Comparison of Two Strains of Kalanchoë Latent Virus, Carlavirus Group

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ABSTRACT

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A carlavirus isolated from *Kalanchoë blossfeldiana* 'Texas Sunset' and designated KV-2 was compared with a previously characterized carlavirus isolated from cultivar Rotkappchen and designated KV-1. The isolates had similar host ranges, thermal inactivations (~75 C), buoyant densities (1.30 g/cc), and capsid protein molecular masses (33,000–34,000 daltons). KV-2 was distinguished from KV-1 in that KV-2: systemically infected *Chenopodium* spp. that were only locally infected by KV-1; had a normal length that was about 30 nm longer than that of KV-1; and was degraded by reagents such as 1% Triton X-100, 5 mM EDTA, and chloroform-carbon tetrachloride that did not degrade KV-1. KV-2 was purified from *C. quinoa* by clarification of phosphate-buffered crude sap extracts with 1,1,2-trichloro-1,2,2-trifluoroethane (Freon), precipitation with 6% polyethylene glycol, and centrifugation on 10–40% linear CsCl density gradients. SDS-degraded KV-1 and KV-2 formed lines of partial fusion in Ouchterlony gel double diffusion tests. Isolate-specific antibodies were found in each homologous antiserum in intragel cross-absorption tests. Both isolates reacted in indirect ELISA with antisera to carnation latent, chrysanthemum B, lily symptomless, and potato S viruses, but not to elderberry A, hippeastrum latent, red clover vein mosaic, or narcissus latent viruses. Since only minor differences were found in most of the properties of the two viruses, KV-1 and KV-2 are concluded to be strains of the same carlavirus, here designated kalanchoë latent virus.

Additional key words: fusiform virus inclusions, cytopathology.

Kalanchoë virus 1 (KV-1), with properties typical of a carlavirus, has been isolated from *Kalanchoë blossfeldiana* Poelln. 'Rotkappchen' and partially characterized (5). Subsequently, a virus with a slightly flexuous rod morphology was isolated from *K. blossfeldiana* 'Texas Sunset' and found also to have properties typical of a carlavirus. This isolate, designated kalanchoë virus 2 (KV-2), was distinguished from KV-1 by the systemic reaction that KV-2 produced in *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn. A preliminary report described some properties of KV-2 (6).

MATERIALS AND METHODS

Virus. Isolate KV-2 was obtained from leaf homogenates of Texas Sunset made in 0.1 M phosphate buffer, pH 8, containing 0.02 M sodium sulfite and 1% polyvinylpyrrolidone (MW 10,000) (PVP-10). The homogenate was clarified with a chloroform/butanol mixture (1:1, v/v) added to 20% (v/v), and the supernatant obtained by low-speed centrifugation was treated with polyethylene glycol (MW 6,000) (PEG-6000) adjusted to a final concentration of 4% (w/v). The precipitate was collected by low-speed centrifugation, suspended in phosphate buffer, and inoculated to corundum-dusted test seedlings. Seedlings of *C. quinoa* developed chlorotic local lesions 2 wk after inoculation and systemic infection in a few apical leaves 3–4 wk after inoculation. Systemically infected leaves were triturated in neutral phosphate buffer and mechanically inoculated to seedlings of *C. quinoa*. The isolate was subsequently maintained in *C. quinoa* by mechanical inoculations. KV-1 also was maintained in *C. quinoa* by mechanical passage.

Purified KV-1 and KV-2 were each mechanically inoculated to Texas Sunset seedlings previously found to be free of KV-1 and KV-2 by bioassay to *C. quinoa* and by enzyme-linked immunosorbent assay (ELISA). Inoculated kalanchoë seedlings were observed for symptom development and indexed for infection.

Methods used in determining host range, physical properties, particle lengths, biophysical and biochemical properties, serological properties, and cytopathological effects were those previously described (5), unless otherwise described herein.

Purification. The purification procedure developed for KV-1 (5) resulted in low yields when used for KV-2. Components in the KV-1 purification protocol were evaluated individually for deleterious effects on KV-2. Inoculated leaves of *C. quinoa* that showed numerous local lesions were ground in a Waring Blendor with 0.1 M neutral phosphate buffer (1:2, w/v), and the slurry was filtered through cheesecloth. Test reagents were added to aliquots of this crude sap preparation to obtain the desired final concentrations and incubated for 1–2 hr at room temperature. Sap was then assayed for viral infectivity by inoculation to seedlings of *C. quinoa*. Control aliquots received no additives or equal amounts of distilled water. Aliquots of sap were also clarified with 1,1,2-trichloro-1,2,2-trifluoroethane (Freon) added to 50% or chloroform-carbon tetrachloride (1:1, v/v) added to 20%, and the aqueous phases recovered from low-speed centrifugation were treated with 6% PEG-6000. Precipitates collected by centrifugation were suspended in one-third the original volume of 0.1 M phosphate buffer and inoculated to *C. quinoa*.

The purification procedure developed for KV-1 was then modified in the following manner to achieve purification of KV-2. Inoculated leaves of *C. quinoa* were chilled and homogenized in two volumes of cold 0.1 or 0.2 M K_2HPO_4 containing 0.2% sodium sulfite and 2 mM ethylenediaminetetraacetic acid, sodium salt (EDTA). An equal volume of Freon was added while blending. The slurry was centrifuged in a Sorvall GSA rotor at 10,000 *g* for 15 min. The upper, aqueous phase was decanted through a disposable paper cleaning tissue (Kimwipe) or a glass wool plug in a funnel. The filtrate was centrifuged at 8,000 *g* for 10 min. PEG-6000 was added with stirring to the supernatant to a final concentration of 6%. The precipitate was collected by centrifugation at 10,000 *g* for 15 min after incubation on ice 1.5–2.0 hr. Pellets were suspended overnight in extraction buffer and clarified by low-speed

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centrifugation. The preparation was precipitated twice by addition of PEG-6000 to 6%, and the final pellets were suspended in 0.02 M K_2HPO_4 with or without 2 mM EDTA.

