurrence of flexuous virus-like particles, ca. 670 nm long, in *Nerine bowdenii*. As they were also found in *N. bowdenii* plants without virus symptoms, he named the virus *Nerine* latent virus (NeLV). Filamentous virus-like particles in *Nerine* have also been reported by Brunt et al. (1970), Koenig et al. (1973), Hakkaart et al. (1975) and Maat (1976), some of the particles being comparable in length to those of NeLV.

The present study further describes NeLV and its serological relationship to other carlaviruses. Its serological detectability in *N. bowdenii* was also investigated in order to enable inspection for certification purposes.

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Materials and methods

Viruses and source plants. Leaves from *N. bowdenii* '63' showing no virus symptoms and from *N. bowdenii* 'Van Roon' with severe mosaic were collected at the Research Station for Floriculture, Aalsmeer, and at commercial nurseries. They were stored at −20°C until use. These, together with artificially infected *Hippeastrum*, were sources for NeLV. Also some plants of *N. bowdenii*, *N. sarniensis*, *N. flexuosa* 'Alba', and *N.* 'Manselii' were collected. They were maintained in a greenhouse of the Research Institute for Plant Protection, Wageningen.

An isolate of carnation latent virus (CaLV) was provided by the Abteilung für pflanzliche Virusforschung der Biologischen Bundesanstalt at Brunswick, Germany. This virus was maintained and propagated in *Dianthus barbatus*.

A preparation of *Hippeastrum* latent virus (HLV) and naturally infected *Hippeastrum* material were from the Phytopathologisch Laboratorium 'Willie Commelin Scholten', Baarn, the Netherlands, and from a commercial nursery, respectively.

Host range tests. When *Nerine* or *Hippeastrum* plants were inoculum sources for mechanical transmission, partially purified preparations were used (see Virus Purification). Sap from other plants was used as such. Plants were grown in an insect-proof, temperature-controlled greenhouse at about 20°C. Virus-free seedlings of *N. bowdenii* were from the symptomless cultivar '63' and those of *Hippeastrum* from 'Scarlet Globe'.

Presence of virus in the inoculated and non-inoculated leaves of test plants was tested with the electron microscope (EM), using clarified extracts as prepared for serological testing of individual *Nerine* plants with the micro-precipitin test (see Serology).

Aphid-transmission tests. Virus-free *Myzus persicae* were starved for 4 h and then fed on mechanically infected *Hippeastrum* plants for 5 min. Thereafter, they were transmitted to virus-free *Hippeastrum* seedlings, 15–20 aphids per plant.

Virus purification. Leaf material was homogenized in a Waring blendor, using buffers and organic solvents chilled at 3°C. Unless otherwise stated, centrifuging at low speed was at 8000 rev/min for 10 min in a Sorvall RC2-B centrifuge, the rotor used depending on the quantity of material. Centrifuging at high speed to sediment the virus was in a Beckman ultracentrifuge for 2 h at 20000 rev/min, 1½ h at 24000 rev/min or 2 h at 25000 rev/min using rotors 21, 30 or 35, respectively, or for 3 h at 25000 rev/min (rotor 30) when the virus was in sucrose (see below).

For the first sucrose-gradient centrifuging 20 ml of partially purified virus, obtained from 1000 g of leaf material, was centrifuged for 1 h at 40000 rev/min in a Ti-14 zonal rotor containing a sucrose gradient from 10–40% (weight/volume). These gradients were prepared linear with volume using a Beckman gradient pump. Sucrose-containing virus zones, isolated with the aid of a Beckman gradient pump and an LKB-Uvicord absorption meter, were diluted 1:1 with buffer or distilled water before centrifuging to concentrate the virus. For the second sucrose-gradient centrifuging in the purification procedure, the material obtained from 1000 g of leaf material was divided into six equal portions and layered on top of a sucrose gradient from 10–40%

in tubes of an SW27 rotor. These were then spun for 1½ h at 24000 rev/min. The gradients were prepared linear with volume, using an LKB-Ultrograd gradient mixer. Virus zones were isolated with an ISCO density-gradient fractionator. Again sucrose-containing virus zones were diluted before concentrating.

