Host Range, Purification, and Properties of a Flexuous Rod-Shaped Virus Isolated from Carrot

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ABSTRACT

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A virus was isolated from carrots and purified utilizing chloroform, differential ultracentrifugation, polyethylene glycol precipitation, and rate-zonal sucrose density-gradient centrifugation. Flexuous rod-shaped particles with a modal length of 736 nm were found in purified preparations. The virus was transmitted by aphids *(Myzus persicae* and *Cavariella aegopodii)* given 5- to 10-minute acquisition and transmission feeding periods. Properties in crude sap included: longevity in vitro = 2 days, thermal inactivation point = 50-55 C, and dilution end point = greater than 10^{-5}.

The virus was named carrot thin-leaf virus (CTLV). Virus morphology, vector relationships, and properties in crude sap suggest that CTLV belongs to the potyvirus group. Differences in host range, symptomatology, and serology distinguish CTLV from other members of the potyvirus group.

Additional key words: umbelliferous virus.

During the summer of 1973 we isolated a virus from carrots [*Daucus carota* L. var. *sativum* (Hoffm.)] grown commercially in central Washington that differed in one or more characteristics from other viruses that infect carrot (2). This report describes the host range, purification, and some properties of this apparently new virus, which we call carrot thin-leaf virus (CTLV).

MATERIALS AND METHODS

Virus source.—The virus isolate used for this study was obtained from a commercial carrot plant collected during 1973. After three serial local lesion transfers on *Chenopodium quinoa* Willd., the isolate was maintained in inoculated carrot seedlings, cultivar Imperator-58.

Culture of test plants.—Plants used for host range, assay, and purification were grown in a Corrulux greenhouse at 22-28 C in pots containing a mixture of loam, sand, and peat moss (2:2:1, v/v). Seed of these plants either were germinated in vermiculite and transplanted to 7.6-cm square plastic pots or were direct-seeded in 10-cm diameter clay pots, depending upon the growth characteristics of the species.

Chenopodium quinoa and *C. amaranticolor* Coste & Reyn. used for purification and assay were transplanted to 7.6-cm square pots, shaded with two layers of cheesecloth, and provided 16-hour photoperiods of supplemental light at 4,300 lx from cool-white fluorescent tubes.

Mechanical inoculations.—Inoculations for purification were made by rubbing tissue extracts in 0.01 M potassium phosphate buffer, pH 7.0, onto *C. quinoa* having 7-8 fully expanded leaves. Plants used for host range studies were inoculated when 1-4 weeks old. Control plants were rubbed with phosphate buffer. All plants were dusted with 20-μm (600-mesh) Carborundum before inoculation.

Lesion assays were made on vigorously growing *C. quinoa* or *C. amaranticolor* trimmed to the two youngest fully expanded leaves. These leaves were dusted with Carborundum and rubbed with cotton swab dipped in inoculum. The assays were applied in a random design to four to six half-leaves.

Prior to inoculation, all plants were given 24-hour dark treatment. After inoculation, symptom development was observed in growth chambers maintained at 24 C under 5,400 lx illumination for 16-hour photoperiods.

Insect culture.—Nonviruliferous cultures of *Myzus persicae* Sulz., and *Cavariella aegopodii* Scopoli were established from single new-born nymphs and were maintained on caged radish and carrot plants, respectively. These plants were illuminated with 4,300 lx for 12-hour photoperiods at 24 C. Louise M. Russell, Systemic Entomology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland, confirmed the identity of each aphid culture.

Virus purification.—Several clarification methods were tested as follows: Nine grams of infected, senescent *C. quinoa* leaves were triturated in 18 ml of 0.01 M phosphate buffer, pH 7. The liquid was expressed through cheesecloth and divided into nine samples which were treated with various amounts of *n*-butanol, charcoal, chloroform, acetic acid (10%), or Mg-bentonite solution. After treatment, all samples were centrifuged at 6,600 *g* for 10 minutes and then concentrated for 1.5 hours at 80,000 *g* in a Spinco Model L preparative ultracentrifuge. Each pellet was resuspended in 1 ml of buffer and assayed for infectivity on *C. quinoa*.

