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Guo, T., Zhang, J. L., Christie, P., & Li, X. L. (2007). Pungency of spring onion as affected by inoculation with arbuscular mycorrhizal fungi and sulfur supply. *Journal of Plant Nutrition*, 30(7), 1023-1034.  
<https://doi.org/10.1080/01904160701394311>

**Published in:**  
Journal of Plant Nutrition

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## Pungency of Spring Onion as Affected by Inoculation with Arbuscular Mycorrhizal Fungi and Sulfur Supply

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### ABSTRACT

The influence of sulfur (S) supply and mycorrhizal colonization on the growth and pungency of spring onion (*Allium fistulosum* L.) seedlings grown for four months in Perlite and nutrient solution containing three levels of sulfate [0.1, 1.75, and 4 mM sulfate ( $\text{SO}_4^{2-}$ )] were investigated. Plants were inoculated with *Glomus etunicatum*, *Glomus intraradices*, or *Glomus mosseae*, and uninoculated controls were included. Shoot and root dry weights of mycorrhizal and control plants supplied with 4 mM  $\text{SO}_4^{2+}$  were higher than with 0.1 or 1.75 mM  $\text{SO}_4^{2-}$ . Enzyme produced pyruvic acid (EPY) and plant sulfur concentrations increased with increasing  $\text{SO}_4^{2-}$  supply. The EPY and plant S concentrations were usually higher in mycorrhizal plants than controls irrespective of S supply, and shoot total S concentrations and EPY were strongly correlated. Arbuscular mycorrhizal colonization appeared to make a substantial contribution to the sulfur status of spring onion, and may thus have had a strong influence on its flavor characteristics.

**Keywords:** enzyme produced pyruvate, sulfur supply level, arbuscular mycorrhizal fungi, *Allium fistulosum*

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Received 13 July 2006; accepted 15 August 2006.

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## INTRODUCTION

*Allium* species make an important contribution to the human diet because of their unique flavor compounds and their potential benefits to human health (Amagase et al., 2001). The functional flavor components of *Allium* plants are organo-sulfur (S) compounds that are synthesized from a common precursor, the S-alk(en)yl cysteine sulfoxides (ACSOs). After disruption the enzyme alliinase acts upon the ACSOs in the cytoplasm, giving rise to the volatile compounds responsible for the characteristic flavor and aroma of the plants (Lancaster and Collin, 1981) with the formation of pyruvic acid and ammonia as by-products. The composition and total concentrations of ACSOs determine the flavor profile of the plants, differ among *Allium* species and cultivars (Yoo and Pike, 1998), and vary with the external environment (Randle and Lancaster, 2002). As the direct measurement of the flavor precursors or the active flavor compounds is laborious, technically demanding and costly for large scale routine agronomic or breeding studies (McCallum et al., 2005), enzymatically generated pyruvic acid (EPY) has been used to determine the gross pungency of *Allium* plants due to the simplicity of the measurement. Early studies showed that the flavor intensity of onion plants correlated well with the levels of EPY in the juice (Schwimmer and Weston, 1961).

The influence of environmental factors on the flavor components of onion has been intensively investigated. Studies have shown that water supply (Freeman and Mossedeghi, 1973), temperature (Randle et al., 1993), harvest time (Bloem et al., 2004), S supply (Randle, 1992; Randle et al., 1999), the amount (Coolong et al., 2004) and the forms of nitrogen (N) supplied (Gamiely et al., 1991), and N and S interactions (Coolong and Randle, 2003; Bloem et al., 2004; McCallum et al., 2005) can affect plant growth, maturity, yield, flavor attributes, and bulb firmness. However, S supply in the substrate is considered to be the primary factor because S is directly involved in the synthesis of the ACSOs and is a major constituent of the flavor compounds. Higher available S in the substrate generally results in greater gross flavor intensity (Randle, 1992; Randle et al., 1999) and alters the composition of the ACSOs and the biosynthetic intermediates in onion plants (Randle et al., 1995; Coolong and Randle, 2003).

