Spinach and pepper response to nitrogen and sulphur fertilization

M. Smatanová[1], R. Richter[2], J. Hlušek[2]

[1]*Central Institute for Supervising and Testing in Agriculture in Brno, Czech Republic*
[2]*Mendel University of Agriculture and Forestry in Brno, Czech Republic*

ABSTRACT

A vegetation pot experiment was established to explore the effect of two doses of nitrogen (0.6 and 0.9 g N in the form of ammonium sulphate) and two doses of sulphur (20.6 and 30.6 mg/kg of soil) on the yields and quality of spinach and pepper in comparison with a natural level (7.85 mg/kg). The results of the experiments confirmed that the application of sulphur by means of $(NH_4)_2SO_4$ in combination with nitrogen had a positive effect on yields and also on the quality of the vegetables. In the sulphur-free variants of spinach the effect was statistically significant and also when the levels of S in the soil were higher. Lower doses of nitrogen under increased levels of sulphur increased the yields statistically significantly (on average by 47%) and the sulphur concentration in the plants increased. The N:S ratio became narrower in proportion with the level of sulphur, particularly under a lower N level. The nitrate content in spinach corresponded with the applied dose of nitrogen and the nitrogen concentration. The sulphur level did not influence the content of C vitamin, but had a positive effect on the content of the essential amino acids cysteine and methionine. A mean level of S_1 in combination with a N_1 dose significantly increased pepper yields, narrowed the N:S ratio and was reflected in dry matter production per 1 g of N. The highest pepper yields were achieved with a dose of S_1, which resulted in the highest dry matter production in the fruit per 1 g of N under both levels of N. At the same time increasing the sulphur level reduced the content of nitrate and increased the level of cysteine from 0.11 to 0.305 g/kg.

Keywords: sulphur; nitrogen; spinach; pepper; yield; quality

Optimal nutrition is essential for high yields and good quality of horticultural crops. Compared to other agricultural crops, vegetables have very high demands for available nutrients in the soil.

Due to the reduction of air pollution, attention has recently been again devoted to sulphur as its action is very similar to the effect of nitrogen (Mengel and Kirkby 1978). The requirements of the various crops for S are dependent on the production of organic matter, on the content of proteins and of primary and secondary metabolites. Brassicas have the highest demands for sulphur fertilisation as they need sulphur for isothiocyanate synthesis (mustard oils); among the root vegetables species producing essential oil it is horseradish and radish; among the liliaceous it is onion and garlic; and among leaf vegetables spinach, and pepper from the group of fruit vegetables.

SO_2 is taken up from the atmosphere and utilised by aboveground parts of higher plants; however, the most important source of sulphur are sulphates taken up by the roots. Within the physiological span of pH a relatively low proportion of the SO_4^{2-} anion is taken up by the roots and is transported particularly in the xylem (Marchner 1995). The mobility of sulphur in the plants is lower. The transport of sulphur is acropetal, from the roots into the young leaves and meristems; sulphur cannot be transported from the older leaves into the young ones. The SO_4^{2-}content may be an indicator of the plants supply of sulphur. Within the Brassicaceae family most of the available data are on rape. According to Schnug and Haneklaus (1994) the reduction of S below 0.3% appears as hidden or evident symptoms of S deficiency.

The first step in the exploitation of S in the plant is the activation of the sulphate ion, which must be transformed by the ATP-sulphate adenine transferase enzyme, which replaces two phosphate groups with the ATP sulphuryl group, and leads to the production of adenosine phospho-sulphate (APS) and pyrophosphate. The APS sulphate is the initial substance for the incorporation of sulphur into the organic compounds in the plant. According to Marchner (1995) this incorporation of sulphur proceeds in two ways: by synthesis of sulphate

Supported by the Faculty of Agronomy, Mendel University of Agriculture and Forestry in Brno, Project No. CEZ-J 08/98: 432100001/11.

esters (path 1) and by reduction of sulphates (path 2). The result of the reaction is phosphoadenosine phosphate (PAPS). This activated sulphate is bound to the organic compounds, lipids, polysaccharides or is used for the production of glucosinolates (Booth et al. 1991).