CsCl-gradient centrifugings were performed in an SW50.1 rotor, which was spun for 16 h at 35000 rev/min. In each of the six tubes 1.4 ml of virus suspension was mixed with 3.5 ml of a CsCl solution in distilled water containing 0.623 g/ml. Virus zones at about 30–35 mm from the bottom of the tube were isolated by puncturing the bottom. They were dialysed against buffer, pH 9, to remove the CsCl.

For host range tests frozen leaves of *N. bowdenii* or *Hippeastrum* were homogenized with a buffer, pH 9 (see below), carbon tetrachloride, and chloroform at a ratio of about 4:16:1:1. The homogenate was centrifuged at low speed and the supernatant obtained centrifuged at high speed. The sedimented material was resuspended in buffer, pH 9, about 1/25–1/5 of the original weight of leaf material and used for inoculation or again centrifuged at low and high speed before use. The buffers for homogenization were 0.1 M tris (= tris(hydroxymethyl) aminomethane) adjusted to pH 9 with thioglycolic acid or 0.2 M tris adjusted to pH 9 with citric acid (0.2 M) and containing 0.1% sodium thioglycolate and 0.02 M sodium diethyldithiocarbamate. Sediments were resuspended in 0.1 M tris adjusted to pH 9 with HCl or in 0.2 M tris adjusted to pH 9 with citric acid.

Several methods were tried for antiserum production, using frozen leaves of *N. bowdenii* (green or mosaic-diseased) or NeLV-infected *Hippeastrum*. The above-mentioned buffers or phosphate-citric acid, pH 7, or tris-citric acid, pH 8, were applied. They contained reducing agents when used for homogenizing. Clarifying agents were chloroform, diethyl ether and carbon tetrachloride. To concentrate the virus, polyethylene glycol 6000 (5–8%) without and with NaCl (0.15–0.5 M) and ultracentrifuging were applied, followed by sucrose-gradient centrifuging (one or two cycles) and sometimes CsCl-gradient centrifuging.

The methods finally selected for antiserum production and for some other purposes (see below) were as follows. Portions of 500 g of frozen leaves of NeLV-infected *Hippeastrum* were homogenized with 1500 ml of 0.1 M tris-thioglycolic acid, pH 9, 250 ml of carbon tetrachloride and 250 ml of chloroform in a 1 gallon Waring blendor. The homogenate was centrifuged at low speed. The supernatant (A) was then centrifuged at high speed. The sedimented material thus obtained from 1000 g of leaf material was resuspended in 400 ml of 0.1 M tris-HCl, pH 9. The suspension was stirred overnight at 3°C and subsequently centrifuged at low speed. The supernatant was centrifuged at high speed and the sediment thus obtained resuspended in 20 ml of buffer (pH 9). After thoroughly stirring during at least 1 h this material was centrifuged at low speed and the supernatant subjected to sucrose-gradient centrifuging. After concentrating the virus from the sucrose, CsCl-gradient centrifuging was applied. Then a second sucrose-gradient centrifuging was performed, and after concentrating by ultracentrifuging, the sediment resuspended in 4 ml of 0.1 M tris-HCl, pH 9 (for serological purposes), or in distilled water (for determination of sedimentation coefficient and buoyant density). When the virus had to be stored for serological purposes, this was done at −20°C after addition of an equal volume of glycerol. In one of the two experiments performed to determine the sedimentation coefficient, the second sucrose-gradient centrifuging was omitted and the virus was

concentrated from the CsCl solution and resuspended in distilled water.

Control preparations of virus-free *Nerine* and *Hippeastrum* plants for serological experiments were prepared according to the purification method described, omitting the sucrose- and CsCl-gradient centrifugings. Moreover, the high-speed centrifugings were for 5 h at 20000 rev/min (rotor 21) and for 2 h at 40000 rev/min (rotor 50).

Serology. Rabbits were injected with NeLV preparations purified from *N. bowdenii* or from *Hippeastrum*. In general two intravenous injections given with a three-day interval were followed two weeks later by an intramuscular injection. For the latter, virus was emulsified with an equal volume of Freund's incomplete adjuvant. After another fortnight the titer was determined and when found too low, additional intravenous and/or intramuscular injections were given. Per injection the virus obtained from 200–400 g of leaf material was administered.

