Neth. J. Pl. Path. 81 (1975) 14–21

Lily symptomless virus in tulip

A. F. L. M. DERKS and C. J. ASJES

Bulb Research Centre, Lisse

Accepted 30 May 1974

Abstract

Lily symptomless virus (LSV) was transmitted mechanically to only two out of 53 plant species (18 families) tested, i.e. *Lilium formosanum* and tulip 'Rose Copland' (Liliaceae). The aphid species *Macrosiphum euphorbiae*, *Myzus persicae*, and *Aphis gossypii* transmitted LSV in a non-persistent manner from *Lilium* Mid-century hybrid 'Enchantment' to tulip 'Rose Copland'. *M. euphorbiae* transmitted the virus more efficiently than *M. persicae* and *A. gossypii*. The yield of LSV-infected tulips 'Peerless Pink' was slightly reduced compared with that of healthy tulips. LSV was observed incidentally in naturally infected commercial stocks. Two procedures to purify LSV from tulips were applied, one using crude sap and the other sap treated with chloroform. The length distribution of LSV particles in tulip showed a higher percentage of particles shorter than 600 nm as in lily.

Introduction

Lily symptomless virus (LSV: R/*:*/8:E/E:S/Ap; Allen, 1972; carlavirus group) is widespread in lilies (Asjes et al., 1973), and has been reported to occur naturally in tulips (Allen, 1971). Persistent and non-persistent modes of transmission of the virus by aphids from lily to lily were found (Brierley and Smith, 1944; Mowat and Štefanac, 1970, 1974; Derks, 1973). In the Netherlands LSV was transmitted mechanically to tulip 'Rose Copland', but the transmission by aphids from *Lilium* spp. to *Tulipa* spp. was unknown and the virus had not been reported in commercial stocks (Asjes et al., 1973). Some features of LSV infection in tulips are reported in this paper.

Materials and methods

Mechanical inoculation. Plants of 53 species belonging to 18 families were inoculated manually after dusting with carborundum (500 mesh), using LSV-infected leaves of *Lilium* Mid-century hybrid 'Enchantment' homogenized in a double quantity of 1/30 M phosphate buffer (pH 7.0) containing 1% Na_2SO_3. Local and systemic infection was evaluated electron-microscopically at weekly intervals for 6–9 weeks after inoculation.

Inoculation with aphids. Myzus persicae (Sulz.) was reared on *Gomphrena globosa*, *Macrosiphum euphorbiae* (Thos.) on *Chenopodium quinoa*, and *Aphis gossypii* Glov. on *Cucumis sativus* at about 20°C. Three modes of transmission were applied:
1. After a 5 sec to 3 min acquisition time on lily 'Enchantment', the apterous aphids

(10 to 15 per plant) were transferred to tulips. The aphids were killed with 0.2% nicotine 1 to 2 h later.
2. After a 20 to 22 h acquisition time on lily 'Enchantment', the apterous aphids (10 to 15 per plant) were transferred to tulips. The aphids were killed with nicotine 1 to 3 h later.
3. After a 2 to 3 day acquisition time on LSV-infected leaves of lily 'Enchantment', the apterous aphids of *M. persicae* and *M. euphorbiae* were first transferred with a paintbrush to *G. globosa* and those of *A. gossypii* to *C. sativus* and then 2 to 3 days later to tulips ('Rose Copland'). The aphids (10 to 15 per plant) were killed with nicotine after 3 days.

Transmission experiments with *M. persicae* and *M. euphorbiae* were carried out in March 1972 and with *A. gossypii* in March 1973. Field-grown and forced tulips were inoculated in a special room at about 20°C. The tulips (about 20 per treatment) had two unfolded leaves (about 5 cm long). The field-grown tulips, planted in boxes under cages, flowered 8 to 10 weeks after inoculation and the forced tulips, grown at 15°C under glass, after 6 weeks. Plants with current-season symptoms were marked. The progeny bulbs of plants with and without current-season symptoms were planted separately in 1973 (*M. persicae* and *M. euphorbiae*) and 1974 (*A. gossypii*). The presence of LSV in plants from progeny bulbs was determined electron-microscopically and serologically.

