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Further characterization of celery latent virus

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Abstract

Celery latent virus has been isolated from plants of celeriac (*Apium graveolens* var. *rapaceum*). *Chenopodium amaranticolor* and *C. quinoa* are good assay plants. Celery (*A. graveolens* var. *dulce*) and celeriac (13 cultivars tested) did not react with visible symptoms.

Fourteen new artificial hosts were found. New systemic symptomless hosts are *Anthriscus cerefolium*, *Nicotiana megalosiphon*, *Pisum sativum*, *Spinacia oleracea* and *Trifolium incarnatum*. Systemic symptoms were caused in the pea cultivars Cicero and Dark Skin Perfection.

Five aphid species, including *Cavariella aegopodii*, four of which not tested with the virus before, were unable to transmit the virus.

Seed transmission was confirmed in celeriac (up to 34%) and in *C. quinoa* (up to 67%) and detected for the first time in *Amaranthus caudatus*. Detection is easier in seedlings and germinated seeds than in dry seeds.

Results of purification were erratic but best at high pH (8 or 9). Sedimentation coefficient was 161 S.

An antiserum reacted with purified virus in micro-precipitin tests (titer 256) but, especially at high salt concentrations also in agar gel (titer 64), presumably because of easy degradation of virus protein. Reactions in agar gel also occurred with crude extracts from virus-infected *C. quinoa* and celeriac.

With the electron microscope flexuous virus particles were found in low concentration in crude sap and in high concentration in purified preparations. Particle measurements revealed an average length of 885 nm (in one preparation 940 nm).

Light microscopy did not show inclusion bodies in epidermal strips nor did electron microscopy of ultrathin sections reveal pinwheels and other structures typical of the potyvirus group. The virus evidently belongs to a new morphological group, possibly together with some other viruses hitherto insufficiently studied.

The virus seems of potential economic importance only, but it is advised to use virus-free mother plants of celery for seed production.

Introduction

In the Netherlands celeriac (*Apium graveolens* var. *rapaceum*) is a vegetable usually grown as an arable crop especially in the South West. There, celery mosaic virus (CeMV) sometimes causes total crop loss on certain fields and cucumber mosaic and tobacco rattle viruses may also occur.

A fourth sap-transmissible virus was detected rather often (Bos, 1973). It resembled the 860 nm long celery latent virus (CeLV), first but incompletely described by

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Brandes and Luisoni (1966) and Luisoni (1966) from *A. graveolens* var. *dulce* (celery) from a vegetable garden near Torino, Italy. This virus was soon found to be seed-borne in *Amaranthus hybridus* var. *hypochondriacus* (1 out of 300) and *Trigonella foenum-graecum* (1 out of 51), in *Chenopodium quinoa* (up to 89%) (Luisoni and Lisa, 1969) and in its natural host celeriac (Bos, 1973).

The virus was therefore considered of sufficient potential importance to be studied in some detail. However, purification and serology were difficult. Verhoyen et al. (1976) have recently published some additional data on host range, electron microscopy and seed transmission in celeriac, and found the virus able to cause up to 38% yield reduction in celery. We now present information to further characterize the virus.

Materials and general methods

Virus isolates. The virus was isolated several times by sap inoculation onto *Chenopodium amaranticolor* and *C. quinoa* from field-collected leaf samples of celeriac as well as from plants grown in the greenhouse from seeds and once from tuber tissue. It was also isolated together with CeMV from (leaf) celery imported as a vegetable from Italy (Orta Nova). Since no appreciable differences were detected between isolates, nearly all work was with an isolate collected from a celeriac plant in the province of Zeeland, October 1971 (code Se3). For serological comparison an Italian isolate was provided in seed of *C. quinoa* by Dr E. Luisoni, Laboratorio di Fitovirologia Applicata del CNR, Torino, Italy. An isolate of CeMV also from celeriac (Se16) was used for a cross-protection and for a double-infection experiment.

Maintenance and propagation usually was in *C. quinoa*, showing systemic symptoms in 10 to 13 days. Long-term storage in small amounts was in leaf material of *C. quinoa*, dried and stored over $CaCl_2$ at 4°C and in seed of this host.

Virus transmission in sap, host range tests, and determination of persistence of infectivity in expressed sap were performed in the conventional ways, often using 0.01 M phosphate buffer, pH 7, as a diluent and always carborundum as an abrasive. Plants were grown and kept in an insect-proof glasshouse at 18–22°C. Assay and indicator hosts were *C. quinoa*, for its sensitive systemic reaction, and *C. amaranticolor*, for its typical local reaction. Insect-transmission tests were with virus-free aphid cultures provided by the IPO Department of Entomology.

Virus purification was from inoculated and systemically infected leaves of *C. quinoa* 15–20 days after inoculation. Leaf material, chilled at 3°C, was homogenized in a Waring blendor, using buffers and organic solvents also chilled.

The buffer used was either 0.18 M phosphate citric acid, pH 7, 0.1 M tris (= tris-(hydroxymethyl)aminomethane) citric acid, pH 8, or 0.1 M tris thioglycolic (or hydrochloric) acid, pH 9. The first two buffers contained 0.1% sodium thioglycolate and 0.02 M sodium diethyldithiocarbamate when used for homogenizing and sometimes also for resuspending the first pellet. The third buffer was adjusted to pH 9 using thioglycolic acid when used for homogenizing and HCl when used for other purposes.

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, rotor 30, 1 h at 30000 rev/min or, in later experiments, 1 h at 20000 rev/min, or for 3 h at 25000 rev/min when the virus was in sucrose (see below).

For sucrose-gradient centrifuging 10 ml of partially purified virus, obtained from 500 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). The 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 LBK-Uvicord absorption meter, were diluted 1:1 with buffer or distilled water before centrifuging to concentrate the virus.

Centrifuging of virus through a layer of sucrose was for 4 h at 25000 rev/min in a SW27 rotor. Ten ml of virus was layered on 25 ml of a solution containing 25% (weight/volume) sucrose in tris buffer, pH 8.

The purification procedure was as follows. One hundred grams of leaf material were homogenized with 200 ml of buffer, 25 ml of diethyl ether and 25 ml of carbon tetrachloride. The homogenate was centrifuged at low speed and 4% (weight/volume) of polyethylene glycol 6000 (PEG) was added to the supernatant. The solution was stirred for 0.5–1 h and centrifuged at 10000 rev/min for 15 min. The sediment was resuspended in 30–35 ml of buffer (with reducing agents), stirred for 1 h and centrifuged at low speed. The supernatant thus obtained was centrifuged at high speed and the sedimented material then resuspended in 2 ml of buffer and stirred overnight. After centrifuging at low speed the supernatants from 500 g of leaf material were combined and subjected to sucrose-gradient centrifuging. From the sucrose the virus was concentrated to 2 ml, 2 ml of glycerol was added, and the material stored at −20°C until use.

Sedimentation coefficient. The sedimentation coefficient was determined by the graphical method of Markham (1960) and using a Spinco Model E ultracentrifuge with schlieren optics. In the purification procedure tris buffer, pH 8, was used. The virus, after clarification, was precipitated with 5% PEG, and after resuspending and centrifuging at low speed it was centrifuged through a layer of sucrose. The sediment thus obtained was resuspended in 0.002 M tris buffer, pH 8, and dialysed against this buffer.