A purification method based on the procedure described by Knesek et al. (3) was adopted. Fifty grams of senescent *C. quinoa* leaves were harvested 11-14 days after inoculation and frozen. The tissue was later homogenized in 150 ml of neutral 0.01 M phosphate buffer for two minutes at room temperature in a Waring Blendor previously coated with a silicone antifoam spray. The resultant liquid was expressed through cheesecloth, stored 15 minutes at 4 C, and then emulsified with an equal volume of chloroform for 30 minutes in an overhead stirrer at room temperature. The emulsion was broken by centrifugation in a Sorvall SS-1 rotor at 3,000 *g* for 30 minutes. The upper phase was removed and centrifuged at 71,000 *g* for 1.5 hours in a Spinco Model L preparative ultracentrifuge. Each pellet was suspended overnight in 4 ml of distilled water adjusted to pH 7.0. The suspension was centrifuged for 15 minutes at 6,000 *g* and the supernatant was layered in Beckman SW 25.1 tubes on 10 ml of distilled water (pH 7) containing 4% polyethylene glycol (MW = 6,000), 30% sucrose, and 0.12 M NaCl. Neutral distilled water was used to bring the volume of each tube to 30 ml. The tubes were centrifuged 2 hours at 60,000 *g*. Each pellet was resuspended overnight at 4 C in 2.5 ml of 0.01 M neutral phosphate buffer. After centrifugation at 6,600 *g* for 10 minutes, each supernatant was layered on rate-zonal sucrose density-gradient tubes and centrifuged at 60,000 *g* for 2 hours. The gradient tubes were prepared in SW 25.1 tubes by layering 4, 7, 7, and 7 ml of neutral distilled water solutions containing 10, 20, 30, and 40% sucrose, respectively. The tubes were equilibrated for 18 hours at 4 C prior to use.

Association of infectivity with rate density-gradient zones.—The density-gradient tubes were fractionated in 0.5 ml aliquots on an ISCO Model-D fractionator. Each aliquot was diluted with 11.5 ml of neutral distilled water and centrifuged at 80,000 *g* for 1.5 hours. Each pellet was resuspended overnight at 4 C in 1 ml of neutral distilled water and assayed on *C. amaranticolor*.

Serology.—Carrot thin-leaf virus antiserum was obtained from a rabbit given four 2-ml intravenous injections of virus-sucrose solution obtained from rate density-gradient columns. The second, third, and fourth injections were given 3, 6, and 10 days, respectively, after the initial injection.

Antisera for the relationship tests were kindly provided by A. F. Murant, Scottish Horticultural Research Institute, Dundee, parsnip mosaic virus (titer = 1/128); R. N. Campbell, University of California, Davis, celery mosaic virus (CeMV) type strain, poison hemlock virus strain of CeMV (1/1,256), and parsley virus strain of CeMV; R. Stace-Smith, Agriculture Canada, Vancouver, B.C., CeMV.

Antigen for standard microprecipitin serology tests was obtained from infected and healthy *C. quinoa* tissue. The tissue was purified in the usual manner except that the density-gradient centrifugation step was omitted.

Electron microscopy.—Electron microscopy was done at the Electron Microscope Center, Washington State University, Pullman, using an Hitachi Model HU-125

TABLE 1. Symptom expression and virus distribution in carrot thin-leaf virus-infected plant species

