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Volatile Isothiocyanates and Nitriles from Glucosinolates in Rutabaga and Turnip[1]

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Abstract. **Glucosinolates extracted from seeds of 2 rutabaga (*Brassica napus* L. Napobrassica group) and 2 turnip (*B. rapa* L. Rapifera group) cultivars, and also from roots sampled at 2-week intervals during growth and development on 2 soil types, were hydrolyzed and the individual volatile products identified by gas chromatography and mass spectrometry. Four isothiocyanates (3-butenyl-, 4-pentenyl-, 4-methylthiobutyl-, and 2-phenylethyl-isothiocyanates), and 2 nitriles (1-cyano-4-methylthiobutane and 2-phenylethylnitrile), were identified. Yields of each constituent varied considerably between cultivars, and also seasonally in root tissue, but generally were quantitatively similar in trend within cultivar grown on loam and organic soils; 2-phenylethyl-isothiocyanate was predominant in roots.**

There is concern about the levels and types of natural plant products in food crops, especially with regards to their toxic and biological effects (4, 10, 14). Cruciferous plants contain glucosinolates (thioglucosides) which form various enzymatic hydrolytic products when plant cells are ruptured. Some of these products are known to be toxic: thiocyanate ion and goitrin (5-vinyl-oxazolidine-2-thione) are goitrogenic; and certain nitriles may suppress growth (7, 15, 18). Isothiocyanates (mustard oils) contribute significantly to the flavour and aroma of cruciferous vegetable crops (8, 9) and may be involved in insect predation and allelochemic properties of these plants (6, 7, 19).

Previously, Ju et al. (12) reported variation in glucosinolate composition, expressed in terms of the hydrolysis products, goitrin, thiocyanate ion, and volatile isothiocyanates, in seeds and in roots of rutabaga ('The Laurentian' and Altasweet') and summer turnips ('Tokyo Cross' and 'Snow Ball'), during growth and development on both loam and organic soils. The seasonal yields of these glucosinolate products tended to be higher in roots of these crops grown on organic soil (12). In this related communication, we examined further some individual glucosinolates, as identified by gas chromatography and mass spectrometry (GC-MS) of their hydrolysis products in seeds and in selected root samples from these crops during growth and development on 2 soil types.

Materials and Methods

Field plots. On May 27, 1978, 'The Laurentian' and 'Altasweet' rutabaga and 'Tokyo Cross' and 'Snow Ball' turnip were direct-seeded in both loam and organic soils in rows 2.1-m-long, spaced 0.9-m-apart, arranged in a randomized block design with 3 replications. Seedlings were thinned 2 weeks after seeding (cotyledon stage) to within-row spacing of 15 cm. Standard recommendations for pesticide and other cultural practices were followed.

Sampling procedures and extractions. Samples of seed (triplicate 10-g samples), and whole roots of 2-week seedlings (100 per replication), and representative wedge-shaped 100-g subsamples of root (6 plants per replication) were collected from each cultivar at 2-week intervals until 16 weeks after seeding. Seed samples were ground in an electric coffee grinder and defatted for 24 hr in a Soxhlet extraction apparatus using hexane. The defatted material was air-dried at room temperature and the powder stored at 2°C in tightly capped glass bottles. Root samples were cut into small pieces, immediately frozen in liquid nitrogen, and stored in a freezer at -20°. After freeze-drying, samples were pulverized and restored in the freezer until analyzed.

For each sample, 500 mg of powder was weighed into a 50-ml screwcapped test tube. Tubes were placed in a water bath at 95 to 100°C for 20 min. Five ml of boiling citrate-phospate buffer (pH 7.0) were added to each tube which was reheated to 95 to 100° for 30 min. After cooling, 10 ml of freshly mixed myrosinase solution (5 mg/ml), obtained from seeds of white mustard (*B. hirta* Moench), were added (17). This mixture was then extracted with 10 ml of methylene chloride by shaking on a reciprocating shaker (180 excursions/min, Eberback Co., Mich.) for 2 hr. The tube was then centrifuged at 5000 rpm for 3 to 5 min, after which the extract separated into 2 layers. Five ml of the lower layer (methylene chloride extract) were transferred to a 10-ml graduated tube and evaporated to 1 ml with a slow stream of nitrogen keeping the graduated tube placed in an ice bath to prevent loss of the more volatile isothiocyanates and nitriles.

