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Lilac ring mottle virus: isolation from lilac, some properties, and relation to lilac ringspot disease

F. A. VAN DER MEER, H. HUTTINGA and D. Z. MAAT

Institute of Phytopathological Research (IPO), Wageningen

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Abstract

A new virus of lilac is described, for which the name lilac ring mottle virus is proposed. The virus can be mechanically transmitted to various herbaceous hosts. Symptoms on the most important hosts are described. The virus is inactivated in crude sap in 10 min at 63–65 °C, after dilution to 10^{-4} and after storage for 5 h at 20 °C. The virus is seed-borne in three herbaceous hosts. It is not transmitted by *Myzus persicae*.

The virus particles are sensitive to high ionic strength and can only be seen in the electron microscope after fixation with glutaraldehyde. They appear as rather irregularly shaped isometric particles with an average diameter of about 27 nm. In rate-zonal centrifuging the virus precipitates in two zones. The bottom component is infectious, the top component is not.

An antiserum with a titre of 1024 was prepared. Serological tests revealed that lilac ring mottle virus is not related to any of 32 isometric plant viruses tested.

Introduction

Lilac *(Syringa vulgaris)* is a woody ornamental that is propagated vegetatively. Hence, virus infections are likely to occur, and in fact several virus diseases have been already reported from lilac (Schmelzer and Schmidt, 1966). To improve the quality of commercial planting material in the Netherlands attempts were made to develop reliable indexing methods for viruses in lilacs which would enable the selection of virus-free stock. Sap inoculations from a number of lilacs to herbaceous plants revealed that some lilacs contain a virus hitherto undescribed. The present paper reports on the herbaceous hosts of this virus, some of its properties in vitro, and its relation to lilac ringspot which shows some symptomatological resemblance. Lilac ringspot is a typical virus-like syndrome but the virus or viruses concerned have not yet been identified. It was first described in Bulgaria by Atanasoff (1935) and was later reported from the Netherlands (Van Katwijk 1955), many other European countries (Schmelzer and Schmidt, 1966) and from the USA (Beale and Beale, 1952). Symptoms consist of leaf deformations, rings, lines and line patterns, all varying much in size and shape. According to this variation and the results of his own transmission experiments to herbaceous hosts, Novák (1969) differentiated between ringspot, chlorotic ring and yellow ring. He judged the three syndromes to be similar to the lilac ringspot descriptions of Atanasoff (1935), Beale and Beale (1952) and Kochman et al. (1964), respectively. Unfortunately, Novák did not further identify the three viruses, isolated from his different sources. Kochman et al. (1964) were the first to transmit a virus from a ringspot-diseased lilac to herbaceous hosts. Mechanical

inoculation of lilac seedlings with the virus did not result in ringspot symptoms. No back inoculations were made from these seedlings. Schmelzer (1970) isolated *Arabis* mosaic virus together with tomato black ring virus from lilac seedlings with ringspot symptoms resulting from grafting (budding) with the lilac ringspot source of Kochman et al. (1964). With many other lilac ringspot sources, however, Schmelzer's results were negative.

Materials and methods

Source plants. Part of the source plants used in experiments during 1972, 1973, and 1974 were ringspot-diseased lilacs collected during yearly field inspections by the Netherlands Plant Protection Service and which they had replanted in Wageningen for further observations. Other sources were two-year-old lilac bushes planted at an interspace of 2 m at an experimental field in spring 1973 to allow careful visual examination during 1973, 1974, and 1975.

Test and assay hosts. Chenopodium quinoa was used as a test and assay host. It was grown in a growth chamber under artificial light at 22 °C and a day length of 16 h. Four weeks after sowing, plants of about 10 cm high were potted in pots 12 cm in diameter and placed in a glasshouse at 20 to 24 °C. Additional light was given between October 15 and March 31 to a total day length of 16 h.

Lilac seedlings were from a symptomless white lilac bush of unknown variety in which no viruses could be detected by means of sap inoculation to *C. quinoa.* Potted seedlings were grown in the just-mentioned glasshouse and inoculated when three to five weeks old and still in the cotyledon stage.

Mechanical transmission. Inoculum was prepared from young lilac leaves and from just expanding or still dormant buds from which the leathery outer scales were removed. This material was macerated in 1 % nicotine in water (0.1 g in 1 ml) and immediately inoculated with finger tips to carborundum-dusted *C. quinoa* plants. No nicotine or other additives were used in sap inoculations between herbaceous hosts, however, sap of *C. quinoa* was always diluted one to ten with water when inoculated to plants of other families.