Spring onion (*Allium fistulosum*), *A. sativum*, *A. cepa*, and *A. tuberosum* are the four most widely cultivated *Allium* species in China. Spring onion accounted for 3.0% of the total cultivated area of vegetables and 3.4% of vegetable production in China in 2004 (Chinese Ministry of Agriculture, 2004). It is used for its contribution to the flavor and texture of most Chinese dishes. However, there is limited information on the flavor attributes of spring onion in contrast to the large amount of available data on plant yield. The growth of spring onion is influenced by N fertilization (Inden et al., 2000; 2001) and is promoted by mycorrhizal colonization (Tawaraya et al., 2001). In previous work, the effect of mycorrhizal inoculation and N and S fertilization on the pungency of spring

onion grown in soil was investigated. The EPY production tended to be mediated by mycorrhizal colonization, but not by N or S supply level (Guo et al., 2006b). The aim of the present study was to investigate further the effect of mycorrhizal colonization and S supply on the pungency of spring onion in order to give more insight into the flavor regulation of the spring onion plants. Plants were grown in Perlite and irrigated with three levels of sulfate to represent low, intermediate, and high S supply levels.

## MATERIALS AND METHODS

Perlite (1–3 mm) was washed with tap water over 1-mm nylon mesh to remove the fine powder, then sun dried and heated in an oven at 110°C for 24 h before use. Aliquots of the Perlite (1000 cm<sup>3</sup>) were then placed in round plastic pots (1.5 L capacity; 15 cm diameter). Firstly, about 250 cm<sup>3</sup> was placed in the bottom of each pot, followed by 500 cm<sup>3</sup> of Perlite mixed thoroughly with mycorrhizal (AM) inoculum, and then the remainder was placed on top. Mycorrhizal treatments received 100 g AM inoculum and non-mycorrhizal controls received an equivalent amount of steam-sterilized inoculum together with a filtrate (0.45 μm pore size) of unsterilized soil to provide a similar microflora except for the absence of the AM fungus. The inoculum consisted of loose soil from pot cultures containing spores and maize root pieces colonized by *Glomus etunicatum* BEG 168, *Glomus intraradices* BEG 141 or *Glomus mosseae* BEG 167.

Seeds of spring onion (*Allium fistulosum* L. cv. 'Zhangqiu') were surface sterilized in a 10% (v/v) solution of hydrogen peroxide for 10 min, then sown in sterilized peat and irrigated with deionized water. Six seedlings of similar size were transplanted into each pot at the three-leaf stage. A thin layer of sterilized sand was placed on the surface of the substrate to minimize evaporation. The seedlings grew in water with 1/10 strength nutrient solution for the first three weeks and thereafter in full strength solution with the different experimental treatments. The nutrient solution followed Hawkins and George (1999) with the following composition: ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) 4 mM, monosodium phosphate (NaH<sub>2</sub>PO<sub>4</sub>) 0.094 mM, disodium phosphate (Na<sub>2</sub>HPO<sub>4</sub>) 0.006 mM, potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) 1 mM, calcium chloride (CaCl<sub>2</sub>) 2 mM, magnesium sulfate (MgSO<sub>4</sub>) 0.75 mM, boric acid (H<sub>3</sub>BO<sub>3</sub>) 69 μM, copper sulfate (CuSO<sub>4</sub>) 1.7 μM, zinc sulfate (ZnSO<sub>4</sub>) 1.2 μM, manganese sulfate (MnSO<sub>4</sub>) 10.4 μM, sodium molybdate (NaMoO<sub>4</sub>) 0.13 μM, iron-ethylene diamine tetraacetic acid (Fe-EDTA) 0.3 μM. Sulfur concentrations in the solution were varied by adjusting the ratios of MgSO<sub>4</sub> to magnesium chloride (MgCl<sub>2</sub>) and K<sub>2</sub>SO<sub>4</sub> to KCl to obtain 0.1, 1.75, and 4 mM SO<sub>4</sub><sup>2-</sup>. The pH of the nutrient solution was adjusted to 6.0 and MES buffer was added. Seedlings were watered once every three days with the nutrient solution. The Perlite was washed thoroughly once each week to minimize accumulation of salts.

There were four mycorrhiza treatments (including the uninoculated controls)  $\times$  three S supply levels  $\times$  five replicates, giving a total of 60 pots. Plants were grown for 20 weeks from April 2004 to September 2004 in the glasshouse of the Department of Plant Nutrition at China Agricultural University without supplementary illumination and with a daytime temperature range of 20–34°C and a night temperature range of 15–26°C.

