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Effect of cultivar and fertilization on garlic yield and allicin content in bulbs at harvest and during storage

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Abstract: Allicin, one of the most important flavor compounds in garlic bulbs, is affected by genotypic characteristics modified by ontogenesis, environmental conditions, fertilization practices, and storage duration. Therefore, the effects of fertilization practices and cultivar on garlic yield and allicin content in garlic bulbs were evaluated at harvest and during storage. One autochthonous (Ptujski spomladanski) and three commercial cultivars (Garcua, Gardacho, and Gardos) were used, and three fertilization treatments (N90S0 – 90 kg N ha^{-1}, N90S60 – 90 kg N ha^{-1} +60 kg S ha^{-1}, and N0S0 – control) were performed during two growing seasons. After the harvest, 32 bulbs per treatment were stored for 4 months at a constant temperature of 15–18 °C and relative humidity of 45% in a shaded and ventilated room. A representative subsample of 8 bulbs per treatment was taken immediately after harvesting, and then 45, 90, and 120 days after harvest (DAH). Clear effects of nitrogen (N) and sulfur (S) supply were expressed, with measurements of the yield being higher on fertilized plots compared to the unfertilized ones, and the impact was cultivar-dependent. N and S fertilization significantly increased the allicin content of Gardos bulbs, while for other cultivars, the allicin content was dependent on weather conditions, revealed in decreased allicin in the year with lower temperature and excessive precipitation. When garlic was stored at 15–18 °C for 120 days, a sharp increase was observed in allicin content between 45 and 90 DAH, while the S status in garlic bulbs was unchanged, which indicated a conversion effect of γ-glutamyl peptides to sulfoxides, a precursor compound for allicin. Changes in the allicin content during storage showed an N fertilization-related pattern, being correlated with the modification of bulb dormancy.

Key words: *Allium sativum* L., allicin content, cultivar, fertilization practice, storage period

1. Introduction

Garlic (*Allium sativum* L.) is a bulbous perennial plant of the family Alliaceae, genus *Allium*, which includes more than 700 species, widely distributed all over the world and valued for their flavor, easy growth, and long storage time (Fritsch and Friesen, 2002). Many plant species of the genus *Allium* are characterized by a rich content of sulfur compounds, which contributes to their antioxidant, antimicrobial, insecticidal, and larvicidal activity (Yin et al., 2002; Tunaz et al., 2009; Lanzotti, 2012; Iqbal et al., 2018). Many of the health benefits associated with garlic consumption have been attributed to thiosulfinates (allicin in the case of garlic), the single most abundant class of organosulfur compounds found in freshly chopped or crushed garlic (Block et al., 2010; Mnayer et al., 2014). Molecules of thiosulfinates originate from alliin (S-alk(en)yl-L-cystein-S-oxide) located in the cytoplasm, through an enzymatic reaction catalyzed by alliinase, which is present in the vacuoles of the vascular bundle sheath cells around the phloem (Ellmore and Feldberg, 1994). Garlic accumulates large quantities of sulfur, which enters the plants actively as sulfate (SO_4^{2-}) and is transported to the leaves, where it can be stored in the vacuole or reduced to sulfide and assimilated into cystein in the chloroplast (Hell, 1997). Cystein is quickly incorporated into proteins or used in the synthesis of methionine and glutathione (Leustek, 2000). In garlic plants, cysteine and glutathione serve as precursor to all biological compounds that contain reduced sulfur including alk(en)yl cysteine sulfoxides (ACSs) (Randle and Lancaster, 2002). The major flavor precursor in garlic is alliin, which yields a series of thiosulfinates, allyl sulfides, dithiines, and ajoenes, after the action of alliinase and subsequent chemical decomposition (Jones et al., 2004). The remaining sulfur compounds within the

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bulbs, which comprise 1%–5% of dry weight together with S-alk(en)yl cysteine sulfoxides (Lancaster and Kelly, 1983), are γ-glutamyl peptides. The major γ-glutamyl peptides in garlic bulbs are γ-glutamyl allyl cysteine and γ-glutamyl isoallyl cysteine (Jones et al., 2004). Their presence in dormant bulbs and seeds in large quantities suggests that these peptides may function as storage sources of nitrogen and sulfur for use in sprouting and germination (Randle and Lancaster, 2002).

The flavor compounds in garlic bulbs are affected by genotypic characteristics modified by ontogenesis, environmental conditions, cultivation practices, and storage duration (Randle and Lancaster, 2002; Ichikawa et al., 2006; Bloem et al., 2011; Montaño et al., 2011). Differences between the cultivars in the total plant S and in the content of flavor compounds probably arise due to the cultivar-dependent variability in sulfur uptake and their ability to reduce sulfate (SO_4^{2-}) so that it can enter the flavor biosynthetic pathway (Randle et al., 1992; Huchette et al., 2005). For example, pungent cultivars of onion are more efficient at sulfate reducing, whereas mild onion cultivars store more of the absorbed S as sulfate in the cell vacuoles, thereby excluding it from the flavor pathway (Randle and Lancaster, 2002). The cultivar-dependent effect of N and S fertility on flavor compounds in spring and autumn garlic cultivars, which was observed by Huchette et al. (2005), suggested that in spring cultivars, a part of S, absorbed by roots, was stored in the vacuoles of cells in the form of sulfate instead of being used through the S-alk(en)yl-cystein sulfoxides pathway. On the other hand, the autumn cultivar was found to be more efficient than the other two (spring) cultivars at accumulating S for flavor (Huchette et al., 2007). Variability in flavor compounds in garlic bulbs in terms of the growth stage was revealed in the large increase of γ-glutamil peptides as cloves matured during the last month of growth (Matsuura et al., 1996).

Environmental factors, such as water stress, light quality, and duration, and the average growing and root zone temperatures have a notable impact on biosynthesis of organosulfur compounds in *Allium* crops (Coolong and Randle, 2003, 2006; Huchette et al., 2005). In garlic plants, distinct cropping temperatures produced a different profile of organosulfur compounds among accessions from Central Asia, when grown in two different climatic regions (Kamenetsky et al., 2004). Changes in flavor quality in response to temperature changes probably occurred due to the increase of absorbed and metabolized S at increased temperatures, and at low temperatures due to S accumulation as SO_4^{2-}, which did not enter the flavor biosynthetic pathway (Coolong and Randle, 2003). The fertilization schedule and water supply play an important role in ensuring a high yield and a good quality of bulbs, since garlic is a demanding crop in terms of nutrient requirements (Martins et al., 2016). S fertility has a pronounced impact on how organic S accumulates and is metabolized through the various precursors of the flavor pathway. In onion plants, at low S fertility, nearly 95% of the total bulb S could be accounted for in compounds of the flavor pathway and does not accumulate in peptide intermediates. At increased S fertility, different rates of peptide sulfoxide began to accumulate at higher concentrations (Randle et al., 1995). In garlic plants, S fertilization doubled alliin content in bulbs (Bloem et al., 2004, 2010), as was also found by Huchette et al. (2007), although despite the wide range of the S fertility used in their greenhouse experiment, the effect on garlic flavor compounds was less than expected, suggesting that overfertilization did not result in the metabolism of organosulfur compounds. Many studies have investigated how N besides S nutrition affects flavor in onion and garlic (Coolong et al., 2003; Huchette et al., 2007; Bloem et al., 2010). In some investigations, increased N fertility had an adverse effect on alliin content in garlic bulbs (Bloem et al., 2004, 2010) while, in another one, the effect of nitrogen on S compounds was cultivar-dependent (Huchette et al., 2007). Although there have been several reports on improving the health value of garlic bulbs, considering genetic and environmental factors as well as the interaction between S and N fertilization, little is known about the variation of allicin content during storage conditions, as a result of agricultural measures and growing conditions during the garlic production period. However, in a study by Bloem et al. (2011), the best quality during the entire storage time, in terms of high alliin contents in bulbs was obtained at a minimum sulfur level of 30 kg ha^{-1}, if no nitrogen was applied. Taking into account the high instability of organosulfur compounds (Ichikawa et al., 2006), proper storage conditions are crucial to retain the high quality of garlic bulbs (Martins et al., 2016).

