Neth. J. Pl. Path. 86 (1980) 239–250

Purification of lily symptomless virus. Use and value of antisera against intact and pyrrolidine-degraded virus for testing lilies and tulips

A. F. L. M. DERKS and JENNY L. VINK-VAN DEN ABEELE

Bulb Research Centre, Lisse

Accepted 31 January 1980

Abstract

Lily symptomless virus (LSV) was purified by clarification with chloroform, precipitation with polyethylene glycol and NaCl, and differential centrifugation. The influence of the source material and some buffers on virus yields were determined.

Antisera were prepared against intact and pyrrolidine degraded LSV. It was concluded that intact and degraded LSV have very few antigenic determinants in common or none at all. The sensitivities of the micro-precipitin test and the single immunodiffusion drop test were about the same, but lower than that of electron microscopy.

In the testing of lilies for LSV the most reliable results in leaves were obtained during the period from two weeks after flowering until close to the end of the growing season, and in leaves growing at a level about one-fourth of the distance from the top of the stem. In contrast to secondary infections, primary infections were detected more successfully in stored bulbs than in leaves taken from plants in the preceding growing season.

In the testing of tulips, LSV was detected better in flowers than in leaves. Detection of primary infections was almost impossible. Except for those with a pink flower, experimentally infected tulips remained symptomless.

Additional keywords: *Lilium* Mid-Century hybrid 'Enchantment', *L. speciosum*, *L. longiflorum*, *L. formosanum*, tulip breaking virus, mature plant resistance.

Introduction

Production of virus-free lily stocks via tissue culture (Allen, 1974; Asjes et al., 1974) and subsequent certification (Asjes, 1974; Segers, 1978) require a sensitive, reliable, and quick test for the detection of lily symptomless virus (LSV; R/*:*/8:E/E:S/Ap; Allen, 1972; carlavirus group). In the Netherlands the single immunodiffusion drop test (Van Slogteren, 1976; Van Slogteren et al., 1976) is used for this purpose, but this test can be replaced by ELISA (Beijersbergen and Van der Hulst, 1980).

LSV has been purified in various ways. Clarification with bentonite of butanol (Civerolo et al., 1968), ether/tetra (Van Slogteren and De Vos, 1972), or chloroform (Derks and Asjes, 1975) has been used in combination with differential centrifugation. All of these methods have the disadvantage that host constituents are present in the purified suspension, whether lilies or tulips are used as source.

This paper reports a useful purification method for LSV, the production of antisera

against intact and pyrrolidine-degraded LSV, and a comparative study on the use of serological tests and electron microscopy at different growth stages of lilies and tulips.

Material and methods

Purification. Leaf material was collected when plants were in flower or later from stocks of *Lilium* Mid-Century hybrid 'Enchantment', both grown under glass or in the field from white bulbs (with LSV alone) or bulbs with brown ring symptoms (with LSV and tulip breaking virus (TBV); Asjes et al., 1973).

Frozen leaves were homogenized with a blender in 0.5 M borate buffer (pH 8.2) containing 1 % sodium sulfite (2–3 ml per gram tissue). LSV was purified by clarification with chloroform, precipitation with polyethylene glycol (PEG) and NaCl, and differential centrifugation as described for potato virus M (Shepard, 1972). High-speed centrifugation was performed at 90000 *g* for 90 min. Some modifications of the purification procedure were tested.

Analytical ultracentrifugation. Sedimentation coefficients were determined by the graphical method of Markham (1960) using a Phywé analytical ultracentrifuge.

Spectrophotometry. For the determination of virus concentrations, an extinction coefficient $E_{260}^{0.1\%} = 3.0$ was used, as has been done for other carlaviruses (Shepard, 1972; Veerisetty and Brakke, 1978).

Electron microscopy. Purified suspensions in 0.05 M borate buffer (pH 8.2) and leaf or flower preparations in 0.067 M phosphate buffer (pH 7.2) were negatively stained with 2.5 % PTA (pH 7.2) and examined in a Philips EM 201. For measurements, tobacco mosaic virus was used as an internal length standard.

Serology. For the single immunodiffusion drop test (Van Slogteren, 1976), antisera against degraded virus were prepared. A purified suspension (ca. 5 mg LSV per ml) was mixed with an equal volume of 5 % pyrrolidine in distilled water at room temperature (Shepard, 1972). After opalescence disappeared, the suspension was dialyzed at 9 °C for 48 h against 0.05 M borate buffer (pH 8.2) containing 0.37 % formaldehyde. After emulsification of the dialyzed suspension with an equal volume of Freund's incomplete adjuvant, rabbits were injected intramuscularly (2 ml per hind leg) 5 times at weekly intervals. The rabbits were bled weekly, starting 2–3 weeks after the last injection. When the antiserum titre declined, the rabbits received a booster injection intramuscularly. Titres were determined with the single immunodiffusion drop test using leaves of LSV-infected 'Enchantment' treated with pyrrolidine (Van Slogteren, 1976; Van Slogteren et al., 1976).

