Changes of *S*-alk(en)ylcysteine Sulfoxide Levels During the Growth of Different Garlic Morphotypes

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Abstract

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The contents of three *S*-alk(en)ylcysteine sulfoxides (alliin, methiin, and isoalliin) were determined in the leaves, pseudostems, and bulbs of six garlic genotypes (two flowering plant morphotypes, two semi bolters, and two scape absent morphotypes) cultivated for five consecutive years at the same location. The average levels of alliin, methiin, and isoalliin were found to be as follows: 1.92, 0.44, and 0.07 mg/g fw in the leaves, 1.57, 0.27, and 0.08 mg/g fw in the pseudostems, and 1.71, 0.20 and 0.13 mg/g fw in the bulbs, respectively. No statistically significant year-to-year differences were observed between the samples. Furthermore, the total contents and relative proportions of *S*-alk(en)ylcysteine sulfoxides in various parts of the plants (leaves, pseudostems, bulbs and roots) were evaluated in detail during the whole vegetation period. It was observed that the total content of these amino acids gradually decreased in all parts except for the bulbs. In the bulbs, the total content initially decreased after planting but significantly increased in June and culminated before harvest. Analogous trends were also observed for alliin and methiin concentrations. On the other hand, isoalliin levels steadily decreased during the whole vegetation period in all parts of the plants.

Keywords: garlic; *Allium sativum*; *S*-alk(en)ylcysteine sulfoxides; alliin; methiin; isoalliin

Garlic (*Allium sativum* L., Alliaceae) has been frequently used for culinary and medicinal purposes since ancient times. Thanks to its unique flavour, it belongs to the most popular condiments widely used in many cuisines. Thus, it is not surprising that the worldwide production of garlic has been gradually increasing over the past few decades, reaching nearly 22.3 million tons in 2009 (FAO 2010). The characteristic alliaceous smell, taste, and numerous health beneficial effects of garlic can be primarily attributed to the compounds enzymatically formed from unique non-protein amino acids, *S*-alk(en)yl substituted cysteine sulfoxides. Four individual derivatives, namely *S*-methyl-, *S*-ethyl-, *S*-allyl- and *S*-(*E*)-(1-propenyl)cysteine sulfoxides (trivially called methiin, ethiin, alliin, and isoalliin, respectively), typically occur in garlic (Figure 1). Alliin is always the major derivative, accounting for about 80% of the cysteine sulfoxide pool. On the other hand, ethiin is usually present

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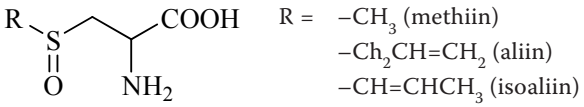

Figure 1. Structure of the *S*-alk(en)ylcysteine sulfoxides monitored in this study

only in trace quantities (Kubec *et al*. 1999; Kubec & Dadáková 2009).

Over many years of selection and cultivation, garlic has lost the ability to produce fertile seeds and some varieties do not even form flower stalks and flowers, which resulted in obtaining numerous garlic genotypes (Volk *et al*. 2004; Kamenetsky *et al*. 2005). Most taxonomic systems divide garlic subspecies grown in Europe into bolting types (flowering plants) and nonbolting (scape absent)/incomplete bolting types (semi bolters). These two groups are taxonomically referred to as *A. sativum* ssp. *ophioscorodon* (syn. *A. sativum* ssp. *sagittatum* KUZN) and *A. sativum* ssp. *sativum*, respectively. Furthermore, garlic growers often classify garlic varieties according to their phenotypic character into hardneck garlic and softneck garlic (IPGRI 2001; Stavělíková & Fáberová 2005; Brewster 2008). Flowering plants belonging to the group *A. sativum* ssp. *ophioscorodon* include hardneck garlic morphotypes that produce scapes or flower stalks and are also known as bolting or hardneck morphotypes. The group *A. sativum* ssp. *sativum* includes softneck morphotypes that are either nonbolting (scape absent) or produce only very weak stalks or do not form flower stalks at all. Under stressful conditions, bulbils can develop within the false pseudostems. Genotypes of the semi bolter morphotypes produce flower stalks, but not flowers (Volk *et al.* 2004; Stavělíková & Fáberová 2005; Brewster 2008).

