10690 J. Agric. Food Chem. 2010, 58, 10690–10696
DOI:10.1021/jf102009j

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Influence of Fertilizer Practices on S-Containing Metabolites in Garlic (*Allium sativum* L.) under Field Conditions

ELKE BLOEM,* SILVIA HANEKLAUS, AND EWALD SCHNUG

Institute for Crop and Soil Science, Federal Research Centre for Cultivated Plants (JKI), Bundesallee 50, D-38116 Braunschweig, Germany

Cysteine sulfoxides (e.g., alliin) are the characteristic sulfur-containing secondary compounds in garlic, which account for taste and pharmaceutical quality. It was the aim of the present study to investigate the influence of sulfur and nitrogen supply under field conditions on the alliin content and cysteine and glutathione as possible precursors. Sulfur and nitrogen were applied in four different rates, and five samplings were conducted. Sulfur fertilization significantly increased the cysteine, glutathione, and alliin contents of leaves and bulbs, while nitrogen fertilization had no significant influence. Cysteine increased by a factor of 1.3−1.5 in leaves and 1.0−2.0 in bulbs. Glutathione increased significantly in bulbs by a factor of 0.9−1.6 but only at main growth and not at maturity. The alliin concentration in bulbs increased with S fertilization significantly at all harvesting dates and at maturity from 5.1 to 11.2 mg g^{-1} of dry weight. High sulfur application in combination with low nitrogen fertilization increased the alliin concentration in garlic significantly during main growth until the beginning of ripening. At the last harvest, 15 kg ha^{-1} S resulted in high-quality garlic suitable for consumption and use in plant protection or pharmaceutical industries.

KEYWORDS: Alliin; cysteine; garlic (*Allium sativum* L.); glutathione; nitrogen; sulfur

INTRODUCTION

Garlic is one of the oldest known crops, having been mentioned in Egyptian, Babylonian, and Indian writings, which are more than 5000 years old, and has been cultivated by humans since ancient times. The plant is grown worldwide on about 1 million hectares (*1*) as a spice and for medical purposes because it is active against a broad range of medical disorders (*2*, *3*). The spectrum of biological activities includes antimicrobial, anthelminthic, antiprotozoal, antifungal, insecticidal, antithrombotic, anticancer, antiarthritic, hypolipidemic, and hypoglycemic properties (*4*). The pharmacological value of garlic is attributed to its *S*-alk(en)yl-L-cysteine sulfoxide content (*5*, *6*). Four types of cysteine sulfoxides were described in *Allium* species from which alliin (*S*-2-propenyl-L-cysteine sulfoxide) is the predominant form found in garlic (*7*). Garlic bulbs also contain a smaller amount of γ-glutamyl-*S*-allylcysteine, *S*-methylcysteine sulfoxide, *S*-*trans*-1-propenylcysteine sulfoxide, *S*-2-carboxypropylglutathione, and *S*-allylcysteine (*8*).

The biochemical pathway of alliin biosynthesis is still speculative, but two possible routes are proposed. The first implies serine and an allyl source, and the second implies glutathione and an allyl source (*9*). Central to both possible pathways is cysteine as a linkage between carbon (C) and nitrogen (N) metabolism and as the precursor for reduced sulfur (S). S fertilization is well-known to increase the glutathione and cysteine concentrations in different crops under field conditions (*10*).

*To whom correspondence should be addressed. Telephone: 00495315962200. Fax: 00495315962199. E-mail: elke.bloem@jki.bund.de.

The characteristic flavor of garlic is released after enzymatic cleavage of alliin to allicin and its derivatives. Allicin is thought to cause the antimicrobial activity because it easily crosses cell membranes and undergoes thiol−disulfide exchange reactions with free thiol groups, the possible mode of action against microorganisms (*11*). These specific properties of garlic suggest a potential of garlic preparations in organic farming as well as horticulture for promoting plant health (*12*). Natural plant products, such as garlic juice, are generally easily biodegradable and do not tend to persist in the environment. Portz et al. (*12*) used garlic juice successfully against the infection of cucumber with *Phytophthora infestans* and *Pseudoperonospora cubensis*. Schüder et al. (*13*) demonstrated that a solution prepared from garlic had a very high potential to control slugs and snails.

The antimicrobial mode of action of garlic can be used in animal nutrition as well. Garlic preparations may be used as prophylactics and antimicrobial feed additives to overcome problems caused by restrictions in the use of synthesized antibiotics and by resistance to such antibiotics (*14*, *15*). The advantages of herbal remedies are that they do not contaminate meat products with chemical substances, and thus far, no resistances have been developed.

From the viewpoint of consumers and pharmaceutical industries, there is a high interest to increase the content of bioactive compounds, such as cysteine, glutathione, and alliin, in garlic to obtain a high valuable basic product. In previous trials, the impact of S fertilization on the alliin content of garlic was investigated under controlled greenhouse conditions, which best reflect dose/effect relationships (*16*). The reason is that, in pot experiments, no nutrient losses of highly mobile elements, such as

N and S, will influence the nutritional status and, with sand as the culture substrate, the added rates of plant-available nutrients can be precisely adjusted. Other advantages are that neither abiotic nor biotic factors may cause stress or limit crop productivity, so that the pure dose/effect interdependence is measured. Haneklaus (*17*) describes for instance how the efficacy of S fertilization on Sr uptake of crop plants is blurred from pot to lysimeter and finally to field scale. These studies showed that the S rates had to be increased manifold to obtain similar effects.