Virus purification. Leaves from infected *C. quinoa* with severe symptoms were stored overnight at 4 °C. Portions of 100 g were then homogenized with a Waring blendor in a mixture of 35 ml of carbon tetrachloride, 35 ml of chloroform, 15 ml of diethyl ether, and 270 ml of 0.018 M phosphate-citric acid buffer pH 7 (PCA buffer) containing 0.1 % thioglycolic acid. The homogenate was centrifuged for 10 min at 6000 *g*. The resulting upper phase was decanted, brought to 8 % with polyethylene glycol 6000 and stirred for 1 h at 4 °C. The precipitate was collected by centrifuging for 15 min at 16300 *g*, and resuspended in about a quarter of the original volume of PCA buffer using a 'Potter'-homogenizer. The debris which was not resuspended within 1 h, was removed by centrifuging for 10 min at 10400 *g*. The clear supernatant was centrifuged for 2 h at 105000 *g*. The high-speed pellets were resuspended in PCA buffer and after 1 h at 4 °C another cycle of low- and high-speed centrifuging was applied. Then contaminating plant material of low molecular mass was removed by a

sucrose-gradient centrifuging and consequently concentrating the virus by high-speed centrifuging. Finally the suspension was centrifuged for 10 min at 12500 *g* and stored at 4 °C after addition of 4×10^{-4}M NaN_3 for physical studies. For antiserum preparation sucrose-gradient centrifuging was repeated. After concentrating, the virus was resuspended in 0.3 ml of PCA buffer for every 100 g of starting material, mixed with an equal volume of 4% glutaraldehyde and stored overnight. It was then subjected to isopycnic centrifuging in a CsCl gradient. The virus zone was isolated with a syringe and dialyzed against PCA buffer for at least 8 h. Rate-zonal density-gradient centrifuging was done in a Beckman SW27 rotor on a linear 10–40% sucrose gradient. The gradients were loaded with 0.1–0.5 ml of virus suspension originating from about 100 g of leaf material and spun for 2 h at 137000 *g*. Fractions were recovered by an Isco density-gradient fractionator with an absorbance monitor. Isopycnic centrifuging was done in a Beckman SW41 Ti rotor during 18 h at 152000 *g*. In each tube 0.6 ml of glutaraldehyde-fixed virus suspension was mixed with 2.4 ml of a CsCl solution in water (623 mg CsCl/ml). The tubes were filled up with mineral oil. Centrifugal forces *(g)* given are at maximum radii.

Electron microscopy. Samples were negatively stained with 1% potassium phosphotungstate pH 6.5 in water. Preparations were examined in a Philips EM 300.

Antiserum preparation and serology. A rabbit was given two intravenous injections of 1 ml of purified virus suspension each, with an interval of one week. Two weeks later an intramuscular injection with an emulsion of 2 ml of virus suspension and of 2 ml of Freund's incomplete adjuvant was administered. Another intravenous injection of 1.5 ml of virus suspension was given 6 weeks later. Every ml of virus suspension represented the amount of virus obtained from about 500 g of infected leaf material.

The Ouchterlony double-diffusion test was applied. As antigens partially purified, purified fixed, and purified unfixed preparations, as well as crude sap from *C. quinoa* were used. For testing lilac bushes, just expanding buds from which the leathery outer scales were removed were macerated in 1% nicotine in water (0.1 g material in 1 ml). The macerate was taken up in cotton wool and pressed out with a hand press.

Antisera to *Pelargonium* leaf curl and bushy stunt viruses were supplied by dr M. Hollings, England and against tobacco streak virus by dr R. W. Fulton, USA. Dr A. F. Murant, Scotland, provided antisera to raspberry bushy dwarf virus, dr J. Dunez, France, antiserum to myrobolan latent ringspot virus, dr G. Morvan, France, antiserum to sowbane mosaic virus, dr A. T. Jones, Scotland, antiserum to elm mottle virus and dr J. C. Devergne, France, antiserum to a mosaic virus (MF) from broad bean.

Results

Symptoms on source plants and isolation of the virus

Sap inoculation experiments carried out in 1972, 1973, and 1974 included 204 lilac bushes 45 of which exhibited symptoms of lilac ringspot. Symptoms showed the same variation as mentioned by Van Katwijk (1955). Rings and line patterns on some plants were very faint and difficult to detect. In several bushes symptoms were restricted to only a few leaves. From 11 plants with, and 8 plants without ringspot

symptoms a virus was transmitted to *C. quinoa* which we tentatively name lilac ring mottle virus (LRMV). Transmission experiments were repeated twice with all of the 204 lilac bushes; all bushes with ringspot symptoms and those in which LRMV was detected were tested at least four times. LRMV, however, was always detected in the same 19 plants and only occasionally one or two of them escaped detection.

Symptoms on herbaceous hosts

C. quinoa. Depending on the concentration of the virus in the inoculum *C. quinoa* showed its first symptoms between six and twenty days after inoculation. They consisted of a faint vein mottling in the basal half of one or two tip leaves, soon followed by epinasty of these and later leaves, and cessation of growth of the whole plant. Plants often showed tip necrosis, and seldom recovered. If formed at all seeds were of poor quality. No typical local lesions have been noticed. However, necrotic patches sometimes appeared three to five weeks after inoculation, when the plant was slowly declining. Between November and March symptoms were much more severe than in May and June.