At harvest, plants were separated into shoots and roots. Root samples (0.5 g fresh weight) were cut into 1 cm-long segments to determine the proportion of root length colonized by AM fungi as described by Giovannetti and Mosse (1980). Pungency as an indicator of onion flavor was determined by the pyruvic acid method (Randle, 1992; Randle et al., 1995), i.e., by measuring enzyme produced pyruvate (EPY). Briefly, the reaction of 2,4-dinitrophenylhydrazine (DNPH) with NaOH was used to detect the presence of pyruvic acid (enzymatically formed pyruvate). A sample of shoot tissue (5 g fresh weight) was blended with an equal volume of water for 3–5 min. Equal aliquots of trichloroacetic acid and onion slurry were combined after 20 min, incubated for 1 h, and then filtered through cheese cloth. One mL of the filtrate was added to 1 mL 0.0125% 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid (HCl) plus 1 mL water and incubated at 37°C for 10 min. Five mL sodium hydroxide (NaOH) were then added before the absorbance was read at 420 nm on a spectrophotometer. Pyruvate determinations were made against a sodium pyruvate standard curve.

The remainder of the plant material was dried in an air-forced oven at 70°C for 48 h and weighed. Oven-dried samples were milled with a high-speed multi-function micro-pulverizer (Whirl Type Model Y-60, Hebei, China) prior to elemental analysis. For the determination of phosphorus (P), sub-samples (about 0.3 g) were firstly wet digested on a block digester (Kjeldatherm, Gerhardt GmbH, Germany) with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Plant P concentration was determined by the vanado-molybdate method (Colwell, 1965) using a Shimadzu Model UV-120-02 spectrophotometer. Total S concentration was determined as described by Gaines and Mitchell (1979). Briefly, plant tissue was dried at 70°C for 72 h in a forced-air oven and then ground to pass through a 0.5-mm screen. Total S was oxidized to SO<sub>4</sub><sup>2-</sup> by heating the dried tissue with magnesium nitrate [Mg(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O]. The samples were then ashed in a muffle furnace. Sulfate was extracted by dissolving the ash in hot dilute HCl. The dissolved SO<sub>4</sub><sup>2-</sup> was precipitated with barium chloride and percent total S was determined turbidimetrically with a spectrophotometer at 420 nm against a calcium sulfate (CaSO<sub>4</sub>·2H<sub>2</sub>O) standard curve.

Dependency of total P uptake and S uptake on arbuscular mycorrhizal fungi for each fungal isolate was calculated based on Plenchette et al. (1983) as follows:

$$\text{Dependency of P uptake} = (\text{P content (+M)} - \text{P content (-M)}) / \text{P content} \\ \times (+M) \times 100;$$

$$\text{Dependency of S uptake} = (\text{S content (+M)} - \text{S content (-M)}) / \text{S content} \\ \times (+M) \times 100,$$

where +M denotes inoculated plants and -M uninoculated plants)

Two-way analysis of variance with replication was performed using the SAS statistical software package (Version 6.12; SAS Institute, Cary, NC) to determine the effects of mycorrhizal inoculation and sulfur supply level in the substrate.

## RESULTS

No AM colonization was observed on the roots of the uninoculated control plants (Table 1). Roots of inoculated plants were extensively mycorrhizal with a mean proportion of root length colonized ranging from 30 to 62%. The proportion of root length colonized was not significantly affected by sulfur supply

Table 1  
Mean shoot dry weight (g), root dry weight (g), shoot-to-root ratio and colonization rate (proportion of root length colonized by AM) of spring onion

Sulfur supply and mycorrhizal status	Shoot dry weight (g)	Root dry weight (g)	Shoot/Root	Colonization rate (%)
<b>0.1 mM SO<sub>4</sub><sup>2-</sup></b>				
Non-mycorrhizal	4.23	1.09	4.12	ND
<i>G. etunicatum</i>	4.55	1.48	3.31	35
<i>G. intraradices</i>	4.40	1.25	3.86	60
<i>G. mosseae</i>	4.40	1.45	2.94	57
<b>1.75 mM SO<sub>4</sub><sup>2-</sup></b>				
Non-mycorrhizal	4.18	0.97	4.50	ND
<i>G. etunicatum</i>	4.18	1.44	2.96	37
<i>G. intraradices</i>	4.45	1.15	3.87	62
<i>G. mosseae</i>	4.32	1.34	3.26	51
<b>4 mM SO<sub>4</sub><sup>2-</sup></b>				
Non-mycorrhizal	5.25	1.29	3.89	ND
<i>G. etunicatum</i>	5.11	1.55	3.08	30
<i>G. intraradices</i>	4.93	1.28	3.59	53
<i>G. mosseae</i>	4.99	1.70	3.08	60
Significance <sup>a</sup> due to:				
S supply	***	***	NS	NS
Inoculation treatment	NS	***	***	***
Inoculation × S supply	NS	NS	NS	**

<sup>a</sup>By analysis of variance; \*\*\*  $P < 0.001$ ; \*  $P < 0.05$ ; NS, not significant; ND, not determined (no colonization observed on roots of uninoculated controls).

and *G. etunicatum* produced the lowest colonization rate among the three AM fungi investigated.

