Effect of Turnip Mosaic Virus Infection on the Development, Virus Titer, Glucosinolate Concentrations, and Storability of Rutabaga Roots

L. W. STOBBS, Agriculture Canada, Research Station, Vineland Station, Ontario L0R 2E0; and V. I. SHATTUCK and B. J. SHELP, Department of Horticultural Sciences, University of Guelph, Guelph, Ontario N1G 2W1

ABSTRACT

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Rows of rutabaga plants, grown in a greenhouse groundbed, were inoculated with turnip mosaic virus (TuMV) at 2-wk intervals during root development. Plants inoculated within 12 wk after seeding produced unmarketable roots that were small and elongated with severe goosenecking of the upper root and stem. Dry matter content of healthy roots and roots infected at different stages in their development did not differ significantly. Infected roots contained low levels of virus, independent of the stage of root development at which they were inoculated. The presence of TuMV in roots and decay of the roots in storage were not correlated. Roots infected early in their development contained higher concentrations of total glucosinolates, compared with roots infected later. These glucosinolates decreased in concentration as the root size increased. The concentration of 2-hydroxy-3-butenyl glucosinolate was higher in inoculated plants than in uninoculated plants, whereas other glucosinolates were unaffected or reduced in concentration in inoculated plants. Such alterations in glucosinolate metabolism may result in flavor changes and metabolic breakdown products, such as 5-vinyloxazolidine-2-thione, resulting from the hydrolysis of 2-hydroxy-3-butenyl glucosinolate, a potent goitrogen.

In recent years, turnip mosaic virus (TuMV) has become a persistent problem for the rutabaga industry in southern Ontario. Plants infected early in their development become stunted and generally do not produce roots of marketable size (18). Roots from plants infected with TuMV reportedly store poorly (1), possibly a consequence of altered mineral composition in the roots (29) or from plants being grown in soil with excessive soil nitrogen concentration (18). In addition, studies have shown that when rutabaga tissues are ruptured through injury or insect damage, various secondary products are produced from the hydrolytic breakdown of endogenous glucosinolates (11). These secondary products may adversely affect root flavor (32) and possibly have adverse health effects (2,11,24,39). This study was initiated to investigate the effects of TuMV on root development, levels of virus in root tissues, changes in glucosinolate concentrations, and storability of roots of plants infected at various stages of development.

MATERIALS AND METHODS

Rutabaga (*Brassica napus* ssp. *rapifera* (Metzger) Sinsk 'Laurentien') seed was dusted with captan and seeded in a groundbed in 3.5-m rows spaced 1 m apart in an enclosed greenhouse (12.3 × 9.2 m). The bed consisted of a well-drained sandy loam soil (pH 6.4), which was top-dressed with 35 kg N/ha (NH_4NO_3). The greenhouse mean temperature was 26 ± 5 C with a relative humidity (RH) of 70 ± 5%. Four replicate rows were used for each treatment. Seedlings were thinned to 15 cm and given bimonthly foliar applications of Solubor (0.5 kg B/ha; Oligosol Ltd., Beloeill, Quebec) according to product recommendations (25). The greenhouse was fumigated weekly with Sulfatep (sulfotepp, Plant Fume No. 103 insecticide, Plant Products Co. Ltd., Brampton, Ontario) for aphid control. Oil sprays (20 L of Sunspray oil 7E per 1,000 L of water per hectare) were used initially but were found to be unnecessary for aphid control and caused some phytotoxicity when the greenhouse temperature rose over 28 C. Furadan (carbofuran) drenches (5 L of Furadan per 1,000 L of water per hectare) (Chemagro Ltd., Mississauga, Ontario) were applied at 3-wk intervals. Weeds were removed manually.