Antisera against the intact virus were prepared by injecting the rabbits intravenously with a purified suspension (ca. 1 mg LSV per ml) in 0.9 % NaCl and one week later intramuscularly. The rabbits were bled and received a booster injection as described above. Titres were determined with the microprecipitin test (Van Slogteren, 1955) using leaves of 'Enchantment' (with LSV alone), homogenized with 0.067 M phosphate buffer (pH 7.0, w/v = 1/1), pressed through cheese cloth, and centrifuged for 10 min at 1500 *g*. Spontaneous reactions were usually prevented by additional centri-

fugation for 10 min at 9000 *g* (Derks and Asjes, 1975).

Purified preparations of LSV, intact or degraded, were tested for the presence of LSV and TBV (Van Slogteren and De Vos, 1966) with the microprecipitin test and/or agar gel diffusion method. Determinations of the presence and/or dilution endpoints of LSV in lily and tulip leaves, flowers, or bulbs were performed in tissue frozen at –20 °C (homogenized and centrifuged as described above) with the single immunodiffusion drop test and/or the microprecipitin test. For the determination of the dilution endpoints, LSV-infected stocks of 'Enchantment' and *Lilium speciosum* 'Uchida' were grown under glass at about 20–30 °C. Leaves of 'Uchida' were picked about half-way up the stems from the same 7 plants throughout the season. The dilution endpoints of LSV in leaves of 'Enchantment' were determined (Van Slogteren et al., 1976) in different plants for each sampling date; the lilies, grown from bulbs of size 9/10, were sampled at various heights.

Transmission experiments. Virus-free plants of 'Enchantment' were inoculated with LSV by aphids (*Macrosiphum euphorbiae* and *Myzus persicae*); after a short acquisition time (Derks and Asjes, 1975), the aphids were placed on the top leaves of the plants to be inoculated. Inoculation was done at four stages of growth: sprouts 2–5 cm long (7 weeks before flowering), first flower buds just visible (4 weeks before flowering), in the middle of the flowering period, and 2 weeks after flowering. In 1975 bulbs of size 9/10 (20 per treatment) were used and in 1976 small bulblets with a weight of 0.5–1.0 gram (ca. 40 per treatment) supplemented with bulbs of size 9/10 to mark the flowering period. The lilies were grown in insect-proof glasshouses at about 20 °C except in 1975, when the temperature was often 30–35 °C. The lilies inoculated in 1975 were examined with the electron microscope on the basis of leaves picked at different levels on the plants. In the year of inoculation the lilies were examined, if possible, at the beginning and end of the flowering period (July), and again 2 and 6 weeks after flowering; in the year after inoculation only at flowering time. The leaves were also tested serologically with the single immunodiffusion drop test or microprecipitin test 2 weeks after flowering in the year of inoculation and at flowering time in the next year. Extracts of tips of the outer bulb scales were tested with the single immunodiffusion drop test at the end of the storage period (February). The lilies inoculated in 1976 were periodically examined with the electron microscope, i.e. part of the leaves. In the first year of growth the leaves and bulblets were too small to give sufficient material for the single immunodiffusion drop test.

In 1974 and 1976, fifteen tulip cultivars with different flower colours were inoculated (Derks and Asjes, 1975), viz. Aladdin, Apeldoorn, Bellona, Brilliant Star, Gander, Golden Harvest, Karel Doorman, Lustige Witwe, Madame Lefeber, Olga, Paul Richter, Pax, Peach Blossom, Prominence, and Queen of the Night. The tulips (18 bulbs per cultivar) were grown in the field under cages, and flowered 4–6 weeks after inoculation. The plants were examined visually, electron-microscopically, and serologically during flowering time in the year of inoculation and the following year.

Results

Purification. Electron-microscopical examination of leaf preparations of several cultivars of *L. speciosum*, *L. longiflorum*, *L. formosanum*, and Mid-Century hybrids

Table 1. Effect of source and various buffers on virus yield. The values are means (in mg LSV per 100 gram leaf tissue of lily 'Enchantment') with the standard error.

