Neth. J. Pl. Path. 97 (1991) 381-399

Mite-borne virus isolates from cultivated Allium species, and their classification into two new rymoviruses in the family Potyviridae

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Accepted 12 September 1991

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Abstract

While testing several samples of onion and of vegetatively propagated garlic, sand leek and shallot from a number of countries, virus isolates with unusually flexuous particles were obtained by mite (*Aceria tulipae*) or sap transmissions. No aphid-borne poty- or carlavirus was transmitted by mites, and mite-borne virus isolates could not be transmitted by aphids. The mite-borne isolates did not react with antisera to aphid-borne potyviruses of *Allium* spp. or with the Agdia potyvirus group monoclonal. In contrast to the mite-borne onion and garlic mosaic viruses reported in the literature, our mite-borne isolates induced no visible or only very mild symptoms in *Allium* spp., except isolates from shallot 'Santé' which caused diffuse striping. Heavily mite-infested test plants or plant samples showed streaking and malformation due to mite feeding (tangle-top).

The mite-borne virus isolates could be classified with test plants and a discriminating antiserum into three groups, representing two viruses and a strain of one of them. They are tentatively named onion mite-borne latent virus (OMbLV), garlic strain of this virus (OMbLV-G), and shallot mite-borne latent virus (SMbLV). Mite transmission, length of virus particles (ca. 700 to 800 nm), and the presence of granular inclusion bodies in infected tissue indicate that the viruses belong to the mite-borne genus *Rymovirus* of the family *Potyviridae*. OMbLV from shallot and onion, and OMbLV-G from garlic and sand leek, can be assayed on *Chenopodium murale* but differ in their natural hosts. They are very common. SMbLV, to which *C. murale* does not react, was isolated from shallot originating from Asia and Russia.

Additional keywords: Aceria tulipae, dry bulb mite, garlic, garlic latent virus, garlic mosaic virus, garlic yellow streak virus, onion, onion mite-borne latent virus, onion mosaic virus, onion yellow dwarf virus, potyvirus-specific IgG, sand leek, shallot, shallot mite-borne latent virus, tangle-top, wheat curl mite.

Introduction

Crops of *Allium* spp. are vulnerable to virus infection. Viruses are prevalent in vegetatively propagated species such as garlic (*A. sativum*) and shallot (*A. cepa* var. *ascalonicum*). They may become epidemic in onion (*A. cepa*) when replanted from previous year's sets or from bulbs for seed, and in leek (*A. ampeloprasum* var. *porrum*) when grown the year around.

Some years ago the aphid-borne leek yellow stripe (LYSV) and onion yellow dwarf

(OYDV) potyviruses (Bos et al., 1978a) and shallot latent (SLV) carlavirus (Bos et al., 1978b) were characterized, and antisera to them became available. Meanwhile the identity of several other *Allium* viruses remained obscure (Bos, 1983; Walkey, 1990). These include two garlic mosaic potyviruses and a garlic latent carlavirus in Japan (Lee et al., 1979; Abiko et al., 1980), garlic yellow streak potyvirus in New Zealand (Mohamed and Young, 1981), and a garlic latent virus in Germany (Graichen and Leistner, 1987). Two other viruses of uncertain identity, the so-called onion mosaic virus in Russia (Razvjazkina et al., 1969; Razvjazkina, 1971; Tulegenev, 1972; Cheremushkina, 1974, 1975, 1982) and a garlic mosaic virus in the Philippines (Ahmed and Benigno, 1984, 1985a, b), with flexuous particles of 675 nm and 696 nm, respectively, were reported to be transmitted by *Aceria* (*Eriophyes*) *tulipae* Keifer, the dry bulb mite or wheat curl mite (Lange and Mann, 1960; Slykhuis, 1980; Cheremushkina, 1982; Hafez and Maksoud, 1983).

During our research on viruses of *Allium* crops, mite-borne virus isolates were obtained from vegetatively propagated garlic, sand leek (*A. scorodoprasum*) and shallot from different parts of the world, and from Russian onion. The particles of these isolates were more flexuous than those of aphid-borne poty- and carlaviruses. This report characterizes such isolates by host range and symptoms, identifies them as two new members of the proposed *Rymovirus* genus of the family *Potyviridae* (Barnett, 1991, personal communication 1991), and distinguishes a strain of one of these viruses.

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' and 'Rostovski' were from bulbs grown at IPO from seed. Bulbs or plants of crow garlic (*Allium vineale*) were collected in the field, and plants of garlic 'Germidour' and 'Thermidrôme' and rakkyo (*A. chinense*) landrace Utrechtse Sint-Jan's-ui were from bulbs obtained from commercially grown plants with very mild symptoms. Virus-free garlic plants 'Rocambole' were obtained by meristem-tip culture. *Amaranthus bicolor* 'Perfecta', *A. caudatus*, *A. cruentes* 'Marianne', *A. hypochondrianum* 'Green Thumb', *A. tricolor* 'Molton Fire', *Atriplex hortensis* 'Red Plume', broad bean (*Vicia faba*) 'Compacta' and 'Dubbele Witte', *Celosia argentea* var. *plumosa* 'Geisha', *Gomphrena globosa*, leek 'Zwitserse Reuzen' selection Jolant, sand leek (*Allium scorodoprasum*; erroneously named garlic), spinach (*Spinacia oleracea*) 'Nores', *Tetragonia expansa*, and Welsh onion (*A. fistulosum*) 'Kyoto Market' were all grown from commercial seed. Chive (*A. schoenoprasum*), Chinese chive (*A. tuberosum*) and *Lilium formosanum* were raised from seed provided by the former Institute for Horticultural Plant Breeding, 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 accession P1, originally obtained from Kings Park and Botanic Garden, West Perth, Australia. *Chenopodium* spp. and *N. benthamiana* were from seed stocks regularly used at IPO. Plants were grown in aphid-proof glasshouses at 18-20 °C.

Virus isolates. Virus isolates, method of their isolation, and data on the infected *Allium* samples from which they were obtained, are specified in Table 1. Several representative isolates have been preserved for reference and further characterization (Table 1).