Family and Species	Common name	Symptoms[a]	Virus distribution[b]	
			Inoculated leaves	Systemic tissue
Chenopodiaceae:				
Atriplex hortensis L.		S	+	–
Chenopodium amaranticolor Coste & Reyn.		CLL,RLL	+	–
C. ambrosioides L.	wormseed goosefoot	LCA	+	–
C. foliosum (Moench) Asch.		LCA	+	–
C. murale L.	nettleleaf goosefoot	LM	+	–
C. quinoa Willd.		CLL,NLL	+	–
Compositae:				
Cichorium endiva L.	endive	S	+	–
Zinnia elegans Jacq.	zinnia	S	+	–
Leguminosae:				
Lathyrus ordoratus L.	sweetpea	S	+	–
Pisum sativum L.	pea			
-OSU-42 & Alaska		Y,NLL	+	–
-447, Ranger, Little Marvel, Perfected Freezer, P.I. 193835		S	+	–
Early Sweet 11, and Perfected Wales				
Solanaceae:				
Nicotiana clevelandii Gray	tobacco	CM	+	+
Umbelliferae:				
Anthriscus cerefolium (L.) Hoffm.	chervil	T,W	+	+
Apium australe Thon.	sea celery	C,N,D,	+	+
Coriandrum sativum L.	coriander	T,W,M,V	+	+
Daucus carota L. var. *sativum*	carrot (5 cultivars)	T,W,M,V	+	+
Pastinaca sativa L.	parsnip	S	+	+
Petroselinum hortense Hoffm.	parsley	S	+	+

[a]A = area, C = chlorotic, D = death of outer leaves, L = local, LL = local lesion, M = mottle, N = necrotic, R = red ring, S = symptomless, T = thread-like leaflets, V = vein-clearing, W = twisted leaves, Y = yellow vein banding.

[b]Virus recovered (+) or not recovered (–) from inoculated leaves or noninoculated young systemic tissue by rub indexing on *C. quinoa*.

electron microscope. Virus for electron microscopy was obtained from the infectious density-gradient zones, concentrated in a Spinco No. 40 rotor at 80,000 *g* for 1.5 hours, and suspended at 4 C for 24 hours in 0.5 ml of buffer. The virus was fixed at room temperature with an equal volume of 3.5% glutaraldehyde and negatively stained with phosphotungstic acid at pH 7. Tobacco mosaic virus (TMV) particles (assumed to be 15 nm in diameter) were included in some preparations as a size reference.

RESULTS

Host range.—Carrot thin-leaf virus was recovered from the inoculated and young uninoculated leaves of the following species: *Nicotiana clevelandii* Gray, *Anthriscus cerefolium* (L.) Hoffm. (chervil), *Apium australe* Thon., *Coriandrum sativum* L. (coriander), *D. carota* var. *sativum, Pastinaca sativa* L. (parsnip), *Petroselinum hortense* Hoffm. (parsley), and from the inoculated leaves of ten species belonging to the Chenopodiaceae, Leguminosae, and Compositae (Table 1).

Fig. 1. *Left:* Carrot leaf infected with carrot thin-leaf virus showing chlorotic spots and the characteristic twisted, thread-like leaflets. *Right:* Healthy carrot leaf.

Most Chenopodiaceae developed local reactions 4-6 days after inoculation. *Chenopodium quinoa* and *C. amaranticolor* developed discrete chlorotic local lesions. *Nicotiana clevelandii* developed a faint chlorotic mottle whereas parsley and parsnip, even though systemically infected, showed no symptoms. Leaves of *A. australe* became highly chlorotic and the older leaves eventually developed necrosis and died.

Characteristic symptoms on carrot, chervil, and coriander appeared 2-3 weeks after inoculation. Most leaflet lobes of newly formed leaves were distorted and narrow (Fig. 1). Vein-clearing, chlorotic spots, and occasional faint mottling could be seen on some of the wider leaflet lobes of carrot and coriander.

Pea cultivar reactions to CTLV inoculation were varied. Two cultivars were nonhosts. The inoculated leaves of nine cultivars became infected; two of these displayed localized symptoms, but the other seven were symptomless.

Sixty-one species (from 17 families) which produced no symptoms after inoculation, and from which CTLV was not recovered by back inoculation to *C. quinoa,* were as follows: AIZOACEAE: *Tetragonia expansa* Thunb. AMARANTHACEAE: *Amaranthus gangeticus* L., and *Gomphrena globosa* L. APOCYNACEAE: *Vinca rosea* L. BALSAMINACEAE: *Impatiens balsamina* L. BORAGINACEAE: *Myosotis* sp. CARYOPHYLLACEAE: *Dianthus caryophyllus* L. CHENOPODIACEAE: *Beta vulgaris* L., *B. vulgaris* L. var. *cicla,* and *Spinacia oleracea* L. COMPOSITAE: *Ambrosia artemisifolia* L., *Articium minus* Bernh., *Lactuca sativa* L., and *Tagetes erecta* L. CONVOLVULACEAE: *Convolvulus arvensis* L. CRUCIFERAE: *Brassica nigra* (L.) Koch, *B. oleracea* L. var. *virides, B. oleracea* L. var. *botrytis, B. oleracea* L. var. *gemmifera* DC., *Capsella bursa-pastoris* (L.) Medik., *Descurainia sophia* (L.) Webb, *Lepidium sativum* L., and

