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Characterization of a carlavirus from dandelion (Taraxacum officinale)

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Abstract

A carlavirus was isolated from leaves of a dandelion plant raised in the experimental garden of the Hugo de Vries Laboratory in Amsterdam. The virus was readily sap-transmissible and infected 24 out of the 52 plant species and cultivars tested, with visible symptoms in 18 of them. *Myzus persicae* and *Cuscuta subinclusa* (dodder) did not transmit the virus. In addition the virus was not seed-transmitted in dandelion. Dilution end-point was 10^{-5}, thermal inactivation occurred at between 80-85 °C and longevity in vitro was approximately 24 h. The virus had a sedimentation coefficient of 136 S. Polyacrylamide gel electrophoresis of the coat protein gave two bands, consisting of proteins with molecular masses ranging from 37 000 to 34 300 Da (band I) and from 34 000 to 32 800 Da (band II). The molecular mass of the RNA was 2.84 x 10^6 Da. The average buoyant density of the virus was 1.306 gcm^{-3} and the average A_{260}/A_{280} ratio 1.16. The virus particles had a normal length of 668 nm. With the light microscope, large mainly vacuolate inclusions were observed in the epidermal cells of infected *Nicotiana clevelandii* leaves. In ultra-thin sections of systemically infected leaves of *N. clevelandii,* bundles of aggregated virus particles were detected, whereas in infected dandelion leaves there were fewer aggregates and more scattered virus particles. There was a close serological relationship to dandelion latent virus, chrysanthemum virus B and potato virus S and a more distant one to carnation latent virus, elderberry carlavirus, *Helenium* virus S and potato virus M. The occurrence of the virus was found to be restricted to dandelion plants in the experimental garden in Amsterdam. On the basis of large differences in host range, symptomatology and lack of transmission by *M. persicae* it was decided that the virus could not be considered a strain of either dandelion latent virus, chrysanthemum virus B or potato virus S. We therefore propose that it be called dandelion carlavirus.

Additional keywords: carnation latent virus, chrysanthemum virus B, dandelion carlavirus, dandelion latent virus, elderberry carlavirus, *Helenium* virus S, potato virus M, potato virus S.

Introduction

In August 1981 the Plant Protection Service (PD) at Wageningen received four dandelion plants *(Taraxacum officinale* Weber) from the Hugo de Vries Laboratory in Amsterdam. In the latter laboratory, biosystematic studies on dandelion were marred by the abnormal appearance of some of these plants, which consisted of slight mottling and some malformation of the leaves, this being observed both in the glasshouse and in the experimental garden.

Plants of *Chenopodium amaranticolor,* inoculated at the PD with crude sap from

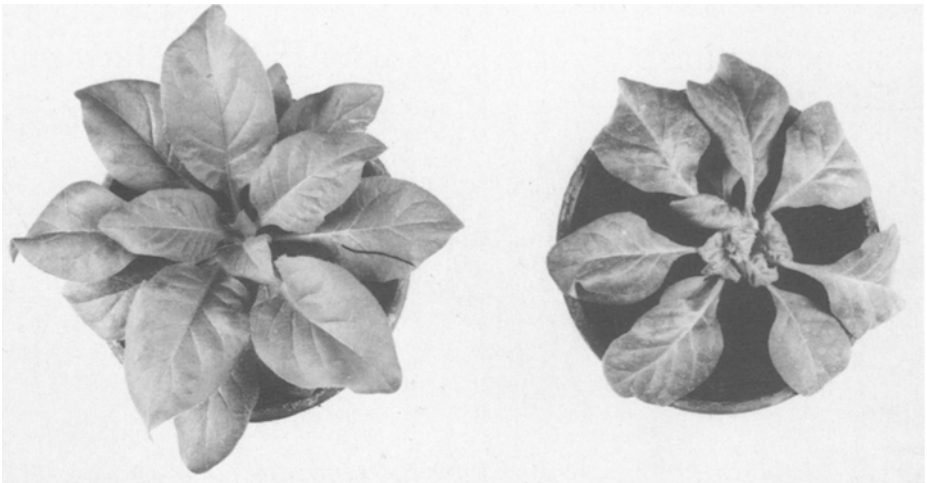

Fig. 1. Leaf curling and stunting in *Nicotiana clevelandii* after inoculation with the virus from dandelion. Left, healthy control.

leaves of one of the four dandelion plants (the other three had died because of unfavourable growing conditions), showed big, greyish-brown necrotic local lesions. Using these local lesion-showing leaves as an inoculum, a small number of plant species and cultivars were tested. The same type of local lesions were obtained on *C. amaranticolor,* as well as brownish-orange local lesions on *C. quinoa,* systemic vein yellowing, leaf curling, stunting and malformation in *Nicotiana clevelandii* (Fig. 1) and yellow spots, net necrosis, leaf curling and apical necrosis of plants of *N. benthamiana* (Fig. 2). Similar results were obtained when we used leaf material from the original dandelion plant as a source of inoculum, although by that time (January 1982) there

