common, whereas *D. lotus, D. virginiana,* and *D. rhombifolia* had the other three bands Rm 0.39, 0.46, and 0.52. The banding pattern observed in *D. rhodocalyx* was conspicuously different from the others.

There was no evidence of environmental effect on the isozyme banding patterns of GPI and MDH. Leaf tissues from young and old plants of the same cultivar gave similar isozyme patterns. The phenotypes were also constant when 'Hiratanenashi' trees grafted on seedling rootstocks of Japanese persimmon were compared with those on *D. rhombifolia*. Season did not appear to influence isozyme banding patterns, since periodic samplings of leaf tissue from 'Hiratanenashi' or 'Fuyu' trees gave consistent isozyme phenotypes from May through October.

Of the two isozyme systems examined, GPI seemed to be particularly useful in discriminating among cultivars because of a variety of banding patterns. The GPI banding pattern of each cultivar is presented in Table 1. 'Yamato Gosho', 'Onihei', 'Iwasedo', 'Kikumanju', 'Tohachi', and 'Shogatsu' had unique banding patterns. These cultivars could be identified by the GPI system alone. On the other hand, the MDH system showed less variation, and none of the cultivars was classified uniquely. However, when the GPI and MDH systems were taken together, 40 classes resulted, and 12 cultivars, 'Kunitomi', 'Anzai', 'Aizu Mishirazu', 'Yokono', 'Nigorokonashiba', 'Oyotsumizo', 'Haze Gosho', 'Dojohachiya', 'Emon', 'Hana Gosho', 'Kyara', and 'Nagara' (originated in Tokushima Prefecture), were newly identified. When isozyme phenotypes were supplemented with other characters, such as morphological and physiological traits, cultivar identification was facilitated. Japanese persimmon cultivars usually are classified into four groups according to fruit type: a) pollination-constant and non astringent (PCNA), b) pollination-constant and astringent (PCA), c) pollination-variant and non-astringent (PVNA), and d) pollination variant and astringent (PVA) (5). Taking these fruit types into account, most of the cultivars could be classified into smaller groups (Table 1).

In the absence of mutations affecting enzyme mobility, cultivars derived by sporting from a common original cultivar would be expected to have an identical isozyme phenotype (10). Attempts to differentiate sports of cultivars using GPI and MDH isozymes were unsuccessful. 'Sugita Wase', 'Tone Wase', and 'Spur Type Hiratanenashi', all derived from 'Hiratanenashi', could not be distinguished from the original 'Hiratanenashi'. Similarly, a dwarf strain of 'Nishimura Wase' exhibited the same isozyme phenotype as 'Nishimura Wase'. Further, a sport of 'Fuyu', 'Matsumoto Wase Fuyu', was not separable from 'Fuyu' by isozyme phenotype nor were two sports of 'Jiro', 'Maekawa Jiro' and 'Koyo', from the original. It would appear that these genetically well-defined enzyme systems would not be useful in distinguishing sports from a common original cultivar.

The following sets of cultivars assumed as identical or similar showed identical isozyme phenotypes: 'Koshu Hyakume', 'Daishiro', and 'Fuji'; 'Jisha' and 'Wase Jisha'; 'Okozu' and 'Shibu Myotan'; 'Sangokuichi' and 'Yosaburo'; 'Seikan' and 'Toyoka'; 'Yatsudera' and 'Mizushima Gosho'; 'Fuyu' and 'Benisakigake'; 'Tenryubo' and 'Hiroshimashimofuri'; and 'Takase' and 'Hagakushi'. If cultivars assumed as identical or similar in spite of having different names showed the identical isozyme phenotypes, it might indicate that they had the same origin.

Our study demonstrated that polymorphism existed among the Japanese persimmon cultivars. There is a need to explore additional enzyme systems to enable more cultivars to be discriminated, and inheritance should be studied to expand our understanding of the genetics of the hexaploid Japanese persimmon cultivars.

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Evaluation of Rutabaga Cultivars for Turnip Mosaic Virus Resistance and the Inheritance of Resistance

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Abstract. **Rutabaga (*Brassica napus* L. Group *Napobrassica*) cultivars were screened for resistance to turnip mosaic virus (TuMV), and the inheritance of resistance was studied. Immunity and resistance were found in four of 49 rutabaga cultivars evaluated. The cultivar 'Sensation' was immune to the Ontario strain of TuMV. In crosses between susceptible 'Laurentian' and resistant Line 165 and susceptible triazine-resistant 'Laurentian' and Line 165, TuMV resistance was determined by a single dominant gene.**

Rutabagas are a popular table vegetable in North America and many parts of the world. Although rutabagas traditionally have been used for fodder, they are cultivated in North America today primarily for human consumption. In recent years, turnip mosaic virus (TuMV) has become a serious problem for the rutabaga industry in southern Ontario, Canada, which is the major rutabaga-producing area in North America. In 1985 a TuMV epidemic in southern Ontario caused production losses of more than 30%. Rutabaga plants infected with TuMV during early development are stunted and usually fail to produce roots of marketable size. Plants infected during the later stages of development are difficult to harvest mechanically and may produce roots with decreased storability.

