PERGAMON

Phytochemistry 62 (2003) 471–481

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*

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Received 10 September 2002; received in revised form 15 October 2002

Dedicated to Meinhart H. Zenk on the occasion of his 70th birthday

Abstract

The glucosinolate content of various organs of the model plant *Arabidopsis thaliana* (L.) Heynh., Columbia (Col-0) ecotype, was analyzed at different stages during its life cycle. Significant differences were noted among organs in both glucosinolate concentration and composition. Dormant and germinating seeds had the highest concentration (2.5–3.3% by dry weight), followed by inflorescences, siliques (fruits), leaves and roots. While aliphatic glucosinolates predominated in most organs, indole glucosinolates made up nearly half of the total composition in roots and late-stage rosette leaves. Seeds had a very distinctive glucosinolate composition. They possessed much higher concentrations of several types of aliphatic glucosinolates than other organs, including methylthioalkyl and, hydroxyalkyl glucosinolates and compounds with benzoate esters than other organs. From a developmental perspective, older leaves had lower glucosinolate concentrations than younger leaves, but this was not due to decreasing concentrations in individual leaves with age (glucosinolate concentration was stable during leaf expansion). Rather, leaves initiated earlier in development simply had much lower rates of glucosinolate accumulation per dry weight gain throughout their lifetimes. During seed germination and leaf senescence, there were significant declines in glucosinolate concentration. The physiological and ecological significance of these findings is briefly discussed.

Keywords: *Arabidopsis thaliana*; Brassicaceae; Glucosinolate; Quantification; Organ variation; Development; Seed germination; Senescence; Catabolism; Transport

1. Introduction

The glucosinolates are a group of over 130 nitrogen- and sulfur-containing natural products found almost exclusively in plants of the Brassicaceae and other related families of the order Capparales (Brown and Morra, 1997; Fahey et al., 2001). They share a core structure containing a β-D-glucopyranose residue linked via a sulfur atom to a (*Z*)-*N*-hydroximino sulfate ester, and are distinguished from each other by a variable R group derived from one of several amino acids. Species with glucosinolates also contain myrosinase (E.C. 3.2.3.1), a β-thioglucosidase that catalyzes the hydrolysis of glucosinolates to biologically active products, including isothiocyanates, thiocyanates and nitriles, depending on the reaction conditions and presence of associated proteins (Fenwick et al., 1983; Chew, 1988a; Rask et al., 2000; Lambrix et al., 2001). Glucosinolates, myrosinase, their associated proteins, and hydrolysis products constitute a frequently-studied plant defense system that appears to deter generalist herbivores and pathogens, but attracts certain specialist herbivores (Chew, 1988b; Bones and Rossiter, 1996; Renwick, 2001). Glucosinolates have also been frequently investigated for their roles in preventing cancer (Hecht, 2000), flavoring *Brassica* vegetables and controlling the utility and production potential of oilseed rape (Brown and Morra, 1997; Mithen, 2001). In both *Brassica* vegetables and oilseed rape, the abundance and the types of glucosino-

Abbreviations: 4MSOB; 4-methylsulfinylbutyl glucosinolate; 4MTB; 4-methylthiobutyl glucosinolate; I3M; indol-3-ylmethyl glucosinolate; 1MOI3M; 1-methoxyindol-3-ylmethyl glucosinolate.

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PII: S0031-9422(02)00549-6

lates are known to be regulated both developmentally and environmentally in various organs and tissues with pronounced effects on anticancer activity, flavor, and herbivore and pathogen damage (Sang et al., 1984; McGregor, 1988; Clossais-Besnard and Larher, 1991; Fieldsend and Milford, 1994a, b; Rosa et al., 1996). For example, herbivore damage or jasmonic acid treatment induces increases in indole glucosinolates in *Brassica napus* (Bartlet et al., 1999; Bodnaryk, 1994; Doughty et al., 1995) and *B. campestris* (Ludwig-Müller et al., 1997). The stimulatory effects of jasmonic acid on the formation of indole glucosinolates are also seen for many other plant defense metabolites, a seminal discovery made by Meinhart Zenk and his colleagues (Gundlach et al., 1992).

The model plant *Arabidopsis thaliana*, a member of the Brassicaceae, also possesses glucosinolates. Nearly 30 different members of this class have been described from the Columbia (Col-0) ecotype (Hogge et al., 1988; Haughn et al., 1991; Reichelt et al., 2002) with additional compounds found in other ecotypes (Kliebenstein et al., 2001). The genetic and molecular resources available for *A. thaliana* research have helped to advance our understanding of glucosinolate biosynthesis (Halkier and Du, 1997; Wittstock and Halkier, 2002) and to clarify the role of the glucosinolate-myrosinase system in plant interactions with insect herbivores (Jander et al., 2001; Nielsen et al., 2001; Lambrix et al., 2001) and microbial pathogens (Brader et al., 2001; Tierens et al., 2001). Yet, a comprehensive understanding of the glucosinolate profile and content during *A. thaliana* development is still lacking.

Here we report the amounts of individual glucosinolates in various organs of the Columbia ecotype of *A. thaliana* during development with special emphasis on changes occurring during seedling germination and leaf development. As glucosinolate accumulation represents the net effect of biosynthesis, transport and catabolism of glucosinolates, precise knowledge of the accumulation profile in different organs provides a foundation for studying the relative importance of these physiological processes. In addition, a detailed understanding of the glucosinolate content of *A. thaliana* will also help in explaining the role of these compounds in interactions with herbivores and pathogens.

2. Results

2.1. Glucosinolate quantification

The glucosinolates present in *A. thaliana* samples of various organs and developmental stages were extracted in boiling water, bound to an anion-exchange column, eluted after conversion to their corresponding desulfated derivatives, and analyzed by HPLC. Compounds were identified as previously described (Reichelt et al., 2002), and the 20 most abundant substances quantified with respect to an added internal standard using relative response factors derived from pure glucosinolate standards and from values in the literature (Buchner, 1987). Given the widespread interest in glucosinolate analysis, the response factors used are listed here with respect to the commercially available 2-propenyl glucosinolate, the internal standard employed (Table 1). They vary up to six-fold, with hydroxylalkyl glucosinolates having the highest values and indole glucosinolates and compounds possessing benzoate ester moieties having the lowest values. In general, modification of the side chain by elongation, oxidation of the parent thiol function, or alkene formation had little effect on the response factor.

2.2. Variation in total glucosinolate content

Samples were taken of the major organs at five critical phases in the life cycle of this typical winter annual: (1) the vegetative phase, where the shoot meristem produces a basal rosette of leaves with highly reduced internodes, (2) the vegetative-reproductive phase transition, when the shoot meristem first converts to reproductive development and starts to elongate, (3) the primary bolt, when the elongated stem has produced inflorescences which have begun to mature into siliques (fruits), (4) the secondary bolt, when axillary inflorescences proliferate and mature siliques are present, and (5) senescence. The constituent glucosinolates and their concentrations are presented in Table 2. The concentration of total glucosinolates in the different organs varied nearly 100-fold ranging from a high of 63 μmol (g dry wt)$^{-1}$ in the seeds to a low of 0.7 μmol (g dry wt)$^{-1}$ in senescent rosette leaves. Intermediate levels were observed in the inflorescences and siliques, 23–30 μmol (g dry wt)$^{-1}$, and lesser amounts in roots, non-senescent rosette leaves, stems and cauline (stem) leaves, 7–23 μmol (g dry wt)$^{-1}$.

