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Carlavirus isolates from cultivated Allium species represent three viruses

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Abstract

From 12 cultivated and mostly vegetatively propagated *Allium* species and varieties tested for carlavirus infections, 94 virus isolates were obtained which varied greatly on indicator hosts. *Chenopodium amaranticolor*, *C. quinoa*, *Celosia argentea* var. *plumosa* 'Geisha', *Nicotiana hesperis* accession 67A and *N. occidentalis* accession P1 proved valuable for detection, isolation and propagation of part of the isolates. The latter three species are new experimental hosts for carlaviruses of *Allium* species. Other isolates could only be transmitted to *Allium* species such as crow garlic (*A. vineale*) leek (*A. ampeloprasum* var. *porrum*) and onion (*A. cepa* var. *cepa*). The isolates were grouped into three viruses by differential hosts and host reactions and their reaction with four antisera.

Shallot latent virus (SLV) was found in ever-ready onion (*A. cepa* var. *perutile*), grey shallot (unidentified *Allium* species), multiplier onion (*A. cepa* var. *aggregatum*), pearl onion (*A. ampeloprasum* var. *sectivum*), rakkyo (*A. chinense*), shallot (*A. cepa* var. *ascalonicum*), and Welsh onion (*A. fistulosum*). Virus isolates from garlic and Asian shallot, fully reacting with antiserum to SLV but differing in host reactions from the SLV type-isolate, are now described as garlic strain (SLV-G) and Asian shallot strain of the virus, respectively. The 'garlic latent virus' from garlic described in Japan is now considered identical with SLV-G.

A carlavirus almost universal in garlic, and also found in great-headed garlic (*A. ampeloprasum* var. *holmense*), in an unidentified *Allium* species, and occasionally in leek, did not react with the antisera to SLV and the Japanese 'garlic latent virus', and is now described as the new garlic common latent virus (GCLV). It appeared identical to a virus erroneously identified in Germany as garlic latent virus.

The new Sint-Jan's-onion latent virus (SjoLV) from Utrechtse Sint-Jan's onion (unidentified *Allium* species) from the Netherlands and similar crops originating from other countries, did not induce reactions in test plants and could only be detected by electron microscope decoration tests. It reacted equally well with the antisera to SLV and GCLV. It was also present together with SLV in ever-ready onion, pearl onion, rakkyo, shallot, and Welsh onion. 'Garlic latent virus' reported in Japan from hosts other than garlic should be regarded as SLV, SjoLV, or a mixture of these viruses.

The carlaviruses were not detected in wild plants of ramsons (*A. ursinum*), and of the predominantly vegetatively propagated crow garlic (*A. vineale*), field garlic (*A. oleraceum*), and sand leek (*A. scorodoprasum*), collected in the Netherlands.

Severe reactions in the indicator hosts incidentally revealed soil-borne viruses in shallot (the nepoviruses *Arabis* mosaic virus (ArMV) and tomato black ring virus) and crow garlic (ArMV and the tobravirus tobacco rattle virus). Tobacco necrosis virus (necrovirus) was detected in roots of shallot.

Additional keywords: aphid transmission, *Arabis* mosaic virus, carnation latent virus, crow garlic, ever-ready onion, field garlic, garlic, garlic common latent virus, garlic latent virus, grey shallot, great-headed garlic, leek, multiplier onion, narcissus latent virus, pearl onion, ramsons, rakkyo, sand leek, shallot, shallot latent virus, Sint-Jan's-onion latent virus, tobacco necrosis virus, tobacco rattle virus, tomato black ring virus, Utrechtse Sint-Jan's onion, Welsh onion, wild plants.

Introduction

Crops of cultivated *Allium* species are commonly infected with one or more viruses, especially when propagated vegetatively (Bos, 1983; Walkey, 1990). During a virus survey at Wageningen, the Netherlands, of *Allium* samples from different parts of the world, two mite-borne potyviruses (Van Dijk et al., 1991) and four aphid-borne potyviruses (Van Dijk, 1993b) have been identified. Carlavirus isolates that were also present in the investigated samples are the subject of the present paper.

Confusion concerning carlaviruses in *Allium* species has increased with time. Shallot latent virus (SLV) described from shallot (*A. cepa* var. *ascalonicum*) in the Netherlands (Bos et al., 1978), and 'garlic latent virus' described from garlic (*A. sativum*) in Japan (Lee et al., 1979), so far are the only biologically and serologically characterized carlaviruses of *Allium* spp. Their serological relationship was reported to be distant (Bos, 1983). SLV was occasionally found in leek (*A. ampeloprasum* var. *porrum*) and onion (*A. cepa* var. *cepa*) in the Netherlands (Bos et al., 1978). It was also found in leek in Denmark (Paludan, 1980) and in Germany (Lesemann et al., 1991), in sand leek (*A. scorodoprasum*) in Belgium (Verhoyen and Horvat, 1981), in garlic in England (Walkey et al., 1987), and in multiplier onion (*A. cepa* var. *aggregatum*) in Finland (Bremer, 1990). 'Garlic latent virus' was also reported in an ornamental *A. ampeloprasum* (Inouye et al., 1981), rakkyo (*A. chinense*) (Sako, 1989; Sako et al., 1989, 1991) and Welsh onion (*A. fistulosum*) (Fukami et al., 1988) in Japan. Identification of SLV in garlic in England was by electron microscope decoration tests, which also revealed carlavirus particles not reacting with SLV antiserum (Walkey et al., 1987), as earlier observed with the same technique in French garlic (Delecolle and Lot, 1981). Garlic in Germany contained a carlavirus tentatively identified as 'garlic latent virus' (Graichen and Leistner, 1987; Lesemann et al., 1992). It was reported to be generally present in European garlic samples (Abo el-Naga et al., 1989), and was also found in leek (Lesemann et al., 1991). The 'garlic latent virus' in Germany, however, proved at Wageningen to be different from the virus with that name earlier described in Japan (Van Dijk et al., 1991).

The detection of narcissus latent virus in garlic was claimed in England (Walkey, 1990) and antiserum to carnation latent virus decorated virus particles from garlic in Argentina (Conci and Nome, 1991; Conci et al., 1992). In the Netherlands, another undescribed carlavirus was detected in shallot and Utrechtse Sint-Jan's onion (Van Dijk et al., 1991), a species then incorrectly identified as rakkyo (Van Dijk, 1993b).

In this study, a large number of carlavirus isolates from various countries are compared biologically and by electron microscope decoration tests with the few antisera available.

Materials and methods

Test species and cultivars. Plants of onion 'Stuttgarter Riesen' were grown from sets, and those of 'Rijnsburger' selection Oporto were from seed. Plants of shallot 'Noordhollandse Strogele' were from bulbs grown at IPO-DLO from seed. Bulbs or plants of

crow garlic (*Allium vineale*) were collected in the field. Plants of garlic 'Thermidrôme' were from certified bulbs. Broad bean (*Vicia faba*) 'Compacta' and 'Driemaal Wit', *Celosia argentea* var. *plumosa* 'Geisha', French bean (*Phaseolus vulgaris*) 'Bataaf', leek 'Zwitserse Reuzen' selection Jolant, pea (*Pisum sativum*) 'Koroza', spinach (*Spinacia oleracea*) 'Nores', and Welsh onion (*A. fistulosum*) 'Kyoto Market' were all grown from commercial seed. Chinese chive (*A. tuberosum*), chive (*A. schoenoprasum*), and Formosan lily (*Lilium formosanum*) was raised from seed provided by the DLO Centre for Plant Breeding and Reproduction Research, Wageningen. Plants of *Chlorophytum comosum* were raised from rosettes obtained from house plants. *Nicotiana hesperis* was accession 67A from the Tobacco Research Laboratory, Oxford NC, USA, and *N. occidentalis* was IPO-DLO accession P1, originally obtained from Kings Park and Botanic Garden, West Perth, Australia. *Chenopodium* spp., *N. benthamiana* and *N. tabacum* 'White Burley' were from seed stocks regularly used at IPO-DLO. Plants of sesame (*Sesamum indicum*) were from seed supplied by the Agricultural University, Wageningen, the Netherlands, and those were grown in aphid-proof glasshouses at 18–20 °C.

Virus isolates. Carlavirus isolates and additional isolates of soil-borne viruses, the method of their isolation or detection, and data on the *Allium* samples from which they were obtained, are specified in Table 1. Several representative isolates as indicated in the table have been preserved for reference and further characterization. The following two isolates also listed in Table 1 were used for comparison:
- garlic common latent virus (GCLV) (this paper), erroneously identified as 'garlic latent virus' in Germany (Graichen and Leistner, 1987): uncoded garlic isolate (IPO-DLO code As224) supplied by Dr K. Graichen (Institut für Phytopathologie, Aschersleben, Germany);
- shallot latent virus (SLV): isolate Ac3 from shallot of IPO-DLO (Bos et al., 1978), from which Ac3A and the new type-isolate Ac3B were sub-isolated (see under Results).

