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Influence of Temperature and Ontogeny on the Levels of Glucosinolates in Broccoli (*Brassica oleracea* Var. *italica*) Sprouts and Their Effect on the Induction of Mammalian Phase 2 Enzymes

FERNANDA MARIA VALENTE PEREIRA,[†] EDUARDO ROSA,*[,†] JED W. FAHEY,[‡,§] KATHERINE K. STEPHENSON,[‡] ROSA CARVALHO,[†] AND ALFREDO AIRES[†]

Department of Plant Science and Agricultural Engineering, Universidade de Trás-os-Montes e Alto Douro, Apartado 1013, 5001-911 Vila Real, Portugal; The Lewis B. and Dorothy Cullman Cancer Chemoprotection Center and Brassica Chemoprotection Laboratory, Department of Pharmacology and Molecular Sciences, School of Medicine, and Center for Human Nutrition, Bloomberg School of Public Health, The Johns Hopkins University, 725 North Wolfe Street, Baltimore, Maryland 21205

Broccoli inflorescences have been recognized as components of healthy diets on the basis of their high content of fiber, vitamin C, carotenoids, and glucosinolates/isothiocyanates. Broccoli sprouts have been recently shown to have high levels of glucoraphanin (4-methylsulfinylbutyl glucosinolate), the precursor of the chemoprotective isothiocyanate, sulforaphane. This study evaluated the effects of temperature and developmental stage on the glucosinolate content of broccoli sprouts. Seedlings cultivated using a 30/15 °C (day/night) temperature regime had significantly higher glucosinolate levels (measured at six consecutive days postemergence) than did sprouts cultivated at lower temperatures (22/15 and 18/12 °C; $p < 0.001$). Both higher (33.1 °C) and lower (11.3 °C) constant temperatures induced higher glucosinolate levels in sprouts grown to a uniform size. Glucosinolate levels were highest in cotyledons and lowest in roots of sprouts dissected both early and late in the 11 day developmental span investigated. Nongerminated seeds have the highest glucosinolate levels and concordantly greater induction of mammalian phase 2 detoxication enzymes. Levels decline as sprouts germinate and develop, with consistently higher glucosinolate content in younger developmental stages, independent of the temperature regime. Temperature stress or its associated developmental anomalies induce higher glucosinolate levels, specific elevations in glucoraphanin content, and parallel induction of phase 2 chemoprotective enzymes.

KEYWORDS: Glucosinolates; broccoli sprouts; chemoprotection; antioxidant

INTRODUCTION

Cruciferous vegetables have received widespread endorsement as nutritionally beneficial vegetables with a number of significant attributes. Broccoli (*Brassica oleracea* var. *italica*) is a crucifer that is an excellent source of vitamin C, calcium, magnesium, carotenoids, and fiber (*1*−*4*). Broccoli has received particular attention as the best source of glucoraphanin (4-methylsulfinylbutyl glucosinolate; GR), a member of a group of plant secondary metabolites termed glucosinolates, which have been shown to possess health attributes (*15*).

Glucosinolates are sulfur-containing, water-soluble plant defense compounds in which one of over 120 side chains (typically either aliphatic, aromatic, or indolic) are attached to a central carbon that is also linked to both a β-thioglucoside and a thiohydroximate moiety (*12*). Glucosinolates are hydrolyzed by the enzyme β-thioglucoside glucohydrolase (EC 3.2.3.1.), commonly referred to as myrosinase, which is found both in human bowel microflora (*5*) and in the cells of the glucosinolate-containing plants, where it reacts with its glucosinolate substrate only upon loss of cellular integrity (e.g., predation/mastication by humans or animals, freeze−thaw injury, or plant pathogens). The myrosinase reaction results in the liberation of glucose and sulfate and, depending upon the hydrolytic conditions, the formation of biologically active compounds such as isothiocyanates, thiocyanates, and nitriles having diverse effects upon human health. Some of these breakdown products have been shown to be potent inducers of phase 2 detoxication enzymes, and others have been shown to be either inhibitors or inducers of phase 1 enzymes. Sulforaphane, which under physiological conditions is the major

* Author to whom correspondence should be addressed (fax 351 259 350 629; e-mail erosa@utad.pt).
[†] Universidade de Trás-os-Montes e Alto Douro.
[‡] The Lewis. B. and Dorothy Cullman Cancer Chemoprotection Center and Brassica Chemoprotection Laboratory, The Johns Hopkins University.
[§] Center for Human Nutrition, The Johns Hopkins University.