Concentrated virus preparations were layered on a continuous-density gradient of 10–40% CsCl in 0.02 M K_2HPO_4 and centrifuged in a Beckman SW 41 Ti rotor at 34,000 rpm for 3–4 hr or 19–20 hr or in a Beckman SW 27 rotor at 24,000 rpm for 5–6 hr at 20–22 C. Triton X-100 from a 20% stock solution or an equal amount of distilled water was added to the partially purified virus preparations immediately before the virus was layered on the CsCl density gradients in some experiments. Tobacco mosaic virus (TMV) mixed with KV-2 was centrifuged in CsCl gradients in one experiment.

Gradients were scanned and fractionated as previously described (5). Virus-containing fractions were dialyzed against 0.02 M K_2HPO_4 and lyophilized for future use in SDS-PAGE analysis or serological tests.

Serology. Antisera were produced against KV-1 and KV-2 (purified by the Freon method described herein) in New Zealand white rabbits by J. Tew (Virginia Medical College, Richmond, VA). One milligram of lyophilized virus was rehydrated in 1 ml of distilled water, mixed with 1 ml of Freund's complete adjuvant, and injected into the four footpads of a rabbit. Four weeks later, the rabbit was injected in each footpad with an additional 0.25 mg of virus in distilled water. Rabbits were bled 2 and 6 wk after receiving the booster injections.

Ouchterlony gel double diffusion and intragel cross-absorption tests (8) were performed in 0.8% agarose gels containing 0.02 M K_2HPO_4 and 0.02% sodium azide. Purified virus (0.5–1 mg/ml) was degraded in 0.1 M Na_2HPO_4-NaH_2PO_4, pH 7.2, containing 0.1% sodium dodecyl sulfate (SDS) and used as antigen, except as otherwise noted. Antisera wells were precharged with SDS-degraded virus, whole virus, or SDS only, 4 hr before receiving antisera in the intragel cross-absorption tests. Antisera were diluted in phosphate-buffered saline (PBS). Wells were spaced 5 mm apart (edge-to-edge) and filled with 25–28 μl of reagent. Microprecipitin and immunoelectron microscopy tests (IEM) were conducted with whole virus (5).

Indirect ELISA was used to test several antisera to known carlaviruses for reactivity with KV-1 and KV-2. Assays were made in Immunlon I polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA 22314) that were washed with PBS containing 0.5% Tween-20 (PBS-Tween) between each step in the assay. Wells were coated with purified KV-1 or KV-2 (diluted to $A_{260\ nm} = 0.05$) in coating buffer (0.05 M sodium carbonate-bicarbonate buffer, pH 9.6) for 2 hr at 30 C. Wells coated with filtered extracts of healthy leaves of *C. quinoa* triturated in coating buffer (1 g/10 cc) were used as controls. Antisera diluted in PBS were incubated in the plates for 2–3 hr. Antisera that showed strong reactions with healthy plant components were absorbed with extracts from healthy *C. quinoa* made by grinding leaves in PBS (1 g/20 cc) and filtering the homogenate through a Kimwipe. Alkaline phosphatase-labeled, goat-affinity-purified antibody to rabbit IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD 20760) was diluted 1/200 or 1/300 in PBS-Tween and reacted with trapped rabbit antibodies for 2–5 hr before substrate (1 mg *p*-nitrophenylphosphate per milliliter in 97% diethanolamine containing 0.5 mM MgCl, pH 9.8) was added. Absorbance of the reaction products was measured at 405 nm on a Titertek Multiskan.

SDS-PAGE. Molecular weight determinations for capsid proteins of KV-1 and KV-2 were performed in tris-borate buffer (3) on 10 × 14 × 0.15-cm polyacrylamide slab gels. Standards for molecular weight determinations were lysozyme (MW 14,400), soybean trypsin inhibitor (MW 21,500), carbonic anhydrase (MW 31,000), ovalbumin (MW 45,000), bovine serum albumin (MW 66,200), and phosphorylase B (MW 92,500) (Bio-Rad Laboratories, Richmond, CA 94804). Samples were electrophoresed at a constant 100 V and 19–27 mA for 2.5–5.0 hr at room temperature. Protein zones were visualized by staining with Coomassie Brilliant Blue.

Electron microscopy. Particles of KV-2 were measured in negatively stained leaf dip preparations of local lesions on *C. quinoa* and of systemically infected leaves of *C. quinoa* and *C. amaranticolor* (5). The normal lengths (NL) of KV-1 and KV-2 particles from local lesions on *C. quinoa* were compared by simultaneously making negatively stained leaf dip preparations for each virus and then photographing the particles at the same magnification (~×5,000) in a Philips model 200 or JEOL model 100C electron microscope. Negatives were projected at ×10 enlargement and the virus particles were traced on paper and measured. Magnification was determined by using a diffraction grating replica (2,160 lines per millimeter or 54,864 lines per inch) in the Philips instrument or the internal calibration of the JEOL microscope. Seven comparisons were made and 50–100 particles of each virus were measured each time.

Leaf pieces of Texas Sunset, naturally or experimentally infected with KV-2, and local lesions and systemically infected leaf tissue of *C. quinoa* and *C. amaranticolor* were fixed, embedded, and sectioned (5) for ultrastructural studies of the cytopathology. Healthy plant tissue served as controls.