The TuMV-S1 strain (38), common to southern Ontario, was used in all inoculations. Virus was maintained on Laurentian rutabaga in a separate greenhouse, and leaf triturates (1:9, tissue/buffer, 0.01 M phosphate buffered saline [PBS], pH 7.2) were used to manually inoculate Carborundum-dusted leaves of the different inoculation treatments. The first four rows of rutabaga were inoculated when the plants reached the six-leaf stage (6 wk after seeding), and additional four-row sections were subsequently inoculated at 2-wk intervals over 12 wk. Four rows of uninoculated plants served as controls. To minimize any possible virus spread, the virus-inoculated and uninoculated plants were kept at opposite ends of the greenhouse.

All roots were harvested 18 wk after seeding. The fresh weight, diameter, and length of 80 roots were measured for each inoculation. The percentage of dry weights of roots was determined by taking a 5-cm slice of tissue through the center of eight roots from each inoculation, dicing these individually into 2-cm cubes, and drying each sample at 60 C.

Virus assays. Both foliar and root tissues were assayed for virus from each inoculation treatment. Twenty plants were randomly selected from each treatment and the foliage was assayed for virus by enzyme-linked immunosorbent assay (ELISA) (7). A 2.5-cm slice of tissue was sampled through the center of each root and similarly assayed. In addition, root and leaf tissue macerates were assayed for virus by serologically specific electron microscopy (SSEM) and bioassay on *Chenopodium quinoa* Willd. (36).

Preparation of fluorescent antibody conjugate. A monoclonal antiserum (14) prepared against the common strain of TuMV was used for conjugation. The antiserum had a titer of 1:2,048 as determined by the sodium dodecyl sulfate (SDS) agar diffusion test (27). Conjugation of the immunoglobulin G with fluorescein isothiocyanate (FITC) was as described previously (37). The procedure yielded a protein/fluorescein molar ratio of 1:2.5 when the optical density was measured at 280 and 495 nm. Conjugate was stored in aliquots at −20 C.

Fluorescent antibody staining. Root and leaf tissue pieces (0.5 cm^3 and 0.5 cm^2, respectively) were fixed for 12 hr in 2.5% glutaraldehyde in 0.5 M neutral phosphate buffer at 4 C. After rinsing in buffer, the tissues were dehydrated in a tert-butyl alcohol series, embedded in Paraplast Plus, and sectioned at 8 μm on a rotary microtome. Sections were mounted on glass slides with a gelatin adhesive, dried, and the paraffin was removed with xylene. Tissues were rehydrated in a graded ethanol series and stored in buffer until required. Sections were stained with undiluted fluorescent antibody stain for 15 min at 25 C. After destaining for 30 min in several changes

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of PBS (0.15 M neutral phosphate buffer + 0.15 M NaCl), sections were mounted in glycerin/PBS (9:1) and examined with a Leitz Dialux 20 microscope equipped with an HBO 50W mercury burner and Leitz exciter filter-barrier filter set H2 (390–495 nm excitation, 515 nm suppression).

Glucosinolate assays. At the end of the test, six randomly selected roots were selected from among the four rows. They were peeled and a 2.5-cm slice, taken through the center of the root (representing approximately 100 g), was rapid-frozen by immersion in liquid nitrogen. Tissues were promptly sealed in plastic freezer bags and stored at −65 C until they could be freeze-dried. All freeze-dried samples were double-sealed in plastic freezer bags and kept at 4 C until assays could be performed.

Glucosinolates were extracted from freeze-dried root tissue (31), then desulfated on ion-exchange mini-columns (8,28). The desulfoglucosinolate solutions were separated by gradient system high-performance liquid chromatography (HPLC) with a C18 (5 μm) reverse-phase column (25.0 cm × 4.6 mm). The identity of the glucosinolates was confirmed by LC plasma spray mass spectrometry (31). The concentrations of the glucosinolates presented in this study were adjusted according to their response factors (4). Regression analysis was performed between each inoculation time and the level of individual glucosinates. The glucosinolate concentrations in uninoculated roots were compared with those of inoculated roots (35).