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myrosinase breakdown product from glucoraphanin, is one of the most potent naturally occurring inducers of phase 2 enzymes (*6*−*8*). A recent study (*9*) reported a maximum content of 2.85 mmol·100 g DW^{-1} of glucoraphanin within a group of 11 broccoli cultivars, the highest values thus far reported from mature broccoli. Broccoli sprouts, however, are the most potent dietary source of glucoraphanin, exceeding that of mature broccoli (*8*, *10*, *11*). Climatic conditions have been reported to have a significant influence on glucosinolate levels [see the review by Rosa et al. (*12*)]. Whereas soil temperatures of 15−20 °C are reported to be optimal for growth and germination of broccoli (*13*), supraoptimal temperatures have been reported to induce increases in cabbage seedling glucosinolates (*14*). Plant organ and plant developmental stage (climate dependent) also have a significant influence on glucosinolate levels and on the relative distribution of individual glucosinolates [see reviews by Rosa et al. (*12*) and Fahey et al. (*14*)].

This study evaluated the influence of temperature on the levels of total and individual glucosinolates such as glucoraphanin in broccoli sprouts. These effects were examined over the period of early development in order to elucidate the effects of temperature and harvest time on glucoraphanin production. An overview of broccoli sprout glucosinolate profiles in a representative sampling of available cultivars is presented. The relative contribution of plant organs (e.g., hypocotyls, cotyledon, radicle/root, and seed coat) to overall glucosinolate content was assessed at two points in sprout development for one of these cultivars. Although experiments were conducted at two different institutions, using different experimental systems and glucosinolate detection methodology, the results presented herein are consistent and complementary, and we have thus presented them in parallel. One set of experiments addresses the mode of sprout growth and range of temperature conditions that could be used in home and commercial broccoli sprout growing facilities. The second set of conditions more closely mimics environmental conditions to be encountered by seeds sown for broccoli production in a greenhouse or field situation.

MATERIALS AND METHODS

Plant Cultivation and Harvest. The two different methods of growth and harvest used herein approximate the two predominant styles of green sprout production. In most of Europe and in the United States, consumers of sprouts prefer younger sprouts that are typically grown in a rotating drum to produce short, curled sprouts with minimal root development and no root hairs (in particular, this is true for broccoli and alfalfa sprouts). The Japanese, and some Europeans, prefer older sprouts (in particular, Kaiware-daikon), which are typically grown in a tray. Alternatively, they are grown directly in small plastic cups and are "harvested" by the consumer after purchase, by cutting off the matted root mass and eating the "above-ground" (e.g., hypocotyls and cotyledon) portion of the sprouts. On these older sprouts, the roots are quite fibrous and unpalatable.

Older Sprouts. Untreated broccoli seeds (cv. Marathon) obtained from Sakata Seed Corp. (Yokohama, Japan) were sown under 2 cm of rockwool (Grodan, Roermond, The Netherlands) placed in 14 cm Petri dishes and watered. They were maintained in a Conviron E15 growth chamber under a 14 h day/10 h night photoperiod with 220 μmol m^{-2} s^{-1} of photosynthetically active radiation supplied from five fluorescent tubes (Osram Sylvania, Inc., Danvers, MA) under one of three temperature regimes: 30/15, 22/15, or 18/12 °C day/night. Four replicates per treatment were evaluated. Sprouts were harvested for glucosinolate determination at 6 days postsowing and for five consecutive days (sprout age = 6, 7, 8, 9, 10, and 11 days), by cutting at the rockwool level, homogenizing in liquid nitrogen, and freeze-drying.

Younger Sprouts. Untreated broccoli seeds (cultivars DeCicco, Emerald City, Everest, Green Comet, Mariner, Packman, and Saga) were purchased from Johnny's Select Seeds (Albion, ME) and Penn State Seed Co. (Dallas, PA). Seeds were surface sterilized by soaking for 15 min in 50% Clorox bleach (2.13% sodium hypochlorite), rinsed exhaustively with sterile distilled water, and sown onto sterile, semisolid Bacto agar (Difco Laboratories, Detroit, MI) prepared using only distilled water and poured to a depth of ∼2 cm. Growing containers (15 cm Petri plates or 31 × 37 cm plastic trays) were incubated in a plant growth chamber (Percival Inc., Boone, IA) providing 200 μmol m^{-2} s^{-1} of photosynthetically active radiation supplied from cool-white fluorescent tubes (Osram Sylvania, Inc.) either continuously or with a day/night cycle. Temperature extremes (11 and 33 °C), as well as three intermediate temperatures (16, 21.5, and 29 °C) were chosen to bracket the range of temperatures under which one could expect reasonable germination and growth of broccoli seeds. Harvesting was performed by gently pulling the entire sprout (including roots) from the agar and rapidly blotting, weighing, and plunging the sprout into a cold (−50 °C) mixture of equal parts of acetonitrile, dimethyl sulfoxide, and dimethylformamide ("triple solvent"), which was followed by homogenization as detailed by Fahey et al.. (*10*). Germination was scored by counting the percentage of seeds with radicle emergence. Both 100 sprout/seed weights and hypocotyl lengths were also determined. In a separate experiment with only broccoli cv. Saga, sprout organs (cotyledon, hypocotyls, radicle/root, and seed coat) were dissected from 20 sprouts grown as described above, at 22.5 °C with a 16/8 h photoperiod, and immediately plunged into a cold triple-solvent mixture, and homogenized and extracted as described in Fahey et al. (*10*) for bioassay of quinone reductase inducer potential (QRIP).