Antiserum preparation was bij injecting one rabbit with virus purified at pH 8 and sedimented at 30000 rev/min and another with virus purified at pH 9 and sedimented at 20000 rev/min. Two intravenous injections, given with a three-day interval, were followed by an intramuscular injection, two weeks later. For the latter, virus was emulsified with an equal volume of Freund's incomplete adjuvant. After another fortnight the antiserum titer was determined and because it was only 64 in the micro-precipitin test, an additional intravenous and intramuscular injection were given simultaneously.

Light microscopy was applied on epidermal leaf or petiole strips from upper leaves after staining with 1% phloxine and 1% methylene blue in Christie's solution and viewing in water.

Electron microscopy was carried out in crude sap and in purified preparations after negative staining with 2% PTA, pH 6.5. For length measurements tobacco mosaic virus (TMV) in 'White Burley' tobacco was added as an internal standard (Bos, 1975). For in situ studies, small pieces of systemically infected leaves of *C. quinoa* with clear symptoms 20 days after inoculation and of celery 'Golden Selfblanching' without symptoms, 15 and 30 days after inoculation, were fixed for 2 h at 4°C with 6% glutaraldehyde in 0.1 M phosphate buffer pH 6.9, rinsed six times with buffer and post-fixed for 2 h at 4°C in 1% osmium tetroxide in the same buffer. The tissue pieces were then rinsed with distilled water, dehydrated in a graded series of ethanol solutions containing 2% uranyl acetate, and embedded in a mixture of Epon-Araldite. Sections were made with an LKB ultrotome, using a glass knife, stained for 1 h in a 2% aqueous solution of uranyl acetate followed by lead citrate for 10 min. Final preparations were studied with a Philips EM 300 electron microscope.

Further details on special techniques will be given under Results.

Results

Host range and symptoms. Host range tests were with two to eight plants or more per species or cultivar depending on plant size. Back inoculations were made to at least one plant of *C. quinoa* and one of *C. amaranticolor* from inoculated and non-inoculated leaves, at least 14 and 21 days after inoculation, respectively. Species that seemed important as possible natural hosts or indicator plants were tested twice or more often.

Table 1 shows that only 2 out of 7 umbelliferous species tested were systemically susceptible. Out of 29 non-umbelliferous species tested only 8 or 9 were immune, 10 showed a latent local infection only, 5 or 6 other species became systemically infected without producing symptoms, 1 became systemically infected with systemic symptoms (*Pisum sativum*) and 3 produced both local and systemic symptoms (*Chenopodium* spp.).

In *Apium graveolens* var. *rapaceum*, either naturally infected or after inoculation, symptoms were never observed. Most observations were on 'Roem van Zwijndrecht', which was at the start of our investigations almost the only cultivar grown in this country. In a test with 13 cultivars 3 out of 4 plants were inoculated and after one month tests were made for local infection and after 1½ month for systemic infection. No symptoms were observed but virus could be recovered from non-inoculated leaves of all but five cultivars. From three of these a second back inoculation three weeks later revealed the presence of virus. The two cultivars resisting infection (viz. 'José' and 'Neckarland') were again inoculated (15 plants each) and this time became systemically infected. In 'José', four plants showed growth reduction but individual back inoculation did not show a correlation between this deviation and infection. Thus, latent systemic infection occurred in all cultivars tested. From inoculated leaves virus could only be recovered in three cultivars viz. Marmerkogel, Pomona and Zwindra. The other cultivars tested were Albaster, Bronskogel, Ceva, Iram, José, Limburgse, Lustra, Neckarland, Prager Reuzen II, and Roem van Zwijndrecht.

Apium graveolens var. *dulce* 'Golden Selfblanching' easily became infected without showing symptoms. The virus was readily recovered from each of 10 inoculated plants tested individually. In this cultivar there was neither a growth depressing effect nor did the virus aggravate the effect of SeMV (Se16) (Fig. 1).

All three *Chenopodium* species tested readily reacted to infection. Local symptoms were distinct in *C. amaranticolor*. Local lesions were chlorotic, sometimes ringlike and might appear as early as 5 to 6 days after inoculation (Fig. 2, left). If occurring, systemic symptoms started some 5 to 6 days later and consisted of a few or several irregularly shaped chlorotic rings (Fig. 2, right). In *C. quinoa* vague

Table 1. Results of host range tests as compared with data from the literature.

Plant species	Present observations	Brandes and Luisoni (1966)[2]	Verhoyen et al. (1976)[2]
Umbelliferae			
Anethum graveolens	– –[1]		
Anthriscus cerefolium	– s	–	– –
Apium graveolens var. *dulce*	–* s	s (S)	s (S)
Apium graveolens var. *rapaceum*[3]	– s	s (S)	s (S)
Coriandrum sativum	– –	s	s
Daucus carota	– –	–	–
Pastinaca sativa	– –		
Petroselinum crispum	– –		–
Other species			
Amaranthus caudatus	l s	s (S)	
Amaranthus hybridus hypochondriacus		L s (S)	L s
Amaranthus retroflexus		L s	
Atriplex hortense		L S	
Beta vulgaris	l –		
Capsicum annuum	l –	–	–
Celosia plumosa red	l –		
Celosia plumosa yellow	l –		
Chenopodium album	L S	(L)l –	
Chenopodium amaranticolor	L S	L S (s)	S
Chenopodium foetidum		s	
Chenopodium quinoa	L S	L S	S
Cucumis sativus	–* –*	–	–
Gomphrena globosa	l s	L s	
Hyoscyamus niger	l –		
Lactuca sativa	– –		
Nicotiana clevelandii	l s	s	
Nicotiana debneyi	l	–	
Nicotiana glutinosa	l –	–	–
Nicotiana hybrida	– –		
Nicotiana megalosiphon	l s		
Nicotiana rustica	– –	–	
Nicotiana tabacum 'White Burley'	l –		
Nicotiana tabacum 'Xanthi'	l ?		–
Petunia hybrida	– –	(L)l –	–
Phaseolus vulgaris	(l) –	–	–
Pisum sativum[3]	l S	–	
Spinacia oleracea 'Noorman'	l s		
Tetragonia expansa	l –	L –	
Trifolium incarnatum	l s		
Trifolium pratense	– –		
Trifolium repens	– –		
Trigonella foenum graecum		(L)l(S)s	
Vicia faba	l –	–	–
Vigna sinensis	– –	–	–
Zinnia elegans	l –		

[1] L = local symptoms; l = latent local infection as tested by back inoculation onto *C. quinoa*. S = systemic symptoms; s = latent systemic infection as detected with *C. quinoa*.
–* = no symptoms, but not tested by back inoculation; – = no infection; ( ) = sometimes reacting as indicated in parentheses.
[2] These authors do not specify whether plants were back tested separately for local and systemic infection.
[3] A series of cultivars tested; see text.

Tabel 1. Resultaten van het waardplantonderzoek in vergelijking met gegevens uit de literatuur.