Shoot dry weight was significantly affected by S supply levels but not by inoculation treatment (Table 1). Regardless of the inoculation treatment, shoot dry weight was lower with 1.75 mM  $\text{SO}_4^{2-}$  supply (except for plants inoculated with *G. intraradices*) and higher with 4 mM  $\text{SO}_4^{2-}$  compared to 0.1 mM  $\text{SO}_4^{2-}$ . In general, mycorrhizal plants had higher shoot dry weights than the controls at 0.1 and 1.75 mM  $\text{SO}_4^{2-}$  supply, but shoot dry weights of mycorrhizal plants were lower at 4 mM  $\text{SO}_4^{2-}$ . Root dry weight was significantly affected by both mycorrhizal inoculation and S supply level. Inoculation with AMF tended to be associated with higher root dry weight at all three S supply levels except for plants inoculated with *G. intraradices* at 4 mM  $\text{SO}_4^{2-}$  supply. Root dry weights of mycorrhizal and non-mycorrhizal plants followed the sequence  $4 > 0.1 > 1.75$  mM  $\text{SO}_4^{2-}$ . Shoot-to-root ratio was significantly affected by inoculation treatment, but not by S supply level (Table 1). Mycorrhizal colonization resulted in lower shoot-to-root ratio compared to the control. Shoot-to-root ratios of plants inoculated with *G. intraradices* were significantly higher than those of plants inoculated with the other two AM fungi.

Phosphorus concentrations in shoots and roots of mycorrhizal plants were significantly higher than these in non-mycorrhizal plants at all three S levels (Table 2). Shoot and root P concentrations of plants inoculated with *G. etunicatum* were usually lower than those of plants inoculated with *G. intraradices* or *G. mosseae*, whereas root P concentrations usually showed the opposite trend (except at 0.1 mM  $\text{SO}_4^{2-}$  supply). Sulfur levels in the substrate had no detectable influence on shoot or root P concentrations. Mycorrhizal P dependency ranged from 17 to 33%. Plants inoculated with *G. etunicatum* had the lowest mycorrhizal P dependency at 0.1 mM and 4 mM  $\text{SO}_4^{2-}$  supply, while plants inoculated with *G. intraradices* had the highest. At 1.75 mM  $\text{SO}_4^{2-}$  supply the P dependency of plants inoculated with *G. mosseae* was higher than that of plants inoculated with either of the other two fungi.

The EPY and total sulfur concentrations (TSC) in shoots and roots increased with increasing S supply level and were often higher in shoots and roots of mycorrhizal plants than of the controls. Comparing the three fungal inocula, plants inoculated with *G. intraradices* had the lowest EPY and plants inoculated with *G. mosseae* had the highest (except at 0.1 mM  $\text{SO}_4^{2-}$  supply). Shoot TSC values of plants inoculated with *G. etunicatum* were highest at 0.1 and 1.75 mM  $\text{SO}_4^{2-}$  supply, while at 4 mM  $\text{SO}_4^{2-}$  supply, shoot TSC did not differ significantly among the fungal isolates. Inoculation with *G. mosseae* led to the highest root TSC values among the three AM fungal treatments.

Sulfur contents of shoots and roots of plants increased significantly with increasing S supply and were usually significantly higher in mycorrhizal plants than in the controls. Mycorrhizal S dependency ranged from 10 to 35%. At 1.75 and 4 mM  $\text{SO}_4^{2-}$  supply, S dependency values of plants inoculated with *G. intraradices* were lower than those of plants inoculated with *G. etunicatum*,

Table 2  
Phosphorus concentration (%) and mycorrhizal P dependency (%) of spring onion