Glucosinolate Analyses. *Older Sprouts.* The freeze-dried material was reduced to a fine powder, and 200 mg aliquots were extracted by adding ∼3 mL of boiling 90% methanol plus 200 μL of benzyl glucosinolate as an internal standard. After 2 min of boiling, a procedure that simultaneously inactivates myrosinase, extracts were centrifuged and the residue was re-extracted twice with boiling 70% methanol (3 mL). Extracts were combined to give a final volume of 10 mL, and a 2.5 mL aliquot was evaporated to dryness and taken up in 2.5 mL of water. Two milliliters was applied to a small DEAE-Sephadex A 25 column (150 mm), and the adsorbed glucosinolate was desulfated as described by Heaney and Fenwick (*16*). Desulfoglucosinolates were eluted with water and analyzed by high-performance liquid chromatography (HPLC) as described by Spinks et al. (*17*). Glucosinolate concentration was expressed in relation to freeze-dried weight (DW), and moisture content of the sprouts ranged from ∼80.1 to 89.2% moisture at 4 and 9 days postsowing, respectively.

Younger Sprouts. Glucosinolate extracts were made by homogenizing sprouts in cold triple solvent (*10*) using a Polytron homogenizer (Brinkman Instruments, Westbury, NY) at half-speed for 3 min. Extracts were diluted 200-fold into microtiter plates for bioassay as described in Fahey et al. (*10*) and Prochaska and Santamaria (*18*) or analyzed by paired ion HPLC according to the methods of Prestera et al. (*19*). Confirmation of glucosinolate identities was performed on certain samples using a complementary protocol for HPLC of intact glucosinolates (*20*).

Reagents. All reagents used were of analytical or HPLC grade. Benzyl glucosinolate was kindly offered by Dr. Renato Iori (Istituto Sperimentale Colture Industriali, Bologna, Italy); other glucosinolate standards used for HPLC were isolated and identified according to published methods (*10*, *19*, *20*).

Statistics. Statistical analyses were performed using SuperANOVA v. 1.11 (Abacus Concepts Inc., Berkeley, CA) software.

RESULTS AND DISCUSSION

Early sprout growth (up to ∼25 mg/sprout) was most rapid in the temperature range in which broccoli sprouts are already being grown commercially (20−28 °C) (**Figure 1**). Growth was dramatically affected by temperature in a predictable fashion (e.g., it was fastest at "optimal" moderate temperatures and fell off dramatically at temperatures both below and above this range). Thus, it took almost 10 days at 11.3 °C to achieve the same growth increment (25 mg of FW) that was achieved in

Table 1. Glucosinolate Content in Broccoli Sprouts Grown under Three Day/Night Temperature Regimes (30/15, 22/15, and 18/12 °C), throughout 6 Consecutive Days Postsowing