In the present study, the main aim was to evaluate the impact of selected cultivars and fertilization with N and S on the quality parameters of garlic bulbs, by measurement of garlic yield, morphological traits, and allicin content in bulbs at harvest and during the storage period. The study was conducted during two growing seasons with distinct weather conditions, which enabled us to examine the interaction among genotype, fertilization practices, and weather conditions and to evaluate how these factors influence the main quality parameters of bulbs, not only at harvest but also during the storage period. This is important from both pharmaceutical and consumer points of view. It could also be highly useful for garlic breeders, who stress market values as the focus of most garlic breeding efforts, to know the effect of the main agricultural measures on the morphological and physiological mechanisms that are responsible for changes in the traits of bulb yield and bulb storage capacity.

2. Materials and methods

The study was conducted on the experimental field of the Biotechnical Faculty, University of Ljubljana (Ljubljana, Slovenia, 46°03′ N and 14°31′ E, 298 m a. s. l.), in the years 2013/2014 and 2014/2015. Basic soil characteristics, the dates of sowing, fertilization, and harvesting, fertilizer application (the type of fertilizer, fertilizer in g m^{-2}, and nutrient amount in kg ha^{-1}) are presented in Table 1. Soil chemical analysis, which was performed 14 days before the sowing date, suggests moderate application of P and K nutrients. Nitrate nitrogen in soil was analyzed at the beginning of March, before the first N fertilization was performed. The experimental field was divided into 4 blocks, each of which had three main plots, on which fertilization treatments were randomly arranged: unfertilized plots (N0S0), fertilization with 90 kg N ha^{-1} (N90S0), and fertilization with 90 kg N ha^{-1} and 60 kg S ha^{-1} (N90S60). The fertilization treatments approximated to real agricultural practice, so fertilization with sulfur alone (N0S60), without nitrogen, was not included in the study. Within each main plot, 4 subplots (each for 1 cultivar) were randomly arranged. Broadcast fertilization was performed 1 day before planting using Hyperkorn 0:26:0, potassium chloride, and Calcin S 16 (Gypsum) (Table 1). Nitrogen was applied as calcium ammonium nitrate during the growing period in two equal halves, the first half being when plants reached the growth stage of 3 or 4 leaves and the second half when plants developed 7 to 8 leaves (dates are listed in Table 1).

The garlic cloves (*Allium sativum* L.) were planted on plots of 3.0 × 1.2 m, with a spacing of 0.3 m between the rows and 0.15 m between the plants within a row. Each

Table 1. List and description of the soil and the experimental work related to the experiments in the years 2013/2014 and 2014/2015.

Soil description	Gleyic fluvisol* Endogenic fluvisol**				
Soil texture					
Soil NO_3-N (mg kg^{-1})	#6.2* / 7.2**				
Soil P_{Olsen} (mg/100g)	20.0				
Soil K_{exch} (mg/100 g)	26.2				
Soil S (mg/100 g)	1.8				
Organic matter (g/kg)	26.3				
Sowing date	In 2013 / In 2014	28 Oct / 30 Oct (for autumn cvs) 14 Nov /15 Nov (for spring cvs)			
Date of fertilization	In 2013 / In 2014 In 2014 / In 2015	P,K,S: 27 Oct / 29 Oct 13 Nov / 14 Nov N(1/2): 5 Mar / 8 Mar (autumn cvs) 22 Mar / 23 Mar (spring cvs.) N(2/2):17 Apr / 20 Apr (autumn cvs) 3 May / 6 May (spring cvs)			
Type of fertilizer		N:P:K N90S0	N90S60	g fertilizer m^{-2}	N:P:K (kg ha^{-1})
Calcium ammonium nitrate (INA, Kutina, Croatia)		27:0:0	27:0:0	33.3	90
Hyperkorn (Hyperkorn, Timac, Agro, Austria)		0:26:0	0:26:0	35.0	90
Potassium chloride (Adriatica, Italy)		0:0:60	0:0:60	25.0	150
Calcin-S 16 (Cinkarna Celje, Slovenia)			20% Ca 16% S	38.0	60 S
Harvest date	In 2014 / In 2015	10 Jun /17 Jun (autumn cvs) 13 Jul /4 Jul (spring cvs)			

- analysis of NO_3-N in the soil was done before N fertilization treatment: * in 2014; ** in 2015

plot comprised 80 plants (4 rows, 20 plants per row). In both years, 4 garlic cultivars were included: Garcua and Gardacho (Plantas de Navarra S.A. Planasa, Spain) are "white" type, autumn cultivars, and are intended for semilong storage. Gardos (Plantas de Navarra S.A. Planasa, Spain) and Ptujski spomladanski (autochthonous Slovenian cultivar, Semenarna Ljubljana, Slovenia) are "purple" garlic cultivars. These can also produce scape and are spring cultivars. As the name of the autochthonous cultivar already indicates, 'spomladanski' means 'spring'. Gardos and Ptujski spomladanski are intended for longer storage. The plants were managed according to the integrated pest management guidelines (Eur-Lex, 2014).

The plants were manually harvested from the 2 external rows of each plot (40 plants from 1.8 m^{-2}), when approximately 60% of the leaves were senesced. The garlic plants were bunched and after a drying period in an open space for 12 days, the leaves and roots were trimmed and the bulb samples were transported to the laboratory for further morphological and chemical analysis. Eight bulbs per treatment were used for morphological analysis. The diameter of the bulbs was measured using a Powerfix Profi Electronic Vernied Caliper, and bulb fresh weight was weighed on high-precision Kern EW 600-2M scales. The samples were dried at 65 °C, weighed for the determination of dry mass, ground to fine powder with a mill (IKA M 20), and homogenized.

The quantity of the yield was determined by weighing the fresh bulb mass, multiplying it by the number of plants per square meter, calculated on the basis of interrow and intrarow spaces. The yield was expressed in tons per hectare. Then, 30% of the total yield was deduced to account for the tractor wheel paths, where plants would not have been planted under normal field production technology. For sprout development, 10 peeled garlic cloves per treatment were sectioned longitudinally and the length of the sprout was reported as a fraction of the full clove length (sprout ratio). Values above 1.0 indicated sprout emergence.

In addition, to investigate how fertilization practices and cultivar affected bulb quality and the changes of allicin content during storage, 32 bulbs per plot were stored for 4 months at a constant temperature of 15–18 °C and a relative humidity of 45% in a shaded and ventilated room. The first sampling for allicin and dry matter content was carried out immediately after harvesting, then 45, 90, and 120 days after harvest (DAH). A representative subsample of 8 bulbs per treatment was taken each time. The storage conditions were chosen to be equivalent to those found in average household storage rooms (15–20 °C, dry, and dark) in order to determine the effects relevant for consumers.

Meteorological data for the growing periods of the experiments conducted in 2013/2014 and 2014/2015 were taken from Ljubljana meteorological station (Slovenia) (299 m a.s.l., 46°3′56″ N, 14°30′45″ E), which is 3.7 km (air distance) from the experimental field (Monthly…2013, 2014, 2015). The average air temperature, the mean precipitation, and the mean sunshine duration in the study area for the 1981–2011 reference period was 9.26 °C, 1063.54, mm and 1471.9 h, respectively, measured at the same meteorological station. During the growing period, the mean air temperatures were above the 30-year average, by 0.6 °C in 2014 and 2.0 °C in 2015 (Figure 1). Precipitation was also above the 30-year average, with the greatest excess in February, March, and May 2014 (175%, 122%, and 100% above the average) during the first experiment and in November 2014 (108% above average) during the second experiment. The amount of precipitation sufficiently fulfilled the water requirements of garlic plants (60–80 mm, Welbaum, 2015); thus, the irrigation was not performed. The sunshine duration was 10% below the 30-year average in 2014 and 7% above in 2015 (Figure 1).

The total N was determined after incineration at 900 °C in a VarioMAX CN analyzer by a thermal conductivity detector (ISO 13878). The measurement uncertainty was 9%. The determination of the total S was carried out by nondestructive EDXRF (energy dispersive X-ray fluorescence spectrometry). The pellets were prepared with 0.5–1.0 g of the powdered sample material using a pellet die and a hydraulic press. For excitation, the disc radioisotope excitation source of Fe-55 (25 mCi, Eckert & Ziegler, Berlin, Germany) was used. The emitted fluorescent radiation was measured using the EDXRF spectrometer with an XR-100SDD detector (Amptek, Berwyn, PA, USA), a PX5 digital pulse processor (Amptek), and a PC-based, multichannel analyzer software package, DPPMCA (Amptek). The spectrometer was equipped with a vacuum chamber. The energy resolution of the spectrometer was 125 eV at 5.9 keV. The analyses of complex X-ray spectra were performed using the AXIL Spectral Analysis Program (Nečemer et al., 2008, 2011). It was required to include the statistical uncertainties of the measured intensities and the uncertainties of the mathematical fitting procedure. For these purposes, quantification was performed using the Quantitative Analysis of Environmental Samples software that was developed in this study's laboratory (Nečemer et al., 2008, 2011). The estimated uncertainties in the analysis were between 5% and 10%. The relatively high total estimated uncertainty is mainly due to the matrix correction and geometry calibration procedures, which included errors of tabulated fundamental parameters. It could also result from the effects of the spectrum acquisition and analysis. The accuracy of the data was checked using the National Institute of Standards and Technology reference materials 1547 (peach leaves) and 1573a (tomato leaves).