A greenhouse trial was conducted with three levels of S (0, 6.3, and 31.2 mg of S/plant) and three levels of N fertilization (31.2, 62.5, and 125 mg of N/plant), and samplings were conducted at vegetative and main bulb growths (*16*). S fertilization increased the alliin concentration in leaves by 4 mg g^{-1} of dry weight and in bulbs by 2 mg g^{-1} of dry weight. At a maximum, an alliin concentration of 9.1 mg g^{-1} of dry weight was determined in garlic bulbs. This experiment revealed no significant effect of N fertilization on the alliin concentration, although it decreased as a trend. This trial was a first indication that higher S rates can increase the alliin content in garlic bulbs. A disadvantage of greenhouse trials is the artificial crop growth and development. Only field experiments are suitable for evaluating and quantifying effects of fertilizer practices on crop-quality parameters reliably (*17*). It was the aim of this study to investigate the influence of N and S fertilization on the potential precursors of alliin biosynthesis, cysteine and glutathione, and the alliin content in garlic under field conditions.

MATERIALS AND METHODS

Experimental Design. In 2002, a bifactorial field trial was conducted at the experimental station of the Julius Kühn-Institute (JKI) in Braunschweig (E 10°27′, N 52°18′). The climate is temperate and characterized by frequent changes in temperature, humidity, and winds. The soil type is a Cambisol with a loamy sand soil texture (6.5% clay and 47% sand), with a low water retention capacity and a high leaching rate. Therefore, crops show generally a clear response to differences in N and S fertilizer treatments. The trial presented here was part of a stationary trial, where the effect of N and S fertilization on different crops was tested. In 2001, yellow lupines (*Lupinus luteus*) were grown. Mustard (var. Maxi) was sown on Aug 1, 2001 as a catch crop with a density of 25 kg ha^{-1}.

Cloves of garlic (*Allium sativum* L. var. Thermidrome) were planted on March 26th in plots of 3 × 4 m, with an interspace of 10 cm and a distance between rows of 50 cm. N and S were applied 1 week after planting. Four S rates (0, 15, 30, and 45 kg ha^{-1} S) were applied as elemental S, and four N rates (0, 50, 100, and 150 kg ha^{-1} N) were applied as calcium ammonium nitrate. The field trial was performed in a randomized block design, with 16 treatments and 64 plots total. Each treatment had four replicates. The first sampling was carried out 6 weeks after planting, when leaves were developed but bulb growth had not yet started. After the first sampling, samples were taken every 3 weeks until withering of leaves, when bulbs were suitable for harvest. In total, five samplings were carried out. Each time, 6−8 plants were harvested per plot. Plants were separated into leaves and bulbs from the first to the third harvest. The plant material was immediately shock-frozen in liquid N and subsequently freeze-dried to prevent the decomposition of the S-containing metabolites, and the dry matter content was determined. At the fourth and fifth harvest, leaves started to wither, so that only bulbs were collected.

The harvest of garlic was performed manually, and the yield was determined by weighing of 40 bulbs per plot. The yield per acreage was calculated on the basis of inter- and intrarow spaces.

Chemical Analysis. Total N was determined employing the Kjeldahl method. The extraction of total S was performed by microwave digestion with HNO_3/H_2O_2 (4:1) and final determination by inductively coupled plasma−optical emission spectroscopy (ICP−OES, Spectro Flame M120S, Kleve, Germany).

Alliin was extracted from leaves and bulbs of garlic and determined according to Hoppe et al. (*18*) by high-performance liquid chromatography (HPLC, Merck Hitachi, Darmstadt, Germany). Alliin was detected at 337 nm by ultraviolet (UV) detection using a LiChrospher RP-18 column (125 × 4 mm, 5 μm).

Free cysteine and glutathione were determined by HPLC (Merck Hitachi, Darmstadt, Germany) according to Hell and Bergmann (*19*). The extraction was carried out using 20−30 mg of fine-ground freeze-dried plant material and 1 mL of 0.1 M HCl containing 4% Polyvidon-25. After removal of plant debris by subsequent centrifugation, the supernatant was used for reduction in the dark with dithiothreitol. The assay contained 1 M Tris/HCl at pH 8, 10 mM dithiothreitol, 0.08 M NaOH, H_2O, and plant extract or standard, respectively. After 1 h of reduction, sulfhydryl groups were derivatized with 25 μL of 10 mM monobromobimane and subsequently stabilized by the addition of 705 μL of acetic acid (5%). The separation of cysteine and glutathione was carried out by HPLC using a 250 × 4.6 mm Nova-Pak C18 column (4 μm) (Waters 044380). Fluorescence detection was used for the measurement, with an excitation wavelength of 380 nm and an emission wavelength of 480 nm.

Two-way analysis of variation (ANOVA) was used to analyze the results, and means were compared by the Tukey test at a 5% probability level.

RESULTS AND DISCUSSION

Targeted N and S fertilization offers the opportunity to increase the alliin content of garlic. Thus, the market value of garlic for medical purposes may be improved substantially. The efficacy of plant material for combating pests and diseases in organic farming enhanced significantly. The health effect as part of the diet fortified considerably.

Jones et al. (*20*) described the lifecycle of garlic in four growth stages beginning with the germination of the garlic clove (stage 1), continuing with the mass increase in root and leaf tissue (stage 2), the development of the bulb (stage 3), and ending with the leaves dying off and maturity of the bulbs (stage 4). In **Figure 1**, this lifecycle is summarized together with the most important changes in nutrient accumulation and biosynthesis of alliin described by Jones et al. (*20*).