temp regime, day/night (°C)	day	glucosinolates content[a] [± SD, μmol·g^{-1} of DW (FW in parentheses)]															
		GI		GR		4OHGB		GE		GB		GN		totals	
30/15	6	18.1 ± 0.68 (2.2 ± 0.10)	a	49.5 ± 1.94 (5.7 ± 0.30)	a	3.9 ± 0.33 (0.5 ± 0.10)	a	5.3 ± 0.25 (0.7 ± 0.03)	a	2.6 ± 0.27 (0.3 ± 0.04)	a	2.2 ± 0.07 (0.3 ± 0.01)	81.7 ± 3.29 (9.7 ± 0.50)	a
	7	14.4 ± 0.18 (1.6 ± 0.02)	b	40.6 ± 0.45 (4.4 ± 0.10)	b	3.0 ± 0.37 (0.3 ± 0.04)	b	3.9 ± 0.19 (0.4 ± 0.02)	b	3.1 ± 0.12 (0.4 ± 0.01)	a	2.1 ± 0.17 (0.2 ± 0.02)	67.1 ± 0.80 (7.4 ± 0.10)	b
	8	12.9 ± 0.42 (1.4 ± 0.03)	bc	35.8 ± 1.11 (3.8 ± 0.10)	b	2.5 ± 0.06 (0.3 ± 0.00)	c	3.3 ± 0.17 (0.4 ± 0.01)	b	2.6 ± 0.19 (0.3 ± 0.02)	a	2.4 ± 0.06 (0.3 ± 0.01)	59.5 ± 1.7 (6.4 ± 0.10)	bc
	9	10.9 ± 0.11 (1.2 ± 0.10)	cd	29.8 ± 2.82 (3.2 ± 0.30)	c	2.0 ± 0.17 (0.2 ± 0.02)	de	2.5 ± 0.17 (0.3 ± 0.02)	c	2.3 ± 0.12 (0.3 ± 0.01)	a	2.3 ± 0.08 (0.3 ± 0.01)	49.6 ± 4.36 (5.4 ± 0.50)	cd
	10	9.8 ± 0.62 (1.2 ± 0.10)	de	27.2 ± 1.58 (3.1 ± 0.20)	cd	1.7 ± 0.13 (0.2 ± 0.02)	e	2.1 ± 0.21 (0.3 ± 0.02)	c	2.2 ± 0.11 (0.3 ± 0.02)	a	2.4 ± 0.11 (0.3 ± 0.01)	45.3 ± 2.56 (5.3 ± 0.30)	de
	11	7.8 ± 0.78 (1.0 ± 0.10)	e	21.9 ± 1.98 (2.6 ± 0.20)	d	1.1 ± 0.15 (0.1 ± 0.02)	f	1.3 ± 0.20 (0.2 ± 0.02)	d	2.1 ± 0.07 (0.3 ± 0.01)	a	1.8 ± 0.14 (0.2 ± 0.02)	36.1 ± 3.02 (4.4 ± 0.40)	e
22/15	6	15.7 ± 2.00 (2.2 ± 0.30)	a	43.3 ± 5.12 (5.7 ± 0.70)	a	1.0 ± 0.29 (0.1 ± 0.04)	a	3.5 ± 0.63 (0.5 ± 0.10)	a	3.7 ± 0.57 (0.5 ± 0.10)	a	2.5 ± 0.47 (0.3 ± 0.10)	69.8 ± 9.06 (9.4 ± 1.20)	a
	7	15.9 ± 0.18 (2.1 ± 0.02)	a	43.7 ± 0.50 (5.5 ± 0.10)	a	1.2 ± 0.03 (0.2 ± 0.00)	a	3.5 ± 0.10 (0.5 ± 0.01)	a	4.8 ± 0.28 (0.7 ± 0.03)	b	2.9 ± 0.15 (0.4 ± 0.03)	72.1 ± 8.89 (9.3 ± 0.10)	a
	8	8.4 ± 1.31 (1.3 ± 0.10)	b	23.6 ± 3.61 (3.4 ± 0.30)	b	0.6 ± 0.22 (0.1 ± 0.03)	ab	1.5 ± 0.24 (0.2 ± 0.03)	b	2.6 ± 0.59 (0.4 ± 0.10	c	2.4 ± 0.43 (0.4 ± 0.10)	39.2 ± 6.14 (5.7 ± 0.60	b
	9	7.6 ± 0.93 (1.2 ± 0.10)	bc	22.1 ± 2.34 (3.2 ± 0.30)	bc	0.9 ± 0.14 (0.1 ± 0.02)	abc	1.2 ± 0.18 (0.2 ± 0.03)	bc	2.3 ± 0.47 (0.4 ± 0.10)	c	2.3 ± 0.46 (0.4 ± 0.10)	36.7 ± 4.43 (5.5 ± 0.60)	bc
	10	7.3 ± 0.26 (1.1 ± 0.03)	bc	21.0 ± 0.63 (3.1 ± 0.10)	bc	1.2 ± 0.07 (0.2 ± 0.01)	ac	1.0 ± 0.03 (0.2 ± 0.01)	bc	2.7 ± 0.21 (0.4 ± 0.04)	c	2.3 ± 0.26 (0.4 ± 0.05)	35.5 ± 1.21 (5.4 ± 0.20	bc
	11	5.9 ± 0.51 (1.0 ± 0.10)	c	17.4 ± 1.51 (2.7 ± 0.20)	c	0.9 ± 0.07 (0.1 ± 0.01)	abc	0.7 ± 0.10 (0.1 ± 0.02)	c	2.2 ± 0.30 (0.4 ± 0.05)	c	2.1 ± 0.27 (0.3 ± 0.04)	29.2 ± 2.67 (4.6 ± 0.40)	c
18/12	6	14.0 ± 0.44 (2.4 ± 0.10)	a	40.0 ± 1.00 (6.5 ± 0.20)	a	0.1 ± 0.02 (0.025 ± 0.00)	a	2.6 ± 0.22 (0.5 ± 0.04)	a	2.9 ± 0.23 (0.7 ± 0.04)	a	2.1 ± 0.20 (0.4 ± 0.03)	62.6 ± 1.69 (10.4 ± 0.30)	a
	7	11.1 ± 0.33 (1.9 ± 0.10)	b	32.5 ± 0.98 (5.1 ± 0.20)	b	0.07 ± 0.01 (0.012 ± 0.00)	a	1.5 ± 0.07 (0.3 ± 0.01)	b	2.6 ± 0.07 (0.6 ± 0.01)	a	2.0 ± 0.20 (0.3 ± 0.03)	50.8 ± 1.58 (8.2 ± 0.30)	b
	8	10.1 ± 0.23 (1.7 ± 0.03)	bc	30.0 ± 0.74 (4.7 ± 0.10)	bc	1.1 ± 0.0 (0.023 ± 0.01)	a	1.3 ± 0.10 (0.2 ± 0.02)	bc	3.5 ± 0.10 (0.6 ± 0.01)	a	2.3 ± 0.04 (0.4 ± 0.01)	47.4 ± 1.14 (7.6 ± 0.10)	bc
	9	8.1 ± 0.53 (1.4 ± 0.10)	cd	24.1 ± 1.63 (3.9 ± 0.30)	cd	0.3 ± 0.01 (0.048 ± 0.00)	a	0.8 ± 0.10 (0.1 ± 0.02)	cd	3.1 ± 0.18 (0.5 ± 0.03)	a	1.9 ± 0.32 (0.3 ± 0.10)	38.2 ± 2.63 (6.4 ± 0.40)	cd
	10	8.3 ± 0.84 (1.5 ± 0.20)	cd	24.5 ± 2.46 (4.3 ± 0.40)	cd	0.2 ± 0.04 (0.037 ± 0.01)	a	0.9 ± 0.18 (0.2 ± 0.03)	cd	3.0 ± 0.40 (0.6 ± 0.10)	a	2.6 ± 0.41 (0.5 ± 0.10	39.5 ± 4.23 (7.0 ± 0.80)	cd
	11	7.3 ± 0.25 (1.3 ± 0.02)	d	22.0 ± 0.85 (3.8 ± 0.10)	d	0.2 ± 0.01 (0.029 ± 0.00)	a	0.7 ± 0.08 (0.1 ± 0.01)	d	3.4 ± 0.70 (0.6 ± 0.10)	a	2.5 ± 0.38 (0.5 ± 0.10)	36.1 ± 2.17 (6.4 ± 0.20)	d