Yield. Healthy tulip plants, as judged from the absence flower symptoms (Asjes et al., 1973) were identified in a heavily diseased stock (about 70% affected) of the cultivar 'Peerless Pink', and were lifted separately at the end of the growing season. The bulbs (150) of both the healthy and diseased tulips were weighed after storage for two months.

The multiplication rate of 'Rose Copland' tulips with and without flower symptoms was determined in 33 and 48 mother bulbs, respectively.

Purification. Tulip leaves and flowers or sap were frozen at −20°C for variable periods. Homogenization was done in phosphate buffer (0.067 M, pH 7.2; w/v = 1/1) containing 0.1% thioglycollic acid, with an Ultra-Turrax homogenizer, after which the sap was squeezed through cheese cloth. When thawed, the sap was used with or without addition of an equal volume of n-chloroform. The mixture with chloroform was stirred for 30 min and centrifuged for 10 min at 2,500 *g*. The sap, with or without chloroform clarification, was centrifuged in a Spinco L-50 ultracentrifuge for 2 h at 92,000 *g* or 1.5 h at 110,000 *g*. The pellet was resuspended in phosphate buffer, and the suspension centrifuged for 10 min at 2,500 *g* prior to serological testing and electron-microscopical examination.

Serology. Tulip leaves and petals were frozen at −20°C overnight or longer, homogenized with phosphate buffer (0.067 M, pH 7.0; w/v = 1/1), squeezed through cheese cloth, and centrifuged for 10 min at 1,000 *g* before applying the microprecipitin test (Van Slogteren, 1955). Spontaneous reactions were prevented by additional centrifugation for 10 min at 9,000 *g*.

Electron microscopy. Leaf preparations in phosphate buffer (0.067 M, pH 7.2) were

appropriately mixed with an internal length standard of tobacco mosaic virus (TMV; Bos, 1970), and treated with 2% phosphotungstic acid (PTA; pH 7.2). The negatively stained, elongated virus particles were examined in a Philips EM 201S electron microscope. The negatives of the photographs taken at a magnification of about 10,800 times were projected on paper, giving a magnification of about 194,000 times. The particles were traced on paper and the measured lengths grouped into classes of 10 nm. The particle lengths were calculated on the basis of TMV length (300 nm; Asjes et al., 1973).

Results

Symptoms and occurrence. After inoculation of tulip 'Rose Copland' LSV caused dark-pink, narrow veinal streaks on the petals (De Bruyn-Ouboter, unpublished results of 1949; Asjes et al., 1973). The streaks only occurring in part of the petals of current-season or forced tulips were hard to recognize (Fig. 1). Symptoms in the following growing season consisted of streaks covering the whole petal. A few commercial tulip stocks, e.g. of 'Peerless Pink', and 'Azida', were found to have similar symptoms. However, in naturally infected plants of 'Preludium' the streaks on the petals were white (Fig. 2). In all these cultivars LSV was detected serologically.

Host plants. LSV could be transmitted mechanically to tulip 'Rose Copland' (Asjes et al., 1973) and to *L. formosanum* (Liliaceae) only. In *L. formosanum* the virus could

Fig. 1. Dark-pink, narrow veinal streaks on only part of the petals of the tulip 'Rose Copland', caused by lily symptomless virus in the current season. Right: infected; left: healthy.