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

Virus transmission by mites. In initial attempts at virus transmission with mites, mites were transferred by inserting pieces of mite-infested bulb scales from infected samples into cuts made in leaves of test plants of onion. To avoid mechanical transmission, this technique was soon modified, and thin slices of mite-infested bulb scales or whole mite-infested sprouts or leaves from samples were placed inside a paper cone attached around leaves of test plants (Fig. 1). The test plants were usually incubated for several days in a nontransparent paper bag before transfer to the glasshouse. Another way of transmission was by storing sprouting onion sets together with mite-infested bulbs in a paper bag for at least a week in the dark at ca. 30 °C, followed by

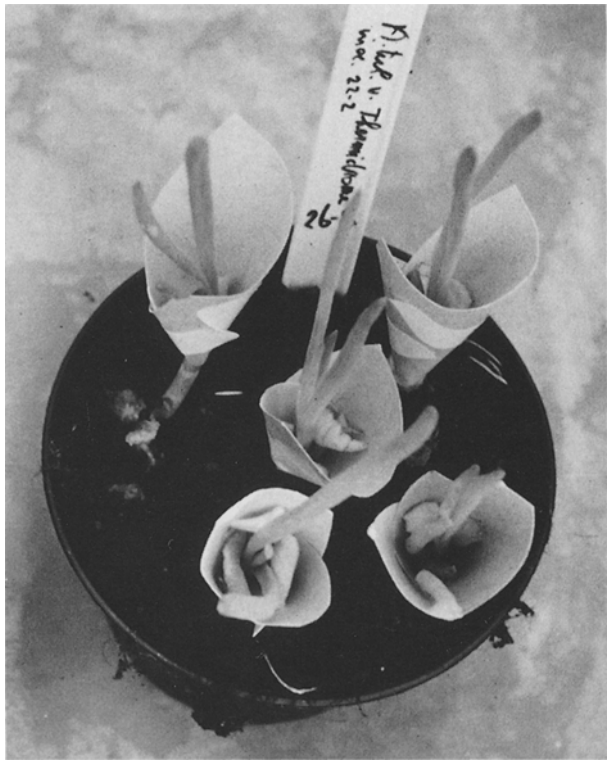

Fig. 1. Inoculation of onion plants with mite-borne virus by placing slices of mite-infested bulb scales in a paper cone around leaves of test plants.

Table 1. Listing and grouping of rymovirus isolates obtained by mite transfer or sap inoculation from samples of garlic (*Allium sativum*), onion (*A. cepa*), sand leek (*A. scorodoprasum*), and shallot (*A. cepa* var. *ascalonicum*) of different origin.

Species sampled *Isolate group* Isolates[1]	Method of virus isolation	Further specification of samples		
		cultivar	country of origin[2]	source[3]
Garlic				
Group B (OMbLV-G)[4]				
As120II[p]	sap: *Chenopodium murale*	unknown	Iran	ICon
As127	mites: onion	unknown	China	GN
As142II	sap: *Chenopodium murale*	Rosé d'Auvergne	France ICer	
As144II[p]	sap: shallot	Thermidrôme	the Netherlands (France)	CulN (ICer)
As178[p]	mites: leek	unknown	Syria	SR
As181[p]	mites: leek	unknown	Syria	SR
As185	mites: onion	Rocambole	the Netherlands (France)	CulN
As219	sap: *Celosia argentea*	Rocambole	the Netherlands (France)	CulN
As221II[p]	mites: leek	Rojo Americano	Chile	SR
As222II[p]	mites: leek	Rosé de Lautrec	Chile	SR
As273[p]	mites: crow garlic	unknown	the Philippines	SR
As292	mites: crow garlic	unknown	Spain	ICon
As297III[p]	mites: crow garlic	unknown	China	ICon
As327II	mites: crow garlic	unknown	Japan	SR
As328II	mites: crow garlic	unknown	Japan	SR
Onion				
Group A (OMbLV)				
Ac163	sap: *Chenopodium murale*	unknown	Russia	SR
Ac185II[p]	sap: *Chenopodium murale*	Strigunovskij	Russia	SR
Ac287[p]	sap: *Chenopodium murale*	Spasskij	Russia	SR
Ac288[p]	sap: *Chenopodium murale*	Spasskij	Russia	SR
Sand leek				
Group B (OMbLV-G)				
Asc10I	sap: *Chenopodium murale*	unknown	Morocco	GN

Shallot				
Group A (OMbLV)				
Ac103[p]	mites: onion	Santé	the Netherlands (Russia)	CulN
Ac111[p]	mites: onion	Ouddorpse Bruine	the Netherlands	CulN
Ac123II[p]	mites: onion	unknown	Spain	ICon
Ac129I[p]	mites: onion	unknown	France	ICon
Ac183I[p]	mites: shallot	unknown	Russia	SR
Ac209	sap: *Chenopodium murale*	Santé	the Netherlands (Russia)	CulN
Ac219	sap: *Chenopodium murale*	unknown	the Netherlands (Indonesia)	GN
Ac220	sap: *Chenopodium murale*	unknown	the Netherlands (Indonesia)	GN
Ac238	sap: *Chenopodium murale*	unknown	the Netherlands	GN
Ac257I[p]	sap: onion	Santé	the Netherlands (Russia)	GN
Ac257II[p]	sap: *Chen. amaranticolor*	Santé	the Netherlands (Russia)	GN
Group C (SMbLV)				
Ac130III	mites: onion	unknown	China	ICon
Ac183III[p]	mites: shallot	unknown	Russia	SR
Ac204II[p]	sap: onion	Santé	the Netherlands (Russia)	CulN
Ac206II[p]	sap: onion	Santé	the Netherlands (Russia)	CulN
Ac268[p]	mites: onion	unknown	Thailand	ICon

[1] Suffix p denotes preservation and storage in the IPO virus collection.
[2] The country mentioned in brackets is the country from which the selection was originally introduced.
[3] Abbreviations of sample sources are as follows: CulN = cultivated in the Netherlands; GN = gene collection in the Netherlands; ICon = imported for consumption in Belgium or the Netherlands; ICer = certified seed imported for cultivation in the Netherlands; SR = supplied by research worker abroad.
[4] OMbLV = onion mite-borne latent virus; OMbLV-G = garlic strain of onion mite-borne latent virus; SMbLV = shallot mite-borne latent virus.

planting of the onion sets. When no mites were naturally present on the plants or bulbs to be tested for infection, transmission was by first exposing the samples to mites from garlic bulbs, and including controls to test whether the mites were originally free of virus. Repeated spraying with Actellic (pyrimifos-methyl) and spatial isolation were used to prevent unwanted migration of mites and contamination by mite-borne virus.

Mite samples of different origin were all identified as *A. tulipae* (Vierbergen, 1989; Fig. 2).