RESULTS

Host range and symptomology. KV-2 infected *C. quinoa*, *C. amaranticolor*, *C. capitatum* (L.) Asch., and *Tetragonia expansa* Murr. Chlorotic local lesions developed on mature green leaves of *C. quinoa* 7–14 days after inoculation with KV-2. Lesions senesced to white papery spots. Green local lesions appeared on lower leaves that yellowed before symptoms developed. Epinasty, leaf cupping, vein yellowing, and chlorotic speckling appeared 2–3 wk after inoculation on a few systemically infected leaves (Fig. 1A). Chlorotic or red local lesions appeared on *C. amaranticolor* 10–18 days after inoculation (Fig. 1B). Systemic infections developed in a

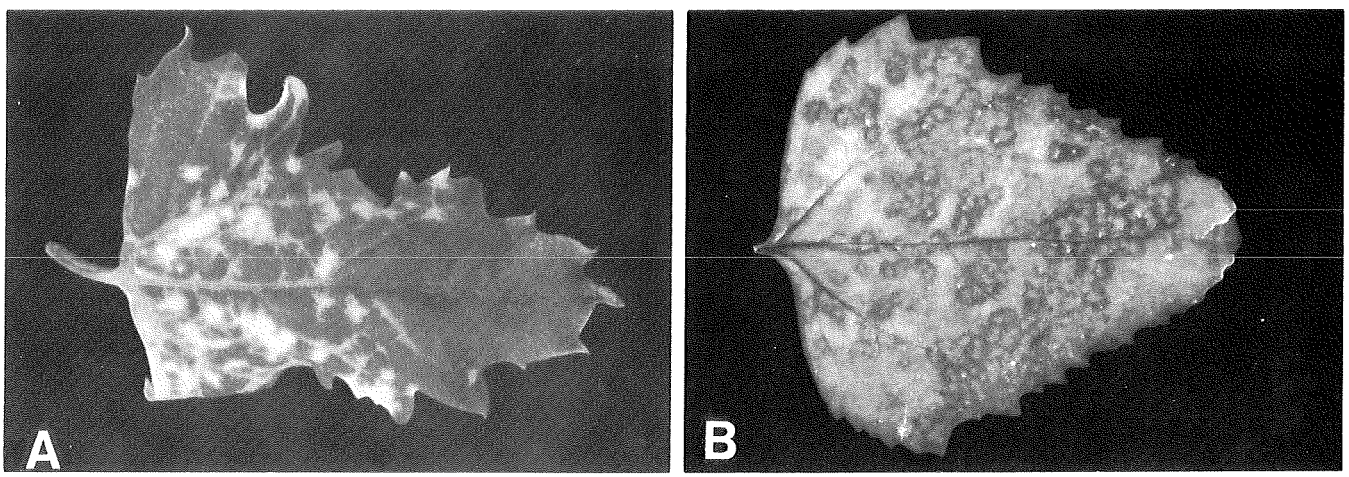

Fig. 1. Symptoms induced by kalanchoë virus isolate KV-2 on **A,** systemically infected leaf of *Chenopodium quinoa* and **B,** inoculated leaf of *C. amaranticolor*.

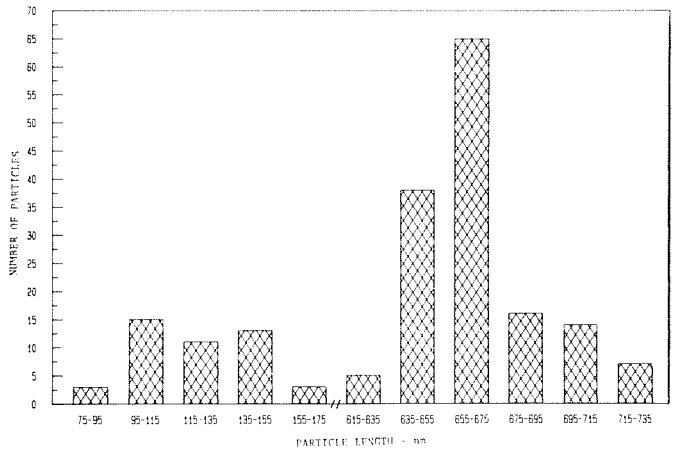

Fig. 2. Histogram of kalanchoë virus isolate KV-2 particle lengths measured in 2% potassium phosphotungstate negatively stained leaf dips of local lesions on *Chenopodium quinoa*.

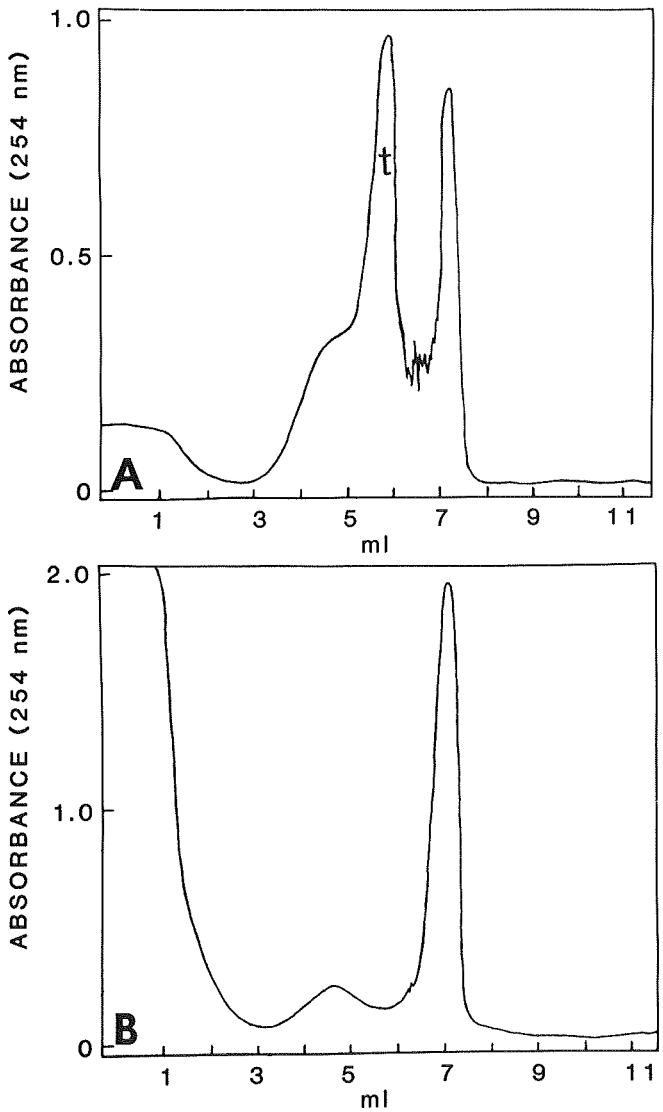

Fig. 3. Ultraviolet absorbance profiles of 10–40% CsCl density gradients that received partially purified preparations of kalanchoë virus isolate KV-2 **A,** mixed with buffer or **B,** mixed with 1% Triton X-100. Gradients were centrifuged at 34,000 rpm for 3 hr at 22 C in a Beckman SW 41 Ti rotor. Gradient B contained a green zone at the meniscus that was not present in gradient A and lacked a green zone (t), at a depth of 5–6 ml, that was present in gradient A. The area of the virus-containing peak was greater in gradient B then in gradient A. This was observed only when preparations were centrifuged into the gradients immediately after adding detergent. Sedimentation was from left to right.

