

Occurrence and Characterization of a Potexvirus Infecting *Arctium minus*

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ABSTRACT

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A previously unidentified potexvirus was isolated from common burdock (*Arctium minus*) in the Niagara peninsula of Ontario, Canada. Infected plants showed a bright yellow mosaic mottling and stunting of growth. The virus was readily transmitted mechanically but was not seedborne. On the basis of host range studies, no similarity could be found between this virus and other described potexviruses, although immunodiffusion tests revealed serological relatedness with potato aucuba mosaic and clover yellow mosaic viruses. The virus in crude sap from *Chenopodium quinoa* was infective to a dilution end point of 10^{-6} . The thermal inactivation point was 58 C, and longevity in vitro at 20 C was up to 13 wk. The virus was characterized by a single sedimenting nucleoprotein (510–540 nm long and 13 nm in diameter), a buoyant density (in CsCl) of 1.304 g/cm³, an ultraviolet extinction coefficient of 2.47, and an A_{260:280} ratio of 1.22. Polyacrylamide gel electrophoresis revealed two proteins with relative molecular masses of 28,300 and 30,500 daltons. Viral RNA, identified by both orcinol and ribonuclease tests, could not be resolved by electrophoresis. The hyperchromic profile (T_m = 74 C) fits that of a single-stranded RNA. Purified viral nucleoprotein had an isoelectric point between pH 4.0 and 4.5.

Arctium minus (Hill) Bernh., or common burdock (4), is a prevalent weed throughout southern Ontario. This species grows abundantly in undisturbed clay soils along the Niagara escarpment, usually in fence rows, along roadsides, or in abandoned fields. In 1985, electron microscopy showed that over 10% of the *A. minus* sampled from the Niagara region was infected with a potexvirus.

Viruses previously reported to infect *A. minus* include a tobnavirus, burdock mottle virus (9); a potexvirus, burdock virus R (10); a closterovirus, burdock yellows virus (13); and several spherical viruses, including burdock mosaic virus (10), cucumber mosaic virus (10), and tobacco ringspot virus (2). The potexvirus reported here is not similar to any of these described viruses and, on the basis of the yellow mosaic symptoms in *A. minus*, has been tentatively named burdock yellow mosaic virus (BdYMV). This paper describes some of the biological properties of BdYMV.

MATERIALS AND METHODS

Virus purification. Virus was isolated from *A. minus* plants collected from fields in the Niagara peninsula near Jordan, Ont. A single-lesion isolate of the virus was obtained from mechanically inoculated leaves of *Chenopodium amaranticolor* Coste & Reyn. The virus was propagated and subsequently maintained in *C. quinoa* Willd.

Purified virus preparations were obtained from systemically infected leaves of *C. quinoa*. Tissue was homogenized in 0.5 M neutral potassium phosphate buffer containing 0.5% ascorbic acid. The homogenate was expressed through cheesecloth and the sap clarified by stirring for 3 min with 8.5% chloroform. After centrifugation at 10,000 g for 15 min, the virus was extracted from the aqueous fraction by two cycles of differential centrifugation with high-speed runs at 86,000 and 180,000 g for 2 and 1.5 hr, respectively. Virus pellets were resuspended in 0.1 M neutral phosphate buffer. The final high-speed pellets were resuspended in 0.01 M neutral phosphate buffer. Further purification of the virus was achieved by centrifugation at 104,000 g for 2 hr in 10–40% linear sucrose density gradients. The single virus band was collected with an ISCO density gradient fractionator (Instrumentation Specialties, Lincoln, NE), and virus was recovered by centrifugation at 86,000 g for 1.5 hr. Purified virus was assayed for infectivity on *C. quinoa*.

Host range. Infected leaves of *A. minus* were triturated in 0.01 M neutral phosphate buffer (tissue:buffer, 1:9), and the extract was rubbed onto Carborundum-dusted leaves of eight of each of the following plants: *A. minus*, *Beta vulgaris* L. 'White King,' *Brassica napus* L. 'Laurentien,' *Callistephus chinensis* Nees, *Capsicum annuum* L. 'Early Hybrid,' *Cheiranthus cheiri* L. 'Allioni,' *C. amaranticolor*, *Chenopodium murale* L., *C. quinoa*, *Chrysanthemum carinatum* L. 'Manatee Iceberg,' *Cucumis sativus* L. 'Improved Long Green,' *Datura stramonium* L., *Gomphrena globosa* L.

