Occurrence and Characterization of a Turnip Mosaic Virus Isolate Infecting *Alliaria petiolata* in Ontario, Canada

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ABSTRACT

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An aphid-transmissible virus infecting *Alliaria petiolata* in southern Ontario was identified as turnip mosaic virus (TuMV). Host range studies characterized the *A. petiolata* virus isolate (TuMV-Al) as distinct from the Ontario rutabaga isolate (TuMV-Br). TuMV-Al was not transmissible to most Cruciferae tested, and *A. petiolata* did not act as a reservoir for the TuMV-Br strain that infects commercially grown *Brassica* crops in the area. The virus was not detected in seed or seedlings grown from vernalized seed from infected plants of *A. petiolata* when tested by enzyme-linked immunosorbent assay (ELISA) or sap-inoculation onto herbaceous indicator plants. Indigenous populations of *Myzus persicae* collected from infected plants transmitted virus in a nonpersistent manner for up to 8 hr after acquisition from *A. petiolata*. Both TuMV isolates were serologically related, and particles of both viruses were morphologically similar. Polyacrylamide gel electrophoresis failed to resolve differences between either proteins or nucleic acids of the TuMV-Al and TuMV-Br isolates. On the basis of these studies, TuMV-Al is a related strain of TuMV.

Additional key words: garlic mustard

Alliaria petiolata (M. Bieb.) Cavara & Grande, commonly referred to as garlic mustard (3), is a prevalent weed throughout southern Ontario. This species occurs abundantly in Middlesex and Elgin counties (4) and is found in the damp, shaded soils along the Niagara escarpment. In 1985, more than 30% of the *A. petiolata* that were tested from southern Ontario were infected with a strain of turnip mosaic virus (TuMV-Al).

Although this virus has been reported in garlic mustard in Europe (2,10,12, 14,17), this is the first report of TuMV in this host in Ontario. Because this weed attains maximum abundance in Middlesex County (4), where most of the rutabaga (*Brassica napus* subsp. *napobrassica* (L.) Reichb. 'Laurentien') and canola (*B. napus* subsp. *oleifera* 'Tandem') industry is concentrated, we considered it important to determine whether it may be an overwintering reservoir of virus and thus a potential threat to *Brassica* crops. The relationship of this isolate with the rutabaga strain of TuMV reported in Ontario (TuMV-Br) was examined by comparing host range, serological and structural relatedness, and field transmissibility.

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MATERIALS AND METHODS

Viruses and antiserum. The rutabaga isolate of TuMV (TuMV-Br), collected in 1985 from infected rutabaga fields in Middlesex and Huron counties, Ontario, Canada, has been described (8,9). Virus was maintained in systemically infected *B. napus* 'Laurentien.' The *A. petiolata* isolate of TuMV (TuMV-Al) was collected in 1984 on the Niagara escarpment near St. Catharines, Ontario, and was maintained in systemically infected plants. Virus was extracted from each host by the purification procedure of Choi et al (5). Virus particle aggregration was further minimized by decreasing the molarity of the extraction buffer to 0.05 M and amending it with 0.5 M urea. Virus was purified by rate-zonal density-gradient centrifugation at 61,000 *g* for 120 min in 5, 15, 25, and 35% sucrose in 0.05 M potassium phosphate buffer, pH 7.5, with 0.5 M urea.

Antiserum against TuMV-Br was prepared by injecting New Zealand white rabbits intramuscularly with virus preparations emulsified with an equal volume of Freund's complete adjuvant. Four 2-mg intramuscular injections were given at 10-day intervals, and the rabbits were bled 2 wk after the last injection. The titer of the antiserum was 1/512 as determined by double diffusion (15) in 0.7% Bacto agar containing 0.85% NaCl, 3% sodium dodecyl sulfate (SDS), and 0.1% NaN_3. Before immunodiffusion testing, virus was disrupted by sonication on ice for 4 min at 20 kc/sec (Biosonik, Bronwill Scientific, Rochester, NY). Antiserum was not prepared against TuMV-Al because it was not possible to remove all traces of host protein from the virus preparations.