The micro-precipitin test under paraffin oil and the microplate method of enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977) were applied. Results of micro-precipitin tests were read after about 20 h at room temperature. With ELISA the plates were coated overnight at 6°C. Incubation with sap and with enzyme-antiserum conjugate was for 2 h and with enzyme substrate for 1 h at room temperature. Between two reaction stages plates were intensively washed with a gentle jet of tap water followed by rinsing with distilled water.

Serological relationships were investigated with the micro-precipitin test, using purified preparations. Dilutions of antigens and sera were made with 0.1 M tris-HCl, pH 9, or with 0.1 M tris-citric acid, pH 8, both containing 0.05% NaN_3.

Antisera to potato viruses S and M (PVS, PVM) and to a *Nerine*-virus preparation were provided by the Abteilung für pflanzliche Virusforschung der Biologischen Bundesanstalt at Brunswick, Germany, antisera to narcissus latent virus (NaLV) and CaLV were from Dr A. A. Brunt, Littlehampton, England, and antisera to poplar mosaic virus (PoMV), NaLV, 'Iris Bont', CaLV, and lily symptomless virus (LSV) were from the Bulb Research Centre, Lisse, the Netherlands. The other antisera used were from own stock.

To test individual *Nerine* plants, clarified extracts prepared in different ways were used. The methods finally selected for routine testing were as follows. Leaves, stored at −20°C for at least some days, were thawed and sap was prepared with a power-driven crusher. For the micro-precipitin test four drops of crude sap were collected in a small centrifuge tube containing 0.2 ml of a 1% solution of Na_2SO_3 in 0.1 M tris-citric acid, pH 8, containing 0.05% NaN_3. After storage for 1 h at room temperature, the tubes were centrifuged during 10 min at 6000 rev/min and the supernatants used for serological testing. For these tests sera were dialysed against tris-citric acid buffer, pH 8, 0.01 M, containing 0.05% NaN_3 and diluted 1:15 with the same buffer. For ELISA, three drops of sap were mixed with 0.5 ml of PBS-Tween (Clark and Adams, 1977), containing 2% polyvinylpyrrolidon 10000 and 0.2% ovalbumin.

Electron microscopy. Clarified extracts of *Nerine* and NeLV-infected *Hippeastrum*, prepared as A (see Virus Purification) or according to a method similar to that used for testing of individual *Nerine* plants with the micro-precipitin test, were examined with a Philips EM 300 after negative staining with 2% phosphotungstic acid in distilled water adjusted with KOH to pH 6.5. In a few cases clarified extracts were concentrated

by one cycle of differential centrifuging. Chop preparations with TMV were photographed alternately with preparations containing the *Nerine* viruses. Particles were measured in photographic negatives, using a low-power binocular microscope with objective lens × 1 and a micrometer eyepiece × 12.5.

To calculate particle lengths, for every series of photographs the average length expressed in micrometers was calculated and compared with the average length of TMV particles measured from photographs taken alternately with photographs of a *Nerine*, *Nicotiana clevelandii* or *Hippeastrum* specimen. The average length of TMV was assumed to be 300 nm.

Sedimentation coefficient and buoyant density. The sedimentation coefficient at infinite dilution in water at 20°C was determined by the graphical method of Markham (1960) and using a Spinco Model E ultracentrifuge with schlieren optics.

The buoyant density was determined in CsCl. For this purpose 0.95 ml of virus suspension was mixed with 2 ml of CsCl solution (0.623 g/ml), and overlaid with paraffin oil. The CsCl gradients were centrifuged for 19 h at 5°C at 30000 rev/min in a Beckman SW41 rotor. Three-drop fractions (about 0.1 ml) were taken from the bottom of the tubes, and 1 ml of water was added to alternate fractions which were then monitored at 260 nm. Refractive indices at 25°C (n_D^{25}) were measured on the other fractions, and the buoyant density at 25°C (ρ^{25}) of the virus was determined by using the relation $\rho^{25} = 10.2402 n_D^{25} - 12.6483$ (Bruner and Vinograd, 1965).

