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Further characterization of dandelion yellow mosaic virus from lettuce and dandelion

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Abstract

A damaging virus isolated in the Netherlands from lettuce was studied and compared with a virus isolated from dandelion orginating from Czechoslovakia. It was found to biologically resemble dandelion yellow mosaic virus incompletely described from dandelion and lettuce in Great Britain (Kassanis, 1944, 1947) and from dandelion in Germany (Hein, 1963).

Mechanical transmission was greatly improved by buffer solution and transmission by *Myzus persicae* seemed to be in the non-persistent manner. Longevity in vitro of the virus hardly exceeded one day. Thermal inactivation was between 60 and 65 °C and the dilution end-point was between 10 000 and 100 000. It was still infectious in leaf material dried and stored over $CaCl_2$ at 4 °C for 6½ years.

The virus was isolated and purified with difficulty and was found to consist of one type of spherical particle of ca 30 nm diameter, with a sedimentation coefficient of 159 S, a buoyant density of 1.42 $g.cm^{-3}$ and an A_{260}/A_{280} ratio of 1.67. An antiserum was prepared with a titre of 256 in the agar double-diffusion test. The virus could be identified in crude extracts from lettuce and *Chenopodium amaranticolor* by enzyme-linked immunosorbent assay (ELISA), but not by agar double diffusion. It could only be visualized in crude sap in the electron microscope after trapping of virus particles on antiserum-coated grids. The virus cannot yet be assigned to any known virus group.

It is of potential economic importance to lettuce because of its occurrence in widely differing regions in Europe, its aggressiveness and virulence on 22 out of 23 lettuce cultivars tested (and on endive) and its pathogenicity to *Lactuca* genotypes which are resistant to lettuce mosaic virus and other important pathogens of lettuce. 'Laibacher Eis' was the only cultivar showing some tolerance.

Additional keywords: Resistance to virus infection, virus infection of wild plants.

Introduction

Since 1972 the two senior authors now and then obtained stunted lettuce plants with small, narrow and often asymmetrical young leaves. From such plants a pathogen could be mechanically transmitted. Biologically it resembled dandelion yellow mosaic virus (DYMV) wich had been described in the literature without information on its intrinsic properties. Since our 'virus' soon appeared to be aggressive and virulent on lettuce cultivars and on other *Lactuca* spp. that are resistant to lettuce mosaic virus and other pathogens of lettuce, we have attempted to further characterize it and study its effect on some other cultivated and wild plant species.

Literature

The disease had first been reported from different parts of Britain in lettuce plants suffering from severe disease and in plants of dandelion *(Taraxacum officinale)* with a vivid yellow mottle, fairly common in the vicinity of Rothamsted and also occurring in several other districts. In lettuce the young leaves became bronzed as a result of fine brown necrosis along the veins and in interveinal areas. In the glasshouse, this was usually followed by chlorosis, dwarfing and malformation of the whole plant. In the open, necrosis was the major symptom and affected plants became worthless (Kassanis, 1944, 1947). In Cos varieties of lettuce the disease may have occurred since 1933, first in Sussex and later in other parts of the country (Moore, 1946).

In Britain the disease incitant was difficult to transmit mechanically, hence no properties in vitro were determined. It could be transferred with *Aulacorthum solani, Myzus ascalonicus* and *M. ornatus,* but not with *M. persicae,* after acquisition feeds of some hours, though the aphids thereafter ceased to be infective within an hour (Kassanis, 1947).

An apparently similar pathogen was later found once in a dense stand of dandelion with brightly yellow mottled leaves in Fischenich, West Germany. Hein (1963) succeeded in mechanically transmitting it, though also with difficulty. Most inoculated lettuce cultivars reacted with severe necrosis, but such symptoms could not be observed in many lettuce fields checked for natural occurrence. The German 'virus' lost infectivity in sap between 2 and 3 h of storage at room temperature and was therefore not studied further for its physical properties.

DYMV has been mentioned to occur in Norway and presumably Denmark and Sweden (Rønde Kristensen et al., 1965; Rønde Kristensen, 1966).

A few reports from Czechoslovakia are confusing. A virus found in dandelion in South Bohemia was concluded to closely resemble DYMV (Brčák and Polák, 1966), but was later named dandelion necrotic ringspot virus or *Taraxacum* mosaic virus (Čech and Branišová, 1973) and dandelion necrotic blotch or dandelion yellow mosaic virus (Brčák, 1979). The virus or viruses has or have been found to be transmissible in sap, but not by the aphid *Aulacorthum solani* (Brčák, 1979). The dilution endpoint was higher than 5000 in lettuce sap and over 625 in dandelion sap, and longevity in vitro at room temperature was 12 days (Brčák and Polák, 1966) or less than 3 days (Brčák, 1979). Twenty-five Czechoslovak and 6 Scandinavian isolates from Norway, Sweden and Finland were sap transmissible with difficulty. All but 2 Finnish isolates caused systemic symptoms including necrosis in lettuce. Several isolates incited local lesions in *Chenopodium quinoa* and a few isolates systemic symptoms as well. In crude sap no reaction was obtained with antisera to *Arabis* mosaic, cucumber mosaic, tobacco necrosis and tomato necrotic ringspot viruses (Brčák, 1979). The *Taraxacum* mosaic virus was partially purified by Čech and Branišová (1973) and found to have spherical particles of 32 nm with an A_{260}/A_{280} ratio of 1.64. Later a clump of spherical particles, ca 30 nm in diameter, could be observed with the electron microscope in ultrathin sections of one plant from Finland with an 'isolate resembling DYMV' (Brčák, 1979), but in that report no reference was made to the work on *Taraxacum* mosaic virus by Čech and Branišová (1973).

