

Etiology

Characterization and Ultrastructural Studies of a Nepovirus from *Euonymus*

R. C. Larsen, R. C. Gergerich, and K. S. Kim

Department of Plant Pathology, University of Arkansas, Fayetteville 72701.

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ABSTRACT

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A virus of *Euonymus europaeus*, causing leaf mottling, stunting, dieback, and witches'-broom, was discovered in northwest Arkansas. The virus produced ringspots and chlorotic local lesions and later became systemic in *Chenopodium quinoa* and *Nicotiana rustica*. The virus was seed transmitted in *C. quinoa*. Purified virus preparations exhibited three centrifugal components, and buoyant densities of virus particles were 1.481 and 1.500 g/cm³ for middle and bottom components, respectively. The virus particles were isometric and averaged 28 nm in diameter. The $A_{260/280\text{nm}}$ ratio of purified virus was typically 1.9. The percentage of RNA was 42% in the bottom component and 36% in the middle component.

Analysis of virion proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis showed a single viral protein with a relative mass (M_r) of about 53,000. Nucleic acids of virus particles analyzed under denaturing conditions by horizontal agarose gel electrophoresis consisted of two ssRNA bands of M_r 2.2×10^6 and 2.6×10^6 , for RNA-2 and RNA-1, respectively. Ultrastructural examination of infected plants revealed cytopathic effects characteristic of nepoviruses. This virus is closely related serologically to the elm and the birch strains of cherry leaf roll virus and will be referred to as the *Euonymus* strain of cherry leaf roll virus.

Additional keywords: cytopathology, electron microscopy.

Few virus diseases have been reported on various species of the horticultural shrub, *Euonymus*, a member of the family Celastraceae, and these viruses were primarily reported in Europe, Japan, and the United States. A virus disease of *Euonymus* from

Europe, *euonymus* mosaic, is caused by a strain of tobacco necrosis virus (18). An unnamed rhabdovirus (12), a strain of cucumber mosaic virus (2), strawberry latent ringspot (23), and a filamentous virus have also been reported (21). In 1985, *euonymus* chlorotic ringspot was reported in the United States by Puffinberger and Corbett (22) to be caused by a strain of tomato ringspot virus (TomRSV-Eu).

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Recently, a diseased *Euonymus europaeus* L. plant exhibiting symptoms of leaf mottling, interveinal chlorosis, stunting, witches'-broom, and dieback was found in northwest Arkansas (17). Preliminary examination indicated a previously undescribed virus, which was later found to be a strain of cherry leaf roll virus (CLRV).

This paper describes the isolation, characterization, serology, and ultrastructure of this isolate referred to as the euonymus strain of CLRV (CLRV-Eu).

MATERIALS AND METHODS

Virus source and maintenance. The virus was isolated from *E. europaeus* by macerating symptomatic leaf tissue with 0.1 M phosphate buffer, pH 7.2, containing 10 mM Na₂SO₃, and rubbing the extract onto Carborundum-dusted leaves of *Chenopodium quinoa* Willd. and *Nicotiana rustica* L. Subsequent transmissions to *C. quinoa* and other hosts were made using 0.1 M phosphate buffer, pH 7.2, containing 20 mM Na₂SO₃. The isolate used in this study was passed through two successive local lesions in *C. quinoa* and maintained in *C. quinoa* and *N. rustica*.

Host range. The host range was determined by mechanical inoculation of different plant species with extracts from CLRV-Eu infected *N. rustica*. Host plants were evaluated by visual observation. Symptomless plants were assayed for CLRV-Eu infection by Ouchterlony gel diffusion tests and also by back-inoculation to *C. quinoa*.

Nematode and seed transmission. Nematode transmission studies were carried out using *Xiphinema americanum* Cobb (identified by R. Robbins, University of Arkansas, Fayetteville). Source plants of *C. quinoa* and *N. rustica* growing in a sterile mixture of peat and vermiculite were inoculated at the four-leaf stage with CLRV-Eu. After symptoms appeared (in about 6 days), 100 nematodes/pot were added to the soil containing infected or healthy source plants. After a 14-day acquisition period, infected and healthy source plants were excised below the soil level, and two test plants of the same type as the source hosts were planted in the nematode-containing soil. Test plants were observed for symptom expression for 3 wk, and roots and leaves were then tested for virus by back-inoculation to *C. quinoa* and by Ouchterlony gel diffusion tests.

Seed was collected from CLRV-Eu infected *C. quinoa* and sown in pots containing a sterile mixture of peat and vermiculite. Month-old seedlings were tested individually for virus infection by local lesion assay on *C. quinoa*.

Physical properties. Thermal inactivation point, dilution end point, and longevity in vitro of the virus were determined using sap extracted from systemically infected *C. quinoa* 10 days after inoculation. *C. quinoa* served as the local lesion assay host.

Virus purification. A modification of a method previously described for purifying beet curly top virus (16) was used to purify CLRV-Eu. Systemically infected *C. quinoa* or *Nicotiana tabacum* L. 'Kentucky-16' leaves were harvested 10–12 days after inoculation. Tissue was homogenized in a Waring blender using cold 0.1 M phosphate, 20 mM Na₂SO₃ buffer, pH 7.2, in a ratio of 2 ml per gram of tissue. The extract was expressed through cheesecloth, and Triton X-100 was added to a final concentration of 1%. After being stirred overnight (4 C), the sap was clarified by the addition of 15% (v/v) chloroform and stirred for 10 min at 4 C. The phases were separated by centrifugation at 10,000 g for 10 min. The aqueous phase was decanted through cheesecloth and two Kimwipe tissues. The virus was precipitated with 8% (w/v) polyethylene glycol (MW 8000), 0.2 M NaCl, and stirred at 4 C for 1.5 hr. The precipitate was collected by centrifugation for 15 min at 10,000 g and resuspended overnight at 4 C in one tenth the original extract volume in 0.1 M phosphate buffer, pH 7.2, and 0.5% Triton X-100. The suspension was centrifuged for 10 min at 12,000 g, and the supernatant was centrifuged for 1.5 hr at 150,000 g. The pellets were resuspended in 20 ml of 0.1 M phosphate buffer, pH 7.2, stirred overnight at 4 C, and centrifuged for 10 min at 12,000 g. The supernatant was centrifuged for 1 hr at 120,000 g, and the pellets were resuspended in 0.1 M

phosphate buffer, pH 7.2. The virus was further purified by rate zonal centrifugation in linear sucrose gradients (10–40%) for 2.5 hr at 25,000 rpm in a Beckman SW-27 rotor.