2.3. Variation in glucosinolate composition

To better visualize the relationship of plant development and glucosinolate accumulation, the individual glucosinolates in the various organs are shown in Fig. 1 as sectors whose areas are proportional to concentration. Each glucosinolate is identified by a color corresponding to its general class and a pattern. Aliphatic glucosinolates (red, 1–13) were the major class in photosynthetic tissues. 2-Phenylethyl glucosinolate (yellow, 14) and compounds with benzoyloxy substituents (green, 15 and 16) glucosinolates were most abundant in the seed. Indole glucosinolates (blue, 17–20) were most prominent in the root and mature rosette leaves. The

Table 1
Response factors for desulfated glucosinolates at $A_{229\ nm}$ relative to that of desulfo-2-propenyl-glucosinolate (desulfosinigrin)

Glucosinolate (abbreviation)	Trivial name	Relative response factor	Source[a]
2-Propenyl	Sinigrin	1.0	*Iberis umbellata, Brassica juncea*
3-Butenyl	Gluconapin	1.0	*A. thaliana, Alyssum maritimum, B. napus*
Methyl	Glucocapparin	1.0	*Cleome spinosa*
1-Methylethyl		1.0	*Lunaria annua*
3-Hydroxypropyl (3OHP)		2.1	*A. thaliana*
4-Hydroxybutyl (4OHB)		1.4	*A. thaliana*
3-Methylsulfinylpropyl (3MSOP)	Glucoiberin	1.2	*I. umbellata*
4-Methylsulfinylbutyl (4MSOB)	Glucoraphanin	0.9	*Erysimum allioni*
5-Methylsulfinylpentyl (5MSOP)	Glucoalyssin	0.9	*L. annua*
6-Methylsulfinylhexyl (6MSOH)	Glucohesperin	1.0	*Arabis perenans, L. annua*
8-Methylsulfinyloctyl (8MSOO)	Glucohirsutin	1.1	*A. thaliana*
3-Methylsulfonylpropyl	Glucocheirolin	0.9	*Cheiranthus cheiri*
4-Methylsulfinyl-3-butenyl	Glucoraphenin	0.9	*Matthiola incana*
3-Methylthiopropyl (3MTP)	Glucoibervirin	0.8	*I. umbellata*
4-Methylthiobutyl (4MTB)	Glucoerucin	0.9	*A. thaliana*
6-Methylthiohexyl (6MTH)	Glucosquerellin	1.0	*Alyssum maritimum, Arabis perenans*
7-Methylthioheptyl (7MTH)		1.0	*A. thaliana*
8-Methylthiooctyl (8MTO)		1.1	*A. thaliana*
Benzyl	Glucotropaeolin	0.8	*Tropaeolum majus, Lepidium sativum*
2-Phenylethyl (2PE)	Gluconasturtiin	1.0	*Nasturtium officinale*
3-Benzoyloxypropyl (3BzOP)	Glucomalcomiin	0.4	*A. thaliana*
4-Benzoyloxybutyl (4BzOB)		0.3	*A. thaliana*
4-Hydroxybenzyl	Sinalbin	0.4	*Sinapis alba*
Indol-3-ylmethyl (I3M)	Glucobrassicin	0.3[b]	
4-Methoxyindol-3-ylmethyl (4MOI3M)	4-Methoxyglucobrassicin	0.3[b]	
1-Methoxyindol-3-ylmethyl (1MOI3M)	Neoglucobrassicin	0.2[b]	

[a] Plant species from which the desulfated glucosinolate was purified.
[b] Determined by Buchner (1987).

diversity of glucosinolates varied considerably among organs with 20 glucosinolates identified in siliques, 18 in seeds, 15 in inflorescences and cauline leaves, 12 in rosette leaves, and only 10 in roots.

2.4. Leaves

The major class of glucosinolates in the rosette leaves depended on their age. In the vegetative stage, 4-methylsulfinylbutyl glucosinolate (4MSOB) and other aliphatic glucosinolates contributed more than 80% of the total, with indole glucosinolates contributing the balance. However, the proportion of aliphatic glucosinolates declined with age resulting in the predominance of indole glucosinolates, mostly indol-3-ylmethyl glucosinolate (I3M) and its 1-methoxyl derivative (1MOI3M), by the time of senescence. Senescent and dead leaves had only 10 and 4% of the glucosinolate concentration of mature rosette leaves, respectively. The leaves of the oldest plants also contained typical seed glucosinolates, such as 4-methylthiobutyl-, 4-hydroxybutyl- and 4-benzoyloxybutyl-glucosinolates.

To better define the relationship between glucosinolate content and leaf development, replicate rosette leaves of different ages were collected over a 10 day period during the vegetative phase and analyzed. Leaves of all age groups showed an increase in total glucosinolates during expansion (Fig. 2), although this was barely apparent for the oldest cohort of leaves analyzed (circles). Within a cohort, there was a linear relationship between leaf dry weight and glucosinolate content, meaning that as leaves age, glucosinolate accumulation kept pace with dry matter accretion and glucosinolate concentration did not change. However, each successively younger cohort showed a greater rate of glucosinolate accumulation relative to dry weight gain, so that later-initiated rosette leaves had higher glucosinolate concentrations. The oldest leaves measured had only 10 μmol glucosinolate (g dry wt)$^{-1}$ while the youngest leaves had over 60 μmol (g dry wt)$^{-1}$. Therefore, the inner leaves of the rosette have much higher glucosinolate concentrations than the outer ones. A similar pattern of glucosinolate variation was observed in the developing leaves of *Brassica napus* (Porter et al., 1991).

2.5. Roots

The root tissue had a similar glucosinolate pattern to that of the rosette leaves, except that there was a much greater proportion of indole glucosinolates. The two major glucosinolates in the roots were the aliphatic

Table 2
Glucosinolate content (in μmol per g dry weight) of *A. thaliana* (ecotype Columbia, Col-0) at various developmental stages