Results

Host range tests. The results are given in Table 1. Although both cultivars of *N. bowdenii* contained elongate particles of at least two different sizes, only NeLV could be transferred to some of the test plants mentioned. *Chenopodium quinoa*, *C. amaranticolor*, and *Gomphrena globosa* sometimes showed local lesions. On *C. quinoa* small dark green rings or points became visible three weeks after inoculation on inoculated leaves when these turned yellow. On *C. amaranticolor*, tiny, chlorotic, dark-edged lesions developed in the old, still green inoculated leaves three weeks after inoculation. On *G. globosa* small brown necrotic rings might develop about two weeks after inoculation.

N. clevelandii (only locally) and *Hippeastrum* became infected without showing symptoms. In seedlings of *N. bowdenii* '63' inoculated with preparations of NeLV-infected *Hippeastrum* only incidentally a few virus particles could be observed. These plants were also symptomless.

Aphid-transmission experiments. Virus could not be detected with the electron microscope in any of 20 *Hippeastrum* plants during 9 months after the aphids had fed on them.

Serology. In the micro-precipitin test antisera prepared had titers up to 4096 against purified preparations. The first antisera prepared to virus preparations from *N. bowdenii* also contained antibodies to normal plant antigens and to cucumber mosaic virus (CuMV). Antisera prepared to NeLV, purified with methods comparable to that described for NeLV-infected *Hippeastrum* leaves, did not contain antibodies to

Table 1. Results of host range tests with NeLV.

Test plant	Inoculated with extract from[1]	Reaction of test plant	
		local[2]	systemic[3]
Allium cepa	2	–	–
Allium porrum	2	–	–
Apium graveolens var. *rapaceum*	2	–	–
Beta vulgaris	2	–	–
Brassica oleracea var. *botrytis*	2, 3	–	–
Brassica oleracea var. *gemmifera*	2	–	–
Callistephus chinensis	3	–	–
Chenopodium amaranticolor	1, 2, 3	L(2, 3); l(1)	–
Chenopodium quinoa	1, 2, 3	L(1, 3); l(2)	–
Cucumis sativus 'Gele tros'	2, 3	–	–
Dianthus barbatus	1, 3	–	–
Dianthus caryophyllus 'William Sim'	2, 3	–	–
Gomphrena globosa	1, 2, 3	L	–
Hyoscyamus niger	3	–	–
Hippeastrum	1, 2, 3	l	s
Lilium Midcentury-hybrid 'Enchantment'	2	–	–
Lycopersicon esculentum	2, 3	–	–
Nerine bowdenii	2	l*	s*
Nicotiana clevelandii	2, 3	l	–
Nicotiana glutinosa	2, 3	–	–
Nicotiana hybrida 'Christie'	3	–	–
Nicotiana megalosiphon	2	–	–
Nicotiana rustica	2, 3	–	–
Nicotiana tabacum 'Samsun NN'	2	–	–
Nicotiana tabacum 'White Burley'	2, 3	–	–
Petunia hybrida	2, 3	–	–
Phaseolus vulgaris 'Bataaf'	2, 3	–	–
Pisum sativum 'Koroza'	2, 3	–	–
Tetragonia expansa	2	–	–
Trifolium incarnatum	2	–	–
Trifolium pratense	2	–	–
Trifolium repens	2	–	–
Vallota speciosa	2	–	–
Vicia faba 'Compacta'	2, 3	–	–

[1] 1 = *Nerine bowdenii*, 2 = *Hippeastrum*, 3 = *Nicotiana clevelandii*.
[2] – = no infection, l = latent local infection, L = visible local infection.
[3] s = latent systemic infection, * = infection stated with difficulty.

Tabel 1. Resultaten van waardplantproeven met NeLV.

CuMV and only very weakly reacted with extracts from virus-free plants, if at all.