[a] Glucosinolates: GI, methylsulfinylpropyl; GR, 4-methylsulfinylbutyl; 4OHGB, 4-hydroxyindol-3-ylmethyl; GE, 4-methylthiobutyl; GB, indol-3-ylmethyl; GN, 2-phenethyl. Values for each temperature regime in the same column not followed by the same letter are significantly different at $P \leq 0.05$.

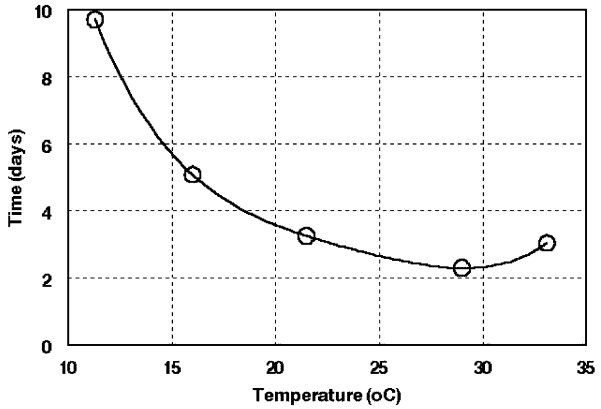

Figure 1. Influence of temperature on the time required to grow broccoli cv. DeCicco sprouts to a uniform size (25 mg/sprout). Each time point was determined by obtaining the average of three replicates of 100 sprouts for which the average blotted weight came to within 5% of the target (25 mg).

<3 days at 28 °C (**Figure 1**). Germination, however, decreased progressively with increasing temperature: percentages ± SD (four 100 seeds replicates) of seeds with radicle emergence were 79 ± 5.3, 69 ± 4.5, 75 ± 9.2, 66 ± 3.0, and 66 ± 4.1 at 11.3, 16.0, 21.5, 29.0, and 33.1 °C, respectively.

Total Glucosinolates. Total glucosinolate levels in broccoli cv. Marathon sprouts varied between 81.7 ± 3.3 and 29.2 ± 2.7 μmol g^{-1} of DW, depending upon the temperature regime and sprout age (**Table 1**). There was a highly significant effect ($p < 0.001$) of temperature (three separate regimes) on total glucosinolate levels. The highest average levels (56.6 μmol g^{-1} of DW) were observed when sprouts were grown at 30/15 °C day/night. These levels were significantly different ($p < 0.01$) from the levels observed under the two other growing conditions, 22/15 °C (47.1 μmol g^{-1} of DW) and 18/12 °C (45.8 μmol g^{-1} of DW), which were not significantly different from each other.

