A Carlavirus from *Kalanchoë blossfeldiana*

S. S. Hearon

Research plant pathologist, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.
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ABSTRACT

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A virus with slightly flexuous, filamentous particles was isolated from a symptomless *Kalanchoë blossfeldiana* 'Rotkappchen' plant. The virus, designated kalanchoë virus-I (KV-I), had a thermal inactivation point between 65 and 70 C, was inactivated by aging in vitro at ambient room temperature for 3–5 days, and had particles with a normal length of 600–620 nm in PTA. The experimental host range was limited to kalanchoë, several *Chenopodium* spp. and *Tetragonia expansa*. *Chenopodium quinoa*, grown at 20–28 C under 19,368–21,520 lux (1,800–2,000 ft-c) of supplemental fluorescent light for 16 hr/day during periods of low natural light intensities, was used for virus bioassay and propagation. The virus was purified by trituration of leaves in 0.1 M K_2HPO_4, containing 0.1% 2-mercaptoethanol, 5 mM EDTA, and 1% Triton X-100; clarification of the sap with chloroform and carbon tetrachloride; and precipitation of the virus with 4% polyethylene glycol-6000. Further purification was attempted by passage of partially purified virus preparations through a column of controlled-pore glass beads or density gradient centrifugation. The buoyant density of KV-I in cesium chloride was 1.311 g/cm^3, the sedimentation coefficient was ~170 S, and the apparent molecular weight of the coat protein was 34,000–36,000 daltons. KV-I is distantly related to carnation latent, chrysanthemum B, lily symptomless, and potato S viruses, and is unrelated to elderberry A, hippeastrum latent, narcissus latent, potato M, or red clover vein mosaic viruses in microprecipitin tests and serologically specific electron microscopy. The properties of KV-I are typical of a carlavirus, but distinct from previously characterized members of this virus group.

Additional key words: cytopathology, fusiform virus inclusions.

Kalanchoë blossfeldiana Poelln. is a short day succulent (Crassulaceae) that is vegetatively propagated and sold as a flowering pot plant. Growers have become aware of virus-type symptoms in most commercial cultivars of kalanchoë now in production. An unidentified, graft-transmissible agent associated with virus-type symptoms in *K. flammea* in Florida has been reported (9). Kalanchoë is a host for potato yellow dwarf virus (11). No other information is presently available on viruses infecting this plant.

Three viruslike agents (singly or in various combinations) have been detected in this laboratory in ultrastructural and transmission studies of naturally infected kalanchoës (13). This paper describes the isolation and partial characterization of a carlavirus, referred to as kalanchoë virus-I (KV-I), from *K. blossfeldiana* 'Rotkappchen.'

MATERIALS AND METHODS

Virus isolation. The virus was isolated from *K. blossfeldiana* 'Rotkappchen' by mechanical inoculation to *Chenopodium quinoa* Willd. with crude sap from leaves triturated in neutral 0.05 M phosphate buffer. *C. quinoa*, grown at 20–28 C under supplemental fluorescent light 19,368–21,520 lux (1,800–2,000 ft-c) on a 16-hr photoperiod, was used for bioassays and virus propagation throughout. Phosphate-buffered crude sap of several other cultivars of kalanchoës were subsequently indexed on *C. quinoa* and *Tetragonia expansa* Murr. to determine the prevalence of KV-I. All kalanchoës were selected from commercial sources.

Host range and physical properties of the virus. Inoculated leaves of *C. quinoa* with more than 25 lesions per leaf were ground in 0.05 or 0.1 M neutral phosphate buffer and mechanically inoculated to Carborundum-dusted leaves of test seedlings maintained in the greenhouse under natural daylight and 16 hr of supplemental fluorescent light. No tests were conducted in July and August when greenhouse temperatures commonly exceeded 30 C. Negatively stained leaf-dip preparations and/or back inoculation to *C. quinoa* were made to detect symptomless local or systemic infections of inoculated seedlings and kalanchoë cultivars.

Thermal inactivation point and aging in vitro were determined in phosphate-buffered crude sap extracts of inoculated *C. quinoa* by using *C. quinoa* as the assay host. Particle length was determined in leaf-dip preparations of *C. quinoa* stained with 2% neutral phosphotungstate (KPTA). Magnification was determined by using a diffraction grating replica (54,864 lines per centimeter).

Purification. Inoculated leaves of *C. quinoa* were collected when the local lesions were chlorotic (ie, prior to necrosis). This necessitated harvesting and chilling the older leaves 1–3 days before the younger ones on which lesions developed more slowly. The period between inoculation and harvest varied from 10–16 days, depending on the ambient light and temperature conditions.

Chilled leaves were homogenized in cold 0.1 M K_2HPO_4 buffer (2 ml per gram of tissue), pH 9.4, containing 0.1% 2-mercaptoethanol, 1% Triton X-100 and 5 mM ethylenediamine tetraacetic acid (EDTA). Chloroform and carbon tetrachloride (one part each solvent and four parts buffer) were blended with the homogenate. The emulsion was centrifuged at 4,800 *g* for 15 min and the aqueous phase was filtered through glass wool. The pulp layer was reprocessed as above in half the volumes of extraction medium and organic solvents.

The clarified preparation was divided into three aliquots, and either 2, 4, or 7% polyethylene glycol-600 (PEG) was added to each, respectively. The precipitates were collected by centrifugation at 11,000 *g* for 15 min in a Sorvall GSA rotor. Pellets were resuspended over a period of 2–16 hr in extraction buffer without mercaptoethanol, then the suspensions were centrifuged in a Sorvall SS-34 rotor at 3,300 *g* for 10 min. The PEG precipitation procedure was repeated twice omitting Triton X-100 in the second resuspension medium and using 0.05 M tris-HCl or K_2HPO_4-NaH_2PO_4, pH 7.5, as the final resuspending medium (one-fortieth of the original buffer volume). Preparations were assessed for virus infectivity and purity using bioassay, $A_{260/280}$, density gradient

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centrifugation in 30% cesium chloride (CsCl), electron microscopic examination of negatively stained preparations, and/or elution profiles from a column of controlled pore glass beads (CPG) (1).