A number of studies have dealt with the contents of *S*-alk(en)ylcysteine sulfoxides in garlic cloves. However, only little is still known about the levels of these sulphur amino acids in other parts of garlic and about the changes in their contents during the vegetation period. For example, it was reported that the content of alliin in garlic bulbs increased more than 18 times during the growth period, changing from 0.55 mg/g fw to 10.33 mg/g fw. The maximum alliin concentration was detected at the stage when the leaves began to wither (Matsuura *et al.* 1996). On the other hand, Bloem *et al.* (2004) observed that the content of alliin in seven-week old bulbs (when leaves were already developed) was 3.0 mg/g fw, but it decreased to 1.1 mg/g fw during the next 14 weeks of the bulb growth. At the beginning of the plant development, the highest alliin levels were found in the leaves (13.1 mg/g fw). With further plant development, alliin was found mainly in the bulbs (5.9 mg/g fw after 14 weeks). However, neither of these two reports dealt with the contents of the other two important cysteine derivatives present in garlic, i.e. methiin and isoalliin.

In this study, we examined the distribution of the three main cysteine derivatives (alliin, methiin, and isoalliin) in the individual parts of different garlic morphotypes and investigated the changes in the contents of these compounds during garlic growth.

MATERIAL AND METHODS

Plant material. The set of six garlic genotypes analysed in this study, namely two flowering plant morphotypes (F), two semi bolters (S) (producing scapes but never developing heads) and two scape absent morphotypes (N), was obtained from the

Table 1. The samples analyzed in this study

No.	Morphotype	Evigez[a]	Accession Name	Donor Institution
1	S	09H0100069	Alan	Czech Republic
2	S	09H0100799	Landrace (Rozhanovce)	Slovak Republic
3	N	09H0100078	Japo	Czech Republic
4	N	09H0101171	Lukan	Czech Republic
5	F	09H0100081	Ropal	Czech Republic
6	F	09H0100035	Chinese giant	P.R. China

[a]Plant Genetic Resources Documentation in the Czech Republic (Fáberová 2009)

S = semi bolters; N = scape absent; F = flowering plants

Crop Research Institute in Prague, Department of Vegetables and Special Crops, Olomouc, Czech Republic (Table 1). All these genotypes are included in the official collection of vegetatively propagated *Alliums* for long day conditions. The cloves were planted in the autumn period (middle of October) and grown under open field conditions according to the general agronomic practice in Olomouc-Holice (49°37'N, 17°17'E, 209 m a.s.l.). The trial plots have soil horizon loam sand to loam and soil type is alluvial and gley soil. The cloves were stained with a combination of iprodian (255 g/l, Rowral Flo), chlorpyrifos-methyl (400 g/l, Reldan 40 EC) and carbendazin (500 g/l, Bavistin WG) for 20 minutes. The insecticides chlorpyrifos-methyl (400 g/l, Reldan 40 EC) and diazion (10%, Basudin 10G) were used to protect the plants against pests. The fertiliser used was Cererit (8% N, 13% P_2O_5, 11% K_2O, 15% SO_4^{2-}) in the amounts of 40 g/m^2 (autumn) and of 20 g/m^2 (spring). Weeds were removed manually from the field in the course of the whole vegetative period. The harvested plants were either immediately used for analysis or dried in a special room with a good air circulation. After 6–8 weeks, the rest of leaves and roots were cut and the garlic was prepared for planting.

The samples were collected on May 16, 2005, May 23, 2006, May 20, 2007, May 17, 2008, and May 18, 2009. The individual plant parts analysed in these samples included leaves (L), pseudostems (P), and bulbs (B). For comparison, the same six garlic genotypes were sampled eight times during the whole vegetation period in 2009. In 2009, the samples were collected on March 10 (I), March 30 (II), April 20 (III), May 5 (IV), May 18 (V), June 1 (VI), June 15 (VII), and June 29 (VIII). The plant parts analysed in these samples were leaves (L), pseudostems (P), bulbs (B), and roots (R). The bulbification started in the middle of May within 5 days difference between the individual morphotypes. Furthermore, the spathes with bulbils (T) were evaluated in the flowering plant morphotypes. All samples were analysed in duplicate using two distinct plants. The content of cysteine sulfoxides was also determined in the fully developed garlic bulbs one week after harvest (July 7, samples No. IX). The climatic data for years 2005–2009, including average air temperature, sunshine duration, and total precipitation, were obtained from the Czech Hydrometeorological Institute (2010). The detailed climatic factors for years 2005–2008 are described by Horníčková *el al.* (2010).