Host range. The following plants were tested in the greenhouse to determine their reactions to both TuMV-Al and TuMV-Br: *A. petiolata, Beta vulgaris* L. 'White King,' *Brassica oleracea* L. var. *capitata* L., *B. oleracea* var. *botrytis* L., *B. napus* var. *napobrassica* 'Laurentien,' *B. napus* subsp. *oleifera* L. 'Tandem,' *Capsicum annuum* L. 'Early Hybrid,' *Cheiranthus cheiri* L. 'Allioni,' *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Cucumis sativus* L. 'Improved Long Green,' *Datura stramonium* L., *Hesperis matronalis* L., *Gomphrena globosa* L., *Lycopersicum esculentum* Mill. 'Glamour,' *Matthiola incana* R. Br., *Nicotiana glutinosa* L., *N. tabacum* L. 'Harrow Velvet,' *Ocimum basilicum* L. 'Dark Opal,' *Petunia hybrida* (Vilm.) 'Calypso,' *Phaseolus vulgaris* L. 'Frenchie,' and *Vigna unguiculata* (L.) Walp 'Queen Anne Blackeye.'

Leaves infected with TuMV-Al and TuMV-Br isolates, from systemically infected *A. petiolata* and *B. napus*, respectively, were triturated in 0.01 M neutral phosphate buffer (tissue:buffer, 1:9). The extract was used to inoculate Carborundum-dusted leaves of eight test plants of each species. Inoculated plants were maintained under greenhouse conditions (25 C) with supplementary high-pressure sodium vapor light (350 μE m^{-2} s^{-1}, 16-hr photoperiod) and examined daily. After 4 wk, plants were checked for the presence of virus by enzyme-linked immunosorbent assay (ELISA) (7) and by back-inoculation into *C. quinoa*.

Seed investigations. Mature seed pods were sampled from 30 healthy and 30 TuMV-infected greenhouse-grown *A. petiolata* plants. Pods were measured, dried, and the number of seeds per pod and seed diameter recorded. To enhance germination, seeds were vernalized for 8 mo at 2 C, the seed coats scarified with a razor blade, and the seeds surface-sterilized for 10 min in 0.5% aqueous

sodium hypochlorite. Seeds then were washed in three changes of sterile distilled water and transferred to sterile filter paper in glass petri dishes. The paper was moistened with 5 ml of 0.1% sterile gibberellic acid (Sigma), and the dishes were sealed with Parafilm. Dishes were maintained at 20 C and received a 16-hr photoperiod for 300 $\mu E\ m^{-2}\ s^{-1}$. Percent seed germination was recorded after 2 mo, and 30 seedlings were transplanted to a sand:soil:peat (1:1:1) potting mix and maintained in a glasshouse. Mature plants were tested by ELISA for TuMV.

Serology. The double-antibody sandwich ELISA was used, as described by Clark and Adams (7). All tests were done in Immulon 2 flat-bottom Removawell plates (Dynatech) with 200 μl of liquid used for each of the four steps. Wells were coated with purified TuMV-Br immunoglobulin (Ig) at 1 μg/ml diluted in 0.05 M sodium carbonate buffer, pH 9.6 (1:1,000, v/v), and incubated for 4 hr at 38 C. Plant samples were incubated in wells for 4 hr at 38 C, and after washing, TuMV Ig (1 μg of protein per milliliter) conjugated with alkaline phosphatase (type 7, Sigma) at an enzyme:protein ratio of 2.5:1 (v/v) with 0.06% glutaraldehyde, was added for 3 hr at 38 C. Substrate reactions were stopped by adding 20 μl of 3 M NaOH, and the absorbance was measured at 405 nm in a Beckman DU-8 spectrophotometer fitted with a microplate accessory (Beckman Instruments). Test samples were considered positive if absorbance values exceeded twice that of healthy control samples.