'Dwarf Buddy,' *Lycopersicon esculentum* Mill. 'Glamour,' *Matthiola incana* (L.) R.Br., *Nicotiana clevelandii* Gray, *N. glutinosa* L., *N. rustica* L., *N. tabacum* L. 'Harrow Velvet,' *Petunia hybrida* Vilm. 'Calypso,' *Phaseolus vulgaris* L. 'Frenchie,' *Spinacia oleracea* L. 'Longstanding Bloomsdale Dark Green,' *Vigna unguiculata* L. Walp. 'Queen Anne Blackeye,' and *Zinnia elegans* Jacq. 'Peter Pan.'

Inoculated plants were maintained under greenhouse conditions (25 C) with supplementary light and examined daily. Four weeks after inoculation, plants were checked for the presence of virus by examining leaf triturates in the electron microscope and by back-inoculation onto *C. quinoa*.

Seed transmission. Seed was field-collected from 15 naturally infected *A. minus* plants, sown in a sand:soil:peat (1:1:1) mix, and placed in a growth cabinet with a day temperature of 26 C (16 hr), night temperature of 16 C (8 hr), and photoperiod of 16 hr at 300 $\mu\text{Es}^{-1}\text{m}^{-2}$. Fifteen seedlings from each parent plant were individually transplanted to 12-cm pots and maintained in a greenhouse for 8 wk. Apical leaves from each plant were then tested for virus infection by electron microscopy, using leaf dip preparations, and by bioassay on *C. quinoa*.

Serology. Serological relationships were examined by the Ouchterlony double-diffusion technique (14) using 1% BactoAgar (Difco Co., Detroit, MI) containing 0.85% NaCl, 2% sodium dodecyl sulfate (SDS), and 0.1% NaN₃. All viruses were disrupted by sonication on ice for 4 min at 20 kc/sec (Biosonik, Bronwill Scientific, Rochester, NY). Center wells were charged with 100 μl of purified BdYMV containing 20–400 μg of virus; outer wells were charged with 100 μl of antisera against clover yellow mosaic virus (CYMV), cymbidium mosaic virus (CyMV), potato aucuba mosaic virus (PAMV), potato virus N (PVN), potato virus X (PVX), and white clover mosaic virus (WCMV). In other tests, each antiserum was checked against its homologous virus. Antisera and viruses were provided by R. Stace-Smith, Agriculture Canada Research Station, Vancouver.

In vitro tests. Dilution end point. Aliquot samples of crude sap extracts from BdYMV-infected *C. quinoa* were diluted in a 10-fold dilution series in 0.01 M neutral potassium phosphate buffer.

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Infectivity of the diluted samples was assayed on *C. quinoa*.

Thermal inactivation point (TIP) and longevity in vitro (LIV). Both determinations were made using crude sap from infected *C. quinoa*. For TIP determinations, the sap was diluted 1:1 in 0.01 M neutral potassium phosphate buffer. Aliquots of 1 ml of buffered sap were heated for 10 min at selected temperatures in a circulating water bath controlled to 1 C. The heated solutions were cooled in an ice bath, and each was assayed by mechanical inoculation on two *C. quinoa* plants.

For LIV determinations, 1-ml aliquots of infectious crude sap were stored in sealed glass tubes at 20 C. A control sample was assayed immediately. At predetermined time intervals, an aliquot was assayed by mechanical inoculation on two *C. quinoa* indicators.

Electron microscopy. Virus particles were examined in *A. minus* tissue titurates using the leaf dip procedure as previously described (18). Measurements were made from 250 virus particles.

Sedimentation in cesium chloride. The buoyant density of BdYMV was determined by equilibrium banding in CsCl gradients, as described by Allen and Dias (1). Gradient columns were scanned at A_{254nm} , and the refractive index of alternate drops was determined at 25 C in an Abbe refractometer (Carl Zeiss, West Germany). Conversion of the refractive index to density was done from tables (17). Infectivity tests were made on *C. quinoa*.

Determination of ultraviolet extinction coefficient. The extinction coefficient of sucrose gradient-purified virus was determined as previously described (16).

