

Purification and Partial Characterization of a Carlavirus from *Taraxacum officinale*

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ABSTRACT

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A carlavirus was isolated from naturally infected dandelion (*Taraxacum officinale*) in the Okanagan Valley, British Columbia. In a survey of potential hosts, this virus was found to produce local and systemic symptoms in *Chenopodium amaranticolor* and *C. quinoa* and local lesions in *Gomphrena globosa*. The carlavirus for which the name dandelion latent virus (DLV) is proposed, has flexuous particles with normal length 640 nm and width 12–13 nm. A purification schedule was developed that yielded 20–30 mg of DLV per kilogram of infected *C. quinoa* tissue. Partially purified preparations of DLV have a single nucleoprotein component in sucrose and cesium chloride density gradients. The molecular weight of the RNA is 2.5×10^6 . The UV absorption spectrum has a maximum at 259 nm and a minimum at 245 nm. The A_{\max}/A_{\min} is approximately 1.1 and the

A_{260}/A_{280} is 1.4. In sap from DLV-infected *C. quinoa*, the thermal inactivation point was 75–80 C, the infectivity dilution end point was 10^{-5} – 10^{-6} , the longevity in vitro was 4–5 days at 23 C, 28–56 days at 4 C, and >2 yr in a lyophilized state. DLV was transmitted nonpersistently by the aphid, *Myzus persicae*. It was not seed transmitted in dandelion or *C. quinoa*. By tube precipitin serology, antiserum prepared against DLV had a maximum homologous titer of 40,960. Two carlaviruses that caused similar systemic chlorosis in *C. quinoa*, a Peruvian strain of potato virus S and helenium virus S, were purified for comparative serological testing. DLV is related serologically to potato virus S and distantly related to chrysanthemum virus B, helenium virus S, and narcissus latent virus.

The common dandelion (*Taraxacum officinale* Weber) is widespread throughout the world, but the available literature suggests that dandelion is not a host for many viruses that are known to have wide natural host ranges, except for those viruses transmitted by nematodes. In such cases, the nepoviruses are seed-transmitted at a low level in dandelion seedlings and the seedlings remain symptomless (10).

Preliminary examination of a leaf dip from infected dandelion revealed slightly flexuous, rod-shaped virus particles ~650 nm long. Based on particle size and morphology, the virus (for which the name dandelion latent virus [DLV] is proposed) was tentatively considered a member of the carlavirus group (7). Whether it was a new virus or related to a recognized virus was not known.

The objectives of this study were to describe the distribution and some of the biological, physicochemical, and serological properties of DLV and to compare three carlaviruses from geographically isolated and distinct areas: DLV from Canada, helenium virus S from Germany, and Peru virus S from Peru.

MATERIALS AND METHODS

Field occurrence and virus isolates. DLV was isolated from dandelions in the Okanagan Valley, British Columbia, and a culture was obtained from A. J. Hansen, Summerland, British Columbia. Field occurrence of DLV was determined by indexing dandelion samples from various sites in British Columbia. Initially, samples were indexed on *Chenopodium quinoa* Willd., but after an antiserum had been produced, all indexing was by enzyme-linked immunosorbent assay (ELISA) (1). Doubtful ELISA results were clarified by inoculating the sap on *C. quinoa*.

From descriptions in the literature, the most likely virus to be related to DLV was a strain of potato virus S from potato in Peru (5). A culture of this virus, here designated Peru virus S (PeVS), was obtained from A. M. Lekeu, Lima, Peru. A carlavirus that caused systemic symptoms in *C. quinoa* was reported from *Helenium amarum* hybrids in Germany (6), and a culture of

helenium virus S (HVS) was obtained from R. Koenig, Braunschweig, Germany.