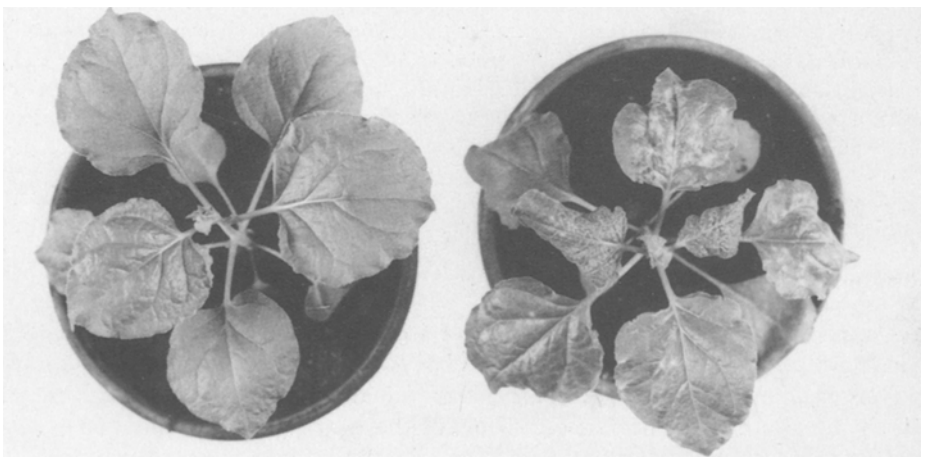

Fig. 2. Local chlorotic-necrotic spots systemic net necrosis and leaf curling in *Nicotiana benthamiana* after inoculation with the virus from dandelion. Left, healthy control.

were no visible symptoms anymore, suggesting that the original symptoms were due to physiological disorders.

Chop preparations for the electron microscope of local lesions in infected leaves of *C. amaranticolor,* and of systemically infected leaves of *N. clevelandii,* revealed the presence of filamentous virus-like particles, about 670 nm long.

On the basis of particle size and morphology it appeared that we might be dealing with a carlavirus.

The aim of this study was to determine the identity of this virus from its biological, physico-chemical and serological properties. In the course of this investigation a paper appeared (Johns, 1982) giving certain characteristics of a carlavirus isolated from dandelion in Canada, and named dandelion latent virus. Therefore, in the first instance, we compared the virus isolated by us with that described by Johns (1982).

Materials and methods

Isolation, maintenance and propagation of the virus. The source of the virus was a dandelion plant, code-numbered THM 57-11-5 (Den Nijs and Sterk, 1984a), raised from seed in a glasshouse and planted in the experimental garden (the 'Overtuin') of the Hugo de Vries Laboratory in Amsterdam. The seed originated from a plant in a moist, poor, *Carex*-rich hay-field at Fresne-St. Mamès (France). From this dandelion plant the virus was isolated by mechanical transmission to *C. amaranticolor, C. quinoa, N. benthamiana* and *N. clevelandii.* Maintenance and propagation of the virus was in *N. clevelandii.*

Inoculation. Manual inoculations were done using either purified virus or water-diluted crude sap from virus-infected plants using carborundum (600 mesh) as abrasive.

Plant material. All test and assay plants were grown in sterilized soil in the glasshouse at 20-30 °C. Dandelion plants were raised from two batches of seed, code-numbered THM 56-1 and THM 56-3, respectively, which originated from plants in a rich grassland at Etuz, France (Den Nijs and Sterk, 1984a).

Host range. In host range tests, 3 plants of each of 52 species and cultivars were inoculated either with water-diluted crude sap of symptom-showing leaves of *N. clevelandii,* or with purified virus suspensions. Water-inoculated plants of the same species and cultivars served as controls. After about four weeks return inoculations to *C. amaranticolor* and *C. quinoa* were made from inoculated and from non-inoculated leaves of the test plants.

Persistence of infectivity in crude sap. Sap from infected *N. benthamiana* or *N. clevelandii* leaves was used as inoculum. Infectivity assay was done using leaves of *C. amaranticolor* and *C. quinoa.*

Transmission by aphids. Transmission experiments with *Myzus persicae* were performed with apterous aphids from virus-free cultures. Aphids were starved for 90 min and then given an acquisition access on young, diseased plants of *N. benthamiana* and

N. clevelandii for about 30 min. Thereafter, these aphids were transferred to young, healthy plants of *N. benthamiana* and *N. clevelandii,* respectively, for an inoculation feeding period of about 90 min (6 plants with 10-25 aphids per plant). Finally, they were killed with an insecticide (pirimicarb). For experiments on transmission in the persistent manner, aphids were given an acquisition access period of 7 days, after which they were transferred to healthy plants (6 plants with 10-25 aphids per plant) for inoculation feeding for another period of 7 days. From these plants aphids were transferred to another series of healthy plants. After 7 days, the aphids were killed with an insecticide (pirimicarb).

Transmission by dodder. Shoots of *Cuscuta subinclusa* established on young, symptom-showing plants of *N. benthamiana* were trained on to young, healthy plants of *N. benthamiana.* Presence of the virus in the latter plants was determined about six weeks after the dodder had established itself on the basis of visible symptoms. In the absence of symptoms the plants were both indexed on *C. amaranticolor, C. quinoa* and *N. clevelandii,* and examined electron microscopically.

Transmission through seed. For seed transmission experiments 30 seeds of inoculated, infected dandelion plants were sown. The same number of seeds from non-inoculated dandelion plants were used as controls. Presence of the virus in the young seedlings was determined by bio-assay on the two above-mentioned species of *Chenopodium* and on *N. clevelandii.*

Field occurrence. Field occurrence of the virus was determined by indexing dandelion samples from different places in the Netherlands, viz. sites in the provinces of Noord-Holland, Zuid-Holland, Drente and Gelderland. In the experimental garden of the Hugo de Vries Laboratory leaf samples were taken from 22 dandelion plants growing at various distances from the place where the original infected dandelion plant was found. Indexing of the plants was carried out in September 1983 and April 1984 by bio-assay on *C. amaranticolor, C. quinoa* and *N. clevelandii,* and by enzyme-linked immunosorbent assay (ELISA). Along with the above samples purified virus suspensions and sap from infected dandelion plants in the glasshouse were used for comparison.

Virus purification. The virus was purified from systemically infected leaves of *N. clevelandii* both by the method of Veerisetty and Brakke (1978), for the purification of alfalfa latent virus using minor modifications, and by a method described by Tavantzis (1983). The partially purified virus suspensions obtained were subjected to density gradient centrifugation for further purification.