Sucrose density gradient centrifugation. Virus was fixed by dialyzing partially purified suspensions overnight against buffered 0.1% glutaraldehyde and then against buffer only. Fixed or unfixed virus was then layered on 10–40% linear sucrose density gradients in 0.01 M tris-HCl buffer, pH 7.5, and centrifuged at 95,000 *g* for 3 hr at 5 C. Gradients were scanned at 254 nm and fractionated by using an ISCO Model UA-5 analyzer and Model 640 fractionator. Fractions were assayed for infectivity in *C. quinoa* and/or examined for the presence of particles by electron microscopy.

Permeation chromatography on a controlled-pore glass bead column. Partially purified virus (0.5–2.0 ml) was layered on the top of an M 15/90 Pharmacia glass column loaded with CPG of 70 nm pore size (±10%) and (120–200 mesh) (Sigma CPG-700) prepared according to Barton (1). The eluant was collected in 10–12 ml fractions and monitored at 254 nm. Samples were assayed in *C. quinoa*, then fractions were centrifuged at 27,000 rpm in a Type 30 Beckman rotor for 1.5–2.0 hr. Resuspended pellets were examined by electron microscopy and virus concentration was determined spectrophotometrically by using an extinction coefficient ($E_{1cm,\ 260nm}^{0.1\%}$) equal to 3.

Biophysical and biochemical properties. Preparations from 4 and 7% PEG treatments were diluted to approximately 1 mg/ml in tris-HCl buffer, pH 7.5. Then 0.05 ml was mixed with 5.0 ml of CsCl, initial density of 1.2858 g/cm^3 in 0.01 M tris-HCl buffer, pH 7.5 and centrifuged at 150,000 *g* for 17–20 hr at 20–25 C. Purified tobacco mosaic virus (TMV) from R. Steere was centrifuged alone or mixed with the 4% PEG preparation of KV-I in companion gradients. Gradients were scanned at 254 nm. Refractive indices of 0.1- or 0.2-ml fractions were determined with a Bausch and Lomb Abbé refractometer and CsCl densities were calculated using conversion tables (12). Diluted fractions were examined by electron microscopy and each assayed for infectivity.

SDS-PAGE of capsid protein was carried out on 6% slab gels (10 cm × 14 cm × 3.0 mm) using 0.1 M phosphate buffer, pH 7.5 (22). Standards for molecular weight determination were from a Pharmacia electrophoresis calibration kit (Lot #9P018T). The suspension of purified virus (~1 mg/ml) was mixed 1:1 (v/v) with an aqueous solution containing 2% 2-mercaptoethanol, 8 M urea, and 2% sodium dodecylsulfate (SDS), then heated for 2–5 min in boiling water. Samples (10–50 µl) were electrophoresed at 140 ma constant current and 45–50 V for 4–5 hr at ambient room temperature (20–30 C). Gels were stained with Coomassie Brilliant Blue R-250.

Serology. Antisera were prepared by J. Tew, Richmond School of Medicine, against purified virus fixed with 0.3% formalin. Virus (~0.75 mg) was emulsified with an equal volume of Freund's incomplete adjuvant and injected into the hind foot pads and behind the front foot pads of each of two rabbits. The rabbits were reinjected similarly at the same sites 4 wk later, then bled 2 wk after the last injection.

Purified KV-I and clarified crude sap from healthy *C. quinoa* were reacted with antisera to carnation latent (CLV) chrysanthemum B (CBV), lily symptomless (LSV), elderberry A (EVA), red clover vein mosaic (RCVMV), hippeastrum latent (HiLV), and narcissus latent (NLV) viruses, (all contributed by A. A. Brunt), and potato virus S (PVS) (American Type Culture Collection PVAS-103) in microprecipitin tests. Leaf-dip immunoelectron microscopy was also used to detect particle decoration with KV-I, PVS, and potato virus M (PVM) (16). Local lesions on *C. quinoa* inoculated with KV-I, PVS, or PVM were dipped in 7 µl of distilled water on carbon-stabilized formvar coated grids. Seven microliters of test antisera diluted in 0.05 M tris-HCl buffer, pH 9, containing saline was added to the droplet, incubated for 30–40 min at 32–36 C, and stained with KPTA.

Cytopathology. Leaf pieces from naturally infected Rotkappchen and local lesions from mechanically inoculated *C. quinoa* and *T. expansa* were fixed with a 2% glutaraldehyde-1.5% acrolein mixture, postfixed with 1% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in Epon 812 plastic according to a technique in which propylene oxide is used to facilitate infiltration. Ultrathin sections were stained with uranyl acetate and lead citrate.

RESULTS

Host range. KV-I induced pinpoint necrotic or chlorotic local lesions that become necrotic on old, then young, inoculated leaves of *C. quinoa* (Fig. 1a), *C. amaranticolor* Coste & Reyn., *C. album* L., and *C. murale* L. without systemic infection. Large chlorotic local lesions, 4–6 mm in diameter, also formed on *T. expansa* (Fig. 1b) 2–3 wk after inoculation. Reinoculation of KV-I to *K. blossfeldiana* 'Conquistador,' 'Mace,' 'Montezuma,' and 'Princess' resulted in symptomless systemic infections of the plants 6 wk or more after inoculation. No virus was detected by electron microscopic examination of leaf-dip preparations of the test plants prior to inoculation. Tip cuttings taken immediately before inoculation of the test plants were rooted to serve as controls. A virus with particles of similar size and morphology and that produced local lesions on *C. quinoa* and *T. expansa* was isolated from naturally infected cultivars of kalanchoës including 'Korall,' 'Feurzauber,' and 'Texas Sunset.'

