

Host Range, General Properties, Purification, and Electron Microscopy of Hop Latent Virus

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ABSTRACT

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Hop latent virus was latent in mosaic-sensitive hop seedlings and produced a systemic chlorotic flecking in cultivar Cluster hop seedlings. The virus produced pinpoint necrotic local lesions in the primary leaves of *Phaseolus vulgaris* 'Kinghorn', *Chenopodium album*, *C. hybridum*, *C. quinoa*, *C. urbicum*, and *Atriplex hortensis* var. *cupreata* also were susceptible. The virus was extracted from hop

seedlings by acid precipitation, sucrose-polyethylene glycol-NaCl precipitation, and sucrose density-gradient centrifugation. One visible zone appeared 26-29 mm below the meniscus. The sedimentation coefficient was 176S and the normal length of the rod-shaped particle was 610 nm. The virus reacted with antiserum to potato virus M.

Virus diseases of hop, *Humulus lupulus* L., are common in hop yards throughout the world and many virus symptoms have been reported (11, 12). However, with few exceptions, the specific virus or virus combination responsible for a particular syndrome has not been determined. Several rod-shaped viruses have been reported in hop plants (10) but their pathogenicity has not been established.

Preliminary to a project to characterize hop viruses and relate them to diseases of hop, we have isolated and differentiated three rod-shaped viruses from hop in the state of Washington (9, 10). The following report describes the general properties, host range, purification, electron microscopy, serology, and symptomatology in hops of hop latent virus (HLV). A preliminary report was made on the purification of this virus (8).

MATERIALS AND METHODS

The virus used in this investigation was transmitted from a symptomless hop plant of the cultivar Petham Golding to *Chenopodium murale* L. (10). Then the virus was transferred from systemically-infected *C. murale* tissue back to cuttings of a virus-free hop seedling. The virus reached a higher concentration in this seedling than in others tested (7).

Hop seeds were obtained from open-pollinated commercial plantings of Early Cluster and Late Cluster hop cultivars and stratified in moist sand at 4 C for 90 days, germinated in vermiculite, and the seedlings were

transplanted into 15-cm diameter plastic pots. Softwood cuttings were taken after the seedlings were approximately 2.5 m tall. All plants were grown in a greenhouse in which the temperature ranged from 20-27 C.

Primary leaves of *Phaseolus vulgaris* L. 'Kinghorn', a local lesion host, were used to assay virus infectivity (8). Postinoculation conditions were those of the greenhouse except when determining the physical properties where conditions were 13,000 lux, 25 C, for a 16-hr photoperiod.

Host range.—Inoculum consisted of stabilized crude extract (see below) or clarified extracts in which virus was concentrated by high speed centrifugation. Inoculated test plants were indexed on Kinghorn bean after 3-4 wk using crude juice or partially clarified juice of which the contents were concentrated and examined with an electron microscope for the presence of rod-shaped particles prior to assay.

Preparation of stabilized crude extract.—It was necessary to stabilize the virus in hop juice prior to most experiments. Stability was obtained by triturating hop tissue in a Waring Blendor for 2 min with 0.05 M sodium phosphate buffer, pH 8.0, (6 ml/g tissue) containing 0.2% nicotine alkaloid, 0.2% ascorbic acid, and 0.5 g polyvinylpyrrolidone (PVP) per gram of tissue. The homogenate was filtered through cheesecloth and the liquid portion was held at -19 C for 1-4 days, after which the extract was thawed and centrifuged at 2,000 g for 10 min. This supernatant liquid is referred to as stabilized crude extract.

Clarification.—Hop plants were used as a virus source for clarification and purification (Table 2). The virus was precipitated from stabilized crude extracts by

polyethylene glycol (PEG) MW 6,000, 5 g/100 ml extract, and was assayed as a concentrated preparation in 0.3 ml of 0.01 M sodium phosphate buffer, pH 8.0. The pH of stabilized crude extracts was adjusted to pH values ranging from 7.3 to 3.2 with 2 M acetic acid. The supernatant liquid (hereafter called *the supernatant*) and precipitate were tested for infectivity. After storage at 4 C for 30-60 min at the designated pH values, the supernatants and precipitates were suspended in the original volume of buffer and both precipitate and supernatant adjusted to pH 7.3 with 0.25 M NaOH and assayed.