C. album. One week after inoculation a slight bending of the tip of the plants was noticed together with epinasty of the tip leaves. Leaves still expanding at the time of inoculation, and of which some were inoculated, often exhibited a bright vein chlorosis between three and five weeks after inoculation of the plants (Fig. 1). Inoculated leaves sometimes showed many faint light green spots which later disappeared. Plants usually recovered to a high degree.

C. amaranticolor. First symptoms appeared from seven to ten days after inoculation. The first one or two systemically affected leaves showed mottling at their basal halves. They were followed by one or two very small, severely deformed leaves. Inoculated leaves and leaves not inoculated because of their being too small at the time of inoculation exhibited a yellow vein banding in about four weeks. Such leaves became very brittle, their petioles thick and fleshy, and the veins at the lower-surface surrounded by newly-formed tissue. Plants did not show renewed extension growth, although from some lateral buds weak and short shoots developed that produced flowers and seeds of poor quality. This indicates a direct or indirect influence of the virus on flower production, because healthy *C. amaranticolor* plants never produced any flowers under the same circumstances.

Celosia plumosa. The inoculated and the systemically affected leaves showed light flecks about ten days after inoculation. Later leaves exhibited an overall chlorosis while plant growth was slightly reduced.

Celosia argentea. Symptoms resembled those on *C. plumosa*, except that the local flecks were less pronounced, whereas systemically infected leaves were brighter yellow in colour.

Phaseolus vulgaris 'Beka'. In one experiment inoculated plants exhibited a systemic mottling followed by epinasty in younger leaves. The virus could be recovered from the plant tips by back inoculation to *C. quinoa.* In another experiment with the same isolate no symptoms were obtained and the virus could only be detected in inoculated leaves.

Nicotiana megalosiphon. Necrotic local lesions appeared seven to ten days after infection, soon followed by a systemic mottling.

N. tabacum cvs White Burley, Samsun NN and Xanthi, *N. glutinosa* and *N. clevelandii.*

Fig. 1. Epinasty of some tip leaves and vein chlorosis in older leaves of *Chenopodium album*, caused by lilac ring mottle virus.

Fig. 1. Epinastie van de topbladeren en nerfvergeling in oudere bladeren van Chenopodium album, veroorzaakt door kringvlekkerigheidsvirus van sering.

Fig. 2. Temporary wilting of tip leaves of *Nicotiana tabacum* 'White Burley', caused by lilac ring mottle virus.

Fig. 2. Tijdelijke verwelking van enkele topbladeren van Nicotiana tabacum 'White Burley', veroorzaakt door kringvlekkerigheidsvirus van sering.

The only symptom observed was wilting of one or two of the tip leaves, between two and three weeks after inoculation. Later on such leaves recovered, but they remained somewhat smaller than later leaves (Fig. 2).
N. rustica, *C. foetidum*, *C. botrys*, *Gomphrena globosa*, *Vigna sinensis*, and *Vinca rosea* were systemically invaded by the virus without showing symptoms.
Amaranthus caudatus, *Cucumis sativus*, *Lactuca serriola*, *Urtica urens*, *Capsicum annuum*, and *Lycopersicon esculentum* did not react and no virus could be recovered.

Persistence of infectivity in crude sap
In several experiments with five isolates infectivity in crude sap of *C. quinoa* was retained after three to five but not after six h at 20 °C. In an experiment with isolate S25-5-3 crude sap from LRMV-infected *C. quinoa* was still infective after 10 min at 63 °C but not after 10 min at 65 °C and higher temperatures. With the same isolate the dilution end point was 10^{-4}, however, only four out of ten *C. quinoa* plants became infected with the final dilution. With the longest storing periods, the highest temperature treatment and the highest dilutions, the first symptoms appeared after 12 to 18 days whereas plants inoculated with fresh sap of *C. quinoa* always showed symptoms after six to seven days.

Seed transmission
Seeds of LRMV-infected *C. quinoa* produced plants that were much weaker than those from seeds of healthy *C. quinoa*. Three out of 23 plants from one seed lot were severely stunted and were found to be infected with LRMV. From another sample only 87 out of 188 seeds germinated and the virus was detected in 11 plants. In this case infected plants were weaker than healthy plants, but they were not stunted. From 548 plants obtained from seeds of infected *C. amaranticolor* plants, 477 were severely stunted with asymmetric leaves and 101 did not show symptoms. LRMV was found in all of the 25 stunted plants tested, and in 6 out of 25 seemingly healthy plants. Seed

transmission was also detected in *Celosia argentea*. With two isolates 3 out of 21 and 4 out of 8 seedlings, respectively, were infected. They did not show symptoms.