Chemicals and reagents. *S*-Alk(en)ylcysteine sulfoxides (alliin, methiin, and isoalliin) were prepared/isolated as described elsewhere (Kubec & Dadáková 2008). The OPA derivatisation reagent was prepared by dissolving 140 mg of *o*-phthaldiadehyde in 5 ml of methanol. After the addition of 100 µl of *tert*-butylthiol (2-methylpropane-2-thiol), the solution was adjusted to 50 ml with 50mM KH_2PO_4 buffer (pH 9.5) (Velíšek *et al*. 1993).

Sample preparation. Fresh garlic plants of each genotype sampled in years 2005–2008 were divided into three parts (bulbs, pseudostems, and leaves), whereas the plants collected in 2009 were divided into four parts (roots, bulbs, pseudostems, and leaves) and the samples of flowering plants collected in June 2009 were divided into roots, bulbs, pseudostems, leaves, and spathes with bulbils and flowers. The samples (about 5 g) were placed in 50 ml of methanol and gently boiled for 10 min to inactivate alliinase. Norleucine (10 mg) was added to the sample as an internal standard. The sample was then homogenised using a blender and filtered through a 0.45 µm cellulose acetate syringe-tip filter (Alltech). An aliquot (100 µl) of the filtrate obtained was mixed with 900 µl of the OPA reagent and analysed by HPLC. The analysis of pseudostems, leaves, roots, spathes, and bulb was done only once (twice of the samples gathered in July 7, sample No. IX) using a single plant. The limits of quantification (LOQ) were determined to be 0.01 mg/g fw for all three amino acids monitored.

High-performance liquid chromatography. HPLC separations were performed on a Consta-Metric binary pump system (Watrex, San Francisco, USA), employing an AS 100 autosampler (20 µl injections), a SpectroMonitor UV detector (Thermo Scientific, Inc., Waltham, USA) and a C-18 reverse phase column (Synergi POLAR-RP 80Å, 250 × 4.6 mm, 4 µm; Phenomenex, Torrance, USA). The chromatographic conditions were as follows: (A) 50mM KH_2PO_4 buffer (pH 6.5, solvent A) and methanol (solvent B), flow rate of 0.8 ml/min and the gradient A/B 59/41 (0 min), 25/75 (in 37 min), 25/75 (in 39 min) and 59/41 (in 50 min), detection wavelength of 337 nm. The column temperature was maintained at 37°C.

Statistical analysis. Descriptive statistics and linear discrimination analysis were performed using the software SPSS for Windows, Release 11.0.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

The levels of alliin, methiin, and isoalliin were determined in six garlic genotypes representing three different garlic morphotype groups, namely flowering plants (F), semi bolters (S), and scape absent plants (N) (Table 1). To minimise the possible year-to-year variation caused by the climatic conditions, the samples were cultivated in five successive years (2005–2009) at the same location. The samples were collected approximately in the middle of the growth cycle and three different parts of the plants, i.e. leaves (L), pseudostems (P), and bulbs (B), were analysed. The results obtained are shown in Table 2.

As can be seen, the total amounts of cysteine sulfoxides in the three parts analysed varied in quite a narrow range of 1.66–2.69 mg/g fw (average of 2005–2009). The leaves generally contained some-

Table 2. The content of *S*-alk(en)ylcysteine sulfoxides (ACSO, in mg/g fw) in various garlic morphotypes in 2005–2009