Garlic was sampled in total 5 times during the vegetation period: at first when vegetative growth started, then 2 times during the main growth of leaves and bulbs, and 2 times after the foliage had died off. N and S fertilization did not affect the yield. A mean bulb yield of 6.1 tons ha^{-1} was obtained. N and S fertilization had no influence on the fresh or dry weight of the bulbs. During the vegetation period, the dry matter content of leaves and bulbs was affected only little by N or S fertilization in such a way that the dry matter content increased only slightly by N fertilization (**Table 1**). The reason is most likely the fact that a significant amount of N and S bound in the mustard biomass was not leached during winter after freezing off of the crop, so that this reservoir contributed significantly to the mineral nutrition of the garlic.

At the first harvest, a medium bulb yield of 3.6 g of fresh weight/plant and a medium leaf yield of 4.0 g of fresh weight/plant was determined, which were affected by neither N nor S fertilization [least significant difference ($LSD_{5\%}$) for bulbs was 0.4, and $LSD_{5\%}$ for leaves was 0.5]. Therefore, at the following samplings with the exception of the last harvest, only the dry matter content was determined. It is obvious from the data in **Table 1** that also at harvest the bulb yield was not affected by N and S fertilization. This indicates that enough N and S were available in the soil to fulfill the demand of garlic. Mustard as a catch crop is able to deliver enough N for the following crop. Kolbe et al. (*21*) reported a N fixation in the above ground biomass of mustard of 84 kg ha^{-1}, which is high enough to fulfill the N uptake of garlic.

The results presented in **Tables 2** and **3** agree with changes in the mineral nutrient concentration and alliin content described by Jones et al. (*20*). The nutrient and alliin concentrations were

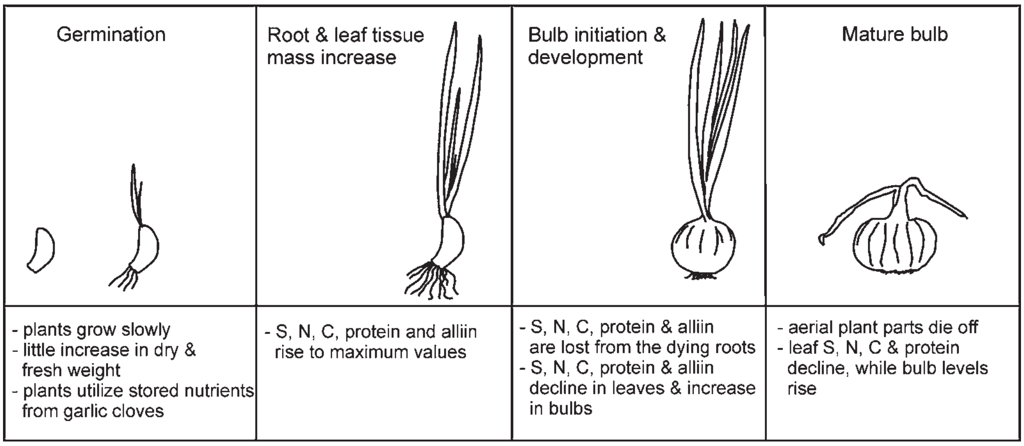

Figure 1. Lifecycle of *A. sativum* L. and the most important changes in nutrient and alliin accumulation (according to ref *20*).

Table 1. Influence of N and S Fertilization on the Dry Matter Content of Leaves and Bulbs of Garlic at Different Growth Stages and on the Bulb Yield at Harvest[a]

		first harvest		second harvest		third harvest		fourth harvest	fifth harvest	fifth harvest
		leaf	bulb	leaf	bulb	leaf	bulb	bulb	bulb	bulb
fertilization (kg ha^{-1})		dry matter content (%)								yield (g of FW plant^{-1})
N	0	10.4	9.2	12.3	13.1	14.6	19.7	30.2	32.0	29.5
	50	10.4	8.7	12.3	12.4	15.3	19.3	31.8	31.1	31.8
	100	10.4	8.6	12.1	12.9	15.3	19.8	31.5	31.6	30.6
	150	10.4	8.9	12.4	13.3	14.9	20.2	32.2	32.3	30.7
$LSD_{5\%}$		0.4	1.0	1.1	0.7*	1.6	0.5*	1.7*	1.2	2.4
S	0	10.5	8.5	12.0	13.0	15.2	20.1	31.8	32.4	29.8
	15	10.5	8.2	12.4	13.1	15.0	19.8	31.4	31.2	31.0
	30	10.4	9.6	12.0	12.7	14.9	19.8	30.4	31.6	30.8
	45	10.2	9.1	12.7	12.8	15.0	19.4	32.0	31.8	31.1
$LSD_{5\%}$		0.4	1.0*	1.1	0.7	1.6	0.5*	1.7	1.2	2.4

[a] First harvest, vegetative growth started, but bulb growth had not yet started; second and third harvest, main growth of leaves and bulbs; fourth harvest, foliage starts to die off; fifth harvest, foliage has almost died off. Two-factorial ANOVA was used to analyze the results, and the means were compared by the Tukey test at a 5% probability level. Significance levels were coded in the following way: (*) significant, $p < 0.05$; (**) highly significant, $p < 0.01$; (***) very highly significant, $p < 0.001$. $n = 16$.