Virus transmission by aphids. Transmission experiments with aphids (*Acyrthosiphon pisum*, *Myzus ascalonicus* and *M. persicae*), including controls with OYDV isolates, were with at least 50 aphids per isolate, and by transfer of aphids immediately after an access probing time of five min at maximum. Aphid species were identified by Ms J.D. Prinsen (IPO-DLO).

Serology and virus identification. Most tests were with antisera to LYSV and OYDV from IPO (Bos et al., 1978a). Antiserum to SLV also was from IPO (Bos et al., 1978b). Antisera to a Japanese garlic mosaic potyvirus (Abiko et al., 1980), garlic yellow streak potyvirus (Mohamed and Young, 1981), a Japanese isolate of LYSV (Noda and Inouye, 1989), the Japanese garlic latent carlavirus (Lee et al., 1979), and antiserum to and virus sample of the German garlic latent virus (Graichen and Leistner, 1987), were kindly supplied by Dr K. Abiko (Chugoku National Agricultural Experiment Station, Fukuyama, Japan), Dr N.A. Mohamed (Plant Protection Centre, MAF, Lincoln, New Zealand), Dr N. Inouye (Research Institute for Bioresources, Okayama University, Kurashiki, Japan), Dr T. Inouye (University of Osaka Prefecture, College

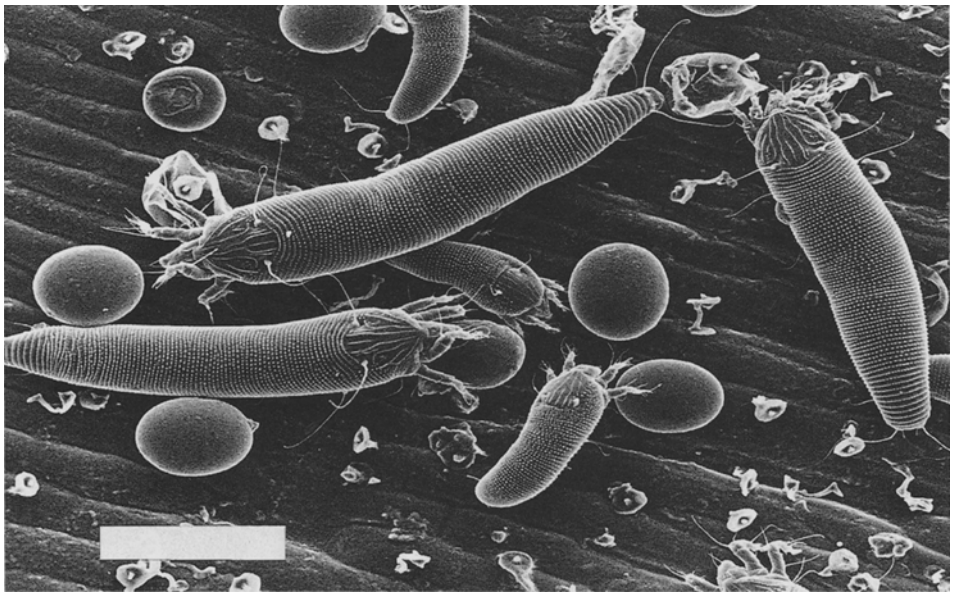

Fig. 2. Scanning electron micrograph of different stages of the dry bulb mite (*Aceria tulipae*) infesting a garlic clove. (Bar represents 100 μm.)

of Agriculture, Osaka, Japan), and Dr K. Graichen (Institut für Phytopathologie, Aschersleben, Germany), respectively. The Agdia potyvirus group test was used according to instructions of Agdia (Agdia Inc., Elkhart, USA), with LYSV and OYDV as positive controls. Identification of LYSV, OYDV and SLV was by comparison with type-isolates and serology. The German garlic latent virus proved to be different from the Japanese virus of the same name (P. van Dijk, unpublished results), and they will therefore be referred to as German GLV and Japanese GLV, respectively. They were identified by serology and comparison with the German type isolate and host reactions of the Japanese virus reported by Lee et al. (1979).

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 sulfite, incubation of the sap on a grid for ca. one min, and floating the grid for one min on drops of the sodium sulfite solution and of a 2 % (w/v) solution of sodium phosphotungstate (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 sulfite solution, prior to staining. Particle length determination was by measuring 50 particles, with TMV as an internal standard.

Light microscopy. For study of intracytoplasmic inclusion bodies, epidermal strips were stained with a mixture of four parts methylene blue and one part phloxine red, both 1% (w/v) in Christie's solution (Cellosolve (ethylglycolmonoethyl ether) : ethanol : water = 2 : 1 : 1).

Results

Several bulb samples of garlic and shallot were found to be infested with *A. tulipae* (Fig. 2) when visually inspected with a binocular microscope. The number of mites on individual bulbs varied largely between samples, and greatly increased after storage of the bulbs for several months. In preliminary mite-transfer tests, OYDV and SLV were sometimes transmitted to onion test plants, apparently mechanically as a result of the insertion of infested bulb scales. In later tests designed to prevent such mechanical transmission, no mite transmission was obtained of OYDV, a second and undescribed aphid-borne potyvirus (P. van Dijk, unpublished results), and SLV in shallot, or potyvirus isolates serologically related to LYSV or OYDV, German GLV and Japanese GLV in garlic, even when very large numbers of mites were used. Instead, the mites transmitted a number of virus isolates with elongate particles, clearly differing in degree of flexibility and in structure from those of aphid-borne potyviruses and carlaviruses. Similar isolates were obtained by sap inoculation (Fig. 3).

The isolates, when studied further, could be classified into three groups, according to host range, symptoms, and reaction with a discriminating antiserum. Table 1 lists all 36 isolates, together with method of their isolation and data on their origin. Nineteen isolates, three or more of each group, were transmitted by mites. Test plants infested with large numbers of mites developed clear yellow to white streaks and malformation; such symptoms were shown to be caused by mite feeding and not by virus infection (see below). Isolate Ac257I from shallot (group A), Ascl0I from sand