For rate-zonal centrifugation gradients were made with 10-40% (w/v) sucrose in 0.0165 M disodium phosphate – 0.0018 M trisodium citrate buffer, pH 9.0 (phosphate-citrate buffer). After layering 2-5 ml of the partially purified virus suspension (1-3 mg ml^{-1}) the gradients were centrifuged at 82 000 *g* in a Beckman SW 27 or 28 rotor for 2.5 h. Virus zones were isolated using an LKB Uvicord II absorbance monitor.

Ultraviolet light absorption. The ultraviolet light absorption of purified virus suspensions was determined with a Gilford 2400-2 self-recording spectrophotometer. Absorbance values were not corrected for light scattering. Virus concentrations were deter-

mined using an arbitrary extinction coefficient $E_{260}{}^{0.1\%}_{1\,cm}$ of 3.0 (Veerisetty and Brakke, 1978).

Sedimentation coefficient. The sedimentation coefficient was determined on a Spinco Model E ultracentrifuge with Schlieren optics using a purified virus suspension (2 mg ml^{-1} in phosphate-citrate buffer of pH 9.0 at 20 °C); values were determined by the graphical method developed by Markham (1960).

Buoyant density. The buoyant density of purified virus suspensions was determined in CsCl. About 0.5 ml of a virus suspension (0.5 mg ml^{-1}) was layered on a solution of 0.149 g CsCl ml^{-1} in phosphate-citrate buffer in a tube of a Beckman SW 50.1 rotor and centrifuged at 114 000 *g* for 18 h. The gradient was fractionated in samples of 0.5 ml each and the solute concentration in each sample determined in a refractometer. Densities were calculated by using the relation $\varrho^{25°} = 10.2402\,\eta_D^{25°} - 12.6483$ for densities between 1.00 and 1.38 and $\varrho^{25°} = 10.8601\,\eta_D^{25°} - 13.4974$ for densities higher than 1.37 (Bruner and Vinograd, 1965).

Molecular mass of the capsid protein. The molecular mass of the capsid protein was determined by polyacrylamide gel electrophoresis on 10% slab gels (16 cm x 18 cm x 1.5 mm). In most of the experiments a purified virus suspension (0.1 mg ml^{-1}) was mixed 1:1 (v/v) with an aqueous solution containing 2% 2-mercaptoethanol (v/v), 8 M urea and 2% (w/v) sodium dodecyl sulphate (SDS) and then heated in boiling water for 2 min. Samples of degraded virus (20 μl) were subjected to electrophoresis at 200 V constant voltage at about 16 °C for 5-6 h. Gels were stained with Coomassie Brilliant Blue. The following proteins were used as markers (their molecular masses in daltons are given in parentheses): phosphorylase b (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000) and soybean trypsin inhibitor (20 000).

Molecular mass of the virus nucleic acid. Extraction of RNA from purified suspensions of the virus was done as described by Van den Hurk et al. (1977). RNA preparations were analysed on horizontal 1% agarose gels containing 5 mM methylmercury according to the method described by Bailey and Davidson (1976). Samples of RNA (2-5μg in 40 μl) in electrophoresis buffer with 10 mM methylmercury and 10% glycerol were applied to the gels. Electrophoresis was at about 150 V at room temperature for 3.5-4 h. The gels were stained with a solution of 1 μg ethidium bromide ml^{-1} of 0.5 M NH_4-acetate and the bands were made visible under ultraviolet light. The four nucleic acid species of cowpea chlorotic mottle virus (CCMV-RNA_1, CCMV-RNA_2, CCMV-RNA_3, CCMV-RNA_4) and the two nucleic acid species of cowpea mosaic virus (CPMV-RNA_1, CPMV-RNA_2) were used as markers, with their molecular masses (x 10^{-6}) taken as: 1.20, 1.07, 0.81, 0.25, 2.02 and 1.37 Da, respectively.

Light microscopy. Epidermal strips of infected leaves of *N. benthamiana* and *N. clevelandii* were prepared for light microscopy according to the method described by Christie and Edwardson (1977) using Azure A stain.

Electron microscopy. The virus was examined, under the electron microscope, in preparations of crude sap and in (partially) purified virus suspensions. For crude sap preparations, both symptom-showing leaves of *C. amaranticolor* and of *N. clevelandii,* and symptomless leaves of dandelion were chopped. The carbon-reinforced formvar-coated copper grids were floated on a drop of sap from the chopped material

for 15 sec and then transferred to a drop of 3% glutaraldehyde for fixation. After 5 min, the specimen was stained with 2% potassium phosphotungstate (PTA) for 1 min. Purified virus suspensions were directly placed on grids for 1-2 min and then stained with 2% PTA. The suspensions were examined with a Siemens Elmiskop 101 or with a Zeiss 109 electron microscope. For length measurements of the virus particles, fixed catalase crystals with a lattice spacing of 8.6 ± 0.22 nm were used as an internal size standard (Wrigley, 1968). Particle measurements were made from negatives with a binocular microscope at x 12.5 using a micrometer eyepiece.

For in situ studies, symptom-showing leaves of *N. clevelandii* and symptomless infected leaves of dandelion were prepared as described by Jayasinghe and Dijkstra (1979).