Table 2. Influence of N and S Fertilization on the N and S Concentrations in Leaves and Bulbs of Garlic at Different Growth Stages[a]

		first harvest		second harvest		third harvest		fourth harvest	fifth harvest
fertilization (kg ha^{-1})		leaf	bulb	leaf	bulb	leaf	bulb	bulb	bulb
		Nitrogen Concentration (mg of N g^{-1} of DW)							
N	0	47.7	30.0	29.9	16.6	27.2	17.1	21.2	27.1
	50	51.8	34.2	32.6	19.5	28.2	17.6	20.8	27.4
	100	52.8	36.6	32.5	19.1	27.8	17.5	20.8	27.5
	150	53.8	35.1	33.4	19.0	28.2	17.2	21.1	27.1
$LSD_{5\%}$		1.5***	2.2***	1.4***	1.5**	0.9	0.6	1.1	1.1
S	0	51.3	33.8	31.5	18.0	26.8	17.0	21.6	27.5
	15	52.1	34.2	31.8	18.0	27.9	17.4	20.9	27.8
	30	50.9	34.0	32.2	18.6	28.4	17.4	20.9	27.3
	45	51.8	33.8	32.9	19.7	28.4	17.6	20.5	26.6
$LSD_{5\%}$		1.5	2.2	1.4	1.5*	0.9**	0.6	1.1	1.1
		Sulfur Concentration (mg of S g^{-1} of DW)							
N	0	16.2	10.7	8.3	5.4	6.6	5.8	6.8	8.2
	50	15.8	11.2	7.8	5.7	6.3	5.7	6.4	7.8
	100	15.8	11.0	7.1	5.1	5.8	5.9	6.1	7.6
	150	15.4	10.8	7.3	5.3	5.9	5.8	6.2	7.3
$LSD_{5\%}$		0.9	0.8	0.4***	0.5	0.4***	0.6	0.4**	0.5**
S	0	14.6	10.2	5.1	3.3	4.2	4.0	4.3	5.2
	15	16.0	10.9	7.6	5.0	6.2	5.6	6.4	7.8
	30	16.1	11.2	8.7	6.3	6.8	6.6	7.3	8.9
	45	16.6	11.4	9.1	6.8	7.3	7.0	7.5	9.0
$LSD_{5\%}$		0.9***	0.8*	0.4***	0.5***	0.3***	0.6***	0.4***	0.5***

[a] First harvest, vegetative growth started, but bulb growth had not yet started; second and third harvest, main growth of leaves and bulbs; fourth harvest, foliage starts to die off; fifth harvest, foliage has almost died off. Two-factorial ANOVA was used to analyze the results, and the means were compared by the Tukey test at a 5% probability level. Significance levels were coded in the following way: (*) significant, $p < 0.05$; (**) highly significant, $p < 0.01$; (***) very highly significant, $p < 0.001$. $n = 16$.

Table 3. Influence of N and S Fertilization on the Alliin, Cysteine, and Glutathione Concentrations in Leaves and Bulbs of Garlic at Different Growth Stages[a]

		first harvest		second harvest		third harvest		fourth harvest	fifth harvest
fertilization (kg ha^{-1})		leaf	bulb	leaf	bulb	leaf	bulb	bulb	bulb
				Cysteine (μmol g^{-1} of DW)					
N	0	1.97	0.84	0.53	0.27	0.59	0.30	0.48	0.43
	50	1.92	0.83	0.62	0.29	0.56	0.31	0.46	0.42
	100	1.92	0.75	0.62	0.28	0.60	0.28	0.47	0.48
	150	1.77	0.79	0.61	0.26	0.59	0.30	0.46	0.43
$LSD_{5\%}$		0.43	0.08	0.08	0.03	0.06	0.05	0.15	0.11
S	0	1.60	0.78	0.46	0.19	0.50	0.18	0.46	0.33
	15	1.80	0.76	0.56	0.28	0.57	0.30	0.44	0.38
	30	1.83	0.84	0.68	0.29	0.62	0.35	0.48	0.49
	45	2.35	0.83	0.68	0.34	0.66	0.36	0.48	0.55
$LSD_{5\%}$		0.43**	0.08	0.08***	0.03***	0.06***	0.05***	0.15	0.11**
				Glutathione (μmol g^{-1} of DW)					
N	0	3.36	1.90	2.18	1.92	1.98	1.23	1.04	0.70
	50	3.85	1.96	2.46	2.13	1.90	1.28	1.02	0.51
	100	4.00	1.66	2.49	2.06	2.02	1.17	1.06	0.62
	150	3.88	2.04	2.64	2.06	1.97	1.25	1.13	0.63
$LSD_{5\%}$		0.49	0.55	0.25**	0.16	0.14	0.18	0.18	0.17
S	0	3.72	1.97	2.47	1.77	1.91	0.92	1.15	0.61
	15	3.69	1.78	2.39	1.98	1.97	1.22	0.90	0.66
	30	3.69	1.68	2.53	2.05	1.96	1.30	1.21	0.58
	45	3.98	2.13	2.38	2.36	2.03	1.48	1.00	0.61
$LSD_{5\%}$		0.49	0.55	0.25	0.16***	0.14	0.18***	0.18**	0.17
				Alliin (mg g^{-1} of DW)					
N	0	41.2	26.6	16.9	16.2	14.5	16.9	13.0	10.4
	50	41.9	27.3	16.3	16.7	14.3	16.2	13.3	8.3
	100	43.4	26.8	16.1	14.4	13.5	16.2	12.7	10.5
	150	43.0	26.9	17.1	15.7	13.4	15.9	12.4	7.4
$LSD_{5\%}$		4.4	3.5	1.8	1.6*	1.1	1.8	2.0	3.6
S	0	42.0	27.3	12.8	9.4	9.5	10.2	7.9	5.1
	15	43.4	27.3	17.7	15.9	15.1	16.7	12.5	10.0
	30	41.4	26.2	17.7	18.8	15.3	17.3	15.2	11.2
	45	42.7	26.7	18.1	18.9	15.7	21.0	15.7	10.4
$LSD_{5\%}$		4.4	3.5	1.8***	1.6***	1.1***	1.8***	2.0***	3.6**