As previously shown with broccoli cv. Saga by Fahey et al. (*10*), glucosinolate levels in the present study dropped dramatically with age of sprouts; all harvest and cultivation systems examined had highly significant differences between harvest dates. Highest total glucosinolate levels were measured at the earliest time of harvest and decreased steadily with time (**Table 1**; **Figures 2** and **4**).

Individual Glucosinolates. The major glucosinolate found in both older and younger sprouts in this study was glucoraphanin (4-methylsulfinylbutyl GS; GR). GR and glucoiberin (3-methylsulfinylpropyl GS; GI) on average represented 61.3 and 21.6% of the total glucosinolate content, respectively, of cv. Marathon sprouts. The indole glucosinolates (4-hydroxyindol-3-ylmethyl plus indol-3-ylmethyl glucosinolate) represented as

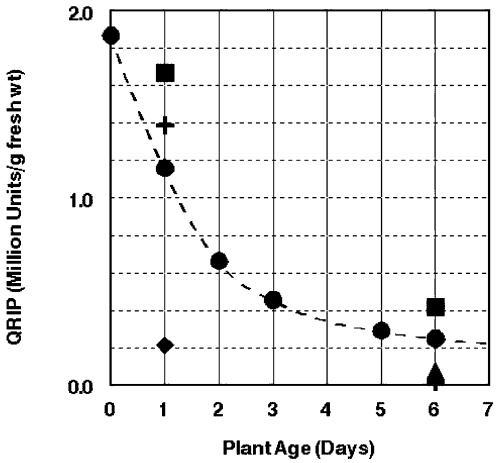

Figure 2. QRIP of whole broccoli cv. Saga sprouts at 0 (seed), 1, 2, 3, 5, and 6 days postsowing: (●) whole sprout; (■) cotyledons; (▲) hypocotyls; (+) radical, roots; (◆) seed coats. Bioassays were conducted in microtiter plates using Hepa 1c1c7 murine hepatoma cells as described by Fahey et al. (*10*) and Prochaska and Santamaria (*18*), and purified myrosinase was added directly to sprout extracts immediately prior to assay. Sprouts were grown at 22.5 °C with a 16:8 h photoperiod, and sprout parts were dissected from 20 representative plants and pooled for analysis at both 1 and 6 days postsowing.

much as 8.4% of the total content. Glucoerucin (GE; 4-methylthiobutyl glucosinolate) and gluconasturtiin (GN; 2-phenylethyl glucosinolate) were present at even lower levels and accounted for only 4.2 and 4.6% of the total glucosinolate content of cv. Marathon sprouts, respectively (**Table 1**). Total methylthioalkyl glucosinolates (GR + GI + GE) in cv. DeCicco sprouts grown to a size of 25 mg FW ranged from 8.8 to 15.1 μmol/g of FW, and total indole glucosinolates ranged from 0.66 to 2.8 μmol/g oF FW (**Figure 3A**).

The general glucosinolate profile of broccoli sprouts in this study (**Table 1**; **Figures 3A**, **4**, and **5**) is similar to that reported by Fahey et al. (*10*), who found that indole glucosinolates accounted for <10% of total glucosinolates, with high levels of GR and lesser quantities of GI and GE in 3-day-old sprouts of >70 cultivars. They did not identify GN as a component of broccoli sprouts, whereas we have shown that it is present at very low levels in sprouts of broccoli cv. Marathon (**Table 1**). The glucosinolate profiles, expressed as a percent of total glucosinolates, are compared in **Figure 5** for 3-day-old sprouts of seven representative broccoli cultivars. GR and GI make up the vast majority of the glucosinolates in all but one of these cultivars, Green Comet, which presents a striking comparison. The presence of a pronounced glucosinolate peak corresponding to what had tentatively been identified as GN was repeated with multiple accessions of this cultivar and had not been observed by Fahey and Stephenson (*23*) in any of the >70 other cultivars examined. When inflorescences at the vegetable or heading stage from plants of this variety were examined, a similar anomaly persisted (data not shown). Thus, with this notable exception, large differences in glucosinolate profiles as a function of plant genotype are relatively minor compared to sprout age and temperature effects and are under investigation in the authors' laboratories. Age effects shown herein (**Table 1**; **Figures 2** and **4**), however, are entirely consistent with those previously shown by Fahey et al. (*10*) with 0−10-day-old broccoli sprouts of cv. Saga. Once light-grown sprouts emerge from the seed (e.g., in the 48 h period following initiation of imbibition), they rapidly develop functional chloroplasts and begin to photosynthesize. At this point they cease being dependent upon their cotyledonary