Isoelectric point. Electroosmophoresis was done on Gelman (Gelman Instruments, Montreal, Quebec) Sephaphore III cellulose polyacetate strips (2.5 × 17.1 cm). Strips were briefly soaked in 0.02 M Tris-Na₂HPO₄ buffer titrated to various pH values (3.0–6.5) with citric acid. The pH of the tank and strip buffer was the same. CsCl gradient-purified virus (5 μl) was applied to each side of a strip, 1 cm from the strip center. Electrophoresis was done for 2 hr at a constant current of 0.6 mA/cm across the width of the strip. Virus bands were detected with 0.002% Nigrosine in 2% acetic acid, and strips were destained in 5% acetic acid. Tobacco mosaic virus (TMV) was used as a standard.

Polyacrylamide gel electrophoresis (PAGE) of protein subunits. Gels (75 × 5 mm) of 10% polyacrylamide were prepared as described by Allen and Dias (1). Virus dissociation and electrophoresis were done as described previously (18). Ovalbumin (*M_r* 43,000 daltons), myoglobin (*M_r* 16,890 daltons), and cytochrome C (*M_r* 13,370 daltons), 50 mg each, were used as markers for relative molecular mass determination.

PAGE of nucleic acid. Nucleic acid

was separated from virus particles by using: 1) dissociation buffer as described, 2) a cold water-saturated phenol procedure (7), and 3) protease digestion of virus (0.15% protease in 0.01 M potassium phosphate buffer, pH 7). In each case, nucleic acid was concentrated by ethanol precipitation. Nucleic acid was electrophoresed in each of three buffer systems: 1) 0.12 M Tris-HCl containing 0.06 M sodium acetate and 0.003 M sodium ethylenediaminetetraacetic acid (NaEDTA), pH 7.2 (buffer E, Choi et al [5]); 2) 0.1 M sodium phosphate buffer, pH 7, containing 0.05% SDS; and 3) 0.2 M Tris/0.2 M borate/0.002 M EDTA buffer, pH 7.9 (Peacock and Dingman [15]) at double (2×) concentration. Nucleic acid was electrophoresed on 2.4% polyacrylamide gels (75 × 5 mm) at 150 V for intervals ranging from 0.5 to 3 hr. RNA standards were extracted from tomato blackring (G strain, 2.40 × 10⁶ daltons and 1.65 × 10⁶ daltons), tomato bushy stunt (1.50 × 10⁶ daltons), and tobacco mosaic (2.05 × 10⁶ daltons) viruses. Gels were scanned for absorbance at 265 nm and stained in 0.01% toluidine blue in 40% 2-methoxyethanol.

Thermal denaturation experiments. The thermal denaturation profile of BdYMV RNA (50 μg) in Tris-borate-EDTA electrophoresis buffer (15) containing 8 M urea was determined as described by Dias and Allen (6). Absorbance was corrected for light scattering by extrapolation of the absorbance detected at 360–600 nm (8). The graphic determination of the midpoint of hyperchromic transition (*T_m*) was made as described.

Effect of RNase on viral nucleic acid. Virus (2 mg/ml in 0.01 M neutral phosphate buffer) was added to an equal volume of dissociation buffer and allowed to incubate at 50 C for 10 min. At this time, 5 μg of pancreatic ribonuclease (type 3A, Sigma Chemical Co., St. Louis, MO) was added to an aliquot of the dissociated virus. Undissociated virus, RNase, and dissociated virus minus RNase were assayed by mechanical inoculation on *C. quinoa*.

RESULTS AND DISCUSSION

The potexvirus described here appears to be dissimilar to other viruses reported in *A. minus*. On the basis of the symptomatology of the Ontario isolate in burdock, the virus has been tentatively referred to as burdock yellow mosaic virus (BdYMV). The most prominent symptoms associated with virus-infected *A. minus* were a generalized stunting and bright yellow mosaic evident on the basal leaves of the plants. Yellow flecking was commonly seen on the apical leaves and often assumed irregular triangular interveinal patches. Symptoms were more pronounced on plants growing in open, sunny locations than on those

growing in partial or full shade. The brilliant yellow mosaic symptoms are similar to those described on *A. lappa* L. for burdock mosaic virus (BdMV), a spherical virus reported by Inouye and Mitsuhashi (10). Symptoms incited by BdYMV were very pronounced in *A. minus* in the summer, and necrotic rings were never observed on naturally infected or sap-inoculated plants. *A. minus* was easily infected by mechanical inoculation with sap from infected plants and with purified virus. The host range of BdYMV was dissimilar to that of other viruses isolated from *A. lappa* and could not be compared with the only other potexvirus reported in *A. minus*, burdock virus R (Bd-R), since the authors (10) were unable to obtain a pure isolate of that virus. Local lesions were produced by BdYMV on inoculated leaves of *C. amaranticolor*, *G. globosa*, and *N. tabacum* 'Harrow Velvet,' and *C. murale* and *C. quinoa* were infected latently and systemically, respectively.