[a] First harvest, vegetative growth started, but bulb growth had not yet started; second and third harvest, main growth of leaves and bulbs; fourth harvest, foliage starts to die off; fifth harvest, foliage has almost died off. Two-factorial ANOVA was used to analyze the results, and the means were compared by the Tukey test at a 5% probability level. Significance levels were coded in the following way: (*) significant, $p < 0.05$; (**) highly significant, $p < 0.01$; (***) very highly significant, $p < 0.001$. $n = 16$.

extremely high at first sampling (stage 2). During bulb development at the second and third harvest, the N and S concentrations decreased as well as the cysteine, glutathione, and alliin concentrations in leaves and bulbs. This may indicate translocation processes of nutrients and S-containing compounds. During ripening of the bulbs at the fourth and fifth harvest, leaves started to die off and S and N contents in bulbs increased correspondingly (**Table 2**).

The statistical analysis revealed that no interactions between N and S fertilization were observed. Thus, in the results, only the single effects of N and S application rates are shown (**Tables 1−3**).

N and S fertilization increased the N and S concentrations of garlic leaves and bulbs significantly (**Table 2**). The N concentration of leaves and bulbs increased strongest during vegetative growth, while after the foliage had died off, the different treatments had no significant influence on the N concentration of the bulbs. In contrast, S fertilization increased the S concentration in leaves and bulbs consistently during the whole vegetation period. Leaves and bulbs showed the highest S concentration at the first harvest, when vegetative mass growth of the plants was high but bulb growth had not yet started. There was only a weak significant relationship between S fertilization and the S concentration of the bulbs at that time because S in bulbs originated largely from the planted garlic clove.

Graded N fertilization affected the S concentration in leaves and bulbs in such a way that the S concentration of leaves decreased significantly at the second and third harvest and that in bulbs at the fourth and fifth harvest (**Table 2**). In comparison, graded S fertilization had only a minor effect on the N concentration of bulbs and leaves, which increased at the second and third harvest.

In general, later growth stages reflected the nutrient uptake of garlic from the soil at best. Leaf S decreased from the second to the third harvest. At the same time, bulb S increased continuously until the fifth harvest.

A higher S uptake yielded a large increase in S-containing compounds, such as alliin (**Table 3**). Alliin constituted up to 27% of the total S in bulbs at harvest. S fertilization increased the cysteine and glutathione concentrations at different growth stages (**Table 3**) as well, but their proportion did not exceed 2% of the total S.

N fertilization had only a minor effect on all S-containing metabolites. Glutathione in leaves increased with an increasing N supply only at the second harvest (**Table 3**). Cysteine was not affected by N fertilization.

It was shown before in greenhouse experiments that N fertilization did not significantly affect the iso-alliin or alliin content of onions and garlic (*16*). Huchette and co-workers (*22*) obtained

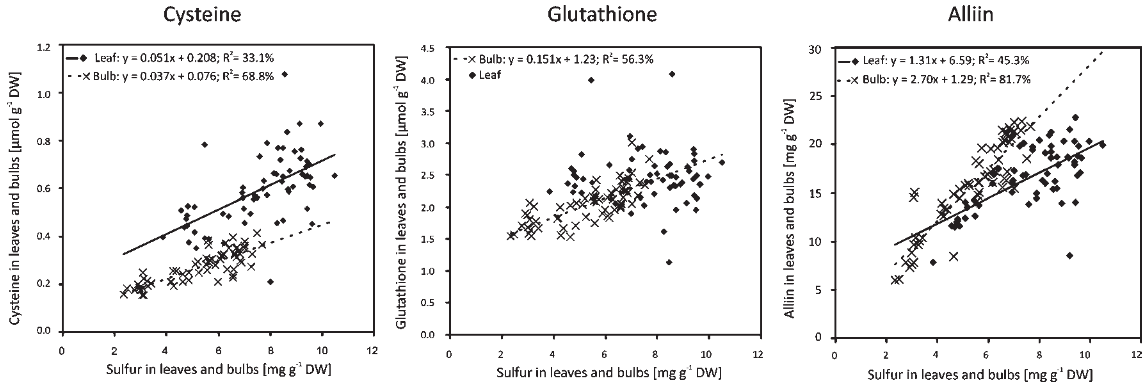

Figure 2. Relationship between the S concentrations in leaves and bulbs of garlic and the alliin, cysteine, and glutathione concentrations at main vegetative growth (second harvest).

comparable results, and Liu et al. (*23*) determined a decreasing pungency in spring onions when excessive N was supplied. In this study, the alliin concentration decreased slightly in bulbs at the second harvest with an increasing N supply (**Table 3**).

S fertilization significantly increased the concentrations of all investigated S-containing metabolites. Cysteine increased in leaves and bulbs. This increase was most pronounced during main growth (second and third harvest). At this time, cysteine increased by a factor of 1.3−1.5 in leaves and 1.8−2.0 in bulbs.

S fertilization increased the glutathione concentration of bulbs significantly at the second, third, and fourth harvest. During ripening, glutathione decreased in bulbs from about 2 μmol g^{-1} of dry weight at the second harvest to less than 0.7 μmol g^{-1} of dry weight at the fifth harvest. Glutathione is discussed as a precursor for alliin biosynthesis (*20*).