Buoyant density determination. Purified virus preparations were adjusted to a density of 5 g/cm³ with CsCl and were centrifuged for 20 hr at 150,000 g. Virus bands were removed, and the refractive index determined using an ABBE Mark II Digital Refractometer (A. O. Reichert, Buffalo, NY). Buoyant density was determined as described by Vinograd and Hearst (27). The percentage of RNA was calculated using the method of Sehgal et al (24).

Antiserum production and serology. A rabbit was given four subcutaneous injections of 1 mg/ml of purified virus at weekly intervals. The first injection was prepared by emulsifying the virus with an equal volume of Freund's complete adjuvant (Difco). Virus for subsequent injections was prepared using Freund's incomplete adjuvant. Antiserum titers were determined by the Ouchterlony gel diffusion test using expressed sap from both healthy and CLRV-Eu infected *C. quinoa* as antigens.

Serological relationships were determined by Ouchterlony gel diffusion tests using antisera against TomRSV-Eu (M. K. Corbett, University of Maryland), CLRV-Eu, the elm isolate of cherry leaf roll virus (CLRV-Elm), tobacco ringspot virus (TobRSV), and TomRSV (ATCC No. 13). J. I. Cooper (Institute of Virology, Oxford, England) kindly supplied the antigen and antiserum to the birch isolate of cherry leaf roll virus (CLRV-Birch).

Protein and nucleic acid analysis. Capsid protein from purified CLRV-Eu was prepared as described previously (6,7) and labeled with 2-methoxy-2,4-diphenyl-3[2H]-furanone (15). Preparations were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 0.8-mm vertical mini-slab gel (Idea Scientific, Corvallis, OR) for 60 min at 200 V (6,14).

Nucleic acids were extracted from purified CLRV-Eu by a modification of a procedure by Falk et al (5). An equal volume of buffer ([0.2 M Tris-Cl, 1.5% SDS, 1.5% *N*-lauroylsarcosine, pH 8.0] and 1/20 volume of bentonite [8]) was added to the virus preparation and shaken occasionally at room temperature for 30 min. The sample was extracted twice using one volume of water-saturated phenol, pH 8.0, containing 0.1% 8-hydroxyquinoline and one volume of chloroform:pentanol (24:1, v/v). The sample was vortexed to form an emulsion and kept on ice for 10 min. The emulsion was broken by low-speed centrifugation, the aqueous phase was subjected to a second extraction with chloroform/phenol, and nucleic acids were precipitated from the aqueous phase with 0.5 volume of 7.5 M ammonium acetate, 2.5 volumes of ethanol, and stored overnight at -20 C. Nucleic acids were collected by centrifugation at 12,000 g for 10 min, and the pellets washed in 70% ethanol and dried. Viral nucleic acids were then resuspended in sterile glass-distilled water and stored at -20 C.

The type of viral nucleic acid was determined by treating samples with DNase I at 10 µg/ml (Sigma) in the presence of 0.2 M NaCl, 0.1 M Tris, and 0.01 M MgCl₂, pH 7.5, for 30 min, or RNase A at 10 µg/ml (Sigma) in water for 30 min at room temperature. Nuclease reactions were stopped by the addition of proteinase K at 5 µg/ml (Sigma) in 2× SSC buffer (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) containing 0.05% SDS and incubation for 30 min at room temperature.

The single- or double-stranded nature of the nucleic acids was determined by the previously described lithium chloride fractionation method (1). One volume of the nucleic acid sample was added to one volume of 4 M LiCl and stored overnight at 4 C. The sample was centrifuged at 17,000 g for 10 min, and the pellet and supernatant were collected and saved separately. The pellet was resuspended in glass-distilled water, and the nucleic acids were precipitated with ethanol. Viral nucleic acid sizes were estimated by electrophoresis using a glyoxal denaturing system (19). The relative masses of CLRV-Eu nucleic acids were compared to those of the walnut strain of CLRV (CLRV-Walnut; kindly supplied by Dr. Adib Rowhani, University of California, Davis). An RNA ladder (Bethesda Research Laboratories, Gaithersburg, MD), tobacco mosaic virus RNA, and brome mosaic virus RNA

served as internal standards. Samples were analyzed on 1% horizontal agarose gels at 10 V/cm. The gels were denatured for 15 min in 50 mM NaOH, followed by neutralization in 50 mM sodium acetate, pH 4.8. The gels were stained in ethidium bromide (0.5 µg/ml) and photographed.

Ultrastructure and electron microscopy. Purified virus was negatively stained for 5 min on 300-mesh, carbon-coated Formvar grids using 2% uranyl acetate containing 1% ethanol and 1% acetic acid. Purified TMV particles were mixed with CLRV-Eu particles for a size reference. Leaf tissue from *Euonymus europaeus* exhibiting typical CLRV-Eu symptoms as well as tissue from *C. quinoa* and *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Monarch' showing systemic infection were prepared for thin-section electron microscopy as described previously (13,25). Sections were double stained for 15 min in a 2% aqueous solution of uranyl acetate and for 5 min in lead citrate. Specimens were examined using a JEOL 100 CX electron microscope.