Host range and symptomatology. Infected dandelion leaves were ground in inoculation buffer: 0.1 M potassium phosphate, pH 7.4, with 1% polyvinyl pyrrolidone and 1% nicotine. For inoculations to determine the host range, the effect of inhibitors in the sap of *Chenopodium* was reduced by clarifying the extract from systemically infected *C. quinoa* leaves with calcium phosphate (11). DLV was concentrated twofold by centrifugation at 26,000 rpm for 90 min (Beckman No. 30 rotor) through 20% (w/v) sucrose, and the pellet was resuspended in inoculation buffer. Plants were lightly dusted with carborundum, and the inoculum was applied with a foam pad. The following families were represented in the host-range study: Amaranthaceae, Apocynaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Gramineae, Leguminosae, Plantaginaceae, Rosaceae, and Solanaceae. A negative check was included for each test by similarly rubbing a plant with inoculation buffer only. Immediately after inoculation, the plants were rinsed with tap water. The test plants were assayed 2 wk later by mechanically inoculating *C. quinoa* plants with sap prepared from the test plants.

Stability in vitro. Systemically infected *C. quinoa* leaves were ground (1:2, w/v) with inoculation buffer and centrifuged at 5,000 rpm for 5 min (Sorvall SS-34 rotor). The supernatant was dispensed in 1-ml aliquots and stored at 4 or 23 C. At intervals, infectivity was tested by inoculating *C. quinoa*, and symptoms were recorded 3 wk after inoculation. Lyophilized tissue was stored at room temperature and infectivity was checked every 4 mo by inoculating *C. quinoa*.

To determine the dilution end point, systemically infected *C. quinoa* leaves were ground (1:10, w/v) with inoculation buffer, and the resulting sap was diluted in 10-fold increments to 10^{-10} . Three *C. quinoa* plants were inoculated with 1 ml of each dilution, and the symptoms were recorded 14 and 21 days after inoculation.

The thermal inactivation point was determined with sap from systemically infected leaves of *C. quinoa* ground (1:2, w/v) with inoculation buffer. The sap was incubated in a water bath for 10 min after it had reached the treatment temperature, and aliquots were used to inoculate plants of *C. quinoa*. Symptoms were recorded 14 and 21 days after inoculation.

Seed transmission. *C. quinoa* seeds were collected from infected plants, germinated on moist filter paper, and separated into two lots for testing. Seedlings and seed coats were separated and ground

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in inoculation buffer for indexing on healthy *C. quinoa*, or seedlings were transplanted to soil and indexed at the six-leaf stage by ELISA and by inoculation of *C. quinoa* with the sap. Seeds from infected dandelions were allowed to age before they were tested. Of 64 seedlings tested by ELISA, 13 were indexed on *C. quinoa*.

Aphid transmission. A colony of *Myzus persicae* (Sulz.) was established on healthy *C. quinoa* and maintained at 10–15 C with a day length of 16 hr. Aphids were collected and starved for 1 hr. After exposure to infected *C. quinoa* leaves, the aphids were transferred to healthy *C. quinoa* plants for 18 hr. Symptoms were recorded 10 days after inoculation.

Virus purification. Systemically infected *C. quinoa* plants were homogenized in a Waring Blendor with 2 volumes (w/v) of cold 0.2 M sodium borate buffer (pH 9.0) containing 1% mercaptoethanol and 0.2% sodium diethyldithiocarbamate. The homogenate was expressed through nylon cloth, and the sap was centrifuged at 10,000 rpm for 20 min (Sorvall GSA rotor). The supernatant was filtered through a layer of Mira cloth, left at 4 C overnight, and then centrifuged at 10,000 rpm for 20 min (Sorvall GSA rotor). The resulting supernatant was centrifuged at 26,000 rpm for 90 min (Beckman No. 30 rotor). The pellets consisted of a bottom layer containing the virus and a top layer of green debris. The upper green layer was allowed to slide off the virus pellet by inverting the centrifuge tubes for about 10 min. Distilled water was used to rinse the remaining green debris from the virus pellet. The virus was resuspended in a small amount of 0.02 M borate buffer, pH 8.5, for 1–2 hr at 4 C. The suspension was centrifuged at 5,000 rpm for 5 min (Beckman No. 40 rotor) and the supernatant applied to sucrose density gradients. Continuous density gradients were made in polyallomer tubes with 10–40% (w/v) sucrose in 0.02 M borate buffer, pH 8.5. The virus was applied to the gradients and centrifuged at 38,000 rpm for 90 min (Beckman SW 41 rotor), and the absorbance profiles at 254 nm were recorded with an ISCO density gradient monitor.