In one serological experiment, some carlavirus antisera, when tested with purified virus preparations of PVS, PVM, red clover vein-mosaic virus (RCVMV), pea streak virus (PSV), chrysanthemum virus B (CVB), and NeLV that had been stored for several years at −20°C, and contained 50% glycerol, reacted to much higher titers than they originally did. Also when dilutions of both antisera and antigens were made with tris-citric acid buffer, pH 8, some antiserum titers were higher than when made at pH 9, and more non-specific reactions occurred at pH 8. With the exception of the

Table 2. Results of micro-precipitin tests to determine the relationship of NeLV to other carlaviruses.

Antisera[1]	Antigens[2]													
	NeLV Nerine pH 8		Nerine virus-free pH 8		NeLV *Hippeastrum* pH 8	*Hippeastrum* virus-free pH 8	HLV pH 8	NeLV *Hippeastrum* pH 9		*Hippeastrum* virus-free pH 9	CaLV *Dianthus barbatus*	*Dianthus barbatus* virus-free	Homologous titers
NeLV 7556	1024	4096	–	4	4096	16	1024	1024		4			
NeLV 7253	1024		–				256						
NeLV 75516		4096		4	4096	1		1024		–	64	4	
PVS	64	64	4	16	16	1	64	64		4			4096/8
PVM	16	16	1	16	16	4	4	16		4			4096/16
PVS-Germany		64		4	64	1		4		–			
PVM-Germany		16		16	16	1		4		–			4096/4
RCVMV	1	16	4	4	16	4	4	4		4			1024
PSV	16	16	1	–	16	4	16	4		–			4096
CVB	16	64	4	4	64	4	64	16		16			4096/4
SLV	1		4		1	1	1	–		16			1024
LonVS	4	16	–	1	16	1	4	–		–			1024
CaLV-Brunt		1024		16	1024	–		1024	64	–	1024	–	
CaLV-Lisse	+*		–*				+*		64		4096	4	
PoMV	1	4	–	–	1	–	4	4		–			256
Iris Bont V		8		8	–	–							80
LSV	64	64	–	16	16	4	16	16		4			1280
NaLV-Brunt	–		–		–	1	1						4000
NaLV-Lisse	4	–	–	–	–	1	1						640
Nerine-Germany	256		–				256						
PVX		64		4	64	4		16		–			4096
PVY		16		4	4	4		–		–			1024
FrMV		4		4	4	4		4		4			4096
NS 1	–	–	–	–	1	1	1	–	–	–	–	4	
NS 2	–	–	–	–	1	4	4	–		–			
NS 3	–	1	–	–	1	4	1	–		–			

[1] PVS, PVM, PVX, PVY = potato viruses S, M, X, and Y respectively; RCVMV = red clover vein mosaic virus; PSV = pea streak virus; CVB = chrysanthemum virus B; SLV = shallot latent virus; LonVS = a carlavirus from *Lonicera*; CaLV = carnation latent virus; PoMV = poplar mosaic virus; LSV = lily symptomless virus; HLV = *Hippeastrum* latent virus; NaLV = narcissus latent virus; FrMV = freesia mosaic virus; NS = normal serum.
[2] – = no reaction; * = reaction with absorbed, diluted serum.

Tabel 2. Resultaten van micro-precipitatieproeven ter bepaling van de verwantschap van NeLV met andere carlavirussen.

Table 3. Results of serological tests with NeLV-infected plants of *N. bowdenii*.

Cultivar	Grown in glasshouse or outdoors	Number of samples tested	Planting date	Sampling date	Micro-precipitin test Number (%) of samples reacting		ELISA Number (%) of positive reactions
					specifically	non-specifically	
63	glasshouse	50		76–12–24	43 (86%)	0	
G[1]	glasshouse	13		76–12–24	13 (100%)	0	
63	glasshouse	50	Aug. '76	77–02–08	31 (62%)	2	
63	glasshouse	50	March '77	77–07–29	43 (86%)	2	
63	outdoors	50	March '76	77–07–29	24 (48%)	1	
63	glasshouse	50	March '77	77–10–03	37 (74%)	0	50 (100%)
Van Roon	glasshouse	50		76–12–24	49 (98%)	0	
Van Roon	glasshouse	48	Nov. '76	77–02–08	32 (67%)	12	
Van Roon	glasshouse	50	March '77	77–07–29	48 (96%)	0	
Van Roon	glasshouse	50	Juni '77	77–07–29	17 (34%)	30	
Van Roon	outdoors	50	April '76	77–07–29	48 (96%)	2	
Van Roon	outdoors	50	April '77	77–07–29	44 (88%)	4	
Van Roon	glasshouse	50	March '77	77–10–03	50 (100%)	0	49 (98%)

[1] G: unknown cultivar with green leaves, but otherwise deviating from '63'.