RESULTS

Host range and symptoms. Leaves of *E. europaeus* infected with CLRV-Eu exhibited systemic mottling and interveinal chlorosis (Fig. 1A). Many leaves were rolled upward, a common symptom of cherry leaf roll virus (3,4). No leaves of infected euonymus plants showed ringspot symptoms associated with TomRSV-Eu (22) or strawberry latent ringspot virus (23) in *Euonymus*. Inoculated plants of *C. quinoa* showed chlorotic local lesions (Fig. 1B) with occasional ringspots in 4-5 days, followed by systemic infection with chlorosis and severe leaf distortion, tip dieback, and stunting of the entire plant. Chlorotic ringspots, a characteristic of nepoviruses (9), appeared on inoculated leaves of *N. rustica* accompanied by ringspots on subsequent leaves (Fig. 1C).

Plants susceptible to CLRV-Eu by mechanical transmission were: Aizoaceae—*Tetragonia expansa* Murr.; Amaranthaceae—*Gomphrena globosa* L.; Chenopodiaceae—*Beta macrocarpa* Guss., *B. vulgaris* L., *Chenopodium amaranticolor* Coste & Reyn., *C. murale* L., *C. quinoa* Willd., *Spinacea oleracea* L.; Cucurbitaceae—*Cucumis melo* L., *C. sativus* L., *Cucurbita maxima* Dene., *C. moschata* Dene., *C. pepo* L.; Leguminosae—*Glycine max* (L.) Merr., *Phaseolus vulgaris* L. 'Black Valentine', 'Cherokee Wax', and 'Pinto', *Vigna unguiculata* subsp. *unguiculata* 'Georgia 21' and 'Monarch'; Solanaceae—*Nicotiana clevelandii* Gray., *N. glutinosa* L., *N. rustica*, *N. tabacum* 'Kentucky-16', *Petunia hybrida* Vilm.

Plants not infected by CLRV-Eu were: Compositae—*Zinnia elegans* Jacq.; Malvaceae—*Gossypium hirsutum* L., *Hibiscus esculentus* L., *Malva parvifolia* L.; Solanaceae—*Datura stramonium* L., *Lycopersicon esculentum* Mill., *Physalis alkekengi* L., *Solanum melongena* L.

Nematode and seed transmission. *Xiphinema americanum* failed to transmit CLRV-Eu to *N. rustica* and *C. quinoa*. Serological assays and back-inoculation tests of sap from roots and leaves of the test plants were negative.

None of the germinated seed of *C. quinoa* tested for seed transmission of CLRV-Eu exhibited symptoms of infection. However, after inoculation with sap from the seedlings to *C. quinoa* indicator plants, typical CLRV-Eu symptoms occurred. Seed transmission occurred in 47/50 (94%) of the seedlings of *C. quinoa* grown.

Physical properties. Thermal inactivation of CLRV-Eu occurred in plant sap after 10 min at 55 C but not at 50 C. Sap remained infectious after storage at room temperature for 4 days but not after 5 days. The dilution end point assay indicated that crude sap was infectious at 10^{-3} but not at 10^{-4} .

Virus purification. Rate zonal centrifugation of CLRV-Eu produced two light-scattering bands in the lower portion of sucrose density gradients, whereas a broad golden band was observed near the top of the gradient. This phytoferritin band was eliminated from subsequent virus preparations before rate zonal centrifugation by absorption of the preparation with antiserum made against purified phytoferritin (R. C. Larsen and J. E. Duffus,