The first stabile organic compound in path 2 is cysteine, from which another amino acid arises, i.e. methionine, which serves to produce enzymes and co-enzymes. The reduction of sulphates takes place in the chloroplasts and is activated by light, which is important for the detoxication of SO_2 in the leaves (Marchner 1995). This reaction leads to the production of glutathione, an important transport substance; according to De Kok and Stulen (1993) it acts as an antioxidant regulating the level of cysteine in the cell and is the precursor of phytochelatins.

Organic sulphur in the plants is the precursor of flavours, aromatic and taste substances, which are characteristic for some vegetables. Of no lesser importance are the effects of isothiocyanates, which are both toxic and pharmacological (anti-bacterial, anti-fungicidal) and can inhibit carcinomas (Hlušek et al. 2002, Shaw et al. 2003).

MATERIAL AND METHODS

In a vegetation pot trial we investigated the effect of three increasing doses of sulphur under two levels of nitrogen on the yields and quality of spinach and pepper. Spinach was grown as a preceding crop, followed by pepper.

The trial was established in plastic pots containing 11 kg of light sandy loam (fluvizem); the agrochemical characteristics are given in Table 1.

The experiment was carried out in the vegetation hall of the Department of Soil Agrochemistry and Plant Nutrition of the Central Institute for Supervising and Testing in Agriculture Brno, Czech Republic. Fertilisation was performed on April 4, 2002. All the fertilisers were applied prior to spinach sowing. The natural content of sulphur in the soil was 7.85 mg/kg in variants No. 1 and 2. The application of $(NH_4)_2SO_4$ modified the level of water-soluble S to 20.6 mg/kg (variants 3 and 4) and 30.6 mg/kg (variants 5 and 6); the data are given in Table 2. Nitrogen was supplemented in the form of NH_4NO_3. Spinach was supplemented with a dose of nitrogen on the level of N_1 = 0.6 g and N_2 = 0.9 g and after its harvesting pepper was supplemented with identical doses too. The content of calcium was increased with $CaCO_3$, and MgO was applied to supply magnesium. Each variant of fertilising had five replications.

Spinach, variety Monores was sown out on April 10, 2002, i.e. 12 seeds into each pot. Full emergence was reported on April 18; in the stage of two true leaves the pot was thinned to six plants and harvested in the stage of table maturity on May 22, 2002. After the spinach was harvested the soil in the pots was loosened and mixed and before the peppers were planted was additionally fertilised with nitrogen in doses of N_1 = 0.6 g and N_2 = 0.9 g. The 15 cm high seedlings were then planted out, (pairs) of peppers, variety Folik, which had been pre-cultivated in the soil used for the establishment of the trial. During vegetation Karate and Pyrimor, preparations for aphid control, were applied (3× and 2×, respectively). Peppers were harvested 3 times during vegetation (on August 5, August 28 and October 1). The experiment with peppers was terminated on October 2, 2002. During the experiment optimal soil moisture was maintained by watering with demineralised water.

Table 1. Agrochemical properties of soil before the establishment of the experiment

pH/ $CaCl_2$	Available nutrients (mg/kg soil) Mehlich III				S_{water} (mg/kg)
	P	K	Mg	Ca	
6.2	222	264	98	1520	7.85

The parameter, which was evaluated in spinach, was the weight of leaves and in pepper the weight of the fruit. The concentration of N and S, content of nitrates, ascorbic acid L and of the amino acids cysteine and methionine was determined in the harvested products.