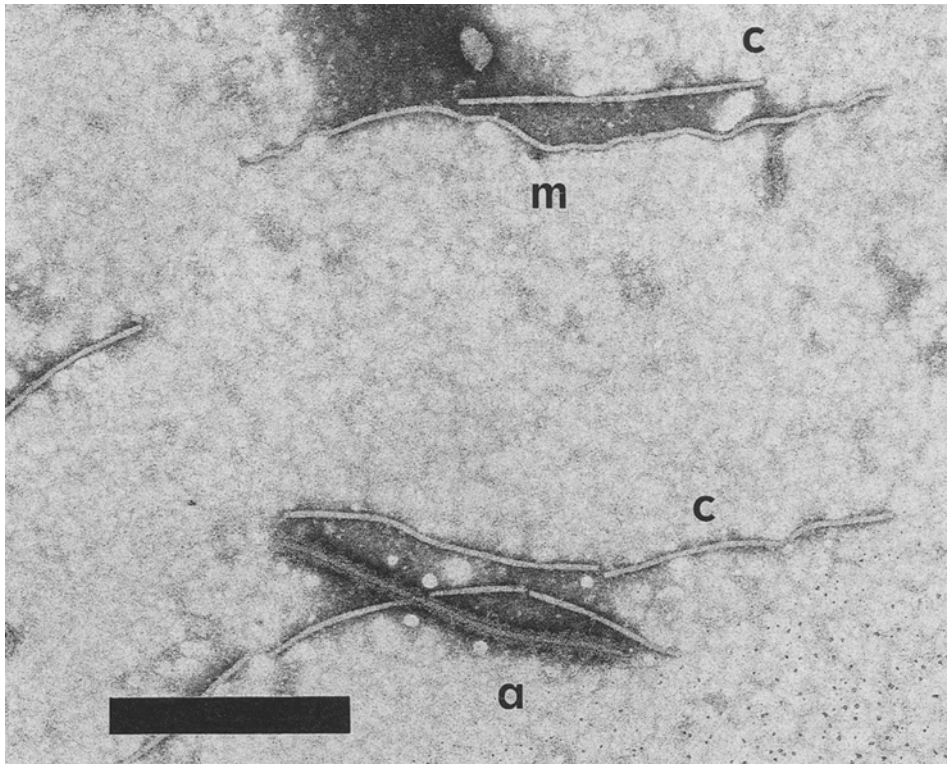

Fig. 3. Strikingly flexuous particle of mite-borne isolate As185 (m), as compared with more rigid particles of aphid-borne potyvirus (a; decorated with antiserum to garlic yellow streak virus) and carlavirus (c) in sap of garlic 'Germidour'. (Bar represents 500 nm.)

leek (group B) and Ac268 from shallot (group C) were shown to be nontransmissible by aphid species such as *Myzus ascalonicus*, *M. persicae* and especially *Acyrthosiphon pisum*, while OYDV was transmitted in control tests. Table 2 summarizes the results obtained with representative isolates of the three groups.

Biological tests

Group A: Isolates from onion and shallot inducing local lesions in Chenopodium murale. Some of the onion or shallot plants, inoculated with mites from bulbs of the Dutch shallot 'Ouddorpse Bruine' (isolate Ac111) and from unnamed shallot cultivars from France (Ac129I), Russia (Ac183I; Table 2), and Spain (Ac123II; Table 2), contained flexuous virus particles but showed no symptoms or a slight mottle only. After further transfer by sap such isolates did not produce symptoms in crow garlic (*A. vineale*), leek or rakkyo (*A. chinense*). Garlic did not become infected upon inoculation. Clearly defined small rings, often with a necrotic centre, were observed in *Chenopodium murale* (Fig. 4, left) 2-2½ weeks after inoculation. Similar local lesions appeared in *Atriplex hortensis* (Fig. 4, right), *C. foliosum* and *C. opulifolium*, but later or less defined than in *C. murale*. *C. amaranticolor* and *Gomphrena globosa* reacted poorly, if at all. *C. quinoa*, *Celosia argentea* and *Nicotiana occidentalis* – indicator

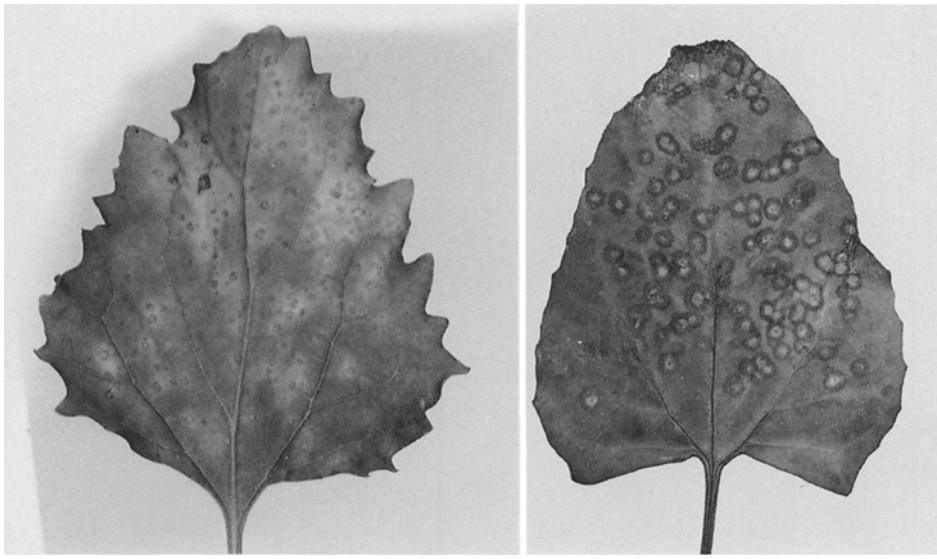

Fig. 4. Local reaction of *Chenopodium murale* (left) three weeks after sap inoculation with Spanish shallot isolate Ac123II, and of *Atriplex hortensis* 'Red Plume' (right) four weeks after sap inoculation with isolate Ac103 from shallot 'Santé'.

plants for SLV – did not show symptoms, nor did *Amaranthus bicolor*, *A. caudatus*, *A. cruentes*, *A. hypochondrianum*, *A. tricolor*, *Chenopodium schraderianum* (*C. foetidum*), broad bean, *Chlorophytum comosum*, *Lilium formosanum*, *N. benthamiana*, *N. hesperis*, spinach or *Tetragonia expansa*. Bio-assay on *C. murale* and *Atriplex hortensis* of original shallot samples for the mite-borne virus, was usually not possible because of natural contamination of the samples with SLV, also reacting in these indicator species. Isolate Ac103 was transmitted from leaves of shallot 'Santé' with mites from garlic bulbs. It induced rather diffuse yellow stripes in relatively old leaves and flowering stalk of onion (Fig. 5) similar to those observed in old but not in young leaves of 'Santé'. Two more deviating isolates were sap-transmitted from the same cultivar. The relatively virulent Ac257I (Table 2) from a plant showing striking yellow streaks caused similar symptoms in onion, while isolate Ac257II from the same plant induced clear yellow local lesions in *C. amaranticolor*. Ac257I did not infect Chinese chive (*Allium tuberosum*), chive (*A. schoenoprasum*), garlic or Welsh onion (*A. fistulosum*).