Organ	Rosette Lvs	Rosette Lvs	Rosette Lvs	Rosette Lvs	Rosette Lvs	Senescing Rosette Lvs	Dead Rosette Lvs	Cauline Lvs	Cauline Lvs	Cauline Lvs	Siliques	Siliques	Siliques	Inflorescences	Inflorescences	Roots	Seeds
Stage of development	Vegetative	Transition	1° Bolt	2° Bolt	Senescence	2° Bolt and senescence	2° Bolt and senescence	1° Bolt	2° Bolt	Senescence	1° Bolt	2° Bolt	Senescence	1° Bolt	2° Bolt	2° Bolt	Senescence
Glucosinolate abbreviation[a]																	
1. 3MSOP	1.56±0.25	1.05±0.42	1.03±0.35	0.55±0.16	0.41±0.05	0.04±0.01	0.02±0.00	2.46±0.65	1.81±0.42	0.82±0.12	2.35±0.36	2.54±0.53	1.15±0.18	2.60±1.01	3.16±0.84	1.78±0.84	0.16±0.05
2. 4MSOB	10.63±1.75	7.45±3.10	5.97±2.17	2.95±0.81	2.47±0.37	0.30±0.11	0.14±0.02	16.52±4.37	10.93±2.29	5.13±0.60	15.48±2.69	18.89±4.05	9.71±0.95	18.50±7.02	21.80±6.80	7.48±3.71	2.43±1.07
3. 5MSOP	0.46±0.13	0.37±0.18	0.33±0.11	0.26±0.03	0.26±0.1	n.d.[c]	n.d.	0.71±0.10	0.57±0.05	0.34±0.20	0.76±0.12	0.86±0.27	0.58±0.06	0.85±0.41	0.76±0.31	0.33±0.09	0.27±0.12
4. 6MSOH	0.04±0.01	0.03±0.02	0.01±0.02	n.d.	n.d.	n.d.	n.d.	0.07±0.11	n.d.	0.05±0.07	0.05±0.02	0.07±0.03	0.09±0.02	0.04±0.04	0.03±0.03	n.d.	0.14±0.04
5. 7MSOH	0.22±0.05	0.19±0.04	0.08±0.02	0.04±0.03	0.02±0.03	n.d.	n.d.	0.18±0.02	0.08±0.03	0.03±0.04	0.25±0.07	0.41±0.14	0.51±0.15	0.31±0.18	0.32±0.34	0.22±0.01	1.11±0.26
6. 8MSOO	1.28±0.42	1.21±0.32	0.35±0.07	0.14±0.04	0.15±0.14	n.d.	n.d.	0.72±0.18	0.30±0.07	0.12±0.06	0.94±0.21	1.55±0.30	1.85±0.46	1.17±0.31	0.91±0.20	0.27±0.32	6.31±1.31
7. 3MTP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.09±0.12	0.06±0.09	0.01±0.02	0.13±0.21	0.08±0.19	n.d.	0.05±0.02
8. 4MTB	0.84±0.87	0.37±0.16	0.18±0.2	0.08±0.03	0.16±0.13	n.d.	n.d.	0.42±0.35	0.32±0.14	0.28±0.15	0.51±0.47	1.5±1.26	4.80±0.83	0.76±1.19	0.67±1.09	0.33±0.12	26.03±5.60
9. 5MTP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03±0.03	0.09±0.09	0.36±0.10	0.02±0.06	0.03±0.04	n.d.	1.82±0.42
10. 7MTH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01±0.02	0.07±0.06	0.40±0.10	n.d.	n.d.	n.d.	2.98±0.28
11. 8MTO	0.09±0.13	0.01±0.03	n.d.	n.d.	0.01±0.02	n.d.	n.d.	n.d.	n.d.	0.01±0.01	0.01±0.03	0.09±0.09	0.49±0.16	n.d.	n.d.	n.d.	4.43±0.30
12. 3OHP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02±0.03	0.12±0.06	0.23±0.15	0.56±0.03	0.15±0.18	0.09±0.04	n.d.	0.83±0.30
13. 4OHB	n.d.	n.d.	n.d.	n.d.	0.01±0.01	n.d.	n.d.	n.d.	n.d.	0.03±0.05	0.17±0.11	0.59±0.41	1.36±0.50	n.d.	n.d.	n.d.	3.70±0.93
14. 2PE	0.02±0.20	0.01±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02±0.03	0.06±0.05	0.11±0.01	0.02±0.04	0.03±0.05	n.d.	0.34±0.06
15. 3BZOP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.08±0.06	0.27±0.22	0.72±0.18	n.d.	n.d.	n.d.	3.86±0.43
16. 4BZOB	n.d.	0.01±0.01	0.01±0.01	n.d.	0.03±0.02	n.d.	0±0.01	n.d.	0±0.01	0.05±0.02	0.10±0.08	0.54±0.45	1.31±0.63	n.d.	n.d.	n.d.	7.43±0.92
17. I3M	2.13±0.07	2.17±0.38	2.17±1.15	1.70±0.17	1.43±0.91	0.14±0.05	0.03±0.01	2.22±1.56	1.14±0.18	1.46±1.17	1.77±0.84	1.84±0.43	1.16±0.29	1.99±1.20	2.21±0.89	0.64±0.04	1.48±0.43
18. 4MOI3M	0.20±0.01	0.22±0.03	0.33±0.17	0.43±0.05	0.29±0.12	0.23±0.07	0.02±0.005	0.09±0.06	0.07±0.01	0.09±0.05	0.01±0.01	0.02±0.02	0.02±0.03	0.01±0.01	0.01±0.02	1.15±0.52	n.d.
19. 1MOI3M	0.44±0.28	0.74±0.48	0.53±0.38	1.14±0.39	2.65±1.8	n.d.	0.05±0.03	0.09±0.07	0.06±0.02	0.17±0.09	0.01±0.01	0.02±0.02	n.d.	0.05±0.04	0.05±0.03	6.33±2.43	n.d.
20. 4OHI3M	0.01±0.01	0.0±0.01	0.01±0.01	0.08±0.07	0.09±0.07	0.03±0.02	n.d.	0.06±0.05	0.1±0.08	0.09±0.13	0.04±0.02	0.07±0.09	0.1±0.06	0.08±0.05	0.07±0.04	0.08±0.01	0.01±0.01
Total μmol (g dry wt)$^{-1}$	17.9	13.8	11.0	7.4	8.0	0.7	0.3	23.5	15.4	8.7	22.8	15.4	25.3	26.7	30.2	18.6	63.4
Organ weight, g dry wt plant^{-1}	0.02	0.03	0.06	0.05	0.02			0.10	0.21	0.19	0.03	0.21	0.55	0.02	0.03		0.10–0.50[d]
Total[b] μmol organ^{-1}	0.4	0.4	0.7	0.4	0.2			2.4	3.2	1.7	0.7	3.2	13.9	0.5	0.9		

[a] Full name listed in Table 1. Numbers refer to the labeled sectors in Fig. 1.
[b] This quantity represents the total glucosinolate content for this organ when considering a single plant. For example, if all of the rosette leaves of a single plant were harvested at the vegetative stage, they would contain 0.4 μmol of glucosinolates.
[c] n.d.: Not detected.
[d] Typical weight range for seeds harvested from plants grown under similar conditions.