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Table 1. Reaction of rutabaga cultivars to field infection of turnip mosaic virus in Ontario, Canada, 1986.

Cultivar	Plant response[z] S	R	S/R
Acme[y]			+
Ashgrove	+		
Bangholm	+		
Best of All	+		
Calder		+	
Champion	+		
Conqueror	+		
Criffel	+		
Crimson King	+		
Danestone	+		
Doon Major[y]	+		
Doon Spartan	+		
Essex Model	+		
Fortune	+		
Grandmaster	+		
Gofmanskaja Belaja	+		
Harriettfield	+		
Inverquhomery	+		
Kalfafellsrofa	+		
Kiri			+
Large White	+		
Laurentian[y]	+		
Laurentian (Triazine resistant)	+		
Line 165		+[x]	
Magnificent[y]	+		
Mancunian	+		
Marian	+		
Merrick	+		
Mestnaja	+		
Mestnaja Belaja	+		
Mestnaja Belaja Zelenogolovaja	+		
Monkwood	+		
Parkside	+		
Peerless[y]	+		
Premier	+		
Purple King	+		
Ruta Otofte			+
Sator Otofte			+
Scotia	+		
Seefelder	+		
Sensation		+	
Snedkaja Zeltaja	+		
Superlative	+		
Tankard	+		
Tiperary	+		
Victory	+		
Viking	+		
Vogesa		+	
Wilhelmsburger	+		

[z]S = foliar symptoms present, R = foliar symptoms absent, S/R = segregation for symptomless and susceptible plants.
[y]More than one commercial seed source evaluated.
[x]Mild virus symptoms at harvest.

Table 2. Genetic analysis of turnip mosaic virus resistance in seedlings from rutabaga crosses.

Generation	No. plants Resistant	Susceptible	Expected ratio	Goodness of fit (χ^2 test)
Laurentian (P_1)		15		
Triazine Resistant Laurentian (P_2)		15		
Line 165 (P_3)	20			
F_1 ($P_1 \times P_3$)[z]	22			
F_1 ($P_2 \times P_3$)[z]	20			
F_2 ($P_1 \times P_3$)	127	45	3:1	0.80–0.70
F_2 ($P_2 \times P_3$)	150	50	3:1	1.00
BC_1 (F_1 ($P_1 \times P_3$) $\times P_1$)	31	29	1:1	0.90–0.80
BC_2 (F_1 ($P_2 \times P_3$) $\times P_2$)	51	45	1:1	0.70–0.50
BC_1 (F_1 ($P_2 \times P_3$) $\times P_3$)	24			

[z]Reciprocal differences were absent, so the data are pooled for presentation.

TuMV is transmitted by more than 49 species of aphids, and attempts to control these vectors and the spread of TuMV through insecticides have proven ineffective (5). A single strain of TuMV 1 infects Ontario-grown rutabagas, and this strain is more virulent than those occurring in Great Britain (B.H. McNeill, personal communication). In addition, it is not one of the major TuMV strains (C1, C2, and C3) (7) found in New York State (L.W.S. and V.I.S., unpublished data).

The development of resistant cultivars is needed and seems promising in view of the isolation of a rutabaga line in Great Britain possessing immunity to TuMV (Line 165) (8). However, there is no information available on the inheritance of TuMV immunity from this source, which would be useful in planning a breeding program. Therefore, a study was conducted to examine the inheritance of TuMV immunity in rutabaga and also to screen additional rutabaga sources for immunity or resistance to the Ontario strain of TuMV.

Rutabaga cultivars were obtained from the National Vegetable Research Station, Wellesbourne, Warwick, U.K.; the Plant Gene Resources of Canada in Ottawa, Ont. Canada; Stokes Seeds and Dominion Seed House in Ont., Canada; and Ken Proudfoot of the Agriculture Canada Research Station, St. John's, Newfoundland, Canada. Triazine-herbicide-resistant 'Laurentian' was supplied by V. Souza-Machado (Horticultural Dept., Univ. of Guelph) and Line 165 was obtained from J.A. Tomlinson (National Vegetable Research Station, Wellesbourne, Warwick, U.K.). The cultivars, listed in Table 1, were hand-sown on 15 June 1986 at the Horticultural Research Station, Cambridge, Ont. Plants were grown on Fox sandy loam soil (fine loamy over sandy or sandy skeletal, mixed, mesic-typic hapludalfs), pH 6.5, in rows 30 cm apart with 20-cm spacing between plants in rows. The rows were ≈8 m long. The experimental design was completely randomized with one replication per cultivar. To assess the uniformity of TuMV infection, 10 rows of 'Laurentian', a cultivar very susceptible to TuMV, were randomly planted within the trial, and all border rows were also 'Laurentian'. Nitrogen, P, and K fertilizers were applied at rates of 30, 80 and 60 kg·ha^{-1} and were broadcast prior to planting. Boron, as sodium borate, was applied as a foliar spray at 300 ppm once during the season to prevent brown heart. Natural rainfall was supplemented with sprinkler irrigation when required. Insect and disease control followed commercial practices of the region.