All centrifugations were carried out at 4 C, whereas all other manipulations were at room temperature, unless otherwise noted. Yields were calculated based on A_{260} values and an assumed extinction coefficient of 3.0 (7).

Equilibrium density gradient centrifugation was by the method of Purcifull and Hiebert (9). The high-speed pellets obtained by the standard procedure for virus purification were resuspended in 5 ml of 0.02 M potassium phosphate buffer, pH 8.5, and layered onto 7 ml of cesium chloride (1.2858 g/cc) in 0.02 M potassium phosphate buffer, pH 8.5. The gradients were centrifuged at 32,000 rpm for 18 hr (Beckman SW 41 rotor) and the virus band was collected, either by puncturing the cellulose nitrate tube and collecting the opalescent zone by drops, or by displacing the column through an ISCO density gradient monitor.

Polyacrylamide gel electrophoresis of viral RNA. The dissociation buffer used for the preparation of RNA from purified

virus was adapted from Dodds et al (2): 0.08 M tris-HCl buffer, pH 9.0, 0.004 M Na_2EDTA , 4.0 mg/ml bentonite, 4 M urea, 4% SDS, 16% sucrose, and 0.2% mercaptoethanol. Purified virus in dissociation buffer (approximate concentration of virus 0.3 mg/ml) was heated in a water bath at 50 C for 15 min. Samples of 20, 40, and 80 μl were applied to 2.5% polyacrylamide gels (2) which had been pre-electrophoresed at 8 mA per tube for 30 min. The RNA preparation was electrophoresed for 2.5 hr at 8 mA per tube. Gels were stained in 0.01% toluidine blue and destained in distilled water. Standards and their molecular weights were potato virus S (2.4×10^6) and tobacco mosaic virus, cowpea strain (2.0×10^6 and 0.3×10^6).

Particle size. Leaf dips were prepared from systemically infected *C. quinoa* leaves in 2% phosphotungstic acid, pH 6.7. The grids were scanned with a Philips EM 300 and photographs taken as required.

Infectivity of gradient fractions. A partially purified DLV preparation was layered onto continuous sucrose density gradients formed with 10–40% (w/v) sucrose in 0.0165 M disodium phosphate and 0.0018 M trisodium citrate buffer, pH 9.0. After centrifugation at 38,000 rpm for 90 min (Beckman SW 41 rotor), the gradients were fractionated with an ISCO density gradient monitor and fraction collector. Selected fractions were checked for particles by electron microscopy, and all fractions were inoculated onto plants of *C. quinoa*. Symptoms were recorded at 12, 14, and 18 days after inoculation.

Ultraviolet light absorption. The ultraviolet light absorption of purified virus preparations was determined over the range from 230–340 nm at 5-nm intervals with a Beckman DU spectrophotometer. The values were plotted and corrected for light scattering (8).

Serology. A young, white New Zealand rabbit was immunized with four intramuscular injections of purified DLV (1 mg/ml) emulsified 1:1 with Freund's complete adjuvant. Injections were given at 0, 2, 10, and 14 wk. After the second injection, blood was collected at weekly intervals, and the titer of the antiserum was determined by tube precipitin test. The relative amount of precipitate formed for each reaction was evaluated as very dense to no visible precipitate. Similar schedules were followed for production of antisera to PeVS and PVS. Antisera to PVS and other carlaviruses were obtained from Agriculture Canada, Research Station, Vancouver, British Columbia. Purified DLV was tested against antisera to other carlaviruses for which homologous antigens were not available: carnation latent virus (CLV), chrysanthemum virus B (CVB), narcissus latent virus (NLV), pea streak virus (PSV), poplar mosaic virus (PopMV), potato virus M (PVM), and red clover vein mosaic virus (RCVMV). Monologous and heterologous tests were conducted with dilutions of DLV, PeVS, and PVS in combination with dilutions of their antisera. All antigens were tested at 80 and 20