Year/analyte	Morphotype S			Morphotype N			Morphotype F		
	L	P	B	L	P	B	L	P	B
2005									
Methiin	0.35	0.20	0.16	0.51	0.38	0.27	0.31	0.25	0.28
Alliin	1.27	1.08	2.05	1.71	1.67	2.58	1.28	1.37	2.18
Isoalliin	0.04	0.04	0.13	0.03	0.04	0.07	0.02	0.04	0.09
Sum of ACSO	1.66	1.32	2.34	2.25	2.09	2.92	1.61	1.66	2.55
2006									
Methiin	0.10	0.06	0.05	0.18	0.08	0.04	0.22	0.17	0.15
Alliin	1.73	1.04	1.10	1.88	1.43	0.94	1.73	1.73	1.58
Isoalliin	0.05	0.05	0.10	0.04	0.07	0.08	0.07	0.12	0.22
Sum of ACSO	1.88	1.15	1.25	2.10	1.58	1.06	2.02	2.02	1.95
2007									
Methiin	0.65	0.28	0.35	0.84	0.35	0.19	0.69	0.31	0.18
Alliin	2.12	1.56	2.17	3.05	2.18	1.86	2.32	1.62	1.62
Isoalliin	0.15	0.15	0.28	0.11	0.12	0.14	0.15	0.11	0.18
Sum of ACSO	2.92	1.99	2.80	4.00	2.65	2.19	3.16	2.04	1.98
2008									
Methiin	0.61	0.63	0.38	0.70	0.49	0.24	0.67	0.39	0.26
Alliin	2.25	2.02	2.34	2.33	1.95	2.05	2.01	1.83	1.63
Isoalliin	0.15	0.16	0.21	0.04	0.03	0.06	0.13	0.13	0.16
Sum of ACSO	3.01	2.81	2.93	3.07	2.47	2.35	2.81	2.35	2.05
2009									
Methiin	0.12	0.07	0.16	0.21	0.13	0.12	0.49	0.27	0.18
Alliin	1.46	0.97	1.36	1.81	1.62	1.11	1.80	1.47	1.08
Isoalliin	0.03	0.04	0.07	0.02	0.03	0.04	0.08	0.10	0.10
Sum of ACSO	1.61	1.08	1.59	2.04	1.78	1.27	2.37	1.84	1.36
Average of 2005–2009									
Methiin	0.36	0.24	0.22	0.49	0.28	0.17	0.47	0.28	0.21
Alliin	1.77	1.33	1.80	2.15	1.77	1.71	1.83	1.60	1.62
Isoalliin	0.08	0.09	0.16	0.05	0.06	0.08	0.09	0.10	0.15
Sum of ACSO	2.21	1.66	2.18	2.69	2.11	1.96	2.39	1.98	1.98

S – semi bolters; N – scape absent; F – flowering plants; L – leaves; P – pseudostems; B – bulbs

Table 3. The content of *S*-alk(en)ylcysteine sulfoxides (ACSO, in mg/g fw) in garlic during vegetation period in 2009