Allicin was analyzed with a slightly modified method of Liang et al. (2013). The bulbs were peeled and the cloves

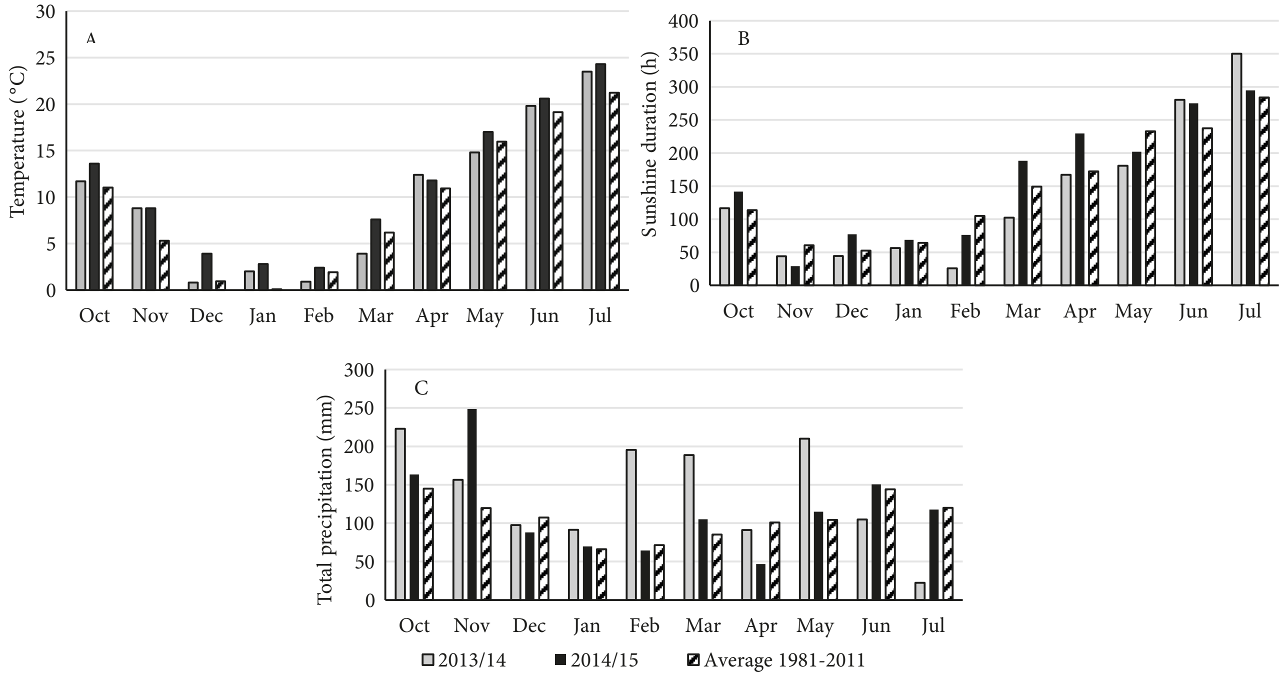

Figure 1. Monthly meteorological data from October to July 2013-2014 and 2014-2015 and for the period 1981–2011 from Ljubljana Meteorological Station: Mean air temperature (°C) (A), Sunshine duration (h) (B); Total precipitation (mm) (C) (Monthly… 2013, 2014, 2015).

were roughly sliced in a small blender. Twenty grams of material were then divided into 4 centrifuge tubes (5 g per tube) and 25 mL of double distilled water was added into each of the tubes. The mixture was homogenized using a T-25 Ultra-Turrax (IKA® - Labortechnik, Staufen, Germany) for 1 min at 11.000 rpm. After homogenization, the samples for allicin determination were transferred onto a shaker at room temperature for 30 min, centrifuged at 10.000 × *g* for 15 min, and filtered through a 0.45-µm polyamide filter Chromafil (Macherey-Nagel, Düren, Germany). The samples were then stored at –80 °C and subsequently lyophilized. Garlic extracts were analyzed using the Agilent 1260 Infinity HPLC system (Agilent Technologies, Inc., Wilmington, DE, USA), consisting of a binary pump (Agilent 1260 Infinity model G1312B), an autosampler (model G1367E), and a diode array detector (model G4212B). The Agilent 1260 Infinity HPLC system was controlled by Agilent HPLC 2D Chemstation SW software (Agilent Technologies, Palo Alto, CA, USA). HPLC analysis was carried out using a C18 column (Zorbax Eclipse Plus; 4.6 × 150 mm, 3.5 mm particle size; Agilent Technologies, Inc., Wilmington, DE, USA) and an analytical guard column (Agilent Eclipse XDB-C18; 4.6 × 12.5 mm, 5 mm particle size). The column was operated in isocratic mode, with a mobile phase of 0.08% formic acid in water: methanol (35:65, v/v) at a flow rate of 0.5 mL/min. The column temperature was maintained at 25 °C and the samples at 4 °C. The injection volume was 10 µL. Instrument control and data acquisition were performed using Agilent HPLC 2D Chemstation SW software (Agilent Technologies, Palo Alto, CA, USA). UV-Vis absorption spectra were recorded in the range from 200 to 400 nm and allicin was recorded at 220 nm. All the extracts were diluted in the mobile phase solution prior to the analysis and filtered through 0.45-µm PTFE syringe filters. Each standard solution and sample solution was analyzed in triplicate. Allicin standard was obtained from LGC (Middlesex, UK), and methanol for liquid chromatography LiChrosolv® was obtained from Merck. For detection of dry matter, 2 g of the frozen sample was freeze-dried for 22 h in a Gamma 2-20 lyophilizer (Christ, Germany) and the water content (%) was calculated from the difference between the masses before and after lyophilization.

The data from all of the analyses, which were performed immediately after the harvest (yield of garlic bulbs, allicin content, morphological traits of bulbs), were tested using multifactorial analysis of variance (ANOVA). Duncan's multiple range test was performed at a significance level of $P < 0.05$ to determine significant differences between means as required. Storage measurements were taken only on the bulb yield from the second year of the study and analyses were based on one year. Data from all of the analyses that were performed during the storage period (changes in allicin, sprout ratio, bulb mass, and dry matter content) were tested for any differences among the sampling dates within the storage period using one-way analysis of variance (LSD test, P < 0.05). Least significant differences tests were performed at a significance level of P>0.05 to determine significant differences between means as required. Statistical analysis of the data was performed using the statistical programs Statgraphic Centurion XVI (Manugistic, Inc.; Rockville, Maryland, USA) and R program (Team, 2011).

3. Results

For garlic yield, ANOVA showed a significant impact of cultivar and fertilization. Moreover, their interaction was also significant (Table 2). Fertilization with N90S0 and N90S60 significantly increased the yield, though only in purple-type, spring cultivars (Figure 2). The yield of Gardos from the N90S0 and N90S60 treatments was significantly higher in both years than the yield of the control plants (N0S0), while the yield of Ptujski spomladanski from the same treatments was higher than the control plants only in 2014. The year of garlic production also influenced the yield, showing higher bulb yield in 2014 than in 2015 with all cultivars except Garcua, with which the opposite was the case. Bulb size, measured in terms of either fresh weight or bulb diameter, was highly dependent on cultivar and fertilization treatment. In addition, their interaction had a significant impact (Table 2, Figure 3). Gardos produced the heaviest bulbs in both years, and the lightest bulbs were produced by Garcua in 2014 and Gardacho in 2015. Plants from the N90S0 treatment produced the heaviest bulbs in both years and the lightest bulbs were produced by the control plants (N0S0) (Table 2, Figure 3).