Materials and methods

Most research was with virus isolates Ls2 and Ls4 from lettuce plants originating from trial fields of breeding companies in the Westland area and Limburg, respectively. The isolates were maintained and multiplied in lettuce and, preferably, in *Chenopodium quinoa.* Some comparative work was later done with isolates Ls12, Ls16 and Ls158 from lettuce and with an isolate (Tar2) from a naturally infected plant of dandelion with chlorotic rings collected in a garden in Prague, Czechoslovakia in 1976 (L. Bos). Most isolates were desiccated and stored in leaf material over $CaCl_2$ at 4 °C.

Virus transmission was in sap by mechanical inoculation, using carborundum as an abrasive. For most transmissions, plant material was ground 1 : 4 (w/v) in 0.03 M phosphate buffer pH7 containing 0.25% sodium disulfite, 0.5% sodium DIECA and 7% Norit SX-1.

In a tentative insect transmission test aphids, *Myzus persicae,* were starved for 8 h, given a few minutes of acquisition on lettuce plants with Ls2, and immediately thereafter ½ h of inoculation access on virus-free lettuce seedlings, 10 aphids per plant, whereafter they were left overnight on a second series of test plants.

Host-range studies were by inoculation of 2 to 9 potted seedlings of each species or cultivar, daily examination for symptoms, and back-inoculation from inoculated leaves and non-inoculated tip leaves, at least 10 and 20 days after inoculation, respectively, onto two plants of *C. quinoa* and/or 3 to 6 young lettuce plants (mostly 'Swift').

Persistence of infectivity in expressed sap was studied in the conventional way using lettuce as a source of virus and *C. quinoa* and *C. amaranticolor* as local-lesion test plants.

Virus purification was with Ls2 from leaves of systemically infected *C. quinoa* plants harvested 13-18 days after inoculation. Portions of 1000 g were homogenized with a Waring blender in 2000 ml of 0.5 M potassium phosphate buffer, pH 7, containing 0.1% thioglycolic acid. The homogenate was filtered through two layers of cheesecloth and the filtrate frozen for at least one night at −20 °C. After thawing, n-butanol was added to the filtrate to a final concentration of 8%. The mixture was stirred for 45 min, left for 30 min at 4 °C and then centrifuged for 10 min at 16 000 *g* (all *g* values are given at R_{max} and all centrifugings were done at 4 °C). The upper phase was decanted and centrifuged for 2½ h at 116 000 *g*. The resulting pellets were resuspended in 210 ml of 0.01 M tris (hydroxymethyl) aminomethane-HCl, pH 9 (tris buffer), stirred overnight at 4 °C and centrifuged for 10 min at 7700 *g*. The supernatant was centrifuged for 2½ h at 116 000 *g*. The pellets were resuspended in 6 ml of tris buffer, stirred for 3 h at 4 °C and centrifuged for 10 min at 7700 *g*.

One-ml portions of the supernatant were mixed in a Beckman SW41 rotor tube with 2.5 ml of a Cs_2SO_4 solution (37.26 g/50 ml), overlaid with paraffin oil and centrifuged for 17-20 h at 30 000 rpm. The virus was recovered by puncturing the bottoms of the tubes and collecting appropriate fractions. The Cs_2SO_4 was removed by dialysis overnight against tris buffer at 4 °C. One-ml portions of the suspension were each centrifuged for 2 h at 24 000 rpm in a Beckman SW27 rotor on a 10-40% sucrose gradient in tris buffer. The virus fractions were recovered with an Isco density-gradient fractionator, diluted 1 : 1 with tris buffer and centrifuged for 4½ h at 241 000*g*. Pellets were resuspended in tris buffer.

The sedimentation coefficient was determined by the graphical method of

Markham (1960) using a Spinco Model E ultracentrifuge with Schlieren optics. The buoyant density in Cs_2SO_4 was determined as described earlier (Bos et al., 1980).

An antiserum to Ls2 was prepared by injecting a rabbit with purified virus. Eight intravenous injections and one subcutaneous injection of 4-5 ml each were administered, regularly divided over a period of 3 weeks. These were followed 2 months later by an intramuscular injection with an emulsion of 2 ml of purified virus and 2 ml of Freund's incomplete adjuvant. Antiserum titres were determined in the agar double-diffusion test using purified virus. The agar concentration was 1% in 0.02 M phosphate-citric acid buffer (pH 7) containing 0.05% sodium azide. Enzyme-linked immunosorbent assay (ELISA) was largely as described by Clark and Adams (1977) or by Flegg and Clark (1979), but preparation of γ-globulin and enzyme conjugate was according to Tóbiás et al. (1982). Coating of microtitre plates was with 1 μg. cm^{-3} of γ-globulin. Enzyme conjugate was used at a final dilution of 1 : 500. Extracts of plants were prepared with the buffer used for mechanical transmission of the virus, but without Norit and with 0.1% Tween 20.