few leaves and caused vein yellowing and leaf distortion. *C. capitatum* showed faint chlorotic local lesions 10–16 days after inoculation and developed a mild vein yellowing on systemically infected leaves. *T. expansa* developed large faint chlorotic local lesions 2–3 wk after inoculation, but no systemic infection. Local lesions induced by KV-2 on *Chenopodium* spp. and *T. expansa* resembled those of KV-1 and required similar incubation periods for development. However, KV-1 never produced systemic symptoms in *Chenopodium* spp. regardless of the concentration of virus inoculum.

Texas Sunset seedlings mechanically inoculated with either KV-1 or KV-2 did not develop leaf symptoms. KV-2 recovered from these plants by bioassay to *C. quinoa* produced systemic symptoms in the latter host after one additional passage to increase the titer. Virus recovered from the KV-1 inoculated kalanchoë seedlings produced only local lesions on *C. quinoa*.

Test seedlings that were not infected by KV-2 included: *Beta vulgaris* L. 'Detroit Ruby Red,' *Capsicum annuum* L., *C. frutescens* L., *Cassia occidentalis* L., *Cucumis sativus* L., *Cucurbita pepo* L., *Datura stramonium* L., *Glycine max* (L.) Merr., *Gomphrena globosa* L., *Hibiscus cannabinus* L., *H. esculentus* L., *Limonium sinuatum* (L.) Mill. 'Midnight Blue,' *Lycopersicon esculentum* Mill., *Nicotiana benthamiana* Domin., *N. clevelandii* Gray, *N. debneyi* Domin., *N. glutinosa* L. × *N. edwardsonii* Christie & Hall, *N. megalosiphon* Heurck & Muell., *N. tabacum* L., *Petunia hybrida* Vilm., *Phaseolus vulgaris* L., *Pisum sativum* L., *Plantago major* L., *Saponaria vaccaria* L. 'Pink Beauty,' *Solanum melongena* L., *Taraxacum officinale* Weber, *Vicia faba* L., *Vigna unguiculata* L., and *Vinca rosea* L.

Virion size and stability. The majority of the KV-1 particles measured 590–640 nm, while the majority of the KV-2 particles measured 620–670 nm in length (Fig. 2). Short particles (75–175 nm) were in all preparations. Their origin was not investigated.

The average NL of KV-1 and KV-2 particles measured in negatively stained leaf dip preparations was 620 and 650 nm, respectively. The absolute value of the NL for KV-1 and KV-2 varied slightly among comparisons; however, in five of seven comparisons, the NL of KV-2 was between 640 and 675 nm, while that of KV-1 was between 600 and 640 nm. The difference between the two values in each comparison was 30–40 nm. In two comparisons, the NL of both viruses was 630–640 nm. The NL of KV-2 particles in systemically infected *Chenopodium* spp. was 660 nm.

In partially purified virus preparations adjusted to an $A_{260\ nm} = 0.1$ (1-cm light path), KV-1 and KV-2 were infectious after heating for 10 min at 75 C, but not after heating at 80 C. However, >95% of the infectivity of both viruses was lost by heating at 75 C for 10 min. The thermal inactivation point for both viruses in crude sap of *C. quinoa* was generally 5–10 C less than that observed in partially purified preparations. Longevity of KV-1 and KV-2 in crude sap extracts of *C. quinoa* in PBS-Tween with 1% PVP (MW 40,000) was 5–7 and 3–5 days, respectively. Infectivity of both viruses was lost 1–2 days sooner in extracts in phosphate buffer only.

Chloroform-carbon tetrachloride treated aliquots of buffered leaf extracts from *C. quinoa* infected by KV-2 gave one-third as many local lesions as those not treated with organic solvent or those clarified with Freon. Infectivity was reduced in aliquots incubated for 2 hr with 5 mM EDTA and essentially lost in aliquots adjusted to 50 mM EDTA. Infectivity was unchanged at all pH intervals tested between pHs 7.0 and 10.2 during a 2-hr incubation period, but was lost at pH 6 or less. Controls that received no detergent produced 10 times more local lesions than aliquots incubated with 1% Triton X-100. Infectivity was reduced in aliquots with 0.5% Triton X-100 and lost in aliquots with 5% Triton X-100.

Virus purification. Preparations of KV-1 contained less contaminating green host material when clarified with chloroform-carbon tetrachloride than when clarified with Freon. The quantities of purified virus obtained by either method were similar after the preparations were subjected to CsCl density gradient centrifugation.

Density gradients that were layered with detergent-treated virus retained a green layer at the meniscus and lacked a green band similar to the one observed near the middle of control gradients (Fig. 3A and B). Yields of KV-2 were increased sometimes with a short 1% Triton X-100 treatment, presumably due to release of virions from aggregates of host material. The virus zone in gradients that received virus treated with 5% detergent was much smaller than that in control gradients, indicating virus degradation by the detergent.

Degradation of KV-1 by detergent was not observed. However, detergent-treated KV-1 and KV-2 were often particulate when recovered from gradients. When examined in the electron microscope by negative staining, the particulate virus fractions were found to contain large aggregates of virus particles and sometimes vesicular or floccular material. Therefore, Triton X-100 was used only in the first steps of KV-1 purification and was omitted from KV-2 purification.