N analysis was performed by mineralisation according to Kjeldahl (Zbíral 1994). Sulphur was determined after the combustion of plant material in HNO_3 and H_2O_2 using the AES-ICP. Nitrates were determined in fresh plants by direct potentiometry using the ion selective electrode. The level of ascorbic acid L was determined using the titra-

Table 2. Experimental scheme

Variant	Experimental scheme	S_{water} (mg/kg)	Nutrient level (g/pot) N	S
			NH_4NO_3	$(NH_4)_2SO_4$
1.	N_1S_0	6.8	0.6	–
2.	N_2S_0		0.9	–
3.	N_1S_1	20.6	0.6	0.63
4.	N_2S_1		0.9	0.63
5.	N_1S_2	30.6	0.6	1.08
6.	N_2S_2		0.9	1.08

Table 3. Average yields of spinach and contents of N and S in dry matter

Variant	Experimental scheme	Weight of spinach leaves per pot			Content in dry matter (%)		N:S	Production of dry matter/1 g N
		fresh		dry matter (g)				
		g	rel. %		N	S		
1.	N_1S_0	84.2	100.0	7.99	4.87	0.201	24.22	20.53
2.	N_2S_0	76.4	90.7	7.18	5.16	0.211	24.45	19.38
3.	N_1S_1	128.4	152.5	11.43	5.40	0.488	11.07	18.52
4.	N_2S_1	124.1	147.5	10.54	5.71	0.514	11.10	17.51
5.	N_1S_2	129.8	154.1	11.55	5.54	0.585	9.47	18.05
6.	N_2S_2	112.9	134.0	11.27	5.62	0.497	1.30	17.79

$DT_{0.05}$ = 6.3, $DT_{0.01}$ = 8.5

tion method according to ISO 6557/2. The amino acids were indicated in fresh matter by oxidative hydrolysis on the Amino Acid Analysator 400. Yields were evaluated statistically by uni-factor variance analysis with tests of the significance of the differences using Tukey's test with 95% reliability and plotted in graphs by means of confidence intervals.

RESULTS AND DISCUSSION

Evaluation of the effect of N and S doses on the yield and quality of spinach

Table 3 and Figure 1 show that higher doses of nitrogen slightly increased yields, and that S on the level of S_1 and S_2 increased yields statistically significantly compared to the variant not fertilised with sulphur. No statistically significant differences were detected between the doses of nitrogen and sulphur.

The highest spinach yields, i.e. 129.8 g, were achieved in the N_1S_2 variant. The relative increase compared to the N_1S_0 variant was 54.1%. In dependence on the level of S_1 = 20.6 mg/kg the second highest yield was reported after N_1S_1 fertilisation – 128.4 g; the relative increase was 52.5%. Table 3 shows that the nitrogen concentration increased in dependence on the N dose and the increased levels of S. Higher levels of N_2 resulted in a higher N content in all the variants. The N content was the highest in the N_2S_2 variant, i.e. 5.71% in the dry matter.

A similar situation was evident in terms of the S concentration. The dose of N_2 nitrogen in variant 4 resulted in a higher S content, but this trend changed in the N_2S_2 variant and a lower dose of nitrogen N_1S_2 – 0.585% S in dry matter had a better effect.

The N:S ratio was lower in plants in dependence on the increasing S level. Increasing the doses of N_2 nitrogen widened the N:S ratio. Dry matter production achieved with 1 g N is a calculated value and this indicator corresponds perfectly with the increasing dose of N. The amount of dry matter was not affected by N and S doses, but the optimal nutrition was reflected in the qualitative parameters (Table 4). The highest content of nitrates was detected in the S_0 variants (without sulphur application) due to the N_1 dose – 4737 mg/kg. The results also confirmed the conclusions of Schnug and Haneklaus (1994) nitrates accumulate in the plants if the S level is low. Increasing the level of S decreased the content of nitrates in spinach, proof of which is also the lowest value – 2307 mg/kg in the N_2S_2 variant. The Decree of the Ministry of Health No. 53/2002 specifies 2500 mg/kg as the highest permitted amount of nitrates for spinach harvested between April 1 and October 31. This limit was not exceeded only in variant 6. Fertilisation of leaf vegetables in terms of the increased content of