Purification.—Fifteen ml of the final supernatant from the acid precipitation process was layered on 10 ml of a mixture of 4% PEG (MW 6,000), 50% sucrose, and 0.7% NaCl in 0.01 M sodium phosphate buffer, pH 8.0, placed in a density-gradient tube (6) and centrifuged for 2 hr at 24,000 rpm in a SW 25.1 rotor.

Electron microscopy.—Electron micrographs were made with a Hitachi HS-8-1 electron microscope and calibrated with a lined grid (2,157 lines per mm). Virus was prepared for particle-length measurements by the following four treatments: (i) negatively stained with 1% PTA, (ii) fixed with 3.5% glutaraldehyde and then stained, (iii) shadow-cast with platinum-palladium (80:20), and (iv) fixed and shadow-cast.

Serology.—Antiserum to HLV was prepared in a rabbit given four intravenous injections of virus from the infectious zone of density-gradient columns. The

injections were administered over a period of 7 days. Bleedings were taken at 2-3 day intervals over a period of 26 days beginning 9 days after the first injection.

Serological comparisons were made with antisera to potato virus S, potato virus M, and carnation latent virus. The homologous titer of each antiserum was 1:2,048.

RESULTS

Host range.—None of the species inoculated with stabilized crude extract from hop was infected as indicated by back inoculations to Kinghorn bean. However, when using concentrated virus as inoculum, the virus was recovered from *Atriplex hortensis* L. var. *cupreata*, *Chenopodium album* L., *C. hybridum* L., *C. murale* L., *C. quinoa* Willd., and *C. urbicum* L. On occasion, chlorotic lesions developed after 3 wk in inoculated leaves of *C. murale* and *C. quinoa* but the infection was usually latent in all of these hosts. Pinpoint necrotic local lesions developed in primary leaves of Kinghorn bean (Fig. 1). Hop latent virus was not recovered from *Apium graveolens* L., *Beta vulgaris* L., *Brassica oleracea* L. var. *capitata*, *B. pekinensis* (Lour.) Rupr., *Capsicum annum* L. 'Long Red', *Chenopodium amaranticolor* Coste & Reyn., *C. ambrosioides* L., *C. bonus-henricus* L., *C. botrys* L., *C. capitatum* (L.) Asch., *C. polyspermum* L., *Convolvulus arvensis* L., *Cucumis sativus* L., *Datura stramonium* L., *Gomphrena globosa* L., *Helianthus annuus* L. 'Russian Mammoth', *Lycopersicon esculentum* Mill., *Malva rotundifolia* L., *Medicago sativa* L. 'Ranger', *Nicotiana clevelandii* Gray, *N. debneyi* Domin., *N. glutinosa* L., *N. rustica* L., *Physalis floridana* Rybd., *Plantago lanceolata* L., *P. virginica* L., *Solanum melongena* L., *Trifolium pratense* L. 'Kenland', *Urtica dioica* L., *Vigna sinensis* (Torner) Savi, or *Vinca rosea*.

All of the Cluster hop seedlings inoculated with HLV developed a systemic chlorotic flecking within 2-6 wk (Fig. 2). Usually one or two leaves on each plant developed chlorosis along one of the side main veins accompanied by downward curling of that side of the leaf (Fig. 2). Many flecks per leaf developed in certain hop seedlings but in others only a few developed. The flecking, once developed, usually remained with the plant during the growing season, and became necrotic as the plant approached maturity. Mosaic-sensitive Eastwell Golding hop seedlings did not develop symptoms when grafted to HLV-infected Cluster seedlings.