TABLE 2. Effect of clarification methods on the removal of host material and on the infectivity of carrot thin-leaf virus

Treatment	Color[a]		Infectivity[d]
	Supernatant[b]	Pellet[c]	
None	lt. g	g	78
Chloroform (50:50)[e]	lt. y	br	75
Charcoal (12% by weight)	dark g	g	60
Chloroform-butanol (50:50)[e]	lt. y	br	3
Acetic acid (to pH 4.8)	clear	br	2
Butanol (8-1/2% by vol.)	lt. y	br	1
Mg-bentonite-43% solution (1 ml/10 g tissue)	lt. y	lt. br	37
Mg-bentonite (1 ml/5 g tissue)	clear	lt. br	29
Mg-bentonite (1 ml/1.25 g tissue)	clear	lt. br	0

[a] g = green, y = yellow, br = brown, lt. = light.
[b] After low speed centrifugation.
[c] After high speed centrifugation.
[d] Average number of lesions on each of six half-leaves of *C. quinoa*.
[e] Emulsified with an overhead stirrer.

Raphanus sativus L. CUCURBITACEAE: *Cucumis melo* L., *C. sativus* L., and *Curcurbita maxima* Dcne. GRAMINEAE: *Echinochloa crus-galli* (L.) Beauv., *Hordeum vulgare* L., *Secale cereale* L., *Triticum aestivum* L., and *Zea mays* L. var. *saccharata*. LEGUMINOSAE: *Arachis hypogaea* L., *Glycine max* (L.) Merr., *Lupinus* sp., *Phaseolus vulgaris* L. (12 cultivars), *Pisum sativum* L. 'Tom Laxton' and 'P.I. 193586'), *Sesbania exaltata* (Raf.) Cory, *Trifolium pratense* L., *Vicia sativa* L., and *Vigna sinensis* (Torner) Savi. MALVACEAE: *Althaea rosea* Cav., *Hibiscus moscheutos* L., and *Malva neglecta* Wallr. PLANTAGINACEAE: *Plantago lanceolata* L., and *P. major* L. var. *resularis*. SOLANACEAE: *Capsicum frutescens* L. var. *grossum, Datura stramonium* L., *Hyoscyamus niger* L., *Lycopersicon esculentum* Mill., *Nicandra physalodes* (L.) Gaertn., *Nicotiana glutinosa* L., *N. tabacum* L., *Petunia hybrida* Vilm, *Physalis drumondi, P. floridana* Rydb., *P. longifolia* Nutt., *Solanum capicastrum* Link, *S. integrifolium* Poir., *S. melongena* L., and *S. tuberosum* L. UMBELLIFERAE: *Apium graveolens* L. var. *dulce*.

Stability of infectivity.—General properties of CTLV in neutral 0.01 M phosphate buffer or distilled water extracts from infected *C. quinoa* leaves were: dilution end point = greater than 10^{-5}, thermal inactivation point between 50 and 55 C, and longevity in vitro at 22 C = 2 days. Carrot thin-leaf virus infectivity was not noticeably altered after storage in crude sap for 2 days or in purified preparations for 1 week at 4 and −20 C, or in leaf tissue for 8 days at 4 C and for 10 months at −20 C. However, infectivity decreased by over 95% after storage in leaf tissue for 1 month at 4 C or in preparations from rate density-gradient columns stored for 1 week at 21 C.

Aphid transmission.—Both aphid species tested transmitted CTLV. Three of five *M. persicae* and four of 10 *C. aegopodii* transmitted CTLV from infected carrots to healthy carrot seedlings after acquisition and transmission feeding times of 5 and 10 minutes, respectively. Symptoms caused by CTLV first appeared on the test plants 16 days after the transmission feeding.