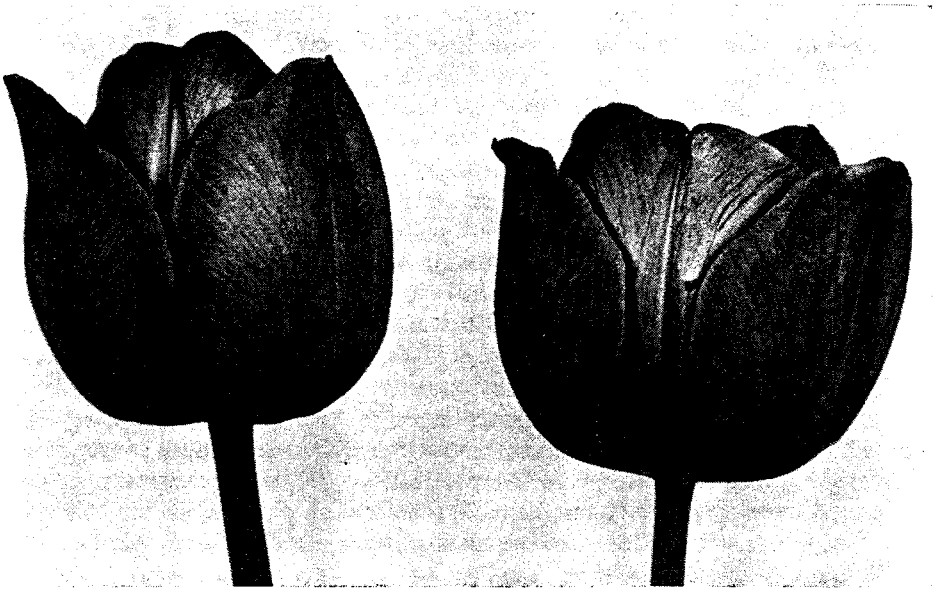

Fig. 1. Donkerrose, fijne strepen op een gedeelte van de nerven van de bloembladen van de tulp 'Rose Copland' in het jaar van infectie met het symptoomloos lelievirus. Rechts: geïnfecteerd; links: gezond.

Fig. 2. White, narrow veinal streaks on tulip petals of 'Preludium', caused by lily symptomless virus. Right: infected; left: healthy.