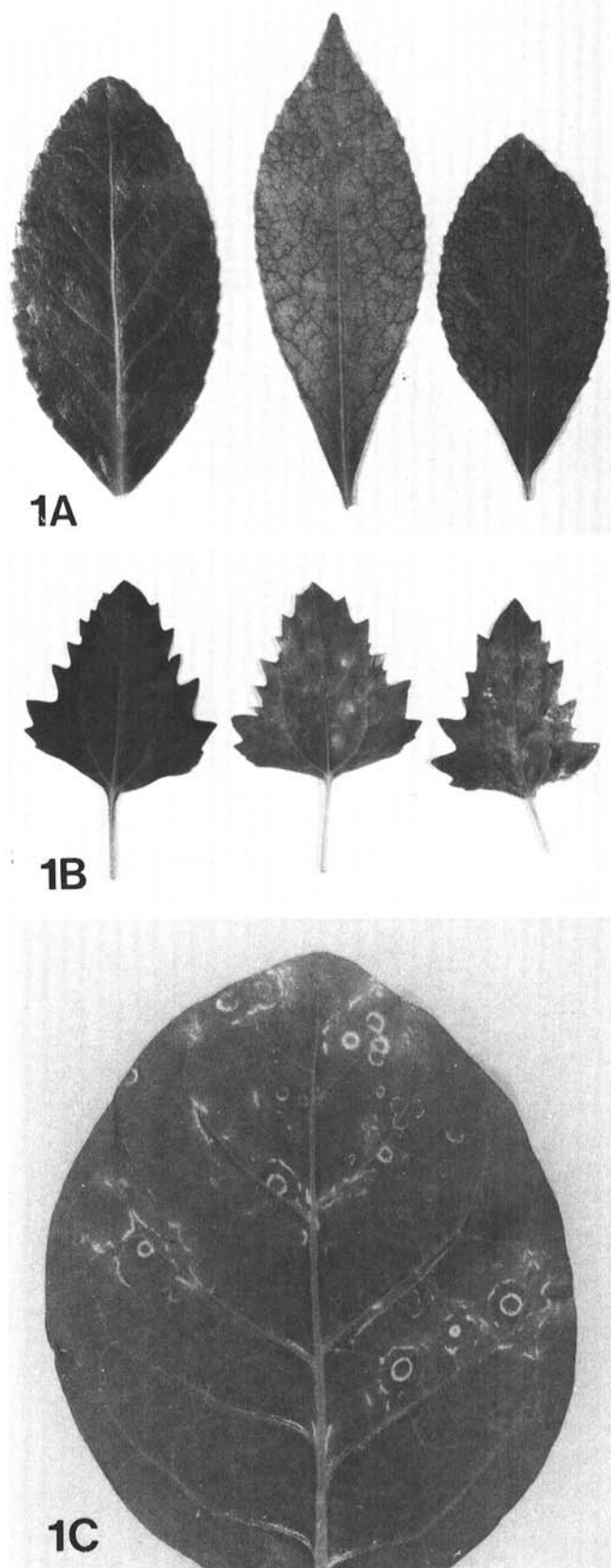


Fig. 1. Leaf samples illustrating symptoms of the euonymus strain of cherry leaf roll virus. **A**, Naturally infected *Euonymus europaeus* exhibiting mottling and interveinal chlorosis. The leaf at left is healthy. **B**, *Chenopodium quinoa* illustrating chlorotic lesions (middle) and characteristic systemic chlorotic mottling symptoms (right). The leaf at left is healthy. **C**, *Nicotiana tabacum* 'Kentucky-16' showing characteristic ringspot symptoms.

unpublished). A faint light-scattering band thought to be virus top component was observed occasionally near the top of the gradient. With an extinction coefficient of $10 \text{ (mg/ml)}^{-1} \text{ cm}^{-1}$ at 260 nm (tobacco ringspot virus), virus yields averaged 30 mg/kg of tissue. The $A_{260/280\text{nm}}$ of purified middle and bottom virus components was 1.9 uncorrected for light-scattering. Buoyant densities of middle and bottom components after reaching equilibrium in CsCl, were 1.481 and 1.500 g/cm^3 , respectively. The percentage of RNA was determined on the basis of buoyant density to be 42 and 36%, respectively, for bottom and middle components.

Antiserum production and serology. Antiserum made against purified CLRV-Eu had a titer of 1/1,024, against CLRV-Eu infected plant sap in gel diffusion tests. No reactions occurred with healthy plant sap. Reciprocal serological tests performed with CLRV-Eu, TobRSV, TomRSV, and their antisera showed no heterologous reactions. Additionally, no reactions occurred in Ouchterlony gel diffusion tests when CLRV-Eu was tested with TomRSV-Eu antiserum. Positive reactions, however, were obtained in reciprocal tests using CLRV-Eu, CLRV-Elm, and CLRV-Birch antigens and antisera (Fig. 2). Spur formation in these tests showed that CLRV-Eu is closely related but serologically distinct from the CLRV-Elm and CLRV-Birch isolates.

Analysis of virus proteins and nucleic acids. When the coat protein of purified CLRV-Eu was analyzed by SDS-PAGE, only a single protein of 53,000 relative mass (M_r) was detected (Fig. 3). Molecular weights were determined from six gel runs using six different virus preparations. Two components of viral nucleic acid were detected by gel electrophoresis. The viral nucleic acids were unaffected by treatment with DNase, but digested by RNase. Both RNA components were precipitated with 2 M LiCl, demonstrating that they are single-stranded RNA. The glyoxal-denaturing RNA gels consistently revealed (three repetitions) comigration of CLRV-Walnut and CLRV-Eu RNAs (Fig. 4) and average sizes of about 7.7 kb ($2.6 \times 10^6 M_r$) for RNA-1 and 6.4 kb ($2.2 \times 10^6 M_r$) for RNA-2, which correspond closely to the published molecular weights of $2.82 \times 10^6 M_r$ and $2.29 \times 10^6 M_r$ for RNA-1 and RNA-2 of CLRV-Walnut, respectively (11,20).

Electron microscopy and cytopathology. Examination of purified virus preparations revealed isometric, electron-dense particles

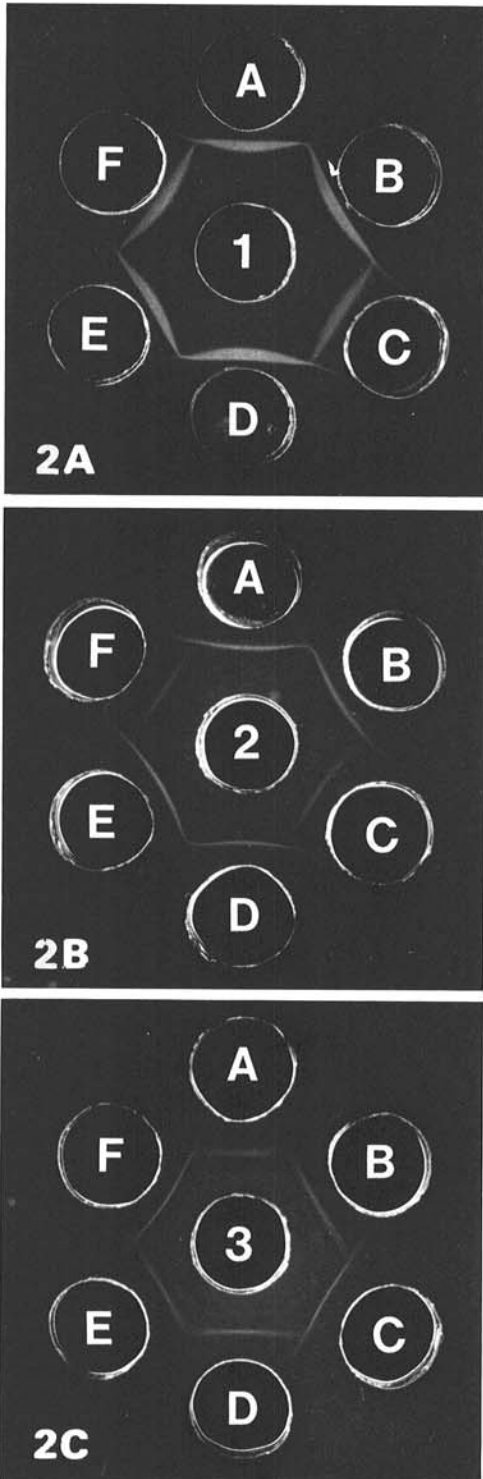


Fig. 2. Ouchterlony double diffusion tests in 1% agarose with antisera prepared against the euonymus strain of cherry leaf roll virus (CLRVEu), the elm isolate of cherry leaf roll virus (CLRVElm), and the birch isolate of cherry leaf roll virus (CLRVBirch). Wells contain: 1 = CLRVEu antiserum; 2 = CLRVElm antiserum; 3 = CLRVBirch antiserum; A and D = CLRVEu antigen; B and E = CLRVElm antigen; C and F = CLRVBirch antigen. Antigens are from crude sap extracted from *Chenopodium quinoa*.