Fig. 5. Diffuse yellow stripes of isolate Ac103 from shallot 'Santé' in flowering stem of onion 'Rijnsburger', systemically infected from bulb.

Table 2. Host reactions and reactions with a differential antiserum of three groups of rymovirus isolates from garlic (As) and shallot (Ac).

Characterization by	Isolate group and representative isolates							
	group A (OMbLV)[1]			group B (OMbLV-G)			group C (SMbLV)	
	Ac123II	Ac183I	Ac257I	As120II	As178	As221II	Ac204II	Ac268
Test plants[2]								
Allium ampeloprasum var. *porrum* (leek)	. –*[3]	. s	. –*	. –*	. s	. s	. –	. –
A. cepa (onion)	. (S)	. s	. S	. –*	. –	. –	. s	. (S)
A. cepa var. *ascalonicum* (shallot)	. s	. s	. S	. .	. –	. .	. s	. s
A. chinense (rakkyo)	. .	. s	. s	. .	. –	. –	. .	. .
A. fistulosum (Welsh onion)	. .	. .	. –	. –*	. –	. .	. –	. –
A. sativum (garlic)	. .	. –	. –	. s	. s	. s	. .	. –
A. schoenoprasum (chive)	. .	. .	. –	. .	. –	. .	. .	. .
A. tuberosum (Chinese chive)	. .	. .	. –	. .	. .	. .	. .	. .
A. vineale (crow garlic)	. .	. s	. .	. .	L s	. .	. s	. s
Amaranthus bicolor	–* –*	. .	. .	–* –*	. .	. .	. .	. .
A. caudatus	–* –*	. .	. .	–* –*	. .	. .	. .	. .
A. cruentes	–* –*	. .	. .	–* –*	. .	. .	. .	. .
A. hypochondrianum	–* –*	. .	. .	–* –*	. .	. .	. .	. .
A. tricolor	–* –*	. .	. .	–* –*	. .	. .	. .	. .
Atriplex hortensis	L –*	(L) –*	(L) –*	L –*	L –*	. .	. .	. .
Celosia argentea var. *plumosa*	– –*	–* –*	–* –*	–* –*	–* –*	–* –*	–* –*	–* –*
Chenopodium amaranticolor	(L) –*	–* –*	–* –*	–* –*	(L) –*	–* –*	–* –*	–* –*
C. murale	L –*	L –*	L –*	L –*	L –*	L –*	–* –*	–* –*
C. quinoa	–* –*	–* –*	–* –*	–* –*	–* –*	–* –*	–* –*	–* –*
C. schraderianum	–* –*	. .	. .	–* –*	. .	. .	. .	. .
Chlorophytum comosum (spider plant)	. .	–* –*	. .	–* –*	–* –	. .	–* –	–* –
Lilium formosanum	. .	. –*	. –	. .	. –	. .	. .	. .
Nicotiana benthamiana	–* –*	–* –*	–* –*	–* –*	(l) –*	. .	–* –*	–* –
N. hesperis	–* –*	–* –*	. .	– –*	. .	. .	–* –*	–* –
N. occidentalis	l –*	–* –*	l –*	l –*	– –*	– –*	–* –*	–* –*

Spinacia oleracea (spinach)	. .	. .	−* −*	. .	−* −*	. .	−* −	−* −
Tetragonia expansa	−* −*	. .	. .	−* −*	. .	. .	. .	. .
Vicia faba (broad bean)	−* −*	. .	−* −*	−* −*	. .	−* −*	−* −*	−* −*
Antiserum[4]	2-3	4	4	4-5	4-5	5	1	1
Particle length[5]	775[6]	.	.	716[7]	.	.	.	767[8]

[1] Acronyms of proposed virus names are as follows: OMbLV = onion mite-borne latent virus; OMbLV-G = garlic strain of onion mite-borne latent virus; SMbLV = shallot mite-borne latent virus.
[2] For cultivars and accessions see 'Materials and methods'.
[3] The first and second symbol denote the reaction of inoculated and non-inoculated leaves, respectively: L = local lesions; l = latent local infection; S = systemic symptoms; s = latent systemic infection; − = no infection; −* = no symptoms, but not tested for latent infection; ( ) = reaction very poor or variable; . = not tested.
[4] German antiserum to a garlic latent virus (see 'Materials and methods') decorating particles increasingly weak from 5 (partial) to 1 (very slight).
[5] Normal particle length in nm (for method of assessment see 'Materials and methods'); . = length not determined.
[6] Length distribution curve of particles showed peaks at 775 nm and 751 nm.
[7] Length distribution curve of particles showed peaks at 716 nm and 687 nm.
[8] Length distribution curve of particles showed peaks at 767 nm and 743 nm.

Further isolates classified into group A were obtained by inoculation of sap from Dutch (Ac209 and Ac238) and Indonesian (Ac219 and Ac220) shallot, and notably from Russian onion (Ac163, Ac185II, Ac287 and Ac288) (Table 1). The virus was not found in about 100 Thai shallot bulbs of two samples tested by manual inoculation and by transfer of mites. No mite-borne virus was detected in five samples of rakkyo (*A. chinense*) from Hong Kong, the Netherlands and the USA, when tested by inoculation onto *C. murale* and by electron microscopy.

Over 100 wild plants of crow garlic (*A. vineale*), collected at Ouddorp (the Netherlands) around fields where shallot 'Ouddorpse Bruine' or French shallot had been grown during the previous season, and 25 plants of crow garlic collected elsewhere, were assayed on *C. murale*. Despite the prevalence of the mite-borne virus and its vector in both shallot cultivars (Ac111 and Ac129I), high experimental susceptibility of crow garlic to the virus (Ac103 and Ac183I), and the predominantly vegetative reproduction of the species, none of the plants contained the virus.