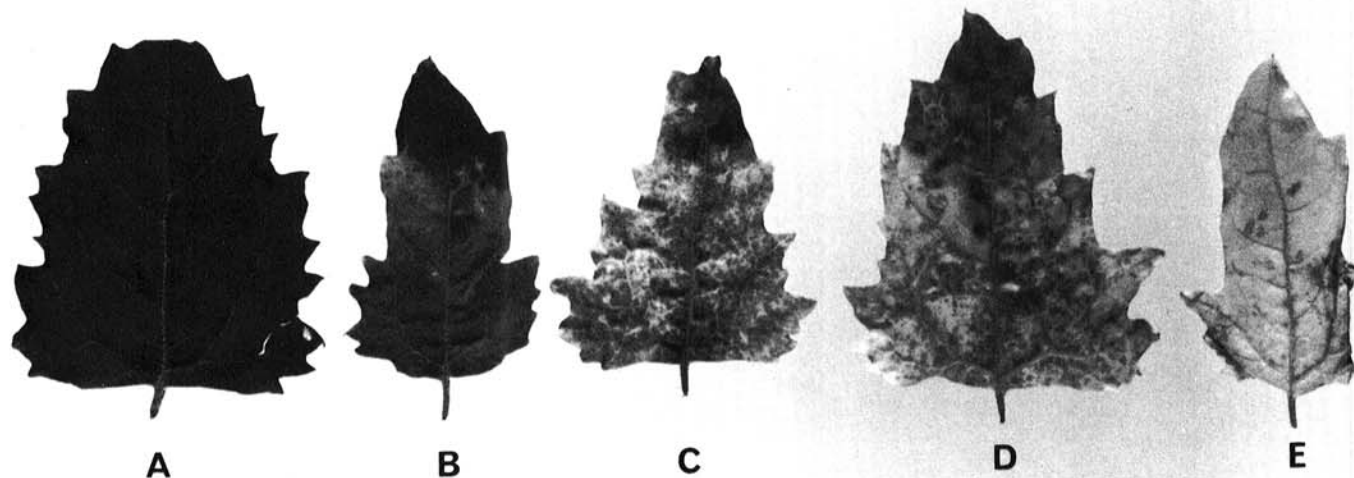


Fig. 1. Symptoms of systemic dandelion latent virus infection in *Chenopodium quinoa*. A, healthy leaf and B–E, progression of symptom development in four top leaves of an inoculated plant.

$\mu\text{g/ml}$ against the appropriate antisera dilutions and the end points determined. All antisera were stored at 4 C with chlorbutol as preservative.

The experimental procedures for the ELISA were those of Clark and Adams (1) with the following modifications: the immunoglobulin G (IgG) was concentrated from crude antiserum by two cycles of ammonium sulfate precipitation, and the first major peak eluted through the DEAE-cellulose column was collected. The conjugate was stored at 4 C without the addition of bovine serum albumin, and the coated microtiter plates (Cooke-Dynatech, Alexandria, VA 22314) were stored at 4 C overnight or longer prior to use. For routine testing, optimal dilutions of coating IgG and conjugated IgG were found to be 1:2,000 and 1:8,000, respectively. A moist environment was maintained throughout the assay by enclosing the plate in a small plastic bag. Test samples were incubated overnight at 4 C rather than at 37 C for 4–6 hr. With this system, all 96 wells on the plate could be used without any edge effect. The color reaction was assessed spectrophotometrically at 405 nm or visually as positive or negative.