Purification.—All clarification procedures removed some green host material from CTLV-infected *C. quinoa* leaf homogenates. However, most treatments also removed much of the infectivity (Table 2). Only chloroform emulsion clarified the homogenate without reducing the infectivity.

When viewed by overhead light against a black background rate zonal density-gradient tubes containing purified preparations from healthy or diseased tissue exhibited a faint visible noninfectious zone located 0-4 mm below the meniscus. Tubes containing diseased preparations also exhibited two discrete visible zones located 27-29 and 30-31 mm from the meniscus (Fig. 2). The 30-31 mm band was light and was encompassed by a light opalescence which occurred 30-50 mm below the meniscus. Infectivity was associated with both lower zones and the related opalescent area. Samples from both

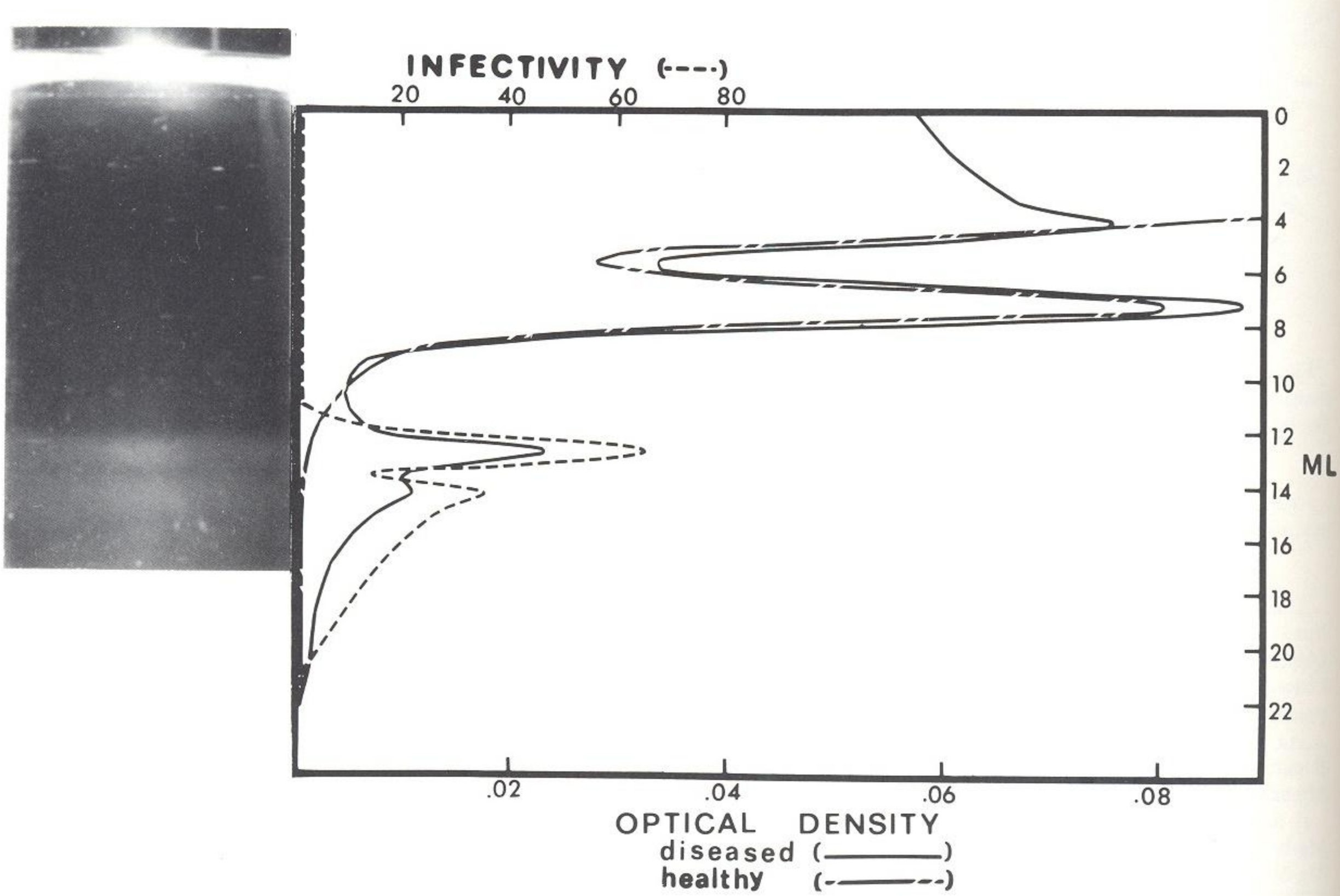

Fig. 2. *Left:* Upper 18 ml of rate density-gradient column after centrifugation of preparation from *Chenopodium quinoa* infected with carrot thin-leaf virus. Note the two opalescent zones which are associated with infectivity. *Right:* Infectivity (local lesions per half-leaf) curve for tube on the left and ISCO adsorption curves for rate density-gradient tubes containing healthy or diseased material.

zones produced typical symptoms on carrot and *C. quinoa*. Electron microscope observations showed that some end-to-end aggregation occurred in samples from the 27-29 mm zone. However, much aggregation occurred in preparations from the bottom zone and the lower opalescent area.

Occasionally a yellow zone was observed in rate density-gradient tubes layered with either healthy or diseased materials. This zone could be observed under room light against a white background 14-17 mm below the meniscus. The yellow band was not visible in Fig. 2 because a dark background was used. However, the yellow pigment did absorb ultraviolet light and consequently was detected during the fractionation on the ISCO Model-D fractionator. This yellow zone always was obtained during initial purification attempts made during late summer, but was absent in trials made with *C. quinoa* grown during the winter.

Light adsorption properties.—Infectious 0.25-ml aliquots were removed by the ISCO fractionator from the 27-31 mm region of rate density-gradient tubes. These solutions had ultraviolet adsorption spectra typical of nucleoproteins with low nucleic acid content. Solutions from both infectious zones exhibited low light scattering above 320 nm and had 260/280 ratios of 1.18 - 1.19, suggesting a nucleic acid content of about 4.2%.