Electron microscopy was in crude sap from lettuce and *C. quinoa* and with purified suspensions from *C. quinoa* after staining with 1% potassium phosphotungstate (PTA), pH 6.5, in water or with 2% uranyl acetate (UA) in water. Immuno electron microscopy (trapping) was by sensitizing carbon-coated grids for 15 min on a drop of antiserum diluted 1 : 100, rinsing them with 20 drops of 0.1 M phosphate buffer pH 7.2 and then by floating them for 30 min or 1 h on a drop of virus-containing sap, prepared by grinding diseased tissue in buffer as for mechanical transmission. Final cleaning was by rinsing with 30 drops of distilled water and staining with 6 drops of 1% UA.

Results

Mechanical transmission and host-range studies. Sap transmission was difficult when water was used as an extraction medium. With buffer, containing disulfite, DIECA and Norit, transmission was successful to most individuals of a susceptible species. The results of host-range tests are recorded in Table 1 along with information from the literature. On several species infection was symptomless.

In the aphid-transmission test, 2 out of 10 lettuce plants upon each of which 10 *M. persicae* had fed for ½ h showed symptoms, and 1 out of 10 upon which they had fed thereafter.

All 23 lettuce cultivars tested were susceptible to the virus. Of these cultivars 19, including 'Gallega de Invierno' highly resistant and several other cultivars tolerant to lettuce mosaic virus, were tested comparatively with the isolates Tar2, Ls2, Ls12, Ls16 and Ls158. All but 'Laibacher Eis' were practically uniformly and highly sensitive to the isolates Tar2, Ls2, Ls16 and Ls158. In spite of poor symptom expression, all isolates could readily be recovered from non-inoculated leaves of this cultivar. *Lactuca serriola,* often used as a parent in breeding for disease resistance particularly *Bremia lactucae,* was also highly sensitive when tested with Ls2 and Ls4, and the same held for 'Hilde × *L. serriola*' tested with Tar2, Ls2, Ls16 and Ls158. Isolate Ls12 was of low virulence and was so uniformly on al cultivars tested. In symptom severity Tar2 was most virulent followed by Ls2, Ls16 and Ls158, respectively (Fig. 1). Later during plant development the latter three were nearly equal in virulence.

Fig. 1. Systemic symptoms in lettuce 'Reskia' 28 days after inoculation with five isolates, from lower left to upper right: Tar2, Ls2, Ls12, Ls16, Ls158 and non-inoculated control. Each pot contains three plants.

Fig. 1. Systemische symptomen in sla, 'Reskia', 28 dagen na inoculatie met vijf isolaten, van linksonder naar rechtsboven: Tar2, Ls2, Ls12, Ls16, Ls158 en niet-geïnoculeerde controle. Elke pot bevat drie planten.

Symptoms in lettuce plants usually appeared 4 days after inoculation. Several cultivars with Tar2 and some with Ls2 started with diffuse necrotic or chlorotic local lesions. Systemic symptoms appeared almost simultaneously and consisted of irregular vein chlorosis and diffuse interveinal spotting to clearing of tissue. These abnormalities were soon followed by a diffuse vein chlorosis or vein banding and by some bronzing on the lower surface of the leaves and some necrosis along the edges of narrow leaves. Most striking were severe leaf narrowing and curling, plant stunting and complete absence of heading (Fig. 2). In some cultivars, such as 'Hilde × *L. serriola*' and 'Balloon', leaves later showed a yellow ringspotting and dark green mottling or mosaic associated with extreme and irregular leaf narrowing (Fig. 3). With Ls12 all cultivars showed a diffuse chlorotic spotting throughout plant development (Fig. 3, right). Seven weeks after inoculation symptoms of this isolate tended to aggravate in most cultivars and to include some leaf narrowing and curling.

Plants of endive *(Cichorium endivia),* only inoculated with Ls4, showed symptoms like those in lettuce, including severe plant stunting (Fig. 4).

Table 1. Results of host-range studies.

Plant species and cultivars tested	Isolates studied						Data from the literature	
	from lettuce					from dandelion	Kassanis (1947)	Hein (1963)
	Ls2	Ls4	Ls12	Ls16	Ls158	Tar2		
Apium graveolens							–	
Calendula officinalis							–	
Cheiranthus cheiri								–
Chenopodium amaranticolor	LS*	L–*	L–	LS*	LS*	LS*		
Chenopodium quinoa	LS	LS	L–	LS*	LS*	LS*		–
Cichorium endivia		–S*						
Cichorium intybus							–	
Coriandrum sativum								–
Cucumis sativus 'Gele tros'	L–	– –						
'Sporu'	L–							
Gomphrena globosa	(L)s	– –*	– –	l–	–s	Ls		–
Datura stramonium								–
Lactuca sativa							(L)S	(L)S
Attractie', 'Balloon', 'Benita', 'Capitan', 'Dolly', 'Flair', 'Fürchtenichts', 'Gallega de Invierno', 'Laibacher Eis', 'Mirena', 'Mondian', 'Palmyran', 'Patty', 'Reskia', 'Soraya', 'Tannex', 'Trocadero', 'Violin', 'Wonder van Voorburg'.	S		S	S	S	S		
'Avires'		S						
'Hilde × Gallega de Invierno'			S			S		
'Hilde × *L. serriola*	–*S		S	S	S	S		
'Merida'	LS	S						
'Portato'	–*S							
'Viruzan'			S			S		