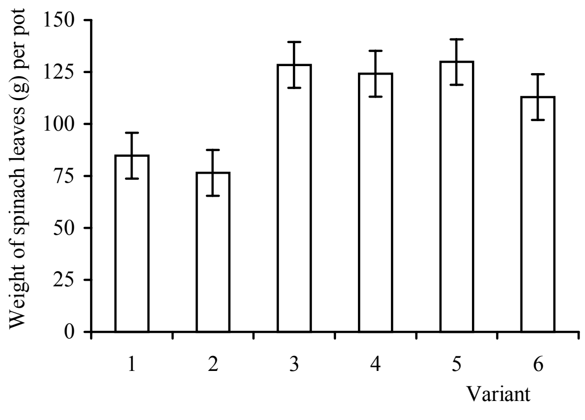

Figure 1. Spinach yields – confidence intervals

Table 4. Content of nitrates, ascorbic acid L, amino acids, N and S consumption (spinach)

Variant	Experimental scheme	Dry matter (%)	Fresh matter: NO_3^- (mg/kg)	Fresh matter: ascorbic acid L (ppm)	Fresh matter: amino acid (g/kg) cysteine	Fresh matter: amino acid (g/kg) methionine	Total consumption of elements (g/pot) N	Total consumption of elements (g/pot) S
1.	N_1S_0	9.5	4737	57.5	0.28	0.46	0.389	0.016
2.	N_2S_0	9.4	3015	51.9	0.32	0.41	0.370	0.015
3.	N_1S_1	8.9	2525	44.8	0.51	0.65	0.617	0.056
4.	N_2S_1	8.5	3540	44.4	0.58	0.76	0.602	0.054
5.	N_1S_2	8.9	4204	49.5	0.53	0.67	0.640	0.068
6.	N_2S_2	9.1	2307	52.7	0.45	0.66	0.577	0.051

nitrates in dependence on the N dose and external conditions is frequently influenced by a number of external factors (Maynard et al. 1976).

The level of ascorbic acid L decreased only slightly after the level of S was increased from S_0 to S_1 and S_2 and ranged within the framework of analytical error.

Of the amino acids, cysteine and methionine responded positively to sulphur fertilisation, in accordance with the findings of Eppendorfer (1968). The difference between the S_1 and S_2 levels was not very evident in the content of the above-mentioned amino acids. According to the results, the level of both amino acids was the highest in the N_2S_1 variant.

The total consumption of S and N by the plants showed that in proportion to the production of matter also the consumption of the two elements increased. It was proved that doses of sulphur at a S_1 and N_1 level ensured optimal matter production. Further increase in S and N doses to a N_2S_2 level did not considerably influence yields and qualitative parameters.

Evaluation of the effect of N and S doses on the yields and quality of pepper

The results (Table 5 and Figure 2) demonstrate that yields in variants 3 and 4 were higher, i.e. where medium doses of S (S_1) were applied under two different levels of nitrogen (N_1 and N_2). With the first dose of nitrogen the yields of fresh and dry matter increased to the S_1 level. A higher content of sulphur in the soil inhibited fruit yields, which was manifested in reduced weight (i.e. 21.6 g – 44.4%). An optimal level of sulphur, i.e. 20.6 g mg/kg of soil under N_1 (variants 3 and 4) had a positive effect on fruit yields compared to variants 5 and 6 where the decrease in yields was statistically significant.

Likewise, the N concentration in fruit increased in proportion to the dose of applied nitrogen. The changes in the sulphur content were more important; in peppers the content of S increased in proportion to the increasing S doses. Increasing the S level in the soil to 30.6 mg/kg did not influence its concentration. It can be assumed that such a dose signalises the beginning of the so-called

Table 5. Average yields of pepper and contents of N and S in dry matter

Variant	Experimental scheme	Weight of fruit per pot: fresh g	Weight of fruit per pot: fresh rel. %	Weight of fruit per pot: dry matter (g)	Content in dry matter (%) N	Content in dry matter (%) S	N:S	Production of dry matter/1 g N
1.	N_1S_0	148.3	100	11.8	2.42	0.110	22.00	41.49
2.	N_2S_0	105.6	71.2	9.9	2.51	0.080	31.34	39.84
3.	N_1S_1	173.1	116.7	16.1	2.13	0.209	10.19	46.95
4.	N_2S_1	147.5	99.3	13.6	2.33	0.228	10.22	42.92
5.	N_1S_2	116.3	78.4	11.2	2.39	0.231	10.35	41.84
6.	N_2S_2	82.5	55.6	8.5	2.57	0.243	10.58	38.91