1. Source	
grown from bulbs with brown ring symptoms	12.5 ± 1.7
grown from white bulbs	4.4 ± 0.1
2. Extraction buffer	
0.5 M borate buffer (pH 8.2) + 1% Na_2SO_3	13.2 ± 2.5
0.5 M borate buffer (pH 8.2)	10.9 ± 5.5
3. Extraction and resuspension buffers	
borate buffers (pH 8.2)	19.3 ± 1.2
tris buffers (pH 8)	10.6 ± 6.5

Tabel 1. Effect van uitgangsmateriaal en buffer op de virusopbrenst. De waarden zijn gemiddelden (in mg LSV per 100 gram blad van lelie 'Enchantment') met de standaardafwijking.

showed the highest number of virus particles (LSV) in 'Enchantment'. Because leaf extracts of this cultivar were also less slimy than those of most of the other lilies, 'Enchantment' was chosen as source for virus purification studies. For 'Enchantment', virus yields of LSV were about three times higher in plants grown from bulbs with brown ring symptoms than in those grown from white bulbs (Table 1). TBV was found in the purified preparation in only one out of 22 cases, as determined serologically, by virus measurements and analytical ultracentrifugation.

The extract was slimier and less virus could be detected with the single immunodiffusion drop test in fresh leaf homogenates than in frozen material.

The addition of 1 % sodium sulfite to the extraction buffer had only a slight effect on virus yields (Table 1), and was done routinely.

Fig. 1. Analytical ultracentrifugation patterns found for LSV (A) and for LSV and TBV (B). Sedimentation from left to right. Pictures were taken after about 12 min of centrifugation at 21 500 rpm.

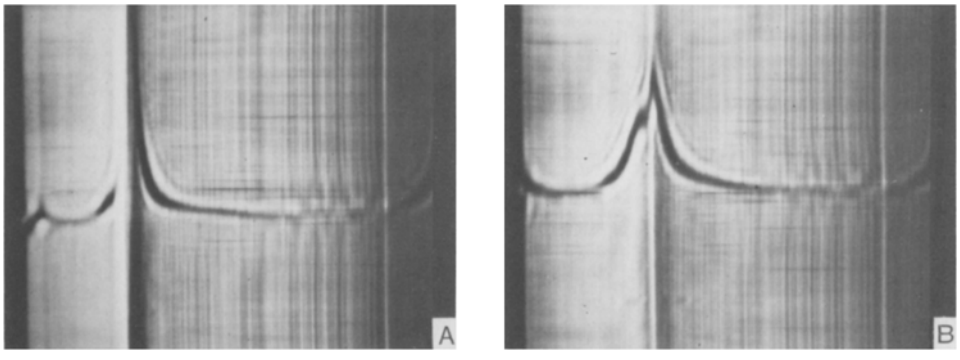

Fig. 1. Analytische ultracentrifugebeelden van LSV (A) en van LSV en TBV (B). Sedimentatie van links naar rechts. De foto's werden genomen na ongeveer 12 min centrifugeren bij 21 500 tpm.

Because the compactness of the pellets after the first high-speed centrifugation hampered resuspension, other buffers were tested. With 0.1 M tris-thioglycollic acid (pH 9) as extraction buffer and 0.1 M tris-HCl (pH 9) as resuspension buffer, the pellets obtained after the first high-speed centrifugation were even stickier than in those obtained with borate buffers. Tris buffers (pH 8) gave easily resuspended pellets but the virus yields were lower than with the borate buffers if the pellets were resuspended with a mixer (Table 1).

Analytical ultracentrifugation. Purified LSV sedimented as a single component with a sedimentation coefficient of about 171 S (mean value of 7 trials) in 0.005 M borate buffer (pH 8.2). One purified suspension showed an additional, smaller peak (shoulder) of TBV with a sedimentation coefficient of about 155 S (mean value of 2 trials; Fig. 1).

Spectrophotometry. UV spectra of the almost colourless purified LSV suspensions showed a minimum at 246 $\pm$ 2 nm and a maximum at 260 nm. The max:min ratio ranged from 1.06 to 1.16.

Electron microscopy. Leaf preparations of 'Enchantment' often showed lateral aggregation of LSV particles, especially when the plants derived from bulbs with brown ring symptoms or were inoculated early in the growing season and grown at about 20°C. Purified LSV suspensions showed no lateral but sometimes a few terminal aggregations (Fig. 2).

Serology. Homologous titres of antisera prepared against pyrrolidine-degraded LSV varied according to the individual rabbit and the number of booster injections. After the first series of 5 injections the titre ranged from 0 to 1/20 with an average of about

Fig. 2. Electron micrographs of unaggregated, purified LSV (A) and of markedly aggregated LSV particles in lily 'Enchantment', grown at about 20°C and inoculated when the first flower buds were just visible (B). Preparations were stained with 2.5% PTA (pH 7.2). Magnification bars represent 500 nm.