Fig. 1. Celery 'Golden Selfblanching' ca. two months after inoculation with CeLV (B), CeMV (C) and CeLV + CeMV (D); (A) non-inoculated control.

Fig. 1. Bleekselderij 'Golden Selfblanching' ongeveer twee maanden na inoculatie met CeLV (B), CeMV (C) en CeLV + CeMV (D); (A) niet-geïnoculeerde controle.

chlorotic, sometimes ringlike local lesions appeared in 6 to 7 days, later developing into diffuse chlorotic flecks. Systemic symptoms were characteristic. They appeared in 10 to 13 days and consisted of irregular chlorotic flecks and rings and slight leaf deformation (Fig. 3). *C. album* reacted with distinct small chlorotic local lesions in some 6 days and with small chlorotic systemic rings in one more week.

Of the two cultivars of pea (*Pisum sativum*) often used in our host-range tests 'Rondo' was immune, but 'Koroza' attained a symptoml :ss systemic infection. Since pea crops are often grown near cele-

Fig. 2. Leaves of *Chenopodium amaranticolor* with local (left) and systemic symptoms (right) three weeks after inoculation with CeLV.

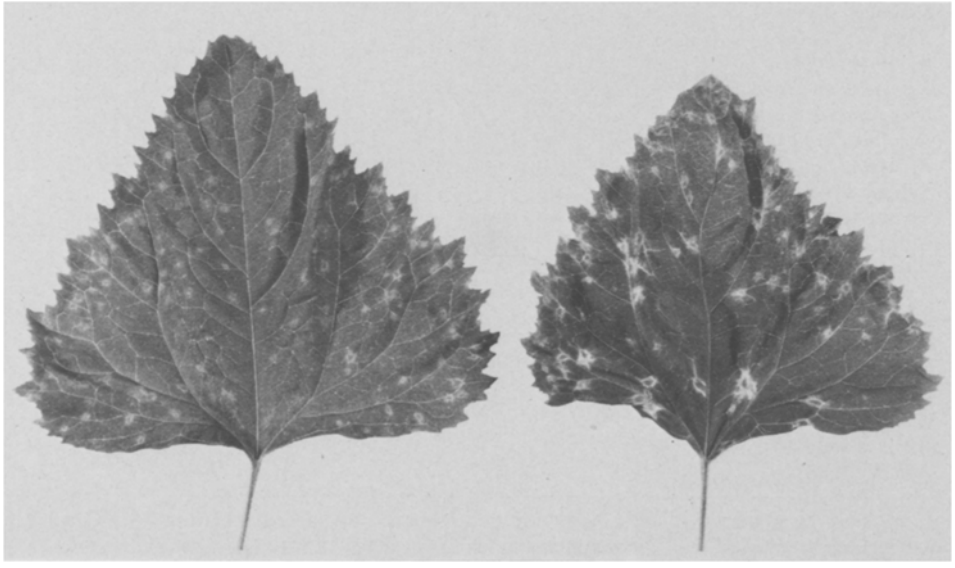

Fig. 2. Bladeren van Chenopodium amaranticolor met lokale (links) en systemische symptomen (rechts) drie weken na inoculatie met CeLV.

Fig. 3. Leaves of *Chenopodium quinoa* with characteristic systemic symptoms of CeLV three weeks after inoculation.

Fig. 3. Bladeren van Chenopodium quinoa met karakteristieke systemische symptomen van CeLV drie weken na inoculatie.

riac and the virus had been reported to be seed transmitted in fenugreek (Luisoni and Lisa, 1969), 22 more pea cultivars were tested. Six of these became systemically infected, viz. Castro, Cicero, Cobri, Dark Skin Perfection, Gloire de Quimper and Perfected Freezer. Cicero and Dark Skin Perfection were the only varieties reacting visibly, the former with unmistakable dwarfing, the latter with a striking chlorotic vein banding and sometimes ringspotting (Fig. 4).

The immune cultivars were Allround, Aurora, Colmo, Dik Trom, Double One, Esmeralda, Green Arrow, Kebby, Kelvedon Wonder, Legio, Onyx, Pauli, Ramto, Recette, Roi des fins verts, and Starolvert.

The six susceptible cultivars were also tested at different temperatures in groups of 12 plants each: (1) in the glasshouse (18 to 22°C), (2) in a climate room at 16 and 12°C (day and night, respectively), and (3) similarly at 24 and 20°C. Symptoms were as described, but absent at the highest temperature. In 'Dark Skin Perfection' vein banding persisted for the entire two months of observation. This time some plants of 'Castro' and 'Perfected Freezer' also showed vein chlorosis in the glasshouse.

Fig. 4. *Pisum sativum* 'Dark Skin Perfection' one month after inoculation with CeLV; left, healthy leaf.

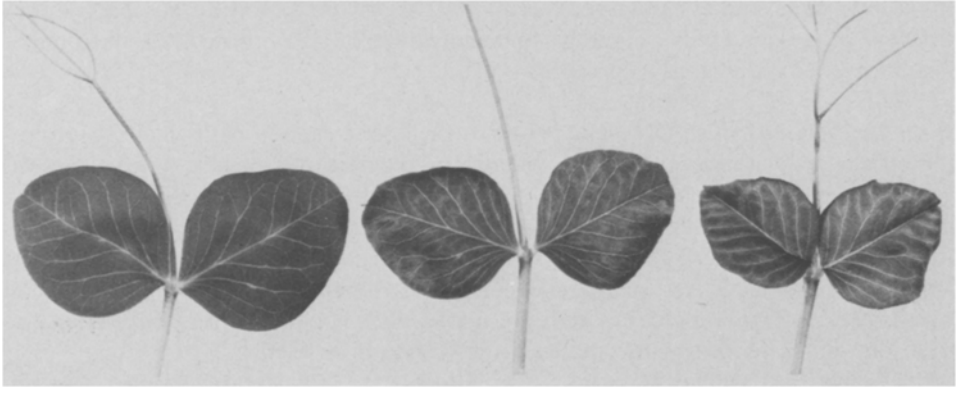

Fig. 4. Pisum sativum 'Dark Skin Perfection' één maand na inoculatie met CeLV; links, gezond blad.

Persistence of infectivity. Determinations were made twice in sap from systemically infected plants of *C. quinoa*, and treated fractions were tested on *C. amaranticolor* and *C. quinoa*. Dilution end-point was between 100 and 1000, and 1000 and 10000, respectively; thermal inactivation was around 50°C (in one experiment between 45 and 50; in the second test at 50°C a late reaction was obtained); ageing in vitro was between 3 and 4 days and 4 and 8 days. Apparently, in the second test the original virus concentration was highest.

Finely cut leaf material of *C. quinoa* dried over $CaCl_2$ at 4°C in 1971, still contained infective virus when tested in 1977.

Insect transmission tests. The virus-free aphids were first starved for 2 h and then allowed to feed for 5 to 10 min on infected plants. Immediately thereafter they were transferred to assay plants (5–10 aphids per plant) where they were left for $^1/_2$ h and then transferred to another series of plants. There they were killed with an aphicide after 24 h. Per test 80 aphids were used. No transmission was obtained with *Acyrthosiphon pisum* (from pea to pea; pea plants were later on assayed by back inoculation onto *C. quinoa*), *Aphis fabae* (from *C. quinoa* to the same), *Cavariella aegopodii* (from *C. quinoa* to the same), *Macrosiphum euphorbiae* (from *C. quinoa* to the same), *Myzus persicae* (from *C. quinoa* to the same and from celery to *C. quinoa*).