For the total S and allicin contents in garlic bulbs, ANOVA showed a significant impact of cultivar and fertilization treatment, and their interaction was also significant (Table 3). The N content in bulbs was influenced only by cultivar, while the fertilization effect was not significant (Table 3). The highest minerals (N and S) and allicin contents (Figure 4) were found in Gardos bulbs in both years, while the lowest contents were found in bulbs of Ptujski spomladanski in 2014 and of Gardacho in 2015. The content of sulfur and allicin differed between cultivars, as they were affected by S or/and N fertilization. We also determined the closest relationship between the S and allicin content in garlic bulbs (Figure 5). In 2014, the N supply significantly increased the allicin content in bulbs of all cultivars (Figure 4), while the addition of S decreased the allicin content in bulbs of three cultivars, the exception being Gardos, with which an increase of allicin was recorded. A significant impact of N and S fertilization was also observed in 2015, revealed in increased allicin content in bulbs of all three Spanish cultivars, although the differences were significant only for Gardos (Figure 4).

Table 2. Average morphometric traits of bulbs and average bulb yield of four garlic cultivars and three fertilization practices, grown in 2014 and 2015; significances are in parentheses.

	Average bulb mass (g)		Average bulb diameter (mm)		Garlic bulb yield (t ha^{-1})	
Year	2014	2015	2014	2015	2014	2015
Cultivar						
Garcua	40.9 c	35.3 b	54.9 c	47.5 b	5.3 c	6.9 b
Gardacho	45.7 b	25.8 d	68.9 a	40.6 d	5.1 c	4.2 d
Gardos	53.2 a	46.9 a	57.5 bc	50.7 a	12.0 a	10.0 a
Ptujski spomladanski	43.2 bc	30.0 c	62.3 b	43.2 c	7.4 b	5.7 c
Fertilization						
'N0S0'	42.7 b	32.1 c	58.5 a	44.2c	6.4 b	6.0 b
'N90S0'	48.6 a	37.2 a	63.1 a	47.1 a	8.0 a	6.4 b
'N90S60'	46.0 ab	34.2 b	61.1 a	45.2 b	7.9 a	7.5 a
ANOVA						
Cultivar (C) (SE)	*** *(1.5)*	*** *(0.6)*	*** *(1.9)*	*** *(0.3)*	*** *(0.3)*	*** *(0.2)*
Fertilization (F) (SE)	*** *(1.3)*	*** *(0.5)*	*NS*	*** *(0.2)*	*** *(0.2)*	*** *(0.2)*
C × F (SE)	*** *(1.4)*	*** *(1.1)*	*** *(1.7)*	*** *(0.1)*	*** *(0.3)*	*** *(0.2)*

Different letters in the column denote significant differences in treatment (Duncan test, P < 0.05). Factors or their interactions are significant at *** P < 0.001, ** P < 0.01, * P < 0.05; NS - not significant.
SE – standard error for significant term

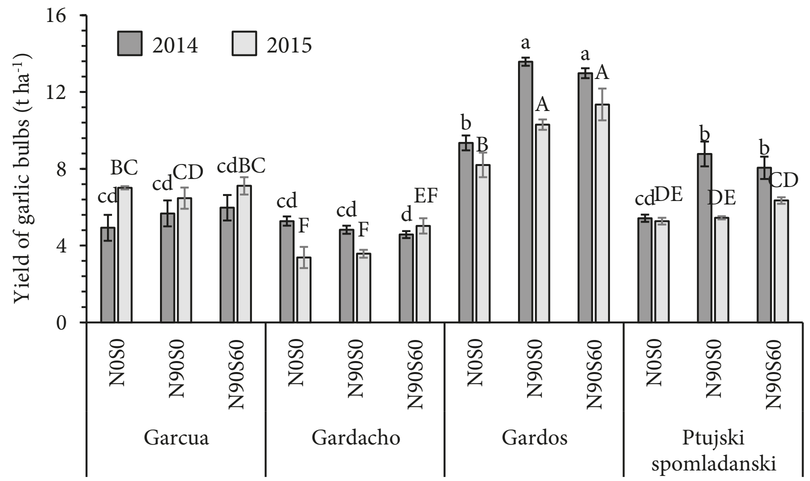

Figure 2. Average yield of garlic bulbs and significant error bars for different cultivars and different fertilization practices harvested in two growing seasons (2014 and 2015). Different lower-case letters (a,b…) denote significant differences among treatments in 2014; different upper-case letters (A,B,…) denote significant differences among treatments in 2015 (Duncan's test, $P < 0.05$).

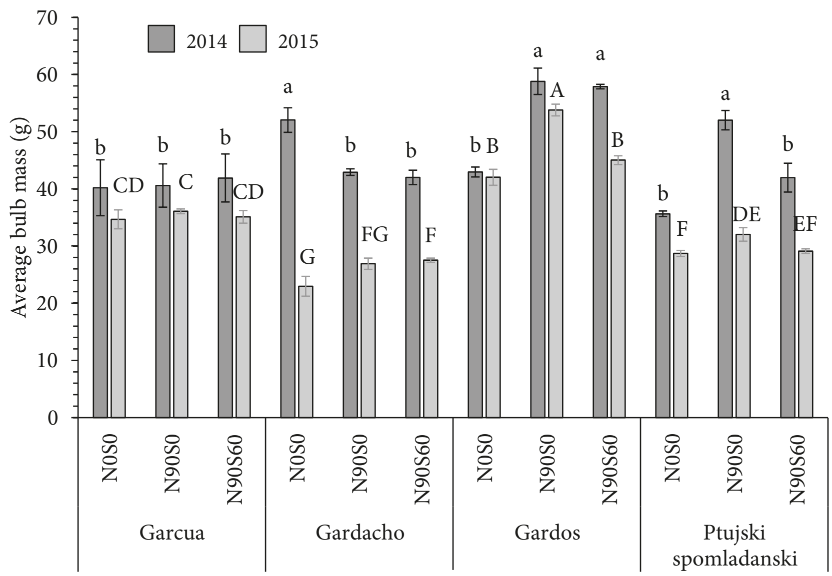

Figure 3. Average bulb mass (g) with significant error bars for different cultivars and different fertilization practices, harvested in two growing seasons (2014 and 2015). Different lower-case letters (a,b…) denote significant differences among treatments in 2014; different upper-case letters (A,B,…) denote significant differences among treatments in 2015 (Duncan's test, $P < 0.05$).

Garlic was stored for 4 months in dry and dark storage containers, where the temperature was automatically controlled. At the end of the storage period, the bulbs had experienced a weight loss of 14% to 35% depending on cultivar and fertilization treatment (Figure 6). Garcua and Gardacho showed a higher weight loss of bulbs from the N90S60 treatment compared to bulbs from the N0S0 and N90S0 treatments. Gardos showed a bulb weight loss of 30% from the N90S0 treatment, and 21% and 17% in bulbs from N0S0 and N90S60 treatments, respectively. Bulbs of Ptujski spomladanski had the lowest weight loss during the storage period. Since the weight of bulbs decreased during the storage period, the dry matter content increased (Figure 7). The highest increase in dry matter content was recorded in bulbs from the control treatment N0S0, and the lowest increase was recorded in bulbs from the N90S0 treatment.

Table 3. Content of the total S, N, and allicin (mg g^{-1} DW) in bulbs at harvest, for four garlic cultivars and three fertilization practices, harvested in the years 2014 and 2015.

	Total S concentration in bulbs (mg g^{-1} DW)		Nitrogen concentration (mg g-1 DW)		Allicin in bulbs (mg g^{-1} DW)	
Year	2014	2015	2014	2015	2014	2015
Cultivar						
Garcua	3.24 a	3.43 ab	24.7 b	33.1 a	3.47 ab	3.84 b
Gardacho	2.42 b	2.82 c	24.2 b	31.3 b	3.04 bc	3.25 c
Gardos	3.15 a	3.89 a	25.7 a	33.9 a	3.82 a	4.17 a
Ptujski spomladanski	2.29 b	3.22 bc	24.9 b	30.9 b	2.91 c	3.75 b
Fertilization						
'N0S0'	2.51 b	2.26 b	24.6 a	31.9 a	2.90 b	2.5 c
'N90S0'	2.92 a	3.73 a	25.2 a	32.7 a	3.65 a	4.21 b
'N90S60'	2.75 b	4.04 a	24.8 a	32.3 a	3.37 a	4.52 a
ANOVA						
Cultivar (C) (SE)	*** *(0.04)*	*** *(0.17)*	* *(0.3)*	* (0.3)	*** (*0.16)*	*** *(0.07)*
Fertilization (F) (SE)	*** (*0.03)*	*** *(0.15)*	NS	NS	*** *(0.14)*	*** *(0.06)*
C × F (SE)	*** *(0.06)*	*** *(0.30)*	NS	NS	*** *(0.28)*	*** *(0.13)*