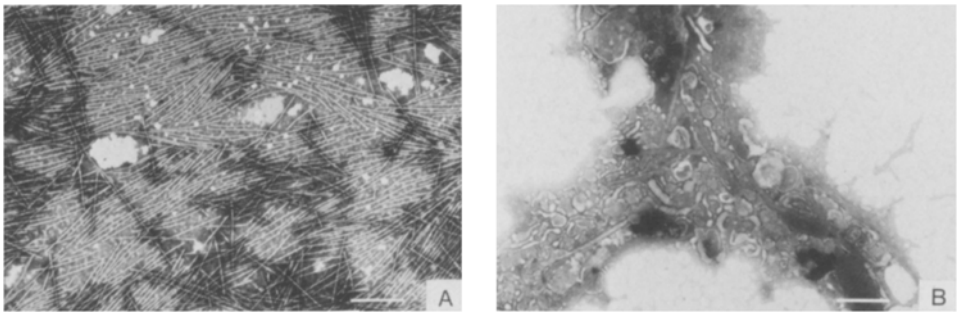

Fig. 2. Elektronenmicroscopische foto's van ongeaggregeerd, gezuiverd LSV (A) en van sterk geaggregeerde LSV-deeltjes in lelie 'Enchantment', welke geteeld werd bij ongeveer 20°C en geïnoculeerd toen de eerste bloemknoppen net zichtbaar waren (B). De preparaten werden negatief gekleurd met 2,5% PTA (pH 7.2). De vergrotingsstaven geven 500 nm weer.

1/8. After each booster injection the average titre rose. After the third, the average titre was 1/40 and none of the rabbits had a titre below 1/20. In some rabbits the titre remained at the same level, 1/40, for six months, whereas in others the value dropped to 1/10 after 2 months. Antisera prepared against intact LSV showed titres between 1/2048 and 1/4096 after the second booster injection. With these antisera, up to about 5 μg LSV per ml could be detected with the micro-pecipitin test.

Before injection into the rabbits, suspensions of degraded LSV (ca. 0.15 mg per ml) did not show any serological reaction in the microprecipitin test with antiserum against intact LSV. However, antisera against pyrrolidine-degraded LSV had titres of more than 1/128 against intact LSV in the microprecipitin test from the first bleeding. Therefore, very concentrated suspensions of degraded LSV (ca. 5 mg per ml) were tested with the agar gel diffusion method. Now, the antiserum against intact LSV gave precipitation just around the well and the antiserum against the degraded virus gave two precipitation lines, one just around the well and the other half-way between the well and the central channel (Fig. 3).

Electron-microscopically, the pyrrolidine-degraded LSV suspensions showed some small fragments and intact particles, which were also found after the addition of a double volume of 5% pyrrolidine to the purified suspension. After centrifugation for

Fig. 3. Serological reactions obtained with the agar gel diffusion method (stained with Amido black); pyrrolidine-degraded LSV in the wells and antiserum against degraded LSV (top) or against intact LSV (bottom) in the central channel.

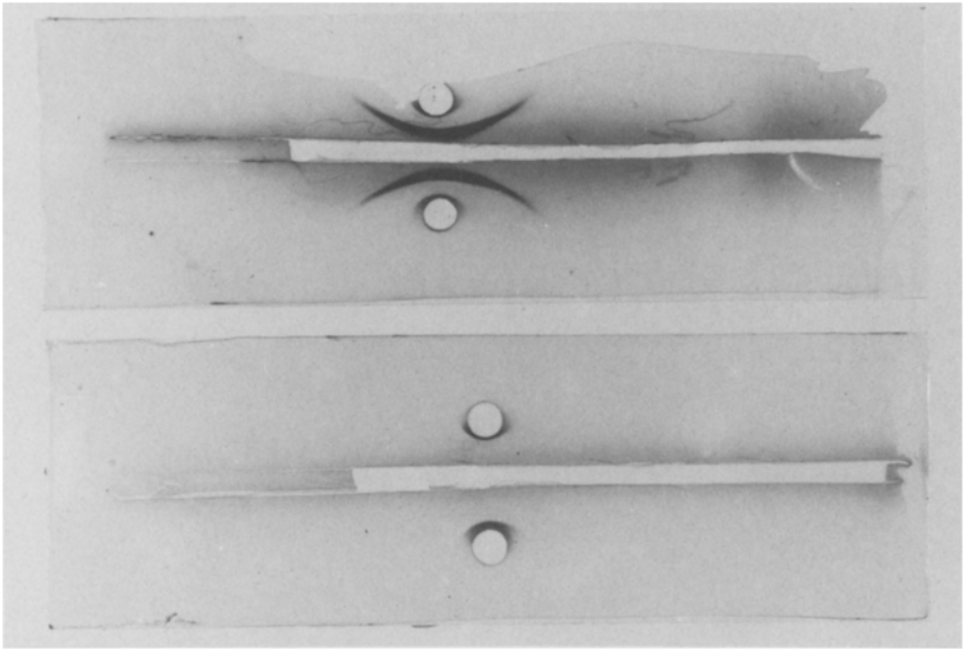

Fig. 3. Serologische reacties met de agargel-diffusiemethode (gekleurd met Amido black); met pyrrolidine afgebroken LSV in de putjes en antiserum tegen afgebroken LSV (boven) of tegen intact LSV (onder) in het middenkanaal.