Antisera. The antisera to carla-, nepo-, and potyviruses used are listed below (quotation marks indicate reidentification of the carlavirus in this paper or contamination of the potyvirus with carlavirus (Van Dijk, 1993b)) and with original reference and antiserum supplier.
- *Arabis* mosaic virus (nepovirus) (Murant, 1970a); Mr D.Z. Maat (IPO-DLO);
- carnation latent virus (CLV) (carlavirus) (Wetter, 1971); Mr D.Z. Maat (IPO-DLO);
- garlic common latent virus (GCLV) (carlavirus) (this paper), erroneously identified as 'garlic latent virus' in Germany (Graichen and Leistner, 1987), and earlier provisionally named 'German garlic latent virus' (Van Dijk et al., 1991); Dr K. Graichen, Institut für Phytopathologie, Aschersleben, Germany;
- 'garlic latent virus' (carlavirus) (Lee et al., 1979), earlier provisionally called 'Japanese garlic latent virus' (Van Dijk et al., 1991), and further described in this paper as the garlic strain of shallot latent virus; Dr T. Inouye, University of Osaka Prefecture, College of Agriculture, Osaka, Japan;
- 'garlic yellow stripe virus' (potyvirus) (De Carvalho, 1980, 1981), consisting of a virus complex including garlic common latent virus (Van Dijk, 1993b); Dr T.T. Matsumoto, State of California, Department of Food and Agriculture, Sacramento, California, USA;
- narcissus latent virus (NLV) (carlavirus) (Brunt, 1976); Mr D.Z. Maat, IPO-DLO;
- shallot latent virus (SLV) (carlavirus) (Bos, 1982); Mr D.Z. Maat, IPO-DLO;
- tomato black ring virus (nepovirus) (Murant, 1970b); Mr D.Z. Maat, IPO-DLO.

Table 1. Isolates of three carlaviruses and four soil-borne viruses from twelve cultivated and one wild *Allium* species and varieties of different origin.

Cultivated/wild species	Host used for virus isolation[3]	Further specification of samples		
Species sampled				
Virus[1]		Cultivar or selection	Country of origin[4]	Source[5]
Isolate[2]				
Cultivated species				
Ever-ready onion (*A. cepa* var. *perutile*)				
SLV				
Ac319II	[ever-ready onion]	no cultivar name	the Netherlands (England)	GN
SjoLV				
Ac319III	[ever-ready onion]	no cultivar name	the Netherlands (England)	GN
Garlic (*A. sativum*)				
GCLV				
As100[p]	*Nicotiana occidentalis*	Germidour	France	ICer
As102	*Nicotiana occidentalis*	Thermidrôme	France	ICer
As114	*Nicotiana occidentalis*	unknown	France	ICon
As121	crow garlic	Germidour	France	ICer
As122	*Celosia argentea*	Messidrôme	France	ICer
As123II[p]	*Nicotiana occidentalis*	Thermidrôme	France	ICer
As128III	*Nicotiana occidentalis*	unknown	India	GN
As131III[p]	*Celosia argentea*	unknown	Indonesia	GN
As140II	*Chenopodium quinoa*	unknown	Argentina	ICon
As142III	*Chenopodium quinoa*	Rose d'Auvergne	France	ICer
As144III	*Nicotiana occidentalis*	Thermidrôme	the Netherlands (France)	CulN (Icer)
As154III[p]	*Chenopodium murale*	unknown	Indonesia	SR
As168[p]	*Nicotiana occidentalis*	unknown	Czechoslovakia	SR
As171III	*Nicotiana occidentalis*	unknown	Czechoslovakia	SR
As182III	onion; *Nic. occidentalis*	Jubilejnij Gribowskij	former USSR (Uzbekistan)	SR
As186I	*Nicotiana occidentalis*	Rocambole	the Netherlands (France)	CulN
As186II	*Chenopodium quinoa*	Rocambole	the Netherlands (France)	CulN
As187	*Celosia argentea*	Germidour	the Netherlands (France)	GN
As188II	*Nicotiana occidentalis*	Messidrôme	the Netherlands (France)	CulN (ICer)
As199III[p]	onion; *Nic. occidentalis*	unknown	the Netherlands (former DDR)	GN

Table 1. (Continued).

Cultivated/wild species Species sampled *Virus*[1] Isolate[2]	Host used for virus isolation[3]	Further specification of samples		
		Cultivar or selection	Country of origin[4]	Source[5]
As222III	*Chenopodium quinoa*	Rose de Lautrec	Chile	SR
As224[p,6]	*Nicotiana occidentalis*	unknown	former DDR	SR
As336IV	[garlic]	unknown	Argentina	ICon
As381III	*Chenopodium quinoa*	unknown	China	ICon
SLV-G				
As126III	*Chenopodium amaranticolor*	unknown	Egypt	GN
As182IV	onion; *Chen. amaranticolor*	Jubilejnij Gribowskij	former USSR (Uzbekistan)	SR
As199II[p]	onion; *Chen. amaranticolor*	unknown	the Netherlands (former DDR)	GN
As223IV	*Celosia argentea*	Rosado Argentino	Chile	SR
As237II	*Chenopodium quinoa*	unknown	the Netherlands (Bolivia)	GN
As328III	onion	unknown	Japan	SR
As391III[p]	*Nicotiana occidentalis*	Lumbu Hijau	Indonesia	CulI
Great-headed garlic (*A. ampeloprasum* var. *holmense*)				
GCLV				
Aa127II	*Celosia argentea*	unknown	the Neth. (former Yugoslavia)	CulN
Aa127III	*Celosia argentea*	unknown	the Neth. (former Yugoslavia)	CulN
Aa128III[p]	*Celosia argentea*	unknown	Israel	ICon
Aa129	*Celosia argentea*	unknown	Japan	SR
Grey shallot (unidentified *Allium* sp.)				
SLV				
Ax114	*Celosia argentea*	Griselle	France	SR
Ax115	*Chenopodium quinoa*	Grise de la Drôme	the Netherlands (France)	GN
Leek (*A. ampeloprasum* var. *porrum*)				
GCLV				
Aa105[p]	*Nicotiana occidentalis*	Bulgaarse Reuzen	the Netherlands	CulN

Table 1. (Continued).

Cultivated/wild species Species sampled *Virus*[1] Isolate[2]	Host used for virus isolation[3]	Further specification of samples Cultivar or selection	Country of origin[4]	Source[5]
Multiplier onion (*A. cepa* var. *aggregatum*)				
SLV				
Ac251II[P]	*Celosia argentea*	unknown	the Netherlands (the Philippines)	GN
Ac318	onion	unknown	the Netherlands (Poland)	GN
Pearl onion (*A. ampeloprasum* var. *sectivum*)				
SLV				
Aa144II	*Nicotiana hesperis*	unknown	Indonesia	CuII
SLV^{Asia}				
Aa112[P]	*Nicotiana hesperis*	Anak	the Netherlands (Indonesia)	GN
Aa145I	*Nicotiana occidentalis*	Anak	Indonesia	CuII
SjoLV				
Aa144III	[pearl onion]	unknown	Indonesia	CuII
Aa145II	[pearl onion]	Anak	Indonesia	CuII
Rakkyo (*A. chinense*)				
SLV				
Ach14I	*Chenopodium quinoa*	unknown	the Netherlands (Thailand)	GN
Ach15I	*Chenopodium quinoa*	unknown	the Netherlands (Indonesia)	GN
Ach17I	*Chenopodium quinoa*	unknown	Indonesia	CuII
SjoLV				
Ach14II	[rakkyo]	unknown	the Netherlands (Thailand)	GN
Shallot (*A. cepa* var. *ascalonicum*)				
ArMV				
Ac179	*Chenopodium quinoa*	Santé	the Netherlands (former USSR)	CuIN
Ac180[P]	*Chenopodium quinoa*	Santé	the Netherlands (former USSR)	CuIN
Ac181	*Chenopodium quinoa*	Santé	the Netherlands (former USSR)	CuIN
SLV				
Ac3A[7]	*Chenopodium amaranticolor*	unknown	the Netherlands	CuIN
Ac3B[P,7]	*Chenopodium amaranticolor*	unknown	the Netherlands	CuIN

Table 1. (Continued).

Cultivated/wild species **Species sampled** *Virus*[1] Isolate[2]	Host used for virus isolation[3]	Further specification of samples		
		Cultivar or selection	Country of origin[4]	Source[5]
Ac110	*Chenopodium quinoa*	Gourmet	the Netherlands	GN
Ac115^{P}	*Nicotiana occidentalis*	Noordhollandse Strogele	the Netherlands	CulN
Ac116II	*Vicia faba*	Noordhollandse Strogele	the Netherlands	CulN
Ac119II	*Celosia argentea*	Ouddorpse Bruine	the Netherlands	CulN
Ac125III	*Nicotiana occidentalis*	unknown	Indonesia	SR
Ac127III	*Celosia argentea*	Sumenep	Indonesia	SR
Ac129II^{P}	*Sesamum indicum*	Échalote Bretonne Longue	France	ICon
Ac130IV	*Celosia argentea*	unknown	China	ICon
Ac132II^{P}	onion[8]	unknown	Spain	ICon
Ac133II	*Chenopodium amaranticolor*	Noordhollandse Strogele	the Netherlands	CulN
Ac137^{P}	*Nicotiana occidentalis*	Germinor	the Netherlands (France)	GN
Ac140II^{P}	*Nicotiana occidentalis*	unknown	Spain	PB
Ac157II	*Chenopodium amaranticolor*	unknown	the Netherlands (France)	CulN
Ac162II^{P}	*Vicia faba*	Tagar	former USSR (Mongolia)	SR
Ac162V^{P}	*Nicotiana occidentalis*	Tagar	former USSR (Mongolia)	SR
Ac174II	[shallot]	Noordhollandse Strogele	the Netherlands	CulN
Ac183II^{P}	*Chenopodium amaranticolor*	Tagar	former USSR (Mongolia)	SR
Ac203^{P}	*Chenopodium quinoa*	Ouddorpse Bruine	the Netherlands	CulN
Ac205^{P}	*Chenopodium quinoa*	Santé	the Netherlands (former USSR)	CulN
Ac207^{P}	*Nicotiana hesperis*	Santé	the Netherlands (former USSR)	CulN
Ac208	*Celosia argentea*	Santé	the Netherlands (former USSR)	CulN
Ac234	*Chenopodium quinoa*	unknown	Norway	GN
SLV^{Asia}				
Ac127II^{P}	*Nicotiana hesperis*	Sumenep	Indonesia	SR
Ac130II	*Chenopodium quinoa*	unknown	China	ICon
Ac142	*Chenopodium quinoa*	unknown	Indonesia	GN
Ac177II	*Nicotiana occidentalis*	unknown	Thailand	ICon
Ac188^{P}	*Nicotiana hesperis*	unknown	Thailand	ICon
Ac266II	*Nicotiana occidentalis*	unknown	Thailand	ICon

Table 1. (Continued).