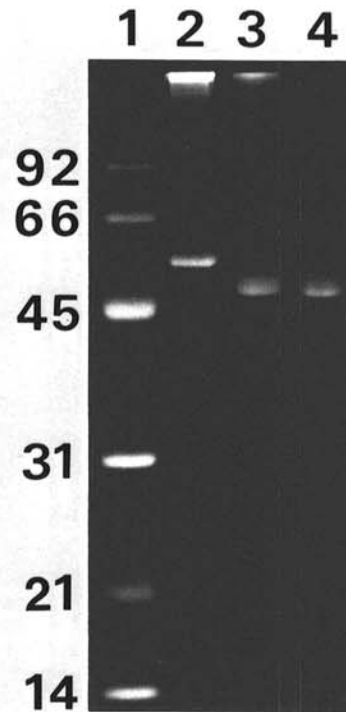


Fig. 3. An SDS-polyacrylamide (12% acrylamide) vertical slab gel showing the capsid protein of the euonymus strain of the cherry leaf roll virus (CLRVEu). Lane 1 consists of molecular weight standards with their corresponding relative masses ($M_r \times 10^3$) shown at left. Standards are: phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme in decreasing order of M_r . Lane 2 shows the virion capsid protein of CLRVEu. Lane 3 shows the capsid protein of CLRVEu middle and bottom components. Lane 4 shows capsid protein of the CLRVEu top component.

about 28 nm in diameter (Fig. 5A) when the middle and bottom components from sucrose density gradients were examined. Isometric, empty particles about 28 nm in diameter that were penetrated by the stain were observed from the top component (Fig. 5B). Highly concentrated CLRV-Eu occasionally formed crystal lattices on the grids.

Cytopathological effects caused by CLRV-Eu infection were, in general, similar to those of other members of the nepovirus

group, but some structural features were distinct from other nepoviruses. Particles averaging 28 nm in diameter, which were assumed to be the virus and which will be referred to as the virus particles, occurred only in the cytoplasm.

In infected leaf cells of *E. europaeus*, densely stained viroplasmic inclusions of circular profile occurred (Fig. 6). These inclusions, which were not observed in other hosts studied, consisted of two types of viruslike particles, one type with an electron-dense core and the other with an electron-lucent core that may represent nucleocapsids and protein shells of the virus, respectively. Viruslike particles with dense cores also occurred within the cytoplasm aligned in rows along membranes that were continuous with the membranes of the endoplasmic reticulum (Fig. 7). In all host plants, virus particles also occurred in rows in membranous tubules that were often continuous with the plasmodesmata (Fig. 8). In many cases, canals of the plasmodesmata in cell walls were greatly enlarged, containing aggregates of irregularly packed virus particles (Fig. 9). Another common cytopathic feature in all host plants examined was the presence of fingerlike cell wall protrusions of various sizes into the cytoplasm (Fig. 10). Virus particles aligned in a row within tubules were often embedded in the middle of the protruded cell walls.

DISCUSSION

The properties of CLRV-Eu presented here are consistent with those of cherry leaf roll virus, a member of the nepovirus group. Virus diseases affecting *Euonymus* species have been reported (2,12,18,21-23); however, this is the first report of *Euonymus* infected by a strain of CLRV.

All attempts to transmit CLRV-Eu by the nematode *Xiphinema americanum* were unsuccessful. This agrees with reports that transmission of CLRV strains by nematodes is extremely rare (3,11). The high percentage of seed transmission that was obtained

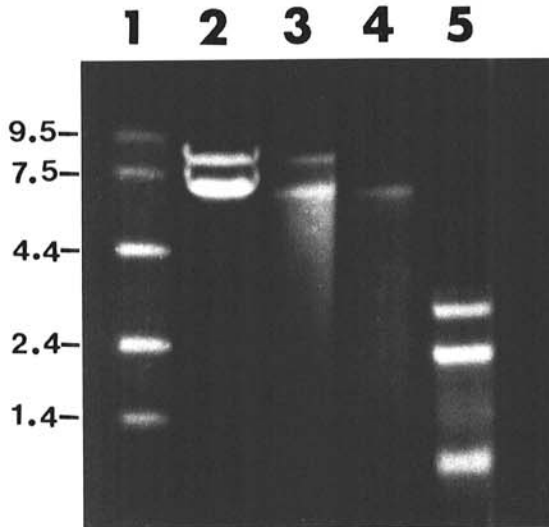


Fig. 4. Molecular weight determination of CLRV-Eu RNAs in a 1% agarose horizontal glyoxal denaturing gel stained with EtBr and visualized by UV transillumination. Lane 1, Molecular weight markers (kb); Lane 2, CLRV-Eu RNAs; Lane 3, CLRV-Walnut RNAs; Lane 4, TMV RNA; Lane 5, BMV RNAs.