Serology. For antiserum production, a mixture of 1 ml of a purified virus suspension (1 mg ml^{-1}) and 1 ml of Freund's complete adjuvant was injected into a rabbit subcutaneously. After 15 days, a second injection was given intramuscularly with 1 mg virus emulsified with Freund's complete adjuvant. Twenty days later, the rabbit was bled. Purified virus was tested against antiserum to the following carlaviruses (homologous titres according to the donors are given in parentheses): alfalfa latent virus (32), carnation latent virus (1024), chrysanthemum virus B (128), dandelion latent virus (10 000), elderberry carlavirus (512), *Helenium* virus S (512), poplar mosaic virus (1024), potato virus M (1024), potato virus S (1024). Antiserum to dandelion latent virus (DLV) and to *Helenium* virus S were provided by Dr R. Stace-Smith, Plant Pathology Section Research Station, Vancouver (Canada) and by Dr Renate Koenig, Institut für Viruskrankheiten der Pflanzen, Brunswick (GFR), respectively. All the other antisera except that to elderberry carlavirus (which was from own stock) were provided by Ing. D.Z. Maat, Research Institute for Plant Protection, Wageningen.

The microprecipitin test according to Van Slogteren (1954) and the microplate method of ELISA (Clark and Adams, 1977) were used for serological tests.

Microprecipitin test. Dilutions of purified virus suspensions (0.5 mg ml^{-1}) were made with 0.1 M tris (= tris (hydroxymethyl) aminomethane)-citric acid, pH 8.0. The reactions were evaluated after an incubation period of 6-18 h at room temperature.

ELISA. For ELISA young leaf material (3 g) was ground in 12 ml phosphate buffered saline containing 0.05% Tween 20, with or without replacement of 0.01 M KH_2PO_4 by 0.01 M KCN, and strained through cheesecloth. Coating of the microplates was with 1 μg of γ-globulin ml^{-1}. The final dilution of the conjugated γ-globulin used was 1:500 in most of the experiments. The colour reaction was assessed colorimetrically at a wavelength of 405 nm.

Results

Reaction of test plants and host range. Mechanical inoculation of dandelion seedlings with purified virus resulted in symptomless infection in most of the experiments. Only once some yellowing in the upper part of leaves on plants inoculated with the virus was observed, whereas the water-inoculated controls did not show this symptom. The symptoms shown by the original infected dandelion plant from the PD were never observed in experiments on mechanical transmission, which strengthened our surmise that these symptoms were due to physiological disorders. Occasionally both in-

oculated and non-inoculated dandelion seedlings showed slight mottling. The latter was possibly due to nutritional stress caused by improper soil or too high a temperature. The composition of the soil proved to be critical for a good growth; only soil consisting of peat and a large amount of clay enabled the seedlings to grow well.

Out of the 52 plant species and cultivars tested 18 showed symptoms, 6 had latent infections and the remainder were not infected at all (Table 1). *Chrysanthemum morifolium* showed local spots (Fig. 3), local symptomless infection or no infection at all, depending on the seedling used.

Persistence of infectivity in crude sap. In sap from infected *N. benthamiana* or *N. clevelandii* leaves, infectivity was lost after dilution to 10^{-5} or after heating for 10 min at 80-85 °C. Storage of the sap at 20 °C resulted in 90-98% of the infectivity being lost within 16 h, the number of local lesions dropping from about 600 per leaf with freshly expressed sap to about 12 after storage. Upon longer storage, the lesion number slowly decreased and was zero at about 24 h.

Transmission by aphids. Myzus persicae did not transmit the virus in the six experiments carried out.

Transmission by dodder. No virus transmission was obtained from systemically infected plants of *N. benthamiana* to healthy plants of the same species.

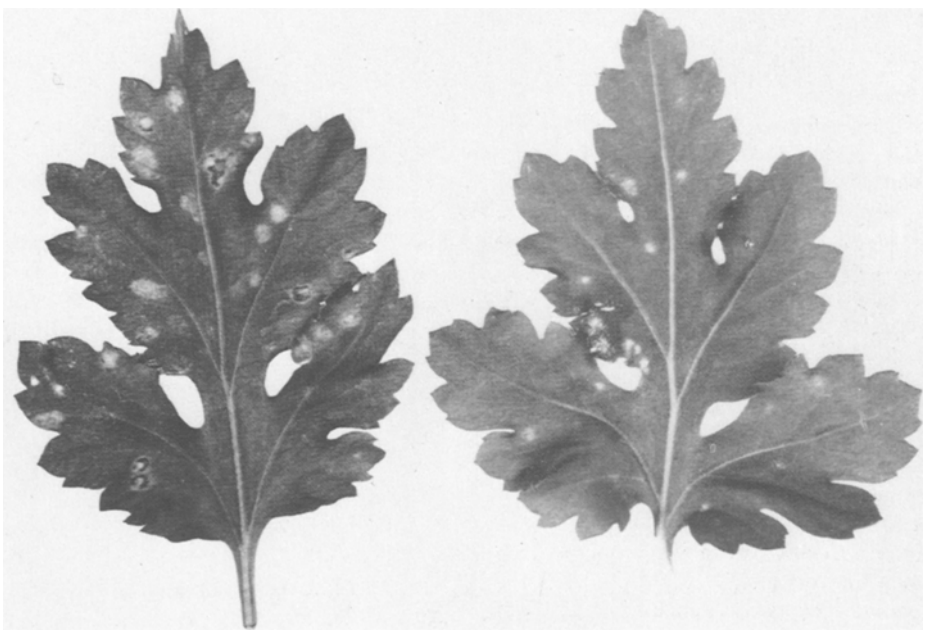

Fig. 3. Two leaves of a seedling of *Chrysanthemum morifolium* with chlorotic and necrotic spots after inoculation with the virus from dandelion.