Plants not infected by KV-I as determined by electron microscopic examination of negatively stained leaf-dip preparations and/or back inoculations to *C. quinoa* were: *Beta vulgaris* L. 'Detroit Ruby Red'; *Calendula officinalis* L.; *Capsicum*

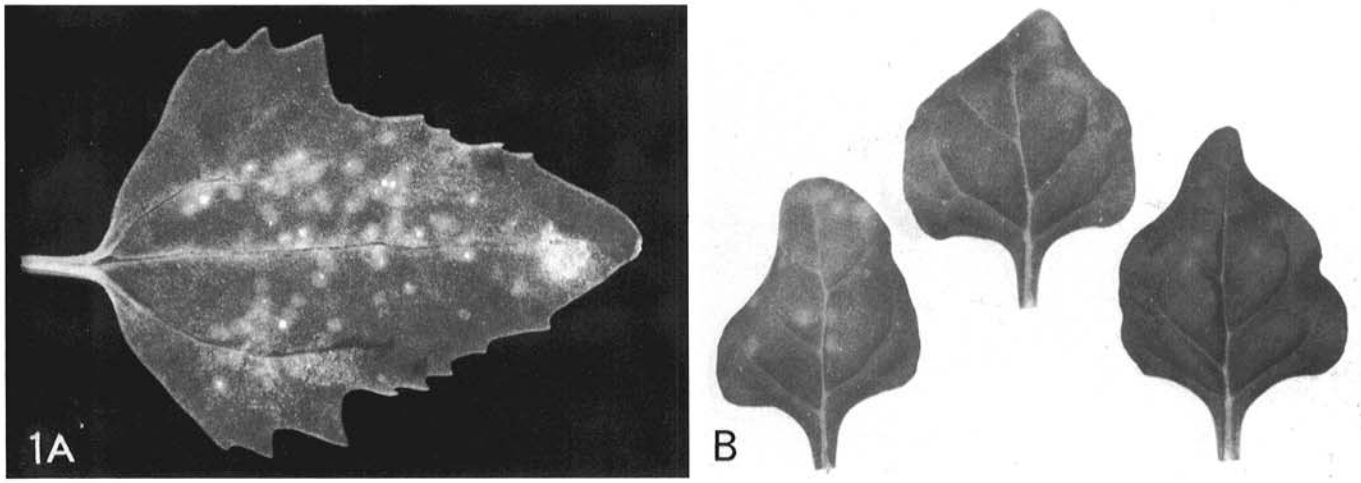


Fig. 1. Chlorotic local lesions on leaves of **A,** *Chenopodium quinoa* at 11 days and **B,** *Tetragonia expansa* at 3 wk (left and right) after inoculation with kalanchoë virus-I and an uninoculated leaf of *T. expansa* (center).

annuum L. '*Yolo Wonder*'; *C. frutescens* L.; *Cassia occidentalis* L.; *Cucumis sativum* L. 'Caserta Bush,' 'Lemon,' and 'National Pickling'; *Cucurbita pepo* L. 'Buttercup'; *Dahlia variabilis* (Willd.) Desf.; *Datura stramonium* L.; *D. metel* L.; *Fagopyrum esculentum* Moench; *Glycine max* (L.) Merr. 'Lee'; *Gomphrena globosa* L.; *Lilium longiflorum* Thunb.; *Lycopersicon esculentum* Mill. 'Rutgers'; *Mirabilis jalapa* L.; *Nicotiana benthamiana* Domin.; *N. clevelandii* Gray; *N. debneyi* Domin.; *N. glutinosa* L.; *N. clevelandii* × *N. glutinosa* ('Christie's Hybrid'); *N. megalosiphon* Heurck & Muell.; *N. rustica* L.; *N. tabacum* L. 'Samsun,' 'Samsun NN,' and 'Xanthi'; *Petunia hybrida* Vilm. 'Pink Cascade'; *Phaseolus vulgaris* L. 'Black Turtle Soup,' 'Pinto,' and 'Bountiful'; *Pisum sativum* L. 'Alaska'; *Scabiosa atropurpurea* L.; *Solanum rostatum* Dunal; *Spinacia oleracea* L. 'Spinach 424'; *Tropaeolum majus* L.; *Vicia faba* L. 'Major,' 'Minor,' and 'Long Pod'; *Vigna unguiculata* (L.) Walp.; *Vinca rosea* L.; *Viola tricolor* L.; and *Zinnia elegans* Jacq. 'Golden Orange.'

Physical properties and normal particle length. The virus was inactivated by heating for 10 min at 70, but not 65 C. Phosphate buffered crude sap retained infectivity at room temperatures for 3–5 days. The normal length of more than 200 measured particles in newly formed local lesions was 620 nm with 70% of the particles 580–640 nm long. As the lesions aged the range of particle sizes increased and particles 1,200–1,800 nm in length and particles shorter than 580 nm became more common.

Virus purification. Attempts to purify KV-I at neutral pH resulted in the formation of aggregates of virus and plant debris that were pelleted by low-speed centrifugation. Aggregation and adsorption of phytoferritin to the virions was overcome by adding EDTA and Triton X-100 to 0.1 M dibasic sodium or potassium phosphate. Reextraction of the leaf pulp increased virus yields 50%. Yields of ~10 mg of virus per 100 g of tissue were achieved by using PEG-6000 precipitation and avoiding high-speed centrifugation.