RESULTS

Field occurrence. Dandelions were indexed from 44 collection sites throughout British Columbia, and only those from the Okanagan Valley were found to be infected. The age and condition of the plant material used for ELISA were critical. Old leaves or slightly decaying leaves were not suitable because they produced high levels of background color. Samples that produced anomalous ELISA results were indexed on *C. quinoa*. Of 518 plants indexed, 71 were infected with DLV. In affected areas of a field, many plants were infected, but in between, there were large areas where no infection could be detected. Dandelions from noncultivated areas were not infected while those from cultivated areas were more likely to be infected.

Host range and symptomatology. Infected dandelions in the field exhibited no visible symptoms. Similarly, dandelion seedlings rub-inoculated with DLV showed no symptoms, but the virus could be recovered by indexing to *C. quinoa*. *Chenopodium quinoa* and *C. amaranticolor* developed distinct chlorotic local lesions 5–7 days after inoculation and progressed from mild vein clearing to complete chlorosis and epinasty (Fig. 1). Axial shoots also exhibited chlorosis. Plant collapse often prevented flowering and seed set. Local lesions developed on *Gomphrena globosa* 12 days after inoculation and no systemic symptoms developed.

Virus infection, or lack thereof, was assayed in both inoculated and uninoculated leaves by back inoculation of *C. quinoa* for all plants in the host range survey. DLV did not infect *Brassica juncea* (L.) Coss; *B. pekinensis* (Lour.) Rupr. 'Petai'; *Capsicum frutescens* L. var. *grossum*; *Cucumis sativus* L.; *Datura stramonium* L.; *Dianthus barbatus* L.; *Fragaria vesca* L. 'Alpine'; *Helianthus annuus* L.; *Hordeum vulgare* L.; *Lactuca sativa* L. var. *capitatum*; *Lycopersicon esculentum* Mill. 'Rutgers' and 'Subarctic'; *Nicotiana clevelandii* Gray; *N. debneyi* Domin.; *N. glutinosa* L.; *N. rustica* L.; *N. tabacum* L. 'Haranova,' 'Havana,' 'Samsun,' 'Silvestris,' 'White Burley,' and 'Xanthi'; *Petunia hybrida* Vilm.; *Phaseolus vulgaris* L. 'Bountiful' and 'Pinto'; *Pisum sativum* L. 'Perfection'; *Plantago lanceolata* L.; *P. major* L.; *Solanum tuberosum* L. 'Milton Pearl' and 'Red Pontiac'; *Verbena encelioides* (Car.) Benth. & Hook.; *Vicia faba* L.; *Vigna sinensis* Erdl. 'Black Eye'; *Vinca rosea* L.; or *Zinnia elegans* Jacq.

Stability in vitro. Infectivity was retained in crude *C. quinoa* sap between 4 and 5 days at 23 C, between 28 and 56 days at 4 C, and more than 2 yr in a lyophilized state at 23 C. The dilution end point was between 10^{-5} and 10^{-6} . The temperature range of inactivation for DLV in sap of *C. quinoa* was 75–80 C.

Seed transmission. The germination rate of seeds from healthy and infected *C. quinoa* on moist filter paper was 95–100%. Inocula prepared from seed coats and seedlings produced no symptoms of infection on *C. quinoa*. These seedlings matured with no symptoms and no latent infection was detected by ELISA or back inoculation of *C. quinoa*. The germination rate of seeds from healthy and

infected dandelions on moist filter paper and on soil was 64%. No latent infection was detected by ELISA or back inoculation of *C. quinoa*.

Aphid transmission. DLV was transmitted from inoculated *C. quinoa* to healthy *C. quinoa* by *M. persicae* after access periods ≥ 5 min. No transmission was obtained when aphids were allowed to feed on healthy *C. quinoa*.

Virus purification. Various methods of clarifying plant extracts and concentrating virus were undertaken, and a purification schedule was developed that was efficient for purifying DLV, PeVS, and HVS from *C. quinoa*. Virus yields obtained by this method were 20–30 mg/kg of plant material. The effectiveness of each method was determined by a comparison of the gradient absorbance profiles and virus yield (Fig. 2).