$DT_{0.05}$ = 22.6, $DT_{0.01}$ = 30.4

Table 6. Content of nitrates, ascorbic acid L, amino acids, N and S consumption (pepper)

Variant	Experimental scheme	Dry matter (%)	Fresh matter				Total consumption of elements (g/pot)	
			NO_3^- (mg/kg)	ascorbic acid L (ppm)	amino acid (g/kg)			
					cysteine	methionine	N	S
1.	N_1S_0	8.0	55.6	175.0	0.12	0.11	0.287	0.013
2.	N_2S_0	9.4	114.6	203.9	0.11	0.11	0.249	0.008
3.	N_1S_1	9.3	31.1	129.9	0.23	0.12	0.343	0.034
4.	N_2S_1	9.2	43.1	147.9	0.35	0.15	0.316	0.031
5.	N_1S_2	9.6	43.5	124.5	0.29	0.12	0.267	0.026
6.	N_2S_2	10.3	51.9	110.9	0.35	0.16	0.218	0.021

luxury uptake by the plant, with no effect on further production of matter. The increased sulphur concentration in the fruit was reflected also in a lower N:S ratio, which decreased from 22 (variant 1) to 10.19 (variant 3) and from 31.3 (variant 2) to 10.22 (variant 4).

Table 6 shows that increasing levels of nitrogen increased the nitrate concentration (N_2) and the applied sulphur considerably decreased the nitrate content, which was most evident in variant 3 (N_1S_1) where it reached 31.1 mg/kg of fresh matter. The Decree of the Ministry of Health No. 53/2002 specified 200 mg/kg as the limit for the nitrate content in peppers and this limit was not exceeded in any of the experimental variants. Due to the increasing doses of sulphur in the soil the content of ascorbic acid L considerably decreased. The highest level was discovered in variant 2 (N_2S_0), i.e. 203.9 mg/kg, while the average value in the sulphur variants was 128.3 mg.

The content of cysteine, as an essential amino acid, increased after the application of sulphur on average from 0.11 to 0.35 g/kg, while methionine increased from 0.11 to 0.16 g/kg. The trend in the content of cysteine is similar as in spinach where it increased considerably after the application of sulphur (by as much as 100%). However, only slight differences in cysteine were discovered between the S_1 and S_2 levels. In the case of methionine, fertilisation with nitrogen and sulphur (variant 4) resulted in only negligible differences compared to variants 1 and 2.

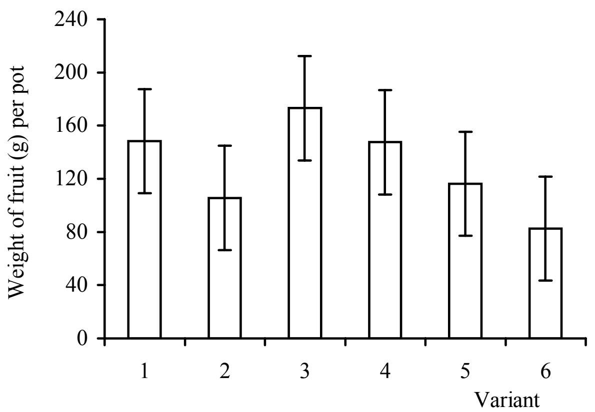

Figure 2. Total yields of pepper – confidence intervals

REFERENCES

Booth E., Walker K.C., Schnug E. (1991): The effect of site, foliar sulphur and nitrogen application on glucosinolate content and yield of oilseed rape. Proc. Int. Rapeseed Congr., Saskatoon, *2*: 567–572.