Lactuca serriola	S	S					S	S
Lactuca virosa							S	
Lycopersicon esculentum 'Ailsa Craig'	–*–*							
Nicotiana benthamiana	ls		–s	ls	–s	ls		
Nicotiana clevelandii	ls		– –	–s	– –	ls		
Nicotiana glutinosa	(l)–	– –	– –	l–	– –	l–	–	
Nicotiana megalosiphon		– –						
Nicotiana tabacum 'White Burley'	(l)(s)	l–	– –*	l–	– –	l–		
Nicotiana rustica	(l)(s)	l–	– –	l–	– –	l–		
Pastinaca sativa							–	
Petunia hybrida	ls	l–						
Petroselinum sativum							–	
Phaseolus vulgaris 'Bataaf'	–*–*	– –						
'Saxa'								–
Pisum sativum 'Koroza'		–*–*	– –*					
'Rondo'	– –	– –	– –*					
Senecio vulgaris								–
Sonchus oleraceus							–	
Spinacia oleracea 'Noorman'	–*S*							
'Nores'		l–						
'Virosa'		– –						
'Wintra'		–s						
Taraxacum officinale		ls					S	–
Vicia faba	– –	l–	– –	– –	– –	L–		
Vigna sinensis								–

L = local symptoms; l = latent local infection; S = systemic symptoms; s = latent systemic infection.
– = no infection; –* = no symptoms, but not tested by back inoculation.
Symbol in parentheses = reaction erratic.

Tabel 1. Resultaten van het waardplantonderzoek.

Fig. 2. Systemic symptoms in lettuce 'Hilde × *L. serriola*' 28 days after inoculation with isolates Tar2 (left) and Ls158 (middle); right, healthy control plants. Each pot contains three plants.

Fig. 2. Systemische symptomen in sla, 'Hilde × L. serriola', 28 dagen na inoculatie met isolaten Tar2 (links) en Ls 158 (midden); rechts, gezonde controleplanten. Elke pot bevat drie planten.

Fig. 3. Systemic symptoms in lettuce, from left to right, two leaves of 'Hilde × *L. serriola*', one leaf of 'Balloon' and one leaf of 'Patty' with isolates Ls2, Ls158, Ls16 and Ls12, respectively, 6 weeks after inoculation.

Fig. 3. Systemische symptomen in sla, van links naar rechts, twee bladeren van 'Hilde × L. serriola', één blad van 'Balloon' en één blad van 'Patty', met achtereenvolgens de isolaten Ls2, Ls158, Ls16 en Ls12, 6 weken na inoculatie.

Fig. 4. Systemic symptoms, including severe stunting, by Ls4 in endive 14 days after inoculation; left, healthy control plants.

Fig. 4. Systemische symptomen, inclusief ernstige dwerggroei, van Ls4 in andijvie 14 dagen na inoculatie; links, gezonde controleplanten.

In *Chenopodium amaranticolor* and *C. quinoa* many pinpoint and slightly larger chlorotic local lesions, respectively, appeared 6 to 7 days after inoculation (Fig. 5, left). With all isolates but Ls12 these were followed a few days later by a systemic stippling (Fig. 5, right) and sometimes a slight malformation of the leaves.

In *Gomphrena globosa,* Tar2 was the only isolate regularly producing large diffuse chlorotic and later reddening rings and arcs, first appearing some days after inoculation (Fig. 6).

Vicia faba 'Kompakta' only reacted to Tar2 with a few necrotic local lesions in some of the plants inoculated (Fig. 6, right).

Persistence of infectivity. At serial 10-fold dilution of crude sap from lettuce plants infected by Ls2 infectivity steeply declined at dilutions between $\times 100$ and $\times 10\,000$ and was completely lost at $\times$ 100 000. Thermal inactivation of Ls2 was between 60 and 65 °C. Longevity in vitro of Ls2 and Tar2 hardly exceeded 24 h. Infectivity rapidly diminished between 2 and 7 h of storage and after 24 h only few local lesions per plant were formed.

All five isolates dried and stored over $CaCl_2$ were still infectious after extended periods of storage, which was 3½ years for Ls158, 6 years for Tar2 and 6½ years for Ls2, Ls12 and Ls16.

Virus purification and determination of biophysical properties. The method described resulted in pure virus preparations but virus yield was very low. In sucrose gradients

Fig. 5. *Chenopodium quinoa* (above) and *C. amaranticolor* (below) with local lesions 9 days after inoculation with isolate Tar2 (left) and systemic stippling 23 days after inoculation with isolate Ls16 (right).