Comparisons of preparations from 4 and 7% PEG precipitation showed 4% PEG precipitated comparable amounts of virus with less coprecipitation of plant material than did 7% PEG (see the sections titled permeation chromatography and biophysical and biochemical properties that follow). Not all of the virus was precipitated by 2% PEG.

Sucrose density gradient sedimentation. Sucrose density gradient sedimentation of fixed or unfixed virus resulted in the formation of multiple virus-containing zones in the gradients (Fig. 2) and large pellets. Electron microscopy showed zones to contain (from top to bottom of the gradient) debris with virion fragments; virion fragments; monomers, dimers, and polymers; and large aggregates of virions in plant contaminates. Only 10–20% of the virus (as estimated by A_{260} of reconcentrated fractions) occurred in the gradient zone composed primarily of monomers. The sedimentation coefficient of KV-I was estimated (4) as 170 S, based on a sedimentation coefficient of 194 S (27) for TMV in companion gradients.

Permeation chromatography on a controlled-pore glass bead column. When preparations containing 2–4 mg of virus per milliliter were chromatographed on the CPG column, the virus eluted in 0–24 ml after the void volume (63 ml) with a sharp front boundary and a slightly elongated trailing edge (Fig. 3, bottom). Normal plant contaminants eluted 20–60 ml after the void volume. The trailing side of the virus peak and the leading edge of the plant debris peak overlapped in heavily contaminated preparations (Fig. 3, top). The resulting reconcentrated 0–24 ml virus fraction usually had an $A_{260/280}$ ratio of 1.33–1.38 (uncorrected for light scattering) with maximum absorption at 258 nm and a minimum absorption at 244 nm.

In the elutions of some preparations, a shoulder occurred on the trailing edge of the virus peak or the virus elution was delayed. These fractions contained large amounts of virus and plant debris. Retention of debris in these fractions may result from the passage of large, stable debris-containing aggregates around the CPG.

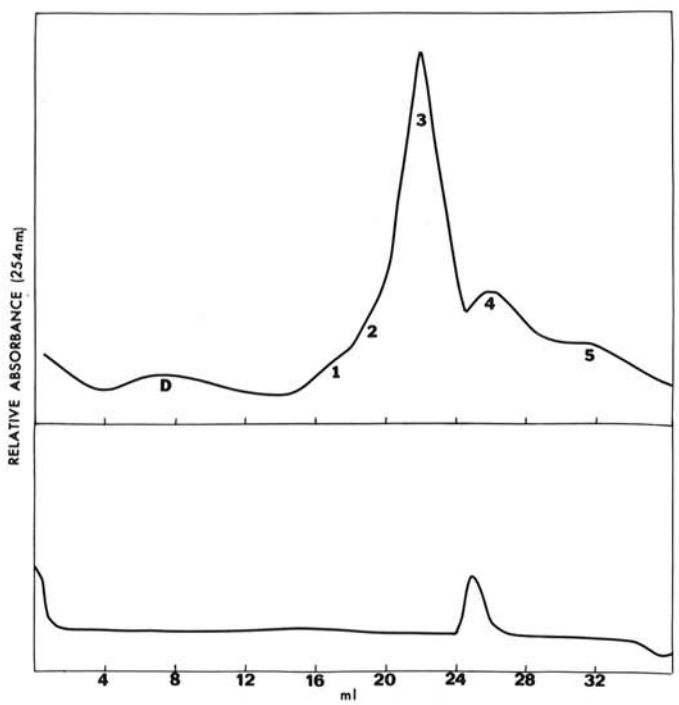


Fig. 2. Spectrophotometric profiles of 10–40% linear sucrose density gradients in 0.01 M tris-HCl, pH 7.5, buffer that were layered with 1.5 ml of glutaraldehyde-fixed kalanchoë virus-I (KV-I) (top) or tobacco mosaic virus (bottom) and centrifuged in a Beckman SW 27 rotor at 27,000 rpm for 3 hr at 4 C. The tube receiving KV-I contained a diffuse light-scattering band at 46–47 mm below the meniscus; the tube receiving TMV contained a light-scattering band (UV absorbing band 24–25 ml) 52 mm below the meniscus. Negatively stained preparations of the KV-I gradient showed that D fractions contained predominantly small contaminant and virion fragments, fractions 1 and 2 contained virus fragments larger than in D and small aggregates of contaminants, fraction 3 contained predominantly virions 580–640 mm long, and fractions 4 and 5 contained predominantly virion dimers and trimers and large aggregates of contaminating material. A large pellet in the bottom of the tube contained large aggregates of virus in cellular debris. Sedimentation is from left to right.

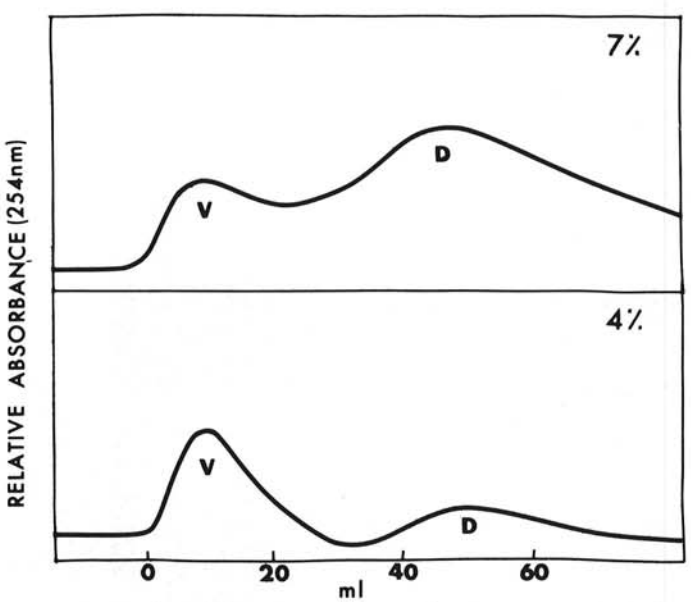


Fig. 3. Comparison of the molecular permeation chromatography elution profiles of a partially purified virus preparation obtained after precipitation with 7% (top) or 4% (bottom) polyethylene glycol-6000 (PEG). Virus (V) eluted predominantly in the 24 1-ml fractions after the column void volume (63 ml) while UV-absorbing host contaminants and some virus (D) eluted in the succeeding 24–60 1-ml fractions. The resuspended 4 and 7% PEG-precipitated preparations contained approximately equal quantities of virus, but the 7% PEG preparation contained more contaminating materials.