KV-1 and KV-2 sedimented as single components in CsCl density gradients when they were centrifuged to equilibrium. If the centrifugation was halted prior to this stage, the virus was contained in two bands in the gradients (Fig. 4A and B).

The average buoyant density of KV-2 in CsCl in six experiments was 1.304 g/cc, compared to 1.311 g/cc for KV-1. KV-2 was not separated from KV-1, but both banded above TMV in CsCl density gradients. In one experiment, the buoyant density of KV-2 in Cs_2SO_4 was 1.269 g/cc.

Purified KV-2, like KV-1, had a maximum absorption value at a wavelength of 258 nm and a $A_{260/280}$ of 1.32–1.34, uncorrected for light scattering. Virus yields of 10–30 mg/kg of plant tissue were obtained.

Serology. KV-1 and KV-2 were closely related serologically. KV-1 and KV-2 antisera in homologous and heterologous reactions had end points of 1/256–1/512 in microprecipitin tests. The serological differentiation indices (9) were 1 or less.

Ammonium sulfate precipitation of the immune serum gave globulin yields of 5.4 and 3.9 mg/ml (based on $E_{280\ nm} = 1.8$) from the rabbits immunized with KV-1 and KV-2, respectively. KV-1 antiserum was diluted 7:3 with distilled water or PBS, and virus preparations were diluted to comparable $A_{260\ nm}$ values before use in ELISA or gel double diffusion tests.

Precipitin lines of partial fusion formed with SDS-degraded virus in balanced antigen and antibody conditions in Ouchterlony gel double diffusion tests. Precipitin lines formed spurs in the direction of the homologous reaction (Fig. 5). Intragel cross-absorption tests showed the presence of isolate-specific antibodies in each antiserum (Fig. 6A and B). These tests were successful only when antisera wells were precharged with SDS-treated virus. Precharging of antisera wells with intact virus or SDS alone did not eliminate the homologous or heterologous precipitin bands. Precharging of the antisera wells with the SDS-treated homologous virus eliminated both homologous and heterologous precipitin bands.

KV-2 particles were decorated by 1/50 dilutions of antisera to KV-1, carnation latent, potato S, and lily symptomless viruses in IEM tests. Decoration of KV-2 particles was not observed with antisera to elderberry A, hippeastrum latent, narcissus latent, or red clover vein mosaic viruses or normal serum at 1/50 dilutions.

KV-1 and KV-2 reacted with homologous and heterologous antisera in the double-antibody sandwich form of ELISA, but the $A_{405\ nm}$ was approximately twofold greater in the homologous as compared to the heterologous reaction. In indirect ELISA, both viruses reacted strongly with antisera to carnation latent, lily symptomless, potato S, and chrysanthemum B viruses (Table 1). Neither reacted with 1/500 dilutions of antisera to elderberry A, hippeastrum latent, narcissus latent, or red clover vein mosaic viruses.

Absorption of some of the antisera with healthy plant sap was necessary to visualize the virus-specific reactions. Since the amount of healthy component in the virus preparation did not necessarily match that in the healthy crude sap controls, subtracting the $A_{405\ nm}$ reaction value of the antiserum with healthy component from that of antiserum with virus did not adequately measure the virus-

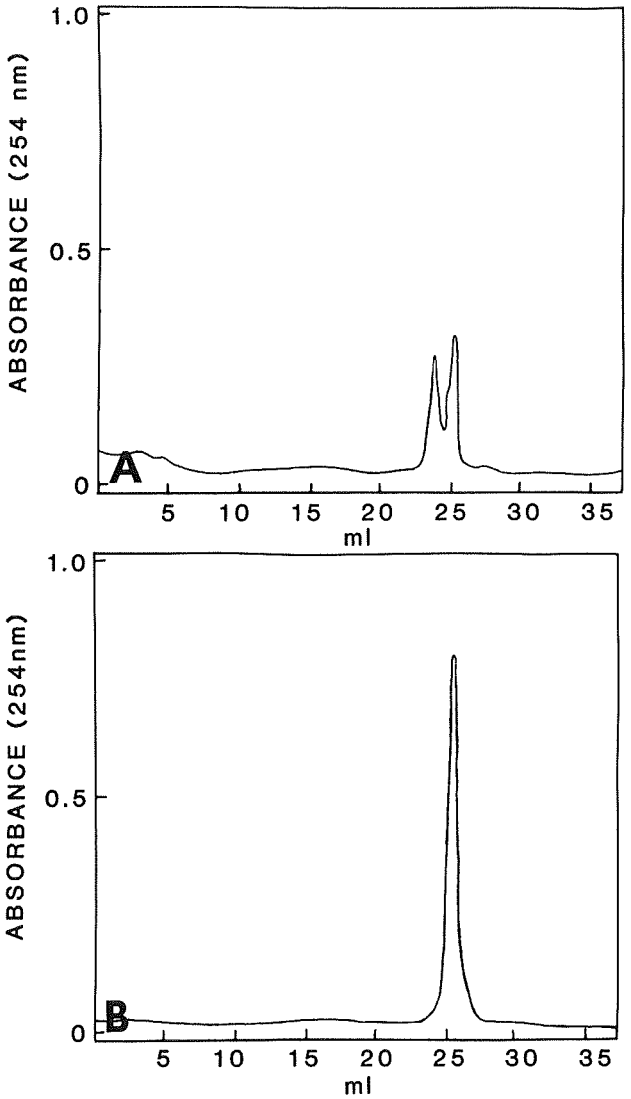

Fig. 4. Ultraviolet absorbance profiles of 10–40% CsCl density gradients that were layered with kalanchoë virus isolate KV-2 and centrifuged at 25,000 rpm for **A**, 3 hr and **B**, 5 hr at 22 C in a Beckman SW 27 rotor. Profiles of gradients that were centrifuged for 20 hr resembled those of gradients centrifuged for 5 hr, and showed that the virus reached equilibrium in 5 hr, but not in 3 hr, if preformed gradients were used. Sedimentation was from left to right.