Tabel 3. Resultaten van serologische toetsingen uitgevoerd met planten van N. bowdenii, geïnfecteerd met NeLV.

experiment with the old antigens, the results of the tests performed to determine serological relationships of NeLV to other carlaviruses are summarized in Table 2. Two preparations of HLV reacted also with NeLV antiserum, one of them being included in Table 2. The results show that NeLV is rather closely related to HLV, CaLV, and to a lesser extent to PVS, PVM, PSV, CVB, and LSV. Furthermore, the PVX antiserum rather strongly reacted with NeLV. In a separate test, PVX did not react with NeLV antiserum.

In preliminary experiments with the micro-precipitin test, much non-specific precipitation occurred when individual *Nerine* plants were tested, using 0.85% NaCl as a serum diluent. This occurred to a lesser extent, when the sera had been dialysed against 0.1 M tris-citric acid buffer, pH 8, containing 0.05% NaN_3 and diluting the sap samples with the same buffer before centrifuging. Still better results were obtained when 1% Na_2SO_3 was added to the buffer used to dilute the sap and when the preparations were left for 1 h before centrifuging. The results of testing completely infected stocks of *N. bowdenii* (mainly '63' and 'Van Roon') with the micro-precipitin test and/or ELISA are given in Table 3. The samples were taken at different times of the year and from plants of different ages.

With the micro-precipitin test in two lots of 'Van Roon', sampled 2–3 months after planting, much non-specific precipitation occurred. When these are excluded, the average % of samples reacting specifically for the presence of NeLV was 95.6% for 'Van Roon', and 72.6% for '63' (G included). Only 100 samples were tested with ELISA. Extinction values measured at 405 nm for virus-free control plants were from 0.05–0.07. For the 50 samples of *N. bowdenii* '63' the extinction values varied from 0.13–1 and for those of the cv. Van Roon from 0.13–∞, except one that had only 0.07, ELISA thus giving a reliability of 100 and 98% respectively. In general, in both tests reactions with 'Van Roon' were much stronger than with '63'. With ELISA positive reactions were also obtained with extracts from *N. flexuosa* 'Alba' and from naturally infected *Hippeastrum hybridum* plants.

Electron microscopy. The results of particle length measurements are given in Table 4. The average length of 1399 particles measured was 664 nm. In preparations of 'Van Roon', the virus concentration was much higher than in those of '63'. In both cultivars 780 nm particles were also observed (not included in Table 4). Usually the highest concentration of NeLV particles was found in preparations of *Hippeastrum*. In *N. flexuosa* 'Alba', *N.* 'Mansellii', and *N. sarniensis* also particles were found with an average length of about 660 nm.

Sedimentation coefficient and buoyant density. The virus sedimented as a single component. Its sedimentation coefficient at infinite dilution in water at 20°C was 155 S in the two experiments performed.

In CsCl-gradient centrifuging NeLV reached equilibrium in a single band. The buoyant density of NeLV calculated from these experiments was 1.298 g/cm^3.

Discussion

Host plant reactions of NeLV (Table 1) are similar to those of HLV (Brölman-Hupkes, 1975). However, *C. quinoa* did not consistently react with local lesions after

Table 4. Results of particle length measurements of NeLV, using TMV as an external standard (300 nm).