Virus stability and general properties.—Leaf tissue used to determine virus general properties was taken from the sixth node below the growing tips of young hop plants that had been systemically-infected for at least three mo. These plants were grown at 22 C in a glasshouse shaded to 13,000 lux at midday and a 12-hr photoperiod. Stability was tested by triturating with mortar and pestle 1 g of tissue in 2 ml of distilled water and incubating at 22 C. The virus was very unstable in crude hop juice and all infectivity usually was lost after 30 min. The use of buffer containing antioxidants and PVP and freezing the extract markedly preserved infectivity (Table 1). The stabilizing effect of PVP added to the buffer at rates of 0.5, 0.75, and 1.0 g/g tissue was tested. Use of PVP levels greater than 0.5 g/g tissue did not increase infectivity.

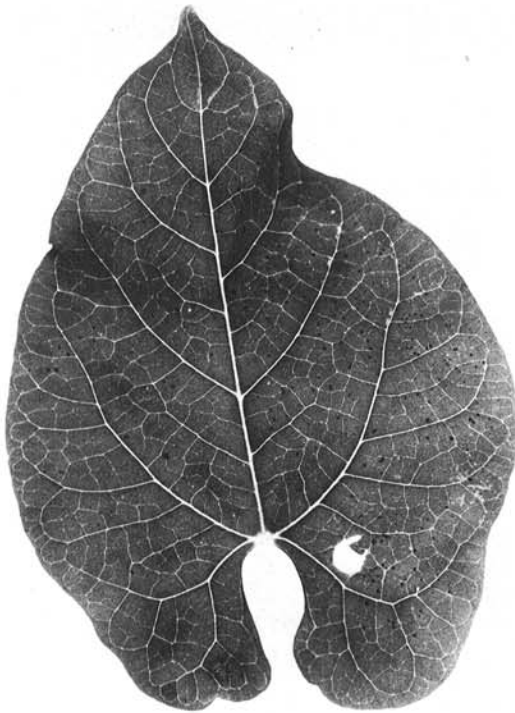


Fig. 1. Pinpoint necrotic local lesions produced by hop latent virus in inoculated primary leaves of *Phaseolus vulgaris* 'Kinghorn'.

The rapid inactivation of HLV in crude hop juice necessitated the use of stabilized crude extract for determining the dilution end-point and thermal inactivation point. In stabilized crude extract the dilution end-point was between 1:100 and 1:1,000, the thermal inactivation point was between 70 and 75 C and the virus

remained infective for at least 25 days at -19 C. The thermal inactivation point of purified virus was 71 C.

Clarification.—Clarification of hop juice was attempted using a number of standard procedures (Table 2) and by PEG and acid precipitation. The ether:carbon tetrachloride and charcoal treatments were the only treatments that did not reduce infectivity but neither provided sufficient clarification. Precipitation by PEG resulted in a relatively pure final preparation but infectivity was low. At pH values of 5.0 to 3.2 all infectivity was in the precipitate. A double cycle of precipitation at pH 5.0 finally was selected for clarification of HLV from hop tissue.

Purification procedure.—Stabilized crude extract from 20-40 g of infected hop tissue was adjusted to pH 5.0 with 12.0% acetic acid, held for 30 min at 4 C, and

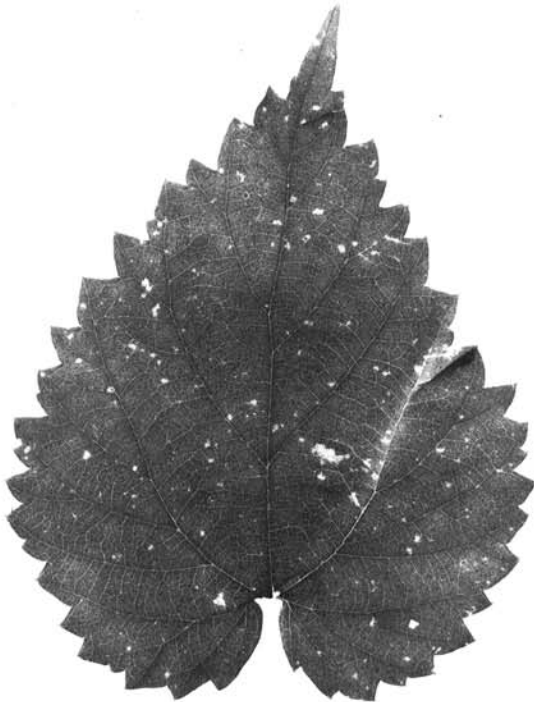


Fig. 2. Systemic chlorotic flecking and chlorosis along leaf vein of a hop seedling inoculated with hop latent virus.