The trial was examined visually three times during the season for the presence of TuMV infection, with no attempt made to rate the severity of foliar symptoms. On 21 Oct., the trial was harvested and roots for each cultivar bulked for an estimation of fresh weight. Young leaves from 'Calder', Line 165, 'Sensation', and 'Vogesa' were collected and assayed for the presence of TuMV using the enzyme-linked immunosorbent assay (ELISA) technique of Clark and Adams (4) as modified by Lister (6). All tests were done in polystyrene Removawell plates (Immulon 2, Dynateck Laboratories, Alexandria, Va.) with 200 µl of liquid used for each of the four steps. Wells were coated with purified TuMV immunoglobulin (Ig) at 2 µg·ml^{-1} at 38°C for 3 hr. After washing, 200 µl of crude healthy or test plant tissue macerates [1 tissue : 19 ELISA extraction buffer ratio (v/v)] were each loaded into five replicate wells for each sample. Alkaline phosphatase (Type 7, Sigma) conjugated to TuMV Ig at a 2.5 enzyme : 1 Ig ratio (w/w) was added for 3 hr at 38°. 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. For immune electron microscopy (IEM), TuMV-sensitized, carbon-stabilized, collodion-coated copper grids (38 µm) were floated on tissue macerates for 30 min. Grids were rinsed in buffer, stained in 2% phosphotungstic acid (pH 6.5), and examined with a Philips EM201 electron microscope.

During 1985 and 1986, greenhouse tests were conducted to evaluate the inheritance of TuMV immunity previously reported for Line 165 (8). The parents used in this study included 'Laurentian', triazine-resistant 'Laurentian' ('TRL') and Line 165. 'Laurentian' was chosen as a parent because it is the most important rutabaga cultivar grown commercially in North America. 'TRL' was developed by V. Souza-Machado at the Univ. of Guelph by transferring the genome of 'Laurentian' into the cytoplasm of the weed Bird's Rape (*Brassica campestris* L.). This cultivar is very desirable for commercial production, because triazine herbicides as α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-p-toluidine (trifluralin) can be used with 'TRL' but not 'Laurentian' at the pre- and post-emergence stages for control of germinating annual grasses and broadleaf weeds. Line 165 is a selection originating from the cultivar 'Ruta Otofte', possessing immunity to a strain of TuMV in Warwickshire, U.K. (8).

Parental plants were sown in the greenhouse on 10 Dec. 1985 in 15-cm pots con-

taining 2 peatmoss : 1 soil : 1 perlite (by volume) for 8 weeks under natural lighting supplemented by high-intensity sodium vapor lamps yielding a quantum flux at pot level of 150 $\mu mol \cdot s^{-1} \cdot m^{-2}$ when measured by a LI-COR Quantum Photometer (Model LI-185). Plants were maintained on a 16-hr light period, and a 20N–20P–20K (3.0 $g \cdot liter^{-1}$) fertilizer solution was applied weekly. Night and day temperatures were 15° and 25°C, respectively. The 8-week-old plants were vernalized for 15 weeks (2) and then returned to the greenhouse for flowering and subsequent crossing. 'Laurentian' and 'TRL' were crossed reciprocally to Line 165 to obtain the F_1, F_2, and backcross generations. Inadequate seed number was common when Line 165 was used as the maternal parent in crosses, despite the use of bud pollination. Insufficient population size resulted for several of the generations, and they are not reported. Seeds from the controlled pollinations were planted and grown under the conditions described for the parental plants. After 5 weeks, leaves of each seedling were dusted with celite and then rub-inoculated with the diluted leaf sap from TuMV-infected rutabaga plants collected from Cambridge, Ont. Each seedling was assessed visually for the absence (R) or presence (S) of TuMV leaf symptoms 23 days following inoculation. A random sample of 52 symptomless plants of the F_2 generation were evaluated further for the presence or absence of TuMV by ELISA. The F_2 generation of the cross 'TRL' × Line 165 also was evaluated for trifluraline-resistance using chlorophyll fluorescence (1) prior to TuMV inoculation.