Storage study. Roots from the various treatments were washed, air-dried, and stored according to accepted commercial storage recommendations (16). Roots were stored in bushel baskets in cold storage (2 ± 2 C, 90 ± 4% RH) over 8 mo, and 10 roots from each treatment were examined bimonthly for signs of brown internal discoloration or secondary decay. After 6 mo in storage, eight roots from each inoculation treatment were potted and placed in the greenhouse. New growth was assayed for TuMV by ELISA and SSEM.

RESULTS

Rutabaga roots infected with TuMV early in their development were small and elongated at harvest (Fig. 1). Most exhibited the characteristic "gooseneck" appearance resulting from accelerated abscission and replacement of leaves along the stem (Fig. 2). As plants were inoculated progressively later in their development, roots were correspondingly larger and more spherical in shape (Fig. 1, Table 1). Root fresh weight and diameter increased with each 2-wk delay in rutabaga inoculation (Table 1). No significant differences were found in percentage of dry matter of roots from any of the inoculation periods when compared with the control (Table 1).

Low levels of virus were detected by ELISA in root tissue (Table 2). Substrate absorbance values for root tissue were about 20% of those recorded for leaf tissue (Table 2). Few lesions (three to five) were produced on *C. quinoa* leaves inoculated with root triturates compared with foliar tissue triturates (120–130 lesions per leaf). Similarly, there were fewer virus particles in root tissue (two to four particles per field) compared with those in foliar tissue (50–80 particles per field) when viewed under the electron microscope at the same magnification (×16,350) (Table 2). In all assays, no quantitative differences in virus titer were apparent in the roots from the different inoculation times.

Fluorescent antibody staining of paraffin-embedded sections failed to reveal virus in the roots. Fluorescent antibody staining detected virus-specific fluorescence in foliar tissues that was largely associated with mesophyll and epidermal cells. Macerates from root vascular tissues had similar numbers of virus particles as undifferentiated parenchymal tissue when examined under the electron microscope. Particle counts remained low (approximately two to three particles per field).

The principal glucosinolates in rutabaga roots are presented in Table 3. Eight principal compounds were identified in roots, which accounted for more than 95% of the total glucosinolate concentration. Roots of both uninoculated and inoculated plants were rich in glucosinolates, with 2-hydroxy-3-butenyl and 4-methylthiobutyl glucosinolates being the most abundant compounds.

Regression analysis indicated that the concentrations of 4-methylthiobutyl, 3-indolylmethyl, 2-phenylethyl, and 1-methoxy-3-indolymethyl glucosinolates in inoculated roots at harvest were dependent on inoculation time (Table 3). In general, the concentration of these glucosinolates decreased linearily and/or quadratically as the inoculation date was delayed. The concentration of 2-hydroxy-3-butenyl glucosinolate in inoculated roots was independent of the time of inoculation. Inoculated roots contained a significantly higher concentration of this glucosinolate at all inoculation times than the control with mean concentrations ranging from 22.04 to 17.07 μM/g of dry tissue (Table 3). Although significant concentration differences ($P \leq 0.05$) for 4-hydroxy-3-indolylmethyl glucosinolate were noted between uninoculated and inoculated roots, the concentration of this glucosinolate over the dates of inoculation did not follow any regression pattern.

The total glucosinolate concentration in inoculated roots at harvest decreased

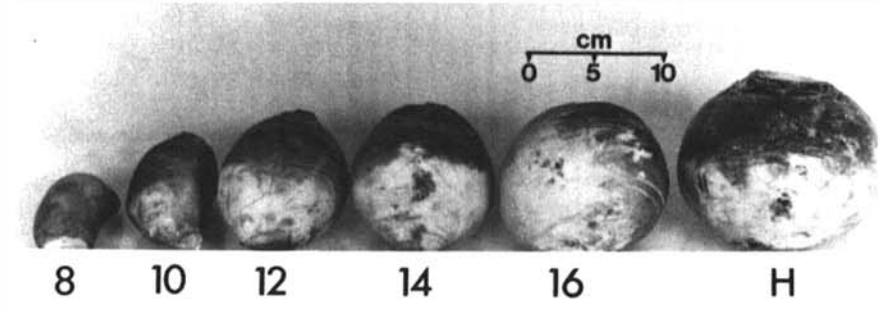

Fig. 1. Effect of turnip mosaic virus on root development. Numbers indicated the time of inoculation (weeks) after seeding. H = healthy control. All roots were harvested 18 wk after seeding.