90 min at 90000 *g*, the supernatant only showed one precipitation line with the antiserum against the degraded virus (half-way between the well and the central channel) and none with the antiserum against the intact virus. The resuspended pellet reacted as the intact virus, i.e. only a small precipitation line around the wells with both antisera. Both antisera, i.e. against degraded and intact LSV, did not react with sap from virus-free lilies and tulips or from TBV-infected tulips.

Findings in lilies. In secondarily infected 'Uchida' grown under glass, LSV could be detected most reliably between flowering and the end of the growing season because of the highest average dilution endpoint, the smallest spread in results and the fact that all plants were detected (Fig. 4). For 'Enchantment' grown under glass from bulbs of size

Fig. 4. Relative virus concentrations in leaves from the middle of plants of *L. speciosum* 'Uchida' during the growing season (average dilution endpoints of clarified sap from 7 plants and spread).

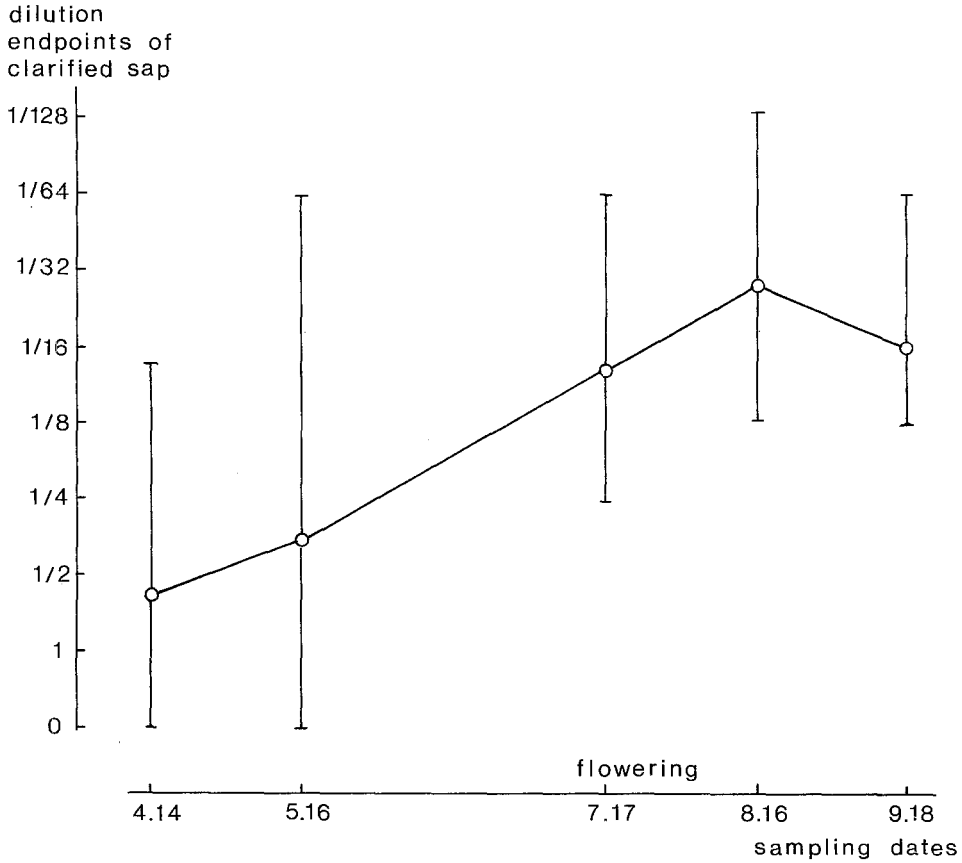

Fig. 4. Relatieve virusconcentraties in bladeren van het midden van de plant van L. speciosum 'Uchida' gedurende het groeiseizoen (gemiddeld verdunningseindpunt van geklaard sap van 7 planten en de spreiding).

Table 2. Percentages of LSV infection in plants of lily 'Enchantment' detectable in leaves and/or bulbs with the electron microscope (EM), the single immunodiffusion drop test (SID), and the microprecipitin test (MP), in the year of inoculation (1975 or 1976) and the following years.