Back transmission from herbaceous hosts to lilac seedlings

In March 1972 20 potted lilac seedlings in the glasshouse were inoculated with sap from systemically infected *C. quinoa* leaves. Two months later in five of them LRMV could be detected by sap inoculation to *C. quinoa*. However, none of the twenty plants showed any symptom during the frist growing season. In January 1973 they were all planted in the open and in the following October three of the five infected seedlings exhibited small faint rings on leaves of some shoot tips, still growing at that time (Fig. 3). In May 1974 and May 1975 this symptom was noticed on each of the five infected seedlings. During high summer symptoms were almost invisible. Between

Fig. 3. Small faint rings on leaves of lilac seedlings that were inoculated with sap of lilac ring mottle virus-infected *Chenopodium quinoa*.

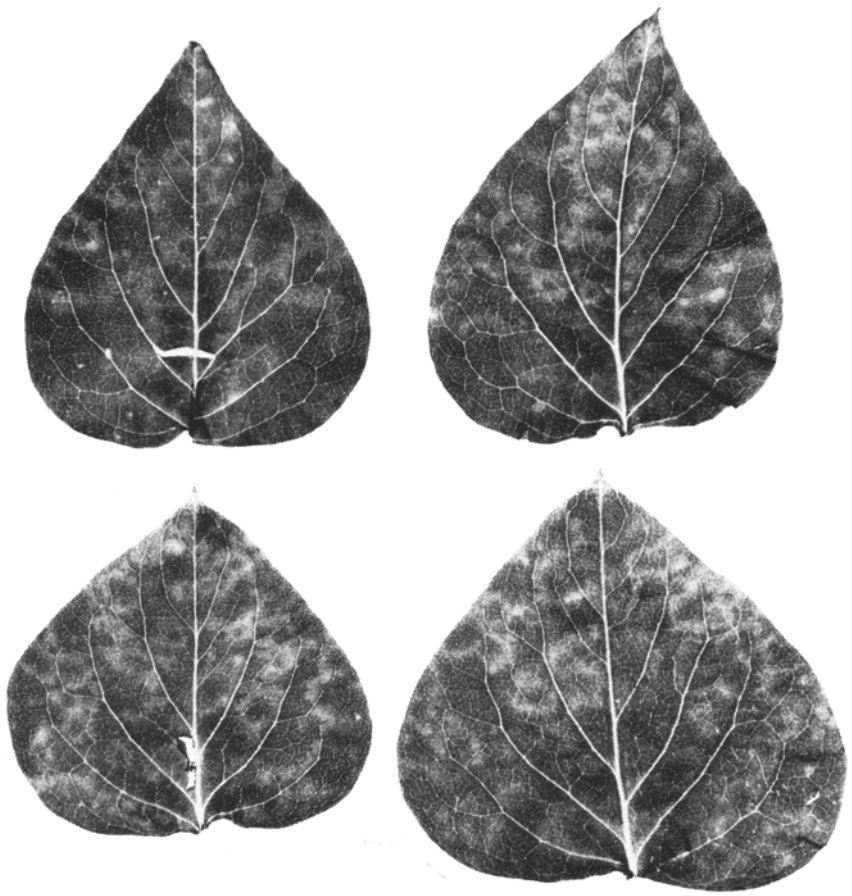

Fig. 3. Kleine vage kringen op bladeren van seringezaailingen, die geïnoculeerd werden met sap van met kringvlekkerigheidsvirus van sering geïnfecteerde Chenopodium quinoa.

May 1973 and May 1975 the 20 plants were repeatedly indexed on *C. quinoa* and LRMV was always detected in the same five plants only. No symptoms were observed in 30 non-inoculated lilac seedlings which originated from the same seed lot and had been treated in the same way, nor could any virus be transmitted from those plants to *C. quinoa*.

Field observations on naturally infected plants

The already mentioned results of the sap inoculations from lilac did not suggest a causal relationship between LRMV and lilac ringspot. However, lilacs showing ringspot appeared more often infected (11 out of 45) than symptomless plants did (8 out of 154). Moreover, the ring mottle symptom obtained on artificially infected lilac seedlings shows much resemblance to the upper left leave of Fig. 1 in the lilac ringspot description by Schmelzer and Schmidt (1966). Similar symptoms were also noticed on some leaves of three lilacs that were included in the sap inoculation experiments. One of these, a LRMV-infected plant, did not show other symptoms. The other two exhibited also typical lilac ringspot symptoms, i.e. rings and lines and leaf deformations (Fig. 4) but in spite of repeated attempts no LRMV could be isolated from them. To get more information about a possible correlation between LRMV and lilac ringspot, 660 one-year-old lilac plants were indexed on *C. quinoa* in February and March 1975. During field observations in May, June and July those plants were carefully examined for symptoms. The results, represented in Table 1 do not give any further evidence for a correlation between LRMV and lilac ringspot. Only two of the 65 LRMV-infected plants exhibited typical ring mottle symptoms, but these were only present on shoots from the *Syringa vulgaris* seedling rootstock. Rootstocks of the other 63 infected plants did not produce shoots and no ring mottle was observed on rootstock shoots of LRMV-free plants.