Table 1. Reactions of test plants to inoculation with the virus from *Taraxacum officinale.*

Test plant	Symptoms[1]	
	local	systemic
Ageratum conyzoides	--	--
Amaranthus caudatus	--	--
Begonia semperflorens	--	--
Beta vulgaris 'Groeningia'	YRSp, YSp	--
Browallia speciosa	--	--
Calceolaria integrifolia	--	--
Calendula officinalis	--	--
Callistephus chinensis	--	--
Chenopodium album	NL, YL	--
Chenopodium amaranticolor	NL	--
Chenopodium capitatum	--	--
Chenopodium quinoa	NL, YL	Ch, LDf
Chrysanthemum morifolium	ChSp, NSp, +, --	--
Crotalaria juncea	--	--
Cucumis sativus 'Lange Gele Tros'	--	--
Cyamopsis tetragonoloba	--	--
Dahlia variabilis 'Cactus'	−	Mot
Datura stramonium	+	--
Dianthus barbatus	--	--
Gazania rigens	+	+
Gomphrena globosa	NL	--
Helianthus annuus 'Sungold'	+	VY, YSp
Impatiens glandulifera	+	+
Lablab niger	--	--
Lavatera trimestris	+	Mos, Mot, VCh
Lycopersicum esculentum 'Moneymaker'	--	--
Nicotiana benthamiana	ChSp, NSp	Ch, LC, NN,Wlt
Nicotiana clevelandii	Ch, ChSp, LC, NSp, VCh	Ch, LC, LDf, St, VCh
Nicotiana debneyi	--	--
Nicotiana glutinosa	ChSp	--
Nicotiana megalosiphon	ChSp	Ch, Et, LC, LDf
Nicotiana rustica	--	--
Nicotiana tabacum 'Samsun NN'	--	--
Nicotiana tabacum 'White Burley'	--	--
Petunia hybrida	ChSp, NSp	--
Phaseolus vulgaris 'Noordhollandse Bruine'	--	--
Physalis floridana		--
Pisum sativum 'Koroza'	--	--
Salvia splendens	--	--
Solanum melongena 'Lange Violette'	--	--
Solanum rostratum	+	+
Sonchus oleracea	--	N, Y
Tagetes patula	−	--

Table 1. Continued.

Test plant	Symptoms[1]	
	local	systemic
Taraxacum officinale	+	+
Tetragonia expansa	NSp	--
Trifolium incarnatum	--	--
Verbesina encelioides	--	--
Vicia faba 'Driemaal Wit'	--	--
Vigna cylindrica	+	YRSp, YSp
Vigna unguiculata 'California Blackeye'	--	--
Vinca rosea	--	--
Zinnia elegans 'Persian Carpet'	+	Ch, Ep

[1] Ch = chlorosis; ChSp = chlorotic spots; Ep = epinasty; Et = etching; LC = leaf curling; LDf = leaf deformation; Mos = mosaic; Mot = mottling; N = necrosis; NL = necrotic lesions; NN = net necrosis; NSp = necrotic spots; St = stunting; VCh = veinal chlorosis; VY = vein yellowing; Y = yellowing; YL = yellow lesions; YRSp = yellow ring spots; YSp = yellow spots; Wlt = wilting.
+ = symptomless infection; -- = no infection; – = no symptoms.

Transmission through seed. None of the plants raised from the seed of infected dandelion plants was infected.

Field occurrence. The results obtained from bio-assay and ELISA showed that the non-cultivated dandelions from the provinces of Drente, Gelderland, Noord-Holland and Zuid-Holland were not infected, whereas, by contrast, 21 out of the 22 dandelion plants from the experimental garden in Amsterdam proved to be infected (Table 2).

As one side of the experimental garden was bordered by a hedge of *Ligustrum vulgare,* this plant species was also indexed. Although sap from *Ligustrum* leaves gave positive reactions on *C. quinoa* and *N. clevelandii,* the symptoms on these test plants were quite different to those caused by our virus from *Taraxacum.* Moreover, in ELISA it did not give a reaction. Electron microscope examination of the local lesions on *C. quinoa* and the mottled leaves of *N. clevelandii* revealed the presence of an isometric virus, most probably cucumber mosaic virus.

Virus purification and ultraviolet light absorption. Purified virus preparations, obtained after rate-zonal centrifugation, had an absorption maximum at 260 nm and minimum at 249 nm. Without correction for light scattering the A_{max}/A_{min} absorption ratio varied from 1.03 to 1.14 (average 1.08) and the A_{260}/A_{280} from 1.12 to 1.24 (average 1.16). The yield was 2-3 mg virus per 100 g leaf material.

Sedimentation coefficient. The virus sedimented as a single component. The average sedimentation coefficient of the virus at a concentration of 2 mg ml^{-1} in phosphate-citrate buffer at pH 9.0 at 20 °C was 136 S.

Buoyant density. The buoyant density of the virus calculated from three experiments was 1.306 g cm^{-3}.

Table 2. Results of ELISA and bio-assay with sap from leaves of 22 plants of *Taraxacum* from the experimental garden of the Hugo de Vries Laboratory in Amsterdam.