Morphotype	Part analyzed	Analyte	Sample No.								
			I	II	III	IV	V	VI	VII	VIII	IX
Semi bolters (S)	Leaves (L)	methiin	0.77	0.82	0.26	0.19	0.12	0.15	0.09	0.01	
		alliin	4.02	4.02	1.60	1.62	1.46	1.70	1.52	0.64	
		isoalliin	0.41	0.18	0.04	0.04	0.03	0.02	0.01	n.d.	
		sum of ACSO	5.20	5.02	1.90	1.85	1.61	1.87	1.62	0.65	
	Pseudostems (P)	methiin	0.71	0.81	0.31	0.15	0.07	0.09	0.04	0.02	
		alliin	3.95	3.63	1.55	1.39	0.97	1.17	1.14	1.04	
		isoalliin	0.38	0.14	0.04	0.06	0.04	0.03	0.01	0.02	
		sum of ACSO	5.04	4.58	1.90	1.60	1.08	1.29	1.19	1.08	
	Bulbs (B)	methiin	0.35	0.33	0.24	0.09	0.16	0.63	0.33	0.33	0.45
		alliin	3.28	2.69	1.96	1.15	1.36	3.89	3.19	3.97	4.64
		isoalliin	0.29	0.09	0.07	0.06	0.07	0.14	0.03	0.03	0.04
		sum of ACSO	3.92	3.11	2.27	1.30	1.59	4.66	3.55	4.33	5.13
	Roots (R)	methiin	0.20	0.11	0.09	0.08	0.06	0.05	0.02	0.01	
		alliin	2.64	0.88	1.60	1.79	1.30	1.03	1.20	1.37	
		isoalliin	0.53	0.08	0.22	0.20	0.13	0.04	0.04	0.06	
		sum of ACSO	3.37	1.07	1.91	2.07	1.49	1.12	1.26	1.44	
Scape absent (N)	Leaves (L)	methiin	0.91	1.03	0.69	0.45	0.21	0.50	0.30	0.11	
		alliin	4.72	4.11	2.42	2.36	1.81	2.90	2.53	1.93	
		isoalliin	0.37	0.09	0.04	0.03	0.02	0.03	0.01	0.01	
		sum of ACSO	6.00	5.23	3.15	2.84	2.04	3.43	2.84	2.05	
	Pseudostems (P)	methiin	1.04	1.02	0.59	0.28	0.13	0.23	0.12	0.01	
		alliin	4.75	3.88	2.42	1.87	1.62	1.80	1.58	2.05	
		isoalliin	0.28	0.10	0.04	0.04	0.03	0.03	0.05	0.06	
		sum of ACSO	6.07	5.00	3.05	2.19	1.78	2.06	1.75	2.12	
	Bulbs (B)	methiin	0.60	0.36	0.33	0.18	0.12	0.39	0.33	0.59	0.78
		alliin	3.97	2.16	2.23	1.83	1.11	2.51	2.38	5.90	6.22
		isoalliin	0.22	0.06	0.05	0.06	0.04	0.06	0.10	0.09	0.03
		sum of ACSO	4.79	2.58	2.61	2.07	1.27	2.96	2.81	6.58	7.03
	Roots (R)	methiin	0.35	0.35	0.16	0.12	0.06	0.19	0.04	0.01	
		alliin	3.00	1.73	1.37	1.94	1.68	2.44	1.24	1.47	
		isoalliin	0.44	0.12	0.10	0.16	0.08	0.06	0.13	0.09	
		sum of ACSO	3.79	2.20	1.63	2.22	1.82	2.69	1.41	1.57	
Flowering plants (F)	Leaves (L)	methiin	0.92	1.03	0.60	0.60	0.49	0.89	0.65	0.42	
		alliin	3.99	3.67	1.97	1.87	1.80	2.64	2.29	1.30	
		isoalliin	0.36	0.43	0.04	0.08	0.08	0.20	0.24	0.17	
		sum of ACSO	5.27	5.13	2.61	2.55	2.37	3.73	3.18	1.89	
	Pseudostems (P)	methiin	0.87	0.87	0.60	0.39	0.27	0.36	0.17	0.25	
		alliin	3.63	3.33	1.84	1.68	1.47	1.69	1.53	1.30	
		isoalliin	0.29	0.37	0.05	0.12	0.10	0.11	0.08	0.06	
		sum of ACSO	4.79	4.57	2.49	2.19	1.84	2.16	1.78	1.61	
	Bulbs (B)	methiin	0.56	0.36	0.43	0.23	0.18	0.67	0.41	0.92	1.30
		alliin	3.05	1.95	1.72	1.36	1.08	2.58	2.32	3.68	5.77
		isoalliin	0.17	0.15	0.05	0.16	0.10	0.22	0.14	0.11	0.07
		sum of ACSO	3.78	2.46	2.20	1.75	1.36	3.47	2.87	4.71	7.14
	Roots (R)	Methiin	0.35	0.48	0.35	0.33	0.22	0.48	0.19	0.19	
		Alliin	3.24	2.48	1.92	2.10	2.09	1.79	2.38	2.02	
		Isoalliin	0.37	0.16	0.09	0.35	0.33	0.14	0.27	0.22	
		Sum of ACSO	3.96	3.12	2.36	2.78	2.64	2.41	2.84	2.43	
	Spathes with bulbils (T)	methiin						1.50	1.39	0.96	
		alliin						6.77	4.07	2.24	
		isoalliin						0.67	0.23	0.20	
		sum of ACSO						8.94	5.69	3.40	