Table 1. Occurrence of ringspot on LRMV-infected lilacs and LRMV-free lilacs (see text).

Cultivar	Number of plants indexed	Ringspot on LRMV-infected plants	Ringspot on LRMV-free plants
Andenken an Ludwig Spath	100	0/5	0/95
Michel Buchner	100	0/24	0/76
Mme Lemoine	100	0/9	6/91
Mad. Flor. Stepman	100	1/5	0/95
G. J. Baardse	100	0/1	0/99
Esther Staley	100	1/13	4/87
Alphonse Lavallée	50	0/0	1/50
Ruhm von Horstenstein	10	0/8	0/20
Total	660	2/65	11/595

Numerator: number of plants showing ringspot. Denominator: number of LRMV-infected plants (column 3) or LRMV-free plants (column 4).

Tabel 1. Het vóórkomen van kringvlekken op seringen die wel, en seringen die niet geïnfecteerd zijn met kringvlekkerigheidsvirus.

Fig. 4. Typical symptoms of lilac ringspot, i.e. rings and lines and leaf deformations, as described by several authors.

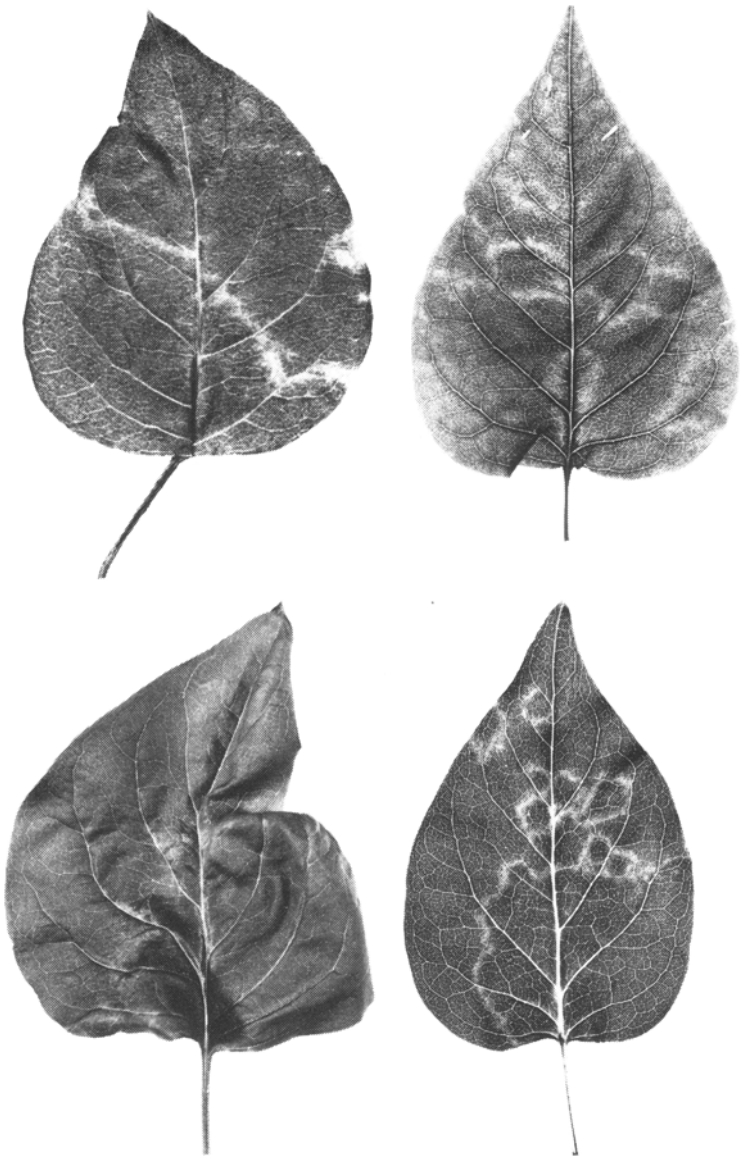

Fig. 4. Typische symptomen van kringvlekken bij sering, namelijk kringen en lijnen en bladmisvormingen, zoals ze door verscheidene auteurs zijn beschreven.

Aphid transmission

Myzus persicae from a virus-free colony were allowed to feed on LRMV-infected plants of *C. quinoa* and *N. clevelandii* for one to seven days. They were then transferred to 36 healthy *C. quinoa* plants and allowed to feed for one week. Between 30 and 50 aphids were placed on each test plant. After insecticide treatment the plants were kept under observation in a glasshouse for four weeks. No symptoms were noticed.