Host	Cultivar	Range measured (nm)	Total number of particles measured	Average particle length (nm)	Top at (nm)	Number of particles at top
Nerine bowdenii	63	626–709	27	662	661	9
		615–699	91	658	664	37
		640–695	28	666	661	13
		604–699	61	652	652	28
		625–713	61	665	661	32
		611–714	23	668	677	7
	G*	635–693	37	663	657	21
	Van Roon	618–700	23	665		
		619–705	114	656	656	45
		619–705	31	671	684	10
		615–712	185	671	677	58
		626–695	49	667	674	18
		615–712	113	664	664	35
		610–705	77	660	658	26
		594–711	147	667	660	56
		618–715	173	657	667	63
Hippeastrum	Scarlet	621–707	90	670	671	44
	Globe**	634–707	64	669	671	30
Nicotiana clevelandii			5	668	668	5
Total and average			1399	664		

* symptomless, but different from 63; ** inoculated seedlings.

Tabel 4. Resultaten van de deeltjeslengtemetingen van NeLV, bij gebruik van TMV als externe standaard (300 nm).

inoculation with NeLV, but this may have been due to growing conditions. NeLV and HLV were also found closely related serologically (Table 2, and Results) and NeLV was easily transmitted to *Hippeastrum* in which it reached a high concentration. Particles of NeLV (664 nm, Table 4) are longer than those of HLV (584–611 nm) as reported by Brölman-Hupkes (1975). Our results strongly indicate that HLV and NeLV are closely related, if not identical. Unfortunately, the description of HLV (Brölman-Hupkes, 1975) was only tentative, no serology had been applied, and no calibration standard had been used in particle-length measurements. This resulted in classing HLV into the potexvirus group. Therefore, we now think that the name *Nerine* latent virus should have priority, even though its early descriptions (Hakkaart, 1972; Hakkaart et al., 1975) were far from complete. We consider HLV as an isolate of NeLV.

Crude extracts from *N. bowdenii* sometimes caused non-viral yellowing of test plants on mechanical inoculation, thus possibly masking virus symptoms (Maat, Hakkaart & Huttinga, unpublished). This, in addition to the inconsistent occurrence of local lesions, makes *C. amaranticolor*, *C. quinoa*, and *G. globosa* unsuitable for testing *Nerine* plants for the presence of NeLV.

Virus particles could only incidentally be observed in seedlings of *N. bowdenii* '63' inoculated with NeLV. They were easily found in *Hippeastrum* seedlings and in *N. clevelandii* (locally) inoculated with the same preparation. This suggests that *N. bowdenii* '63' seedlings resisted infection upon mechanical inoculation, resulting in an extremely low concentration of NeLV in these plants. The virus concentration was also much lower in naturally infected *N. bowdenii* '63' than in 'Van Roon'. This was revealed with the EM as well as serologically. In seedlings of '63', inoculated mechanically or with aphids with *Nerine* virus X, narcissus mosaic virus, or two potyviruses, hardly any or no particles could be found, even in plants that had shown symptoms of the 780 nm virus, or in which virus particles had been found in earlier experiments (Maat, Hakkaart & Huttinga, unpublished). How seedlings of *N. bowdenii* 'Van Roon' react in transmission experiments remains to be investigated. So far the same viruses (CuMV, NeLV, and a 780 nm virus) have been found in both cultivars (Hakkaart et al., 1975), but '63' does not show virus symptoms and its concentration of elongate particles is lower than that of 'Van Roon'. This suggests '63' is more tolerant to the viruses and may be more resistant to infection than 'Van Roon'.

Table 3 shows that NeLV could be demonstrated rather reliably in certain lots of 'Van Roon', and much less so in '63', using the micro-precipitin test. So far only a limited number of samples have been tested with ELISA. The results, especially with '63', were better than those obtained with the micro-precipitin test. The problem of non-specific reactions may also be less with ELISA. Why one of the samples of 'Van Roon', reacting positively in the micro-precipitin test, did not react in ELISA is not clear. In seedlings of '63' inoculated with NeLV, even less NeLV particles were observed than in naturally infected plants in which always more than one virus occurred. Therefore testing of *N. bowdenii* plants carrying only NeLV will require a very sensitive test method.

Using virus preparations that had been stored at $-20°C$ during some years and that contained 50% glycerol, some of the antiserum titers were higher than originally. This may have been due to aggregation in these preparations. In the experiments performed (Table 2), non-specific precipitation occurred frequently. Whether the reaction of the PVX antiserum with NeLV indicates a real relationship or is non-specific is a question that requires further investigation. Anyhow, PVX did not react with a NeLV antiserum tested. However, NeLV is closely related to CaLV. CaLV (Wetter, 1971) and NeLV differ in host plant reactions and therefore can be considered as different viruses.