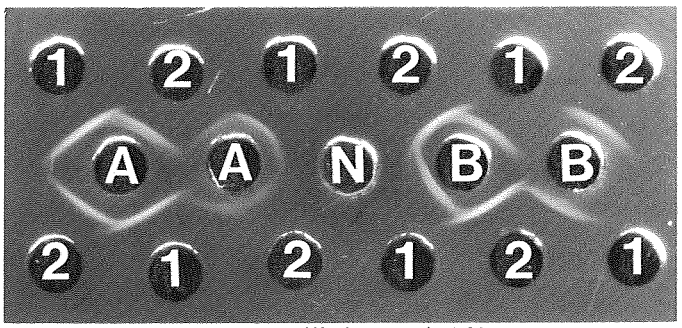

Fig. 5. Ouchterlony gel double diffusion tests in 0.8% agarose containing 0.02 M dibasic potassium phosphate and 0.01% sodium azide. Wells labeled 1 and 2 received kalanchoë virus isolates KV-1 and KV-2, respectively. Virus (0.5 mg/ml) was mixed 1:1 with 0.1% SDS in sodium phosphate buffer, pH 7.3, before being placed in the wells. Middle row: first and second wells from the left (labeled A) received KV-1 antiserum diluted 1/2 and 1/4, respectively; the center well (labeled N) received normal serum diluted 1/2; and the fourth and fifth wells from the left (labeled B) received KV-2 antiserum diluted 1/2 and 1/4, respectively. Note that spur formation is evident under balanced antigen-antibody conditions, ie, around antiserum wells 1 and 4.

specific reaction. For instance, the reaction of chrysanthemum virus B antiserum with healthy sap was greater than that with purified KV-1. The $A_{405\ nm}$ value of the antisera and healthy sap reaction was reduced from 1.59 to 0.14 by absorbing the antiserum with healthy sap preparations. In comparison, the $A_{405\ nm}$ reaction value of the chrysanthemum B virus antiserum with KV-1 was only reduced from 1.296 to 0.897. The 0.897 value is thereby a more reliable estimate of the virus-specific antiserum reaction than the −0.294 value obtained by subtracting the unabsorbed antiserum reaction with healthy sap from that of the unabsorbed antiserum with virus.

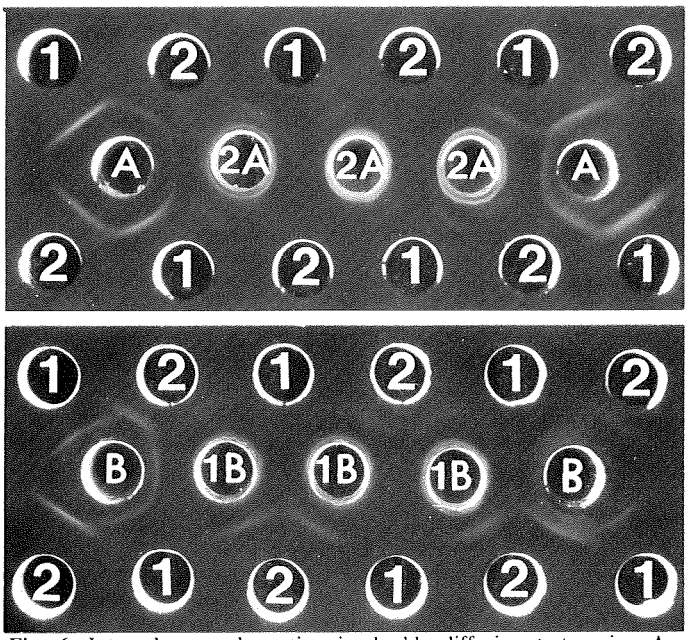

Fig. 6. Intragel cross-absorption in double diffusion tests using **A,** antiserum to kalanchoë virus isolate KV-1 or **B,** antiserum to kalanchoë virus isolate KV-2. In **A,** and **B,** wells labeled 1 or 2 in the top and bottom rows received SDS-degraded KV-1 or KV-2, respectively. In **A,** wells in the middle row were precharged with untreated KV-2 (labeled A) or SDS-degraded KV-2 (labeled 2A) 4 hr before they were charged with KV-1 antiserum. In **B,** wells in the middle row were precharged with untreated KV-1 (labeled B) or SDS-degraded KV-1 (labeled 1B) 4 hr before they were charged with KV-2 antiserum. A precipitin zone is visible with the homologous reactions but not the heterologous reaction after cross-absorption with the SDS-degraded heterologous antigen. Cross-absorption with intact virus did not eliminate the homologous or heterologous reactions.

SDS-PAGE. KV-1 and KV-2 virion capsid proteins were calculated to have a molecular mass of 33,000–34,000 daltons on 6 and 12% polyacrylamide gels (Fig. 7). Minor bands were detected in almost all preparations of both viruses. These bands occurred at positions corresponding to proteins with molecular masses of 31,000, 32,000, 35,000, and 66,000 daltons. While the band corresponding to a protein of molecular mass of 66,000 daltons may represent dimers of the major viral capsid protein, the origin of the other minor bands is unknown.

Cytopathology. Ultrathin sections of local lesions and systemically infected leaves from *C. quinoa* and *C. amaranticolor* inoculated with KV-2 contained similar viruslike particles (hereafter called "virus particles" or simply "particles") and inclusions. Inclusions and particles were most abundant, however, in the local lesions of *C. quinoa*. Fusiform inclusions consisted of massive aggregates of parallel virus particles and pockets of cytoplasmic materials, vesicles, or microbodies (Fig. 8A). Smaller aggregates of virus also occurred along organelle membranes and the tonoplast. These inclusions and aggregates resembled those induced by KV-1 (5) and are typical of inclusions observed in tissues infected by carlaviruses. Bands of virus particles, measuring 600–680 nm in width, and "fingerprintlike" inclusions (Fig. 8B) also were observed in cells within the local lesions. Except for the width, these inclusions resembled those observed commonly by others in cells infected by potexviruses. Large quantities of endoplasmic reticulum (often dilated), vesicular bodies, and large vacuoles were observed in the cytoplasm surrounding virus inclusions.