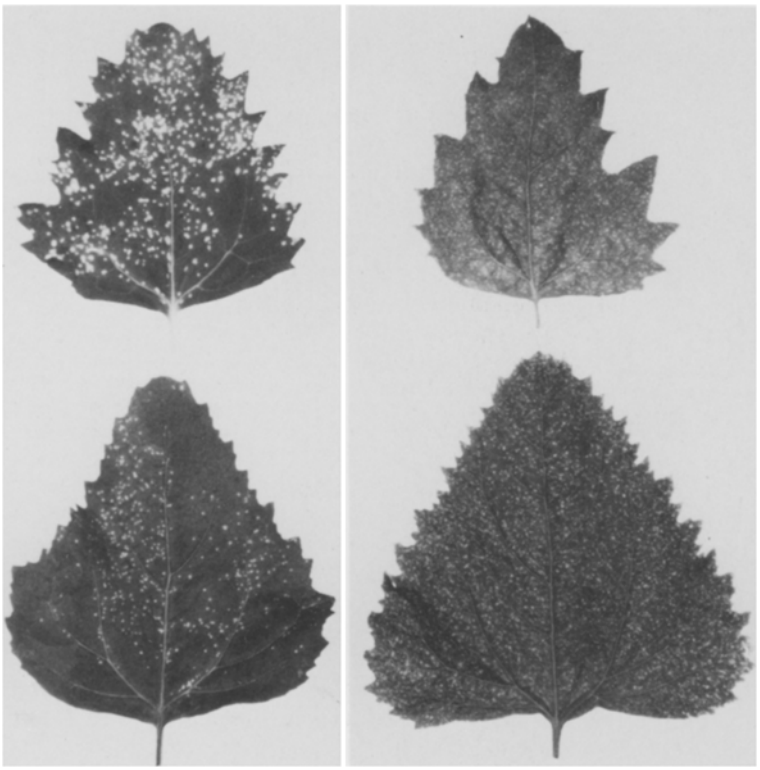

Fig. 5. Chenopodium quinoa (boven) en C. amaranticolor (onder) met lokale lesies 9 dagen na inoculatie met isolaat Tar2 (links) en systemische spikkeling 23 dagen na inoculatie met isolaat Ls16 (rechts).

the virus sedimented in three zones (Fig. 7). The material in these zones all had the same A_{260}/A_{280} ratio of 1.67 (corrected for light scattering). All zones were infectious on *C. quinoa.* When examined with the electron microscope they all contained one type of spherical particles (Fig. 8) though many particles were obviously damaged. The virus reached equilibrium in a single zone in Cs_2SO_4 gradients. The buoyant density in Cs_2SO_4 was 1.42 $g.cm^{-3}$. Furthermore only one main peak was found at analytical ultracentrifuging. The sedimentation coefficient in 0.01 M tris buffer (pH 9), at 20 °C and at infinite dilution was 159 S.

Serology. Two weeks after the series of intravenous and subcutaneous injections the antiserum titre was 64 when tested with purified virus. It rapidly decreased to 16 in the next 4 weeks. Two weeks after the intramuscular injection the titre was again 64 and increased to 256 in the next 4 weeks. No positive reactions were obtained when

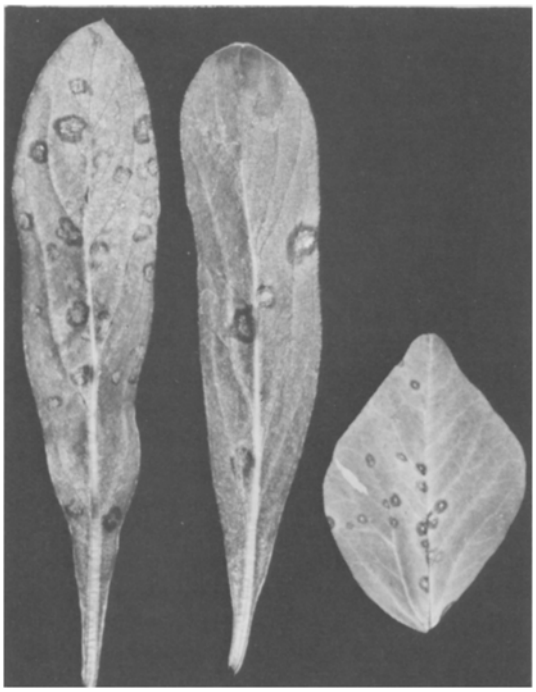

Fig. 6. *Gomphrena globosa* and *Vicia faba* 'Kompakta' with local lesions 19 days after inoculation with isolate Tar2.

Fig. 6. Gomphrena globosa en Vicia faba 'Kompakta' met lokale lesies 19 dagen na inoculatie met isolaat Tar2.

the agar was in saline. The antiserum did not react in agar gel with sap of lettuce plants containing Ls2, of healthy plants of lettuce, *C. amaranticolor* or *C. quinoa,* or of these plant species containing Tar2.

The modified procedure of ELISA (Flegg and Clark, 1979) gave much better results than the standard procedure. With the former, four of the five isolates tested (Ls2, Tar2, Ls16, Ls158) gave clearly positive reactions using extracts from infected plants

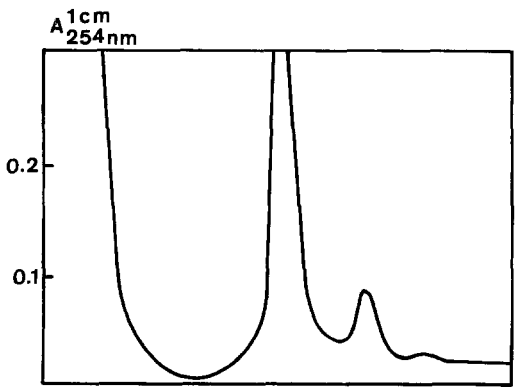

Fig. 7. UV-absorption pattern after ultracentrifugation of the virus isolate Ls2 in a sucrose gradient. Sedimentation is from left to right.