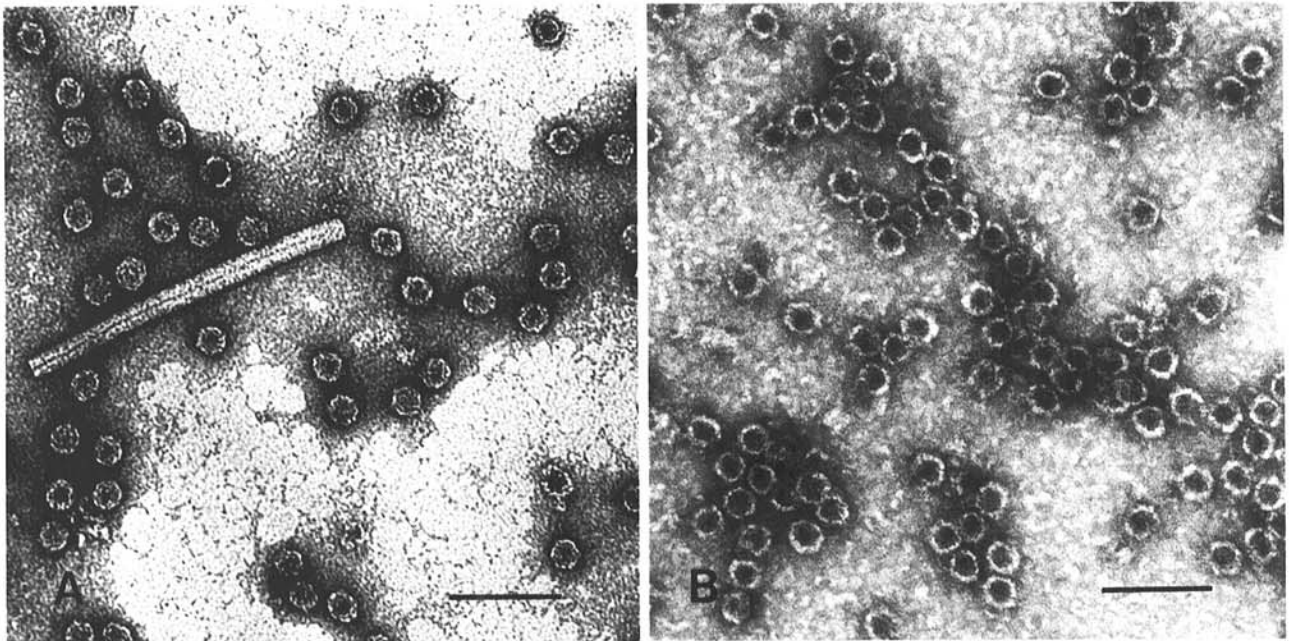
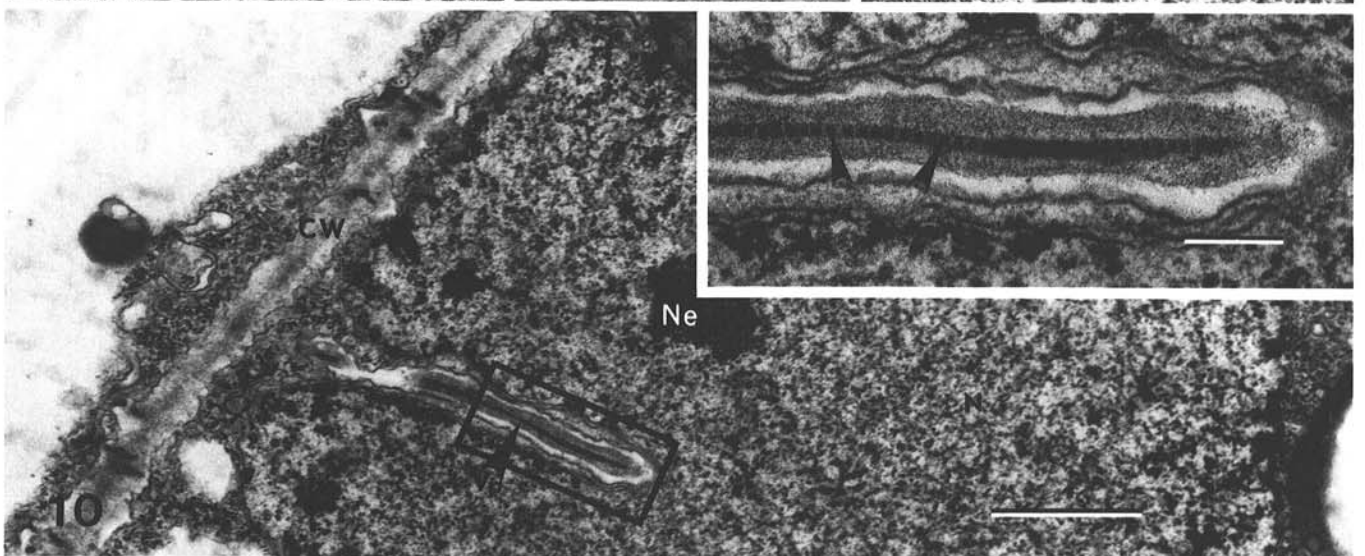
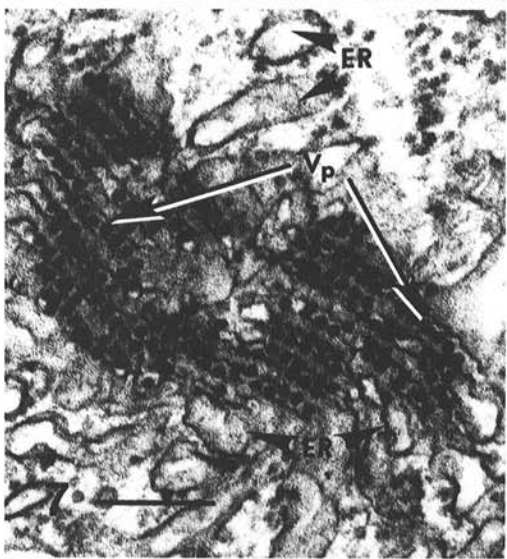
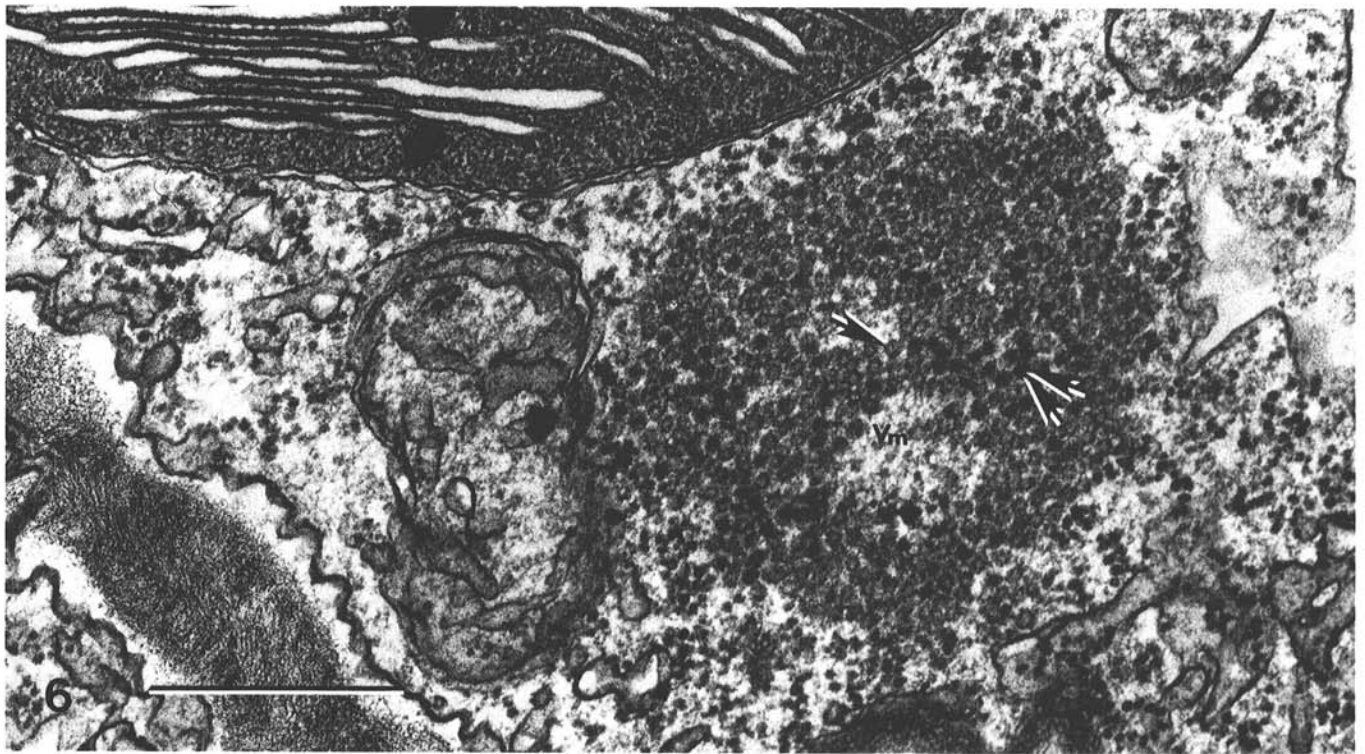