S fertilization increased the alliin concentration at all growth stages, with exception of the first harvest. During main growth and ripening (from the second to the fifth harvest), the alliin concentration in bulbs was twice as high in the fertilized plots (45 kg ha^{-1} S) compared to the unfertilized plots. From second to third harvest, alliin in leaves decreased, while at the same time, an increase in bulbs occurred. This may indicate the translocation of alliin from leaves to bulbs. Leaves are the main site of cysteine sulfoxide biosynthesis (*24*), which was translocated to cloves during bulb development (*25*, *26*). At later growth stages when the foliage had almost died off (fourth and fifth harvest), the alliin concentration in bulbs decreased again. At that time, biosynthesis of alliin had ceased, while mass growth of the bulbs continued (**Table 3**).

Freeman and Mossadeghi (*27*) were the first to show the positive effect of S fertilization on flavor compounds in *Allium* under controlled greenhouse conditions. They also demonstrated that a saturation point existed, so that any additional sulfate resulted in only a little response to the pungency of onion. Thus, S fertilization is unlikely to enhance pungency on soils that contain ample sulfate (*28*, *29*).

The strongest effect of S fertilization on the content of S-containing metabolites in leaves and bulbs was found during main growth (**Table 3**). Correspondingly, the closest relationship between the S status of the plant and the metabolite concentration was determined (**Figure 2**). This stresses the need for a sufficiently high S supply at this time for achieving the best quality of garlic in terms of high alliin contents. Such a close relationship between S fertilization and S-containing compounds was shown before for several crop plants and components (*10*, *30*, *31*). In this context, it is important to mention that such a close relationship can be masked at later growth stages, for instance, because of interfering processes by pests and diseases or other environmental conditions, which cause the degradation or conversion of active compounds.

The S supply is a key factor determining the cysteine and alliin contents in leaves and bulbs of garlic. S nutrition influenced the glutathione content in garlic bulbs strongly during main vegetative growth. However, when the foliage died off, this relationship was no longer visible (**Table 3**).

The close relationship between total S and alliin in bulbs of garlic is visualized in **Figure 3** in relation to different combinations of N and S rates at main vegetative growth and harvest time. Changes in the alliin concentration were directly reflected in corresponding changes in the total S, irrespective of the fertilizer treatment (**Figure 3**). This finding supports the need for a sufficiently high S supply to improve crop quality. Results were less consistent for conformity between S and glutathione, S and bulb yield, and alliin and bulb yield (**Figure 3**).

The results clearly reveal that S fertilization is a suitable measure to increase the alliin concentration of garlic on S-deficient soils. This has a significant impact on expected health effects. The officially recommended daily dosage is 4 g of fresh garlic to yield a therapeutic effect (*32*). Without S fertilization, the consumption of 4 g of fresh garlic bulbs equals an uptake of 6.4 mg of alliin, while with S fertilization (45 kg ha^{-1} S), an alliin uptake of 13.1 mg of alliin could be realized. Recommendations for daily alliin or allicin uptake vary widely between 4 and 12 mg of alliin and 2−5 mg of allicin.

Nasim et al. (*33*) demonstrated in a study with diabetic rats the higher therapeutic efficiency of garlic, which has been previously fertilized with S in comparison to garlic material without additional S fertilization. The authors attributed the effect to significant differences in the alliin content of the used plant material.

In the present study, it was possible to double the alliin concentration in bulbs of garlic by S fertilization. The highest S rate also yielded the highest alliin concentration in leaves and bulbs of garlic until the fourth harvest, but no saturation point was obtained. The highest alliin concentration of 21 mg g^{-1} of dry weight was determined in bulbs at main growth when the leaves were still photosynthetically active. At the last harvest, the alliin concentration increased with S fertilization as well; however, the rate of S application did not increase the alliin concentration more. This result might indicate that the lowest S fertilizer level of 15 kg ha^{-1} S was high enough to obtain maximum alliin concentrations in bulbs of garlic. However, it is important to consider that garlic bulbs are usually stored before usage and that the alliin content further accumulates during storage of the bulbs (*26*). Garlic bulbs fertilized with a high level of S may have a higher potential to accumulate alliin after harvest because of