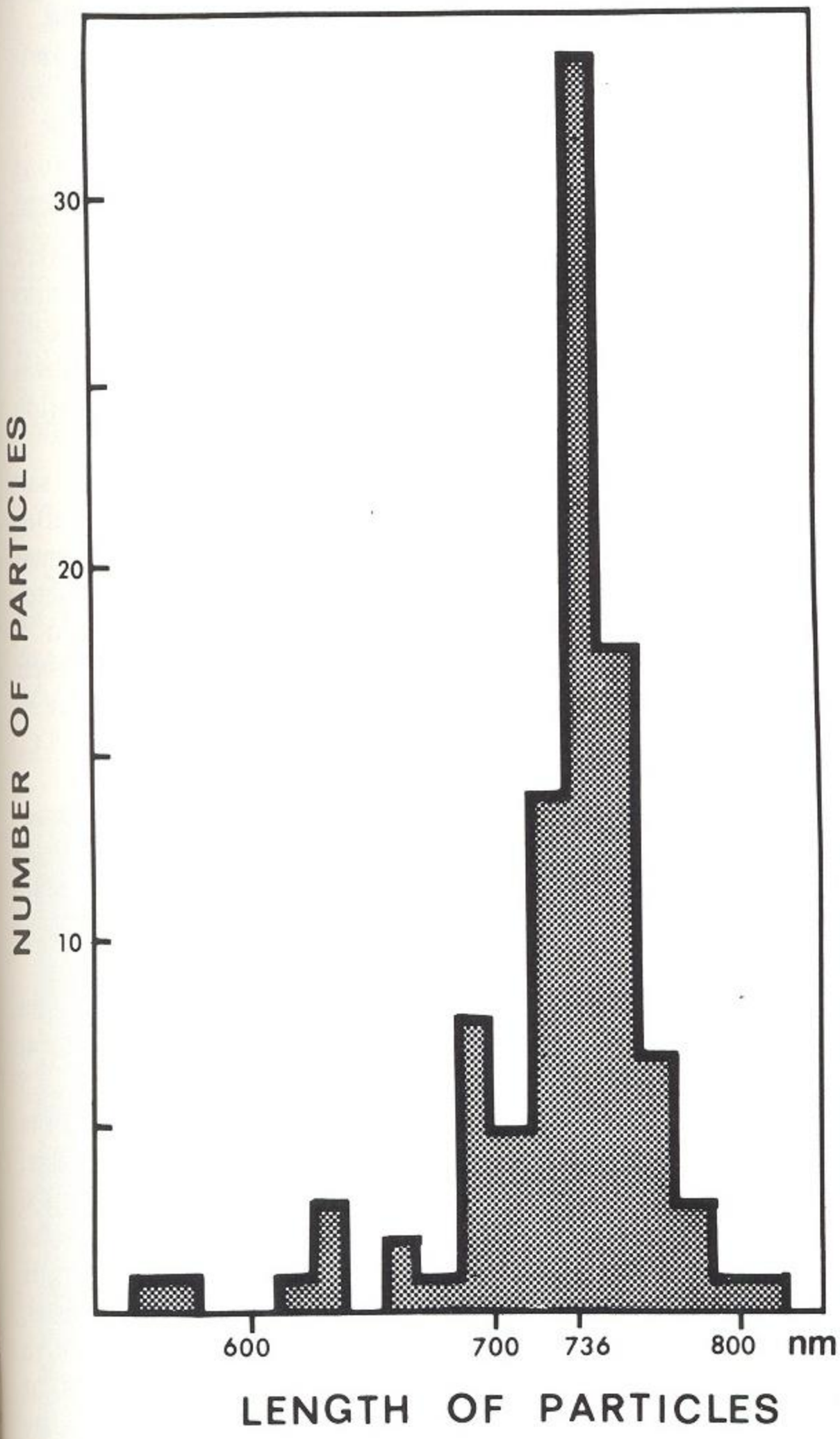

Fig. 3. Histogram showing the length distribution of 100 carrot thin-leaf virus particles from rate density-gradient zones in the 550 to 820 nm range. Particles in this range represented 78% of all measured particles.

Electron microscopy.—Electron micrographs of preparations from density-gradient virus zones showed flexuous rod-shaped particles measuring about 11-nm wide and from 175 to 1,600 nm in length. The length of 78% of 253 measured particles ranged between 550 and 820 nm. The remaining 22% had widely varied lengths. The histogram (Fig. 3) of 100 particles in the 550- to 820-nm range showed the modal length (736 nm) and represented 34% of the population.

Serology.—In microprecipitin serology tests, CTLV formed white flocculent precipitates with its homologous antiserum diluted up to 1/64. The virus did not react with normal serum, buffer, or antiserum prepared against parsnip mosaic virus (PMV), celery mosaic virus (CeMV), poison hemlock strain (PHV) of CeMV, or parsley virus strain (PV) of CeMV.

DISCUSSION

General properties in crude sap, particle morphology, estimated percent nucleic acid, and vector relationships suggest that CTLV is a member of the potyvirus group. Several members of this group can infect various species of the Umbelliferae. These are carrot mosaic virus (CarMV) (1), PMV (4), and celery mosaic virus: type strain (CeMV) (5, 6), PHV strain (6), and PV strain (6).

Carrot thin-leaf virus differed from PMV and the three CeMV strains in host range, symptomatology, and serology. Under our conditions, only CTLV infected *N. clevelandii* and produced the extremely thin leaves on carrot and coriander. On the other hand, CTLV did not infect celery or *Spinacea oleracea* which are hosts for CeMV and PMV, respectively. In our tests, antisera to PMV and to three strains of CeMV failed to react with purified CTLV. We were unable to test a possible serological relationship between CTLV and CarMV. However, reported differences in both host range and symptomatology (1) suggest that the two viruses may be unrelated.

Although CTLV appears to be a previously undescribed virus, preliminary surveys suggest that it frequently occurs in carrots grown in Washington.

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