Seed-transmission test. Seed transmission of the virus in celeriac was already suggested by observations made while studying celery mosaic virus in 'Roem van Zwijndrecht'. The latent virus was soon detected in plants on isolated trial plots, in seedlings on hotbeds (in frames) and in glasshouse-grown test plants inoculated with celery mosaic virus. In 1972 it was first proved by back inoculation from seedlings sown for this purpose, and found to occur in 32% of the seeds. Because of its potential importance seed transmission was then further tested in celeriac and some other hosts. Results are summarized in Table 2. Seed transmission was also observed in *Amaranthus caudatus* and *C. quinoa*.

Cross protection. 'Roem van Zwijndrecht' and 'Golden Selfblanching' were used as systemic hosts for both CeLV and CeMV (Se16). Plants were inoculated first with either one of the viruses and 18 days later with the other as a challenge. Presence of the second virus could be observed directly with CeMV and by back inoculation onto *Chenopodium* with CeLV. There was no indication of cross protection when either CeLV or CeMV was used as protector virus.

Virus purification. Since *C. quinoa* is one of the hosts reacting with clear symptoms, this species was chosen for virus propagation. To determine the best time of harvest, virus concentration was determined at a series of times after inoculation by back inoculation onto *C. amaranticolor*. The highest concentration was found ca. 15 days after inoculation (Fig. 5) and maximum concentration was maintained for at least 15 days. The older leaves yielded cleaner preparations. Consequently, purification was preferably from inoculated leaves of *C. quinoa* harvested 15 to 20 days after inoculation, but systemically infected leaves have also been used.

Several buffers with pH's from 4.5 to 9 were tried. Reasonable results were obtained with those at pH 7, 8 and 9. At pH 7 more aggregation of virus particles oc-

Table 2. Survey of seed-transmission tests.

Host species and cultivar	Pretreatment	Way of testing	Results
Apium graveolens rapaceum			
'Roem van Zwijndrecht' commercial seed 1971	sowing[1]	leaf samples from individual plants	16/50[2] (32%)
		do	17/50 (34%)
	dry seed	one seed	0/1
		one group of 10	0/1
		one group of 100	0/1
	soaking for 1–2 days	in groups of 10	4/10
		in groups of 100	1/2
	germination on filter paper for 1 week	germs in groups of 10	4/9[3]
		in groups of 100	5/5
	do	do individually	8/25 (32%)
		in groups of 10	7/10
		in groups of 50	2/2
		in groups of 100	2/2
commercial seed 1972, Nr. 6	dry seed	in groups of 10	5/10[4]
	germination on filter paper for 10–11 days	germs in groups of 10	5/5
		in groups of 50	1/1
Nr. 8	do	do in groups of 10	0/5
		in groups of 50	0/1
'Pomona'[5]	do	do in groups of 10	1/5
		in groups of 50	0/1
Amaranthus caudatus (artificial infection)	sowing	leaf samples from individual plants	0/5
	germination on filter paper	whole seeds in groups of 10	3/5
Chenopodium q uinoa (artificial infection)	sowing	visual observation and back inoculation of abnormal seedlings	2/50 (4%)
other origin	dry seed	in groups of 10	4/10
	germination for 3 days	whole seeds in groups of 10	6/10
other origin and other virus isolate	sowing	visual observation and back inoculation from individual seedlings	27/40 (67%)
Nicotiana clevelandii (artificial infection)	sowing	leaf samples from groups of 10	0/7
Pisum sativum (artificial infection)			
'Castro', 'Cicero', 'Cobri', 'Dark Skin Perfection', 'Gloire de Quimper', 'Koroza', 'Perfected Freezer'	soaking for 1–4 days	whole seeds in 5 groups of 5	0/5[6]
'Cicero'	sowing	leaf samples from individual plants	0/6
'Dark Skin Perfection'	do	do	0/4
'Koroza'	do	do	0/12

[1] Sowing always was in compost soil in the glasshouse.
[2] Number of plants or groups reacting over number of plants or groups tested on *C. amaranticolor* and *C. quinoa*.
[3] Strawberry latent ringspot virus was isolated from one group of 10 germinated seeds not containing CeLV.
[4] In case of positive reaction symptoms weak and occurring late.
[5] Seed from the cultivars Albaster, Bronskogel, Ceva, Iram, José, Limburgse, Lustra, Marmerkogel, Neckarland, Prager Reuzen II, and Zwindra all tested in the same way proved free of virus.
[6] For each cultivar 5 groups of 5 seeds were tested with identical results.

Tabel 2. Overzicht van de zaadoverdrachtsproeven.

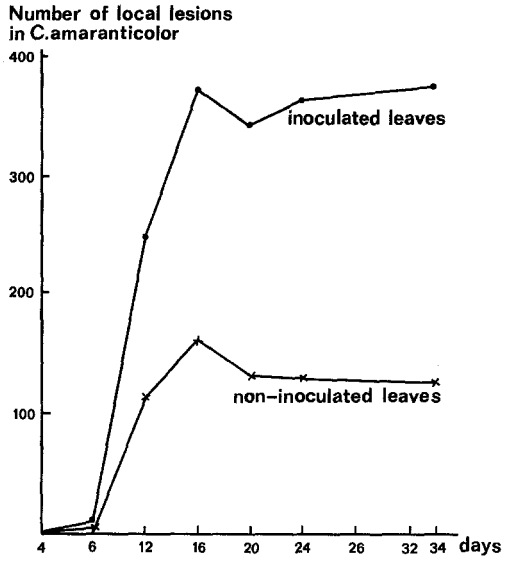

Fig. 5. Course of co ıcentration of CeLV in inoculated (upper line) and non-inoculated leaves (lower line) of *C. quinoa* as determined by back inoculation onto *C. amaranticolor*.

Fig. 5. Concentratieverloop van het CeLV in geïnoculeerde (bovenste lijn) en niet-geïnoculeerde bladeren (onderste lijn) van C. quinoa, bepaald door teruginoculatie op C. amaranticolor.

curred than at pH 8 or 9, whereas at pH 9 there seemed to be more broken particles than at pH 7 or 8.

Fig. 7 shows the results of a purification at pH 8, with some end-to-end particle aggregation. After inoculation of such preparations onto *C. amaraticolor* and *C. quinoa* and sometimes other test species, only the two *Chenopodium* spp. reacted and did so in the characteristic way. These results indicate that the particles seen are indeed the celery latent virus.

Sedimentation coefficient. The virus sedimented as a single component. Its sedimentation coefficient at infinite dilution in 0.002 M tris buffer, pH 8, at 20°C was 161 S.

Serology. The antiserum prepared with the virus purified at pH 8 reacted strongly with normal plant material. The one prepared with the virus purified at pH 9 had a titer of 256 against purified virus in the micro-precipitin test and 64 in the Ouchterlony double-diffusion test. This antiserum reacted up to an antiserum dilution of 1/16 with crude extracts of infected *C. quinoa* and celeriac but not with sap from non-infected plants, using the Ouchterlony test.