Different letters in column denote significant differences in treatment (Duncan test, $P < 0.05$). Factors or their interactions are significant at *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; NS - not significant.
SE – standard error for significant term

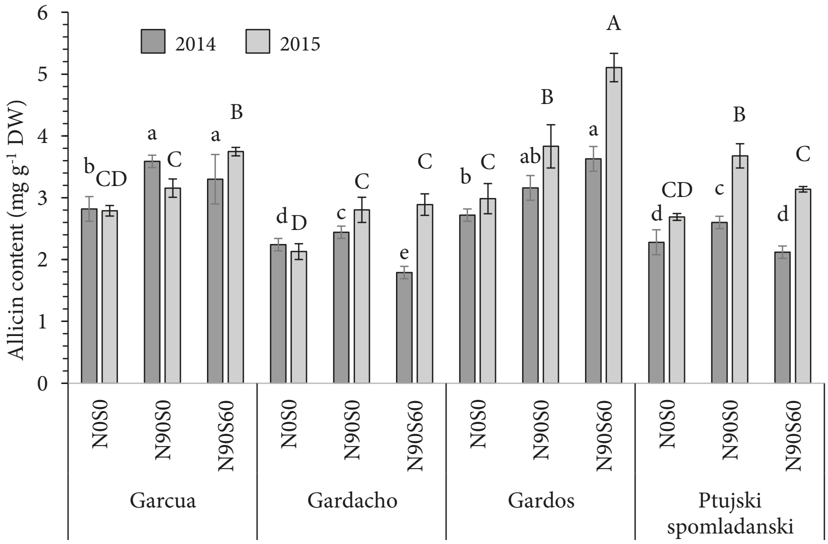

Figure 4. Average allicin content (mg g^{-1} DW) in bulbs at harvest, for four garlic cultivars and three fertilization practices, harvested in the years 2014 and 2015. Different lowercase letters denote significant differences among treatments in 2014; different capital letters denote significant differences among treatments in 2015 (Duncan test, $P < 0.05$).

Sulfur and nitrogen (data not shown) status in garlic bulbs during the storage period remained constant, with slight, nonsignificant alterations between the sampling dates, while the allicin concentration (Figure 8) significantly increased during the storage period, reaching the highest concentration on 90 DAH and then slightly decreased or

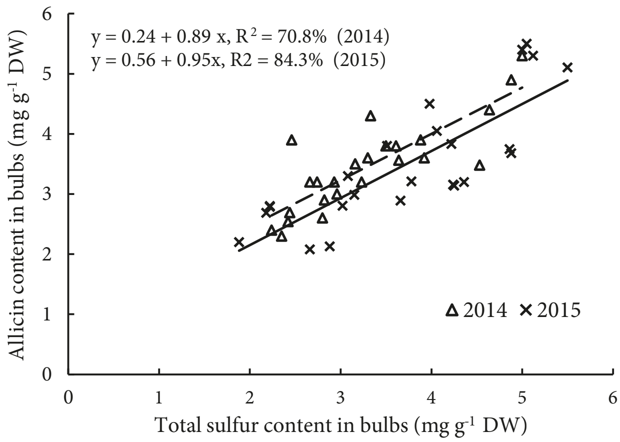

Figure 5. Relationship between the S concentration and allicin content in bulbs of four garlic cultivars, fertilized with N and S and harvested in the years 2014 and 2015.

stayed constant (with the cultivar Ptujski spomladanski). The highest increase in allicin concentration was recorded in bulbs from the N90S0 treatment.

4. Discussion

Among the preharvest factors that affect the morphological traits and chemical composition of garlic bulbs, the selection of genotype in terms of soil type and environmental conditions is of great importance, allowing a high-quality product without compromising the total yield (Martins et al., 2016), which is particularly important from the farmer's point of view. The cultivars in our study were selected on the basis of their economically important characteristics, which was proven during official testing in experiments managed by the National Institute of Agriculture of Slovenia (Ugrinovic and Skof, 2012). In addition to foreign cultivars that have been introduced

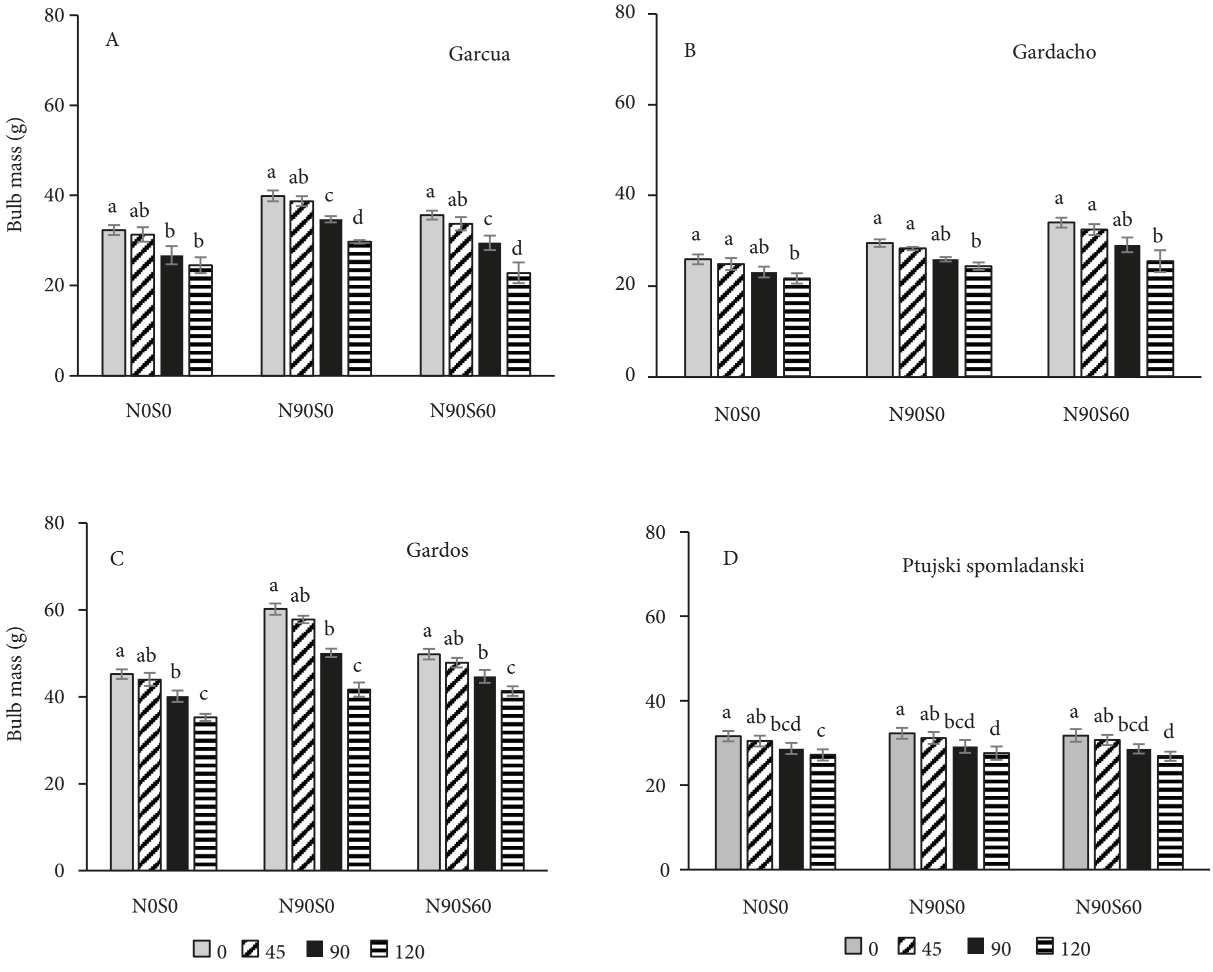

Figure 6. Average bulb mass, measured 0, 45, 90, and 120 days after harvest, for four garlic cultivars and three fertilization practices. Data consist of the average of 8 bulbs, at each measured date. Different letters denote significant differences among treatments (LSD test, $P < 0.05$).