Cultivated/wild species Species sampled *Virus*[1] Isolate[2]	Host used for virus isolation[3]	Further specification of samples Cultivar or selection	Country of origin[4]	Source[5]
SjoLV				
Ac174III	[shallot]	Noordhollandse Strogele	the Netherlands	CulN
Ac204I[p]	crow garlic	Santé	the Netherlands (former USSR)	CulN
Ac206I[p]	[shallot]	Santé	the Netherlands (former USSR)	CulN
TNV				
Ac190	*Nicotiana benthamiana*	unknown	Thailand	ICon
Ac191	*Nicotiana benthamiana*	unknown	Thailand	ICon
TBRV				
Ac170II[p]	*Chenopodium quinoa*	Ouddorpse Bruine	the Netherlands	CulN
Unidentified *Allium* species[9]				
GCLV				
Ax11III	*Chenopodium quinoa*	unknown	the Netherlands (Morocco)	GN
Utrechtse Sint-Jan's- onion and similar crops (unidentified *Allium* sp.)				
SLV				
Ax111I	*Chenopodium quinoa*	Papago l'Itoi	the Netherlands (USA)	GN
SjoLV				
Ax101[p]	leek	Utrechtse Sint-Jan's onion	the Netherlands	GN
Ax103[p]	crow garlic	Utrechtse Sint-Jan's onion	the Netherlands	CulN
Ax111II	onion	Papago l'Itoi	the Netherlands (USA)	GN
Ax112	[unidentified *Allium* sp.]	unknown	the Netherlands (Hongkong)	GN
Ax113	onion	unknown	the Netherlands (Hongkong)	GN
Welsh onion (*A. fistulosum*)				
SLV				
Af12II	crow garlic[8]	unknown	Japan	SR
Af19II	[Welsh onion]	Papak	Indonesia	CulI
SjoLV				
Af19III	[Welsh onion]	Papak	Indonesia	CulI

Table 1. (Continued).

Cultivated/wild species **Species sampled** *Virus*[1] Isolate[2]	Host used for virus isolation[3]	Further specification of samples Cultivar or selection	Country of origin[4]	Source[5]
Wild species				
Crow garlic (*A. vineale*)				
ArMV				
Av155[P]	*Celosia argentea*	wild species	the Netherlands	ColW
TRV				
Av213I	*Nicotiana occidentalis*	wild species	the Netherlands	ColW

[1] Acronyms represent virus names as follows: ArMV = *Arabis* mosaic virus (nepovirus); GCLV = garlic common latent virus (carlavirus); SLV = common strain of shallot latent virus (carlavirus); SLV-G = garlic strain of SLV; SLV^{Asia} = Asian shallot strain of SLV; SjoLV = Sint-Jan's-onion latent virus (carlavirus); TBRV = tomato black ring virus (nepovirus); TNV = tobacco necrosis virus (necrovirus); TRV = tobacco rattle virus (tobravirus).

[2] Suffix [P] denotes preservation and storage in the IPO-DLO virus collection.

[3] Isolation by mechanical inoculation of indicated host (except for isolates Ac132II and Af12II, see footnote 8), unless brackets are used to indicate the original plant sample in which the virus was detected without isolation.

[4] The country mentioned in parentheses is the country from which the selection was introduced.

[5] Abbreviations of sample sources are as follows: ColW = collected in the wild; CulN = cultivated in the Netherlands; GN = gene collection in the Netherlands; ICer = certified bulbs imported for cultivation in the Netherlands; ICon = imported for consumption in Belgium or the Netherlands; PB = material from plant breeder in the Netherlands; SR = supplied by research worker abroad.

[6] Isolate supplied by Dr K. Graichen (Institut für Phytopathologie, Aschersleben, Germany) and identified there as 'garlic latent virus'.

[7] Ac3A and new type-isolate Ac3B now sub-isolated from previous type-isolate Ac3 (Bos et al., 1978).

[8] Transmission by aphids in complex with potyvirus (see text).

[9] A species resembling garlic but forming true seed, earlier incorrectly identified as sand leek (Van Dijk et al., 1991).

Virus transmission by sap inoculation. Transmission in sap usually was with 0.05M potassium phosphate, pH 7.7, with 0.1% (w/v) cysteine hydrochloride, and with carborundum 500 mesh as an abrasive.

Electron microscopy. Preparations for electron microscopy were usually made by grinding small pieces of fresh or dried leaves in a 0.2% (w/v) solution of sodium sulphite, incubating the sap on a grid for c. 1 min, and floating the grid for 1 min on drops of the sodium-sulphite solution and of a 2% (w/v) solution of phosphotungstate acid (PTA), successively. Decoration was by floating the grid on antiserum (dilution 1:3 or 1:10, and incubation for 15 min or 5 min with antisera of low or high titre, respectively), and thereafter on sodium-sulphite solution, prior to staining.

Results and discussion

Initially, a large number of plant species and selections, including 29 *Nicotiana* accessions mostly from Australia, were inoculated with sap of different garlic and shallot samples to investigate their value as an indicator for carlaviruses present in those samples (P. van Dijk, unpublished results). Part of the isolates soon appeared to induce local lesions in *Celosia argentea*, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Nicotiana hesperis*, *N. occidentalis*, *Pisum sativum* (sweet pea) and *Vicia faba* (broad bean), or a systemic reaction in *N. occidentalis*. The genotypes *Celosia argentea* var. *plumosa* 'Geisha', *N. hesperis* accession 67A, and *N. occidentalis* accession P1 were selected for routine use because of their clear reactions, while other cultivars or accessions of these species gave unsatisfactory or negative results. Other carlavirus isolates did not react in any plant species, and could only be transmitted to *Allium* spp. such as crow garlic, onion, and shallot. Severe reactions, occasionally observed in indicator hosts, were of soil-borne viruses.

Investigation of over 100 samples of 12 cultivated species and varieties revealed the presence of 94 carlavirus isolates. Samples of vegetatively propagated crops only rarely harboured no carlavirus, whereas only one of the samples of seed-grown crops contained a carlavirus. No carlaviruses were detected in any of 167 plants of crow garlic (*A. vineale*), 9 of field garlic (*A. oleraceum*), 27 of ramsons (*A. ursinum*), and 9 of sand leek (*A. scorodoprasum*), all collected in the wild in the Netherlands.

Carlavirus isolates, when occurring in mixed infections, were singled out by repeated transfer in single lesions, by passage through non-inoculated leaves of *N. occidentalis*, or by accidental single transfer in sap to test plants. Single isolates greatly varied in host range and symptoms. According to their decoration by four antisera these virus isolates were arranged into three groups, viz. SLV, the new garlic common latent virus (GCLV), and the new Sint-Jan's-onion latent virus (SjoLV). Two biologically deviating groups of SLV isolates were identified as two strains, one representing the 'Japanese garlic latent virus'.

Table 1 lists the isolates, together with plant species used for their isolation and data on their origin, and presents an overview of the viruses and strains isolated from each cultivated and wild *Allium* species. Table 2 records host reactions and serological decoration of selected carlavirus isolates. Occurrence, incidence (where known), biological variability, and serological properties of the carlaviruses and strains are now presented for each virus, with reference to the literature. The soil-borne viruses are briefly described first, to give full account of the virus survey, and to facilitate the identification of carlaviruses by test plant reactions.

Soil-borne viruses

Arabis mosaic virus (ArMV) and tomato black ring virus (TBRV) (nepovirus)
Severe systemic reactions, infrequently observed in *Celosia argentea*, *Chenopodium amaranticolor*, *C. murale*, and *C. quinoa*, were caused by nepovirus infection. The isolates were serologically identified as *Arabis* mosaic virus (ArMV) (from three plants of shallot 'Santé' and one of crow garlic) and tomato black ring virus (TBRV) (from one shallot 'Ouddorpse Bruine') (Table 1). ArMV was earlier reported from tree onion (*A. cepa* var. *viviparum*) (Graichen, 1975), but not from crow garlic and shallot. TBRV had been found in leek and onion in Northern Ireland (Calvert and Harrison, 1963), and in *A. flavum*, chive (*A. schoenoprasum*), leek, shallot, and tree onion in Germany (Graichen, 1975).