Fig. 7. UV-absorptiepatroon na ultracentrifugering van het virus (isolaat Ls2) in een suikergradiënt. Sedimentatie is van links naar rechts.

Fig. 8. Electron micrographs of purified virus (isolate Ls2) after staining with 1% PTA, pH 6.5. Inset, tubule with virus particles. Bar represents 100 nm.

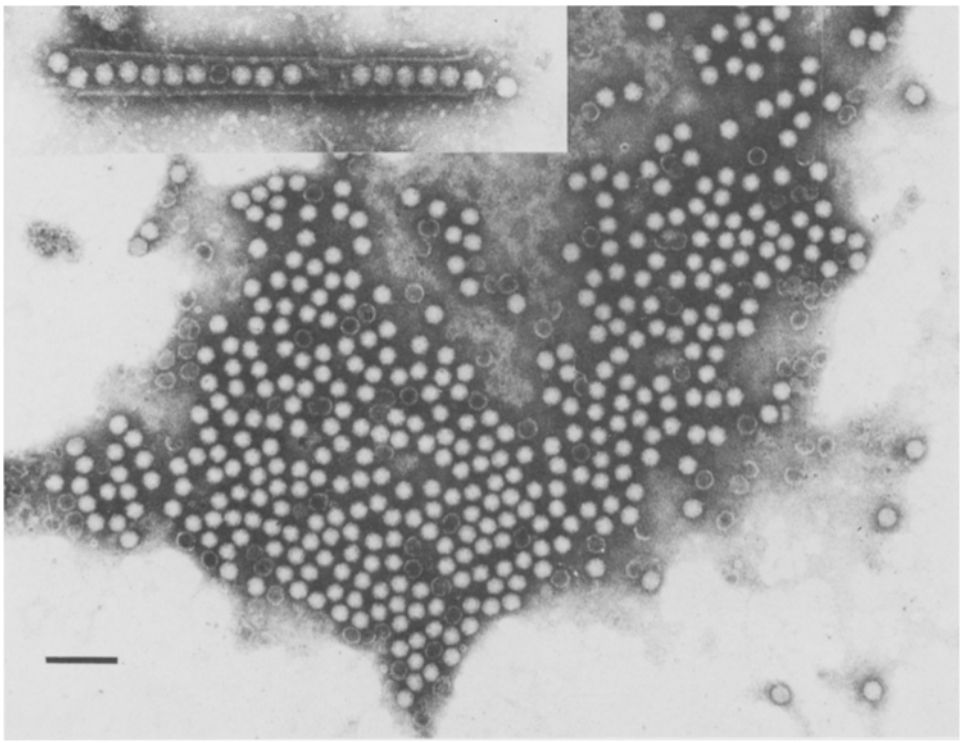

Fig. 8. Elektronenmicroscopische foto's van gezuiverd virus (isolaat Ls2) na contrastering met 1% kaliumfosforwolframaat, pH 6.5. Inzet, buisje met virusdeeltjes. Vergrotingsstaaf geeft 100 nm weer.

of lettuce (cv. Swift) and *C. amaranticolor.* Positive reactions were also obtained with the standard method using extracts from systemically infected *C. amaranticolor*. The isolate Ls12 did not react in any of the tests performed, neither from locally infected *C. quinoa,* nor from lettuce (cvs Myrena, Violin and Swift).

Electron microscopy. Purified preparations of Ls2 contained many spherical particles which were ca 30 nm in diameter based on the width of TMV particles used as internal magnification standard. Such preparations also seemed to contain empty particles of similar size but many of these were obviously damaged (Fig. 8). In partially purified preparations tubules, containing a row of virus particles, were observed (Fig. 8, inset).

No particles have been observed in several preparations of crude sap from plants of lettuce and *C. quinoa* stained with PTA or UA. Particles of Ls2, Ls16, Ls158 and Tar2, could be trapped from sap of inoculated lettuce plants onto grids sensitized with antiserum to Ls2 (Fig. 9). This method failed, however, for Ls12.

Fig. 9. Electron micrographs of particles of isolates Tar2 (left) and Ls2 (right) trapped from crude sap of lettuce onto grids first sentitized with antiserum to isolate Ls2. Bar represents 100 nm.