what higher average concentrations (2.43 mg/g fw) of these amino acids compared to the pseudostems and bulbs (1.92 and 2.04 mg/g fw, respectively). The average relative ratios of methiin/alliin/isoalliin differed slightly between the various plant parts, being 18/79/3 in the leaves, 14/82/4 in the pseudostems, and 10/84/6 in the bulbs. However, no apparent differences were observed between the total levels of cysteine sulfoxides found in the samples of the three garlic morphotypes analysed in this study. Differentiation by linear discrimination analysis (LDA) of the garlic morphotypes according to the contents of the three cysteine sulfoxides in the leaves, pseudostems and bulbs was incomplete, with only 41.1% of the samples being correctly classified. Most significant differences were observed in the total cysteine sulfoxide levels in the scape absent morphotypes (63.3% of samples were correctly classified), whereas the samples of the other two morphotypes (F and S) were classified equally poorly (30.0%). LDA of the individual plant parts (leaves, pseudostems, and bulbs) of the samples collected in 2005–2009 also revealed that the year-to-year differences in cysteine sulfoxide concentrations were not statistically significant. On average, only 19 samples out of 30 (62.2%) were correctly classified (17 samples of leaves, i.e. 56.7%; 19 samples of pseudostems, i.e. 63.3%; 20 samples of bulbs, i.e. 66.7%). LDA between the samples harvested in the individual five years (regardless of the morphotype and plant part) was also performed. On average, only 47.8% of the samples were correctly classified, namely seven samples (38.9%) in 2005, 10 samples (55.6%) in 2006, seven samples (38.9%) in 2007, six samples (33.3%) in 2008, and 13 samples (72.2%) in 2009. These results confirmed our previous observations that the contents of cysteine sulfoxides primarily depend on various genetic factors and post-harvest treatment, whereas ordinary year-to-year differences in the climatic conditions during the growth (e.g. temperature, irrigation) influence their levels to a lesser extent (unless any climatic extremes occur) (Horníčková *et al*. 2010).

We also evaluated the levels of the three *S*-alk(en)-ylcysteine sulfoxides in various parts of the plants during a typical vegetation period of garlic cultivated in the Czech Republic, i.e. between March and July. The same set of six genotypes was evaluated (Table 1). The analysed parts of the samples included leaves (L), pseudostems (P), bulbs (B), and roots (R). Furthermore, the spathes with bulbils (T) of the two genotypes of flowering plant morphotypes were also analysed. The results achieved are summarised in Table 3.

As expected, the contents of cysteine sulfoxides varied considerably during garlic growth, with very similar trends observed in the plants of all three morphotype groups (S, N, and F). In March (samples No. I and II), when the first leaves had already developed, the highest amounts were found in the green parts of the plants. At this stage, total concentrations of all three cysteine sulfoxides in the leaves (Figure 2) and pseudostems (Figure 3) were typically 2–4 times higher compared to those present in the bulbs (Figure 4) and roots (Figure 5). These findings support the general assumption that the green parts of garlic are the most important sites of cysteine sulfoxide biosynthesis, although some biogenesis may also occur in the bulbs (Lancaster & Shaw 1989; Lawson 1996; Hughes *et al*. 2005; Velíšek & Cejpek 2009).

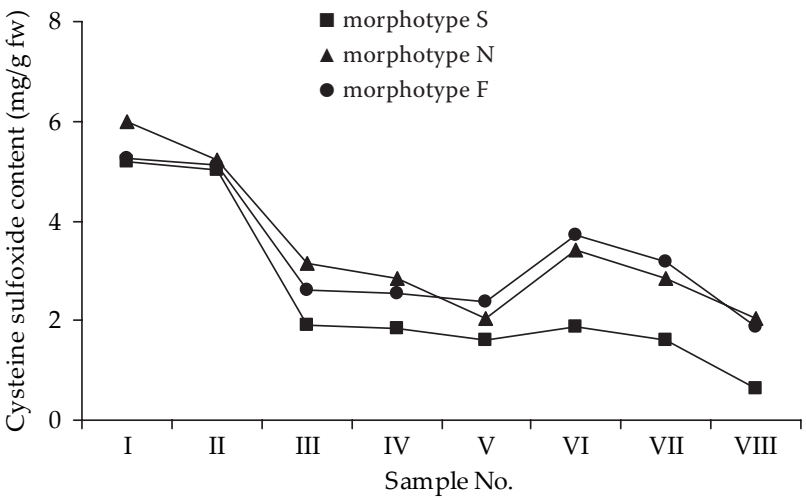

Figure 2. Total amounts of cysteine sulfoxides in the leaves during vegetation in 2009