Tobacco rattle virus (TRV) (tobravirus)
Plants sampled from one natural population of crow garlic in which all plants showed chlorotic and white necrotic stripes on the leaves (Fig. 1), though not containing detectable virus particles when examined in the electron microscope, proved to be infected with a tobravirus by the presence of rod-shaped particles in test plants inoculated with sap from selected plants. The isolate was recognized as tobacco rattle virus (TRV) (Table 1) also by its rapidly enlarging necrotic local lesions in *C. quinoa*, small necrotic local lesions in broad bean, French bean and pea, and severe necrotic symptoms in inoculated and non-inoculated leaves of *N. benthamiana*, *N. occidentalis*, and *N. tabacum*. TRV had earlier been reported in garlic, onion, *A. moly* and *A. ursinum* in Europe (Van Slogteren, 1958; Kristensen and Engsbro, 1966; Graichen, 1975; Bos, 1983), but not in crow garlic. Over hundred of plants of crow garlic from other populations did not reveal

Fig. 1. Plants of crow garlic with chlorotic and necrotic stripes due to natural infection by the tobravirus tobacco rattle virus.

Table 2. Differentiation of representative isolates of garlic common latent virus (GCLV), shallot latent virus host reactions and serology.

Means of characterization	GCLV from garlic						SLV and strains from shallot			
							Common strain			
	As224[1]		As123II		As199III		Ac3B		Ac162II	
Test plants[3]										
Allium ampeloprasum var. *porrum* (leek)	.	.[4]	.	s	.	s	.	s	.	.
A. cepa var. *ascalonicum* (shallot)	.	.	.	.	.	.	.	s	.	s
A. cepa var. *cepa* (onion)	.	.	.	–	.	s	.	s	.	s
A. fistulosum (Welsh onion)	.	.	.	.	.	.	.	.	.	.
A. sativum (garlic)	.	s	.	s	.	s	.	–	.	.
A. tuberosum (Chinese chive)	.	.	.	.	.	.	.	.	.	.
A. vineale (crow garlic)	.	.	.	s	.	.	.	s	.	.
Sint-Jan's onion (unidentified *Allium* sp.)	.	.	.	.	.	.	.	.	.	.
Celosia argentea var. *plumosa*	L_{cn}	–*	L_{cn}	–*	L_{cn}	–*	L_n	–*	L_n	–*
Chenopodium amaranticolor	–*	–*	(L_c)	–*	L_c	–*	L_n	–*	L_n	–*
C. murale	L_c	–*	.	.	L_{cn}	–*	L_{cn}	–*	–*	–*
C. quinoa	L_c	–*	L_{cn}	–*	L_{ce}	–*	L_n	–*	L_n	–*
Nicotiana benthamiana	.	.	–*	–*	l	–*	(l)	–	.	.
N. hesperis	.	.	l	–	l	(s)	(L_{ce})	–	.	.
N. occidentalis	l	(S_n)	l	S_{nc}	l	S_{nc}	(L_c)	–	L_{cn}	–*
Pisum sativum (pea)	–*	–*	.	.	(L_n)	–*	.	.	L_n	–*
Spinacia oleracea (spinach)	.	.	.	.	.	.	.	.	.	.
Vicia faba (broad bean)	–*	–*	–*	–*	–*	–*	(L_n)	–	L_n	(S_n)
Antiserum										
Carnation latent virus (the Netherlands)	.[5]		D±		.		.		.	
Garlic common latent virus (Germany)[6]	D+		D+		D+		D±		.	
'Garlic latent virus' (Japan)	D–		D–		.		D+		.	
'Garlic yellow stripe virus' (USA)[7]	.		.		D+		.		.	
Narcissus latent virus (the Netherlands)	.		D–		.		.		.	
Shallot latent virus (the Netherlands)	D–		D–		D–		D+		D+	

[1] Isolate supplied by Dr K. Graichen (Institut für Phytopathologie, Aschersleben, Germany), erroneously
[2] 'Japanese garlic latent virus' is identical to the garlic strain of shallot latent virus (see text).
[3] For cultivars and accessions see Materials and methods.
[4] The first and second symbol denote the reaction of inoculated and non-inoculated leaves, respectively: L = suffix c = chlorotic or yellow symptoms; suffix e = etching; suffix n = necrotic symptoms; –* = no symp-
[5] Reactions in decoration tests as follows: D+ = strong to fair reaction; D± = weak to very weak reaction; D–
[6] Antiserum to a virus incorrectly identified in Germany as 'garlic latent virus', and described here as the
[7] 'Garlic yellow stripe virus' (potyvirus) is a virus complex including garlic common latent virus (Van Dijk,

(SLV), and Sint-Jan's-onion latent virus (SjoLV) and their strains from different cultivated *Allium* species by

												SjoLV from			
		pearl onion				garlic				Utrechtse S. Jan's-onion		shallot	
		Asian shallot strain				Garlic strain[2]							
Ac205		Ac127II		Aa112		As199II		As391III		Ax101		Ac204I	
.	s	.	.	.	s	.	s	.	.	.	s	.	.
.	s	.	s	.	s	.	s	.	.	.	.	.	s
.	.	.	s	.	s	.	(s)	.	.	.	s	.	s
.	.	.	.	.	.	.	.	.	.	.	s	.	–
.	.	.	.	.	.	.	s	.	s	.	–	.	.
.	.	.	.	.	.	.	.	.	.	.	–	.	.
.	.	.	.	.	.	.	s	.	.	.	s	.	s
.	.	.	.	.	.	.	.	.	.	.	S	.	.
(L_n)	–*	–*	–*	–*	–*	(L_{cn})	–*	.	.	–*	–*	–*	–*
L_n	–*	–*	–*	(L_n)	–*	L_n	–*	L_n	–*	–*	–*	–*	–*
–*	–*	–*	–*	(L_e)	–*	(L_n)	–*	–*	–*	–*	–*	–*	–*
L_{cn}	–*	–*	–*	(L_n)	–*	L_n	–*	(L_n)	–*	–*	–*	–*	–*
–*	–*	–*	–*	–*	–*	(l)	–*	–*	–*	–*	–*	–*	–*
.	.	L_{ce}	–*	L_{ce}	–*	l	–	L_{ce}	–*	–*	–*	–*	–*
.	.	(L_{cn})	–*	(L_c)	–*	l	(s)	L_n	–*	–*	–*	–*	–*
.	.	.	.	.	.	.	.	(L_n)	–*	–*	–*	.	.
.	.	.	.	.	.	.	.	.	.	–*	–*	–*	–*
.	.	–*	–*	.	.	L_n	–*	L_n	–*	–*	–*	–*	–*
.		.		D±		.		D±		.		D–	
D–		.		D±		D±		D±		D+		D+	
D+		.		.		D+		D+		.		D+	
.		.		.		D–		.		D–		.	
.		.		D–		.		D–		.		D–	
D+		D+		D+		D+		D+		D+		D+	

identified as 'garlic latent virus'.

local lesions; l = latent local infection; S = systemic symptoms; s = latent systemic infection; – = no infection;
toms, but not tested for latent infection; ( ) = reaction very poor or variable; . = not tested.
= no reaction; . = not tested.
new garlic common latent virus (see Materials and methods).
1993b).

virus infections when investigated with inoculation tests or in the electron microscope, except for a single infection with ArMV (see before).

Tobacco necrosis virus (TNV) (necrovirus)
Tobacco necrosis virus was detected after inoculation of test species with sap of shallot roots (Table 1). The virus was identified by its characteristic host range and symptoms (necrotic local lesions in *Chenopodium* spp., French bean, *N. benthamiana*, *N. occidentalis*, *N. tabacum*, and additional systemic necrosis in *N. benthamiana*), and its isometric virus particles observed in the electron microscope.

Carlaviruses

Shallot latent virus (SLV)
Common strain. SLV is the first carlavirus of *Allium* spp. described; in the Netherlands it generally infects shallot and occasionally leek and onion (when planted next to shallot), without inducing symptoms (Bos et al., 1978). The type-isolate (Ac3) had been transmitted from a naturally infected shallot plant to leek for its purification and production of an antiserum (Bos et al., 1978). Mixed infection cannot be excluded by this procedure, especially when regarding the vegetative propagation of shallot. The SLV antiserum, however, did not react to mite-borne viruses from shallot (Van Dijk et al., 1991), proving their absence in the SLV culture. Infectivity of the type-material, after 13 years of storage over calcium chloride, appeared extremely low, since several attempts for its transmission to test plants resulted in only two local lesions in *Chenopodium amaranticolor*. Sub-isolates Ac3A and Ac3B, both fully reacting to SLV antiserum in decoration tests, were obtained by repeated single-lesion transfers. Chlorotic local lesions with necrotic

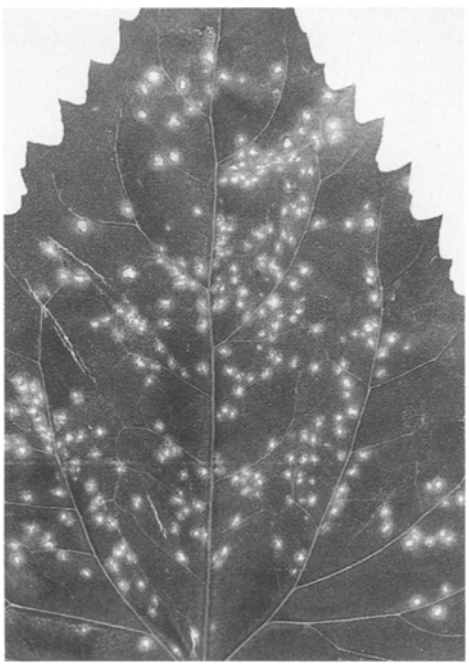

Fig. 2. Desiccating chlorotic local lesions of neotype-isolate Ac3B of shallot latent virus in *Chenopodium amaranticolor* some two weeks after inoculation.

centre of Ac3A in *C. amaranticolor* and *C. quinoa* were low in number and very small in size as compared with those of Ac3B in these species (Fig. 2). *C. murale* and *Celosia argentea* did not react to Ac3A at all, in contrast to their developing clear chlorotic/necrotic lesions with Ac3B. Ac3B could be transferred to onion, reacting with mild chlorosis, in local lesions of *C. quinoa*. Transmission of Ac3B and three other SLV isolates from *C. amaranticolor* to onion or leek failed. *C. amaranticolor* is known to be a poor donor host when no additives in the inoculum are used (Yarwood, 1972). In repeated inoculations, infectivity of Ac3A in sap of *C. quinoa* appeared too low for transfer of the isolate to onion, and it was abandoned.