Electron microscopy. Leaf tissue was macerated in 0.01 M sodium phosphate buffer + 0.15 M NaCl, pH 7.0 (PBS) (1:20 w/v), and carbon-stabilized collodion-coated copper grids (300-mesh) floated on the macerate for 10 min. After washing, the grids were stained with 2% phosphotungstic acid, pH 6.8, in PBS and examined in a Philips 201 electron microscope. Particle measurements were taken from micrographs of each virus and compared with a 462-nm lattice calibration grid (Ladd Industries, Burlington, VA) to establish particle dimensions. Measurements were taken from 100 virus particles of each isolate.

Protein electrophoresis. Gels of 10% polyacrylamide (75 × 5 mm) were prepared as described by Allen and Dias (1). Virus was dissociated by mixing with dissociation buffer (1:1, v/v) and heating for 10 min at 65 C. The dissociation buffer (Peacock and Dingman [16]) consisted of 0.2 M Tris, 0.2 M borate, 0.002 M EDTA, pH 7.9, supplemented with 2% SDS, 3 M urea, 0.2 M mercaptoethanol, and 20% RNase-free sucrose. Electrophoresis of dissociated virus was done at room temperature at constant voltage (100V) for 2 hr in double-strength dissociation buffer. Fifty milligrams each of ovalbumin (M_r 43,000 Da) and tobacco mosaic virus (TMV) subunit protein (M_r 17,500 Da) were used as markers for relative molecular mass determinations. Gels were stained in 0.25% Coomassie brillant blue R in methanol-water-acetic acid (5:5:1, v/v /v), destained, and examined. The migration distances of the stained protein components were measured on a Joyce Loebl Model 400 recording densitometer (Joyce Loebel Inc., Gateshead, England) and relative electrophoretic mobilities calculated.

RNA electrophoresis. RNA was separated from virus particles by mixing virus with dissociation buffer as described. Electrophoresis of dissociated virus was in 0.12 M Tris-HCl containing 60 mM sodium acetate and 3 mM Na EDTA, pH 7.2 [buffer E [6]), in 2.4% polyacrylamide gels (75 × 5 mm) for 3 hr. TMV-RNA (M_r 2.0×10^6 Da) and tomato bushy stunt (TomBSV) RNA (M_r 1.5×10^6 Da) were used as markers. Gels were scanned for absorbance at 265 nm and stained in 0.01% toluidine blue in 40% 2-methoxyethanol.

Aphid transmission studies. The green peach aphid (*Myzus persicae* (Sulz.)) was identified as the predominant aphid species collected from both garlic mustard and rutabaga. For transmission tests, nonviruliferous apterous green peach aphids were reared on healthy *B. napus* 'Laurentien.' Aphids were allowed to feed for 4 hr on either *A. petiolata* or *B. napus*, which were systemically infected with TuMV-Al or TuMV-Br, respectively. After feeding, 10 aphids were transferred to each of 10 healthy *A. petiolata* and *B. napus* plants and allowed to feed for an additional 15 hr. Plants were then sprayed with a 0.026% aqueous solution of pirimicarb (Pirimor) and held for 21 days in a greenhouse at 25 C. Virus infection was confirmed by ELISA.

Retention time of TuMV by single aphids was determined for each virus isolate. Starved aphids were fed on virus-infected leaves of *A. petiolata* or *B. napus* 'Laurentien.' After 30 min of acquisition feeding, aphids were dislodged, transferred to petri dishes, and starved for 0, 0.5, 1, 2, 4, 8, 16, 24, or 36 hr. After postacquisition starvation, aphids were transferred to 10 corresponding healthy test plants for transmission feeding of 12 hr (10 aphids per plant). Plants were then sprayed with pirimicarb and transferred to a greenhouse; symptoms were read after 21 days. Virus infection was confirmed by ELISA.