Group B: Isolates from garlic and sand leek inducing local lesions in Chenopodium murale. Mites from 10 European garlic samples did not transmit virus to onion, but several isolates were obtained in crow garlic or leek when these species were included in the test range. Virus concentration in leek was lower than in crow garlic and garlic. These isolates were As178 (Table 2) and As181 from Syria, As221II (Table 2) and As222II from Chilean samples of 'Rojo Americano' and 'Rosé de Lautrec', respectively, As273 from a symptomlessly infected Philippine cultivar, As292 from Spain, As297III from China and As327II and As328II from Japan. Mite transmission from garlic to onion succeeded only twice (As127 from a Chinese sample, and As185 from Dutch 'Rocambole'), but virus concentration in onion was poor. In contrast to the onion and shallot isolates of group A, not infecting garlic, the garlic isolates were not or only poorly infectious to onion, rakkyo and shallot. They were therefore classified into a second group (B), though they resembled the group A isolates in other host reactions, particularly in *C. murale* (Table 2). All isolates were symptomless in sap-inoculated *Allium* spp., notably Asian isolates in crow garlic.

The mites were present in large numbers on leaves of glasshouse growing plants of 'Rocambole' and they caused severe distortion and streaking as a direct result of feeding (Smalley, 1956; Lange and Mann, 1960; Razvjazkina et al., 1969; Pizarro et al., 1970; Scalopi et al., 1970; Ahmed and Benigno, 1984). Such symptoms were occasionally found in the field (Fig. 6). For this mite toxaemia the descriptive name 'tangle-top' has been used (Pizarro et al., 1970).

The mite-borne virus was more difficult to eliminate from 'Rocambole' by meristem-tip culture than the aphid-borne poty- and carlaviruses also present (M. Verbeek and P. van Dijk, unpublished results).

Deviant isolate As219 of the virus was obtained from 'Rocambole' plantlets grown *in vitro*. It induced faint chlorotic to necrotic local lesions in *Celosia argentea*. Plants of 'Rocambole' infected by the mite-borne virus did not show symptoms. Isolate As120II (Table 2), showing clear cross-banding of particles (Fig. 7), was obtained from symptomless Iranean garlic by sap inoculation onto *A. hortensis*, *C. murale* and *N. occidentalis*, with the latter species showing latent infection of the inoculated leaves only. Likewise, As142II from French certified seed of 'Rosé d'Auvergne' was obtained after sap inoculation in *C. murale*. Testing of samples of other garlic cultivars on the

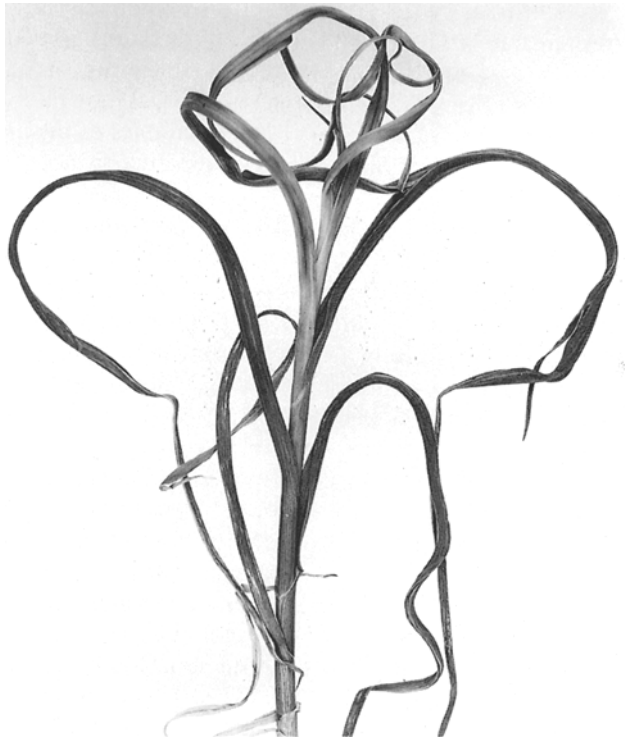

Fig. 6. Field-grown garlic plant with diffuse yellow streaking, leaf distortion and delayed unfolding of leaves (tangle-top) due to feeding by large numbers of mites.

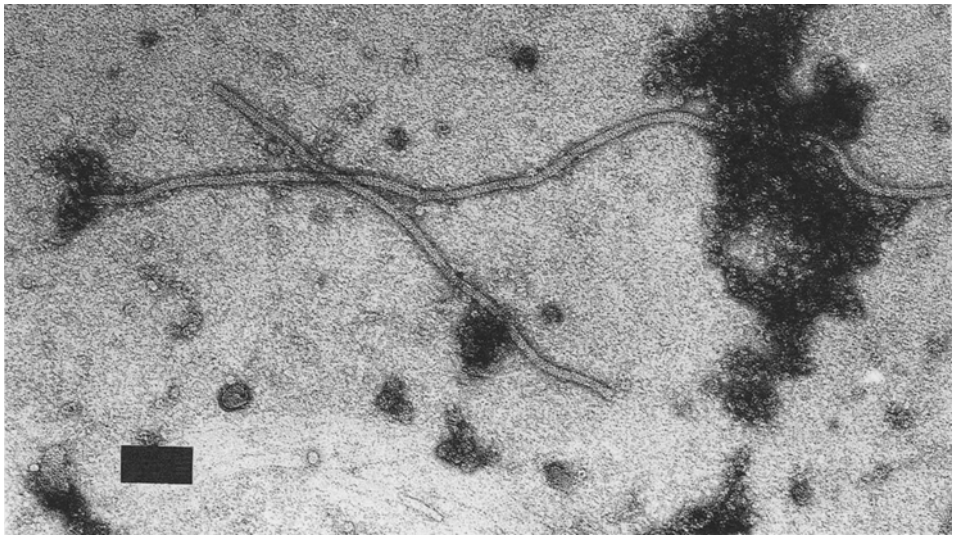

Fig. 7. Electron micrograph of highly flexuous and cross-banded particles of isolate As120II in naturally infected Iranean garlic. (Bar represents 100 nm.)

species mentioned, however, was impeded by the presence of German GLV. As144II from garlic 'Thermidrôme' (propagated from French certified seed) could be separated from German GLV by sap transmission to onion and shallot. It was transmitted further to *C. murale* and thence to sand leek (*A. scorodoprasum*), the latter species appearing very susceptible to the virus. From Moroccan sand leek, Asc10I was transmitted by sap to *C. murale*, and to leek and onion with difficulty.