During Summer 1986, TuMV infection was severe at Cambridge, and no escapes were recorded for 'Laurentian'. TuMV symptoms on the plants were visible by 10 July, which was ≈1 month after sowing. Foliage symptoms for TuMV-infected plants varied from mild chlorotic mottling to marked mosaic patterns along with a reduction in leaf size and increased leaf rugosity. The mean root weights among the cultivars ranged from 533 to 1509 g, with the average being 924.7 ± 36.2 g. The mean root weight obtained for 'Laurentian' ($\bar{x}$ = 612 g) was far lower than what would have been normally expected at Cambridge ($\bar{x}$ = 1000–1100 g) in the absence of TuMV. Line 165 had the highest root weight ($\bar{x}$ = 1509 g) in the trial. In general, genotypes possessing vigourous vegetative growth ('Best of All', 'Doon Major', 'Harrietfield', 'Large White', 'Purple King', 'Wilhelmsburger', and 'Tankard') produced roots well in excess of 1000 g while displaying mild symptoms of TuMV infection. During most of the season, Line 165 remained symptomless, but at harvest, very mild chlorotic foliage symptoms were observed. ELISA revealed the presence of TuMV in Line 165, suggesting that it is resistant but not immune to the Ontario strain of TuMV. However, the high degree of resistance in Line 165 should be sufficient in a breeding program. Of the cultivars evaluated, 'Calder' ($\bar{x}$ = 908 g), 'Sensation' ($\bar{x}$ = 1317 g), and 'Vogessa' ($\bar{x}$ = 1362 g) were symptomless at harvest (Table 1), and TuMV was not detected by ELISA. Subsequent IEM examination of mechanically inoculated 'Sensation' failed to reveal TuMV particles, implying that 'Sensation' is immune to the Ontario TuMV strain. 'Sensation' has been reported (3) to possess considerable resistance to TuMV and aphids. Although no IEM examination was performed on inoculated 'Calder', it also may possess immunity, since 'Calder' was developed from 'Sensation'. Unlike 'Sensation', when 'Vogessa' was manually inoculated with TuMV, IEM examination revealed the presence of virus particles in plant foliage, suggesting that 'Vogessa' possessed high resistance but not immunity to the Ontario strain of TuMV. 'Acme', 'Kiri', 'Ruta Otofte', and 'Sator Otofte' segregated for symptomless and infected plants, suggesting these populations were heterozygous for resistance. Tomlinson (8) observed in Great Britain that 'Acme' and 'Ruta Otofte' populations segregated for TuMV resistance with the frequencies for symptomless plants dependent on the commercial seed source. 'Kiri' originated from complex parentage including 'Sensation'; thus symptomless segregants in 'Kiri' also may possess immunity factor(s) to TuMV. Symptomless 'Ruta Otofte' plants in the trial likely carried the highly resistant gene present in Line 165.

The field trial confirms prior observations that the rutabaga cultivars grown commonly in North America (e.g., 'American Purple Top', 'Fortune', 'Laurentian', 'Purple Top', and 'York') are very susceptible to the Ontario strain of TuMV. However, the data indicate that immunity as well as differences in resistance can be found in germplasm outside of North America. This information offers promise of additional alleles for TuMV resistance, which would be important for rutabaga breeders striving for resistance stability.

Data for the genetic analysis are presented in Table 2. As expected, both 'Laurentian' and triazine-resistant 'Laurentian' developed severe systemic mosaic leaf symptoms and leaf rugosity, while Line 165 remained symptomless after TuMV inoculation. Heterogeneity χ^2 tests for differences between the reciprocal crosses involving 'Laurentian' and Line 165 were nonsignificant, so the data were combined for analysis. All the F_1 plants of this cross were resistant (Table 2). The segregation data of the F_2 population indicated a good χ^2 fit to the expected 3R-1S ratio. The F_1 backcrossed to 'Laurentian' gave further evidence for monogenic control of TuMV resistance, as plants segregated in a resistant to susceptible ratio of 1:1. The segregation ratios for the F_2 and backcross populations fit the hypothesis that a single dominant gene conditions TuMV resistance in this cross. Immune electron microscopy observations revealed a random sample of resistant F_2 plants that possessed trace amounts of TuMV, a finding that supports the field data that Line 165 is not immune to the Ontario strain of TuMV.

Maternal differences also were absent for the cross involving 'TRL' and Line 165 and the 200 F_2 progeny segregated perfectly into 3R:1S (Table 2). The F_2 data and backcross generations indicate that a single dominant nuclear gene conditions resistance (Table 2). The F_2 progeny, which were resistant to TuMV, also were resistant to trifluralin. It appears that the Bird's Rape cytoplasm does not adversely affect the expression of TuMV resistance, and conversely the gene for TuMV resistance does not interfere with the expression of triazine resistance. This information should be useful to breeders attempting to move resistance from Line 165 into other important Brassicas developed with the Bird's Rape cytoplasm. It is suggested the symbol *Tum* for turnip mosaic virus resistance be used for this gene from Line 165.

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