The cytopathology of KV-1 and KV-2 were similar in kalanchoës (5). Cells of infected kalanchoës contained individual virus particles dispersed in the cytoplasm and in small aggregates of particles. However, virus-containing cells were difficult to find in most sections.

DISCUSSION

Kalanchoës are naturally infected or susceptible to infection by several viruses including alfalfa mosaic virus (*unpublished*), a

TABLE 1. Comparison of $A_{405\ nm}$ values obtained from indirect ELISA after rabbit antisera to eight carlaviruses were reacted with kalanchoë virus isolates KV-1 or KV-2, or healthy *Chenopodium quinoa* crude sap antigens coated to wells of microtiter plates and bound antibodies were detected by alkaline phosphatase-conjugated goat antirabbit gamma globulin

		Coating antigen							
		KV-1[a]		Healthy sap[b]		KV-2[a]		Healthy sap[b]	
Antisera (dilution)	Reciprocal of homologous titer according to donor	Antisera absorbed[c]	Antisera non-absorbed[d]	Antisera absorbed[c]	Antisera non-absorbed[d]	Antisera absorbed[c]	Antisera non-absorbed[d]	Antisera absorbed[c]	Antisera non-absorbed[d]
Carnation latent (1/1,000)	8,192	...[e]	1.443	...	0.219	...	1.670	...	0.236
Elderberry A (1/500)	4,096	...	0.131	...	...	...	0.133	...	...
Hippeastrum latent (1/500)	2,048	...	0.098	...	0.227	...	0.050	...	0.295
Narcissus latent (1/500)	1,024	...	0.012	...	...	...	0.021	...	...
Potato S (1/500)	256	...	1.243	...	0	...	1.104	...	0.026
Chrysanthemum B (1/200)	260,000	0.897	1.296	0.140	1.590	1.072	1.782	0.107	1.355
Lily symptomless (1/200)	2,048	1.382	1.647	0.065	0.309	1.391	1.525	0.062	0.338
Red clover vein mosaic (1/200)	4,096	0.299	0.150	0.092	0.451	0.558	0.596	0.091	0.612
KV-1 (1/2,000)	512	1.913	1.874	0.123	0.211	...	...	...	...
KV-2 (1/2,000)	256	...	...	...	...	2	2	0.084	0.140
Normal serum (1/200)		0.168	0.112	0.071	0.183	0.119	0.109	0.086	0.186

[a] Virus was purified, then diluted to $A_{260\ nm} = 0.05$ in coating buffer.
[b] Healthy leaves of *C. quinoa* when ground in coating buffer (1:10, w/v) and filtered through a Kimwipe paper laboratory towel.
[c] Antisera were diluted in a 1:20 (w/v) extract of healthy *C. quinoa* in PBS-Tween and incubated for 2 hr at 30 C prior to being dispensed into microtiter plate wells.
[d] Antisera were diluted in PBS-Tween and incubated for 2 hr at 30 C prior to being dispensed into microtiter plate wells.
[e] ... = Not tested.

strain of tobacco streak virus (R. W. Fulton, *personal communication*), a potyvirus (4), a carlavirus designated KV-1 (5), and a viruslike agent associated with top spotting (7).

Isolation of these viruses has been difficult or unsuccessful, perhaps because of inhibitors in the sap and/or low virus titers. Therefore, isolation by means of inoculating concentrated crude sap extracts from a number of cultivars of kalanchoës to herbaceous seedlings was tried. KV-2 was the only virus, other than KV-1, obtained from kalanchoës in this manner. KV-2 was characterized to determine its relationship to KV-1, which was formerly isolated from cultivar Rotkappchen.

Isolates KV-1 and KV-2 have the properties of a carlavirus, but they differ from each other in some biological properties and sensitivity to some chemicals. They are closely related serologically, but each possesses unique epitopes. Intragel cross-absorption serology using SDS-degraded virus was an effective method for demonstrating serological relationships and specificities with KV-1 and KV-2. Apparently, antiserum made to purified viruses contained significant quantities of antibody to degraded virus. According to the guidelines set forth by Hamilton et al (2), KV-1 and KV-2 appear to be strains of the same carlavirus, herein designated kalanchoë latent virus.

The serological relationships among carlaviruses and their serodiagnosis have been summarized by Wetter and Milne (10). When carnation latent virus antiserum has been used to determine serological relationships among carlaviruses, all viruses except honeysuckle latent, narcissus latent, poplar mosaic, and shallot latent have reacted with carnation latent virus antiserum. The better known members of the group are serologically interrelated. The serological differentiation indices have been derived for several viruses in the group (10).

Indirect ELISA was found to be a sensitive method for detecting serological relationships between KV-1 and KV-2 and other carlaviruses. Adams and Barbara (1) reported the use of $F(ab')_2$-based ELISA in studies of serological relationships among carlaviruses and found carnation latent, lily symptomless, and potato S viruses antigenically similar. The use of $F(ab')_2$ fragments was unneccessary in this study, since wells were easily coated by incubating the plates with virus in coating buffer. This report is thus the second to show strong serological relationships between some carlaviruses in ELISA reactions. Indirect ELISA also appeared to be more sensitive than IEM for demonstrating and quantitating serological relationships between carlaviruses.