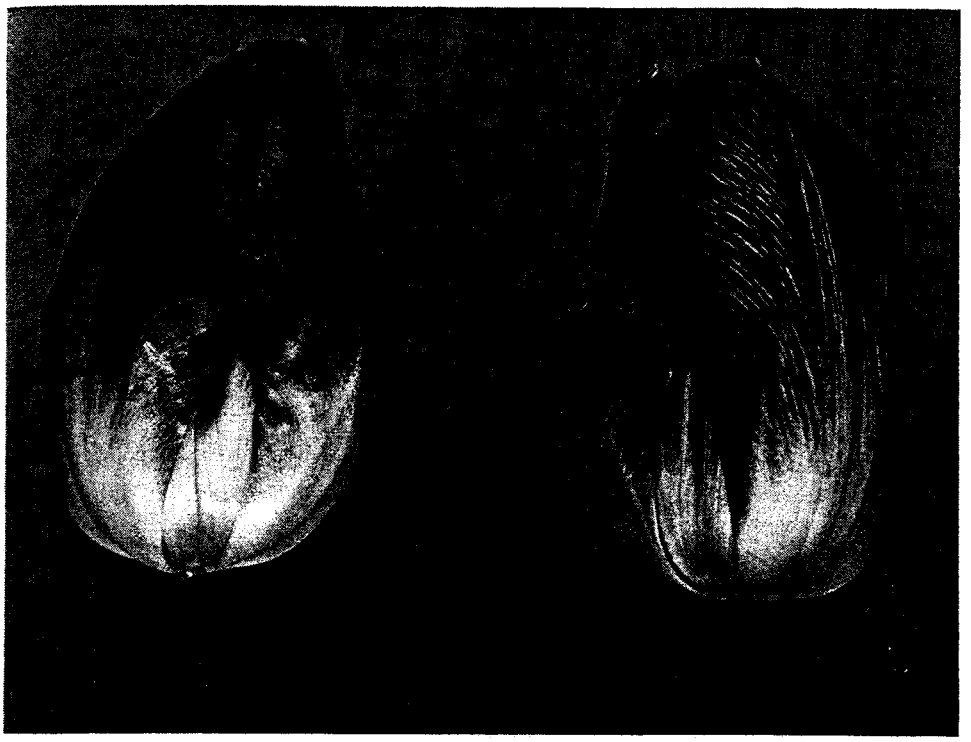

Fig. 2. Witte, fijne strepen op de nerven van bloembladen van de tulp 'Preludium', veroorzaakt door het symptoomloos lelievirus. Rechts: geïnfecteerd; links: gezond.

be detected electron-microscopically 4 to 6 weeks after inoculation. Fifty-one species could not be infected. They belonged to the following families (number of species in parentheses): Aizoaceae (1), Amaranthaceae (3), Apocynaceae (1), Caryophyllaceae (1), Chenopodiaceae (3), Compositae (5), Cruciferae (2), Cucurbitaceae (1), Gramineae (1), Iridaceae (1), Papilionaceae (8), Polemoniaceae (1), Polygonaceae (1), Scrophulariaceae (3), Solanaceae (17), Tropaeolaceae (1), and Umbelliferae (1). Most of the species tested are host plants for other viruses of the carlavirus group (Brandes, 1964).

Aphid transmission. LSV was transmitted to 'Rose Copland' tulips by the 3 aphid species used after a 5 sec to 3 min acquisition time. Results of the transmission to field-grown tulips are shown in Table 1. Elongated virus particles were found in petals with current-season symptoms of forced tulips 6 weeks after inoculation and in those of field-grown tulips after 8 to 10 weeks. The virus particles were not seen in the leaves of tulips showing current-season symptoms on the petals. Almost 100% of the progeny of tulips with current-season symptoms were infected with LSV in the second year of growth. The progeny of tulips without current-season symptoms were

Table 1. Infection percentages of field-grown tulip 'Rose Copland', inoculated with lily symptomless virus from lily 'Enchantment' by various aphid species in a non-persistent manner.

Aphid species	1st year of growth; % plants with current-season symptoms	2nd year of growth; % LSV-infected progeny of plants	
		with current-season symptoms	without current-season symptoms
Macrosiphum euphorbiae	59	96	64
Myzus persicae	33	100	41
Aphis gossypii	25	93	13

Tabel 1. Infectiepercentages van veldtulpen 'Rose Copland', op non-persistente wijze geïnoculeerd met het symptoomloos lelievirus van de lelie 'Enchantment' met behulp van verschillende soorten bladluizen.

infected to a considerable extent, except after inoculation with *A. gossypii*. *M. euphorbiae* transmitted LSV more efficiently than did *M. persicae* under the experimental conditions applied in 1972.

When the aphids were transferred to field-grown tulips after a 20 to 22 h acquisition time, LSV was not transmitted by *M. persicae*, but *M. euphorbiae* gave roughly the same infection percentages as in the experiment with a 5 sec to 3 min acquisition time. In the current season, 58% of the tulips showed flower symptoms. Of the progeny of these tulips 94% were infected with LSV in contrast to 77% of the progeny of the tulips without symptoms.

The virus was not transmitted to 'Rose Copland' tulips by the 3 aphid species used after a 2 to 3 day acquisition time and with the use of an intermediate host.

Yield. The yield of the symptomless tulips was approximately 7% higher than that of the tulips with symptoms. The reduction in yield was not accounted for by the effect of a current-season infection.

The multiplication rate of the 'Rose Copland' tulips with and without flower symptoms did not differ under the experimental conditions. In both cases the average number of progeny bulbs of one mother bulb was 4.1.

Purification. The virus suspension prepared from sap clarified with chloroform showed a serological dilution end-point titre about 10 times lower than the value obtained when no chloroform had been used. The clarification procedure with chloroform yielded relatively less virus from tulips than from comparable quantities of lily leaf material.