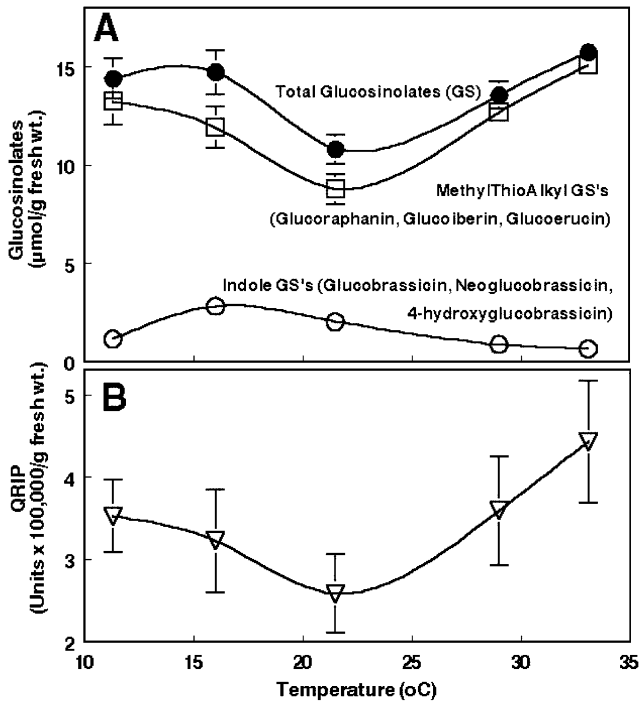

Figure 3. (A) Influence of cultivation temperature on glucosinolate content in broccoli cv. DeCicco sprouts. Each value represents the average of three 100 sprout replicates. All sprouts were grown to a uniform size of 25 mg by controlling the duration of growth at a constant temperature as indicated in **Figure 1**. (B) Influence of cultivation temperature on QRIP in these broccoli sprouts. Bioassays were conducted in microtiter plants using Hepa 1c1c7 murine hepatoma cells as described by Fahey et al. (*10*) and Prochaska and Santamaria (*18*), and purified myrosinase was added directly to sprout extracts immediately prior to assay.

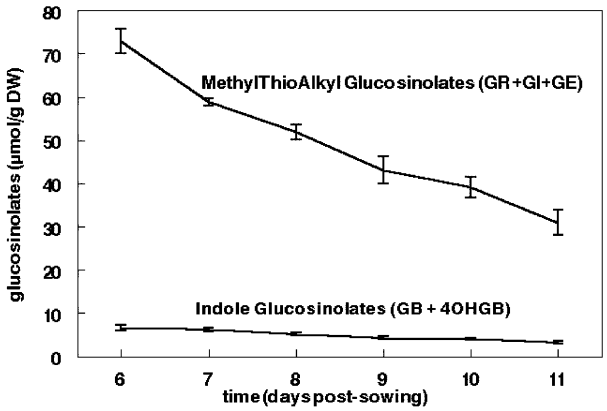

Figure 4. Glucoraphanin content of hypocotyl/epicotyl axes of broccoli cv. Marathon sprouts grown at 30/15 °C (14/10 h day/night cycle).

energy reserves, and ultimately their glucosinolate patterns start to approach those of mature broccoli (*9*, *10*, *21*−*24*) in which indole glucosinolates can account for over half of the total glucosinolate content.

The isothiocyanate derivative of glucoraphanin, known as sulforaphane, has been shown to be the principal, potent, monofunctional inducer of phase 2 detoxication enzymes in broccoli (*7*). It has tumor prevention activity and serves as an indirect antioxidant (*8*, *10*, *11*, *25*). The above-referenced experiments with broccoli sprouts cv. Saga, and other subsequent work with both sprouts and mature broccoli, have all shown highly significant correlations between glucoraphanin content and the induction of quinone reductase (phase 2 enzyme) activity (*5*, *10*, *21*, *23*, *24*). A very strong linear correlation between

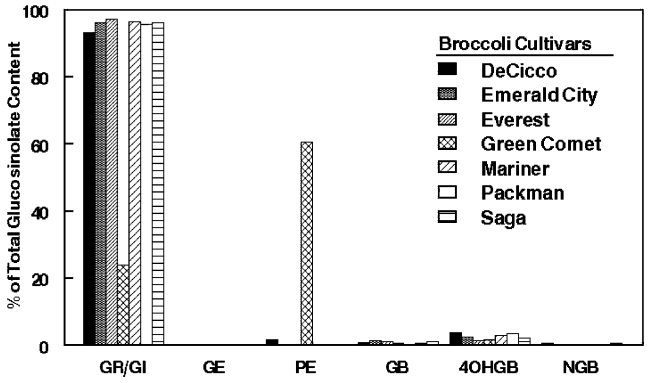

Figure 5. Relative glucosinolate content in 3-day sprouts of seven broccoli cultivars. Total glucosinolate content ranged from 11.1 to 21.3 μmol/g of FW in these seven cultivars. Note that there were two unidentified glucosinolate peaks (not shown) in cv. Green Comet, which accounted for an additional 15% of the total.