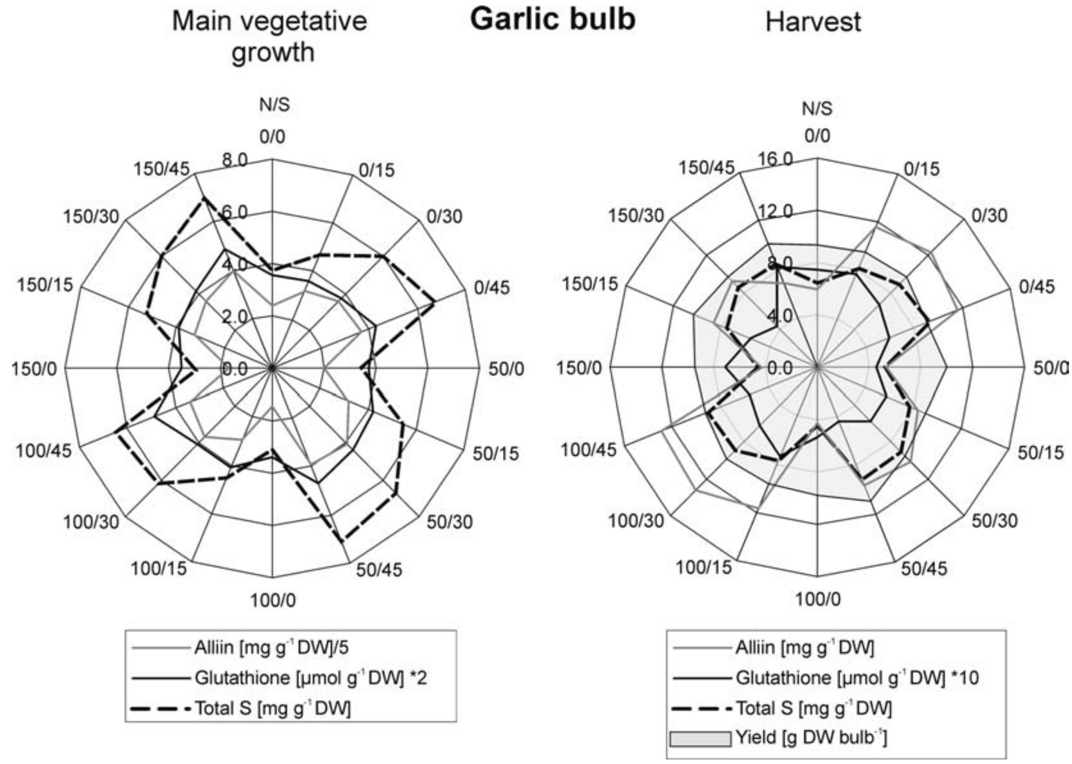

Figure 3. Alliin, glutathione, and total S in bulbs of garlic at main vegetative growth (second harvest) and maturity (fifth harvest) in relation to different N and S application rates. At harvest, an additional bulb yield (grams of dry weight per bulb) is included.

higher contents of alliin precursors. Therefore, a high S fertilizer level may be beneficial to produce high-quality garlic bulbs.

In comparison, N should be fertilized at a lower dose to avoid adverse effects on crop quality, such as lower alliin contents, an enrichment of nitrate and amides, and a higher susceptibility of the crops against fungal diseases. Besides these, N losses through leaching and volatile emissions, for instance, NH_3 and N_2O, with the latter being one of the contributors to global warming, need to be taken into account (*34*).

From the viewpoint of the sensory quality of garlic, it seems conceivable to offer different qualities based on different fertilizer regimes, so that the consumer can choose between bulbs high in alliin with a sharp taste or low in alliin with a milder aroma.

LITERATURE CITED

(1) Konvička, O. *Knoblauch* (*Allium sativum L.*); Konvička-Olomouc Verlag: Buchholz, Germany, 2001; p 202.

(2) Iciek, M.; Kwiecień, I.; Włodek, L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ. Mol. Mutagen.* **2009**, *50*, 247−265.

(3) Keusgen, M. Health and Alliums. In *Allium Crop Science: Recent Advances*; Rabinowitch, H. D., Currah, L., Eds.; CABI Publishing: Wallingford, U.K., 2002; pp 357−378.

(4) Rahman, M. S. Allicin and other functional active components in garlic: Health benefits and bioavailability. *Int. J. Food Prop.* **2007**, *10*, 245−268.

(5) Block, E. The organosulfur chemistry of the genus *Allium*. Implications for organic sulfur chemistry. *Angew. Chem.* **1992**, *31*, 1135−1178.

(6) Collin, H. A. Garlic and cardiovascular disease. In *Functional Foods, Cardiovascular Disease and Diabetes*; Arnoldi, A., Ed.; Woodhead Publishing, Ltd.: Cambridge, U.K., 2004; pp 240−260.

(7) Kawakishi, S.; Morimitsu, Y. Sulfur chemistry of onions and inhibitory factors of the arachidonic acid cascade. In *Food Phytochemicals for Cancer Prevention I*; American Chemical Society: Washington, D.C., 1993; ACS Symposium Series, Vol. 546, Chapter 8, pp 120−127.

(8) Amagase, H. Clarifying the real bioactive constituents of garlic. *J. Nutr.* **2006**, *136*, 716S−725S.

(9) Lawson, L. D. The composition and chemistry of garlic cloves and processed garlic. In *The Science and Therapeutic Application of Allium sativum L. and Related Species*; Koch, H. P., Lawson, L. D., Eds.; Williams and Wilkins: Baltimore, MD, 1996; pp 37−108.

(10) Bloem, E.; Haneklaus, S.; Salac, I.; Wickenhäuser, P.; Schnug, E. Facts and fiction about sulfur metabolism in relation to plant−pathogen interactions. *Plant Biol.* **2007**, *9*, 596−607.

(11) Miron, T.; Rabinkov, A.; Mirelman, D.; Wilchek, M.; Weiner, L. The mode of action of allicin: Its ready permeability through phospholipid membranes may contribute to its biological activity. *Biochim. Biophys. Acta, Biomembr.* **2000**, *1463*, 20−30.

(12) Portz, D.; Koch, E.; Slusarenko, A. J. Effects of garlic (*Allium sativum*) juice containing allicin on *Phytophthora infestans* and downy mildew of cucumber caused by *Pseudoperonospora cubensis*. *Eur. J. Plant Pathol.* **2008**, *122*, 197−206.

(13) Schüder, I.; Port, G.; Bennison, J. Barriers, repellents and antifeedants for slug and snail control. *Crop Prot.* **2003**, *22*, 1033−1038.

(14) Hart, K. J.; Yáñez-Ruiz, D. R.; Duval, S. M.; McEwan, N. R.; Newbold, C. J. Plant extracts to manipulate rumen fermentation. *Anim. Feed Sci. Technol.* **2008**, *147*, 8−35.

(15) Newman, K. Herbs and spices: Their role in modern livestock production. In *Biotechnology in the Feed Industry*; Lyons, T. P., Jacques, K. A., Eds.; Proceedings of Alltech's 13th Annual Symposium, 1997; pp 217−224.