KV-1 and KV-2 occur commonly in many cultivars of *K. blossfeldiana* (*unpublished*). KV-2 may not be distinguished from KV-1 in bioassay tests, because the virus titer in the inoculum prepared directly from kalanchoës may not be sufficient tc give a systemic infection in *Chenopodium* spp. Conversely, KV-1 in a mixed infection with KV-2 may go undetected in bioassays if a systemic reaction is obtained in *Chenopodium* spp., because local

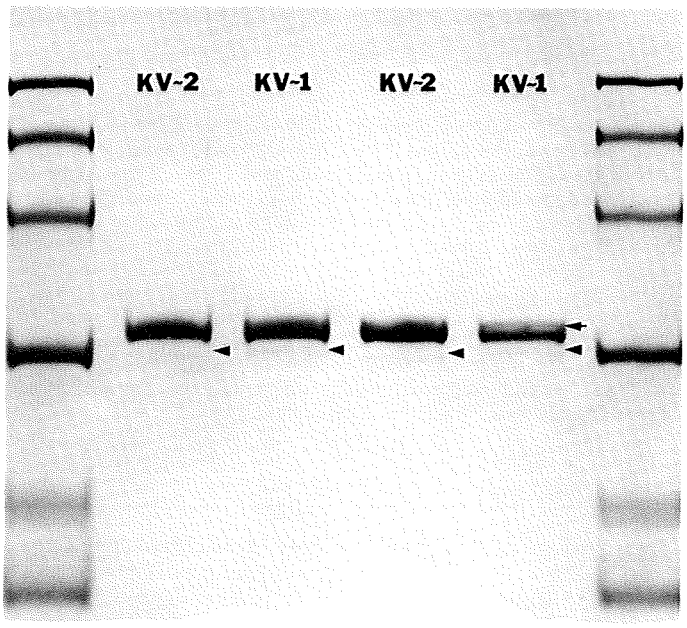

Fig. 7. Gel pattern obtained by electrophoresis of purified kalanchoë virus isolates KV-1 (lanes 3 and 5) and KV-2 (lanes 2 and 4) and molecular weight markers (lanes 1 and 6) on a 12% SDS-polyacrylamide slab gel for 4.5 hr at 20–27 mA and a constant 100V. A faster-migrating protein (◄) was associated with the major viral capsid protein (33–34 kdaltons) in all preparations of KV-1 and KV-2. A slower-migrating protein (–) was also present in one preparation of KV-1 (lane 5). The origin of the minor proteins is unknown.

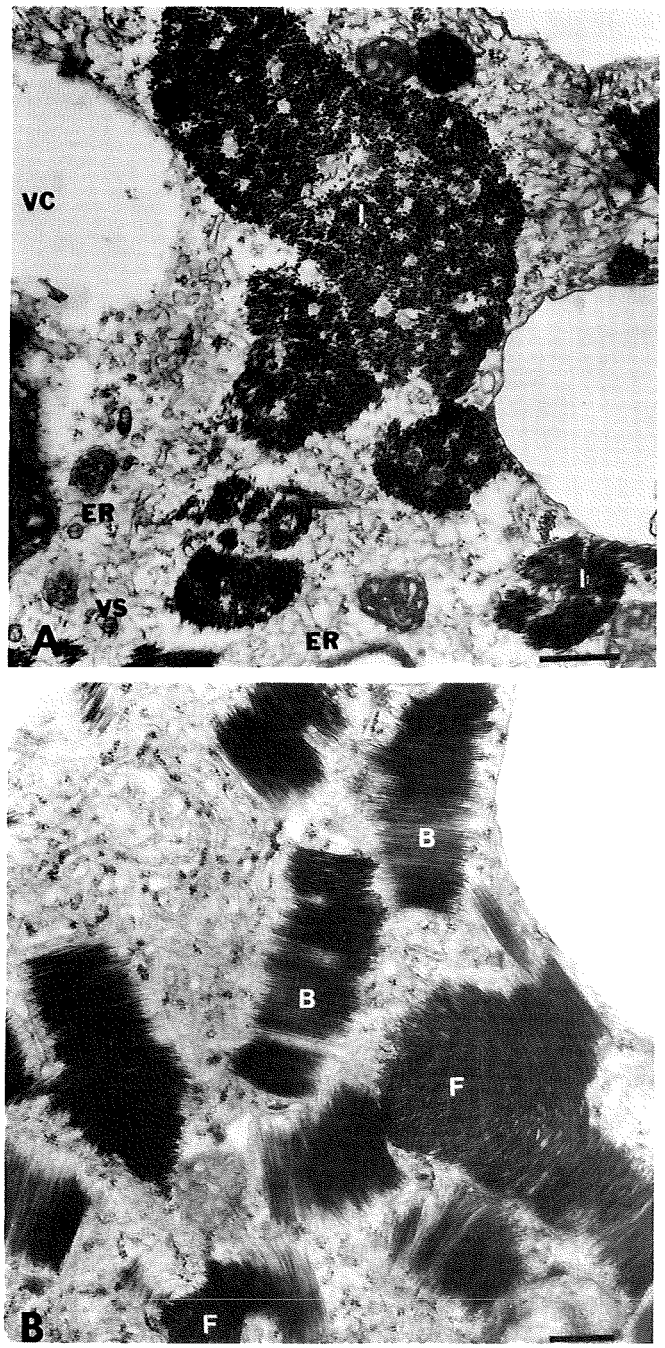

Fig. 8. Electron micrographs of ultrathin sections of local lesions on *Chenopodium quinoa* inoculated with kalanchoë virus isolate KV-2. **A,** Fusiform inclusions (I), cut in cross section or nearly so, consist of virions arranged in parallel but with lengths beginning at offset points along the length of the inclusion. Vesicles (VS), vacuoles (VC), and dilated endoplasmic recticulum (ER) are abundant. **B,** Bands of virions (B), consisting of a single tier of parallel virions with the ends more or less aligned and measuring 600–680 nm, and fingerprintlike (F) inclusions are numerous. Bars = 0.5 μm.

lesions produced by KV-1 and KV-2 are similar. The NL of KV-1 and KV-2 particles were slightly different in most comparative experiments. Because of the variability in the NL absolute values and the small differences involved (~30 nm), NL was not a reliable method for distinguishing the strains. ELISA techniques are under investigation as a means of detecting and differentiating KV-1 and KV-2 in kalanchoës.

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