De Kok L.J., Stulen L. (1993): Functions of glutathione in plants under oxidative stress. In: De Kok L.J., Stulen L., Rennenberg H., Brunold C., Rauser W.E. (eds.): Sulphur nutrition and assimilation in higher plants, regulatory agricultural and environmental aspects. SPB Acad. Publ., The Hague: 125–138.

Eppendorfer W. (1968): The effect of nitrogen and sulphur on changes in nitrogen fractions of barly plants at various stages of growth and on yield and amino acid composition and grain. Plant Soil, *29*: 424–438.

Hlušek J., Richter R., Rigerová L. (2002): Sulphur in the nutrition and fertilization of vegetables. Chem. Inz. Ekol., *11*: 1383–1390.

Marchner H. (1995): Mineral nutrition in higher plants. Acad. Inc., London, UK.

Maynard D., Barker A.V., Minotti P.L., Peck N.H. (1976): Nitrate accumulation in vegetables. Adv. Agron. London, *28*: 71–118.

Mengel K., Kirkby E.A. (1978): Principles of plant nutrition. Int. Potash Inst. CH-3048 Worblaufen-Bern/Switzerland.

Schnug E., Haneklaus S. (1994): Sulphur deficiency in *Brassica napus*. Landb.-Forsch. Völkenrode, Fal. Braunschweig, *144*: 31.

Shaw M., Pither-Joyce M., McManus M., McCallum J. (2003): Purification, characterization, and cloning of γ glutamil transpeptidase. Progress in plant sulfur research 1997–2003: 34.

Zbíral J. (1994): Analýza rostlinného materiálu. Jednotné pracovní postupy. SKZÚZ, Brno.

Received on October 23, 2003

ABSTRAKT

Reakce špenátu a papriky na hnojení dusíkem a sírou

Ve vegetačním nádobovém pokusu byl sledován vliv dvou dávek dusíku (0,6 a 0,9 g N ve formě síranu amonného) a dvou dávek síry (20,6 a 30,6 mg/kg zeminy) ve srovnání s přirozeným obsahem (7,85 mg/kg zeminy) na výnos a kvalitu špenátu a papriky. Výsledky nádobových pokusů prokázaly, že aplikace síry prostřednictvím $(NH_4)_2SO_4$ v kombinaci s dusíkem působila pozitivně jak na výnos, tak na kvalitu zelenin. U špenátu se projevil statisticky průkazný vliv mezi variantami bez síry a s jejími zvýšenými hladinami v půdě. Nižší dávky dusíku při zvýšené hladině síry výnos statisticky průkazně zvyšovaly (nárůst v průměru o 47 %) a v rostlinách vzrůstala koncentrace síry. Poměr N : S se snižoval úměrně s hladinou síry, zejména však u nižší dávky dusíku. Obsah nitrátů korespondoval s aplikovanou dávkou dusíku a se zjištěnou koncentrací dusíku ve špenátu. Hladina síry se nepromítla do obsahu vitaminu C, ale pozitivně ovlivnila obsah esenciálních aminokyselin cysteinu a methioninu. Střední hladina S_1 v kombinaci s dávkou N_1 průkazně zvyšovala výnos plodů paprik, snížila poměr N : S a odrazila se i v produkci sušiny na 1 g N. Nejvyššího výnosu plodů paprik bylo dosaženo u dávky S_1, která při obou dávkách N vedla k nejvyšší produkci sušiny plodu na 1 g dusíku. Současně zvýšení dávky síry snižovalo obsah nitrátů a zvýšilo hladinu cysteinu z 0,11 na 0,305 g/kg.

Klíčová slova: síra; dusík; špenát; paprika; výnos; kvalita

Corresponding author:

Ing. Michaela Smatanová, Ústřední kontrolní a zkušební ústav zemědělský v Brně, Hroznová 2, 656 06 Brno, Česká republika
phone: + 420 543 548 316, fax: + 420 543 548 318, e-mail: michaela.smatanova@ukzuz.cz