Seedlings grown from seed collected from infected *A. minus* plants did not show disease symptoms, and no virus was detected in plant tissues either by electron microscopy or by bioassay on *C. quinoa*. This is consistent with other potexviruses, in which seed transmission is uncommon.

Sucrose gradient-purified virus from *A. minus* showed a strong specific reaction with antiserum to PAMV in immunodiffusion tests, whereas a weak precipitin band was formed against CYMV antiserum. Serological inter-relatedness of this type is common within the potexvirus group (12). No cross-reaction occurred between purified virus and antisera against PVN, PVX, or WCMV, whereas each of these viruses reacted readily with its homologous antiserum. No other similarities were

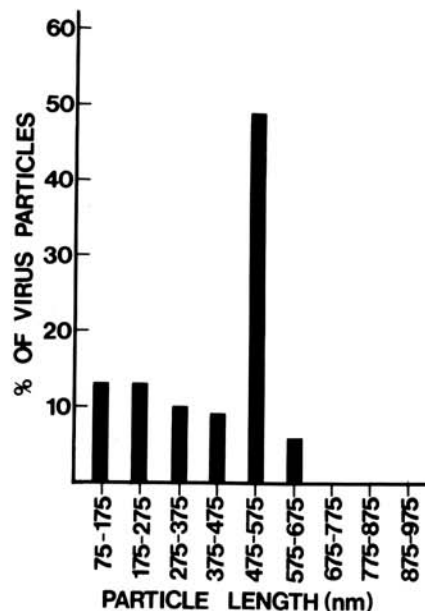


Fig. 1. Particle length of burdock yellow mosaic virus.

apparent between BdYMV and the described properties of PAMV and CYMV (3,11). Serological relatedness of burdock viruses to other potexviruses has not been described.

Extracts from infected *C. quinoa* retained infectivity when diluted to 10^{-5} but not to 10^{-6} . Thermal inactivation was exponential from 50 to 58 C, at which temperature inactivation was complete. Sap from virus-infected *C. quinoa* produced only 70% as many lesions as did controls after 6 wk of storage at 20 C and was noninfective after 13 wk. Infectivity and longevity of BdYMV in vitro were considerably greater than for the burdock virus isolates reported by Inouye and Mitsuhashi (10), whereas the thermal inactivation point was comparable to that of the BdMV isolate.

Electron microscopy of negatively stained samples of crude plant sap from *A. minus* revealed numerous viruslike particles 510–540 nm long (modal length

525–530 nm) and 13 nm in diameter (1,500 particles counted). In purified or sucrose gradient-centrifuged preparations, smaller fragments were evident, representing breakdown segments (Figs. 1 and 2). End-to-end aggregation of virus rods was also more prevalent than in crude plant sap. The Bd-R virus isolate described by Inouye and Mitsuhashi (10) is the only virus of comparable length (600 nm) isolated from burdock, although its diameter (20 nm) is not typical for PVX-type viruses. Also, the Bd-R isolate infecting *A. lappa* was found only in phloem cells. Thin sections of *A. minus* revealed BdYMV virus in cells of all epidermal and mesophyll tissues in high concentrations (*unpublished*).

Purified virus, centrifuged to equilibrium in CsCl, formed a single homogeneous band. The buoyant density (g/cm^3) was calculated to be 1.304. The virus showed uncorrected absorption minimum and maximum at 245 and 265

nm, respectively (Fig. 3). The average uncorrected 260:280 ratio was 1.22. Corrections for light scattering gave an average 280:260 ratio of 0.82. On the basis of the optical density and the dry weight of purified virus, the extinction coefficient ($E_{260}^{0.1\%,1\text{cm}}$) of the virus was calculated at 3.54 mg/cm^2 and was 2.47 after correction for light scattering. Average yields of virus, based on this value, were calculated to be 3.2 mg per 100 g of infected leaf tissue. The isoelectric point was between pH 4.0 and 4.5, compared with 3.5 for TMV.

Two protein components were resolved after virus dissociation. The average relative molecular masses of the components were 28,300 and 30,500 daltons, as calculated by comparison with the protein standards. These components represented 39.1 and 60.9% of the total viral protein, respectively.