Growth stage at inoculation	LSV detection in:						
	first experiment					second experiment	
	1975 leaves		1976		1977	1976	1977
			bulbs	leaves	bulbs	leaves	leaves
	EM	SID	SID	EM/MP	SID	EM	EM/SID
sprouts 2–5 cm long	40	25	60	60	30	48	71
first flower buds just visible	10	5	15	45	5	81	93
middle of flowering period	0	–	10	20	20	7	50
2 weeks after flowering	5	–	5	10	5	0	2

– not determined

Tabel 2. Percentages planten van lelie 'Enchantment', geïnfecteerd met LSV zoals werd vastgesteld met het elektronenmicroscoop (EM), de enkele immunodiffusie-druppeltoets (SID) en de microprecipitatiemethode (MP) in het jaar van inoculatie (1975 of 1976) en de daaropvolgende jaren, gebruik makend van bladeren en/of bollen.

9/10 or from small bulblets producing a single leaf, the most reliable period was between two weeks after flowering of the bulbs with size 9/10 and the end of the growing season, as had been found for field-grown 'Enchantment' (Van Slogteren et al., 1976). The highest average dilution endpoints were found for leaves growing at a level about one-fourth of the distance from the top of the stem.

In the year of infection in both inoculation experiments with 'Enchantment' (1975 and 1976), the highest numbers of virus particles were detected electron-microscopically in the leaves at or just below the top of the plants, irrespective of the time of inoculation and of observation. As a rule, the lowest leaves did not contain virus particles. In the current season of the second experiment leaf preparations showed many more virus particles and much more marked particle aggregation (Fig. 2) than did those of the first experiment.

The percentages of LSV infection detectable in leaves or bulbs with the electron microscope or serologically in the year of inoculation and the following years are shown in Table 2. The percentages of LSV infection detectable with the electron microscope in leaves in the year of inoculation did not change after flowering. These highest percentages are shown in Table 2. The infection percentages mentioned in the fourth column of Table 2 (under 1976, leaves) are the final values of the first experiment. The percentages in the first three columns can be compared with these final values to estimate the effect of the detection method in primary infections.

Findings in tulips. In the year of inoculation not a single plant of all 15 tulip cultivars showed flower symptoms or had virus particles detectable in the petals by the electron microscope. In the second year only the cultivar Olga showed flower symptoms, which consisted of dark-pink veinal streaks, when infected with LSV.

In the second year, as soon as virus particles could be observed electron-microscopically in the petals, serological detection in the petals was also successful with both the single immunodiffusion drop test and the microprecipitin test. However, in leaf extracts of the same plants LSV could not be detected with either serological method in 24 % of the tulips, and in another 8 % LSV detection was uncertain with the microprecipitin test. Electron microscopy in flower and leaf extracts of the same plants revealed that 73 % of the plants showed (many) more LSV particles in flower than in leaf extracts.

Discussion

Civerolo et al. (1968) reported low virus yields after purification of *L. longiflorum* extracts, due to a low concentration of LSV in this host. They also suggested considerable loss of virus during purification. This was possibly due to the fact that *L. longiflorum* produces slimy leaf extracts; Beijersbergen and Van der Hulst (1980) found that slimy (bulb) extracts retained LSV, which could be avoided by adding an enzyme such as hemicellulase. Mechanical inoculation with LSV in leaf extracts of *L. longiflorum* is also difficult (Brierly and Smith, 1944; Allen and McWhorter, 1966). However, the addition of hemicellulase to the extracts facilitates mechanical transmission to *L. formosanum* (Derks, unpublished). Thus, for the choice of a source for purification not only the virus concentration in the leaf tissue is important, but also the ease with which the virus can be freed. This was also concluded by Veerisetty and Brakke (1978), who showed the importance of freezing the tissues to release more virus particles of legume carlaviruses. We found the same for LSV.

It proved possible to purify LSV from 'Enchantment' grown from bulbs with brown ring symptoms, and to eliminate TBV at the same time (with only one exception). Not only freezing of the tissue and precipitation with PEG were disadvantageous for TBV (Derks, unpublished), but also the low concentrations at the start (Asjes et al., 1973).

The antisera against intact and degraded LSV gave no reaction with virus-free lilies and tulips in the single immunodiffusion drop test and the microprecipitin test. Moreover, in ELISA very low extinction values were found for virus-free lilies (Beijersbergen and Van der Hulst, 1980). Thus, a rather easy and quick purification method provided LSV with which antisera could be produced that did not show reaction with host constituents.

Shepard and Secor (1969) mentioned working with degraded-protein sera has the disadvantage that the titres decline rapidly after the last immunizing injection. This holds only partially for pyrrolidine-degraded LSV, because several rabbits showed titres of 1/40 over a period of at least 6 months.