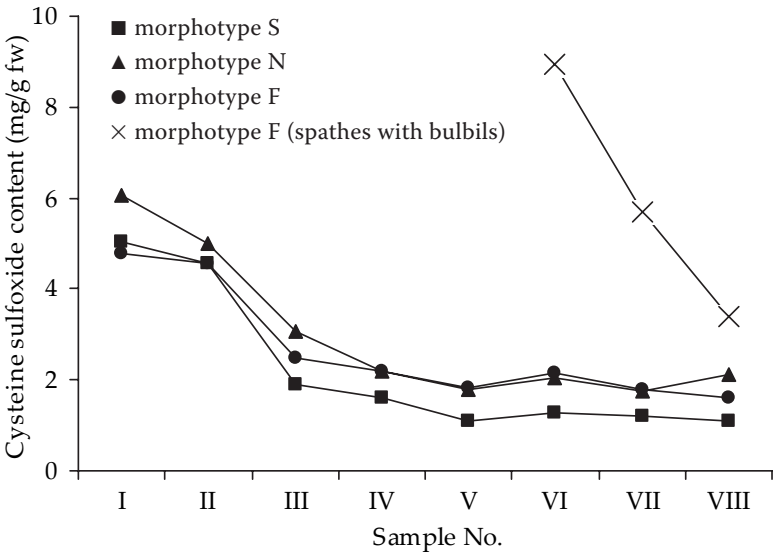

Figure 3. Total amounts of cysteine sulfoxides in the pseudostems and spathes with bulbils during vegetation in 2009

A dramatic decline in the cysteine sulfoxide contents was observed both in the leaves and pseudostems at the beginning of April (between samples No. II and III). The average levels in the leaves sharply decreased from 5.13 mg/g fw to 2.55 mg/g fw, whereas those in the pseudostems changed from 4.72 mg/g fw to 2.48 mg/g fw. In other words, the levels decreased by more than 50% (in L) and 47% (in P) within only two weeks. In the remaining period of vegetation (May–June, samples No. IV–VII), the total content of cysteine sulfoxides in the green parts (L and P) of all morphotypes did not change considerably, staying within a relatively narrow interval of 1.08–3.73 mg/g fw (Figures 2 and 3). At the very end of the growth cycle, when the leaves started to wither (sample No. VIII), the content of cysteine sulfoxides in the green parts further decreased (Figure 2).

Significantly different trends were observed in regard to the changes of cysteine sulfoxide levels in the bulbs (Figure 4). At the beginning of the plant growth (when the leaves and pseudostems were developing) (samples No. I–III), the total content of cysteine sulfoxides rapidly decreased from 4.16 mg/g fw to 2.36 mg/g fw (43% decline within four weeks). In April and May, the levels did not show any extraordinary changes and were comparable to those observed in other parts of the plants. However, the total content of cysteine sulfoxides in the bulbs started to increase dramatically at the beginning of June (approximately five weeks before harvest). For example, the average concentrations found in the bulbs increased more than 2.6 times (from 1.41 to 3.70 mg/g fw) over a two-week period between samples No. V and VI. As bulbing progressed, a continuous increase in the content of cysteine sulfoxides was observed. This accumulation of cysteine sulfoxides may be associated with their possible role as storage compounds of sulphur and nitrogen during dor-

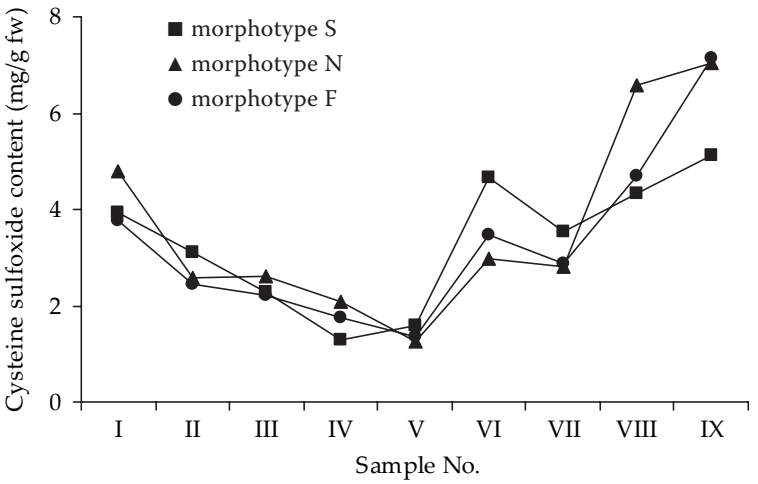

Figure 4. Total amounts of cysteine sulfoxides in the bulbs during vegetation in 2009