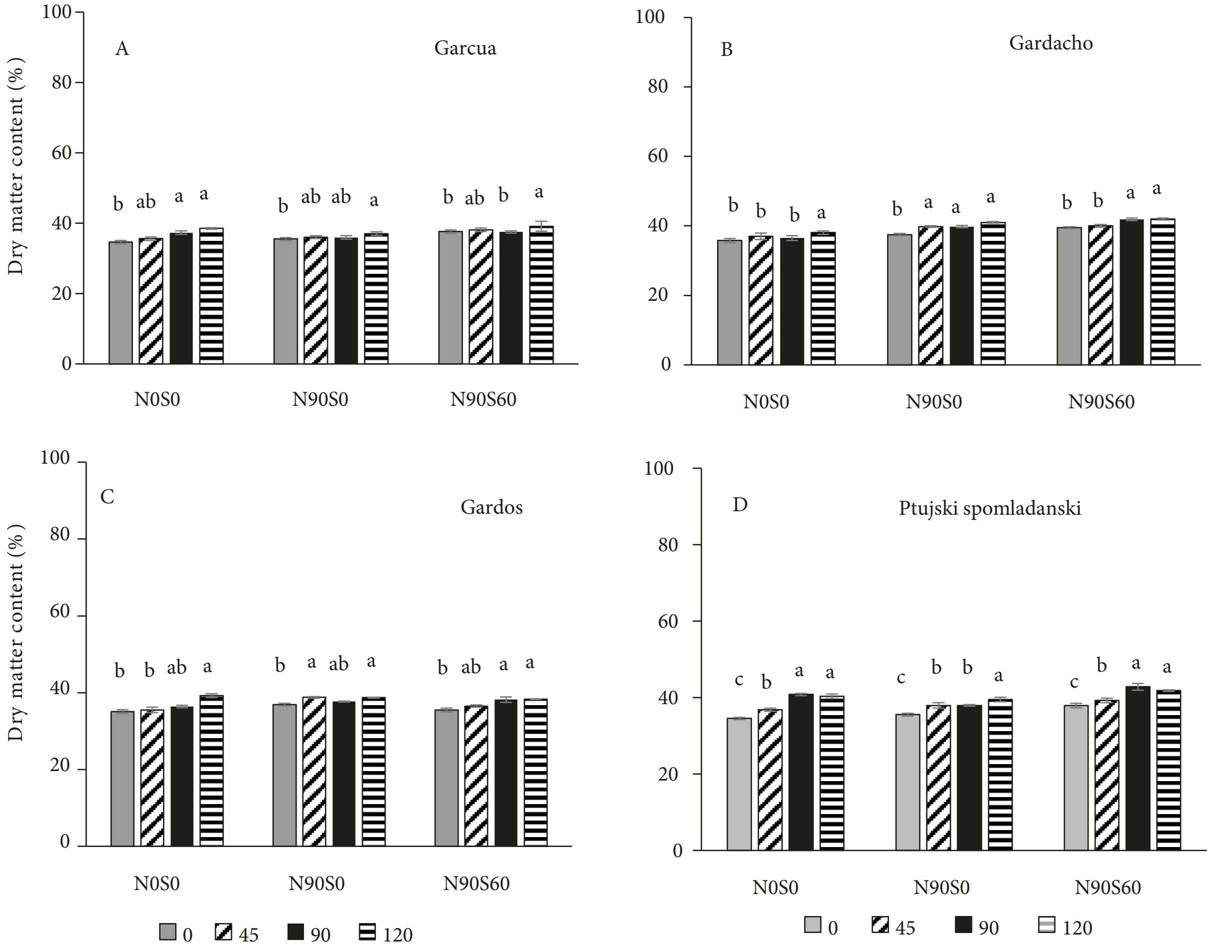

Figure 7. Average dry matter content (%) in bulbs, measured 0, 45, 90, and 120 DAH, for four garlic cultivars and three fertilization practices. Data consist of the average of 8 bulbs, at each measured date. Different letters denote significant differences among treatments (LSD test, P < 0.05).

into Slovenian vegetable production, the majority of cultivars bred from the autochthonous material are still in production because of their particular morphological traits and taste, which are still sought by consumers. Three commercial Spanish cultivars and one autochthonous cultivar Ptujski spomladanski were included in the study for this reason, and also because the authors believe that, in view of the high genetic erosion noticed across the country (Meglic et al., 2001), it is important to improve collections with data of agromorphological and biochemical traits of bulbs of autochthonous cultivars.

The fertilization effect on garlic yield differed in terms of cultivar and the year of production. A positive impact of the N and N+S supply, which was revealed in increased bulb yield in comparison to the control treatment, was observed only in the spring cultivars, Gardos and Ptujski spomladanski (Figure 2). With both cultivars, the addition of S to the N supply (N90S60) slightly decreased bulb yield in 2014 and increased it in 2015 in comparison to the N90S0 treatment. Previous studies on S fertility in allium crop production suggested that the application of sulfur increases the yield and the uptake of other nutrients (N, P, and K) in *Allium* plants (Scherer, 2001; Coolong et al., 2003; Pradhan et al., 2015). Huchette et al. (2007) reported that increased N fertility combined with a high S supply accelerated maturity in autumn and spring garlic cultivars. This may have partly influenced the reduction of positive effect of S fertilization on bulb yield in 2014 together with unpleasant weather conditions in that year.

Fertilization with S is a suitable measure for increasing S-containing metabolites in garlic bulbs, and thus significantly affecting their health effects (Rahman, 2007). Bloem et al. (2011) reported that even with the consumption of 4 g of fresh garlic, which was fertilized with 45 kg S ha^{-1}, the officially recommended daily dosage of alliin (4–12 mg) or allicin (2–5 mg) can be exceeded. This recommendation can also be reached with the consumption of 1 to 2 garlic cloves (about 4 g) from our

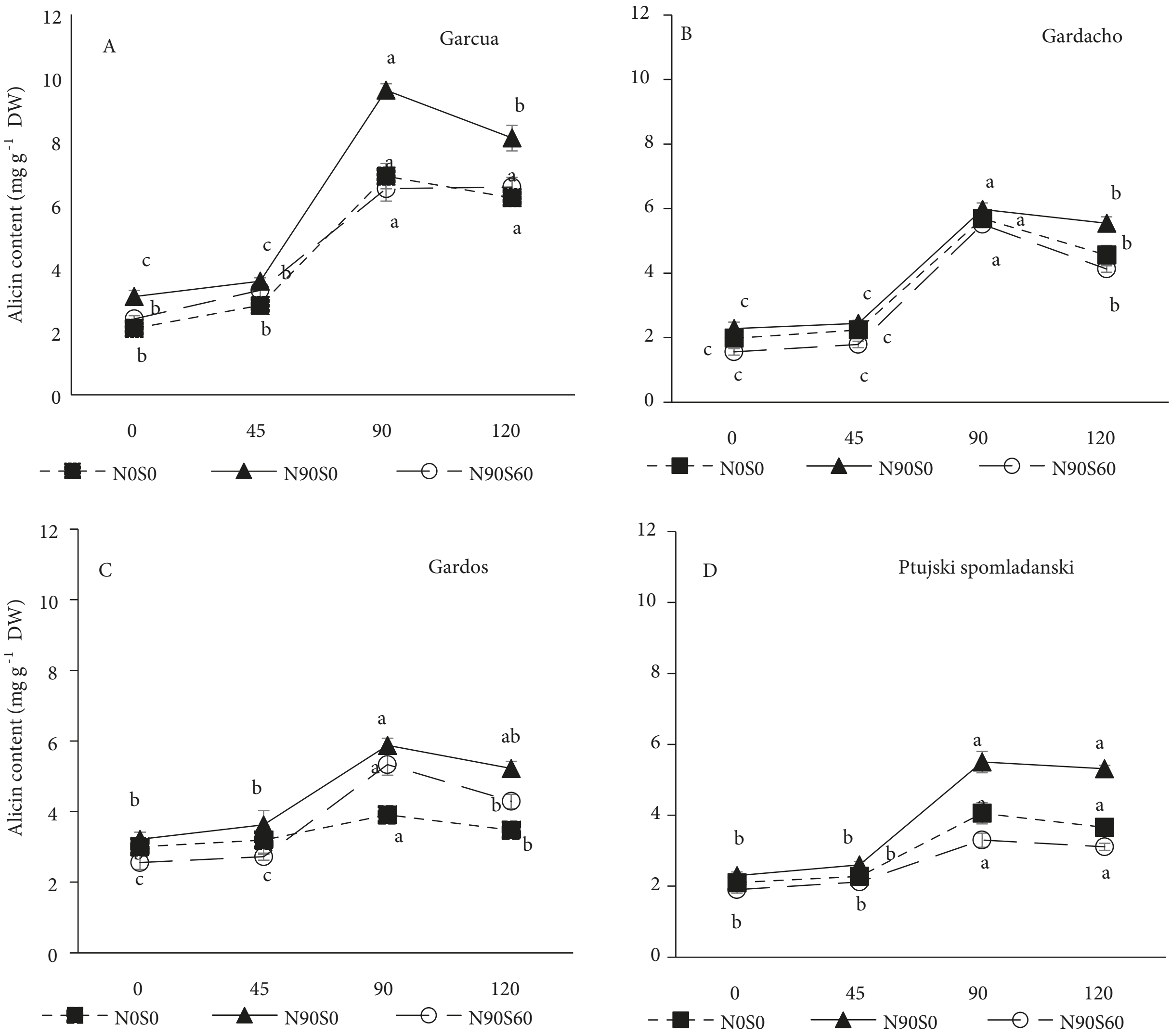

Figure 8. Average allicin content (mg g^{-1} DW) in bulbs, during the storage period, for four cultivars and three fertilization practices. Different letters denote significant differences among treatments (LSD test, $P < 0.05$).