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GC analysis. Based on the method of Mullin et al. (13), 100μl of methyl palmitate (internal standard 0.1 g/100 ml methylene chloride) was added to 0.8 ml of the final extract, and 4 μl of this mixture was injected into a gas chromatograph equipped with dual flame ionization detectors, installed with 2 m × 2 mm I.D. glass columns packed with Chromosorb W HP, 80/100 mesh, and coated with 1.5% OV 17 (Chromatographic Specialities Ltd., Ontario). The temperature was set at 230°C at the injector and 240° for the detector.

The column temperature at injection was 50°C and the programmed rate was 10°/min until an upper limit of 210° was reached and held for 30 min. Each volatile constituent was expressed as μg/g air-dried weight for seeds or freeze-dried weight for root samples. In view of the long duration for these analyses and their good reproducibility, only 1 replicate sample was analyzed, but all determinations were done in duplicate.

MS analysis. To aid in the identification of the volatile constituents, aliquots of sample extracts from seeds of 'Tokyo Cross' turnip only, and from root extracts of 'Altasweet' rutabaga and 'Snow Ball' turnip at the 6-week stage only, were subjected to mass spectrometry using a Finnigan Model 3100 D interfaced with Finnigan 6100 data acquisition system at Agriculture Canada, Ottawa (13). The gas-liquid chromatograph columns had the same dimension and packing materials as described above and the temperature of the columns was set at 50°C for the initial isothermal period of 8 min, then increased at 8° per min to a final temperature of 200°

Results and Discussion

Four isothiocyanates (3-butenyl-, 4-pentenyl-, 4-methylthiobutyl-, and 2-phenylethyl-isothiocyanates) and 2 nitriles (1-cyano-4-methylthiobutane and 2-phenylethylnitrile) were identified in seed and root tissue of rutabagas and turnips by GC-MS analysis (Table 1). Yields of each constituent varied considerably between cultivars in seed (Table 1) and also seasonally in root tissue during growth and development (Fig. 1, Table 2).

In seeds of both turnip cultivars (Table 1), 3-butenyl-isothiocyanate predominated (2561 to 5036 μg/g dry weight), but occurred only in small yields (36 μg/g) in 'The Laurentian' rutabaga, and was not detected in seeds of 'Altasweet' rutabaga. In contrast to seeds, 2-phenylethyl-isothiocyanate was generally the predominant volatile glucosinolate hydrolysis product in roots of both species during growth and development (Fig. 1). The yield of this constituent increased rapidly from the 2- to 4-week stage, or was highest during this period as in the case of 'Tokyo Cross' turnip, followed by a progressively decreasing trend to the 8- to 10-week stage, after which there was little change during the remainder of the growing season. Although the yield of 2-phenylethyl-isothiocyanate was similar in the 2 rutabaga cultivars during development on both soil types, this constituent tended to be lower in turnip cultivars grown on loam soil, particularly during the 2- to 6-week stages (Fig. 1).

Yields of 4-methylthiobutyl-isothiocyanate were relatively large, particularly between the 6- and 10-week stages in both rutabaga cultivars, but were low or not detectable during certain stages of development in turnips (Table 2). In contrast to the predominance of 3-butenyl-isothiocyanate in turnip seeds (Table 1), small but significant yields of this constituent were detected only in 'The Laurentian' rutabaga and in 'Tokyo Cross' turnip on both soil types, with generally trace quantities in 'Snow Ball' turnip and none detected in 'Altasweet' rutabaga (Table 2). 4-Pentenyl-isothiocyanate (Table 2) occurred in small yields but was not consistently present in all cultivars, and tended to occur between the 2- and 8-week stages whenever present.

The presence of the analyzed volatile constituents found in rutabaga and turnip is in agreement with reports of Cole and Phelps (5) and Mullin et al. (13), except for 3-butenyl-isothiocyanate, which was found in rutabaga. In roots of both species, the seasonal variation of the major constituent, 2-phenylethyl-isothiocyanate, was similar to those for volatile isothiocyanates

Table 1. Volatile isothiocyanates and nitriles from glucosinolates as identified in seeds and roots of rutabaga and turnip cultivars.