Since after high-speed centrifugation the pyrrolidine-degraded LSV only reacted in the agar gel diffusion test with the antiserum against degraded LSV and not with the antiserum against intact LSV, we conclude that degraded and intact LSV have very few antigenic determinants in common or none at all.

The sedimentation coefficient of about 171 S we found for LSV is in agreement with

the results of Civerolo et al. (1968), and our sedimentation coefficient of about 155 S for TBV is comparable with that of some other potyviruses (Shepherd and Purcifull, 1971; Brunt, 1973 a, b).

An increase in the number of LSV particles detected with the electron microscope in complex diseases such as necrotic fleck in *L. longiflorum* (Allen and Lyons, 1969), brown ring symptoms in 'Enchantment', and streak mottle in *L. speciosum* (Asjes et al., 1973), was confirmed for 'Enchantment' by comparing virus yields after purification. The increase in the number of virus particles was accompanied by an increase in number and size of particle aggregates, especially lateral aggregates, in crude extracts, as was found in plants of 'Enchantment' which became infected with LSV and were kept at about 20 °C.

For both the single immunodiffusion drop test (Van Slogteren et al., 1976) and the microprecipitin test, about 5 µg LSV per ml was the lowest detectable virus concentration. Electron microscopy is a more sensitive method, but even with this method not all primary infections of LSV in lilies and tulips could be detected at the end of the growing season. Serological detection of primary LSV infections with the single immunodiffusion drop test in bulb scales during storage was even better than electron-microscopical detection in leaves from plants sampled in the preceding growing season. However, bulb scales with secondary infections sometimes gave problems in the single immunodiffusion drop test. Many of these problems can possibly be avoided by using ELISA (Beijersbergen and Van der Hulst, 1980).

Independant from growing in the field (Van Slogteren et al., 1976) or under glass, primary or secondary LSV infection, bulb size and lily species, the most reliable testing period for leaves is between two weeks after flowering and close to the end of the growing season. Moreover, the most reliable results are obtained with leaves growing at a level about one-fourth of the distance from the top of the stem.

When the elongation of stems and leaves stops (about two weeks after flowering; Van der Valk, pers. comm.), the dilution endpoint of LSV in clarified sap of 'Enchantment' reaches an almost constant level (Van Slogteren et al., 1976), and the chance that inoculated LSV can reach the bulb becomes very small. This mature-plant resistance has also been described for other plant-virus combinations (e.g. Beemster, 1961).

Symptoms of LSV in tulips can only be observed on pink cultivars. In the year of infection it is sometimes possible to detect symptoms of LSV on the flowers of the tulip 'Rose Copland' (Asjes et al., 1973; Mowat and Štefanac, 1974; Derks and Asjes, 1975). From the data obtained in this cultivar it can be concluded that to obtain current-season symptoms the plants must become infected with the virus at least six weeks before flowering. This possibly explains why the cultivar Olga did not show current-season symptoms of LSV infection in the experiments described here.

In tulips with current-season symptoms LSV could be detected with electron microscopy in the petals but not in the leaves (Mowat and Štefanac, 1974; Derks and Asjes, 1975). In the second year of growth, LSV could also be detected more easily in the flowers than in the leaves.

Acknowledgments

The authors are indebted to Miss M. H. Bunt for technical assistance and to Mr J. Kamerman (Bulb Inspection Service) for supplying lily bulbs with brown ring symptoms.

Samenvatting

Zuivering van het symptoomloos lelievirus. Het gebruik en de waarde van antisera gemaakt tegen intact en tegen met pyrrolidine afgebroken virus voor het toetsen van lelies en tulpen

Het symptoomloos lelievirus (LSV) werd gezuiverd door klaring met chloroform, precipitatie met polyethyleenglycol en NaCl en differentieel centrifugeren. Het effect van het uitgangsmateriaal en enkele buffers op de virusopbrengst werd nagegaan.

Antisera werden bereid tegen intact en met pyrrolidine afgebroken LSV. Geconcludeerd werd dat intact en afgebroken LSV geen of slechts enkele antigene determinanten gemeenschappelijk hebben.

De gevoeligheid van de microprecipitatietoets en de enkele immuno-diffusie-druppeltoets is ongeveer gelijk. Met het elektronenmicroscoop kunnen echter lagere virusconcentraties worden aangetoond.

Het toetsen van lelies op LSV gaf de betrouwbaarste resultaten met bladeren die op ongeveer driekwart hoogte van de stengel groeien en in de periode tussen 2 weken na de bloei en vrijwel het einde van het groeiseizoen worden geplukt. Primaire infecties konden, in tegenstelling tot secundaire infecties, beter worden vastgesteld aan bolmateriaal tijdens de bewaring dan aan bladeren in het voorafgaande groeiseizoen.