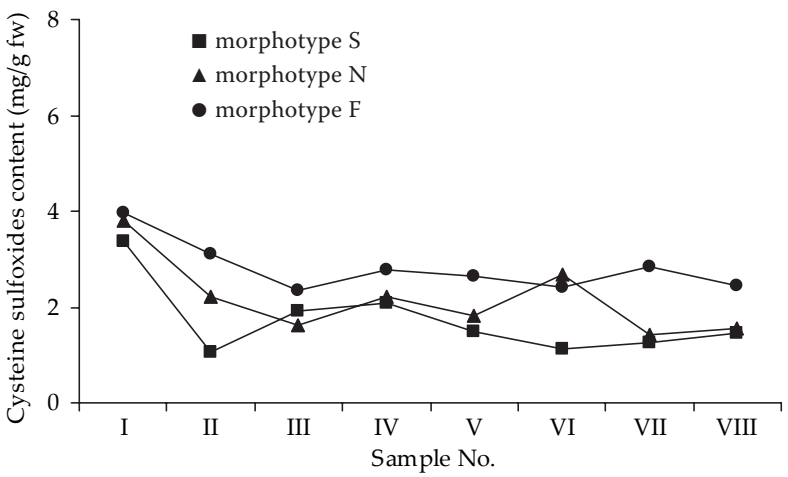

Figure 5. Total amounts of cysteine sulfoxides in the roots during vegetation in 2009

mancy. The bulbs analysed immediately before harvest (sample No. VIII) had the total cysteine sulfoxide content between 4.33 mg/g fw (in S) and 6.58 mg/g fw (in N), with the mean of 5.21 mg/g fw. In accordance with the usual agronomic practice, the harvested bulbs (with the aerial parts still attached to them) were allowed to dry out on the field. During this period, the content of cysteine sulfoxides further increased in all three morphotypes to levels between 5.13 (in S) and 7.14 mg/g fw (in F), with the mean of 6.43 mg/g fw (sample No. IX). These findings are in good agreement with the observations of Matsuura *et al.* (1996) and with our previous results obtained by the evaluation of 58 various garlic genotypes (Horníčková *et al.* 2010).

Spathes with bulbils evolved in the flowering plant morphotypes (F) at the beginning of June. This part of the plants was found to contain very high levels of cysteine sulfoxides (8.94 mg/g fw). However, their content rapidly decreased with further plant development (Table 3).

A significant degree of variation was also observed in the relative proportions of the individual cysteine sulfoxides during garlic growth. For example, the average ratio of methiin/alliin/isoalliin in the bulbs changed from 12/82/6 (sample No. I) to 13/86/1 (sample No. IX). As can be seen, the relative ratio of methiin/alliin did not change considerably, whereas the proportion of isoalliin markedly decreased. This decrease could be explained by the conversion of isoalliin into cycloalliin. Presumably, this cyclisation proceeds spontaneously and results in the accumulation of cycloalliin in the tissue as reported by Ichikawa *et al.* (2006). Interestingly, different methiin/alliin/isoalliin variation patterns were observed in the leaves, pseudostems, and roots. In semi bolting (S) and scape absent species (N), the average relative proportions of methiin/alliin/isoalliin changed from 15/78/7 to 3.5/96/0.5 (in L), from 16/78/6 to 1/97/2 (in P), and from 7/79/14 to 1/94/5 (in R). On the other hand, the corresponding ratios did not change significantly between the individual samples of the flowering plant morphotypes (F). In March (sample No. I), methiin/alliin/isoalliin proportions were found to be 17/76/7 (in L), 18/76/6 (in P), and 9/82/9 (in R), whereas those at the end of June (sample No. VIII) were 22/69/9, 15/81/4, and 8/83/9, respectively.

CONCLUSIONS

Our results show that the total content and relative proportions of the individual *S*-alk(en)ylcysteine sulfoxides in different garlic parts vary to a great extent. It has been found that the contents of these compounds primarily depend on the sampling date, whereas ordinary year-to-year changes in the climatic conditions during the growth (e.g. sunshine duration, precipitation) influence their levels less significantly. In accordance with the previous reports, it has been observed that the contents of the two major *S*-alk(en)ylcysteine sulfoxides (alliin and methiin) in the bulbs decreased during the first stage of the vegetation period. After this initial decline, alliin concentrations in the bulbs increased and the highest amounts were found at the end of June just before harvesting. On the other side, the concentrations of alliin and methiin in the green parts (i.e. pseudostems, leaves, and spathes with bulbils) decreased during the whole vegetation period. The content of

isoalliin, the key precursor of the compounds causing undesirable blue-green discoloration of various garlic products (Kubec & Velíšek 2007), continuously decreased in all plant parts during the vegetation period.

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