RESULTS

Host range and symptomatology. Symptoms of TuMV-Al infection in *A. petiolata* included severe mottling with dark green areas, leaf puckering, marginal downward leafroll, some veinal necrosis, and general stunting of the plant (Fig. 1). Symptoms were more pronounced in plants that received diffuse sun than those growing in full shade. TuMV-Al was also isolated from *H. matronalis* growing in the same habitat and produced only mild mosaic symptoms in infected plants. Seed pods from infected *A. petiolata* plants were smaller, and though the number of seeds per pod remained unchanged, seed size was reduced about 20% (Table 1). Seed germination was not affected by the virus, and all plants grown from vernalized seed obtained from infected plants remained symptomless and tested negative for virus by ELISA.

Reactions of the indicators to mechanical inoculation with TuMV-Al and TuMV-Br are summarized in Table 2. *P.*

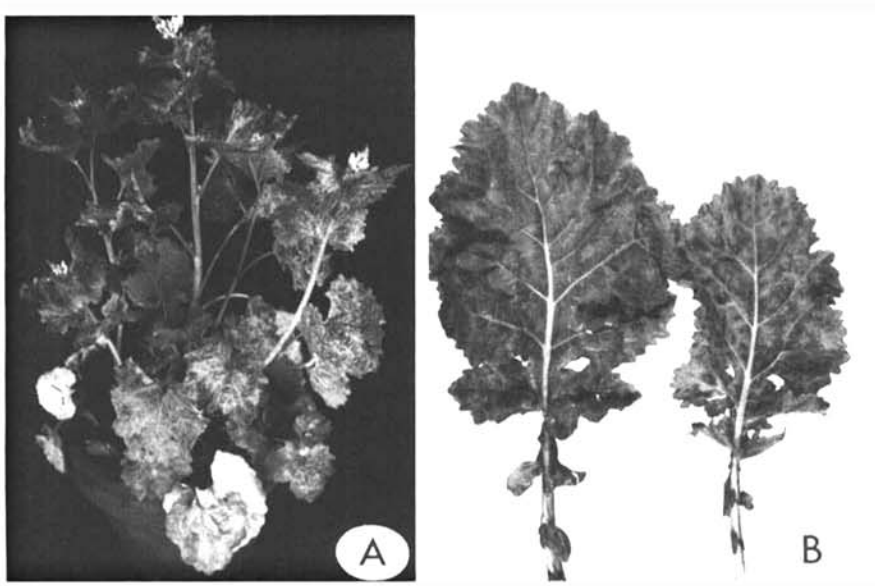

Fig. 1. Symptoms produced by turnip mosaic virus (TuMV) strains. **(A)** *Alliaria petiolata* infected by TuMV-Al and **(B)** *Brassica napus* var. *napobrassica* 'Laurentien' infected by TuMV-Br.

hybrida, N. tabacum, G. globosa, and *P. vulgaris* responded in the same way to both viruses, whereas other hosts reacted differently. Necrotic lesions induced on *Chenopodium* spp. by TuMV-Al were smaller than the chlorotic lesions induced by TuMV-Br. The chlorotic lesions later became necrotic with red-rimmed margins, and leaves showed veinal flecking and spotting. *B. napus* 'Laurentien,' *B. napus* subsp. *oleifera* 'Tandem,' *B. oleracea* var. *botrytis*, and *B. oleracea* var. *capitata* were infected by TuMV-Br but not TuMV-Al, whereas *A. petiolata, H. matronalis,* and *O. basilicum* were infected by TuMV-Al but not TuMV-Br. *B. vulgaris, C. annuum, C. sativus,* and *D. stramonium* were not infected by either virus. *C. cheiri* and *M. incana* reacted to TuMV-Br with severe mosaic and stunting and to TuMV-Al with faint local lesions, mild mosaic, and colorbreaking on flowers. *N. glutinosa* was a useful host for distinguishing the two virus isolates, because the TuMV-Br isolate induced systemic chlorotic lesions while the TuMV-Al isolate failed to induce symptoms. Both viruses were found systemically in this host.

Electron microscopy and serology. Measurements of virus particles of each isolate were not significantly different ($P = 0.01$). The viruses were flexuous rods with mean particle dimensions of 725 × 13 nm. Both showed strong postantibody decoration when treated with TuMV-Br antiserum.