Small contaminants pass through the beads and elute slower than large particles that will not penetrate the pores (1).

Biophysical and biochemical properties. The density of virions precipitated with 4 and 7% PEG were similar; however, 7% PEG preparations contained a larger UV-absorbing zone comprised of virions and plant contamination (Fig. 4), than did the 4% PEG preparations. The buoyant density of KV-I was approximately 1.311 g/cm^3 (Fig. 5).

Electrophoresis of SDS degraded virions on 6% SDS-PAGE slab gels showed either one or two protein bands with apparent molecular weight of approximately 31,000–33,000d and 34,000–36,000d. The larger protein was found in all purified virus preparations. The smaller protein was resolved in only some preparations. The ratio of small to large protein as estimated by band size and staining intensity did not change noticeably with storage of the preparation in glycerol at −20 C over 4–6 mo. The presence of a major, slow and a minor, fast-migrating band has also been noted with other carlaviruses although their origin is unclear (21,26). Faint bands with apparent mol wt of 66–68 × 10d^3 were also often detected with KV-I and may represent dimers of the smaller protein subunits.

Serology. In microprecipitin tests KV-I was related serologically to (but distinct from) CLV, PVS, LSV, and CVB (Table 1). No relationship was detected between KV-I and RCVMV, HiLV, NLV, EVA, or PVM. Microprecipitin test results were confirmed by serologically specific immunoelectron microscopy results (Fig. 6) (Table 1).

Electron microscopy. Ultrathin sections of naturally infected Rotkappchen leaves and local lesions of *T. expansa* leaves contained viruslike flexuous filaments dispersed in the cell cytoplasm or associated with pockets of cytoplasm, vacuoles, endoplasmic reticulum (ER) and ribosomes. Fibrous masses and thin strands also occurred in the cytoplasm (Fig. 7). Small inclusions of viruslike rods were present along the tonoplast and in cytoplasmic bridges across the central vacuole in some cells. Virus aggregates were especially common in vascular parenchyma cells. Distribution of the virus was erratic in mesophyll cells and some sections contained no viruslike particles or inclusions. Tissues harvested in the summer months often contained pockets of cytoplasm, ribosomes, vacuoles, and ER but no viruslike particles.

Ultrathin sections of the local lesions on *C. quinoa* contained cells with viruslike rods dispersed in the cytoplasm and massive fusiform or elongated inclusions of viruslike particles along membranes such as those of the chloroplast, large vacuoles, and tonoplast (Figs. 8–10). Fibrous strand structures like those observed in *T. expansa* and Rotkappchen leaf cells were not found in *C. quinoa*.

DISCUSSION

Particle size and morphology, thermal inactivation point, aging in vitro, buoyant density, sedimentation coefficient, and apparent capsid protein size (as determined by SDS-PAGE) of KV-I were consistent with those of a carlavirus (10,26). Like many carlaviruses, KV-I is symptomless in its naturally infected host, has a narrow host range, and forms fusiform inclusions in infected cells (10). Because KV-I is serologically related to several carlaviruses, there is cogent evidence for classifying it in the carlavirus group.

KV-I can be distinguished from previously described carlaviruses on the basis of host range. Unlike alfalfa latent, cowpea mild mottle, red clover vein mosaic, pea streak, cassia mosaic, and muskmelon vein necrosis viruses (26), KV-I did not infect the legume species included in the host range test. Nor did KV-I infect *S. rostatum* or *N. debneyi* that are hosts for the PVM and PVS (23,24). CVB (14), poplar mosaic (26), NLV (7), and lonicera latent (20) (same as honeysuckle latent [8]) viruses infect tobacco, but KV-I did not. KV-I did not produce local lesion on *G. globosa* as reported for HiLV (6,26) (same as nerine latent [15]), elderberry carlavirus (19), and chicory blotch virus (5). Systemic infection of *C. quinoa* was reported for passiflora latent virus (2), helenium S virus (26), and CLV (25). KV-I did not infect lily seedlings as does LSV (26). Hop latent virus had a narrow host