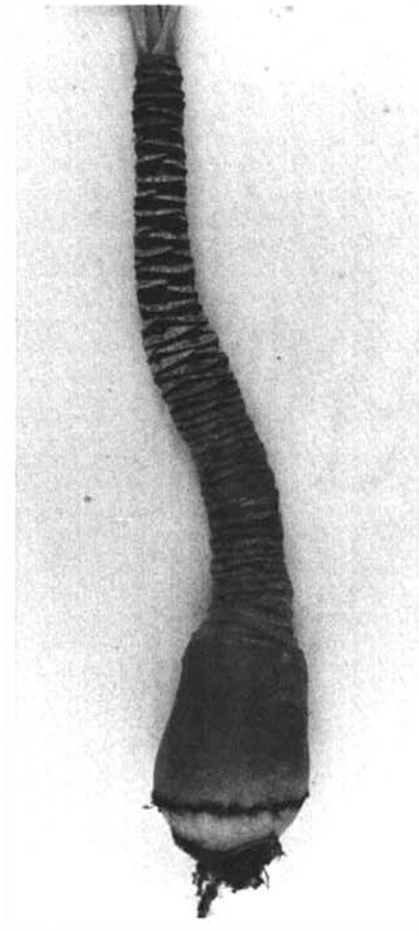

Fig. 2. Goosenecking of the stem caused by accelerated loss and replacement of leaves in plants infected with turnip mosaic virus.

linearly and quadratically as the date of inoculation was delayed. If infection occurred later than 12 wk after seeding, the total glucosinolate content in inoculated roots at harvest did not differ significantly ($P \leq 0.05$) from the controls (Table 3).

No differences in breakdown of roots at the different inoculation times occurred during 8 mo of cold storage. All roots remained firm with no internal discoloration or decay from secondary disease organisms. When individual roots from the different inoculation times were regrown in the greenhouse, new foliar growth showed virus symptoms and assayed positive for virus. Foliage from healthy roots grown under the same conditions was not infected with virus.

DISCUSSION

The effects of TuMV infection in rutabaga were more pronounced in plants infected early in their development. Roots were small, unmarketable, and generally elongated. Loss of foliage associated with early infections resulted in severe goosenecking of the stem and upper root. Canada No. 1 roots for the fresh market (10–15 cm in diameter) were produced when infection of plants was delayed approximately 12 wk after seeding under the conditions of our study. These roots, when trimmed, were globular with a diameter-to-length ratio close to 1 (Table 1). Such roots have been shown to be preferred by consumers (30). In field plantings, early seeding of rutabaga in late May to early June would normally permit roots to reach a marketable size and preferred shape before the introduction of virus by aphids, generally occurring in late July to August in southwestern Ontario (18).

In addition, other studies (17) have reported that rutabaga is more susceptible to aphid transmission of virus at the four-leaf stage where 50–70% of the plants were infected, compared with approximately 5% infection of plants older than 8 wk. Similar findings have been described in potato where young plants were shown to be more susceptible to potato virus Y. Peak susceptibility occurred during rapid plant growth before tuber enlargement (3). Presumably, early field plantings of rutabaga would be less subject to aphid transmission of virus by the time aphid populations peak in Ontario in late July and early August.

In many hosts, virus infection affects photosynthetic rates and the translocation of assimilates (26,34). Reduced photosynthetic capacity resulting from smaller leaf size and premature leaf abscission of infected rutabaga plants was likely the primary determinant in root stunting in early infections. Previous studies suggest that late infection of rutabaga produced only minimal effects on the later stages of assimilate partitioning to the root (30). Plants inoculated early in their development with TuMV produced roots of smaller size with a greater concentration of total glucosinolate than plants inoculated at a later growth stage.