Fig. 5. Electron micrographs of purified virus particles of the euonymus strain of cherry leaf roll virus stained with 2% uranyl acetate. **A**, Virus particles consisting of the middle and bottom components. The elongated particle is tobacco mosaic virus added for a size reference. Bar represents 100 nm. **B**, Virus particles from the top component of the euonymus strain of cherry leaf roll virus. Bar represents 100 nm.

Figs. 6-10. Ultrastructure of host cells infected with the euonymus strain of cherry leaf roll virus. Figs. 6-8 are from *Euonymus europaeus* and 9 and 10 are from *Vigna unguiculata* subsp. *unguiculata* 'Monarch.' **6**, A viroplasmic inclusion (Vm) observed in the cytoplasm. Single arrow shows electron-lucent particles. Double arrows show electron-dense particles. Bar represents 500 nm. **7**, Rows of virus particles (Vp) directly associated with the endoplasmic reticulum (ER). Bar represents 200 nm. **8**, A longitudinal section of a plasmodesma (Pd) traversing the cell wall. Virus particles (Vp) can be observed within this structure. Bar represents 200 nm. **9**, A cross section of a cell wall (CW) containing virus particles (Vp) located within an enlarged lumen of a plasmodesma. Bar represents 200 nm. **10**, A longitudinal section of a cell wall protrusion containing virus particles (Vp) in a line. CW = cell wall. N = nucleus. Ne = nucleolus. Bar represents 1000 nm. Inset: A higher magnification of the wall protrusion within the rectangle. Virus particles are indicated by arrowheads. Bar represents 200 nm.



is also characteristic of CLRV (10,20). Buoyant densities, the $A_{260/280\text{nm}}$ ratio and the physical properties of CLRV-Eu are similar to other strains of CLRV, but differ from members of the tobacco ringspot virus and the tomato black ring virus subgroups (9,10,20,26).

Serological tests indicated no relationship between CLRV-Eu and the TobRSV and TomRSV members of the nepovirus group, nor did any reaction occur against antiserum of TomRSV-Eu. Reciprocal serological tests with CLRV-Eu antigen and antiserum performed against the elm and birch isolates of CLRV produced strong precipitin lines in double diffusion assays. These isolates all shared common antigenic determinants with each other, and, in addition, formed spurs, indicating antigenic sites that are not in common with each other.

The properties shown for CLRV-Eu proteins and nucleic acids are similar to those of CLRV. The molecular weight of the CLRV-Eu capsid protein (53,000) is well within the range of nepovirus proteins, within 2% of that reported for the CLRV subgroup, but smaller than the TRSV capsid protein (9,20). When CLRV-Walnut RNA was used as a typical CLRV representative, the two RNAs co-migrated with the two RNAs of CLRV-Eu.