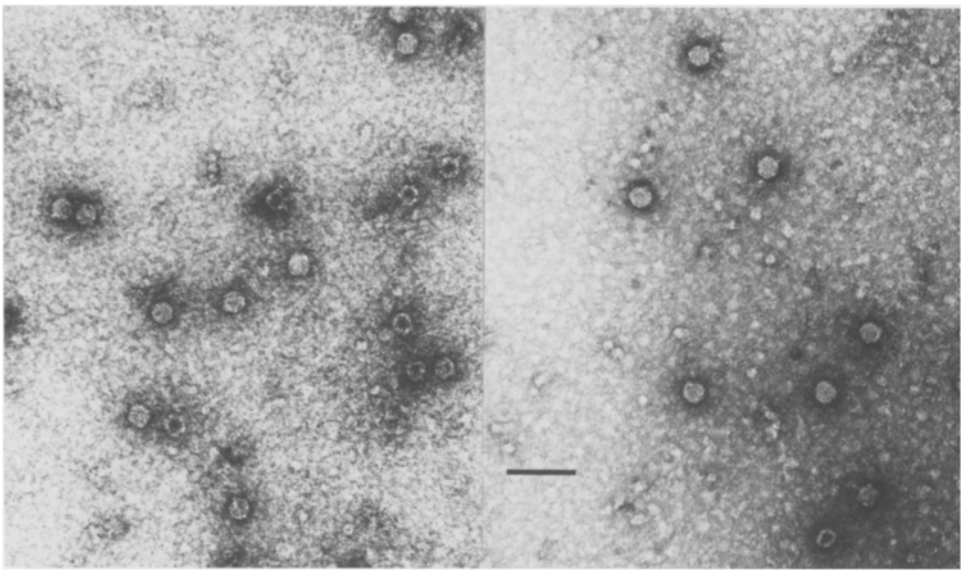

Fig. 9. Elektronenmicroscopische foto's van deeltjes van isolaten Tar2 (links) en Ls2 (rechts) gevangen uit ruw sap van sla op preparaathouders, die eerst gevoelig waren gemaakt met antiserum tegen LS2. Vergrotingsstaaf geeft 100 nm weer.

Discussion

Our investigations have demonstrated the virus nature of the pathogen. Transmission in sap is easy with a special buffer. Transmission by *Myzus persicae* seems to be in the non-persistent manner.

The host ranges and symptoms of the Dutch isolates from lettuce (except Ls12) were similar (Table 1). The Czechoslovak isolate from dandelion (Tar2) produced symptoms on lettuce which were basically identical to our lettuce isolates but it was more virulent. In addition, it was more aggressive, having a wider host range and it was more virulent in soms hosts, such as lettuce, *G. globosa* and *V. faba.* In host range and symptoms on lettuce our virus and Tar2 closely resemble dandelion yellow mosaic virus (DYMV) (Kassanis, 1944, 1947) and 'Salatnekrosevirus' (Hein, 1963) although in our lettuce cultivars tested hardly any necrosis occurred. Kassanis (1947) may have missed the infectivity to *Nicotiana* species due to lack of back-inoculation, since infection with our isolates was latent in most of them. Absence of symptoms in *C. quinoa* plants inoculated by Hein (1963) may have been due to usage of a different selection of the test species or due to a different strain of the virus. We now consider our virus to be DYMV.

The virus had only been described for some of its biological properties because of

its difficult mechanical transmission and low persistence of its infectivity in expressed sap. Attempts by Kassanis (1947) to prepare an antiserum by injecting rabbits with infective sap centrifuged at low speed failed. Now the virus has been characterized for the first time in vitro for its intrinsical properties, and characteristic symptoms have been reproduced in plants of *C. quinoa* with purified virus. Using the modified procedure of ELISA (Flegg and Clark, 1979) four isolates (Ls2, Ls16, Ls158 and Tar2) could be identified in extracts from lettuce and *C. amaranticolor.*

Data obtained by Čech and Branišová (1973) on *Taraxacum* mosaic virus (spherical particles of 32 nm, A_{260}/A_{280} = 1.64, and low virus concentration in plants) resemble our above data, but data on some of the biological properties of their and our viruses are conflicting, no serology was done by them and no proper cross reference was made between the Czech publications on *Taraxacum* mosaic virus and virus isolates resembling DYMV (Brčák, 1979). Our isolate Tar2 from dandelion plants growing in the open in Prague closely resembles our isolates from lettuce but for its higher virulence and aggressiveness, and is considered to be a strain of DYMV.

The relationships of isolate Ls12 to DYMV remain obscure. In host range and eventual effect on lettuce, though of low aggressiveness and virulence, it has features in common with the other isolates studied. Its particles could neither be detected with the electron microscope on grids coated with antiserum to Ls2, nor did the virus react in ELISA with that antiserum. It may be a deviating strain of DYMV.

Difficulty to detect virus particles in crude plant sap, whereas they could easily be seen in purified preparations (Fig. 8), poor mechanical transmission without additives, and low virus yield at purification point to low virus concentrations in infected plants or to instability in expressed crude plant sap.

The single peak observed for Ls2 in Cs_2SO_4 gradients and during analytical ultracentrifuging, and the fact that the different zones observed in sucrose gradients all contained one type of particle, indicate that the virus consists of a single nucleoprotein. The different zones in sucrose gradients may have been composed of monomers, dimers, trimers etc. although this could not be corroborated by electron microscopy. Empty particles, as suggested by Fig. 8, but more clearly so by Fig. 9, did not show up during ultracentrifuging, as was the case with such particles in preparations of cocksfoot mottle virus (Serjeant, 1967). They are likely to be stain-penetrated only because of particle damage, which is obvious in Fig. 8.