Using the same test, positive reactions were also obtained (antiserum titer 4) with crude extracts from *C. quinoa*, inoculated with an Italian isolate of the virus. Absorption of the antiserum with concentrated extracts of virus-free plants did not influence the reaction with infected material. With the electron microscope, in purified preparations only typical elongate virus particles were observed. The reactions in agar gel were strongly influenced by the salt concentration of the agar, especially when the antigen was purified virus. Reactions with purified virus hardly occurred when the agar medium had been prepared in 0.01 M buffer or in 0.085% NaCl, whereas they were very strong when the agar was in 8.5% NaCl.

Light microscopy. No inclusion bodies were found in epidermal strips of *C. quinoa*,

celeriac 'Roem van Zwijndrecht', celery 'Golden Selfblanching' and pea 'Dark Skin Perfection' tested at different times (from 10 to 30 days) after inoculation.

Electron microscopy. Flexuous long particles (Fig. 6, left) could be detected in crude sap from infected leaves of *C. album*, *C. amaranticolor*, *C. quinoa*, celeriac, and 'Dark Skin Perfection' pea. They were usually hard to find and sometimes occurred in groups in vesicles (Fig. 6, right). The average length of nine characteristic particles as compared with TMV was 885 nm.

Many such particles occurred in purified preparations (Fig. 7), but there was much end-to-end aggregation. At high magnification they showed a central hole. Measurements of individual particles of one preparation with TMV as a standard yielded a rather narrow peak with a calculated average length of 940 nm for 117 particles in the three peak classes of ca. 10 nm wide. Calculation of this length for 147 particles in another preparation led to 886 nm.

In sections of diseased *C. quinoa* leaves small aggregates of elongate particles in a more or less parallel array were found in low incidence in the cytoplasm (Fig. 8).

Fig. 6. Particles of CeLV in crude sap of celery 18 days after inoculation, stained with PTA. Bar represents 1000 nm.

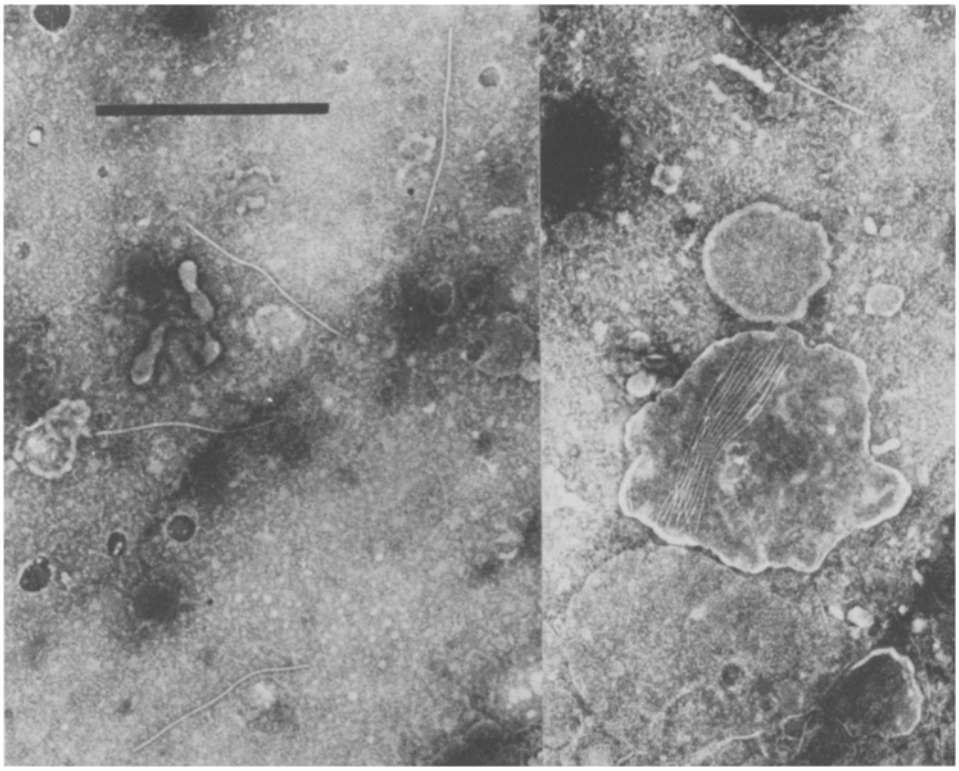

Fig. 6. Deeltjes van het CeLV in ruw sap van selderij 18 dagen na inoculatie, gekleurd met PTA. St aaf geeft 1000 nm weer.

Fig. 7. Electron micrograph of purified preparation of CeLV with particles of tobacco mosaic virus added as an internal magnification standard. Bar represents 1000 nm.

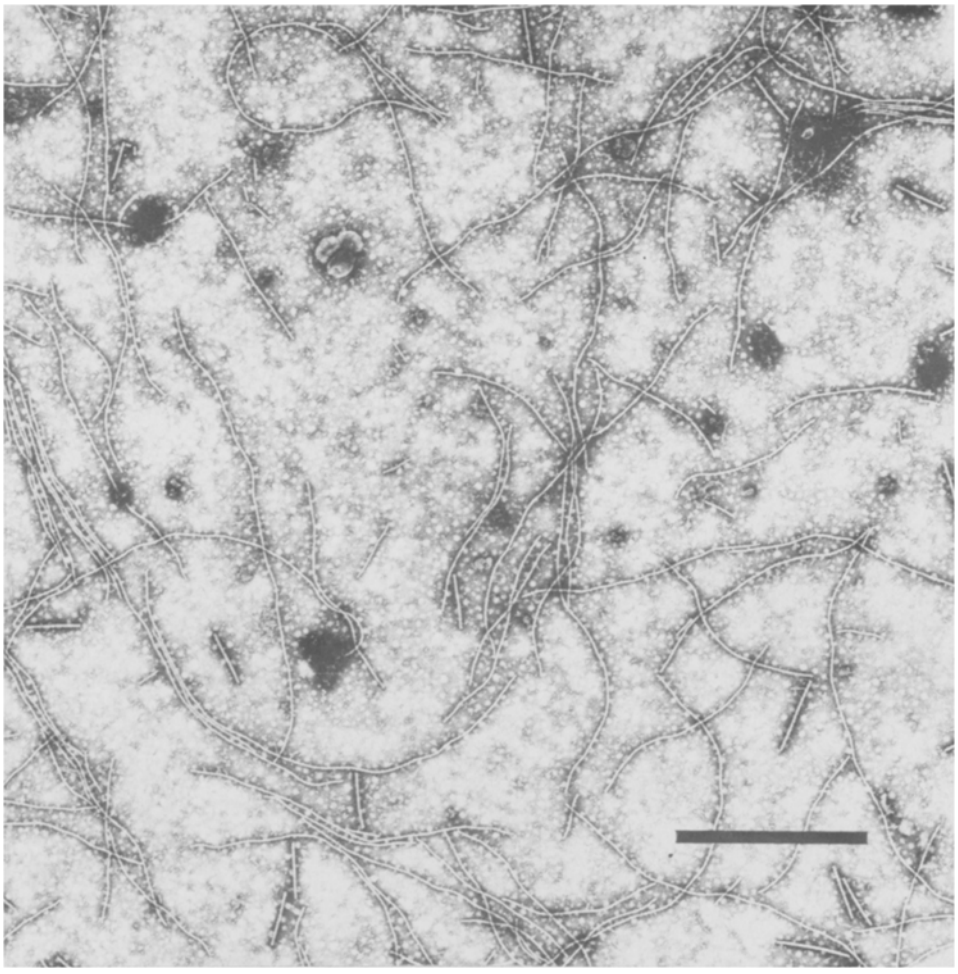

Fig. 7. Elektronenfoto van gezuiverd preparaat van CeLV met deeltjes van tabaksmozaïekvirus als interne vergrotingsstandaard. Staaf geeft 1000 nm weer.