(16) Bloem, E.; Haneklaus, S.; Schnug, E. Influence of nitrogen and sulfur fertilization on the alliin content of onions and garlic. *J. Plant Nutr.* **2004**, *27*, 1827−1839.

(17) Haneklaus, S. Strontiumgehalte in Pflanzen und Böden Schleswig-Holsteins und Bewertung von Düngungsmassnahmen zur Verminderung der Strontiumaufnahme von Kulturpflanzen. Ph.D. Thesis, University of Kiel, Kiel, Germany, 1989.

(18) Hoppe, L.; Bahadir, M.; Haneklaus, S.; Schnug, E. *Sulphur Supply and Alliin Content of Allium Species*; DGQ-Vortragstagung: Kiel, Germany, 1996; Vol. 31, pp 189−192.

(19) Hell, R.; Bergmann, L. γ-Glutamylcysteine synthetase in higher plants: Catalytic properties and subcellular localization. *Planta* **1990**, *180*, 603−612.

(20) Jones, M. G.; Collin, H. A.; Tregova, A.; Trueman, L.; Brown, L.; Cosstick, R.; Hughes, J.; Milne, J.; Wilkinson, M. C.; Tomsett, A. B.; Thomas, B. The biochemical and physiological genesis of alliin in garlic. *Med. Aromat. Plant Sci. Biotechnol.* **2007**, *1*, 21−24.

(21) Kolbe, H.; Schuster, M.; Hänsel, M.; Grünbeck, A.; Schliesser, I.; Köhler, A.; Karalus, W.; Krellig, B.; Pommer, R.; Arp, B. Zwischenfrüchte im ökologischen Landbau. *Sächsische Landesanstalt für Landwirtschaft*, 2004; http://www.landwirtschaft.sachsen.de/LfL.

(22) Huchette, O.; Arnault, I.; Auger, J.; Bellamy, C.; Trueman, L.; Thomas, B.; Ochatt, S. J.; Kahane, R. Genotype, nitrogen fertility and sulphur availability interact to affect flavour in garlic (*Allium sativum* L.). *J. Hortic. Sci. Biotechnol.* **2007**, *82*, 79−88.

(23) Liu, S.; He, H.; Feng, G.; Chen, Q. Effect of nitrogen and sulfur interaction on growth and pungency of different pseudostem types of Chinese spring onion (*Allium fistulosum* L.). *Sci. Hortic.* **2009**, *121*, 12−18.

(24) Nasim, S. A.; Dhir, B.; Kapoor, R.; Fatima, S.; Mahmooduzzafar; Mujib, A. Alliin production in various tissues and organs of *Allium sativum* grown under normal and sulphur-supplemented in vitro conditions. *Plant Cell, Tissue Organ Cult.* **2010**, *101*, 59−63.

(25) Hughes, J.; Tregova, A.; Tomsett, B.; Jones, M. G.; Cosstick, R.; Collin, H. A. Synthesis of flavor precursor, alliin, in garlic tissue cultures. *Phytochemistry* **2005**, *66*, 187−194.

(26) Ichikawa, M.; Ide, N.; Ono, K. Changes in organosulfur compounds in garlic cloves during storage. *J. Agric. Food Chem.* **2006**, *44*, 4849−4854.

(27) Freeman, G. G.; Mossadeghi, N. Effect of sulphate nutrition on flavour components of onion (*Allium cepa*). *J. Sci. Food Agric.* **1970**, *21*, 610−615.

(28) Hamilton, B. K.; Yoo, K. S.; Pike, L. M. Changes in pungency of onions by soil type, sulphur nutrition and bulb maturity. *Sci. Hortic.* **1998**, *74*, 249−256.

(29) Huchette, O.; Kahane, R.; Auger, J.; Arnault, I.; Bellamy, C. Influence of environmental and genetic factors on the alliin content of garlic bulbs. *Acta Hortic.* **2005**, *688*, 93−99.

(30) Haneklaus, S.; Bloem, E.; Schnug, E.; De Kok, L. J.; Stulen, I. Sulphur. In *Handbook of Plant Nutrition*; Barker, A. V., Pilbeam, D. J., Eds.; CRC Press: Boca Raton, FL, 2006; pp 183−238.

(31) Schnug, E. Significance of sulphur for the nutritional and technological quality of domesticated plants. In *Sulfur Metabolismus in Higher Plants—Molecular, Ecophysiological and Nutritional Aspects*; Cram, W. J., De Kok, L. J., Stulen, I., Brunold, C., Rennenberg, H., Eds.; Backhuys Publishers: Leiden, The Netherlands, 1997; pp 109−130.

(32) Blumenthal, M.; Busse, W. R.; Goldberg, A.; Gruenwald, J.; Hall, T.; Riggins, C. W.; Rister, R. S. *The Complete German Commission E Monographs. Therapeutic Guide to Herbal Medicines*; The American Botanical Council in Cooperation with Integrative Medicine Communications: Austin, TX, 1998; p 685.

(33) Nasim, S. A.; Dhir, B.; Samar, F.; Rashmi, K.; Mahmooduzzafar; Mujib, A. Sulphur treatment alters the therapeutic potency of alliin obtained from garlic leaf extract. *Food Chem. Toxicol.* **2009**, *47*, 888−892.

(34) Rolston, D. E.; Venterea, R. T. Gaseous loss of oxides of nitrogen from the agricultural nitrogen cycle. *Proc. Int. Fert. Soc.* **2001**, *464*, 1−36.

Received for review May 26, 2010. Revised manuscript received August 19, 2010. Accepted August 25, 2010.