TABLE 1. Effect of treatments on infectivity of hop latent virus in vitro at 22 C

Treatment ^a	Infectivity at intervals (hr) after preparation					
	0	0.5	5	16	27	42
Distilled water ^b	4 ^c	0	0	0	0	0
Buffer + PVP ^d	31	20	18	0	0	0
Buffer + PVP + freezing ^e	31	31	14	6	5	0

^aSupernatant of 1 g of young hop leaves ground in distilled water, buffer and polyvinylpyrrolidone (PVP) or buffer and PVP plus freezing, and then centrifuged 5 min at 2,340 g.

^bTwo ml of distilled water per 1.0 g tissue.

^cTotal lesions on seven half-leaves of Kinghorn bean primary leaves.

^dFour ml of PO₄ buffer pH 8.0 containing 0.2% nicotine alkaloid, 0.2% ascorbic acid, and 0.5 g PVP per g tissue.

^eSame as d, except the supernatant was frozen for 0.5 hr prior to assay.

TABLE 2. Effects of clarification treatments on hop latent virus infectivity

Treatment ^a	Relative infectivity high-speed pellet ^b	Treatment	Relative infectivity high-speed pellet
Untreated	100	Bentonite ^e	5
Ethanol ^c	7	Hydrated calcium phosphate ^h	0
Heat ^d	35	Activated charcoal ⁱ	100
Chloroform: <i>n</i> -butanol ^e	65	Ether: carbon tetrachloride ^j	100
<i>N</i> -butanol ^f	60		

^aApproximately 10-15 grams of hop tissue was ground in buffer and the extract frozen before use.

^bThe supernatants of a centrifugation at 15,000 g for 15 min were subjected to a 1-hr centrifugation at 100,000 g. Lesion numbers in Kinghorn bean primary leaves.

^cEthanol (90%) added at 50 ml per 100 ml extract and stirred 30 min.

^dThe juice was heated to 65 C in a water bath and left for 10 min then cooled in an ice bath.

^eEqual parts of chloroform and *n*-butanol added at 100 ml per 100 ml extract and stirred 30 min.

^f*N*-butanol added at 8 ml per 100 ml of extract and stirred 30 min.

^gMagnesium bentonite added at 1 ml per 6 g of tissue and swirled (4).

^hHydrated calcium phosphate stirred into extract at 0.9 g per 1 g of tissue (5).

ⁱActivated charcoal added at 10 g per 100 g tissue.

^jVolume of ether equal to extract was added, shaken for 5 min, and centrifuged at 2,340 g for 15 min. The bottom layer was removed and added to an equal volume of carbon tetrachloride, shaken for 5 min, and centrifuged at 15,000 g.

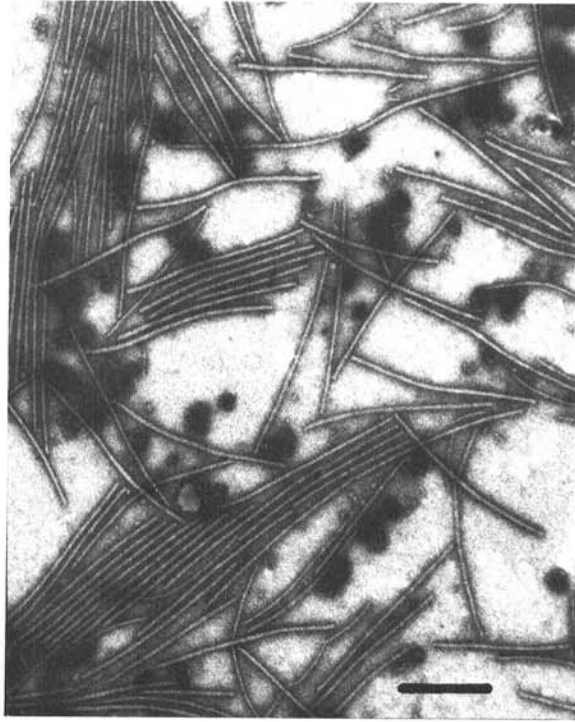


Fig. 3. Negatively-stained virus particles of hop latent virus from density-gradient zone. Bar indicates 300 nm.