Polyacrylamide gel electrophoresis of viral RNA. In polyacrylamide gels, the RNA of DLV co-migrated with PVS-RNA. The mol wt of the single nucleic acid component was estimated as 2.5×10^6 from six determinations.

Virus particle size. The average length of 800 particles from leaf dips was 640 nm (Fig. 3). Purified preparations contained varying amounts of fractured particles, and measurements were confined to those particles that appeared to be intact. The average length of 264 particles was 637 nm, which is in agreement with the measurement of leaf dip preparations. The width of the particles in leaf dips was 12–13 nm.

Infectivity of gradient fractions. No virus particles were detected

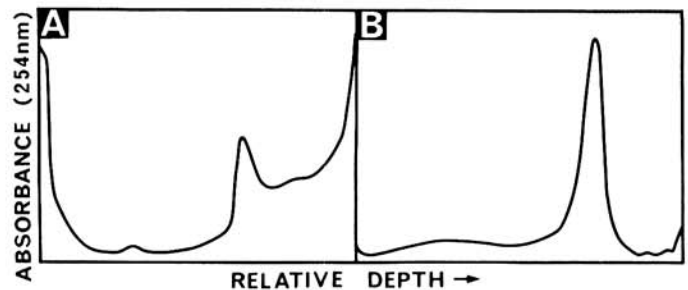


Fig. 2. Density gradient absorbance profiles comparing dandelion latent virus peak (indicated by an arrow) obtained with A, sucrose and B, cesium chloride density gradients after centrifugation at 38,000 rpm for 90 min and 32,000 rpm for 18 hr (Beckman SW 41 rotor), respectively.

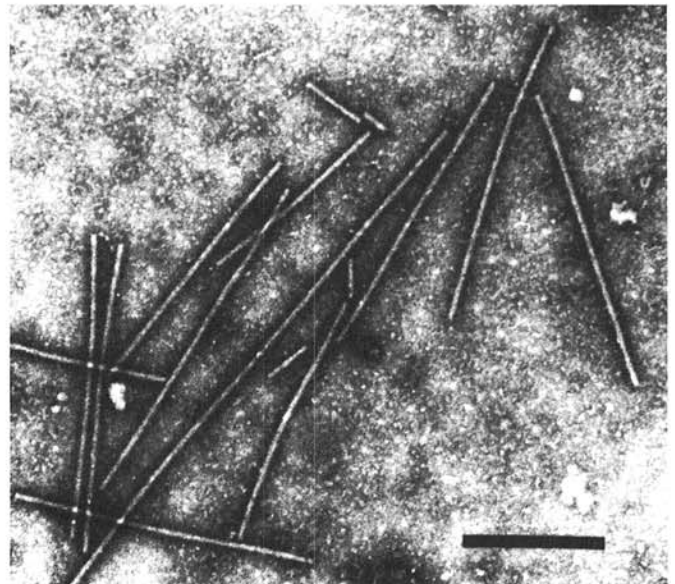


Fig. 3. Flexuous, rod-shaped particles of dandelion latent virus from a leaf dip preparation negatively stained with 2% phosphotungstic acid. Bar = 300 nm.

TABLE 1. Precipitin end points in two-way tube precipitin tests of four carlaviruses and their respective antisera

Antisera	Antigen (20 µg/ml)			
	DLV	PVS	PeVS	HVS
Dandelion latent virus (DLV)	20,480	5,120	2,560	80
Potato virus S (PVS)	1,280	10,240	640	80
Peru virus S (PeVS)	320	5,120	2,560	80
Helenium virus S (HVS)	40	40	20	1,280

in the top quarter of the gradients. The remainder of the gradient contained virus particles and was associated with infectivity. Fractions that contained broken particles were infectious, but symptoms in *C. quinoa* developed slowly, indicating a low inoculum level. Fractions that contained discrete or aggregated complete virions were infectious, and symptoms developed in *C. quinoa* as expected.