Code number	Particulars about the *Taraxacum* plants				Results	
	section	microspecies	ploidy level	country of origin	ELISA	bio-assay
THM 118-11[1]	*Taraxacum*	ND[4]	ND	France	+	+
THM 90-8[1]	*Taraxacum*	ND	ND	France	+	+
THM 118-5[1]	*Taraxacum*	ND	ND	France	+	
THM 104B-7[1]	*Taraxacum*	ND	triploid	France	+	+
THM 85-4[1]	*Taraxacum*	ND	ND	France	+	+
THM 110-2[2]	*Taraxacum*	*Taraxacum jenniskensiae*	diploid	France	+	+
THM 83-8[2]	*Taraxacum*	ND	diploid	France	+	+
THM 111-3[2]	*Taraxacum*	related to *Taraxacum chlorodes*	diploid	France	+	+
50	*Palustria*	*Taraxacum hollandicum*	triploid	Netherlands	+	+
TMJ 95-10[2]	*Taraxacum*	ND	diploid	Netherlands	+ (F_1 plant)	+
H11	*Erythrosperma*	*Taraxacum taeniatum*	triploid	Netherlands	−	−
THM 57-3-b[1]	*Taraxacum*	ND	triploid	France	+	+
THM 57-2-3[1]	*Taraxacum*	ND	triploid	France	+	+
THM 57-4-2[1]	*Taraxacum*	ND	triploid	France	+	+
TB 13-2	ND	ND	ND	Hungary	+	+
TB 13-8	ND	ND	ND	Hungary	+	+
TMJ 67-17[2]	*Taraxacum*	ND	diploid	Netherlands (F_1 plant)	+	+
THM 26-9[2]	*Taraxacum*	*Taraxacum multifidum*	diploid	France	+	+
TPP 23-5[2]	*Taraxacum*	*Taraxacum demotes*	diploid	GFR	+	+
TSH 11-6[2]	*Taraxacum*	*Taraxacum limburgense*	diploid	Netherlands	+	+
THL 33-7[2]	*Taraxacum*	*Taraxacum quadrangulum*	diploid	Switzerland	+	+
THL 120[3]	*Erythrosperma*	*Taraxacum tortilobum*	triploid	GFR	+	+

[1] Den Nijs and Sterk (1984a).
[2] Jenniskens (1984).
[3] Den Nijs and Sterk (1984b).
[4] ND = not determined.

Molecular mass of the capsid protein. In electrophoresis experiments with the coat protein from virus purified 4-6 days after inoculation, two major bands were observed, consisting of proteins with molecular masses ranging from 37 000 to 34 300 Da (band I) and from 34 000 to 32 800 Da (band II). When virus was purified from plants 11 to 22 days after inoculation, band I consisted of protein with a molecular mass of approximately 34 000 Da, whereas band II contained proteins with widely varying molecular masses (from 28 000 tot 23 000 Da in the respective experiments).

Two bands have also been reported for the coat protein of other carlaviruses (Brunt and Kenten, 1973; Boccardo and Milne, 1976; Veerisetty and Brakke, 1977; Hearon, 1982). As it is unlikely that the virus has two different coat proteins, band I may be the entire coat protein and band II a degradation product, as has been suggested by Boccardo and Milne (1976) and Veerisetty and Brakke (1977). The fact that proteins with higher molecular mass values were found mostly in virus purified from leaves 4-6 days after inoculation and the lower values were found in virus preparations from old infections, corroborates the above suggestion.

Molecular mass of the nucleic acid. The molecular mass of the single nucleic acid component was estimated as 2.84×10^6 Da.

Light microscopy. Large vacuolate and ropy inclusions which stained pink with Azure A were observed in the epidermal cells of inoculated *N. clevelandii* leaves with chlorotic spots (Fig. 4). These inclusions frequently occurred in the vicinity of the nucleus, or even adjacent to it.

Electron microscopy. As the number of virus particles in crude sap preparations of either *N. clevelandii* or dandelion was small, the normal length of the virus particles was determined in purified virus suspensions; the average normal length was 668 nm.

In ultra-thin sections of systemically infected leaves of *N. clevelandii,* bundles of aggregated virus particles were frequently observed next to a number of vacuoles (Fig. 5). Most likely these bundles form part of the structures resembling the vacuolate inclusions observed with the light microscope. In addition to bundles there were also scattered virus particles in the cytoplasm (Fig. 5). Similar sections of infected dandelion leaves usually showed fewer aggregates and more scattered virus particles, possibly due to a lower virus concentration.

Serology. Microprecipitin test. The antiserum to the purified virus had a titre of 256. From the results with a number of other antisera (Table 3) it is clear that our dandelion virus is closely related to DLV, chrysanthemum virus B and potato virus S. A distant serological relationship was found with carnation latent virus, elderberry carlavirus, *Helenium* virus S and potato virus M. No visible reactions were obtained with antiserum to alfalfa latent virus and poplar mosaic virus.

ELISA. With the dilution of coating γ-globulin and enzyme-γ-globulin conjugate used, a concentration of 0.1 μg virus ml^{-1} could just be detected. Samples of uninfected dandelion leaves taken in September showed a higher level of background colour than those collected in April (average absorption at 405 nm: 0.23 and 0.05, respectively). This is most likely due to the fact that in September the dandelion plants were at the end of their growing season and therefore had predominantly old leaves.

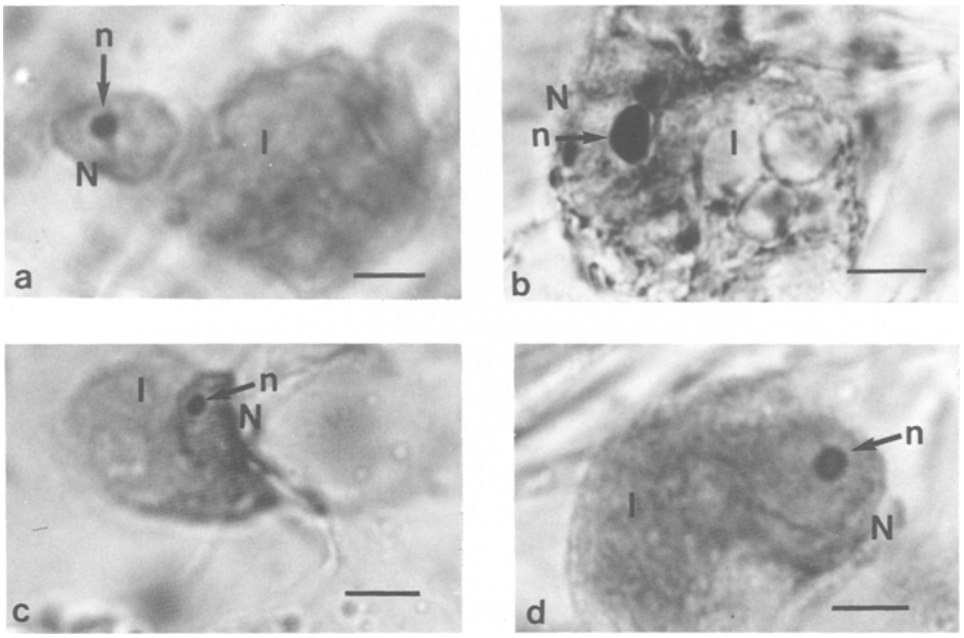

Fig. 4a-d. Inclusions in epidermal cells of *Nicotiana clevelandii* leaves inoculated with the virus from dandelion. I = inclusion; N = nucleus; n = nucleolus. Bars represent 5.5 μm.