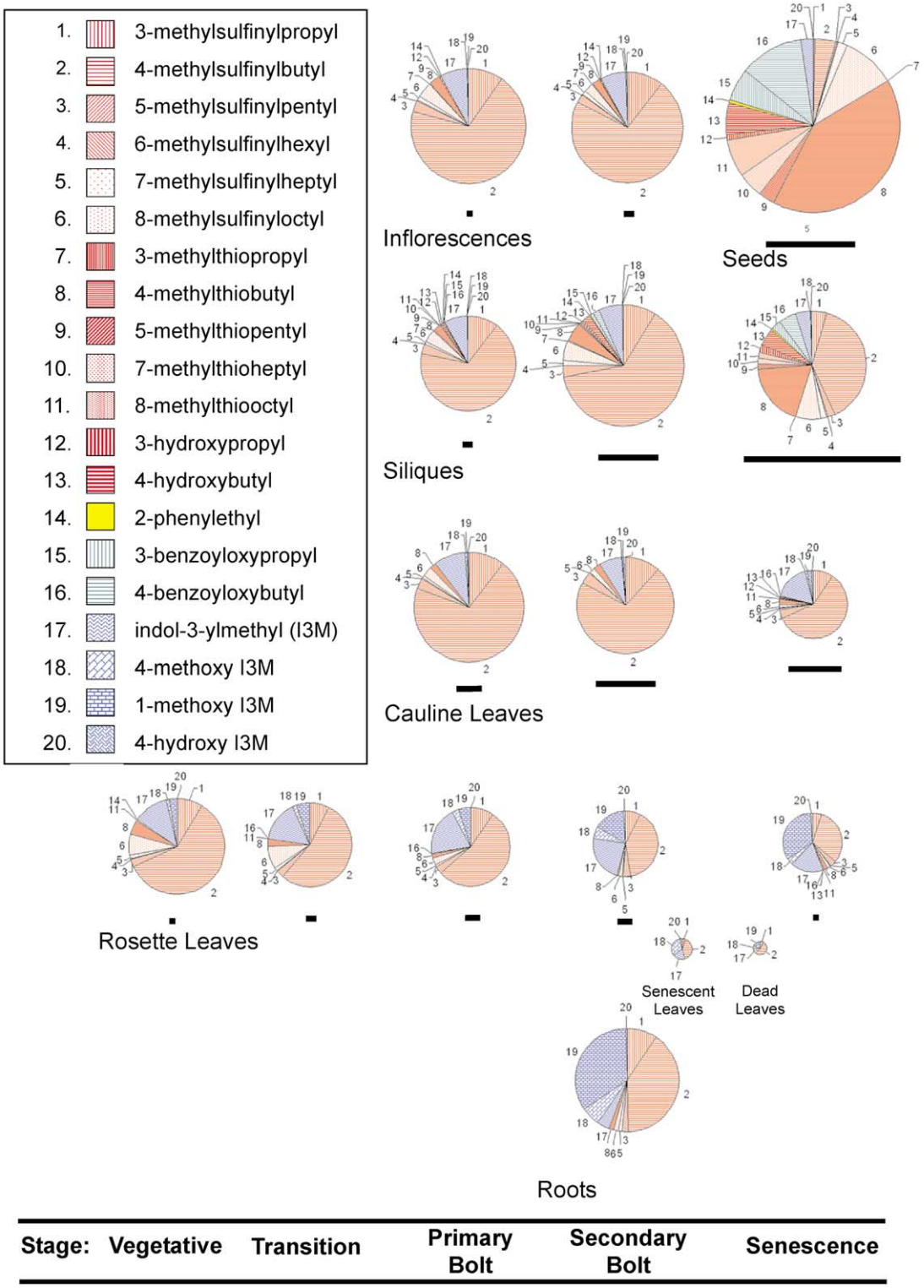

Fig. 1. Glucosinolate profiles of *A. thaliana* organs at various developmental stages. The individual glucosinolates are presented as sectors with areas proportionate to their concentrations in μmol (g dry wt)$^{-1}$. Individual glucosinolates are identified by number and a combination of patterns and color: red for aliphatic (1–13), yellow for benzylic (14); green for benzoyloxy-modified aliphatic (15 and 16) and blue for indole glucosinolates (17–20). The columns correspond to the different developmental stages, the earliest on the left (vegetative) and the oldest, far right (senescence). The rows represent different plant parts arranged by their position on the plant, roots at the bottom, and inflorescences and seeds at the top. The length of the bar under the circles is proportionate to the average dry weight of all organs of each type from a single plant.

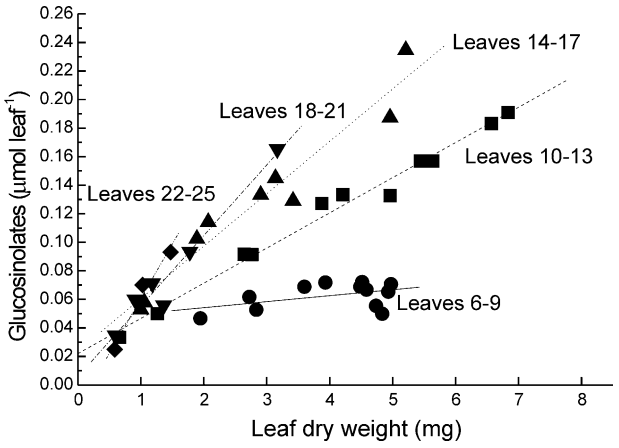

Fig. 2. The accumulation of glucosinolates in expanding *A. thaliana* rosette leaves grouped in cohorts of four according to their order of emergence. The total leaf glucosinolate content, μmol leaf^{-1} was determined by summing the values for the individual compounds. Results are graphed according to leaf dry wt rather than absolute age to allow direct comparison between the different leaf groups from replicate harvests. A linear fit for each leaf cohort was determined: leaves 6–9 (circle, $R=0.47$, $P=0.13$), leaves 10–13 (square, $R=0.99$, $P<0.0001$), leaves 14–17 (upright triangle, $R=0.97$, $P<0.0001$), leaves 18–21 (inverted triangle, $R=0.98$, $P=0.0007$), leaves 22–25 (diamond, $R=0.98$, $P=0.12$).

4MSOB at 7.5 μmol (g dry wt)$^{-1}$ and 1-methoxyindol-3-ylmethyl glucosinolate (1MOI3M) at 6.3 μmol (g dry wt)$^{-1}$. While 4MSOB was found in all organs, 1MOI3M was limited to the roots and senescent rosette leaves (Table 2, Fig. 1).

2.6. Cauline leaves, inflorescences and siliques

The organs attached to the bolting stem varied in glucosinolate content. The cauline leaves and stems had a similar concentration and composition to that of the rosette leaves, and exhibited a comparable decline in aliphatic glucosinolate content with age. The inflorescences also had a composition much like that of rosette leaves, but a higher concentration, 25–30 μmol (g dry wt)$^{-1}$, the second highest concentration in any organ after the seeds. The glucosinolate concentration of the siliques was lower than the inflorescences, 15–25 μmol (g dry wt)$^{-1}$, and the composition was intermediate between those of the rosette leaves and the seeds.

2.7. Seeds

The glucosinolates of *A. thaliana* seeds are distinguished by their high concentration, unique aliphatic constituents and the low level of indole compounds. The seeds contained 63 μmol (g dry wt)$^{-1}$, the highest concentration in any organ with the possible exception of the very youngest leaves. Seeds contained six novel glucosinolates relative to the vegetative tissues, including 7-methylthioheptyl-, 8-methylthiooctyl-, 3-hydroxypropyl-, 4-hydroxybutyl-, 3-benzoyloxypropyl- and 4-benzoyloxybutyl- glucosinolates. Hence it is likely that specific enzymes for the biosynthesis of these glucosinolates, such as those needed for hydroxyl group formation or construction of benzoate esters, are only found in the seeds. The predominant seed glucosinolate, representing 41% of the total, has an aliphatic, butyl-based side chain with a sulfur atom, but is not 4MSOB, the main glucosinolate found in the vegetative parts of the plant, but rather its reduced counterpart, 4-methylthiobutyl glucosinolate (4MTB). The seeds also contain greater amounts of the longer-chain aliphatic glucosinolates, such as 7-methylsulfinylheptyl- and 8-methylsulfinyloctyl- glucosinolate, than are found in the vegetative tissues. The only indole glucosinolates detected in the seeds were indol-3-ylmethyl glucosinolate (I3M) and trace amounts of its 4-hydroxyl derivative, which in total comprised only 2% of the seed glucosinolate content. The absence of modified indole glucosinolates in seeds has also been observed in one cultivar of *Brassica oleracea* (Sang et al., 1984).

2.8. Transition from seed to seedling

Since the seed glucosinolates of *A. thaliana* are so markedly different from those of the vegetative tissue, we investigated the transition from seed to vegetative plant in detail using seedlings planted on solidified Murashige and Skoog growth medium. One day after planting there were no visible changes except for seed hydration. However, two days after planting the radicles emerged, although there was as yet no dry weight accumulation. Four days after planting, the seedlings had cotyledons, 1.5 mm-long shoots, 2.5–3.0 mm-long roots and the dry weight had increased 1.6-fold. Six days after planting, the first leaves were visible and the dry weight had increased 2.1-fold. By 8 days after planting, the first leaves had expanded, the root length was 3.5–4 mm, and the dry weight had increased 2.4-fold.