Electron microscopy. The length distribution of elongated particles of LSV in tulips is different from that obtained in sap preparations of lilies (Fig. 3). Particles shorter than 600 nm account for approximately 70% of all particles in tulip and 12% in lily.

Discussion

Transmission of LSV in a non-persistent manner from lilies to tulips by the 3 aphid species used is in agreement with the results of experiments on transmission to lilies

Fig. 3. Length distribution curves of elongated virus particles in crude sap of tulip 'Peerless Pink' and lily 'Enchantment', infected with lily symptomless virus.

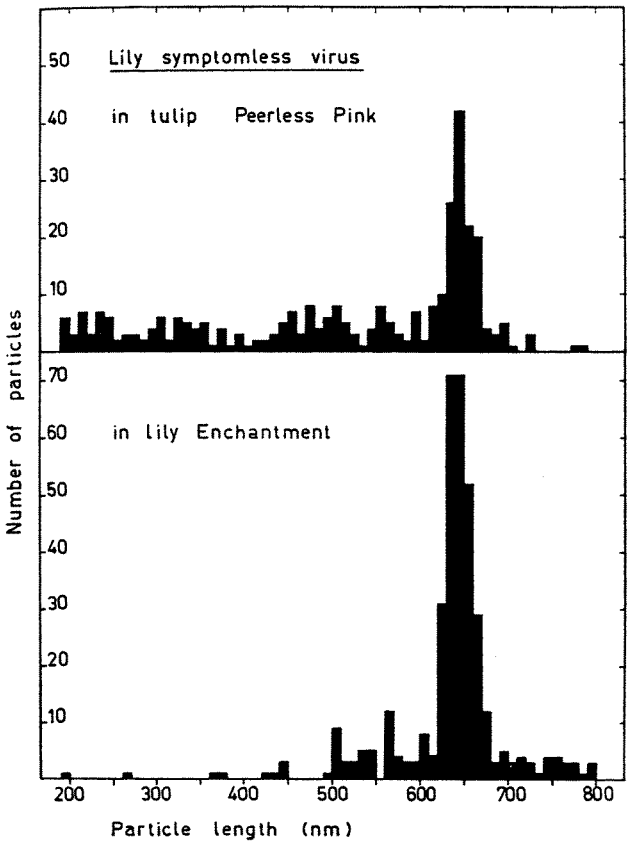

Fig. 3. Lengteverdeling van draadvormige virusdeeltjes in ruw sap van de tulp 'Peerless Pink' en de lelie 'Enchantment', geïnfecteerd met het symptoomloos lelievirus.

with the aphid species *M. persicae*, *M. euphorbiae*, *Aulacorthum solani*, and *Aphis fabae* (Mowat and Štefanac, 1970, 1974; Derks, 1973). With *A. gossypii* we did not find transmission of LSV in a persistent manner (Brierley and Smith, 1944). This disagreement has already been discussed by Mowat and Štefanac (1974), who raised the question of whether Brierley and Smith had been working with a virus not studied since.

The difference in the transmission of LSV between *M. persicae* and *M. euphorbiae* after 20 to 22 h acquisition time, may be attributed to a difference in feeding behaviour. Most *M. persicae* aphids were still feeding on the lily leaves after 20 to 22 h. Transmission of a virus in a non-persistent manner bij feeding aphids is infrequent after an acquisition time of 30 min (Swenson, 1967). In the case of *M. euphorbiae*, however, the aphids were walking around and sometimes probing (not feeding) after 20 to 22 h, which is comparable with a short acquisition time. No transmission of LSV was found

after a 2 to 3 day acquisition time with the use of *G. globosa* as an intermediate host, indicating lack of true persistance of the virus in the aphids.

The relatively successful transmission of LSV by aphids in a non-persistent manner, the rate of infection of the progeny bulbs, and the diagnostic difficulties in identifying affected commercial stocks when tulip flowers are cut too early, indicate the potential danger of LSV for tulip culture if initial sources of infection are found too late.