Group C: Isolates from shallot not inducing reaction in Chenopodium murale. Test plants of onion inoculated with mites from Asian shallots contained very flexuous particles. In contrast to those of group A and B, the isolates Ac130III (from China) and Ac268 (from Thailand; Table 2) did not cause symptoms in *C. murale*, and Ac268 did not infect leek. They were therefore arranged in a third group (C). No symptoms or only very mild striping were observed in onion and shallot. Garlic 'Rocambole' did not become infected after inoculation with Ac268. A third isolate of group C (Ac183III, from Russia), was detected in the culture of group A isolate Ac183I by decoration tests (see below; Fig. 8). The sap-transmitted Ac204II (Table 2) and Ac206II from different samples of Dutch (originally Russian) shallot 'Santé' fully resembled the mite-borne isolates of group C in particle flexibility, host reactions and serology.

Isolates of group C could be differentiated from those of an undescribed carlavirus in rakkyo and shallot 'Santé' with similar host reactions (P. van Dijk, unpublished results), by their more flexuous particles and their not reacting with SLV antiserum (see also below).

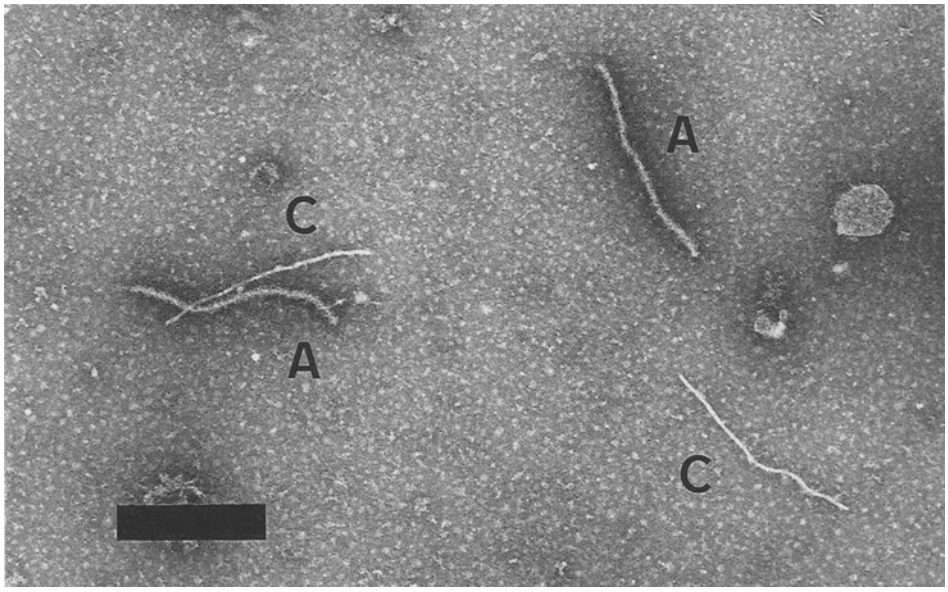

Fig. 8. Particles of group A isolate Ac183I (A) and group C isolate Ac183III (C) after mite transmission of mixture to shallot, and differential decoration with antiserum to German garlic latent virus. (Bar represents 500 nm.)

Serological tests
Practically all isolates of Table 1 were tested in ELISA with the Dutch antisera to LYSV or OYDV, and none of them reacted. Isolates Ac103, Ac183I and Ac257I of group A, As178, As221II and As222II of group B and Ac183III of group C also failed to react with Agdia's potyvirus group antiserum. Decoration tests with different isolates, at least one of each group per antiserum, were negative with antisera to Japanese GLV, garlic mosaic virus (Abiko et al, 1980), garlic yellow streak virus (Fig. 3), LYSV (Dutch and Japanese antisera), OYDV and SLV. The antiserum to German GLV reacted with all three of our isolate groups. It partially decorated isolates of group A and B, although the group A isolates and especially Ac123II were less decorated than group B isolates; group C isolates were only very slightly decorated (Table 2). A very slight reaction with Ac183III, similar to that with Ac268, made it possible to identify Ac183III in a complex with Ac183I (Fig. 8).

Electron microscopy and light microscopy
Normal lengths of particles determined by measuring 50 particles per isolate, were 775 nm, 716 nm and 767 nm for isolates Ac123II (group A), As120II (group B) and Ac268 (group C), respectively. Peculiarly, the particle length distribution curves showed secondary peaks at 24 to 29 nm shorter than the main peaks (Table 2). Particles of the three groups of mite-borne isolates were clearly more flexuous than those of an aphid-borne potyvirus (Figs 3, 7 and 8). Particles of some, but not all of our isolates showed cross-banding (Fig. 7). Although this particle morphology is somewhat suggestive of a closterovirus, granular inclusion bodies characteristic of the family *Potyviridae* were found in shallot 'Santé', naturally infected by isolate Ac103 of group A, but possibly also infected by an isolate of group C (Fig. 9). Similar inclusion bodies were found in shallot plants infected with Ac183I (group A) in complex with Ac183III (group C), in onion infected by Ac257II (group A), Ac204II (group C) or Ac268 (group C), and in leek infected by As178 (group B).

Discussion

Progress in the identification of viruses infecting *Allium* spp. is hampered by their narrow host ranges and common occurrence in complexes, making it difficult to separate and sub-culture them free of other viruses (Walkey, 1990). Use of the dry bulb mite, prevalent and often found in large population densities in garlic and shallot bulbs, especially after storage, enabled us to selectively transmit a number of mite-borne virus isolates. Studies of their host ranges, host reactions, and particle morphology also allowed the detection of similar virus isolates after mechanical isolation.

The Russian onion mosaic virus (Razvjazkina et al., 1969; Razvjazkina, 1971; Cheremushkina, 1974, 1975, 1982) and a Philippine garlic mosaic virus (Ahmed and Benigno, 1984, 1985a, b) are the only viruses of *Allium* spp. reported so far to be mite-borne. A Japanese garlic mosaic virus of Lee et al. (1979) may also be mite-borne, as suggested by high flexibility of the particles photographed in their article. Despite its alleged omnipresence in garlic in Japan (Lee et al., 1979), we could only transmit latent isolates of group B (As327II and As328II) with mites from two Japanese accessions. Mites occurring on other Asian garlic samples, including one from the Philippines,