Particles of the new type-isolate Ac3B were clearly decorated by antiserum to 'Japanese garlic latent virus', and only slightly by that to GCLV (provisionally called 'German garlic latent virus'). Its host range and reactions were similar to those described for SLV (Bos et al., 1978), except the necrotic local lesions in broad bean (*Vicia faba*) by Ac3B. Additional species tested included *Chenopodium foliosum* and *C. schraderianum* (= *C. foetidum*), which did not react, crow garlic (*Allium vineale*), which appeared very susceptible, and *Nicotiana benthamiana*, *N. hesperis* and *N. occidentalis*, the inoculated leaves of which proved symptomlessly infected, the latter two species at high concentration. None of nine plants of garlic 'Thermidrôme', inoculated twice with highly infectious inoculum from leek, became infected (Table 2).

Carlavirus isolates resembling Ac3B in serological properties, but often considerable differing from it in host reactions, could be isolated from shallot plants from different origins (Table 1). The majority of the isolates induced only a poor reaction in *Chenopodium* spp., resembling that of Ac3A. Local lesions in *C. quinoa* appeared as very small green rings with a necrotic centre and few necrotic spots of different size. *Celosia argentea* reacted clearly to most isolates (Fig. 3) but very poorly to Ac205 and not at all to Ac203. Part of the isolates differed from Ac3B by causing yellow to necrotic local

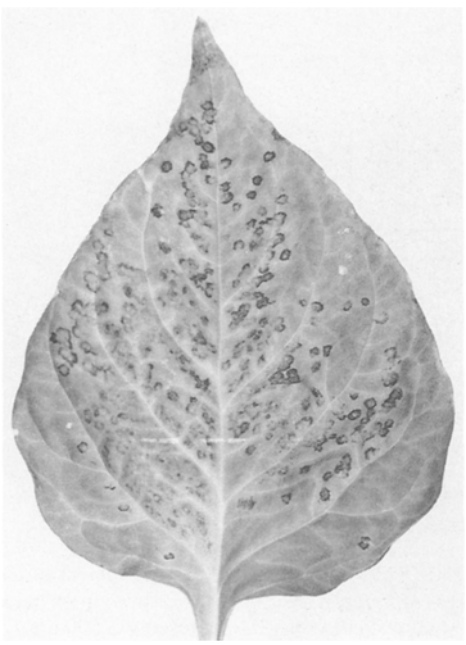

Fig. 3. Local lesions in *Celosia argentea* var. *plumosa* 'Geisha' three weeks after inoculation by shallot latent virus, isolate Ac115.

lesions in *N. occidentalis* (Table 1, 2) - such lesions were not observed in *N. benthamiana* and *N. hesperis* or only very faintly in the latter - but resembled Ac3A or Ac3B in further hosts.

SLV was also found in ever-ready onion (*A. cepa* var. *perutile*) (Fig. 4), grey shallot (unidentified *Allium* species), multiplier onion (*A. cepa* var. *aggregatum*), pearl onion (*A. ampeloprasum* var. *sectivum*), rakkyo (*A. chinense*), and Welsh onion (*A. fistulosum*). Leek and onion, known to occasionally harbour the virus when grown near shallot (Bos et al., 1978), did not contain SLV. This can be explained by the fact that the samples in this survey were not collected in the vicinity of shallot. Occurrence of SLV in leek in complex with the potyvirus leek yellow stripe virus in Denmark and Germany, and aggravating symptoms of the latter virus (Paludan, 1980; Lesemann et al., 1991), was not found in the Netherlands (Van Dijk, 1993b).

Asian strain. Isolates from Chinese (Ac130II), Indonesian (Ac127II and Ac142), and Thai (Ac177II, Ac188 and Ac 266II) shallot, and from Indonesian pearl onion (Aa112) differed markedly from European SLV isolates by not reacting in *Celosia argentea* and *Chenopodium* spp., or inducing only tiny green rings with a necrotic pin-point centre in *C. quinoa*, and causing chlorotic local lesions with etching and gradual desiccation in *N. hesperis* (Fig. 5) and *N. occidentalis* (Table 2). The green rings of Ac130II and Ac142 in *C. quinoa* were not observed when the isolates were propagated in *Nicotiana* spp. Serological reactions resembled those of European SLV isolates (Table 2). Isolates Ac127II and Ac130II, of what may now be called the Asian strain of SLV, occurred in mixed infection with the common strain of the virus (Ac127III and Ac130IV).

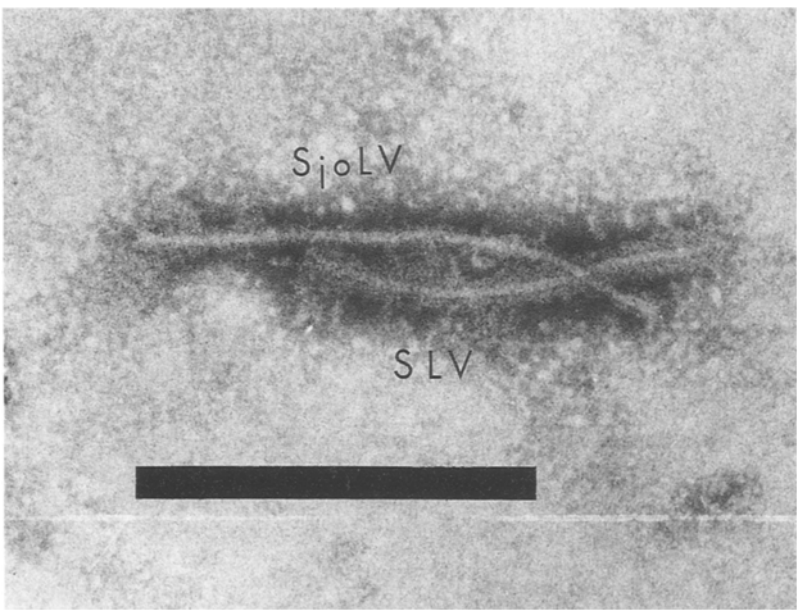

Fig. 4. Particle of shallot latent virus isolate Ac391II (SLV) and of Sint-Jan's-onion latent virus isolate Ac391III (SjoLV) in sap of ever-ready onion (*Allium cepa* var. *perutile*), strongly or only partially decorated, respectively, with antiserum to shallot latent virus. Bar represents c. 500 nm.

Fig. 5. Local lesions in *Nicotiana hesperis* accession 67A, 12 days after inoculation with the Asian strain of shallot latent virus (isolate Ac127II).

Garlic strain (SLV-G). Few isolates from garlic induced local lesions in *Celosia argentea* and *Chenopodium* spp. smaller in size and number and sharper and more necrotic in appearance than those of the carlavirus isolates usually present in garlic (see below under GCLV). Such isolates also differed from GCLV by causing local lesions in *Vicia faba* and not reacting systemically in *N. occidentalis*, and reacting only weakly with antibodies to GCLV (Table 2). Host reactions, especially necrotic local lesions in *C. amaranticolor* and *C. quinoa*, and full decoration by the 'Japanese garlic latent virus' antiserum identify the isolates as the 'garlic latent virus' described by Lee et al. (1979) in Japan. Reactions obtained in my tests differing from those in Japan are infection of onion and *C. murale*, and necrotic local lesions (Fig. 6) instead of systemic necrotic spots in *Vicia faba*. Full decoration by SLV antiserum and failure of SLV to infect garlic (see before), however, further identify 'Japanese garlic latent virus' from garlic as a garlic strain of SLV (SLV-G). This result conflicts with the only distant serological relationship between 'Japanese garlic latent virus' and SLV reported earlier at Wageningen (Bos, 1983), but is agreed upon in Japan (Dr T. Inouye, personal communication, 1993).

Most data were obtained with As199II, separated from GCLV (As199III) by several single-lesion transfers in *C. amaranticolor* and *Celosia argentea*, and subsequent transmission to leek and onion via the highly susceptible crow garlic to raise infectivity of the inoculum. A host-range variant of SLV-G isolated from garlic 'Lumbu Hijau' in Indonesia (Van Dijk and Sutarya, 1992) resembled variants of SLV from shallot in inducing chlorotic/necrotic local lesions in *N. hesperis* and *N. occidentalis* (As391III, Table 1, 2; Fig. 7).