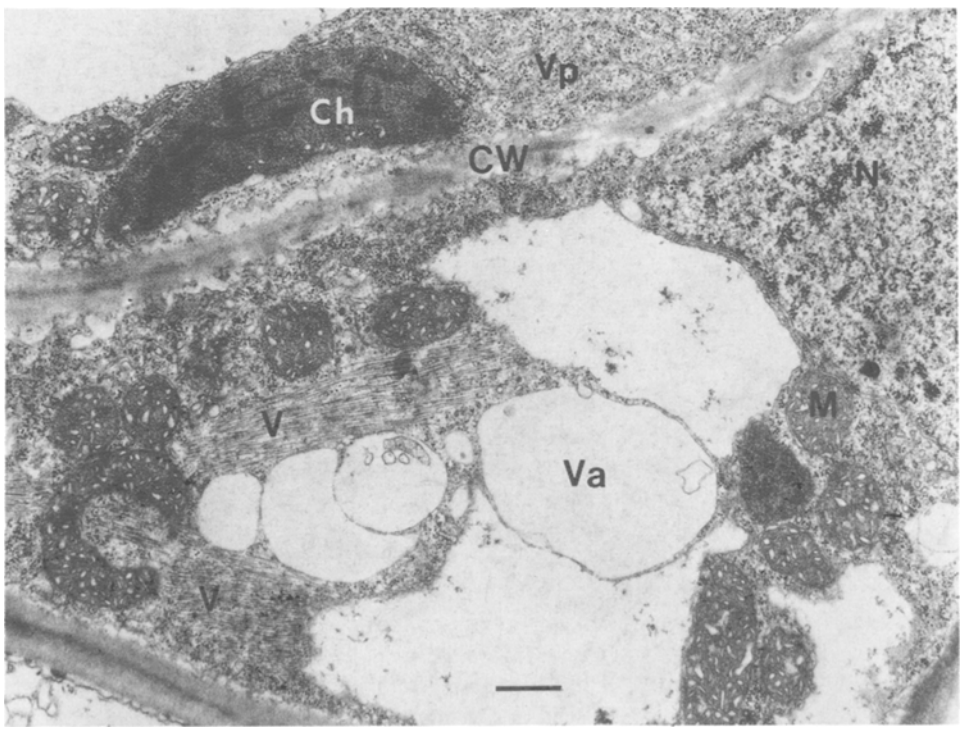

Fig. 5. Ultra-thin section of leaf tissue of *Nicotiana clevelandii* infected with the virus from dandelion. Ch = chloroplast; CW = cell wall; M = mitochondrion; N = nucleus; V = bundles of virus particles; Va = vacuole; Vp = scattered virus particles. Bar represents 500 nm.

Table 3. Results of microprecipitin tests to determine the serological relationship of the virus from *Taraxacum officinale* to carlaviruses.

Antisera	Heterologous titres	Homologous titres (according to the donors)
Alfalfa latent virus	–[1]	32
Carnation latent virus	32	1024
Chrysanthemum virus B	128	128
Dandelion latent virus	128	10 000
Elderberry carlavirus	32	512
Helenium virus S	32/64	512
Poplar mosaic virus	–	1024
Potato virus M	8/16	1024
Potato virus S	64/128	1024

[1] – = no reaction.

It is known that old and decaying leaves of dandelion produced high levels of background colour (Johns, 1982). When KCN was omitted from the extraction medium the background colour was so strong that no reliable results could be obtained.

Discussion

The properties of the dandelion virus described here (in particular its latency in the natural host plant, type of inclusion bodies, particle size and morphology, buoyant density and serological relationship), are typical of a carlavirus. The question now arises whether the virus is identical to, closely related to or distinct from previously characterized members of this group of viruses. Comparing our virus with the DLV described by Johns (1982) (Table 4) it can be seen that it resembles DLV in some of its serological properties, dilution end-point and thermal inactivation point, but differs in its host range, symptomatology, transmission by *M. persicae* and serological cross-reactivities with some of the other carlaviruses tested. We recognize that when it is a matter of differences in degree there is never a hard and fast line between a new virus and a new strain of an already characterized virus. However, as the artificial host ranges, symptomatology and the transmission by *M. persicae* are very dissimilar, we feel that it is justified to classify our virus as a new virus rather than as a new strain of DLV, taking into account the guidelines for the identification and characterization of plant viruses (Hamilton et al., 1981).

The differences in host range and symptomatology between our virus and the two serologically closely related viruses chrysanthemum virus B and potato virus S, are even greater than in case of DLV (chrysanthemum virus B does not infect dandelion and *Chenopodium* spp.). Hence, our virus from dandelion can not be considered a

Table 4. Comparison of properties of the virus from *Taraxacum officinale* (TV) with those of dandelion latent virus (DLV; as reported by Johns, 1982).