The results show that NeLV is a separate carlavirus.

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Samenvatting

Het latente Nerine-virus: enige eigenschappen en serologische aantoonbaarheid in Nerine bowdenii

De teelt van *Nerine* als snijbloem maakt in Nederland snel opgang. Dit bolgewas vertoont dikwijls symptomen, die aan virusinfectie doen denken. In alle tot nu toe onderzochte soorten (*Nerine bowdenii, N. sarniensis, N. flexuosa* 'Alba', *N.* 'Manselii') werden met de elektronenmicroscoop (EM) deeltjes gevonden o.a. met een lengte van ongeveer 670 nm, ook in planten die geen virussymptomen vertoonden. Dit virus was daarom al eerder in een voorlopige mededeling latent *Nerine*-virus (NeLV) genoemd.

NeLV kan op *Chenopodium amaranticolor, C. quinoa* en *Gomphrena globosa* lokale lesies doen ontstaan na mechanische inoculatie, doch doet dit niet altijd. Inoculatie van toetsplanten met ruwe extracten van *N. bowdenii* heeft dikwijls ook beschadigingen tot gevolg (sterke vergeling) die eventuele virussymptomen kunnen maskeren. In *Nicotiana clevelandii* kan NeLV latent aanwezig zijn evenals in *Hippeastrum* (Tabel 1). Hierin komt het overeen met het in de literatuur onvolledig beschreven latente *Hippeastrum*-virus (HLV), waaraan het ook serologisch nauw verwant is (Tabel 2).

NeLV blijkt serologisch tevens nauw verwant te zijn aan het latente anjervirus (CaLV), doch verschilt hiervan in zijn reacties op toetsplanten. NeLV vertoont daarnaast een geringe serologische verwantschap met enkele andere carlavirussen (zie Tabel 2). Hoe de reactie met het aardappelvirus-X-antiserum moet worden verklaard is nog niet duidelijk.

Voor NeLV werd een sedimentatiecoëfficient gevonden van 155 S, terwijl de zweefdichtheid in CsCl 1,298 g/cm^3 bedroeg.

Met behulp van de micro-precipitatietoets kon het NeLV tamelijk betrouwbaar worden aangetoond in bladextracten van bepaalde partijen *N. bowdenii* 'Van Roon'. Bij '63', een symptoomloze cultivar, die echter dezelfde virussen lijkt te bevatten, was dat in mindere mate het geval. Betere resultaten, vooral bij '63', werden verkregen met de ELISA-toets, een zeer gevoelige methode waarbij de resultaten worden afgelezen aan de hand van een enzymatische kleurreactie (Tabel 3). Of deze toetsmethoden dezelfde betrouwbaarheid zullen hebben, wanneer het NeLV als enige virus in *N. bowdenii* zou voorkomen, is nog een vraag.

Het bleek moeilijk zaailingen van *N. bowdenii* '63' met NeLV geïnfecteerd te krijgen, dan wel na inoculatie het virus hierin met behulp van de EM te ontdekken. Ook in natuurlijk geïnfecteerde planten van '63', waarin meerdere virussen tegelijk werden aangetroffen, werden steeds minder deeltjes gevonden dan in preparaten van de mozaïekzieke 'Van Roon'. Dit kan er op duiden, dat '63' een grotere resistentie bezit dan 'Van Roon'. Tot nu toe werden geen proeven gedaan met zaailingen van laatstgenoemde cultivar.

De gemiddelde deeltjeslengte van NeLV werd berekend op 664 nm (Tabel 4).

NeLV kan op grond van de thans beschikbare gegevens worden beschouwd als een lid van de carlavirusgroep. HLV is waarschijnlijk identiek aan NeLV, doch werd evenals NeLV in vorige publikaties, onvolledig beschreven. Op grond van prioriteit beschouwen de auteurs HLV als een isolaat van NeLV.

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