The 16 most abundant glucosinolates showed divergent patterns of change during this time course. During the first two days after planting, there was a general increase in all glucosinolates causing total concentration to rise to almost 1.60 nmol (seedling)$^{-1}$, approximately 3.3% of dry weight (Fig. 3A). After this point, specific increases were observed for glucosinolates associated with vegetative tissues. Increases of at least 6-fold were detected for 4MSOB, 5-methylsulfinylpentyl-, 1MOI3M and 4-methoxyindol-3-ylmethyl- glucosinolate, while 6-methylsulfinylhexyl-, 7-methylsulfinylheptyl-glucosinolate and I3M increased about 2-fold (Fig. 3BC). On the other hand, glucosinolates that predominate in the seed, including 3-hydroxypropyl-, 4-hydroxybutyl-, 3-benzoyloxypropyl-, 4-benzoyloxybutyl-, 4-methylthiobutyl-,

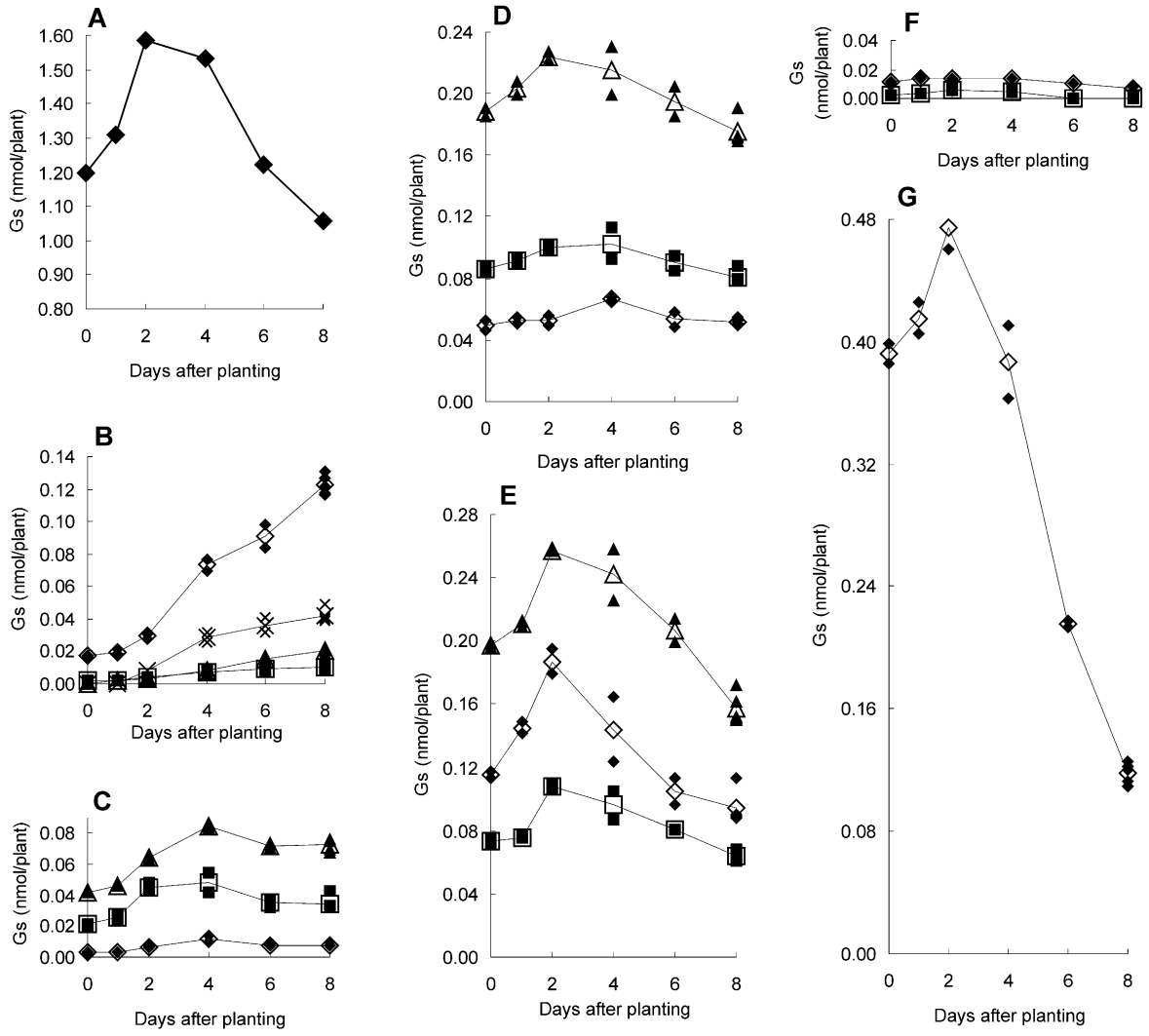

Fig. 3. Changes in *A. thaliana* glucosinolate profile during seed germination. Plants were harvested at 0, 1, 2, 4, and 6 days after planting ($N=2$), and 8 days after planting ($N=4$). Individual data points are plotted with the average values (unfilled symbols) connected by a solid line. (A) Total glucosinolates. (B) Glucosinolates increasing more than 6-fold: 5MSOP (square), 4MOI3M (triangle), 1MOI3M (cross), 4MSOB (diamond). C) Glucosinolates increasing 2-fold: 6MSOH (diamond), 7MSOH (square), I3M (triangle). D) Glucosinolates constant: 3BzOP (square), 4OHB (diamond), 4BzOB (triangle). E) Glucosinolates decreasing slightly: 7MTH (square), 8MSOO (diamond), 8MTO (triangle). F) Glucosinolates decreasing: 4OHI3M (square), 3OHP (diamond). G) 4MTB (diamond). See Table 1 for abbreviations.

7-methylthioheptyl-, 8-methylsulfinyoctyl-, 8-methylthiooctyl-, and 4-hydroxyindol-3-ylmethyl- glucosinolate remained constant or showed a decrease upon seedling germination (Fig. 3D–G). The most significant decrease was exhibited by 4MTB which declined 75% from a maximum of 0.48 nmol (seedling)$^{-1}$ at 2 days after planting to 0.12 nmol (seedling)$^{-1}$ at 8 days after planting (Fig. 3G). Although 4MSOB, a potential metabolite of 4MTB generated by thiol oxidation, increased from 0.02 to 0.12 nmol (seedling)$^{-1}$, this was insufficient to account for all of the 4MTB loss. Thus, young seedlings possess a glucosinolate profile intermediate between seeds and vegetative tissues.

3. Discussion

Plants that produce glucosinolates commonly accumulate them in all vegetative and reproductive parts throughout development (Louda and Mole, 1991). The Columbia ecotype of *A. thaliana* is no exception to this generalization, but the glucosinolate content of individual organs varies considerably. The seeds had the highest concentrations. These organs had over 60 μmol per gram dry weight when mature and up to 80 μmol per gram dry weight during germination (approximately 2.5–3.3%), at least twice as high as all of the other organs except the very youngest rosette leaves. Inflorescences

and siliques had the next highest levels (0.6–1.2%) followed by roots, stems and cauline leaves, and rosette leaves (0.3–1.0%). These patterns are similar to those found in other glucosinolate-containing species studied (Fahey et al., 2001). Thus, *A. thaliana* does not exhibit any diminution of glucosinolate accumulation relative to other species despite its small stature, annual habit and compact genome. Assuming glucosinolates function in defense against herbivores and pathogens, the pattern of differences among organs in *A. thaliana* is consistent with predictions of current theories on optimal distribution of defense substances (Zangerl and Bazzaz, 1993). The reproductive organs, including seeds, flowers and fruits, which contribute most to plant fitness, are expected to have the highest concentrations of defense compounds.