SLV had been detected in 3 out of 12 European garlic samples by its decoration by SLV antiserum in England (Walkey et al., 1987). Practically the same incidence (6 out of 23) was found in my tests with garlic from different parts of the world (Table 1). Conci et al. (1992) did not detect 'Japanese garlic latent virus' or SLV in the three most commonly

Fig. 6. Small necrotic rings in inoculated leaves of broad bean (*Vicia faba*) 'Driemaal Wit' 18 days after inoculation with isolate As199II of the garlic strain of shallot latent virus.

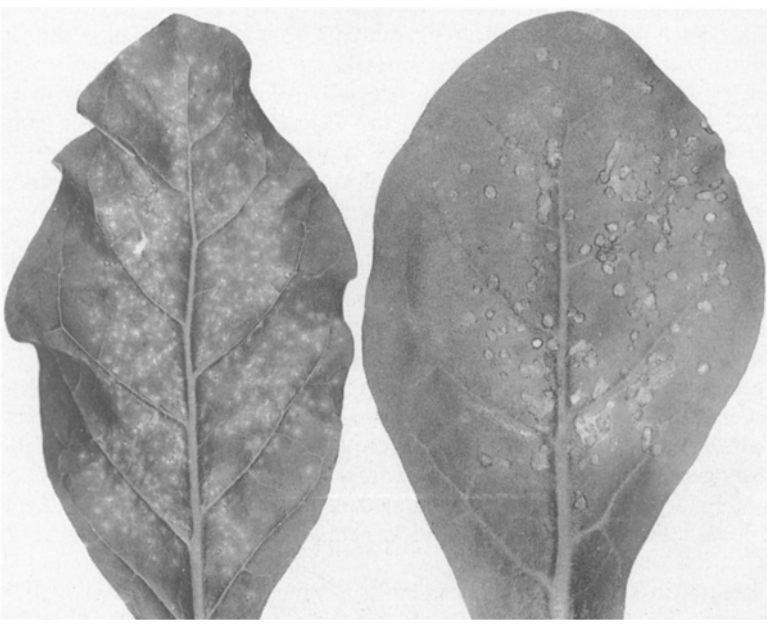

Fig. 7. Local lesions induced by the garlic strain of shallot latent virus (isolate As391III) in *Nicotiana hesperis*-67A (left) and *N. occidentalis*-P1 (right).

grown garlic cultivars in Argentina. In contrast, garlic in Japan was found almost universally infected with 'Japanese garlic latent virus' (Lee et al., 1979), and the only Japanese sample tested also contained the virus (As328III; Table 1).

'Garlic latent virus' in Japan, after its description from garlic, was also reported from ornamental leek (Inouye et al., 1981), rakkyo (Sako, 1989; Sako et al., 1989, 1991) and Welsh onion (Fukami et al., 1988). The 'Japanese garlic latent virus' originally described in garlic did not infect onion and Welsh onion (Lee et al., 1979), whereas isolates from rakkyo infect these species (Sako et al., 1991). The virus reported from Japan should be called SLV because SLV (Bos et al., 1978) was described before the 'Japanese garlic latent virus' (Lee et al., 1979). Serological detection methods for SLV in Japan, however, may not discriminate between SLV and SjoLV, which is usually also present (see below).

Garlic common latent virus (GCLV)

Decoration tests in France showed the general presence in French garlic of a carlavirus not reacting to SLV antiserum, for which the name 'garlic latent virus' was proposed without reference to the Japanese virus of that name (Delecolle and Lot, 1981). These results were confirmed in England and the almost universal presence in European garlic cultivars of carlavirus particles serologically unrelated to SLV was shown (Walkey et al., 1987).

Likewise in my inoculation tests, an apparently undescribed carlavirus could readily be isolated from almost any garlic sample investigated. Chlorotic local lesions or green rings with necrotic etching in *C. quinoa* (Fig. 8) showed up after c. 12 days, and gradually became necrotic. Such lesions in *C. murale* greatly resembled those of the garlic strain of onion mite-borne latent virus (OMbLV-G), and green rings in *C. amaranticolor* appeared late and were faint. *Celosia argentea* also reacted with chlorotic/necrotic local lesions, which were distinct but with some isolates non-necrotic and faint. *N. occidentalis* reacted

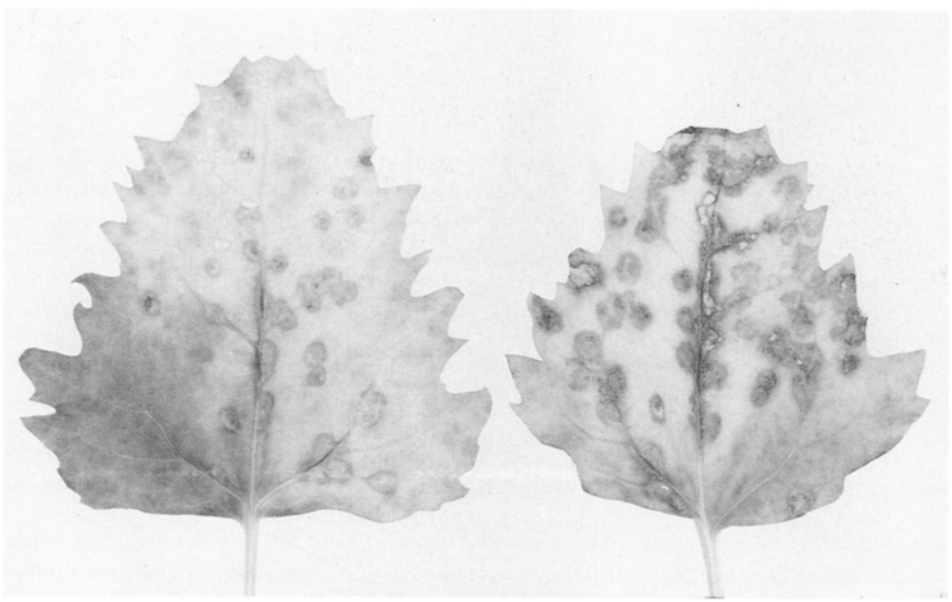

Fig. 8. Local lesions of garlic common latent virus (isolate As121) in *Chenopodium quinoa*: green rings in young leaves (left), and dry rings in old leaves of the same plant (right).

to part of the isolates (Table 1) with systemic vein necrosis of varying severity (Fig. 9) without developing local lesions (Table 2). Onion became infected with only two of the isolates (As182III and As199III) in mixed infection with SLV-G. Multiplication and maintenance of uncontaminated virus cultures, obtained by transfers through *N. occidentalis* or via single lesions of other species, was also possible in leek. The isolate As224 of 'German garlic latent virus', kindly supplied by Dr K. Graichen in Germany, induced similar reactions (Table 1, 2).

The test plant reactions observed obviously differed from the predominantly necrotic reactions in inoculated leaves, also in broad bean, of SLV-G ('Japanese garlic latent virus') (see before). Particles of selected isolates (As123II, As188II, As199III and As224) were not decorated by the antisera to SLV-G (from Japan) and SLV, in contrast to their full decoration by the antiserum of Dr K. Graichen and fair decoration by the antiserum to the 'garlic yellow stripe virus' complex (Table 2). Graichen's antiserum was already reported to contain antibodies to OMbLV-G in addition to those to the carlavirus (Van Dijk et al., 1991). Carlavirus particles in garlic imported from Argentina (As336IV) were decorated to a higher degree and were more rigid than those of OMbLV-G also present (Van Dijk et al., 1991). The present biological and serological observations make it clear that the tentative identification by Graichen and Leistner (1987) in Germany of the carlavirus commonly present in garlic as the 'Japanese garlic latent virus' of Lee et al. (1979) cannot be maintained. The same virus is indicated by the name 'garlic latent virus' used by Abo el-Naga et al. (1989) and Lesemann et al. (1992), although the latter virologists took the name from the French (Delecolle and Lot, 1981; Dr D.-E. Lesemann, personal

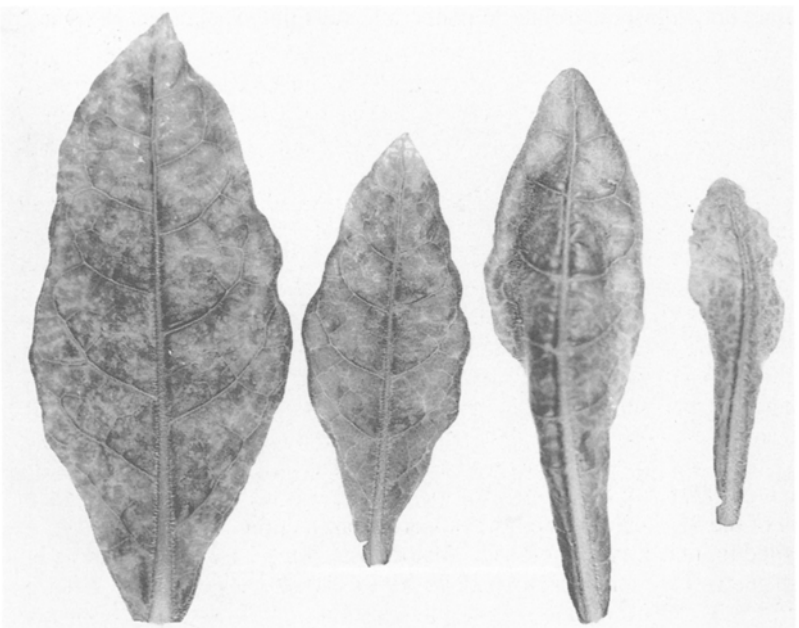

Fig. 9. Vein necrosis and etching induced in non-inoculated leaves of *Nicotiana occidentalis* by garlic common latent virus. Note the more severe necrosis and malformation caused by isolate As199III (middle right and extreme right; 11 days after inoculation) as compared with symptoms of isolate As123II (extreme left and middle left; 23 days after inoculation).

communication, 1993). Rather, it appears that this is a new carlavirus for which the name garlic common latent virus (GCLV) is proposed. This name seems appropriate because the virus does not induce clear symptoms in garlic, if at all, and it is widely present in garlic samples from several parts of the world in contrast with the true 'Japanese garlic latent virus' (SLV-G) (Table 1). Noteworthy in this respect is the absence of GCLV or any other carlavirus in a sample (As120) of Iranian garlic.