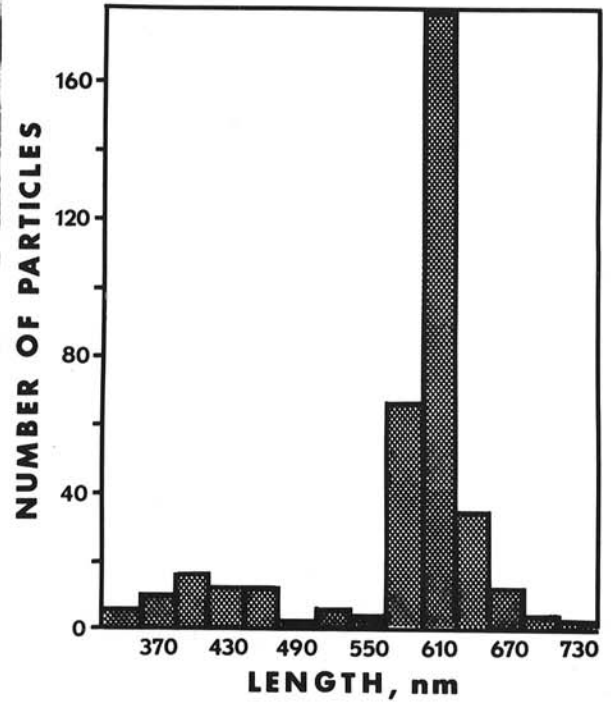


Fig. 4. Histogram showing length distribution of 400 negatively stained particles of purified hop latent virus.