Electrophoresis for viral nucleic acid failed to resolve any distinct bands in any of the electrophoresis buffer systems used. Toluidine blue staining occurred only at the gel origin, and a positive reaction to orcinol reagent identified the nucleic acid as RNA. RNA standards ran normally in each buffer system.

Dissociated virus and ribosomal RNA gave positive reactions to the orcinol reagent, whereas no reaction was evident between dissociated virus and the standard to the diphenylamine reagent. Viral nucleic acid treated with RNase was not infective on *C. quinoa*. Undissociated and dissociated viral nucleic acid not treated with RNase systemically infected all inoculated test plants.

The hyperchromic profile of viral RNA in 0.01 M potassium phosphate buffer, pH 7.2, showed a mean $T_m = 74$ C (Fig. 4). The melting curve fit that of a single-stranded RNA and showed a gradual increase in absorbance at 260 nm with increase in temperature.

The BdYMV isolate appears to be different from any other virus reported from burdock species. Host range, vectors, and prevalence of this virus require further investigation, however.

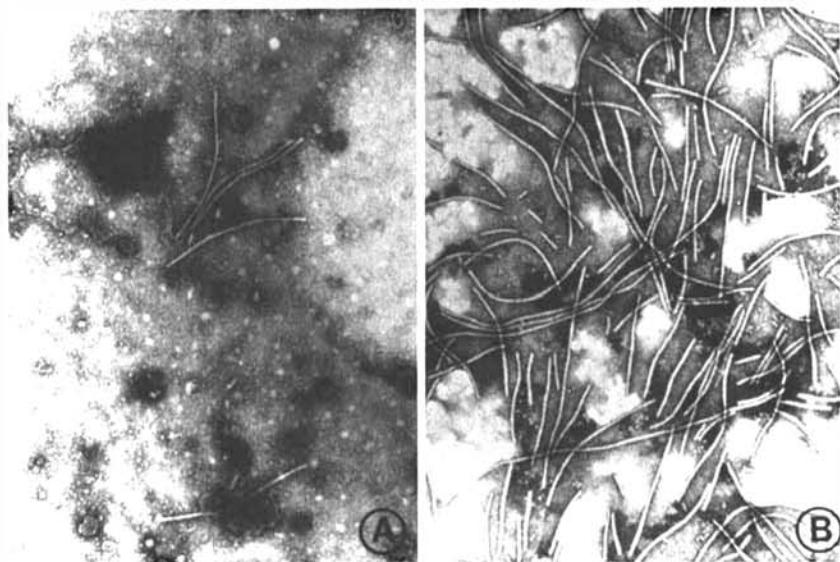


Fig. 2. Electron micrograph of burdock yellow mosaic virus. (A) Leaf dip. (B) CsCl-purified virus showing end-to-end aggregation. ($\times 165,000$)

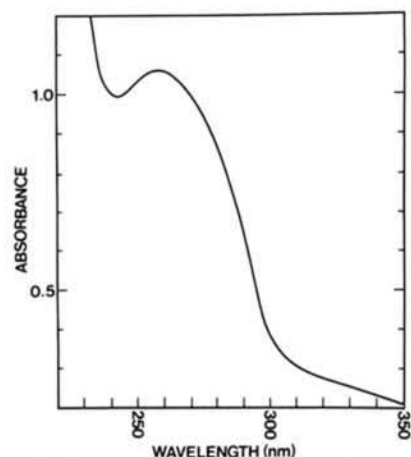


Fig. 3. Ultraviolet absorption spectrum of burdock yellow mosaic virus purified from *Arctium minus*.

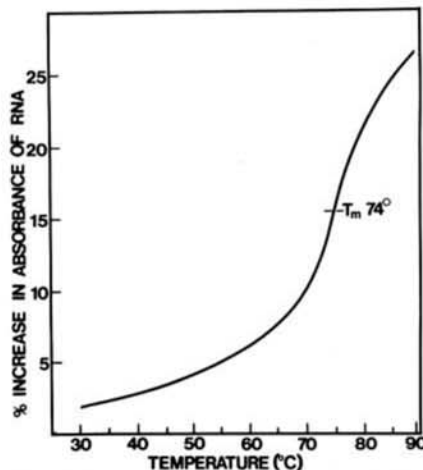


Fig. 4. Absorbance-temperature profile of the RNA component of burdock yellow mosaic virus in 0.01 M $\text{K}_1/\text{K}_2\text{PO}_4$, pH 7.

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