The homologous and heterologous titers of the TuMV-Br isolate antiserum were 1:2,048 and 1:1,024, respectively, as determined by immunodiffusion. Cross-absorption tests were not done because of the lack of a suitable TuMV-Al antiserum.

Electrophoresis. TuMV protein preparations always demonstrated protein heterogeneity when analyzed by SDS polyacrylamide gel electrophoresis. Both TuMV-Al and TuMV-Br were resolved into three components (35,000, 29,000, and 27,000 Da) and designated as slow form (SF), intermediate (IF), and fast form (FF), respectively. Coelectrophoresis of the isolates failed to resolve any differences in the respective component sizes. Two additional proteins, with relative molecular masses of 98,000 and 85,000 Da were present in both virus preparations and, as reported by Choi et al (6), are likely host proteins.

Both TuMV isolates yielded a single RNA component when subjected to polyacrylamide gel electrophoresis. The relative molecular mass of the TuMV-RNA, estimated in comparison with marker RNAs, was 3.1×10^6 Da.

Aphid transmission studies. Mechanical or aphid transmission failed with TuMV-Al from garlic mustard to rutabaga or TuMV-Br from rutabaga to garlic mustard. The TuMV-Al isolate was readily transmitted between garlic mustard plants by aphids and by sap-inoculations with unbuffered plant sap, as was the TuMV-Br isolated between rutabaga plants. Veinal necrosis was observed on leaves of garlic mustard after rub-inoculation with TuMV-Al.

The ability of aphids to retain and transmit each isolate of TuMV was similar (Table 3). *M. persicae* transmitted TuMV after 4–8 hr but was unable to transmit virus when starved for periods exceeding 8 hr.

DISCUSSION

Symptoms induced by TuMV-Al in *A. petiolata* were similar to those described by Lisa and Lovisolo (12). Veinbanding, however, was not seen on naturally infected plants in the field but was the predominant symptom in mechanically inoculated plants. Although Lisa and Lovisolo (12) were unable to find any other natural host for the virus, we also isolated TuMV-Al from *H. matronalis* occurring in the same habitat. Both isolates, when back-inoculated into either *A. petiolata* or *H. matronalis*, produced similar symptoms, whereas TuMV-Br failed to produce infection in either plant.

Differentiation of the two strains of TuMV was possible only by examining the differential reaction of indicator plants to each virus. The TuMV-Al isolate was similar to the type II isolate reported by both Tomlinson (18) and McDonald and Hiebert (13) in that it failed to infect a number of *Brassica* species, specifically *B. oleracea*. The main differences with the TuMV-Br

Table 1. Effects of the *Alliaria* strain of turnip mosaic virus (TuMV-Al) infection of *A. petiolata* on seed characteristics of plants grown in a glasshouse

Plants	Pod length (mm)	Seeds per pod[a] (no.)	Seed diameter[a] (mm)	Seed germination[b] (%)
Healthy	38.0 ± 1.8	9.3 ± 1.1	3.2 ± 0.1	38
TuMV-infected	29.3 ± 1.9	9.2 ± 1.0	2.6 ± 0.2	36

[a] Values represent mean ± standard deviation of measurement of 10 pods pooled from each of 30 plants.
[b] Values represent percentages seed germination from seed pooled from 10 pods from each of 30 plants.

Table 2. Comparative host range of turnip mosaic virus isolates (TuMV-Al and TuMV-Br)