In comparing the glucosinolate composition of different organs, seeds and fruit were found to have the highest diversity of individual compounds which may also reflect the need to maximize the defensive potential of these reproductive organs. The major seed glucosinolates, including compounds with methylthioalkyl side chains and benzoate ester substituents, are also very different than their counterparts in other organs, perhaps as a consequence of the specialized physiology or defense needs of this organ. The roots had higher levels of indole glucosinolates and lower levels of aliphatic glucosinolates than other organs, a trend also seen in *Brassica* species (Rosa, 1997; Kirkegaard and Sarwar, 1998). However, the high concentrations of benzylic glucosinolates seen in *Brassica* roots were completely absent in *A. thaliana*.

The glucosinolate content of *A. thaliana* organs also varies with development. For example, younger rosette leaves typically have higher glucosinolate concentrations than older leaves, but this is not a result of decreases in the concentrations present in individual leaves as they grow. Rosette leaves appear to maintain a fixed concentration during leaf expansion (Fig. 2), but that fixed concentration is much higher in leaves initiated later in development than in those initiated early in development. As a consequence, the center of the rosette appears to be better protected against herbivores and pathogens than the outer portions.

Given a fixed concentration, the balance between glucosinolate metabolism and growth processes, and between glucosinolate synthesis, transport and breakdown, would appear to be unchanged over leaf expansion. However, upon senescence, rosette and cauline leaves show greatly reduced glucosinolate concentrations. This may be ascribed to export to other organs since the onset of senescence in rosette leaves coincides with bolting and the development of the inflorescences, fruits and seeds, which all accumulate high concentrations of glucosinolates. Recent tracer studies have demonstrated the transport of glucosinolates via the phloem from mature leaves to inflorescences and fruits (Brudenell et al., 1999; Chen et al., 2001). On the other hand, the reproductive organs are also likely to synthesize at least some of their own glucosinolates based on precursor feeding studies (Du and Halkier, 1998) and the expression of glucosinolate biosynthetic genes in these organs (Mikkelsen et al., 2000; Reintanz et al., 2001). The divergent glucosinolate composition of the seeds and the fact that the absolute amount of glucosinolates in the seeds may be higher than that in the entire rest of the plant (Bilsborrow et al., 1993) also supports de novo synthesis in these organs.

The disappearance of glucosinolates from senescent leaves may be a consequence of catabolic processes as well as transport. The hydrolysis of glucosinolates by myrosinase after plant wounding is well known, but there is little information about whether or not myrosinase plays a role in glucosinolate breakdown in intact plants. A high rate of catabolism is suggested by studies showing dramatic (two-fold) changes in glucosinolate concentration during a single diurnal cycle in *B. oleracea* (Rosa et al., 1994; Rosa, 1997). Strong evidence for catabolism in *A. thaliana* also comes from our measurements of germinating seeds which showed a net glucosinolate loss of 30% between days 2 and 8 after planting (Fig. 3), a trend shown in other species as well (McGregor, 1988; Clossais-Besnard and Larher, 1991; Hopkins et al., 1998). Curiously, there seems to be a selective loss of particular glucosinolates in *A. thaliana* upon germination, indicating that the degradative system has some specificity.

Now that the patterns of variability in glucosinolate content and composition during *A. thaliana* development have been described, the stage is set for more detailed physiological studies to evaluate the contribution of synthesis, transport and degradation to this variation. Additional studies would also be welcome to understand whether the patterns of glucosinolate accumulation observed in *A. thaliana* are important for plant defense. The use of mutants or transformants with altered allocation patterns may help in explaining the defensive significance of glucosinolate accumulation profiles in this species.

4. Experimental

4.1. Plant material

Seeds of the Columbia (Col-0) ecotype of *A. thaliana* were obtained from Lehle Seeds (Round Rock, Texas, USA). The glucosinolate changes in major organs during a developmental cycle were examined in plants grown in both greenhouse and controlled environment settings. In the greenhouse, seeds were germinated in flats of soil-less potting mix (1:1:1; peat, sand and vermiculite) and after 3–4 weeks transferred to 10 cm diameter pots containing five plants per pot. Plants were treated with a slow-release fertilizer (Osmocote 15:11:13

N:P:K, Scotts Europe) or a general fertilizer (7:3:6 N P K). The controlled environment chamber had a diurnal cycle of 16 h light at 25 °C and 8 h dark at 18 °C. Illumination was with high-pressure sodium lamps at 400 μmol m^{-2} s^{-1}. Under these conditions, developmental times were extended, being about 10 weeks to the secondary bolt flowering stage rather than 7 weeks for greenhouse-grown plants.

Shoots were harvested at five different growth stages: (1) young vegetative (rosette), (2) older rosette at the initiation of bolting, (3) early stage bolting, including flowers and developing fruits (siliques), (4) later stage bolting, including axillary inflorescences and mature siliques, and (5) senescence (Table 1). While only rosette leaves were sampled for the first two stages, plants in the remaining stages were divided into parts containing stem (cauline) leaves, inflorescences or siliques, all with their associated stems. At senescence, seeds were also harvested separately because the siliques readily dehisced upon lyophilization. At the last two harvesting times, samples were also taken of senescing or dead rosette leaves and roots. At the initial stage, 80 plants were combined for harvest, while 45 plants were combined for the remaining stages. The experiment was repeated three times. Harvesting dates were adjusted to match plant growth rates since growth rate varied slightly from experiment to experiment. The initial stage harvests were conducted on flats of seedlings, while harvests at the other stages were of plants transplanted as seedlings to pots. Within each experiment, every organ and developmental stage was replicated three times, and two samples of each replicate were extracted for a total of six samples. For root analyses and to study changes occurring in leaves upon senescence, two replicates of 15 plants each were harvested at the secondary bolt stage and the initiation of leaf senescence. Rosette leaves of two types were harvested from senescing plants: leaves that had lost their green color but were still alive (called "sensescent"), and those that had shriveled and dried (called "dead"). Four samples of each group were taken.

To examine glucosinolate changes during leaf development in detail, plants were grown in a controlled environment chamber with a diurnal cycle of 10 h light at 25 °C and 14 h dark at 18 °C. Illumination was with cool white fluorescent lamps at 120 μmol m^{-2} s^{-1}. Late vegetative phase plants were harvested every 2 days over a 10-day period. At each harvest, the rosette leaves were collected in cohorts of four leaves starting with the sixth leaf formed after the cotyledons and ending with the last complete cohort of four leaves closest to the meristem. The experiment was performed twice with 22 plants each time.

To study glucosinolate changes during seed germination, 14 aliquots of Col-0 seeds (25 mg, approx. 1250 seeds in total) were surface-sterilized by incubations of two min each with 50% ethanol, and 0.5% sodium hypochlorite followed by three washes with sterile water. Seeds were spread onto Petri dishes containing sterile 1×Murashige and Skoog media (Murashige and Skoog, 1962), (Duchefa Biochemie, The Netherlands) in 0.6% Gelrite (Duchefa) and closed with a gas-permeable tape (Leucopore, Duchefa). The plates were maintained in a controlled environment chamber at 22 °C with 120 μmol m^{-2} s^{-1} high pressure sodium lamps and a 16 h light photoperiod. For the first three time points (0, 1 and 2 days after planting), duplicate plates were harvested by flushing seeds from the plate surface with water. Replicate plates were harvested for day 4 (duplicate), day 6 (duplicate) and day 8 (quadruplicate) using forceps to uproot the seedlings. This experiment was performed three times with different batches of seed (all obtained from Lehle Seeds). Although the glucosinolate content of each batch was slightly different, the patterns of change were similar. Data are presented from only one of these experiments.