They were sometimes found near the tonoplast parallel or with their ends perpendicular to it. In transverse section the bundles showed slightly irregular array (Fig. 9).

In celery such virus accumulations were also found, although much more rarely. This may be due to absence of symptoms and the ensuing impossibility to locate infected tissues.

In both hosts no pinwheels, dense bands or other structures characteristic of potyviruses were found.

Fig. 8. Ultrathin section of *C. quinoa*, 20 days after inoculation with CeLV with two rather rare bundles of virus particles (v) in oblique section. *W* cell wall. Bar represents 500 nm.

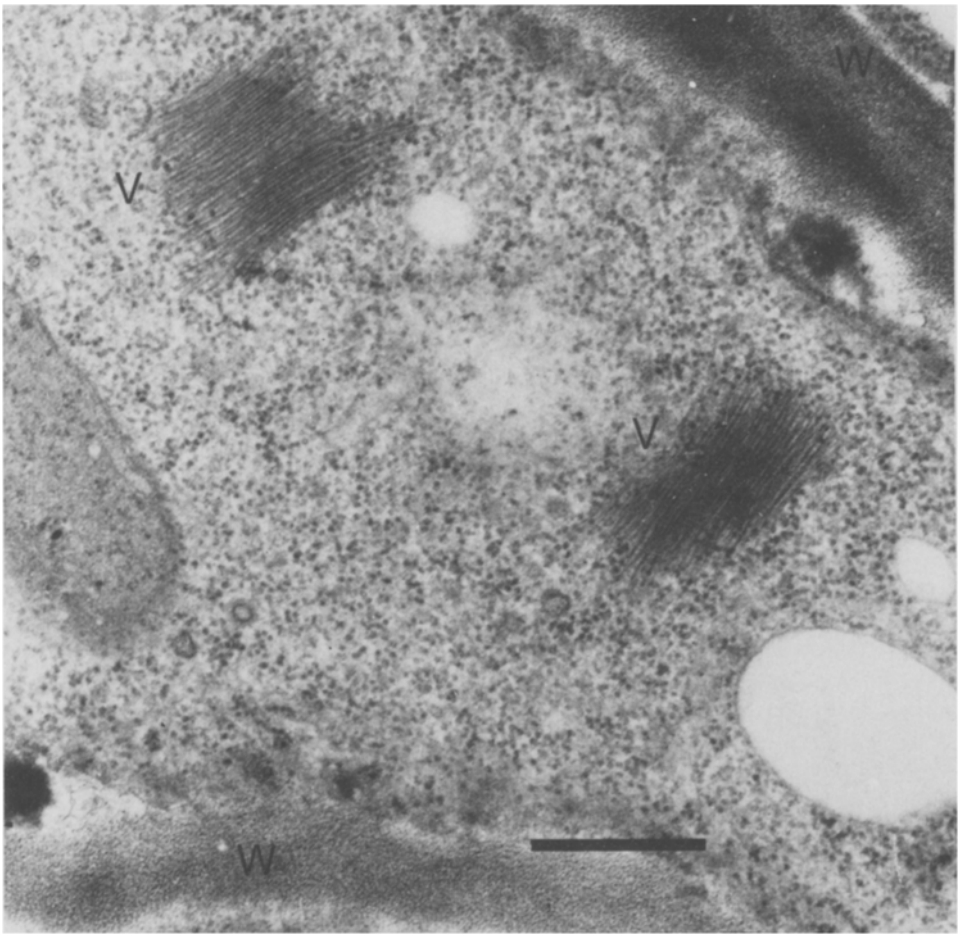

Fig. 8. Ultradunne coupe van C. quinoa, 20 dagen na inoculatie met CeLV met twee vrij zeldzame bundels virusdeeltjes (v) in schuine doorsnede, W celwand. Staaf geeft 500 nm weer.

Discussion

The results obtained here corroborate and further complete those obtained by Brandes and Luisoni (1966) and by Verhoyen et al. (1976).

Table 1 lists all species tested here and some others found to be hosts by the above authors. It also compares our results of host range tests with theirs. We have tested these species for infection, including 14 not used before, by careful back inoculation from inoculated and non-inoculated leaves. Fourteen new suscepts were found, eight of these only contracting local infection, five also becoming systemically infected. Seven species previously reported non-hosts were now also found to become locally

Fig. 9. Text as of Fig. 8, but with one bundle of virus particles (v) nearly in transverse section. Bar represents 500 nm.