GCLV was also isolated from an unidentified *Allium* species resembling garlic but forming true seed (Ax11III) (Van Dijk, 1993b) and from great-headed garlic (*A. ampeloprasum* var. *holmense*) (Table 1). Great-headed garlic isolates Aa128III and Aa129 from Israel and Japan fully resembled garlic GCLV isolates, but Aa127II and Aa127III from former Yugoslavia deviated by their decoration by SLV antiserum. Aa127III also deviated from GCLV by its inducing necrotic local lesions in *Celosia argentea* and *Chenopodium quinoa* resembling those of SLV. Aa127II and Aa127III were tentatively identified as GCLV instead of as SjoLV (see below) because their decoration by GCLV antiserum was stronger than that with SLV antiserum.

GCLV was also occasionally found in leek grown next to garlic (Table 1). The virus had also been found in leek in Germany in complex infection with the potyvirus leek yellow stripe virus (Graichen et al., 1987; Lesemann et al., 1991).

Sint-Jan's-onion latent virus (SjoLV)

Carlavirus particles in symptomless plants of Sint-Jan's onion or Utrechtse Sint-Jan's onion (unidentified *Allium* species, earlier incorrectly identified as rakkyo), grown in Utrecht, the Netherlands (Ax101 and Ax103), and resembling crops originating from Hongkong (Ax112 and Ax113) (Table 1), all became partially or fairly decorated in the electron microscope by both the GCLV and SLV antiserum (Fig. 4). These reactions contrasted with the strong decoration of particles of GCLV and SLV by their homologous antiserum (Fig. 4) and the slight decoration, if at all, by the heterologous antiserum (see above and Table 2). Antibodies to GCLV in the antiserum to 'garlic yellow stripe virus' did not decorate particles of Ax101 (Table 2), but the homologous reaction also was less strong than usual.

When a large number of plants of shallot 'Santé' were tested onto *Celosia argentea*, *Chenopodium* spp. and *Nicotiana* spp., a few 'Santé' plants were found not to contain SLV or OMbLV, as concluded from absence of reaction in these indicators. Electron microscope decoration tests with antisera to SLV and GCLV confirmed the absence of these viruses, and further showed the presence of the same new carlavirus (Ac204I and Ac206I) as that in Utrechtse Sint-Jan's onion, in mixed infection with shallot mite-borne latent virus (SMbLV). The new carlavirus isolates from 'Santé' and those from Utrechtse Sint-Jan's onion greatly resembled SMbLV in host range and symptoms (Van Dijk et al., 1991). Ac204I was separated from SMbLV by chance when inoculated via onion onto crow garlic. Its fair reaction with 'Japanese garlic latent virus' (SLV-G) antiserum (Table 2) makes it unlikely that the reaction of the new virus with SLV antiserum is due to contamination of the SLV culture used for antiserum production.

All isolates failed to induce symptoms in indicator hosts. They could be transmitted to crow garlic and onion, and some but not all of them also to leek and Welsh onion (Table 2). No infection was obtained in Chinese chives, chives, *Chlorophytum comosum*, Formosan lily, and garlic.

Further decoration studies with SLV and GCLV antisera revealed serologically similarly reacting virus particles in combination with SLV particles in ever-ready onion (Fig. 4), Papago l'Itoi (a crop resembling Utrechtse Sint-Jan's onion), pearl onion, rakkyo, shallot 'Noordhollandse Strogele', and Welsh onion (Table 1). The rakkyo samples also

contained non-decorating and highly flexible particles of mite-borne viruses (Van Dijk, 1993a). Earlier use of SLV antiserum at Wageningen had already shown mixtures of fully and partially decorated carlavirus particles in shallot 'Noordhollandse Strogele' (Dr L. Bos, IPO-DLO, pers. comm.). The late discovery that the virus essentially differs from SLV explains why relatively few isolates of the new virus are listed in Table 1.

The only carlavirus thus far reported in rakkyo and Welsh onion is 'Japanese garlic latent virus' in Japan (Fukami et al., 1988; Sako, 1989; Sako et al., 1989, 1991), now regarded as SLV. The virus in Sint-Jan's onion and the serologically similar isolates from other hosts differ from SLV (including 'Japanese garlic latent virus') and GCLV in their fairly close serological relationships to both viruses and in test plant reactions, and hence are identified as a new virus. The virus was provisionally named 'Welsh-onion latent virus' as it was generally detected in Welsh-onion crops in Indonesia (Van Dijk and Sutarya, 1992). The name Sint-Jan's-onion latent virus (SjoLV) seems more appropriate, however, because SjoLV is the only carlavirus present in Sint-Jan's onion.

Transmissibility of carlavirus isolates by aphids

Carlaviruses like potyviruses are known to be transmitted in a non-persistent mode by aphid species. Transmission experiments of SLV have been successful with *Myzus ascalonicus*, but negative or doubtful with *Aphis fabae* and *M. persicae* (Bos et al., 1978).

In attempts to free potyviruses from their association with a carlavirus by transmission with large numbers of aphids (Van Dijk, 1993b), only two out of seven potyvirus isolates remained in complex with the carlavirus: onion yellow dwarf virus isolate Ac132I with SLV isolate Ac132II, and Welsh-onion yellow stripe virus (WoYSV) isolate Af12I with SLV isolate Af12II (Table 1). Aphid transmission does not seem to be a good way to single out carlavirus isolates, since no carlavirus isolate was freed from the potyvirus in these experiments. The stability of the Af12I/Af12II-complex transmitted to two plants of crow garlic sharply contrasts with that of WoYSV isolate Af19I and the SLV and SjoLV isolates (Af19II and Af19III) also present in the Welsh onion source plants, from which only the potyvirus was transmitted to ten plants of crow garlic. It is unknown whether these differences in relative transmissibility of the viruses when in complex are determined by the carlavirus isolates or the potyvirus isolates.

General discussion

The present study of carlaviruses infecting cultivated *Allium* spp. is based on selective isolation and differentiation in susceptible and sensitive indicator hosts. For this, *Celosia argentea* var. *plumosa* 'Geisha', *Nicotiana hesperis* accession 67A and *N. occidentalis* accession P1 have now proved very useful besides the commonly used *Chenopodium* spp. and broad bean. The two *Nicotiana* accessions originate from the arid parts of West Australia, known to be a source of virus-sensitive *Nicotiana* genotypes (Van Dijk et al., 1987). No indicator plant was found for the new carlavirus SjoLV, which could only be detected and identified by electron microscope decoration tests.

The serological identification of biologically different carlavirus isolates is to be regarded tentative because it was based on four antisera to isolates of unknown purity. Further identification therefore requires the preparation of antisera to isolates obtained in pure culture. Some isolates of GCLV and of SLV, including the garlic and Asian shallot strains of SLV, easily infect *Nicotiana* accession 67A and/or accession P1, which will facilitate their separation and subsequent multiplication for purification and antiserum production.

Carlaviruses may show serological cross reactivity (Koenig, 1982). This implies that their serological identification requires comparitive tests with different antisera. For example, the reactions of a carlavirus in garlic in Argentina to carnation latent virus (CLV) antisera (Conci and Nome, 1991; Conci et al., 1992) might be explained by the cross reactivity of GCLV present in Argentinean garlic (As336IV, Table 1) and CLV. In my tests no reaction was found of any of the carlaviruses reported here with narcissus latent virus (NLV) antiserum (Table 2), but SLV earlier reacted to one out of two NLV antisera (Bos et al., 1978). The NLV detected in garlic in England (Walkey, 1990) therefore needs comparison with SLV-G. Likewise, reactions of carlavirus in garlic in China with antisera to potato viruses S and M (Rongchang et al., 1992) needs re-examination. A number of *Allium* species other than garlic known to be infected with SLV (Bos, 1982) or 'Japanese garlic latent virus' (Inouye et al., 1981; Fukami et al., 1988; Sako, 1989; Sako et al., 1989, 1991) now appear to be generally infected with mixtures of SjoLV and SLV (Table 1). The serological tests developed in Japan for rapid detection of 'Japanese garlic latent virus' (SLV) infections in rakkyo and Welsh onion (Fukami et al., 1988; Sako, 1989) have limited value because they probably do not discriminate between SLV and the cross-reacting SjoLV.