study, as 1.0 to 1.8 mg g^{-1} of allicin was found (Figure 4) in bulbs from the S-fertilized treatment, although this trait was cultivar-specific and varied according to the environmental conditions (Huchette et al., 2007). In the bulbs of the Gardos cultivar, the application of S significantly increased the allicin content in comparison to fertilization treatments without S in both years (Figure 4). Montaño et al. (2011) suggested that the levels of individual organosulfur compounds in garlic were affected by cultivar and growing location. On average, the purple-type cultivars showed the highest content of γ-glutamyl peptides and their corresponding sulfoxide derivatives (alliin and methiin). For white-type cultivars, they found the lowest cystein sulfoxide content from among 13 tested cultivars. Huchette et al. (2007) also suggested that the effect of fertilization on flavor compound in garlic bulbs was cultivar-dependent, revealed in high alliin content in bulbs of the spring cultivars, while the autumn cultivar had the highest flavor potential when considering the levels of alliin and γ-glutamil peptides as well.

In relation to the allicin content in garlic bulbs, a notable impact of weather conditions was found on the fertilization effect for Garcua, Gardacho, and Ptujski spomladanski. In all three cultivars, the S supply decreased the allicin content in the year with excessive precipitation and lower temperatures during the spring growing period, while an opposite effect was observed in the following year, with improved conditions for garlic (higher temperatures and moderate precipitation), revealed in the increased allicin content (Figure 4). It was reported for allium crops that the temperature during the growing period influenced S uptake and the amount of S partitioned into

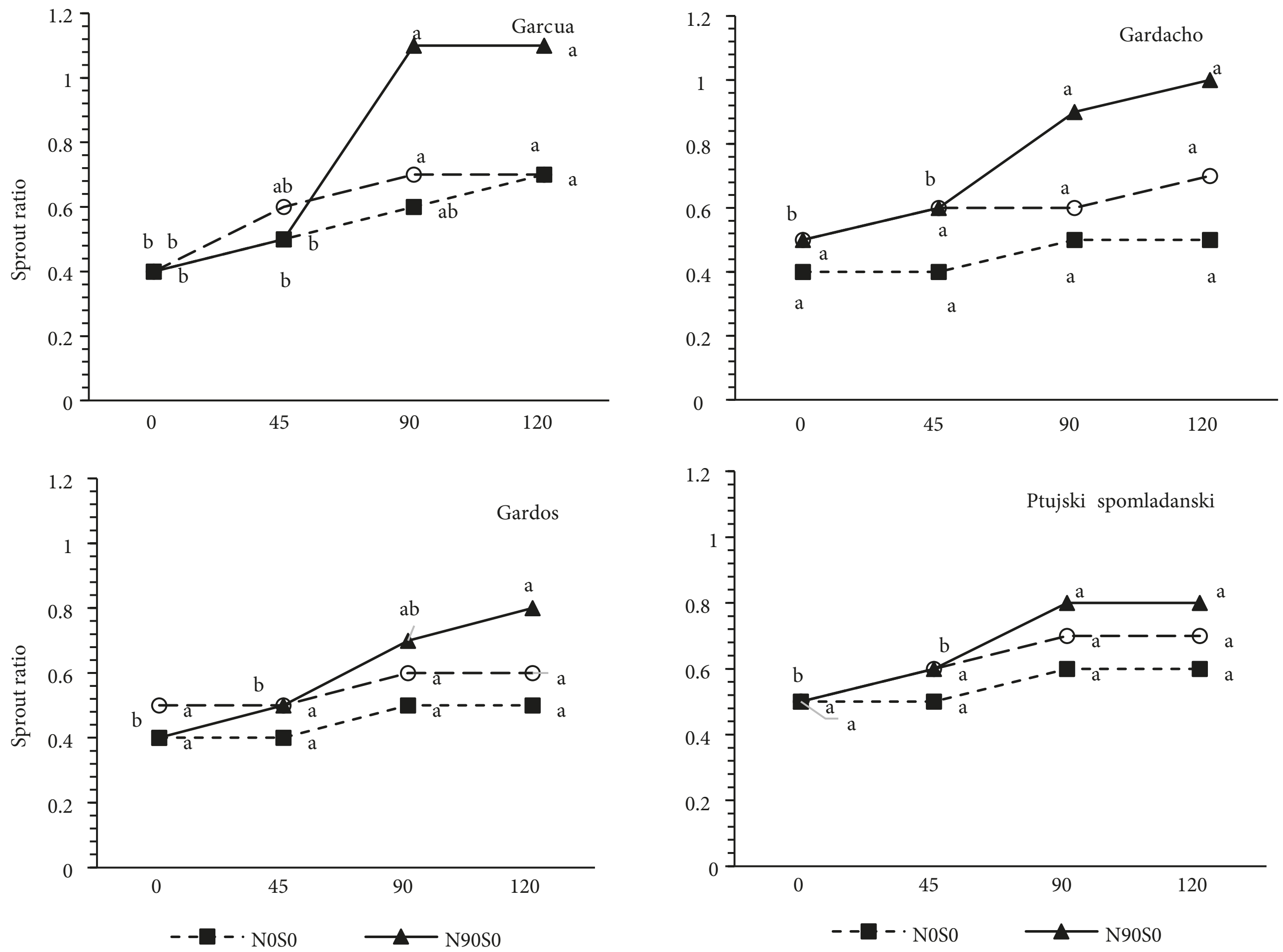

Figure 9. Average sprout growth in garlic cloves, during the storage period, measured 0, 45, 90, and 120 days after harvest, for four garlic cultivars and three fertilization practices. Sprout ratio is the length of the sprout measured in a longitudinal section relative to clove length. Different letters denote significant differences among treatments (LSD test, $P < 0.05$).

organic compounds (Coolong and Randle, 2003). The more or less suitable conditions during the experimental periods (Figure 1) influenced variations in growth and formation of garlic bulbs (Kamenetsky et al., 2004), and the determination of the optimal maturity stage of bulbs at harvest was therefore difficult, especially because cultivars with different lengths of growing period were included in the study. We believe that the positive impact of the S supply on the increased allicin content in garlic bulbs in the second year of the study may also be partly attributed to proper maturity of the garlic bulbs at harvest, since some overripe symptoms, such as slight breaking of the outer wrapper of bulbs of Garcua, Gardacho, and Ptujski spomladanski, were observed at harvest in the first year of the study. Namely, as the main precursor of the pungent flavor that comes from allicin (Block, 1992), the alliin content in garlic bulbs is related to the plant's developmental stages and increases during bulb swelling. When the garlic was overripe and the leaves almost died but the bulbs still remained in the soil, the alliin concentration in the bulbs decreased, mainly due to the cessation of biosynthesis of alliin and continued bulb mass growth (Bloem et al., 2010). On the other hand, a large increase in γ-glutamil peptides and allyl cysteine sulfoxide occurred in garlic bulbs as cloves matured during the last month of growth (Matsuura et al., 1996), thus suggesting a different composition of organosulfur compounds in terms of different bulb maturity stages. Large quantities of γ-glutamyl peptides found in dormant bulbs indicate an important role of these compounds as storage sources of N and S for use in bulb's sprouting (Randle and Lancaster, 2002). The growth and the ripeness of bulbs in the following year were therefore monitored more attentively and the time of harvest was set carefully.