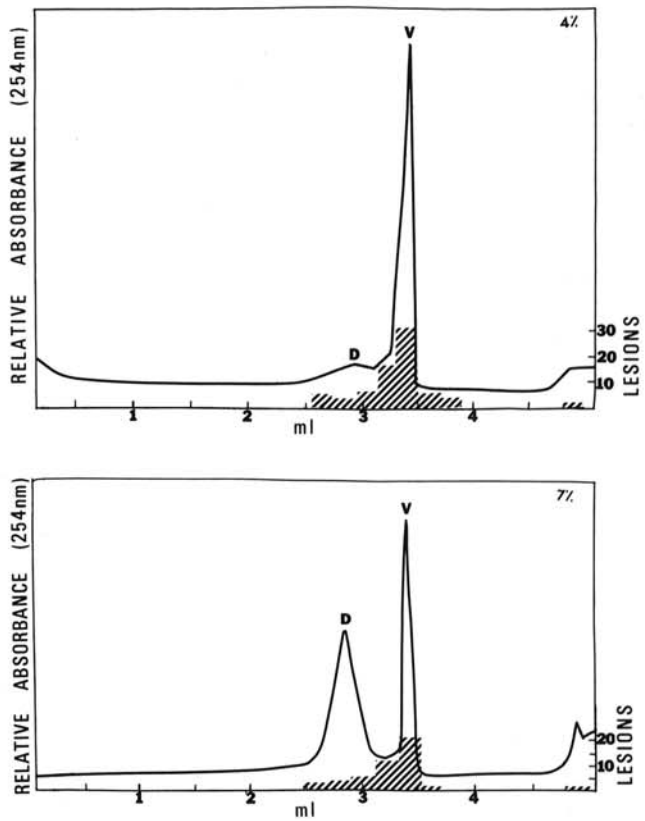


Fig. 4. Spectrophotometric absorbance profiles of kalanchoë virus-I centrifuged on self-forming cesium chloride density gradients and the average number of local lesions induced by 0.2-ml fractions of the gradients assayed in *Chenopodium quinoa*. Gradients received 0.05 ml of a 1 mg/ml virus preparation obtained from 4% (top) or 7% (bottom) polyethylene glycol-6000 (PEG) precipitation and resuspension in 0.05 M tris-HCl, pH 7.5. Gradients were centrifuged at 40,000 rpm in a Beckman SW 50.1 rotor for 18 hr at 20 C. The gradient receiving the 7% PEG preparation contained two visible light scattering zones at 22 and 27 mm below the meniscus, while the gradient receiving the 4% PEG preparation contained one visible zone 27 mm below the meniscus. Negatively stained preparation of the 2.7-ml fractions from both gradients (D) contained virus fragments and debris and the 3.3 ml fractions (V) contained only virus. Infectivity was associated predominantly with the UV-absorbing zones at a depth of 3.3 ml in the gradients. Sedimentation is from left to right.

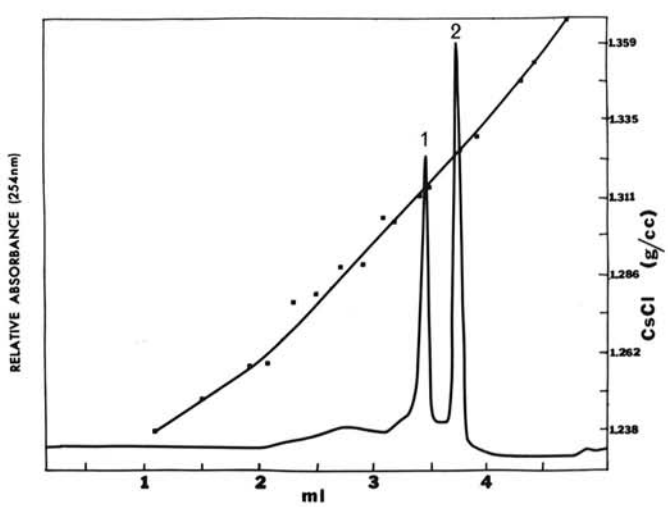
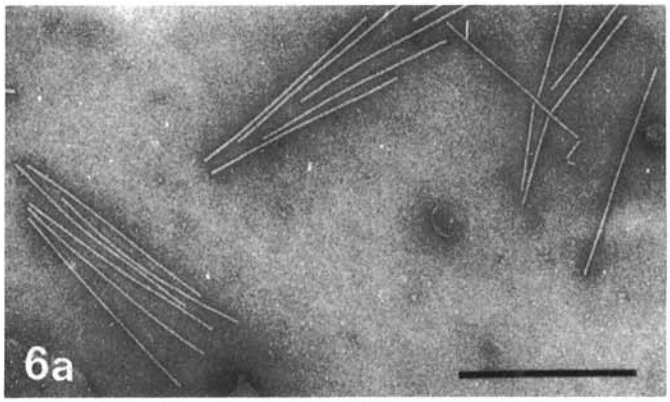