In particle morphology, size and the occurrence of particles in single files within membrane-walled tubules (Fig. 8) our virus resembles nepoviruses (Murant, 1981; Roberts and Harrison, 1970). However, it sediments in one peak only, has a much higher sedimentation coefficient and buoyant density and is aphid-transmitted. Alignment of particles in single files in tubules has also been reported for a number of Phytoreoviruses, but these have much bigger particles with much higher sedimentation coefficients and are leafhopper-borne (Shikata, 1981). They have also been found in ultrathin sections of plants with an ilarvirus (Gérola et al., 1969) and with two comoviruses (Kim and Fulton, 1971; Van der Scheer and Groenewegen, 1971), but these are all multiparticle viruses. DYMV cannot yet be assigned to any known virus group.

So far, we have only occasionally isolated the virus from lettuce plants. Our data and those of Hein (1963) suggest that it may be of little direct importance in lettuce in the Netherlands and Germany. In Great Britain, however, the virus has been found in many parts of the country (Moore, 1946). On occasions considerable numbers of

lettuce plants were affected, but most outbreaks have been in the vicinity of infected dandelion. In field trials, natural spread was rapid from inoculated lettuce plants as infection sources (Kassanis, 1947). The virus may have wide distribution in dandelion in Czechoslovakia and Scandinavia (Brčák, 1979) if indeed identical, and may symptomlessly infect other plant species. It may thus well be of potential economic importance, the more so since it is virulent on all cultivars of lettuce tested but 'Laibacher Eis', and since it does infect *L. sativa* 'Gallega de Invierno', *L. serriola* and *L. virosa,* which are important sources of resistance to lettuce mosaic virus and other pathogens. The lettuce cultivar 'Laibacher Eis' was the only cultivar with some tolerance to the virus. It may also be damaging on endive.

Samenvatting

Verdere karakterisering van het paardebloemegeelmozaïekvirus uit sla en paardebloem

Bij het onderzoek over virusziekten van groentegewassen werden zo nu en dan gedrongen slaplanten ontvangen waaruit met enige moeite een virus met sap kon worden overgebracht naar een beperkt aantal plantesoorten. Een aantal isolaten uit sla werd vergeleken met een isolaat uit paardebloem afkomstig uit Tsjechoslowakije en het virus werd nader gekarakteriseerd.

De overdracht met sap wordt sterk vergemakkelijkt door extractie in bufferoplossing. Overdracht door *Myzus persicae* lijkt non-persistent te zijn. Het virus heeft een houdbaarheid in vitro van ongeveer één dag, een inactiveringstemperatuur tussen 60 en 65 °C en een verdunningseindpunt tussen 10 000 en 100 000. Het kon in blad boven $CaCl_2$ bij 4 °C reeds 6½ jaar worden bewaard. Het vertoont in biologisch opzicht veel overeenkomst met het in Engeland in paardebloem en sla aangetroffen paardebloemegeelmozaïekvirus ('dandelion yellow mosaic virus'), dat waarschijnlijk ook in Duitsland, Tsjechoslowakije, Zweden en Finland in paardebloem voorkomt, maar nog niet op zijn intrinsieke eigenschappen is beschreven.

Het virus kon door ons voor het eerst, zij het met moeite, uit planten worden geïsoleerd en bleek slechts één soort bolvormige deeltjes te bezitten met een diameter van ca 30 nm, een sedimentatiecoëfficiënt van 159 S, een zweefdichtheid van 1,42 g. cm^{-3} en een A_{260}/A_{280} verhouding van 1,67. Het virus lijkt niet te behoren tot enige tot dusver beschreven virusgroep. Een tegen gezuiverd virus bereid antiserum heeft een homologe titer van 256. Het antiserum gaf in agar-gel geen reactie met sap van zieke planten. Met ELISA konden vier van de vijf getoetste isolaten, waaronder het isolaat uit paardebloem, in sap van geïnfecteerde sla en *Chenopodium amaranticolor* worden aangetoond. De beste resultaten werden verkregen met een variant van deze toetsmethode, waarbij sap en enzymconjugaat tegelijkertijd in de putjes van de microtiterplaten worden gedaan. Bedoelde vier isolaten konden ook worden 'gevangen' uit ruw sap van sla op met het gemaakte antiserum voorbehandelde preparaathouders voor de elektronenmicroscoop, terwijl met conventionele methoden de deeltjes niet rechtstreeks aantoonbaar waren in ruw plantesap. Het isolaat Ls12 is van geringe virulentie op sla, heeft een wat afwijkende waardplantreeks en kon serologisch noch elektronenmicroscopisch worden aangetoond.

Hoewel hier en elders in Noordwest en Midden Europa nog slechts incidenteel

voorkomend, lijkt het virus er van potentiële economische betekenis gezien de reeds grote verbreiding in paardebloem en sla in Groot Brittannië en zijn effect op sla. Het is virulent op 22 van de 23 getoetste slarassen en op andijvie en tast ook genotypen aan van sla en het geslacht *Lactuca*, die door hun resistentie tegen het slamozaïekvirus en andere pathogenen worden gebruikt in de resistentieveredeling. De cultivar 'Laibacher Eis' was in het onderzoek de enige cultivar met enige tolerantie.

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