It is possible that the variation in root size between the uninfected and infected plants (Table 1) may have contributed

Table 1. Effect of turnip mosaic virus inoculation at 2-wk intervals on weight and size of rutabaga roots after 18 wk of growth in a greenhousee groundbed

Age at inoculation (wk)	Root measurements			
	Fresh weight (kg)	Dry weight (%)	Diameter (cm)	Diameter/ length ratio
6	0.27 a[z]	9.6 a	4.30 a	0.33 a
8	0.38 a	10.0 a	6.00 b	0.62 b
10	0.55 b	9.7 a	8.29 c	0.74 b
12	0.82 c	9.0 a	11.78 d	0.88 c
14	1.08 d	10.0 a	14.09 e	0.93 d
16	1.41 e	9.5 a	15.96 e	1.03 d
Uninoculated	1.46 e	10.4 a	15.98 e	1.04 d

[z]Means within each column not followed by the same letter differ significantly ($P = 0.05$) according to the t test, $n = 80$.

Table 2. Virus titer in rutabaga root and leaf tissues infected at different ages when grown for 18 wk in a greenhouse groundbed

Age at inoculation (wk)	ELISA (absorbance [405 nm])		Bioassay (no. of lesions)		SSEM (no. of virions)	
	Root	Leaf	Root	Leaf	Root	Leaf
6	0.48 b[z]	2.99 b	4 b	120 b	2 b	80 b
8	0.51 b	3.03 b	3 b	134 b	4 b	54 b
10	0.48 b	3.08 b	3 b	118 b	3 b	72 b
12	0.48 b	3.02 b	4 b	122 b	4 b	68 b
14	0.51 b	2.93 b	3 b	131 b	2 b	59 b
16	0.49 b	2.98 b	5 b	127 b	4 b	73 b
Uninoculated	0.18 a	0.14 a	0 a	0 a	0 a	0 a

[z]Means within columns not followed by the same letter differ significantly (t test, $P = 0.05$). Each value is a mean of 20 observations.

Table 3. Glucosinolate composition[x] of turnip roots from plants infected or uninfected with turnip mosaic virus at various ages when grown for 18 wk in a greenhouse groundbed

Glucosinolate	Infected plants (μmol/g dry weight)						Uninoculated plants	Regression[y]		
	Age when inoculated (wk)									
	6	8	10	12	14	16		L	Q	C
2-hydroxy-3-butenyl	22.04[z]	19.73*	20.25*	21.18*	17.07*	20.04*	12.11	NS	NS	NS
4-methylsulfinyl-butyl	0.67	0.79	0.60	0.49	0.69	0.69	0.72	NS	NS	NS
3-butenyl	0.27	0.24	0.33	0.22	0.29	0.21	0.20	NS	NS	NS
4-hydroxy-3-indolylmethyl	0.37*	0.17	0.38*	0.22	0.25	0.40*	0.26	NS	NS	NS
4-methylthiobutyl	5.66*	5.23*	5.48*	3.60	2.98	3.70	3.26	*	*	NS
3-indolylmethyl	0.14	0.19	0.02*	0.04*	0.03*	0.10*	0.34	*	*	NS
2-phenylethyl	0.15	0.13	0.10	0.10	0.09	0.12	0.07	*	NS	NS
1-methoxy-3-indolylmethyl	0.30*	0.22	0.09	0.11	0.09	0.09	0.17	*	NS	NS
Total glucosinolates	29.60*	26.70*	27.25*	25.96*	21.49	25.35	17.13	*	*	NS

[x]Small amounts of 4-methoxy-3-indolylmethyl and an unidentified saturated butyl glucosinolate detected but not listed.

[y]Linear (L), quadratic (Q), and cubic (C) regression analyses performed on inoculation dates against individual concentration of glucosinolates. NS = nonsignificance, and * = significance at the $P \leq 0.05$ level.