Plants	Local and systemic reaction[a] TuMV-Al	TuMV-Br
Amaranthaceae		
Gomphrena globosa	nll	nll
Chenopodioaceae		
Beta vulgaris	No infection	No infection
Chenopodium amaranticolor	nll	cll, nll, vf
C. quinoa	nll	cll, nll, vf
Cruciferae		
Alliaria petiolata	Mosaic	No infection
Brassica oleracea var. *capitata*[b]	No infection	Mosaic
B. oleracea var. *botrytis*[b]	No infection	Mosaic
B. napus var. *napobrassica* 'Laurentien'[b]	No infection	Mosaic
B. napus subsp. *oleifera* 'Tandem'[b]	No infection	Mosaic
Cheiranthus cheiri	Flower breaking	cll, mosaic
Hesperis matronalis		
Matthiola incana	Flower breaking, mosaic	cll, mosaic
Cucurbitaceae		
Cucumis sativus	No infection	No infection
Labiatae		
Ocimum basilicum[b]	nll	No infection
Leguminosae		
Phasolus vulgaris	cll	cll
Vigna unguiculata	No infection	No infection
Solanaceae		
Capsicum annuum	No infection	No infection
Datura stramonium	No infection	No infection
Lycopersicum esculentum	No infection	No infection
Nicotiana glutinosa[b]	No symptoms	cll
N. tabacum	cll	cll, nll
Petunia hybrida	Mosaic	Mosaic

[a] cll = Chlorotic local lesions, nll = necrotic local lesions, and vf = veinal flecking. "No infection" was confirmed by back-inoculation into *C. quinoa* and by ELISA.
[b] Differential species for the diagnosis of TuMV-Al strains.

Table 3. Retention time of turnip mosaic virus isolates (TuMV-Al and TuMV-Br) by groups of 10 *Myzus persicae* after 30 min of acquisition feeding

Postacquisition starvation time (hr)	No. plants infected of 10 inoculated by aphid feeding	
	TuMV-Al	TuMV-Br
0.0	6	7
0.5	5	5
1.0	3	4
2.0	2	3
4.0	1	2
8.0	1	1
16.0	0	0
24.0	0	0
36.0	0	0

isolate belonging to type I are similar to those outlined by Lisa and Lovisolo (12) with two exceptions. The TuMV-Al isolate failed to infect *B. napus* and no symptoms were visible on *N. glutinosa*, although virus particles were found in low numbers throughout the latter plant. Systemic infection of *N. glutinosa* by an *Alliaria* isolate of TuMV has also been reported by Milicic et al (14).

Contrary to the findings of Brcak and Polak (2), in Ontario, *A. petiolata* does not appear to be a reservoir of TuMV-Br for infection of commercially grown *Brassica* crops. The use of mechanical inoculations to differential hosts in surveys of *Brassica* crops in southwestern Ontario in 1986 (*unpublished*) failed to identify the TuMV-Al isolate of TuMV, whereas the TuMV-Br was commonly associated with field infections.

TuMV was not seedborne in *A. petiolata*. Seed collected from infected plants, though smaller, did not show reduced viability. This is consistent with other reports of TuMV isolates where seed transmission of virus was not reported (18). Overwintering of the virus is reported to occur in underground parts of the plant (2).

Both TuMV isolates were styletborne and transmitted nonpersistently by *M. persicae* to their respective hosts. Retention time by *M. persicae* was similar for each virus isolate, and the aphid remained viruliferous for up to 8 hr after feeding. Transmission of this type is characteristic of the TuMV group (18).

Virus particles of each isolate were similar under the electron microscope and serum to TuMV-Br reacted positively to preparations of TuMV-Al and TuMV-Br. Both viruses were readily detected within their host range by ELISA. Serological comparisons between the TuMV-Al and TuMV-Br isolates (12) indicate that the two isolates are related and can be considered strains of the same virus.

Coelectrophoresis of dissociated protein from TuMV-Al and TuMV-Br failed to resolve any differences in relative relative molecular masses among the three protein fractions. The relative molecular masses of these protein components are approximately 2,000–3,000 Da higher than those reported by Choi et al (6). This slight discrepancy in relative molecular mass may be due to incomplete protein dissociation by SDS, introducing error into relative molecular mass determination using SDS electrophoresis (11). The significance of each of these components has already been discussed (6). Similarly, no differences were found in the RNA component of the two isolates.

On the basis of those similarities, a strong relatedness was shown between the *A. petiolata* and *B. napus* virus isolates, indicating that TuMV-Al is a closely related strain of TuMV.

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