Virus purification and some properties in vitro

The first experiments in which we used three buffers, viz. 0.1 M tris-thioglycolic acid pH 9, 0.18 M phosphate-citric acid pH 7, and 0.018 M phosphate-citric acid pH 7, revealed that the virus is degraded in buffers of high ionic strength. Therefore we used 0.018 M phosphate-citric acid buffer pH 7 (PCA buffer) throughout this study. Doing so the described purification method yielded reasonable amounts of virus, for the preparations were highly infectious. However, in the electron microscope no clear particles could be seen probably because the virus was also degraded in 1% PTA in water. Fixation by addition of 2% glutaraldehyde to the preparations overcame this problem (Fig. 5). The fixed virus particles, however, are still somewhat irregularly shaped. Their average diameter is ca 27 nm.

When the virus was centrifuged on a sucrose gradient two UV-absorbing bands resulted (Fig. 6A). Also in the analytical ultracentrifuge two components were found. The S values (in PCA buffer, at 20°C and at infinite dilution), as determined with the graphical method of Markham (1960), were 98 and 83 S for bottom (B) and top component (T), respectively. In CsCl gradients, however, the virus fixed with glutaraldehyde reached equilibrium in a single band. Unfixed virus degraded in CsCl.

We have compared our purification method with that developed by Jones and Mayo (1973) to purify elm mottle virus from *C. quinoa*. Therefore identical amounts

Fig. 5. Electron micrograph of lilac ring mottle virus after staining with 1% potassium phosphotungstate pH 6.5 in water. Bar represents 200 nm.

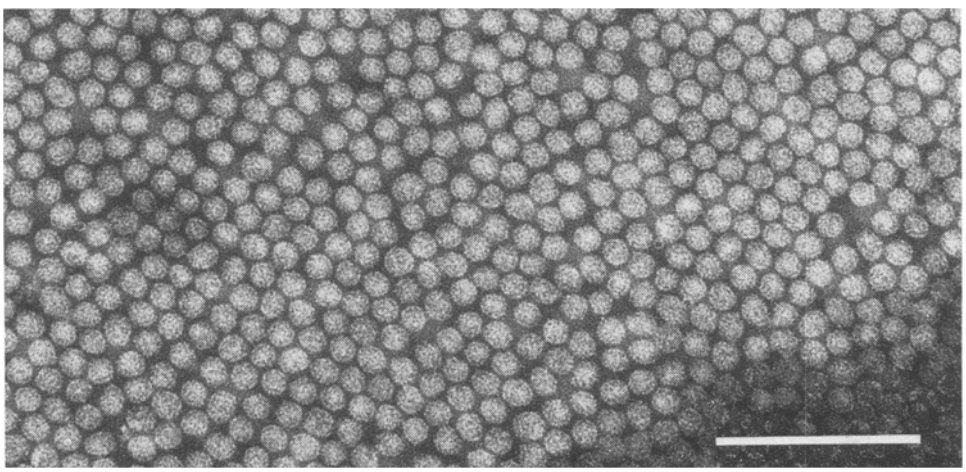

Fig. 5. Elektronenmicroscopische foto van het kringvlekkerigheidsvirus van sering na negatieve kleuring met 1% kaliumfosforwolframaat pH 6,5 in water. De vergrotingsstreep geeft 200 nm weer.

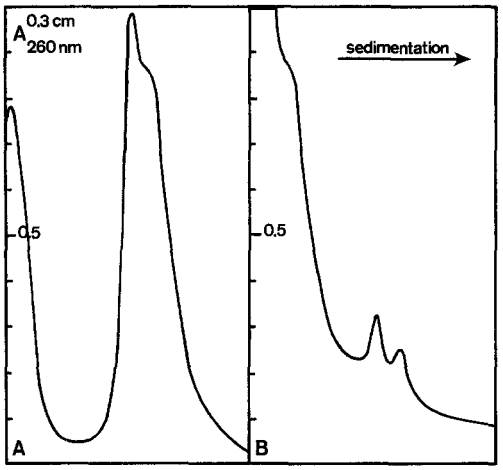

Fig. 6. UV-absorbance patterns of virus preparations after sucrose-gradient centrifuging. A: LRMV purified by the method described in this paper. B: LRMV purified by the method of Jones and Mayo (1973).

Fig. 6. UV-absorptiepatronen van viruspreparaten na het centrifugeren op een suikergradiënt. A: LRMV gezuiverd volgens de methode beschreven in dit artikel. B: LRMV gezuiverd volgens de methode van Jones en Mayo (1973).

of material were purified with both methods and analyzed by sucrose-gradient centrifuging. In Fig. 6 the UV-absorbance patterns show that our method (A) gave higher yields and less contamination with material of low molecular mass than Jones and Mayo's method (B). The ratio of T and B in both preparations is about the same, indicating that it is virus characteristic and not influenced by the purification method used.