4.2. Glucosinolate extraction and analysis

After harvest, plant material was immediately frozen in liquid nitrogen or in a −80 °C freezer, lyophilized to dryness, and ground to a coarse powder with a coffee grinder. Aliquots of lyophilized tissue (20–100 mg) were extracted in 4 ml of boiling water containing 0.05–0.1 μmol of an internal standard (2-propenyl glucosinolate) and 30 μmol $Pb(OAc)_2$ and 30 μmol $Ba(OAc)_2$ to precipitate protein and free sulfate ions. After 10 min boiling and 30 min of additional shaking, samples were cooled to 4 °C and centrifuged at 4000 *g*. The supernatant was then loaded onto an anion-exchange column (DEAE-Sephadex A-25). After washing with 67% (aqueous) methanol and deionized water, the column was capped and treated overnight with a solution of aryl sulfatase (prepared as described by Graser et al., 2001) to convert the glucosinolates to their desulfated derivatives. The desulfated glucosinolates were then eluted from the column with 60% (aqueous) methanol. The eluent was evaporated to dryness under nitrogen at 45 °C and resuspended in water.

Samples were separated by HPLC (Agilent HP1100 Series) fitted with a C-18 reversed-phase column (LiChrospher RP-18, 250×4.6 mm i.d., 5 um particle size, Chrompack, Varian, Darmstadt) using a water (Solvent A)-acetonitrile (Solvent B) gradient at a flow rate of 1 ml min^{-1} at ambient room temperature. The 42 min run consisted of 1.5% B (1 min), 1.5–5.0% B (5 min), 5.0–7.0% B (2 min), 7.0–21.0% B (10 min), 21.0–29.0% B (5 min), 29.0–43.0% B (7 min), 43.0–93.0% B (0.5 min), a 4 min hold at 93.0% B, 93.0–1.5% B (0.5 min), and a 7 min hold at 1.5% B. Eluent was monitored by diode array detection between 190 and 360 nm (2 nm interval). Desulfated glucosinolates were identified by comparison of retention time and UV spectra to

those of purified standards previously extracted from *A. thaliana* (Reichelt et al., 2002) and quantified by A_{229nm} relative to the internal standard.

In order to calculate molar concentrations of individual glucosinolates, relative response factors were used to correct for absorbance differences between the standard and the other components of the extract (Table 1). These were calculated using pure desulfated glucosinolates isolated by preparative HPLC and fractional crystallization and by reference to the literature (Buchner, 1987). In several cases, pure glucosinolates were isolated from plants other than *A. thaliana* that had higher concentrations of specific glucosinolates or less complex mixtures (Table 1). Purity was verified by HPLC and LC–MS, and response factors calculated from A_{229nm} using at least five independently-prepared concentration series for each compound ranging from 0.2 to 50 nmol per 10 μl injection. The response factors for a few of these compounds had already been reported in the literature (Haughn et al., 1991; Fiebig and Arens, 1992), and the values computed in this study showed close agreement. For both pure standards and extracts of experimental material, the lower limit of quantification was 0.1 nmol per injection. The overall recovery of glucosinolates from *A. thaliana* tissue samples in this study was at least 90%.

NOTE: During the preparation of this manuscript, another study describing glucosinolate accumulation in *A. thaliana* appeared (Petersen, B. L., Chen, S., Hansen, C. H., Olsen, C. E., Halkier, B. A., 2002. Composition and content of glucosinolates in developing *Arabidopsis thaliana*. Planta 214, 562–571). This interesting report also describes changes in myrosinase levels during development as well as experiments with a radiolabeled glucosinolate that demonstrate transport and turnover.

Acknowledgements

We thank Neil Oldham for assistance with the mass spectral analyses, and John Allen, Nadine Gallitschke, Shannon Guffey, and Einar Stauber for technical assistance. The research was supported by the Deutsche Forschungsgemeinschaft (grant FOR 383) and the Max Planck Gesellschaft.

References

Bartlet, E., Kiddle, G., Williams, I., Wallsgrove, R., 1999. Wound-induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomol. Exp. Appl. 91, 163–167.

Bilsborrow, P.E., Evans, E.J., Murray, F., Zhao, F.J., 1993. Glucosinolate changes in developing pods of single and double low varieties of autumn-sown oilseed rape (*Brassica napus*). Ann. Appl. Biol. 122, 135–143.

Bodnaryk, R.P., 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry 35, 301–305.

Bones, A.M., Rossiter, J.T., 1996. The myrosinase-glucosinolate system, its organisation and biochemistry. Physiol. Plant. 97, 194–208.

Brader, G., Tas, E., Palva, E.T., 2001. Jasmonate-dependent induction of indole glucosinolates in Arabidopsis by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. Plant Physiol. 126, 849–860.

Brown, P.D., Morra, M.J., 1997. Control of soil-borne plant pests using glucosinolate-containing plants. In: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, San Diego, pp. 167–231.

Brudenell, A.J.P., Griffiths, H., Rossiter, J.T., Baker, D.A., 1999. The phloem mobility of glucosinolates. J. Exp. Bot. 50, 745–756.

Buchner, R., 1987. Approach to determination of HPLC response factors for glucosinolates. In: Wathelet, J.-P. (Ed.), Glucosinolates in Rapeseeds: Analytical Aspects. Martinus Nijhoff Publishers, Boston, pp. 50–58.

Chen, S.X., Petersen, B.L., Olsen, C.E., Schulz, A., Halkier, B.A., 2001. Long-distance phloem transport of glucosinolates in Arabidopsis. Plant Physiol. 127, 194–201.

Chew, F.S., 1988a. Searching for defensive chemistry in the Cruciferae, or, do glucosinolates always control interactions of Cruciferae with their potential herbivores and symbionts? No! In: Spencer, K.C. (Ed.), Chemical Mediation of Coevolution. Academic Press, San Diego, pp. 81–112.

Chew, F.S., 1988b. Biological effects of glucosinolates. In: Cutler, H.G. (Ed.), Biologically Active Natural Products for Potential Use in Agriculture. American Chemical Society, Washington, D.C, pp. 155–181.

Clossais-Besnard, N., Larher, F., 1991. Physiological role of glucosinolates in *Brassica napus*: concentration and distribution pattern of glucosinolates among plant organs during a complete life cycle. J. Sci. Food Agric. 56, 25–38.

Doughty, K.J., Kiddle, G.A., Pye, B.J., Wallsgrove, R.M., Pickett, J.A., 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38, 347–350.

Du, L., Halkier, B.A., 1998. Biosynthesis of glucosinolates in the developing silique walls and seeds of *Sinapis alba*. Phytochemistry 48, 1145–1150.

Fahey, J.W., Zalcmann, A.T., Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56, 5–51.

Fenwick, G.R., Heaney, R.K., Mullin, W.J., 1983. Glucosinolates and their breakdown products in food and food plants. Crit. Rev. Food Sci. Nutr. 18, 123–201.