Fig. 5. Comparison of the buoyant densities of kalanchoë virus-I (band 1) and tobacco mosaic virus (band 2) in 30% cesium chloride density gradient centrifuged to equilibrium as described in Fig. 4. Light-scattering bands occurred at 27 mm and 30 mm below the meniscus. The buoyant densities of TMV and KV-I were approximately 1.324 and 1.311 g/cm^3, respectively. Sedimentation is from left to right.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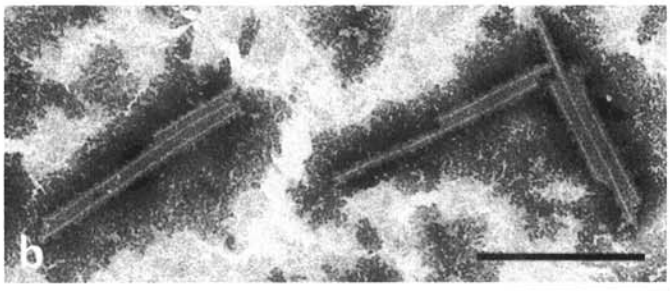
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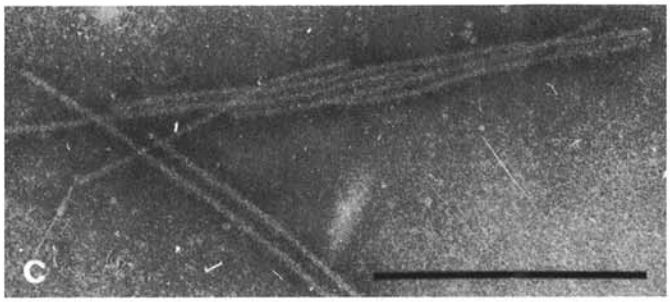
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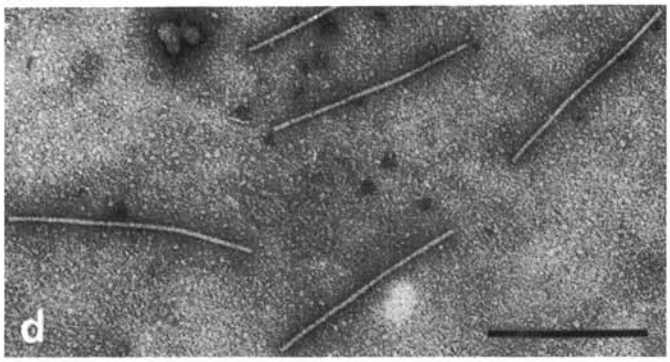
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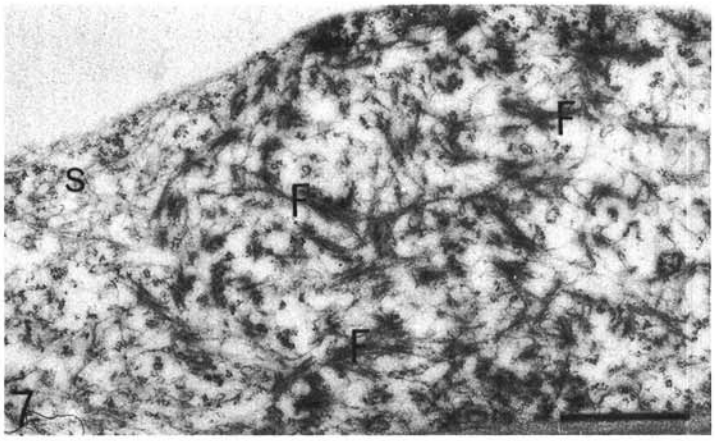
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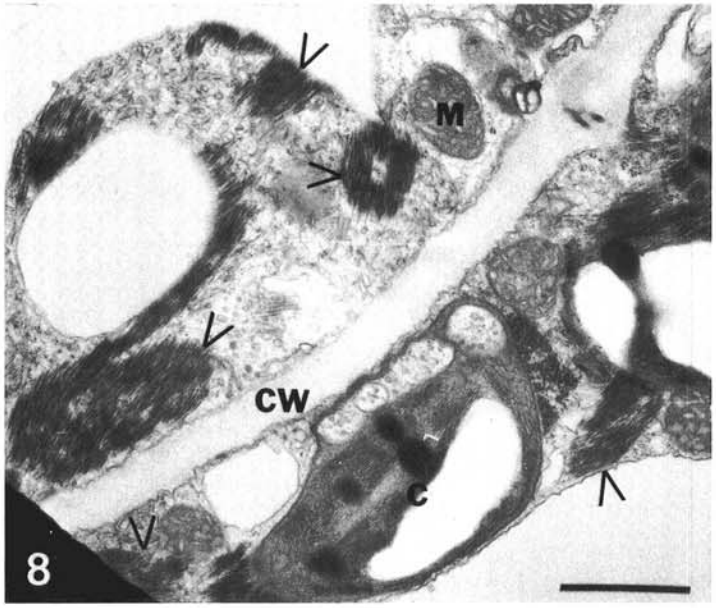
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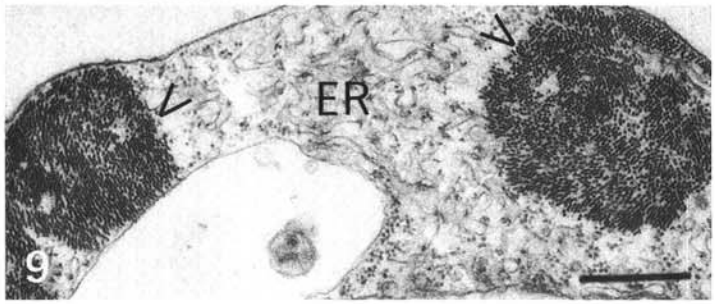
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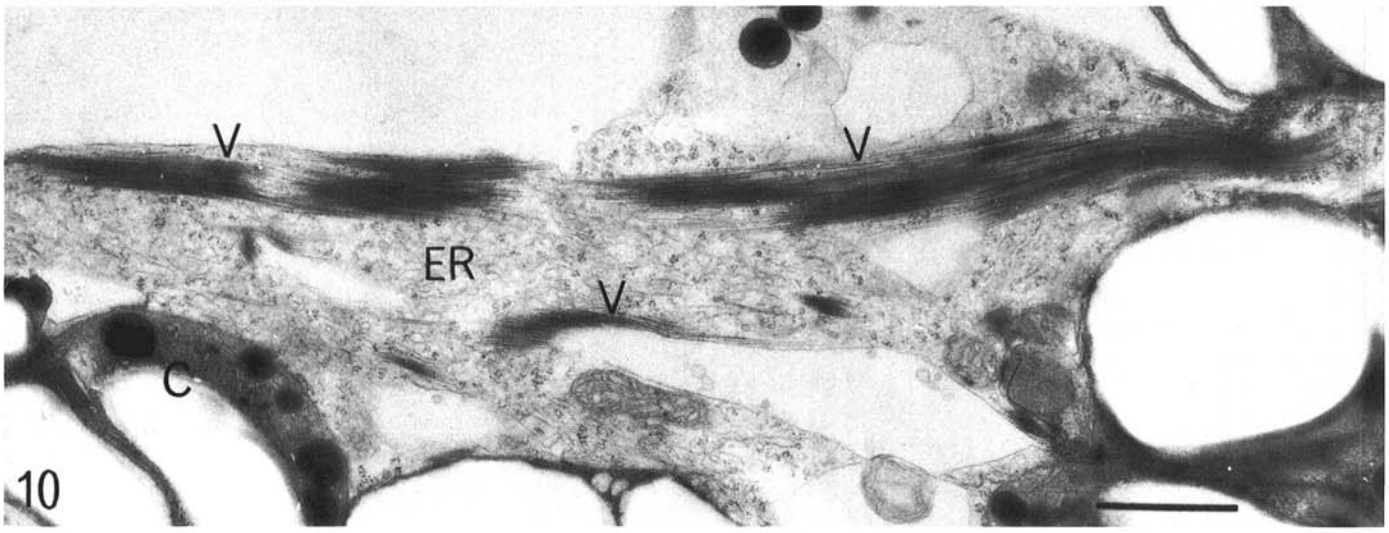
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Figs. 6–10. Electron micrographs of kalanchoë virus-I (KV-I) in negatively stained leaf-dip preparations (**Fig. 6**) and in ultrathin sections of kalanchoë (**Fig. 7**) and *Chenopodium quinoa* (**Figs. 8–10**). **6,** Leaf dips: **a,** treated with PTA negative stain only; **b,** incubated with a 1/400 dilution of antiserum to KV-I for 40 min before negatively staining; **c,** incubated with a 1/50 dilution of antiserum to lily symptomless virus (LSV); and **d,** incubated with a 1/100 dilution of antiserum to narcissus latent virus (NLV). KV-I particles are aggregated and decorated by antibodies in the KV-I and LSV, but not the NLV, antisera. All scale bars = 500 nm. **7,** Cytoplasm of KV-I infected Rotkappchen cell that contains fibrous aggregates (F) and individual thin strands (S). Bar = 1 μm. **8,** Cells in a local lesion on *C. quinoa* inoculated with KV-I that contain numerous aggregates of viruslike particles (<) sectioned in various planes. Note the pockets of cytoplasmic material within the inclusion. Individual viruslike particles are visible in the cytoplasm surrounding the inclusions. CW = cell wall, M = mitochondrion, C = chloroplast, Bar = 1 μm. **9,** Cross section of two inclusions (<) showing the regular arrangement of particles in some areas of the aggregate and voids in other areas. Endoplasmic reticulum (ER) is abundant in the area around the inclusion. Bar = 0.5 μm. **10,** Longitudinal section of inclusions (<) within a cell showing their fusiform or elongated shape, association with accumulations of endoplasmic reticulum (ER) and their tendency to occur along membranes. Bar = 1 μm.