Infectivity of T and B. Components T and B were separated by three successive cycles of sucrose-gradient centrifuging, followed by concentration by high-speed centrifuging. In Fig. 7 this separation is shown by the UV-absorbance patterns of tube contents after gradient centrifugings. Only the indicated fractions of T and B were used for the next steps, so as to make sure that the separation was as complete as possible. The $A^{1}_{260}{}^{cm}_{nm}$ of the final T and B fractions was measured, after which they were diluted to make preparations with $A^{1}_{260}{}^{cm}_{nm} = 0.05$. Also a mixture of T and B was made in which each component had the mentioned concentration. Consequently, from each of the three preparations 4 five-fold dilutions were made. All preparations were then tested for infectivity by inoculating them on two *C. quinoa* plants each. The results of a representative experiment are presented in Table 2. As expected, the T + B was infectious. T was not infectious, but B was and to the same extent as the mixture T + B. Thus it must be concluded that B is infectious by itself and that the infection is not caused by an interaction of B and contamination of the preparation with T, for in that case the mixture T + B should be infectious to a much higher dilution than the B preparation.

Serology

The antiserum prepared had a titre of 1024. It reacted with a single precipitation line in the Ouchterlony double-diffusion test with fixed as well as with unfixed purified virus preparations. It reacted also with crude extracts from infected *C. quinoa* plants and from just expanding buds of LRMV-containing lilac bushes, but not with extracts from healthy plants. Purified preparations of LRMV giving clear reactions with its homologous antiserum did not react with antisera to *Arabis* mosaic virus, raspberry ringspot virus, strawberry latent ringspot virus, tomato black ring

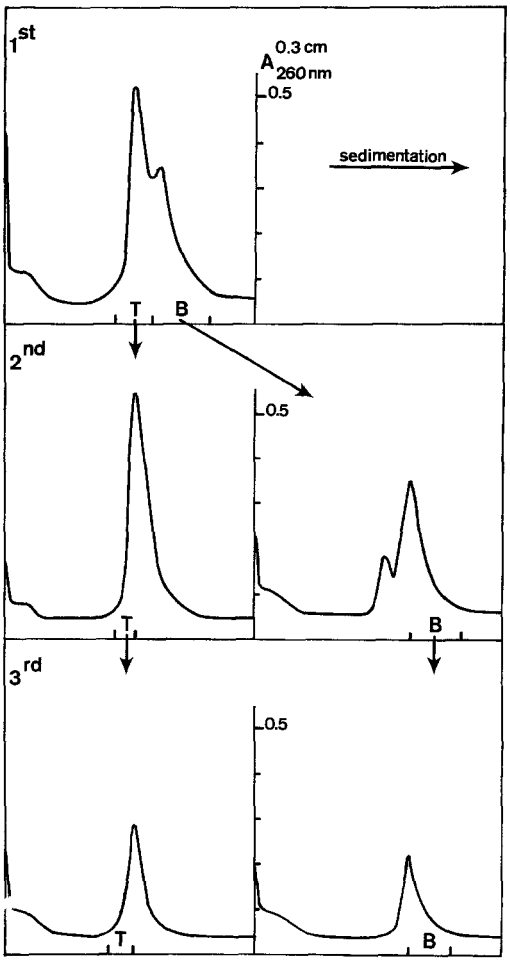

Fig. 7. The separation of top and bottom component of LRMV by three successive cycles of sucrose-gradient centrifuging. Only the indicated fractions were concentrated and used for the next steps.

Fig. 7. De scheiding van top- en bodemcomponent van LRMV door drie opeenvolgende centrifugeringen op suikergradiënt. Alleen de aangeduide fracties werden na concentrering gebruikt voor de volgende bewerkingen.

Table 2. Infectivity of top and bottom component of LRMV as tested on *C. quinoa*.

Components of the inocula	$A_{260\ nm}^{1\ cm}$ of each component in the inocula				
	5×10^{-2}	1×10^{-2}	2×10^{-3}	4×10^{-4}	8×10^{-5}
Top + bottom	++	++	++	−−	−−
Bottom	++	++	++	−−	−−
Top	−−	−−	−−	−−	−−

Each inoculum was tested on two plants. + = the inoculum caused an infection on one plant; − = the inoculum failed to do so.

Tabel 2. Infectiositeit van top- en bodemcomponent van LRMV, getoetst op C. quinoa.

virus, cherry leaf roll virus, tobacco ringspot virus, tomato ringspot virus, myrobolan latent ringspot virus, *Pelargonium* leaf curl virus, tomato bushy stunt virus, raspberry bushy dwarf virus, elm mottle virus, tobacco streak virus, broadbean MF virus, true broadbean mosaic virus, sowbane mosaic virus, broadbean wilt virus, apple mosaic virus, *Prunus* necrotic ringspot virus (cherry isolate), GE36 virus, *Pelargonium* virus L128, cucumber mosaic virus, tomato aspermy virus, bean southern mosaic virus, bean pod mottle virus, tobacco necrosis virus, carnation ringspot virus, carnation mottle virus, red clover mottle virus, two strains of cowpea mosaic virus and turnip yellow mosaic virus.

Discussion

With respect to host plant reactions, serological and some other properties the lilac virus described is not related to other viruses isolated from lilac, such as tomato black ring virus and *Arabis* mosaic virus (Schmelzer, 1970), elm mottle virus (Schmelzer, 1969; Jones and Mayo, 1973) or lilac mottle virus (Waterworth, 1972). Moreover, the virus seems distinct from other plant viruses so far described. Therefore, we name it lilac ring mottle virus (LRMV).