Samenvatting

Het symptoomloos lelievirus in tulp

Bij sapinoculatie van 53 plantesoorten (18 families) bleek het symptoomloos lelievirus (LSV) alleen overgebracht te kunnen worden op *Lilium formosanum* en tulp 'Rose Copland' (Liliaceae). De luizesoorten *Macrosiphum euphorbiae*, *Myzus persicae* en *Aphis gossypii* brachten LSV op non-persistente wijze over van de lelie 'Enchantment' op de tulp 'Rose Copland' (Tabel 1). *M. euphorbiae* bleek een betere vector voor LSV te zijn dan *M. persicae* en *A. gossypii*.

Incidenteel werd LSV te velde aangetroffen bij partijen van enkele cultivars. De symptomen bij 'Rose Copland' tulpen bestaan uit donkerrose, fijne strepen op de nerven van de bloembladen (Fig. 1). Bij 'Preludium' echter waren op de bloembladen witte strepen te zien (Fig. 2).

De opbrengst van 'Peerless Pink' tulpen met symptomen van LSV was iets lager dan die van tulpen zonder symptomen. Het virus werd uit tulp gezuiverd door uit te gaan van ruw sap en van sap behandeld met chloroform. Bij meting van de LSV-deeltjes in tulp bleek het percentage deeltjes, korter dan 600 nm, hoger te zijn dan bij de lelie (Fig. 3).

Acknowledgment

The authors are indebted to Dr D. Peters and H. J. M. van Dorst, for supplying the aphid species, and to Dr D. Hille Ris Lambers, for confirming their identification.

References

Allen, T. C., 1971. Garden Easter lilies infected by viruses. Oregon Ornam. Nursery Dig., Oregon St. Univ. 15: 1–2.

Allen, T. C., 1972. Lily symptomless virus. Commonw. Mycol. Inst./Assoc. Appl. Biol., Descr. Pl. Viruses, No. 96.

Asjes, C. J., Vos, Neeltje P. de & Slogteren, D. H. M. van, 1973. Brown ring formation and streak mottle, two distinct syndromes in Lilies associated with complex infections of lily symptomless virus and tulip breaking virus. Neth. J. Pl. Path. 79: 23–35.

Bos, L., 1970. The identification of three new viruses isolated from *Wisteria* and *Pisum* in the Netherlands, and the problem of variation within the potato virus Y group. Neth. J. Pl. Path. 76: 8–46.

Brandes, J., 1964. Identifizierung von gestreckten pflanzenpathogenen Viren auf morphologischer Grundlage. Mitt. biol. Bund-Anst. Ld- u. Forstw. 110: 5–130.

Brierley, P. & Smith, F. F., 1944. Studies on lily virus diseases: the necrotic-fleck complex in *Lilium longiflorum*. Phytopathology 34: 529–555.

Derks, A. F. L. M., 1973. Virusziekten in lelies. Jversl. Lab. Bloemboll. Onderz., Lisse 1972: 44.

Mowat, W. P. & Štefanac, Z., 1970. Viruses in lilies. Lily Yb., London 33: 251–253.

Mowat, W. P. & Štefanac, Z., 1974. Aphid-transmitted viruses from lilies in Britain. Ann. appl. Biol. 76: 281–287.

Slogteren, D. H. M. van, 1955. Serological micro-reactions with plant viruses under paraffin oil. Proc. 2nd Conf. Pot. Virus Dis., 1954, Lisse/Wageningen: 51–54.
Swenson, K. G., 1967. Plant virus transmission by insects. In: K. Maramorosch and H. Koprowski (Ed.), Methods in virology. Academic Press, New York and London, Vol. I: 267–307.

Address

Laboratorium voor Bloembollenonderzoek, Heereweg 345a, Lisse, the Netherlands.