these two measures ($r^2 = 0.747$) was also found in the present study for broccoli cv. DeCicco sprouts (**Figure 3**). Thus, all available evidence continues to point to broccoli sprouts with higher levels of GR as being most desirable from a cancer preventive and antioxidant perspective.

The other methylthioalkyl glucosinolates in broccoli sprouts, present at much lower concentrations than GR, were glucoiberin (GI; 3-methylsulfinylpropyl glucosinolate) and glucoerucin (GE; 4 methylthiobutyl glucosinolate). The cognate isothiocyanates of these GSs (iberin and erucin, respectively) also elevate phase 2 detoxication enzymes but each at only ~10% the potency of sulforaphane (*6*). Although cancer protective benefits have also been ascribed to indole-3-carbinol, a myrosinase-catalyzed derivative of glucobrassicin (which is also present in the broccoli sprouts described herein), its potential health benefits are now actively being debated (*26*−*28*) and are beyond the scope of this paper. The presence of 2-phenylethyl glucosinolate, although in minor amounts (4.6% of the total glucosinolate content) could also have an additive effect on health protection due to the production of its cognate isothiocyanate, which has been shown to inhibit the induction of several types of cancer (*29*, *30*).

The analysis of variance revealed highly significant differences ($p < 0.001$) in the major individual glucosinolates as a function of both temperature and harvest day. Although indole glucosinolate levels were not affected by temperature during the 6 day experiment of cv. Marathon sprouts, indole glucosinolate levels varied inversely with methylthioalkyl glucosinolates content in cv. DeCicco sprouts grown to a constant size (**Figure 3A**). Although indole glucosinolates are already very low in broccoli sprouts compared to broccoli inflorescences, this fortuitous inverse relationship between indole and methylthioalkyl glucosinolates may be further augmented by judicious selection of genotype and cultural conditions. This possibility is currently under investigation in the authors' laboratories.

The highest levels of the major individual glucosinolates, and thus of total glucosinolates, were observed when broccoli sprouts were grown under the 30/15 °C (day/night) temperature regime (**Table 1**; **Figure 4**) or when they were grown under either high (29 or 33.1 °C) or low (11.3 or 16 °C) constant temperatures (**Figure 3A**). This finding is in agreement with previous experiments with mature broccoli (*14*, *31*) in which supraoptimal temperatures for growth were shown to induce higher levels of glucosinolates.

The cancer protective effect of broccoli sprouts grown at different constant temperatures (bioassayed by the induction of quinone reductase, a representative mammalian phase 2 enzyme) paralleled their methylthioalkyl glucosinolate contents under these growth conditions examined; both higher and lower temperatures yielded enhanced phase 2 enzyme inducer activity (**Figure 3B**), as did earlier developmental stages (**Figure 2**). The results reported herein thus bolster the evidence (*10*) that younger sprouts are the most highly chemoprotective. The harvest of only the aerial (hypocotyls and cotyledon) portion of the sprout is a reasonable practice from the perspective of capturing most of the protective glucosinolates from the plant: young developing roots have only a very small portion of the total glucosinolates and the phase 2 enzyme inducer potential in each sprout (**Figure 2**). Growth at either supra- or suboptimal temperatures caused dramatic (72 and 51%, respectively) and significant increases in the glucoraphanin content and phase 2 enzyme inducer potential of young (25 mg FW) sprouts (**Figure 3b**) in a highly controlled laboratory environment. The wisdom of using such elevated sprouting temperatures for sprouts intended for human consumption is questionable, however, due to the enhanced potential for development of undesirable bacterial flora at these elevated temperatures.

ABBREVIATIONS USED

DW, dry weight; FW, fresh weight; GB, glucobrassicin, indol-3-ylmethyl glucosinolate; GE, glucoerucin, 4-methylthiobutyl glucosinolate; GI, glucoiberin; 3-methylsulfinylpropyl glucosinolate; GN, gluconasturtiin, 2-phenylethyl glucosinolate; GR, glucoraphanin, 4-methylsulfinylbutyl glucosinolate; GS, glucosinolate; NGB, neoglucobrassicin, 1-methoxyindol-3-ylmethyl glucosinolate; QR, quinone reductase; QRIP, quinone reductase inducer potential.

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