The inability to isolate LRMV from many lilacs with ringspot symptoms and the absence of ringspot symptoms on many LRMV-infected lilacs strongly indicate that there is no causal relationship between LRMV and the lilac ringspot syndrome. This syndrome, as described in the literature, consists of ring and line patterns with much variation in size and shape. According to our observations it sometimes included also ring mottle symptoms, almost similar to the ring mottle symptom which LRMV caused on mechanically inoculated seedlings in our experiments. So the presence of LRMV in bushes that exhibit ring mottle symptoms can only be confirmed by sap inoculation to *C. quinoa*. The results of many tests convinced us that indexing lilacs by sap inoculation to *C. quinoa* is fully reliable and offers good possibilities for the selection of LRMV-free stock. The reliability of the serological test has to be further investigated. Transmission of the virus through the seeds of three herbaceous host species indicates that LRMV may be distributed by the use of infected lilac seedling rootstocks.

Samenvatting

Kringvlekkerigheidsvirus van sering: isolatie uit sering, enige eigenschappen en de relatie tot kringvlekkenziekte van sering

Een nieuw virus van sering wordt beschreven. Op grond van de symptomen wordt de naam kringvlekkerigheidsvirus van sering voorgesteld (Fig. 3). Het virus kan mechanisch naar verschillende kruidachtige planten worden overgebracht. De symptomen op de belangrijkste daarvan worden beschreven (Fig. 1 en 2). In ruw sap wordt het virus geïnactiveerd door 10 minuten verhitten tot 63–65 °C, de verdunningsgrens is 10^{-4} en het virus verliest zijn activiteit na 5 uren bewaren bij kamertemperatuur. Het virus kan overgaan met zaad van drie kruidachtige plantesoorten, maar kan niet worden overgebracht door *Myzus persicae*. De relatie tot de kringvlekkenziekte bij sering is onderzocht (Fig. 3; Tabel 1).

De virusdeeltjes zijn gevoelig voor oplossingen met een hoge ionaire sterkte en zijn in de elektronenmicroscoop slechts zichtbaar na fixatie met glutaaraldehyde. Ze zijn tamelijk onregelmatig van vorm en hebben een diameter van ongeveer 27 nm (Fig. 5). Het virus sedimenteert tijdens centrifugering in een dichtheidsgradiënt in twee zones (Fig. 6 en 7). De bodemcomponent is infectieus, de topcomponent is dat niet (Tabel 2).

Een antiserum met een titer van 1024 werd gemaakt. Serologische toetsing wees uit dat het virus niet verwant is aan een van de 32 getoetste bolvormige plantevirussen.

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References

Atanasoff, D., 1935. Old and new virus diseases of trees and shrubs. Phytopath. Z. 8: 197–233.

Beale, J. H. & Beale, H. P., 1952. Transmission of a ringspot-virus disease of *Syringa vulgaris* by grafting. Contr. Boyce Thompson Inst. Pl. Res. 17: 1–6.

Jones, A. T. & Mayo, M. A., 1973. Purification and properties of elm mottle virus. Ann. appl. Biol. 75: 347–357.

Katwijk, W. van, 1955. Ringvlekkenmozaïek bij seringen in Nederland. Meded. Dir. Tuinb. 18: 823–828.

Kochman, J., Kowalska, H. & Szymańska, B., 1964. Untersuchungen über Virosen des Flieders (*Syringa vulgaris* L.) und des Ligusters (*Ligustrum vulgare* L.) Phytopath. Z. 51: 333–350.

Markham, R., 1960. A graphical method for the rapid determination of sedimentation coefficients. Biochem. J. 77: 516–519.

Novák, J. B., 1969. Investigation of virus diseases of lilac. Proc. 6th Conf. Czechoslovak Plant Virologists, Olomouc 1967: 289–299.

Schmelzer, K., 1969. Das Ulmenscheckungs-virus. Phytopath. Z. 64: 39–67.

Schmelzer, K., 1970. Untersuchungen an Viren der Zier- und Wildgehölze. 7. Mitteilung: Weitere Befunde an *Buddleja*, *Viburnum*, *Caryopteris* und *Philadelphus* sowie Viren an *Leycesteria*, *Chionanthus*, *Ribes*, *Hydrangea*, *Syringa*, *Spiraea* und *Catalpa*. Phytopath. Z. 67: 285–326.

Schmelzer, K. & Schmidt, H. E., 1966. Die Viruskrankheiten des Flieders. Arch. Gartenb. 14: 303–314.

Waterworth, H. E., 1972. Purification, serology, and properties of a virus from lilac, *Syringa oblata affinis*. Pl. Dis. Reptr 56: 923–926.

Address

Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, Wageningen, the Netherlands.