Volatile constituents	Structure and molecular weight[z]	Glucosinolate	Source	Presence of volatile constituent			
				Rutabaga		Turnip	
				The Laurentian	Altasweet	Tokyo Cross	Snow Ball
3-Butenyl-isothiocyanate	$CH_2 = {}_{113}CH(CH_2)_2NCS$	3-Butenyl[y] (Gluconapin)[x]	Seed	+[w](36)[v]	−	+(5036)	+(2561)
			Root	+	−	+	+
4-Pentenyl-isothiocyanate	$CH_2 = {}_{127}CH(CH_2)_3NCS$	4-Pentenyl (Glucobrassicanapin)	Seed	−	−	t	t
			Root	−	−	+	+
1-Cyano-4-methylthiobutane	$CH_3S_{129}(CH_2)_4C{\equiv}N$	4-Methylthiobutyl (Glucoerucin)	Seed	−	−	−	−
			Root	+	+	+	+
2-Phenylethyl-nitrile	$C_6H_{5131}CH_2CH_2C{\equiv}N$	Phenylethyl (gluconasturtin)	Seed	+ (26)	+ (42)	+ (31)	+ (44)
			Root	+	+	+	+
4-Methylthiobutyl-isothiocyanate	$HC_3S_{161}(CH_2)_4NCS$	4-Methylthiobutyl (Glucorucin)	Seed	+ (52)	−	−	−
			Root	+	+	+	+
2-Phenylethyl-isothiocyanate	$C_6H_{5163}CH_2CH_2NCS$	Phenylethyl (Gluconasturtin)	Seed	+ (77)	+ (126)	+ (121)	+ (150)
			Root	+	+	+	+

[z]Structure and molecular weight confirmed by MS analysis.
[y]R-group in the glucosinolate.
[x]Trivial name of precursor glucosinolate.
[w]+, present; −, not detected; t, trace quantity.
[v]Numbers in ( ) refer to amounts detected in μg/g dry weight using an arbitrary GC response factor of 1.0 with regards to methyl palmitate excepting 2-phenylethyl-isothiocyanate for which pure standard was available.

Table 2. Seasonal variation of volatile isothiocyanates and nitriles from glucosinolates in roots of rutabaga and turnip during growth and development on two soil types.

Cultivar	Soil type	Volatile constituent (μg/g dry wt) Weeks from seeding 2	4	6	8	10	12	14	16
		4-methylthiobutyl-isothiocyanate							
The Laurentian rutabaga	Loam	– [z]	207	289	178	407	96	47	t
	Organic	–	131	628	277	296	259	251	174
Altasweet rutabaga	Loam	–	334	356	262	278	168	23	37
	Organic	–	81	711	288	209	51	55	47
Tokyo Cross turnip	Loam	–	39	33	–	31	27	–	–
	Organic	–	124	65	–	–	–	–	–
Snow Ball turnip	Loam	–	109	48	t	36	–	–	–
	Organic	–	81	117	13	129	105	111	–
		3-butenyl-isothiocyanate							
The Laurentian rutabaga	Loam	84	176	47	t	16	t	–	–
	Organic	194	132	173	21	24	22	13	8
Altasweet rutabaga	Loam	–	–	–	–	–	–	–	–
	Organic	–	–	–	–	–	–	–	–
Tokyo Cross turnip	Loam	54	96	107	25	84	98	32	87
	Organic	8	303	268	18	379	282	370	57
Snow Ball turnip	Loam	–	t	t	t	–	–	–	–
	Organic	–	–	104	t	t	t	31	t
		4-pentenyl-isothiocyanate							
The Laurentian rutabaga	Loam	–	–	–	–	–	–	–	–
	Organic	–	–	–	–	–	–	–	–
Altasweet rutabaga	Loam	–	–	–	–	–	–	–	–
	Organic	–	–	–	–	–	–	–	–
Tokyo Cross turnip	Loam	–	11	29	–	25	41	–	45
	Organic	–	41	49	–	52	55	31	10
Snow Ball turnip	Loam	–	t	26	15	18	–	–	15
	Organic	–	–	83	49	20	9	t	27
		1-cyano-4-methylthiobutane							
The Laurentian rutabaga	Loam	–	32	112	14	t	t	–	–
	Organic	t	106	25	20	t	t	13	12
Altasweet rutabaga	Loam	18	119	192	38	24	15	20	20
	Organic	–	–	69	21	20	13	12	18
Tokyo Cross turnip	Loam	297	134	96	19	43	11	21	31
	Organic	161	33	31	44	t	t	13	17
Snow Ball turnip	Loam	97	460	198	118	55	39	52	39
	Organic	52	120	45	151	38	65	55	9
		2-phenylethylnitrile							
The Laurentian rutabaga	Loam	–	276	191	51	t	t	–	–
	Organic	t	13	63	40	t	t	t	t
Altasweet rutabaga	Loam	9	562	283	48	17	13	14	18
	Organic	311	142	145	66	29	16	14	16
Tokyo Cross turnip	Loam	–	–	–	t	t	t	t	t
	Organic	–	–	–	t	t	t	t	t
Snow Ball turnip	Loam	–	32	t	–	–	28	–	t
	Organic	–	–	–	–	t	105	111	9

[z] –, not detected; t, trace quantity.