An epidemiologically interesting question is whether wild plants are a virus source for cultivated *Allium* species or not. None of over 200 plants of four wild *Allium* species from the Netherlands tested here contained carlaviruses. These species, except ramsons, propagate mainly vegetatively, and the majority of the samples were from crow garlic collected in the shallot-growing area around Ouddorp, a centre of shallot cultivation. Wild species that were already shown to be no virus source of mite-borne viruses (Van Dijk et al., 1991) or potyviruses (Van Dijk, 1993b) in the Netherlands, appear not to be an infection source of carlaviruses either. Natural transmission of SLV and GCLV from crops of shallot and garlic, respectively, to leek crops (Bos et al., 1978; Paludan, 1980; Graichen et al., 1987; Lesemann et al., 1991; this paper), indicate that carlaviruses are less host-specialized than the mite-borne and aphid-borne potyviruses of *Allium* spp. (Van Dijk et al., 1991; Van Dijk, 1993b). Failure to transmit SLV from shallot to garlic (Table 2), and to transmit 'Japanese garlic latent virus' (SLV-G) from garlic to onion or Welsh onion (Lee et al., 1979), suggests that some host specialization exists.

Three nematode-borne viruses, i.e. ArMV, TBRV, and TRV, have been reported in wild or cultivated *Allium* spp., and these were also found during my survey. Their extremely low incidence in these *Allium* species which are primarily vegetatively propagated is difficult to explain.

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References

Abo el-Naga, M., Schmidt, J. & Fiedler, W., 1989. Virus-free propagation of garlic (*Allium sativum*) through meristem tip culture. Poster abstract of 'Science for Plant Breeding', XII. Eucarpia Congress, 1989. Vorträge für Pflanzenzüchtung 15–II: 26–6, 2 pp.

Bos, L., 1982. Shallot latent virus. CMI/AAB Descriptions of Plant Viruses No. 250, 4 pp.

Bos, L., 1983. Viruses and virus diseases of *Allium* species. Acta Horticulturae 127: 11–29.

Bos, L., Huttinga, H. & Maat, D.Z., 1978. Shallot latent virus, a new carlavirus. Netherlands Journal of Plant Pathology 84: 227–237.

Bremer, K., 1990. Production of virus-free plants of vegetatively propagated onion *Allium cepa* L. Annales Agriculturae Fenniae 29: 1–7.

Brunt, A.A., 1976. Narcissus latent virus. CMI/AAB Descriptions of Plant Viruses No. 170, 4 pp.

Calvert, E.L. & Harrison, B.D., 1963. Outbreaks of tomato black ring virus in onion and leek crops in Northern Ireland. Horticultural Research 2: 115–120.

Conci, V.C. & Nome, S.F., 1991. Virus free garlic (*Allium sativum* L.) plants obtained by thermotherapy and meristem tip culture. Journal of Phytopathology 132: 186–192.

Conci, V.C., Nome, S.F. & Milne, R.G., 1992. Filamentous viruses of garlic in Argentina. Plant Disease 76: 594–596.

Delecolle, B. & Lot, H., 1981. Viroses de l'ail: I. - Mise en évidence et essais de caractérisation par immunoélectromicroscopie d'un complexe de trois virus chez différentes populations d'ail atteintes de mosaïque. Agronomie 1: 763–769.

De Carvalho, M.G., 1980. Identification and properties of viruses in garlic. Dissertation PhD, University of California, Davis, California, 60 pp.

De Carvalho, M.G., 1981. Viroses do alho (Abstract in Portuguese). Fitopatologia Brasileira 6: 299–300.

Fukami, M., Natsuaki, K.T. & Tomaru, K., 1988. Application of gelatin particle agglutination test for simple and rapid detection of garlic latent virus in Welsh onion. Proceedings of the 5th International Congress of Plant Pathology, Kyoto, Japan, 20–27 August 1988: 49.

Graichen, K., 1975. *Allium*-Arten als natürliche Wirte nematodenübertragbarer Viren. Archiv für Phytopathologie und Pflanzenschutz 11: 399–403.

Graichen, K. & Leistner, H.-U., 1987. Zwiebelgelbstreifen-Virus (onion yellow dwarf virus) verursacht Knoblauchmosaik. Archiv für Phytopathologie und Pflanzenschutz 23: 165–168.

Graichen, K., Proll, E., Richter, J. & Leistner, H.-U., 1987. Reinigung und serologischer Nachweis des Porreegelbstreifen-Virus (leek yellow stripe virus). Archiv für Phytopathologie und Pflanzenschutz 23: 501–504.

Inouye, N., Maeda, T. & Mitsuhata, K., 1981. On garlic latent virus isolated from ornamental *Allium ampeloprasum* (Abstract in Japanese). Annals of the Phytopathological Society of Japan 48: 114.

Koenig, R., 1982. Carlavirus group. CMI/AAB Descriptions of Plant Viruses No. 259, 4 pp.

Kristensen, H.R. & Engsbro, B., 1966. Undersøgelser og forsøg vedrørende jordbårne vira. I Rattlevirus. Tidsskrift for Planteavl 70: 353–379.

Lee, Y.W., Yamazaki, S., Osaki, T. & Inouye, T., 1979. Two elongated viruses in garlic, garlic latent virus and garlic mosaic virus. Annals of the Phytopathological Society of Japan 45: 727-734.

Lesemann, D.-E., Vetten, H.J., Maiss, E. & Dalchow, J., 1991. Vorkommen von in Deutschland neu aufgetretenen Viren in Gemüsekulturen. BBA Jahresberichte 1990: H51–H52.

Lesemann, D.-E., Vetten, H.J., Barg, E. & Graichen, K., 1992. Differentiation of carlaviruses infecting *Allium*. In: Recent advances in vegetable virus research. Abstracts of the 7th Conference of the ISHS Vegetable Virus Working Group, Athens, Greece, July 12–16, 1992: 35–36.

Murant, A.F., 1970a. Arabis mosaic virus. CMI/AAB Descriptions of Plant Viruses No. 16, 4 pp.

Murant, A.F., 1970b. Tomato black ring virus. CMI/AAB Descriptions of Plant Viruses No. 38, 4 pp.

Rongchang, C., Xiaolong, L. & Xuezhan, L., 1992. Evaluation of mosaic virus and meristem tissue culture for garlic (In Chinese). Chinese Vegetables 52: 10–14.

Paludan, N., 1980. Virus attack on leek: Survey, diagnosis, tolerance of varieties and winter hardi-

ness. Tidsskrift for Planteavl 84: 371–385.
Sako, I., 1989. Infection of garlic latent virus in rakkyo (*Allium chinense* G. Don) and detection of the virus by serological techniques. Bulletin of the Tottori Prefecture Horticultural Experiment Station 6: 1–16.
Sako, I., Nakasone, W., Okada, K., Osaki, T. & Inouye, T., 1989. Occurrence and incidence of garlic latent virus in rakkyo (*Allium chinense* G. Don). Proceedings of Kansai Plant Protection 31: 23–29.
Sako, I., Nakasone, W., Okada, K., Ohki, S.T., Osaki, T. & Inouye, T., 1991. Yellow streak of rakkyo (*Allium chinense* G. Don), a newly recognized disease caused by garlic latent virus and onion yellow dwarf virus. Annals of the Phytopathological Society of Japan 57: 65–69.
Van Dijk, P., 1993a. Virus diseases of *Allium* species and prospects for their control. Paper presented at the International Symposium 'Alliums for the Tropics', Bangkok, Thailand, 15–19 February 1993. Acta Horticulturae 137: (In press).
Van Dijk, P., 1993b. Survey and characterization of potyviruses and their strains of *Allium* species. Netherlands Journal of Plant Pathology 99, Supplement 2: 1–48.
Van Dijk, P., Van der Meer, F.A. & Piron, P.G.M., 1987. Accessions of Australian *Nicotiana* species suitable as indicator hosts in the diagnosis of plant virus diseases. Netherlands Journal of Plant Pathology 93: 73–85.
Van Dijk, P. & Sutarya, R., 1992. Virus survey of garlic, shallot and Welsh onion in Java, Indonesia. Internal Communication of the DLO Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, the Netherlands, 70 pp.
Van Dijk, P., Verbeek, M. & Bos, L., 1991. Mite-borne virus isolates from cultivated *Allium* species, and their classification into two new rymoviruses in the family *Potyviridae*. Netherlands Journal of Plant Pathology 97: 381–399.
Van Slogteren, D.H.M., 1958. Ratelvirus als oorzaak van ziekten in bloembolgewassen en de mogelijkheden de infectie door middel van grondontsmetting te bestrijden. Tijdschrift over Plantenziekten 64: 452–462.
Verhoyen, M. & Horvat, F., 1981. Identification des virus de l'échalote (*Allium ascalonicum* L.) en Belgique. Parasitica 37: 79–86.
Walkey, D.G.A., 1990. Virus diseases. In: Rabinowitch, H.D. & Brewster, J.L. (Eds), Onions and allied crops, Volume II. CRC Press, Inc., Boca Raton, Florida: 191–212.
Walkey, D.G.A., Webb, M.J.W., Bolland, C.J. & Miller, A., 1987. Production of virus-free garlic (*Allium sativum* L.) and shallot (*A. ascalonicum* L.) by meristem-tip culture. Journal of Horticultural Science 62: 211–220.
Wetter, C., 1971. Carnation latent virus. CMI/AAB Descriptions of Plant Viruses No. 61, 3 pp.
Yarwood, C.E., 1972. Virus transmission from *Chenopodium amaranticolor*. Plant Disease Reporter 56: 1085–1086.