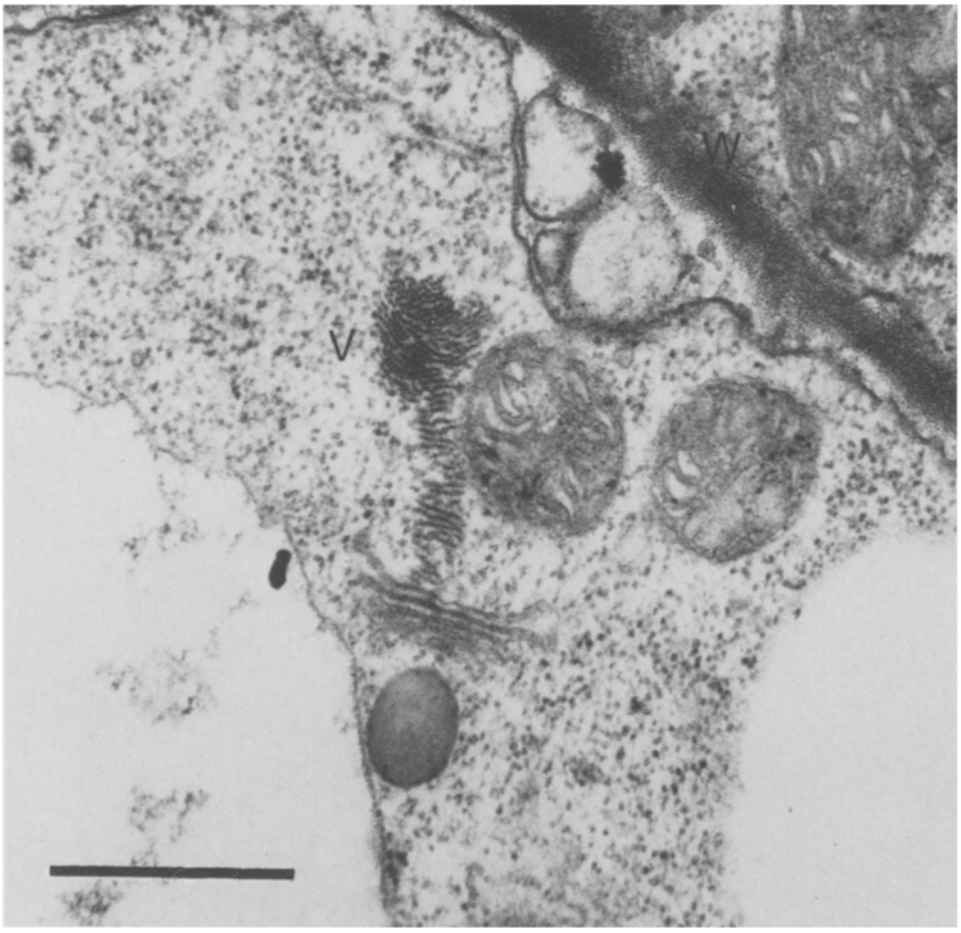

Fig. 9. Tekst als van Fig. 8, maar met één bundel virusdeeltjes (v) nagenoeg in dwarsdoorsnede. Staaf geeft 500 nm weer.

infected and two of these even showed systemic infection. These discrepancies may partly be due to usage of other cultivars or selections, as with *Pisum sativum*. For the sake of completeness species found to be non-hosts by the above authors and not tested by us are listed in Table 3. New latent systemic hosts are *Anthriscus cerefolium*, *Nicotiana megalosiphon*, *Spinacia oleracea* and the legumes *Pisum sativum* and *Trifolium incarnatum*. Two pea cultivars, Cicero and Dark Skin Perfection, even produced symptoms. All 13 celeriac cultivars tested were found susceptible although it was difficult to get 'José' infected and the virus could be recovered from inoculated leaves of three cultivars only. This suggests that susceptibility of celeriac and especially of 'José' is low. Apparent symptoms were not observed in any celeriac cultivar.

Table 3. Plant species not included in Table 1 and not found susceptible when tested with celery latent virus by Brandes and Luisoni (1966: B & L) and by Verhoyen et al. (1976: V).

Umbelliferae	Non-Umbelliferae (continued)
Ammi majus (V)	*Calendula officinalis* (B & L)
A. visnaga (V)	*Cheiranthus allionii* (B & L)
Angelica arcangelica (V)	*Cleome spinosa* (B & L)
A. sylvestris (V)	*Crotalaria juncea* (B & L)
Apium nodiflorum (V)	*Cucurbita pepo* (V)
Carum carvi (V)	*Cucurbita pepo* var. *cucurbitella* (B & L)
Conium maculatum (V)	*Datura metel* (B & L)
Foeniculum vulgare var. *azoricum* (B & L)	*D. stramonium* (B & L; V)
Heracleum sphondylium (V)	*Glycine max* (B & L)
Pimpinella anisum (B & L)	*Lycopersicon esculentum* (B & L)
P. major (V)	*Ocimum basilicum* (B & L)
	Physalis floridana (B & L)
Non-Umbelliferae	*Plantago coronopus* (V)
Amaranthus ascendens (B & L)	*Solanum miniatum* (B & L)
A. deflexus (B & L)	*S. nigrum* (B & L)
Brassica chinensis (B & L)	*Tagetes* sp. (B & L)

Tabel 3. Plantesoorten die niet zijn opgenomen in Tabel 1 en niet vatbaar bleken bij toetsing met latent selderijvirus door Brandes and Luisoni (1966: B & L) en door Verhoyen et al. (1976: V).

In our tests, persistence of infectivity in expressed sap after dilution, heating and ageing was slightly lower than reported by Brandes and Luisoni (1966) and Verhoyen et al. (1976), but this may be due to a difference in original virus concentration.

Brandes and Luisoni (1966) tried in vain to transmit the virus with *Myzus persicae*, and Verhoyen et al. (1976) with *Neomyzus circumflexus*. Our tests with *M. persicae* and four other aphids including *Cavariella aegopodii* were also negative in short feeding times. Sap transmissibility and particle morphology make persistent aphid transmission improbable.

Seed transmission in celeriac, already reported in 1973 (Bos, 1973) was further substantiated and also detected in *Amaranthus caudatus*.

In host range, symptomatology and other biological properties the virus clearly differs from other viruses described so far, including CeMV with which it often occurs in complex. Neither of the two viruses protects celery plants against infection with the other.

Electron microscopy of crude sap and of infected tissue has revealed that the particles of the virus although flexuous are longer than normal for the potyvirus group, that they occur in sparse accumulations, but that pinwheels or cylindrical inclusions characteristic of the potyvirus group are not formed. Nor could typical granular inclusion bodies be detected in the cytoplasm of epidermal strips with the light microscope. These observations agree with the preliminary results by Verhoyen et al. (1976), who do not provide information on the particle concentration in crude sap and infected tissue. The particle size determined by measuring a few particles in crude sap (885 nm) and many in one of the purified preparations (886 nm), while using TMV as a reference, compares reasonably well with that found by Brandes and Luisoni (1966: 858 nm) but is longer than suggested by Verhoyen et al. (1976: 800–850 nm). A length of 940 nm, observed in one of our purified preparations, is puzzling.

Results of purification, although encouraging in some experiments, were poor in

others. Virus concentration in *C. quinoa* was often very low as indicated by the small number of particles found with the electron microscope in crude sap. Moreover, the results of serological tests in agar gel suggest that the virus is easily degraded into small units, especially at high salt concentrations. The tests also indicate the natural occurrence of small, virus-specific antigens in plants infected with CeLV. Such antigens have also been reported for other elongate viruses, e.g. for apple chlorotic leaf spot virus (CLSV) by Chairez and Lister (1973). These authors also found NaCl (0.85%) essential for serology in agar gel. Dunez et al. (1975) used the agar-gel test as a specific diagnostic tool for CLSV. With this test we found the Dutch isolate of CeLV related to an Italian isolate, but not to CLSV using an antiserum to CLSV 'soluble antigen' from Dr M. F. Clark, East Malling Research Station, England. For CLSV also the best culture host was *C. quinoa*, however with similar problems in purification and low virus yields (cf. Chairez and Lister, 1973). In other respects CLSV differs from CeLV, such as in shorter particles (ca. 600 nm) and lower S value (ca. 96 S) (Lister, 1970).

The CeLV resembles sweet potato mild mottle virus (Hollings et al., 1976) which also occurs in low concentration, has a similar persistence of infectivity in expressed sap, is not transmissible by aphids, is 800–950 nm long (depending on $MgCl_2$ or EDTA), and has an S value in water at zero concentration of 155 S. However, it is white-fly borne and differs clearly in host range. Unfortunately, Hollings et al. did not look for pinwheels or cylindrical inclusions, but they did conclude that there was no evidence to support any affinities of their virus to the potyvirus group. Like sweet potato mild mottle virus, CeLV also differs from potyviruses, and it seems that a new group with particles longer than 800 nm is emerging. Further research is needed on the classification of CLSV and its related apple stem grooving virus, now often grouped with closteroviruses.