Properties	TV		DLV	
Symptoms on test plants[1]				
Chenopodium amaranticolor	NL	ni	ChL	Ch, Ep, VC
Chenopodium quinoa	NL, YL	ni	ChL	Ch, Ep, VC
Cucumis sativus	ni	ni	ni	ni
Datura stramonium	si	ni	ni	ni
Gomphrena globosa	NL	ni	NL	ni
Helianthus annuus	si	VY, YSp	ni	ni
Lycopersicum esculentum	ni	ni	ni	ni
Nicotiana clevelandii	Ch, LC, VCh	Ch, LC,	ni	ni
Nicotiana glutinosa	ChSp	ni	ni	ni
Nicotiana rustica	ni	ni	ni	ni
Nicotiana tabacum 'White Burley'	ni	ni	ni	ni
Petunia hybrida	ChSp, NSp	ni	ni	ni
Phaseolus vulgaris	ni	ni	ni	ni
Taraxacum officinale	si	si	si	si
Verbesina encelioides	ni	ni	ni	ni
Vicia faba	ni	ni	ni	ni
Vigna unguiculata	ni	ni	ni	ni
Zinnia elegans	si	Ch, Ep	ni	ni
Stability in sap				
longevity in vitro (days)	1		4 to 5	
thermal inactivation point	80 to 85		75 to 80	
dilution end-point	10^{-5}		10^{-5} to 10^{-6}	
Transmission				
by *Myzus persicae*	no		yes	
through seed	no		no	
Properties of the particles				
A_{260}/A_{280} (uncorrected)	1.16		1.41 ± 0.09	
A_{max}/A_{min} (uncorrected)	1.08		1.09 ± 0.04	
particle length (nm)	668		640	
molecular mass of the nucleic acid (daltons)	2.84×10^6		2.5×10^6	
Serological relationship[2]				
Carnation latent virus	+		−	
Chrysanthemum virus B	++		+	
Dandelion latent virus	++			
Helenium virus S	+		+	
Poplar mosaic virus	−		−	
Potato virus M	+		−	
Potato virus S	++		++	

[1] In each column local reactions are on the left side and systemic reactions on the right side. Ch = chlorosis; ChL = chlorotic lesions; ChSp = chlorotic spots; Ep = epinasty; LC = leaf curling; LDf = leaf deformation; NL = necrotic lesions; NSp = necrotic spots; VC = vein clearing; VY = vein yellowing; YL = yellow lesions; ni = no infection; si = symptomless infection.

[2] ++, + and − = strong, moderate and no visible reactions, respectively.

strain of either chrysanthemum virus B or potato virus S. We, therefore, propose the name dandelion carlavirus (DCV) for the virus isolated in the laboratory.

In contrast to DLV there were no indications that DCV occurred mainly in the vicinity of cultivated orchards. The virus was found only in dandelion plants in the experimental garden where the original infected plant came from. Even dandelion plants in the Botanical Garden on the opposite side of the road along the experimental garden were free from DCV. It is probable that the virus was introduced with plant material (pen roots) collected in GFR, France and Switzerland, and subsequently grown in the experimental garden. As the virus is very readily sap-transmissible, its spread within the garden from the imported dandelion plants to those raised from seed, must have taken place by mechanical contact, for example when sampling material for taxonomic studies.

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Samenvatting

Karakterisering van een carlavirus uit paardebloem (Taraxacum officinale)

Een carlavirus werd geïsoleerd uit een paardebloemplant, die opgekweekt was in de proeftuin van het Hugo de Vries-Laboratorium in Amsterdam. Het virus kon gemakkelijk met sap worden overgebracht en was in staat 24 van de 52 getoetste plantesoorten en -cultivars te infecteren, waarbij op 18 van deze symptomen zichtbaar werden. *Myzus persicae* en warkruid *(Cuscuta subinclusa)* konden het virus niet overbrengen. Evenmin kon het virus met zaad van geïnfecteerde planten van paardebloem overgaan. De verdunningsgrens was 10^{-5}, de inactiveringstemperatuur 80-85 °C en de houdbaarheid in vitro ongeveer 24 uur. Het virus had een sedimentatiecoëfficiënt van 136 S. Polyacrylamide-gelelektroforese van het manteleiwit resulteerde in twee banden, bestaande uit eiwitten met molecuulmassa's die varieerden van 37 000 tot 34 300 Da (band I) en van 34 000 tot 32 800 Da (band II). De molecuulmassa van het RNA was 2,84 x 10^6Da. De gemiddelde zweefdichtheid van het virus bedroeg 1,306 g cm^{-3} en de gemiddelde A_{260}/A_{280} verhouding was 1,16. Het virus had een normale lengte van 668 nm. In de epidermiscellen van geïnfecteerde bladeren van *Nicotiana clevelandii* werden met de lichtmicroscoop insluitsels met draderige en vacuoleachtige structuren waargenomen. In ultradunne coupes van systemisch geïnfecteerde bladeren van *N. clevelandii* waren bundels geaggregeerde virusdeeltjes zichtbaar. In geïnfecteerde bladeren van paardebloem werden daarentegen meer verspreid voorkomende virusdeeltjes gevonden en minder aggregaten. Het virus vertoonde een sterke serologische verwantschap met het 'dandelion latent virus', chrysantevirus B en aardappelvirus S; er was een geringe verwantschap met het latente anjervirus, het carlavirus van vlier, '*Helenium* virus S' en het aardappelvirus M. Het vóórkomen van

het virus bleek beperkt te zijn tot paardebloemen in de proeftuin in Amsterdam. Gezien de grote verschillen in waardplantenreeks, symptomatologie en overdracht met *M. persicae* hebben we gemeend, dat het virus niet slechts als een stam kon worden beschouwd van hetzij het 'dandelion latent virus', hetzij het chrysantevirus B en het aardappelvirus S. We stellen voor de naam carlavirus van paardebloem aan dit virus te geven.

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