Sulfur supply and mycorrhizal status	Shoot P (%)	Root P (%)	Mycorrhizal P dependency (%)
<b>0.1 mM SO<sub>4</sub><sup>2-</sup></b>			
Non-mycorrhizal	0.10	0.11	-
<i>G. etunicatum</i>	0.11	0.15	23
<i>G. intraradices</i>	0.13	0.20	33
<i>G. mosseae</i>	0.12	0.15	26
<b>1.75 mM SO<sub>4</sub><sup>2-</sup></b>			
Non-mycorrhizal	0.10	0.12	-
<i>G. etunicatum</i>	0.11	0.20	28
<i>G. intraradices</i>	0.11	0.19	27
<i>G. mosseae</i>	0.12	0.16	30
<b>4 mM SO<sub>4</sub><sup>2-</sup></b>			
Non-mycorrhizal	0.10	0.13	-
<i>G. etunicatum</i>	0.10	0.18	17
<i>G. intraradices</i>	0.15	0.16	29
<i>G. mosseae</i>	0.12	0.16	25
Significance <sup>a</sup> due to:			
S supply	NS	NS	
Inoculation treatment	***	***	
Inoculation × S supply	NS	**	

<sup>a</sup>By analysis of variance; \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; NS, not significant.

which in turn were lower than those of plants inoculated with *G. mosseae*. At 0.1 mM SO<sub>4</sub><sup>2-</sup> supply, S dependency of plants inoculated with *G. etunicatum* was much higher than of plants inoculated with the other two AM fungi.

## DISCUSSION

The EPY value and sulfur nutritional status of spring onion were significantly enhanced by mycorrhizal colonization (Table 3). Although AM fungal-mediated growth promoting effects have been shown previously in pot experiments (Tawarayama et al., 1999; 2001), to our knowledge the present study is the first in which experimental observation has shown that mycorrhizal colonization can have a large influence on the pungency of spring onion. Previous experiments showed that mycorrhizal fungi tended to affect the pungency of *Allium cepa* (Guo et al., 2006a) and *Allium fistulosum* (Guo et al., 2006b). However, the EPY value did not differ significantly between the mycorrhizal plants and the



Table 3

Enzyme Produced Pyruvate (EPY,  $\mu\text{ mol ml}^{-1}$ ), shoot total sulfur concentration (TSC, %), root total sulfur concentration (%), shoot and root sulfur content ( $\text{mg pot}^{-1}$ ), and mycorrhizal S dependency (%) of spring onion

Sulfur supply and mycorrhizal status	EPY ( $\mu\text{ mol ml}^{-1}$ )	Shoot TSC (%)	Root TSC (%)	Shoot S content (mg)	Root S content (mg)	Mycorrhizal S dependency (%)
<b>0.1 mM <math>\text{SO}_4^{2-}</math></b>						
Non-mycorrhizal	6.56	0.15	0.25	6.44	2.77	-
<i>G. etunicatum</i>	9.56	0.20	0.31	9.00	4.54	32
<i>G. intraradices</i>	8.59	0.17	0.22	7.58	2.78	11
<i>G. mosseae</i>	9.00	0.13	0.31	5.92	4.39	10
<b>1.75 mM <math>\text{SO}_4^{2-}</math></b>						
Non-mycorrhizal	10.02	0.25	0.51	10.47	5.00	-
<i>G. etunicatum</i>	15.80	0.32	0.73	13.23	10.33	34
<i>G. intraradices</i>	14.88	0.28	0.62	12.62	7.12	22
<i>G. mosseae</i>	16.62	0.28	0.87	12.15	11.59	35
<b>4 mM <math>\text{SO}_4^{2-}</math></b>						
Non-mycorrhizal	13.67	0.30	0.94	15.97	12.18	-
<i>G. etunicatum</i>	20.18	0.41	1.09	21.08	16.91	26
<i>G. intraradices</i>	15.49	0.44	1.03	21.94	13.09	20
<i>G. mosseae</i>	21.18	0.42	1.22	20.86	20.72	32
Significance <sup>a</sup> due to:						
S supply	***	***	***	***	***	
Inoculation treatment	***	***	***	***	***	
Inoculation $\times$ S supply	**	***	***	NS	***	

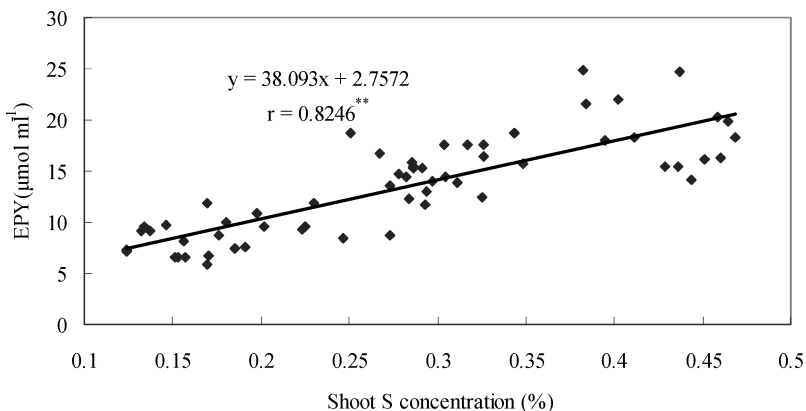
<sup>a</sup>By analysis of variance; \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*, NS, not significant.