Ultraviolet light absorption. The uncorrected and corrected absorption profiles for DLV purified on sucrose density gradients showed a pattern typical for a nucleoprotein, with maximum absorption at 259 nm and minimum at 245 nm. The absorption characteristics were slightly higher when calculated from corrected values than from uncorrected values: A_{\max}/A_{\min} : 1.13 ± 0.04 (corrected) and 1.09 ± 0.04 (uncorrected), and A_{260}/A_{280} : 1.59 ± 0.11 (corrected) and 1.41 ± 0.09 (uncorrected).

Serology. The maximum homologous titer of DLV antiserum determined by tube precipitin tests was 40,960 compared with a titer of 8 for healthy sap. The homologous titer of PeVS antiserum was 10,280 with a titer of 1 for healthy sap. The homologous titer of HVS antiserum from the initial bleeding was 1,280. The end points for monologous and heterologous reactions involving DLV, PVS, PeVS, and HVS antigens and antisera are summarized in Table 1. The optimum concentration of antigen to obtain maximum titers using homologous antiserum was 20 µg/ml. If an excess of antigen was used, the end point was reduced twofold to fourfold and the true titer was not achieved. Heterologous reactions were run using 20 µg of antigen per milliliter because a higher concentration of antigen (80 µg/ml) obscured the degree of relationship. DLV shares more antigenic sites with PVS than with PeVS, and HVS is distantly related to each of the other three viruses tested. Antisera to several other carlaviruses were obtained, and one-way tests were carried out with DLV antigen and the test antisera. Antisera to CVB (1:20) and NLV (1:20) produced a visible precipitate. No reactions were observed with antisera to CLV, PSV, PopMV, PVM, or RCMV.

DISCUSSION

To ensure that the description of a new virus is not that of a known virus under a new name, and that adequate information is available to correctly classify a new virus, a set of guidelines has been developed to aid in the identification and characterization of plant viruses (4). Following these guidelines, DLV virus is considered a typical carlavirus. It has a narrow host range and is latent in its natural host like other carlaviruses. Its particle size and morphology, biochemical properties, and serological relationships also support this classification.

Initially, field samples of dandelions were indexed by inoculating *C. quinoa* plants, but once an antiserum was available, conditions for ELISA were determined with purified DLV preparations.

Samples of young, infected dandelion leaves produced a strong color reaction in ELISA and symptoms of DLV infection in *C. quinoa*. Samples of old, decaying dandelion leaves which produced weak color reactions in ELISA produced no symptoms in *C. quinoa*. Alkaline phosphatase present in the decaying leaves as a result of microbial activity was responsible for the weak background color. All further indexing by ELISA was with young dandelion tissue in order to avoid this background reaction.

The occurrence of DLV in the field was not random. It occurred within a limited area of cultivated orchards and could not be recovered from dandelions in other cultivated areas or from non-cultivated areas. This suggests that the virus may have been introduced as a result of agricultural activity.

The stability of a virus is usually determined by testing its longevity in vitro and its thermal inactivation point. Although DLV had a greater stability than most carlaviruses, the values were close to those expected. The dilution end point was also within range of that expected for a carlavirus. The specific identity of a virus cannot be obtained solely on the basis of these tests because of the inherent variability, but some indication of how well the virus fits into the group can be obtained (3).

Based on differences in host range, symptomatology, and serological reactions, the dandelion virus is distinct from PVS, PeVS, and HVS. Whether the dandelion virus should be classified as a new virus or as a strain of PVS or PeVS is largely a subjective decision. However, the serological data provide sufficient justification to consider the dandelion virus a distinct entity and a new member of the carlavirus group. In considering a suitable designation for the dandelion virus, I am proposing that it be called dandelion latent virus (DLV) in order to designate the natural host of the virus and to give reference to the carlavirus group association.

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