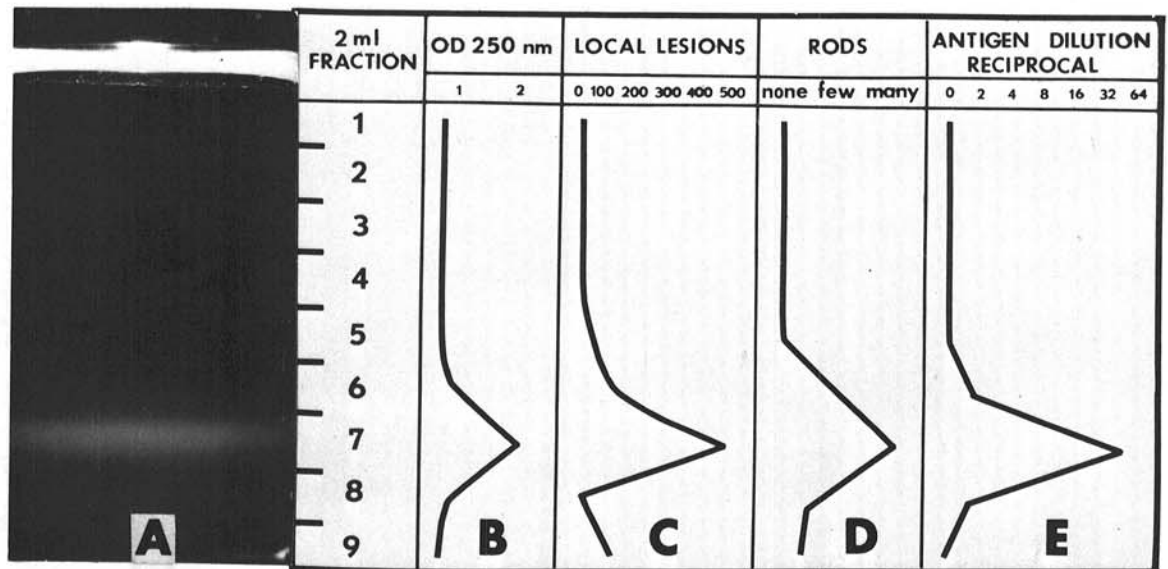


Fig. 5-(A to E). Association of activity with fraction No. 7 of a rate zonal density-gradient tube containing hop latent virus (HLV). A) Rate-zonal density-gradient tube showing visible zone at fraction No. 7. B) Scanning pattern (ISCO) of the tube on left. C) Local lesions produced in primary leaves of Kinghorn bean. D) The presence of rod-shaped particles. E) Serological end-point in microprecipitin tests using a 1/8 dilution of HLV antiserum (titer 1:256).

centrifuged for 10 min at 2,340 g. The precipitate was suspended in 1/15 of the original volume of 0.01 M phosphate buffer pH 8.0, adjusted to pH 6.8 with 1% NaOH, and centrifuged 30 min at 2,340 g. The final supernatant was centrifuged for 2 hr on a PEG-sucrose-NaCl mixture and the pellet was suspended in 2 ml of 0.01 M phosphate buffer (pH 8.0), centrifuged at 7,000 g for 10 minutes, and the supernatant was layered onto standard rate-zonal sucrose density-gradient tubes. After centrifugation for 2 hr at 24,000 rpm in an SW 25.1 rotor one visible zone was present 26-29 mm below the tube meniscus (Fig. 5-A). Healthy hop tissue treated similarly produced no visible zone. Solutions from the visible zone had an $A_{260/280}$ ratio between 1.14 and 1.20. The sedimentation rate (2) of the virus was 1.78 times the rate of the bottom fraction of alfalfa mosaic (AMV) (ATCC-106, J. B. Bancroft). The $S_{20,w}$ of HLV was 176 S if 99 S is used for the $S_{20,w}$ of the bottom component of AMV (1).

Infectivity was associated only with the visible density gradient zone (Fig. 5-C). Similarly, only this zone and the area below contained the slightly flexuous rod-shaped virus particles (Fig. 3, 5-D). No attempt was made to quantify the rod-shaped particles in the density-gradient column. The modal lengths of particles negatively stained or fixed with glutaraldehyde prior to staining were 595-625 nm (Fig. 4) which is slightly shorter than the 625-655 nm modal length obtained for particles which were only shadow-cast. The normal length according to the methods of Brandes and Bercks (3) of negatively stained fixed particles was 610 nm whereas the normal length of particles which were negatively stained without fixing was 620 nm.

Serology.—Antiserum produced against purified HLV had a homologous titer of 1/512 in ring-interface tests. Only the visible density-gradient zone produced reactions in microprecipitin tests with antiserum to HLV (Fig. 5-E). Purified virus reacted in microprecipitin tests with antiserum of potato virus M at dilutions up to and including 1/32. This reaction usually occurred within 6 hr. Reactions with antisera to potato virus S and carnation latent virus were inconclusive. Precipitate usually did not occur. When it did, it was always at dilutions of 1/2 and 1/4 and required 24-36 hr to form.

DISCUSSION

The name hop latent virus emerged in the literature as a means of referring to a 650-nm rod-shaped virus that is present in symptomless mosaic-sensitive hop cultivars (10, 13). We will continue to use the name hop latent virus for the virus described herein because it produces a latent infection in mosaic-sensitive hops and because the name hop latent virus is well established in the literature. However, the fact remains that the virus described in this

investigation produces symptoms in seedlings of Cluster hops, and the possibility exists that there are other rod-shaped viruses which produce latent infections in all hops. At this time we do not know what specific symptoms HLV produces in our commercial Early Cluster and Late Cluster cultivars because we have no commercial Cluster hop free of HLV.

Some characteristics of HLV which should be useful in establishing relationships among the rod-shaped viruses found in hop are as follows: (i) this virus can tolerate -19 C for several weeks and has a relatively high thermal inactivation point; (ii) it is not subject to extensive particle breakage in the presence of *n*-butanol; (iii) the virus remains infective after incubation for at least 1 hr at pH 3.2; and (iv) it has a rather narrow host range.

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