[z]For each glucosinolate, values followed by * indicate significant ($P \leq 0.05$) differences in concentration from the uninoculated controls according to Dunnett's test.

to the differences in glucosinolate concentrations. For example, plants inoculated 6 wk after seeding accumulated far less root dry matter ($\bar{x}$ = 26 g) than the uninoculated control ($\bar{x}$ = 152 g) but were 72% higher in total glucosinolate concentration. Furthermore, as the root size increased, the concentration of total glucosinolates decreased both linearly and/or quadratically. A similar observation has been reported for field-grown turnips (*Brassica rapa* L. ssp. *rapifera* Metzger) where the size of small roots is inversely correlated with glucosinolate concentration (5). Such possible "tissue dilution" of glucosinolates cannot account for the specific differences in concentrations of 2-hydroxy-3-butenyl and 3-indolylmethyl glucosinolates from TuMV infection.

Rutabaga roots contain abundant amounts of glucosinolates (6), which undergo degradation by the enzyme myrosinase to produce various breakdown products that contribute to the flavor and odor of the root. The two major glucosinolates in peeled root tissue were 2-hydroxy-3-butenyl and 4-methylthiobutyl glucosinolate; the concentration of these glucosinolates was altered in response to TuMV infection (Table 3). Bitterness in brussels sprouts has been attributed to the hydrolysis of 2-hydroxy-3-butenyl glucosinolate to 5-vinyloxazolidine-2-thione (10). A radish-like flavor has been reported (21) from the hydrolysis of 4-methylthiobutyl glucosinolate to 4-methylthiobutyl isothiocyanate (15). Thus, roots produced from rutabaga plants infected early in their development might possess an altered flavor. However, it should be noted that besides the products of hydrolysis of glucosinolates, other compounds such as sugars, organic acids, aldehydes, alcohols, ketones (19,20), and other volatiles (13), as well as the conditions of root storage and processing (9), may influence root flavor.

It is noteworthy that the level of 2-hydroxy-3-butenyl glucosinolate was significantly increased in roots of infected plants with the concentration being independent of the time of inoculation and root size. The hydrolysis of 2-hydroxy-3-butenyl glucosinolate forms 5-vinyloxazolidine-2-thione, which contributes to rutabaga root flavor, but it is also a potent goitrogen (24). Interestingly, it has been reported that rutabaga contributes the greatest amount of 2-hydroxy-3-butenyl glucosinolate to Canadian diets (22), and concerns regarding this glucosinolate in human diets has been expressed (22,23).

The cause of root breakdown in storage remains unclear. Several growers and packers have attributed storage problems largely to TuMV infection. Provincial disease control recommendations clearly state that rutabagas infected with virus store poorly and should be marketed as soon as possible (25). No scientific studies have conclusively associated storage breakdown with the presence of TuMV. Mineral imbalances may reduce storability of rutabaga roots (12), although our previous study failed to demonstrate any differences in elemental composition on root storability between infected and healthy roots (30). Roots sampled from the different inoculation times failed to demonstrate any differences in storability; all roots remained firm with no secondary decay over an 8-mo storage period. Dry matter content of the roots did not differ significantly between roots infected at different stages in their development, although dry matter content of roots is not necessarily associated with resistance to storage deterioration (33). Similarly, no significant differences were found in virus titer in roots sampled from the different inoculation periods; virus levels being very low relative to foliar virus titers. Virus levels were close to the detection endpoint of ELISA (10 ng of virus per milliliter of sap) (L. W. Stobbs, *unpublished data*), and virus was not observed by fluorescent antibody staining of root tissues. Because the presence of virus in root tissue could not be correlated with reduced root storability, it is more likely that other factors are involved in storage decay of roots. Cultural practices, presence of soilborne pathogens, and storage conditions need to be examined more closely for causal effect on the breakdown of roots from plants infected with a virus.

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