Bij het toetsen van tulpen werd het LSV met grotere zekerheid vastgesteld in bloemen dan in bladeren. Het vaststellen van primaire infecties was bijna niet mogelijk. Na infectie van tulpen met LSV vertoonden alleen die met een rose bloemkleur symptomen.

References

Allen, T. C., 1972. Lily symptomless virus. CMI/AAB, Descr. Pl. Viruses, No. 96.

Allen, T. C., 1974. Production of virus-free lilies. Acta Hortic. 36: 235–240.

Allen, T. C. & Lyons, A. R., 1969. Electron microscopy of lily symptomless virus and cucumber mosaic virus within fleck diseased lilies. Phytopathology 59: 1318–1322.

Allen, T. C. & McWhorter, F. P., 1966. Virus-like particles associated with curl-stripe disease of *Lilium longiflorum*. Phytopathology 56: 869 (Abstr.).

Asjes, C. J., 1974. The prospect of eliminating virus diseases in lilies in The Netherlands. In: Lilies 1974 and other Liliaceae, 2–12. R. Hort. Soc., London.

Asjes, C. J., Bunt, M. H. & Slogteren, D. H. M. van, 1974. Production of hyacinth mosaic virus-free hyacinths and lily symptomless virus-free lilies by meristem tip culture. Acta Hortic. 36: 223–228.

Asjes, C. J., Vos, N. 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.

Beemster, A. B. R., 1961. Een vergelijking tussen het transport van X-virus en twee verschillende stammen van Y-virus in aardappelplanten. Tijdschr. PlZiekt. 67: 278–279.

Beijersbergen, J. C. M. & Hulst, C. Th. C. van der, 1980. Application of enzymes during bulb tissue extraction for detection of lily symptomless virus by ELISA in *Lilium* spp. Neth. J. Pl. Path. 86: 277–283.

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

Brunt, A. A., 1973a. Iris mild mosaic virus. CMI/AAB, Descr. Pl. Viruses, No. 116.
Brunt, A. A., 1973b. Hippeastrum mosaic virus. CMI/AAB, Descr. Pl. Viruses, No. 117.
Civerolo, E. L., Semancik, J. S. & Weathers, L. G., 1968. Partial purification of viruslike particles associated with the necrotic fleck disease of Easter lily. Phytopathology 58: 1481–1486.
Derks, A. F. L. M. & Asjes, C. J., 1975. Lily symptomless virus in tulip. Neth. J. Pl. Path. 81: 14–21.
Markham, R., 1960. A graphical method for the rapid determination of sedimentation coefficients. Biochem. J. 77: 516–519.
Mowat, W. P. & Štefanac, Z., 1974. Aphid-transmitted viruses from lilies in Britain. Ann. appl. Biol. 76: 281–287.
Segers, C. M., 1978. Teelt van virusvrije lelies in 1978. Bloembollencultuur 88: 758–759.
Shepard, J. F., 1972. Gel-diffusion methods for the serological detection of potato viruses X, S and M. Bull. Mont. agric. Exp. Stn 662, 72 pp.
Shepard, J. F. & Secor, G. A., 1969. Detection of potato virus X in infected plant tissue by radial and double-diffusion tests in agar. Phytopathology 59: 1838–1844.
Shepherd, R. J. & Purcifull, D. E., 1971. Tobacco etch virus. CMI/AAB, Descr. Pl. Viruses, No. 55.
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.
Slogteren, D. H. M. van, 1976. A single immunodiffusion drop test for the detection of lily symptomless virus. Ann. appl. Biol. 82: 91–95.
Slogteren, D. H. M. van, Derks, A. F. L. M., Blom, G. J., Bunt, M. H. & Vink, J. L., 1976. A further simplified single immunodiffusion drop test for the detection of lily symptomless virus in *Lilium* species. Acta Hortic. 59: 29–36.
Slogteren, D. H. M. van & Vos, N. P. de, 1966. Tulip breaking virus, its serological behaviour and serological relationship to a virus isolated from lily. In: A. B. R. Beemster & Jeanne Dijkstra (Eds): Viruses of plants. Proc. int. Conf. Pl. Viruses, July 1965, Wageningen: 320–323.
Slogteren, D. H. M. van & Vos, N. P. de, 1972. Virusziekten in lelies. Jversl. Lab. BloembollOnderz., Lisse 1970–1971: 46.
Veerisetty, V. & Brakke, M. K., 1978. Purification of some legume carlaviruses. Phytopathology 68: 59–64.

Address

Stichting Laboratorium voor Bloembollenonderzoek, Vennestraat 22, 2160 AB Lisse, the Netherlands.