Garlic is not considered a highly perishable product, thanks to a well-preserved dried neck and outer skins, which prolong its shelf life. In addition, garlic cloves are wrapped in a peel, which regulates the transport of

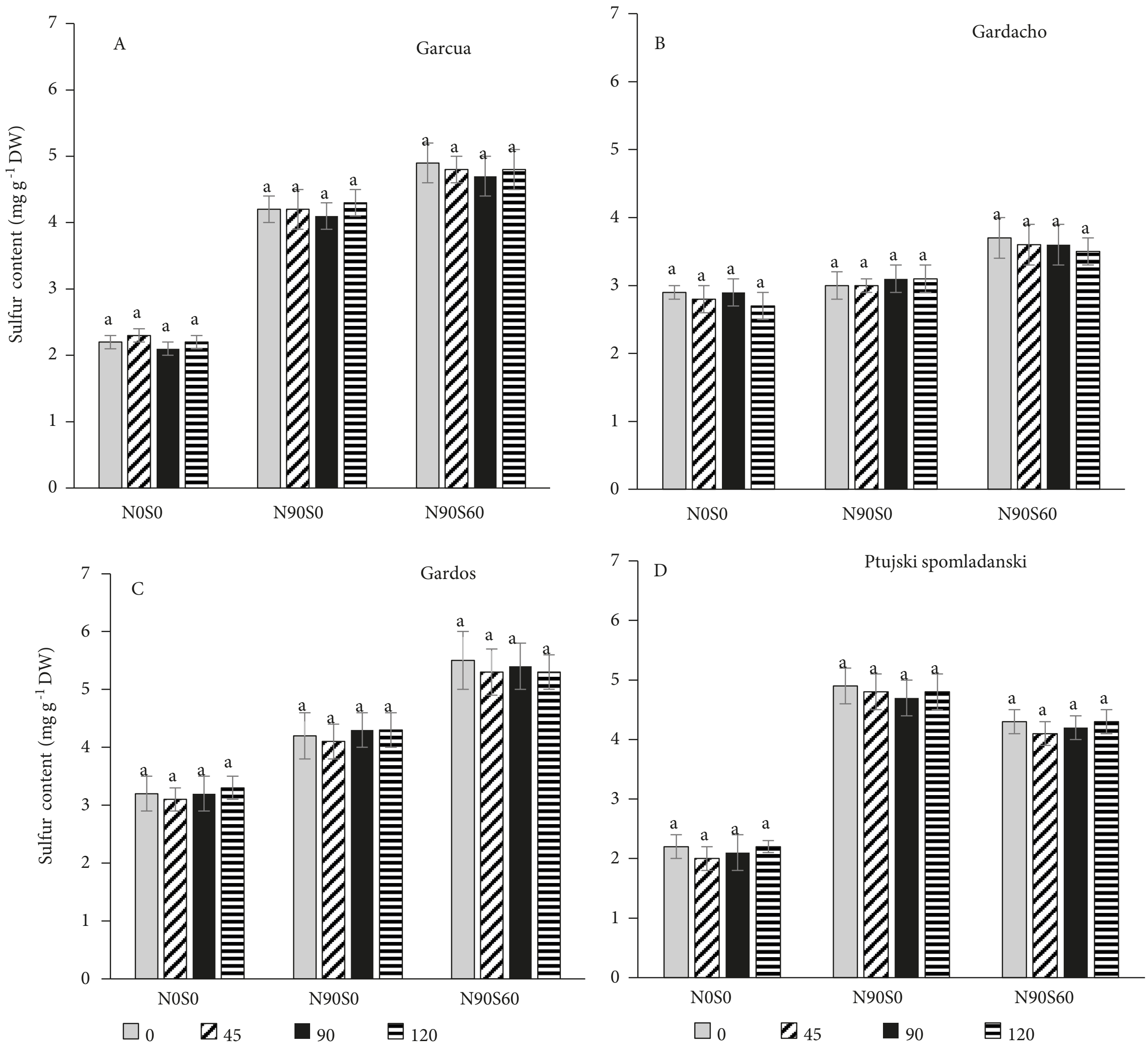

Figure 10. Average S concentration (mg g^{-1} DW) in bulbs, measured 0, 45, 90, and 120 days after harvest (DAH), for four garlic cultivars and three fertilization practices. Data consist of the average of 8 bulbs, at each measured date. Different letters denote significant differences among treatments (LSD test, P < 0.05).

oxygen, carbon dioxide, and moisture but also reduces flavor and aroma (Miller and Krochta, 1997). However, since garlic bulbs are stored for several months in order to ensure year-round supplies for the consumer, storage conditions cause a significant reduction of shelf life and quality, including moisture loss, sprouting, and rooting (Cantwell et al., 2003; Ichikawa et al., 2006), and also significantly influence the composition of organosulfur compounds (Lawson et al., 1992). Numerous studies have dealt with the impact of postharvest factors on bulb storage quality (Vazquez-Barrios et al., 2006; Rosen and Allan, 2007; Bloem et al., 2011), but there are few studies on the effect of agricultural measures, such as genotype selection or fertilization schedule, on the quality of bulbs during storage for crops of *Allium* species. The storage quality of garlic bulbs is closely correlated with the breaking of bulb dormancy, which is adjusted by storage temperature and storage duration (Martins et al., 2016). Studies based merely on morphological observations have shown different influences of storage temperature on garlic sprout development (Ichikawa et al., 2006). At temperatures between 20 and 30 °C, garlic can be stored for about 60 days without any visual changes, while a cooler temperature between 5 and 18 °C affects the breaking of dormancy after 90 and 120 days, signaled by internal development of the sprout (Cantwell et al., 2003; Volk et al., 2004; Vazquez-Barrios et al., 2006; Garcia et al., 2008). In the present study, the temperature of the storage room ranged between 15 and 18 °C and the initial sprout length, which was measured at the beginning of the storage period and was nearly 50% of the clove length (0.5 sprout ratio) (Figure 9), was increased with a longer storage period and also in relation to the fertilization treatments. The sharpest rise of the sprout ratio was recorded with all cultivars between 45 and 90 days after harvest, which may be explained by physiological and biochemical modifications that occur during storage and are usually caused by the interruption of bulb dormancy (Ceci et al., 1991; Lancaster et al., 1998). Sprouting also induces the mobilization and translocation of the flavor compounds into the emerging leaves (Randle and Lancaster, 2002). An increase in the alliin content, which accompanies the breaking of dormancy during the storage period, is a consequence of γ-L-glutamyl transpeptidase activity, which converts γ-glutamyl peptides, γ-L-glutamyl-S-allyl-L-cysteine, and γ-L-glutamyl-S-(*trans*-1-propenyl)-L-cysteine to sulfoxides of alliin and their derivatives (Lawson et al., 1992; Ichikawa et al., 2006; Bloem et al., 2010). Since alliin is a common precursor of allicin (Rahman, 2007; Block et al., 2010), the increase of allicin found in the present study may be because of the increased accumulation of alliin. The increased allicin content (Figure 8) and simultaneously unchanged S status in garlic bulbs (Figure 10) during the whole storage period indicated the conversion of organosulfur compounds in garlic bulbs. The increase of allicin was most pronounced in cloves of Garcua (Figure 8), with which the sprout ratio reached the highest value (1.1) about 90 days after harvest (Figure 9). This may be explained by the cultivar characteristic of a shorter storage life than other cultivars (Plantas de Navarra S.A. Planasa, Spain) and, consequently, earlier onset of sprouting. N application during plant growth also had a marked influence on the storage capacity of garlic bulbs, as shown in Figure 8. Among all the cultivars, the highest allicin content during the storage period was found in bulbs from the N treatment (N90S0), which may indicate that the N supply modified the dormancy of garlic bulbs, since the onset of bulb sprouting is accelerated if plants are grown with a high N supply (Sorensen, 2000). On the other hand, on the basis of the similar variation of allicin content in bulbs from N90S60 and N0S0 treatments during the storage period (Figure 8), it has been suggested that the addition of the S supply to N fertilization may mitigate the N impact on the modification of bulb dormancy of Garcua and Ptujski spomladanski and thus improve the storage capacity of bulbs of these two cultivars.

Our study indicated that preharvest factors, such as cultivar selection, and N and/or S fertility notably affected the changes of yield and quality traits of garlic bulbs at harvest and during storage. We also demonstrated that increasing levels of N and S enhanced the allicin content of the bulbs of Gardos cultivar. Fertilization practice also significantly moderated bulb dormancy and subsequently influenced the conversion of the organosulfur compounds in garlic bulbs during the storage period. To improve the health value of garlic bulbs, genetic factors should be considered as well as the interaction of N and S fertility.

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