Cytopathological studies in two hosts show effects on infected cells common to the nepoviruses. Fingerlike cell wall protrusions containing virus particles, the presence of virus particles in membranous tubules that are often continuous with the plasmodesmata, and the occurrence of virus aggregates in distended plasmodesmata are known to be common cytopathic effects for nepoviruses (9). It is of interest to note that the viroplasmic inclusions found in CLRV-Eu infected *E. europaeus* have not been reported to occur in cells infected with any other nepovirus. In addition, this unique structure was observed only in *E. europaeus* but not in other hosts examined infected with the same virus, and, thus, this inclusion may be due to a host effect. More investigation of this inclusion is needed to determine if it is unique only to CLRV-Eu in *Euonymus* sp., or if it may also occur in other hosts infected with strains of CLRV. We suggest that CLRV-Eu be included as a strain of CLRV in the nepovirus group based on serology, symptomatology, the high percentage of seed transmission, similarities in protein and RNA properties, and the cytopathological features seen in CLRV-Eu infected plants.

LITERATURE CITED

- Baltimore, D. 1966. Purification and properties of poliovirus double-stranded ribonucleic acid. *J. Mol. Biol.* 18:421-428.
- Barnett, O. W., and Baxter, L. W. 1974. Cucumber mosaic virus in *Nandina* and other ornamentals. *Act. Hortic.* 36:65-74.
- Cooper, J. I., and Edwards, M. L. 1980. Cherry leaf roll virus in *Juglans regia* in the United Kingdom. *Forestry* 53:41-50.
- Cropley, R. 1961. Cherry leaf roll virus. *Ann. Appl. Biol.* 49:524-529.
- Falk, B. W., Morris, T. J., and Duffus, J. E. 1979. Unstable infectivity and sedimentable ds-RNA associated with lettuce speckles mottle virus. *Virology* 96:239-248.
- Falk, B. W., and Tsai, J. H. 1983. Physicochemical characterization of maize mosaic virus. *Phytopathology* 73:1536-1539.
- Falk, B. W., and Tsai, J. H. 1984. Identification of single- and double-stranded RNAs associated with maize stripe virus. *Phytopathology* 74:909-915.
- Fraenkel-Conrat, H., Singer, B., and Tsugita, A. 1961. Purification of viral RNA by means of bentonite. *Virology* 14:54-58.
- Harrison, B. D., and Murant, A. F. 1977. Nepovirus Group. No. 185 in: *Descriptions of Plant Viruses*. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
- Jones, A. T. 1985. Cherry leaf roll virus. No. 306 in: *Descriptions of Plant Viruses*. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 6 pp.
- Jones, A. T., McElroy, F. D., and Brown, D. J. F. 1981. Tests for transmission of cherry leaf roll virus using *Longidorus*, *Paralongidorus*, and *Xiphinema* nematodes. *Ann. Appl. Biol.* 99:143-150.
- Jonsson, G. 1974. Localisation nucléaire et cytoplasmique de particules d'allure virale chez des sujets fasciés de Fusains (*Euonymus japonica* Thunb. et *Euonymus japonica* Thunb. var. *microphylla* Jaeger)(1). *Rev. Gen. Bot.* 81:135-150.
- Kim, K. S., and Fulton, R. W. 1984. Ultrastructure of *Datura stramonium* infected with an Euphorbia virus suggestive of a whitefly-transmitted geminivirus. *Phytopathology* 74:236-241.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. *Nature (London)* 227:680-685.
- Lane, L. C., and Cuppels, D. 1981. Detection of plant viruses and nucleic acids by fluorescence. (Abstr.) *Phytopathology* 71:234.
- Larsen, R. C., and Duffus, J. E. 1984. A simplified procedure for the purification of curly top virus and the isolation of its monomer and dimer particles. *Phytopathology* 74:114-118.
- Larsen, R. C., Gergerich, R. C., and Kim, K. S. 1986. Properties and cytopathology of a virus causing mottling symptoms on *Euonymus europaeus*. (Abstr.) *Phytopathology* 76:1075.
- Mali, V. R. 1976. Studies on *Euonymus* mosaic virus disease and its transmission by *Olpidium brassicae* in Czechoslovakia. *Indian Phytopathol.* 29:262-268.
- McMaster, G. K., and Carmichael, G. C. 1977. Analysis of single and double stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Nat. Acad. Sci. USA* 74:4835-4838.
- Murant, A. F. 1981. Nepoviruses. Pages 197-238 in: *Handbook of Plant Virus Infections and Comparative Diagnosis*. E. Kurstak, ed. Elsevier/North-Holland Biomedical Press, Amsterdam.
- Plese, N., and Wrischer, M. 1981. A filamentous virus associated with mosaic of *Euonymus japonica*. *Acta Bot. Croat.* 40:31-34.
- Puffinberger, C. W., and Corbett, M. K. 1985. *Euonymus* chlorotic ringspot disease caused by tomato ringspot virus. *Phytopathology* 75:423-428.
- Schmelzer, K. 1969. Das latente erdbeeringflecken virus aus *Euonymus*, *Robinia* und *Aesculus*. *Phytopathol. Z.* 66:1-24.
- Sehgal, O. P., Jean, J., Bhalla, R. B., Soong, M. M., and Krause, G. F. 1970. Correlation between buoyant density and ribonucleic acid content in viruses. *Phytopathology* 60:1778-1784.
- Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26:31-43.
- Stace-Smith, R. 1985. Tobacco ringspot virus. No. 309 in: *Descriptions of Plant Viruses*. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 6 pp.
- Vinograd, J., and Hearst, J. E. 1962. Equilibrium sedimentation of macromolecules and viruses in a density gradient. *Progr. Chem. Org. Natur. Prod.* 20:372-422.