So far direct economic importance of the virus seems limited since the majority of hosts, including its only known natural host, do not react with visible effects. We have never found symptoms in celeriac and celery after natural or artificial infection. We did not weigh plants as did Verhoyen et al. (1976), who found 38% yield reduction in (leaf)celery, 33% in (blanched)celery and 36% in celeriac. Their experiments were with few plants, but their results suggest that the virus may have a yield-depressing effect. Brandes and Luisoni (1966) incidentally observed some mosaic flecking in artificially infected celery and celeriac plants. So called latent viruses, as of potatoes, may lead to 10–15% yield reduction without showing a visible effect on individual plants. Moreover, latent infections may aggravate the effect of other viruses, although we did not detect this in celery plants doubly infected with CeMV, or it may increase susceptibility to other pathogens. This may also be the potential threat of the virus to other crops, since several species are susceptible but few sensitive. However, there are some, such as two pea cultivars, that do react with variegation and even stunting.

Its potential threat is further enhanced by its seed transmission in diverse plant species up to a high percentage as in celery and up to 89% in *C. quinoa* and even in the legume crop *Trigonella foenum-graecum* (Luisoni and Lisa, 1969). Because of latency, seed transmission is likely to be overlooked. That the virus does not have wider distribution may be due to lack of vectors. Admittedly, absence of aphid vectors does not exclude other ways of spread, but spread through contact and on

hands and implements seems unlikely. Brandes and Luisoni (1966) usually had poor results in mechanical transmission to celeriac.

For gaining insight in possible ways of vector transmission, information on its grouping would be helpful. The reverse also holds. The sweet potato mild mottle virus mentioned above is white-fly transmitted (Hollings et al., 1976).

In view of its potential importance, prevention of spread of CeLV by seed seems justified. Seed of celery and celeriac can easily be tested for infection by sap inoculation to *C. quinoa* from seedlings, from germinated seeds, but preferably not from dry seeds, ground in water or buffer solution. With dry seeds from infected batches of celeriac and *C. quinoa* tested in groups of ten, fewer groups revealed infection than when tested after germination, and test plants reacted later and with weaker symptoms. Seed multiplication schemes should start with mother plants tested for virus-freedom. In one group of the celeriac seeds (Table 2) we detected the strawberry latent ringspot virus, which Walkey and Whittingham-Jones (1970) found transmissible in up to 98–100% of the seeds from infected celery plants. Arabis mosaic virus (Walkey and Mitchell, 1969) and tobacco rattle virus (personal observation) are also known to naturally infect celery and celeriac and to be able to pass via the seed of several hosts. Therefore testing for virus freedom could also include testing for absence of the latter viruses. Here, however, there may be chances of later crop re-infections from the soil.

Samenvatting

Verdere karakterisering van het latente selderijvirus

In Nederland blijkt knolselderij behalve door het veelvuldig voorkomende en schadelijke selderijmozaïekvirus (CeMV) niet zelden door het latente selderijvirus (CeLV) te zijn geïnfecteerd. Het virus is in 1966 voor het eerst in Italië aangetroffen en sinds kort ook bekend in België.

Het virus veroorzaakt in bleekselderij noch in één der 13 getoetste knolselderijrassen symptomen (Fig. 1B) en lijkt in bleekselderij ook het effect van het CeMV niet te versterken (Fig. 1C en D). *Chenopodium amaranticolor* reageert op inoculatie vooral met duidelijke lokale en soms met systemische symptomen (Fig. 2) en *C. quinoa* met systemische symptomen (Fig. 3). Door waardplantonderzoek (Tabel 1) en vergelijking met gegevens uit de literatuur (Tabel 1 en 2) werden veertien nieuwe kunstmatige waardplanten gevonden. Nieuwe systemisch latente waardplanten zijn *Anthriscus cerefolium*, *Nicotiana megalosiphon*, *Pisum sativum*, *Spinacia oleracea* en *Trifolium incarnatum*. In de erwterassen Cicero en Dark Skin Perfection (Fig. 4) ontstonden zelfs symptomen.

De gevonden waarden van houdbaarheid van het infectievermogen bij verdunning (10^3–10^4), bij warmtebehandeling (ca. 50°C) en bij bewaring (4 tot 8 dagen) waren wat lager dan die uit de literatuur. Met vier nog niet eerder met dit virus beproefde bladluissoorten, waaronder *Cavariella aegopodii*, kon het virus niet in korte zuigtijden worden overgebracht.

Zaadoverdracht, die al eerder bij knolselderij was gerapporteerd (Bos, 1973; Verhoyen et al., 1976) kon worden bevestigd (Tabel 2). Het virus werd aangetroffen

in bepaalde herkomsten van 'Roem van Zwijndrecht' (tot 34%) en in 'Pomona'. Overdracht kon ook worden bevestigd in zaad van *C. quinoa* (67%) en werd aangetoond voor zaad van *Amaranthus caudatus*. Het virus kan gemakkelijk door inoculatie op *C. quinoa* worden aangetoond in zaailingen en gekiemde zaden maar minder betrouwbaar in vermalen droge zaden.

De resultaten van viruszuivering vanuit *C. quinoa* (Fig. 5) door klaring met organische oplosmiddelen en verdere zuivering met PEG, differentiële centrifugering en sucrose-gradiënt centrifugering, waren soms redelijk (Fig. 7) maar meestal wisselvallig en het beste bij een buffer-pH van 8 of 9. De gevonden sedimentatiecoëfficiënt was 161 S.

Het na viruszuivering bij pH 8 bereide antiserum reageerde sterk met gezond plantesap. Het na zuivering bij pH 9 bereide antiserum had tegen gezuiverd virus in de micro-precipitatietoets een titer van 256 en in agar-gel van 64. Dit serum reageerde ook met ruw sap van zieke, maar niet van gezonde planten. Waarschijnlijk gaat het hier om een reactie met vooral bij hoge zoutconcentratie vrijgekomen viruseiwit.

In ruw sap (Fig. 6) en in gezuiverde viruspreparaten (Fig. 7) konden karakteristieke flexibele draden worden waargenomen van ongeveer 885 nm lengte (in één gezuiverd preparaat van 940 nm). Met de lichtmicroscoop konden geen celinsluitsels worden waargenomen. In ultradunne coupes kwamen geen schoepenradstructuren en andere, voor de aardappelvirus-Y-groep karakteristieke nieuwvormingen voor. Wel konden sporadisch bundels min of meer parallel gerangschikte draadvormige virusdeeltjes in het cytoplasma worden gevonden (Fig. 8 en 9).

Het virus verschilt duidelijk van virussen van de aardappelvirus-Y-groep en behoort wellicht met enkele andere nog niet volledig beschreven virussen tot een nieuwe morfologische groep.

Door de brede waardplantenreeks, het vermogen om ziekteverschijnselen in bepaalde plantesoorten teweeg te brengen, het veelal symptoomloos voorkomen en de zaadoverdracht is het virus potentieel van praktische betekenis. Het lijkt daarom gewenst bij zaadteelt van selderij uit te gaan van virusvrije moederplanten.

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