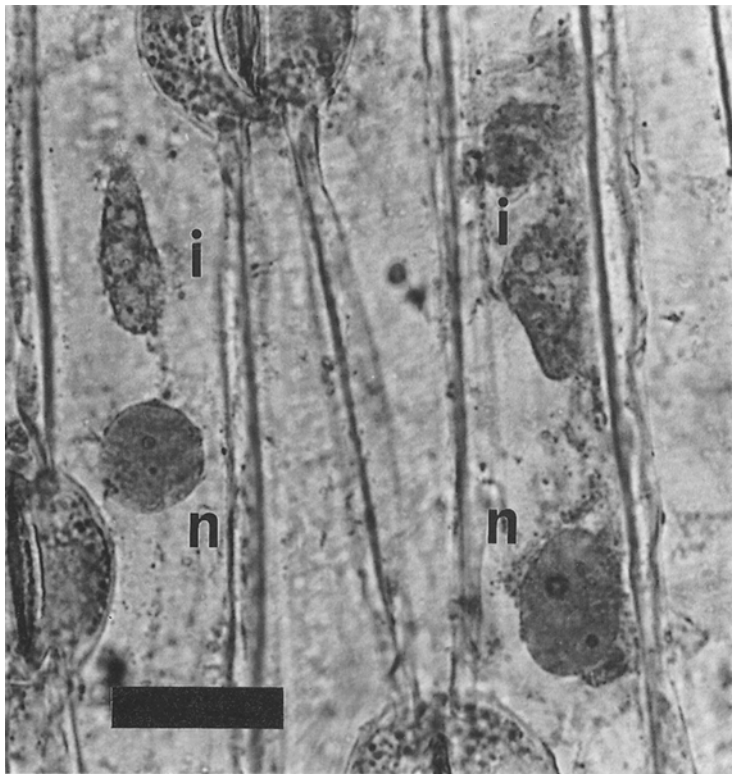

Fig. 9. Granular intracytoplasmic inclusion bodies (i) induced by isolate Ac103 in epidermal cells of shallot 'Santé' (n = nucleus). (Bar represents ca. 25 μm.)

also transmitted such latent isolates (As127, As273 and As297III) but not isolates inducing mosaic-like symptoms in crow garlic, a species that proved highly susceptible to all viruses of *Allium* spp. isolated at IPO (Table 2; P. van Dijk, unpublished results). Likewise, Russian onion and shallot samples appeared infected only by latent mite-borne isolates of group A (Ac163, Ac183I, Ac185II, Ac287 and Ac288) and group C (Ac183III) (Table 1).

Little is known about the mite-borne mosaic viruses of *Allium*, and type material and antisera to them are not available. Reports on onion mosaic virus have long been confusing (Bos, 1983) and its difference from OYDV has been doubted (Schmelzer et al., 1977). Our transmission of type-isolate Ac41 and other isolates of OYDV by aphids, and our failure to transmit OYDV from shallot samples by dry bulb mites, prove that the identification of Ac41 as OYDV is correct, and that the mite-borne onion and garlic mosaic viruses reported in the literature are essentially different from OYDV. From the literature on onion mosaic virus cited here, our own tests on Russian samples, and correspondence with Dr N.P. Cheremushkina (All-Union Research Institute for Vegetable Breeding and Seed Production, Moscow, USSR, 1988-1990), we may now conclude that this virus has not been legitimately described and that the name should be considered a '*nomen nudum*'. Potyvirus-like particles observed in

Russia in mites and in test plants after mite transfer are very likely from isolates fitting in our group A, B and possibly C, while symptoms in test plants probably have been due to mite feeding, and those in source plants due to the presence of OYDV. The existence of a mite-borne garlic mosaic virus reported in Asia (Ahmed and Benigno, 1984, 1985a, b) has not been confirmed.

Latent infections of *Allium* spp. differentiate our three isolate groups from any potyvirus of the genus *Allium* described in the literature. Non-decorated potyvirus particles reported in decoration tests (e.g. Delecolle and Lot, 1981; Walkey et al., 1987) may well have been of a virus fitting in one of our groups. Although further work on physico-chemical characterization and serology remains to be done, we have now established the existence of mite-borne virus isolates of *Allium* spp., infecting such species latently or only with mild symptoms, and having highly flexuous particles ca. 700 to 800 nm long, and not reacting with antisera to aphid-borne potyviruses of *Allium* spp. Obviously, our virus isolates belong to the *Rymovirus* genus of the family *Potyviridae* (Barnett, 1991). Failure of our isolates to react in Agdia's potyvirus test, a test based on a broadly reacting monoclonal antibody which reacts with aphid-borne potyviruses, corroborates the distinction. Shukla et al. (1989) reported another potyvirus antiserum reacting with members of aphid-, mite- and whitefly-borne genera of *Potyviridae*, but their tests may be more sensitive, or their antiserum may have broader specificity than the monoclonal antibody from Agdia.

The reactions of our virus isolates with the German GLV antiserum can be explained by the presence of a group B isolate in the German carlavirus culture. Such contamination may remain unnoticed because of overlapping host reactions. The chlorotic and necrotic local lesions of the New Zealand garlic yellow streak virus, developing late in *Chenopodium murale* and not in other *Chenopodium* spp. (Mohamed and Young, 1981), strongly suggest the presence of a group B isolate in the culture used for host-range studies, but antibodies to such an isolate could not be found in the antiserum (Fig. 3).

Our host-range studies (Table 2) show that isolates of group A and C from onion and shallot are not infectious to garlic, and that isolates of group B from garlic and sand leek are not or only poorly infectious to onion and shallot. Although isolates of group A and B have different natural hosts, they are very similar in their range of other hosts and symptoms, and both react to a considerable degree with the German discriminating antiserum (Table 2). We now propose the names 'onion mite-borne latent virus' (OMbLV) for isolates of group A, and 'the garlic strain of OMbLV' (OMbLV-G) for those of group B. Isolates of group C from shallot differ in host reactions and serological properties from those of the other groups. The name 'shallot mite-borne latent virus' (SMbLV) is therefore now proposed for this virus.

Without the availability of specific antisera, SMbLV would be hard to detect in the presence of OMbLV or OMbLV-G. Because OMbLV is common in European shallot, and its garlic strain in garlic from several parts of the world, the incidence of SMbLV in European shallot and the possible existence of a garlic strain of the virus in garlic remain to be investigated by serology. Such tests are also necessary to further characterize the new viruses and virus strain distinguished here, and to compare them with the rymoviruses of *Gramineae*.

Acknowledgements

We thank Dr N.P. Cheremushkina (All-Union Research Institute for Vegetable Breeding and Seed Production, Moscow, USSR) for supplying Russian plant samples, Ir R. Hooftman (NAK-S, Roelofarendsveen) for testing plant samples in Agdia's potyvirus test, Mr A.J.F. Lieberwerth (Het Hof van Eden, Utrecht) for providing samples of *Allium* species, and Mr G. Vierbergen (Plant Protection Service, Wageningen) for identifying mite samples.

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