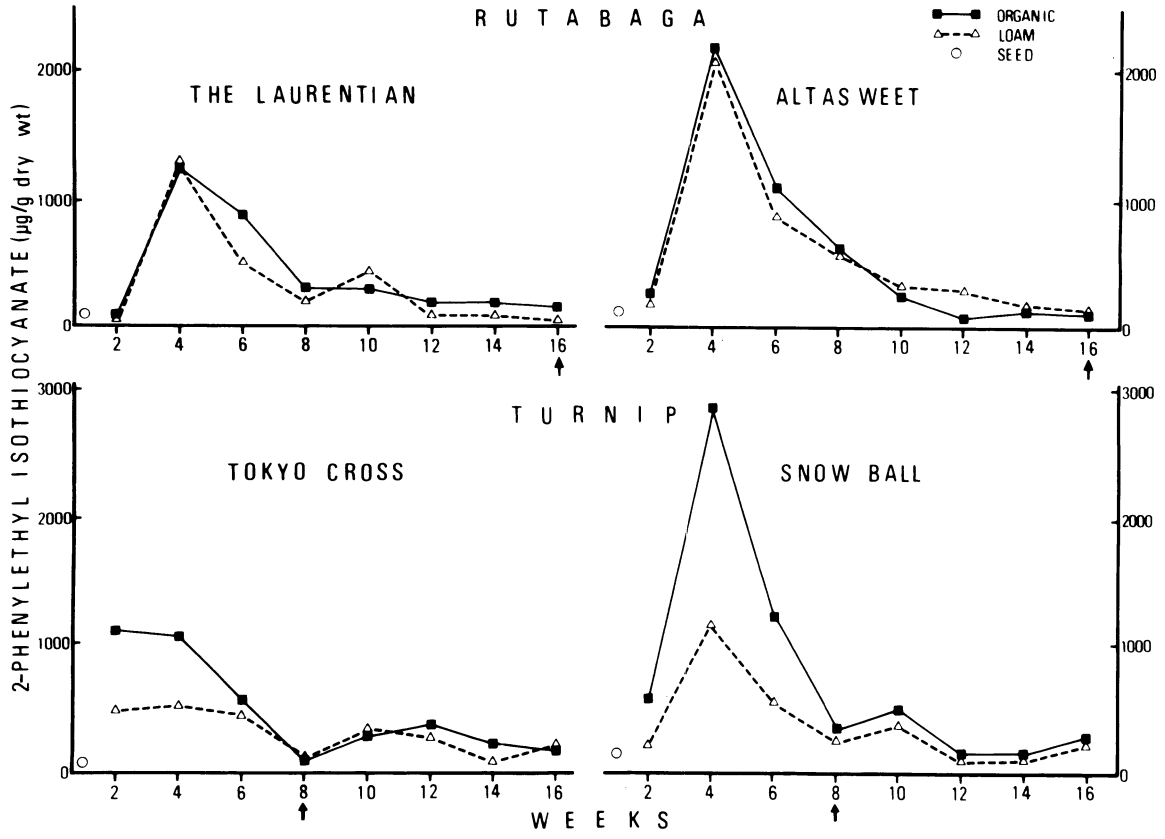

Fig. 1. Variation in 2-phenylethyl-isothiocyanate in root of rutabaga and turnip cultivars during growth and development on 2 soil types. Data represent means from duplicate GC analysis of samples from one replicate. Arrows indicate sampling data closest to optimum marketable stage.

(total fraction) analyzed by UV spectrophotometry (12). Interestingly, correlation analysis indicated that during development on both soil types, the yields of 2-phenylethyl-isothiocyanate in roots of both species were consistently correlated with their corresponding seasonal top/root ratios (Table 3). A similar or consistent relationship was not observed for volatile isothiocyanates (total fraction). Correlative relationships between glucosinolate constituents and top/root ratio (3) and also other parameters have been reported (1, 2). Tookey et al. (18) also reported correlative patterns between seed and corresponding leafy heads in 50 cabbage cultivars, but the present study does not have enough detailed data to allow a similar interpretation.