controls in these earlier studies. In the present experiment, the higher EPY values of mycorrhizal plants may have been a consequence of enhanced S uptake as indicated in Table 3 which shows higher S nutritional status in mycorrhizal plants than in the non-mycorrhizal controls. Mycorrhizal plants had a strong S dependency, ranging from 10 to 35% (Table 3), which is comparable to the mycorrhizal P dependency which ranged from 17 to 33% (Table 2). The results indicate that AM fungi may make a large contribution to plant S uptake and may therefore be involved in the mediation of flavor pathways in spring onion. However, whether the enhanced S uptake by mycorrhizal plants was due to the improved P uptake, or the promotion of S uptake by mycorrhizal hyphae or roots (or even by both mechanisms) cannot be distinguished in the experimental design. Early results of Rhodes and Gerdemann (1978a, b) showed that the increase in S uptake by mycorrhizal plants was attributable to hyphal translocation of S to the host plant. Moreover, it is interesting to note that the EPY values of mycorrhizal plants at lower S supply levels was equivalent to or even

higher than those of non-mycorrhizal plants at higher S supply levels (Table 3). Therefore, it is suggested that effects other than the enhanced sulfur nutritional level due to mycorrhizal colonization may also play a role. The flavor precursors have been generally regarded to be involved in the defense reaction of plants against micro-organisms (Randle and Lancaster, 2002). Wounded plants deliver available sulfur to synthesize defense-related substances by activating genes involved in the metabolism of sulfur (Harada et al., 2000). This situation was highly unlikely to apply in the current experiment. However, the complex nature of plant S metabolism and of the biosynthesis of the flavor compounds indicates that further investigations would be required to elucidate the mechanisms by which AM fungi mediate flavor biosynthesis in spring onion.

The EPY value, shoot and root TSC, and S content of shoots and roots of spring onion all increased with increasing S supply level in the substrate (Table 3). These results were expected and similar results have been reported for onion plants by other investigators (Randle and Lancaster, 2002). Shoot total sulfur concentrations in spring onion ranged from 0.15 to 0.44 %, which falls within the range of S concentrations in onion leaves and bulbs as reported by Randle (1992). The results lead further evidence for the importance of S fertility in the S metabolism, and eventually the flavor intensity, of *Allium* plants.

There was a significant positive correlation between shoot TSC and EPY (Figure 1). Therefore, it is suggested that increased plant pungency is a consequence of elevated shoot total sulfur concentration. In contrast, Randle (1992) reported that the correlation between foliar % S or bulb % S and EPY was poor using 62 onion entries grown under conditions of high and low S fertility. The inconsistency between the experiments may be due to the different growth patterns of the two *Allium* species used. While spring onion plants have only a



**Figure 1.** Linear correlation and regression between shoot EPY and shoot total sulfur concentration in spring onion using the data from all 60 pots in the experiment; \*\*  $P < 0.01$ .

vegetative growth period, onion has periods of both vegetative growth and bulb formation. The flavor biosynthesis process may therefore be more complex in onion than in spring onion due to the translocation of S from the vegetative organs to the bulb at the period of bulb formation. Bloem et al. (2004) reported the translocation of *alliin* from leaves to bulbs at bulb the forming stage, indicating that harvest time had a strong influence on the *alliin* content of onion and garlic.

The strong influence of mycorrhizal colonization on the EPY values in the present experiment indicates the potential for enhancement of the nutritional and organoleptic value of spring onion by inoculation with arbuscular mycorrhizal fungi. Although no significant differences were found in plant EPY value among the three fungal inocula, the lower EPY value of plants inoculated with *G. intraradices* at all S supply levels (Table 3) indicates that AM fungi may differ in their regulatory effect on pungency of spring onion plants. In this context, more fungal taxa require investigation to elucidate the influence of AM fungi on the flavor attributes of the plants. The results also offer prospects for producing more pungent plants by the application of mycorrhizal technology and this may have the additional advantage of lowering sulfur loadings to the environment.

#### ACKNOWLEDGMENTS

This work was funded by the National Natural Science Foundation of China (Project GZ051-11(155)).

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