TABLE 1. Results of microprecipitin and serologically specific electron microscopy (SSEM) tests to investigate the relationship of kalanchoë virus-I (KV-I) to recognized carlaviruses

Antisera	Reciprocals of homologous titers (according to donor)	Antigen	Reciprocals of titer in microprecipitin test after 24 hr using purified virus	Highest dilution showing decoration SSEM using leaf dip
Normal serum		KV-I	8[e]	–[d]
KV-I		KV-I	512	1/400
KV-I		Potato virus M	NT[c]	–[d]
KV-I		Potato virus S	NT	1/100
KV-I		Healthy *C. quinoa* sap	128[e]	NT
Carnation latent[a]	8,192	KV-I	256	1/200
Chrysanthemum B[a]	260,000	KV-I	128	1/50
Elderberry A[a]	4,096	KV-I	–	–
Hippeastrum latent[a]	2,048	KV-I	–	–
Lily symptomless[a]	2,048	KV-I	128	1/50
Narcissus latent[a]	1,024	KV-I	–	–
Potato virus S[b]	256	KV-I	32	NT
Red clover vein mosaic[a]	4,096	KV-I	–	–

[a] Donor: A. A. Brunt.
[b] Source: American Type Culture Collection, PVAS-103 (formerly PVAS-43) by R. Stace-Smith.
[c] NT = not tested.
[d] Minus (–) = negative result.
[e] A granular precipitate was observed here which differed from the floccular precipitate observed in the virus-antibody reactions.

range, but was reported as usually latent in *Chenopodium* species and produced local lesions on *P. vulgaris* (17), unlike KV-I. Shallot latent virus (3) also has a narrow host range, but the reported SLV particle size (650 nm), thermal inactivation point (80 C), sedimentation coefficient (147.5 S), and protein subunit size (23,200d) were different than those of KV-I.

Preliminary investigations indicate that KV-I is common in kalanchoës. However, detection of virus by electron microscopy or by inoculating *C. quinoa* gave inconsistent results and often required repeated testing of the plants. Environmentally induced fluctuations of virus titer, relatively low virus concentration in kalanchoë, and the presence of virus inhibitors in the sap of kalanchoës (18) are probably responsible for lack of consistent detection of KV-I by bioassay. The virus was not consistently detected in infected plants although the buffer, pH, and additives to the crude sap (such as diethylaminoethyl cellulose, polyvinylpyrrolidone, 2-mercaptoethanol, EDTA, or bentonite) were varied.

Purification of KV-I in quantity was only partially successful and results varied from one experiment to the next. Virus aggregation and host component contamination are common difficulties in carlavirus purification. Two types of contamination occurred in KV-I preparations. One type could be eliminated from the virus preparation by column chromatography and consisted of small particles that were not sedimented at 27,000 rpm for 2 hr after separation from the virus. A second type consisted of aggregates of virus and cellular cytoplasmic components. These aggregates passed through CPG column with the virus and produced a shoulder on the trailing end of the virus peak profile or sometimes delayed virus elution. Barton (1) also noted unexplained delays in elution of some preparations of rod viruses from a CPG column. The recommended method for purification of KV-I is 4% PEG precipitation of virus from clarified sap followed by CsCl density gradient centrifugation.

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