The occurrence of nitriles, 1-cyano-4-methylthiobutane in seed (Table 2), and 2-phenylethylnitrile in seed and root tissue (Tables 1 and 2), indicates that accurate analysis of glucosinolates in vegetable material is prone to problems of extraction. If it is assumed that these compounds were derived solely from hydrolysis of glucosinolates, and are not present in the intact plant cells, then they must be formed during sample preparation. Particular care must be taken to prevent autolysis of root samples and low moisture content of seed sample should eliminate hydrolysis during grinding and defatting operations. However, other studies have indicated that these hydrolysis products will be present even though extreme precautions are taken (9, 13). The proportion of nitrile formed relative to the corresponding isothiocyanate was inconsistent, varying with cultivar and species (Tables 1 and 2). Under the strict analytical regime used in this study, consistent nitrile formation would have indicated the presence of a nitrile-forming enzyme unaffected by the heat treatment of the samples. Since epithiospecifier protein, known to be present in turnip (16), was inactivated by heat treatment, it is unlikely that myrosinase or other coenzymes would survive. These results lead to 2 conclusions. First, in any analysis of glucosinolates, nitriles should be determined to give a more accurate result; nitriles do not form thiourea derivatives and are not included in the so-called "total" glucosinolate data (12). If intact glucosinolates are extracted for subsequent analysis in procedures, such as those employed by Helboe et al. (11), the nitriles will not be included either. Second, the data from this study indicate that nitriles may occur *in situ* in cruciferous plant material and may not be entirely due to autolysis or hydrolysis of glucosinolates.

Table 3. Correlation of top/root ratio with the yield of 2-phenylethyl-isothiscyanate in roots of rutabaga and turnip cultivars during growth and development on two soil types.

Cultivar	Soil type	Correlation coefficients (r)
The Laurentian rutabaga	Loam	0.936**
	Organic	0.785*
Altasweet rutabaga	Loam	0.960**
	Organic	0.901**
Tokyo Cross turnip	Loam	0.718*
	Organic	0.830*
Snow Ball turnip	Loam	0.930**
	Organic	0.786*

*,**Significant at 5% (*) or 1% (**) based on 6 df.

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Fertilization, Incorporated Organic Matter, and Early Growth of Rabbiteye Blueberries[1]

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Abstract. **The effect of incorporated sphagnum peatmoss and minimal fertilization on the establishment and subsequent growth of rabbiteye blueberries (*Vaccinium ashei* Reade) was determined in 4 field studies conducted on typical fine sandy loam, upland mineral soils in south Mississippi. Incorporated peatmoss increased plant vigor, plant height, shoot weight, leaf chlorophyll level, and fruit yield and reduced chlorosis symptoms. First- and second-year plant growth and second-year fruit yields were reduced by either slow-release or fast-release granulated fertilizer. Soluble fertilizers produced less plant damage than granulated fertilizers but no more plant growth than no fertilization. There was a close association between over-fertilization and cholorosis symptoms.**

Commercial rabbiteye blueberry plantings are normally established using 2-year-old, bare-rooted plants. Early and continued optimum fruit production depends upon the successful establishment and first-year growth of these plants. General planting instructions include the incorporation of peatmoss into soil in the planting hole and irrigation (1, 5, 8, 9); soil pH should range from 4.5 to 5.5.

Young rabbiteye blueberry plants are very sensitive to fertilizer (5, 7, 8, 9, 10) and inorganic fertilizer placed directly under first-year plants can cause severe damage (3). Fertilization recommendations (N–P–K) for the first year range from none (8, 9) to 5 monthly applications 8–5–7 fertilizer at the rate of 115–150 g/plant (6). A first-year fertilization program in Florida consists of applying 28 g of ammonium sulfate per plant in June and August (1). The recommendation in Georgia is to apply 42 g per plant of 12–2–7 or 16–2–7 slow-release fertilizer when growth begins and again in late July or early August (7). In Arkansas, good first-year growth resulted from the application of 225 g/plant of a complete fertilizer such as 10–9–8 in early spring, followed by 2 applications of 112 kg/ha of ammonium sulfate at 6-week intervals (7).

These studies were conducted to determine the effects of first-

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