Fiebig, H.-J., Arens, M., 1992. Glucosinolate (HPLC-Methode)-Gemeinschaftsarbeiten der DGF, 128. Mitteilung: Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen, 98. Mitt.: Analyse von Fettrohstoffen XII. Fat Sci. Technol. 94, 199–203.

Fieldsend, J., Milford, G.F.J., 1994a. Changes in glucosinolates during crop development in single- and double-low genotypes of winter oilseed rape (*Brassica napus*): I. Production and distribution in vegetative tissues and developing pods during development and potential role in the recycling of sulphur within the crop. Ann. Appl. Biol. 124, 531–542.

Fieldsend, J., Milford, G.F.J., 1994b. Changes in glucosinolates during crop development in single- and double-low genotypes of winter oilseed rape (*Brassica napus*): II. Profiles and tissue-water concentrations in vegetative tissues and developing pods. Ann. Appl. Biol. 124, 543–555.

Graser, G., Oldham, N.J., Brown, P.D., Temp, U., Gershenzon, J., 2001. The biosynthesis of benzoic acid glucosinolate esters in *Arabidopsis thaliana*. Phytochemistry 57, 23–32.

Gundlach, H., Müller, M.J., Kutchan, T.M., Zenk, M.H., 1992. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc. Natl. Acad. Sci., USA 89, 2389–2393.

Halkier, B.A., Du, L.-C., 1997. The biosynthesis of glucosinolates. Trends Plant Sci. 2, 425–431.

Haughn, G.W., Davin, L., Giblin, M., Underhill, E.W., 1991. Biochemical genetics of plant secondary metabolites in *Arabidopsis thaliana*: the glucosinolates. Plant Physiol. 97, 217–226.

Hecht, S.S., 2000. Inhibition of carcinogenesis by isothiocyanates. Drug Metab. Rev. 32, 395–411.

Hogge, L.R., Reed, D.W., Underhill, E.W., Haughn, G.W., 1988. HPLC separation of glucosinolates from leaves and seeds of *Arabidopsis thaliana* and their identification using thermospray liquid chromatography–mass spectrometry. J. Chromatogr. Sci. 26, 551–556.

Hopkins, R.J., Ekbom, B., Henkow, L., 1998. Glucosinolate content and susceptibility for insect attack of three populations of *Sinapis alba*. J. Chem. Ecol. 24, 1203–1216.

Jander, G., Cui, J.P., Nhan, B., Pierce, N.E., Ausubel, F.M., 2001. The TASTY locus on chromosome 1 of Arabidopsis affects feeding of the insect herbivore *Trichoplusia ni*. Plant Physiol. 126, 890–898.

Kirkegaard, J.A., Sarwar, M., 1998. Biofumigation potential of Brassicas. I. Variation in glucosinolate profiles of diverse field-grown Brassicas. Plant Soil 201, 71–89.

Kliebenstein, D.J., Kroymann, J., Brown, P., Figuth, A., Pedersen, D., Gershenzon, J., Mitchell-Olds, T., 2001. Genetic control of natural variation in Arabidopsis glucosinolate accumulation. Plant Physiol. 126, 811–825.

Lambrix, V., Reichelt, M., Mitchell-Olds, T., Kliebenstein, D.J., Gershenzon, J., 2001. The Arabidopsis epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. Plant Cell 13, 2793–2807.

Louda, S., Mole, S., 1991. Glucosinolates: chemistry and ecology. In: Rosenthal, G.A., Berenbaum, M.R. (Eds.), Herbivores: Their Interactions with Secondary Plant Metabolites, 2nd Ed., Vol. 1: The Chemical Participants. Academic Press, San Diego, pp. 123–164.

Ludwig-Müller, J., Schubert, B., Pieper, K., Ihmig, S., Hilgenberg, W., 1997. Glucosinolate content in susceptible and resistant Chinese cabbage varieties during development of clubroot disease. Phytochemistry 44, 407–414.

McGregor, D.I., 1988. Glucosinolate content of developing rapeseed (*Brassica napus* L. 'Midas') seedlings. Can. J. Plant Sci. 68, 367–380.

Mikkelsen, M.D., Hansen, C.H., Wittstock, U., Halkier, B.A., 2000. Cytochrome P450 CYP79B2 from Arabidopsis catalyzes the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. J. Biol. Chem. 275, 33712–33717.

Mithen, R., 2001. Glucosinolates—biochemistry, genetics and biological activity. Plant Growth Regul. 34, 91–103.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.

Nielsen, J.K., Hansen, M.L., Agerbirk, N., Petersen, B.L., Halkier, B.A., 2001. Responses of the flea beetles *Phyllotreta nemorum* and *P. cruciferae* to metabolically engineered *Arabidopsis thaliana* with an altered glucosinolate profile. Chemoecology 11, 75–83.

Porter, A.J.R., Morton, A.M., Kiddle, G., Doughty, K.J., Wallsgrove, R.M., 1991. Variation in the glucosinolate content of oilseed rape *Brassica napus* L. leaves. I. Effect of leaf age and position. Ann. Appl. Biol. 118, 461–468.

Rask, L., Andreasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B., Meijer, J., 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol. Biol. 42, 93–113.

Reichelt, M., Brown, P.D., Schneider, B., Oldham, N.J., Stauber, E., Tokuhisa, J., Kliebenstein, D.J., Mitchell-Olds, T., Gershenzon, J., 2002. Benzoic acid glucosinolate esters and other glucosinolates from *Arabidopsis thaliana*. Phytochemistry 59, 663–671.

Reintanz, B., Lehnen, M., Reichelt, M., Gershenzon, J., Kowalczyk, M., Sandberg, G., Godde, M., Uhl, R., Palme, K., 2001. Bus, a bushy Arabidopsis CYP79F1 knockout mutant with abolished synthesis of short-chain aliphatic glucosinolates. Plant Cell 13, 351–367.

Renwick, J.A.A., 2001. Variable diets and changing taste in plant-insect relationships. J. Chem. Ecol. 27, 1063–1076.

Rosa, E.A.S., Heaney, R.K., Rego, F.C., Fenwick, G.R., 1994. The variation of glucosinolate concentration during a single day in young plants of *Brassica oleracea* var. *acephala* and *capitata*. J. Sci. Food Agric. 66, 457–463.

Rosa, E.A.S., Heaney, R.K., Portas, C.A.M., Fenwick, G.R., 1996. Changes in glucosinolate concentrations in *Brassica* crops (*B. oleracea* and *B. napus*) throughout growing seasons. J. Sci. Food Agric. 71, 237–244.

Rosa, E.A.S., 1997. Daily variation in glucosinolate concentrations in the leaves and roots of cabbage seedlings in two constant temperature regimes. J. Sci. Food Agric. 73, 364–368.

Sang, J.P., Minchinton, I.R., Johnstone, P.K., Truscott, R.J.W., 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. Can. J. Plant Sci. 64, 77–93.

Tierens, K.F.M.J., Thomma, B.P.H., Brouwer, M., Schmidt, J., Kistner, K., Porzel, A., Mauch-Mani, B., Cammue, B.P.A., Broekaert, W.F., 2001. Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of Arabidopsis to microbial pathogens. Plant Physiol. 125, 1688–1699.

Wittstock, U., Halkier, B.A., 2002. Glucosinolate research in the Arabidopsis era. Trends Plant Sci. 7, 263–270.

Zangerl, A.R., Bazzaz, F.A., 1993. Theory and pattern in plant defense allocation. In: Fritz, R.S., Simms, E.L. (Eds.), Plant Resistance to Herbivores and Pathogens. University of Chicago Press, Chicago, pp. 363–391.