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The effect of sulphur fertilization on yield, quality of protein and baking properties of winter wheat

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Abstract. The present paper is based on the data of field and production trials conducted in the years 2004–2007. The trials were carried out in North-Estonia (59° 18' N, 24° 39' E) on break-stony soil and in South-Estonia (58° 27' N, 25° 36' E) on pseudopodzolic soil. The aim was to identify the effect of sulphur fertilization on the yield of winter wheat *(Triticum aestivum L.)* on some of the quality indices of yield and protein quality, including the content of non-replaceable amino acids, and on the baking properties of flour. In the field trials the effect of N and NS fertilization was compared on the nitrogen background of N_{60} + N_{40} kg ha^{-1}. Due to sulphur (in two top dressings in total S_{10} kg ha^{-1}) the yield of winter wheat 'Lars' increased, depending on the weather and soil conditions, in field trials 0.47–1.48 t ha^{-1}, i.e. 7.7–43.0% and in production trials 1.35–2.44 t ha^{-1}, i.e. 39.8–45.5%. The effect of sulphur on the protein and wet gluten contents of wheat grain was not always one-directional, but in all trials the gluten index increased and the quality of protein improved under the influence of sulphur. Sulphur fertilization increased the content of amino acids in the protein of winter wheat in field trials on the average as following: cysteine – 24.5%, methionine – 35.3%, threonine – 14.4% and lysine – 7.7%. In production conditions the fertilization with sulphur increased both the contents of protein and wet gluten and that of major amino acids. Due to sulphur fertilization all major parameters of winter wheat's baking quality improved: stability and quality number of dough, loaf volume and specific volume and round loaf's height to diameter ratio.

Key words: winter wheat, sulphur fertilization, protein, amino acids, baking quality

INTRODUCTION

The incidence of sulphur deficiency has increasingly been reported in cereal crops in Western Europe over the last decade. Decreasing sulphur deposition from the air, and the use of more concentrated phosphate fertilizers that contain less sulphur, has led to reports of sulphur deficiencies in winter wheat. Sulphur deficiency significantly effects the production and quality of winter wheat (Zhao et al., 1999a; McGrath, 2003; Györi, 2005).

Without adequate sulphur, crops can't reach their full potential in terms of yield, quality or protein content; nor can they make efficient use of applied nitrogen (Sahota, 2006). At high nitrogen fertilization levels significant responses to sulphur fertilization were found which emphasised the need for precision application of sulphur in intensive wheat production systems. Continued use of nitrogen fertilizer without supplemental

sulphur on low sulphur soils will reduce flour quality (Ruiter & Martin, 2001; Flaete et al., 2005).

Sulphur does not affect only nitrogen utilisation and grain quality, but it also plays an important part in the formation of the baking quality (Ryant & Hřivna, 2004).

Reproductive growth of wheat appears to be more sensitive to sulphur deficiency than vegetative growth, with decreased grain size under sulphur-limiting conditions. In addition to the effects on yields, the sulphur status of wheat grain is an important parameter for the quality of wheat products (Marschner, 1997; Zhao et al., 1999a; McGrath, 2003; Honermeier & Simioniuc, 2004).

Sulphur deficiency in crop plants has been recognized as a limiting factor not only for crop growth and seed yield but also for poor quality of products, because sulphur is a constituent of several essential compounds such as cysteine, methionine, coenzymes, thioredoxine and sulfolipids. It was shown that sulphur application altered the amino acid composition with a greater proportion of sulphur containing cysteine and methionine (Einfluss ..., 2001; Singh, 2003).

Limiting sulphur availability has been shown to favour the synthesis and accumulation of S-poor or low-S storage proteins at the expense of S-rich proteins. (Zhao et al., 1999a; Flaete et al., 2005).

The protein fraction is known to play the most essential role for bread-making. Mature wheat grains contain 8–20% proteins. The gluten proteins, the gliadins and glutenins, constitute up to 80–85% of total flour protein, and confer properties of elasticity and extensibility that are essential for functionality of wheat flours (Kuktaite, 2004).

Responses of the breadmaking quality of wheat to sulphur are more common than responses in terms of grain yield. Sulphur application did not affect grain protein concentration directly, but tended to increase gel protein weight in flour and the proportion of polymeric proteins. Dough extensibility was increased by sulphur. Correlation and regression analyses showed that grain protein concentration was a poor indicator of loaf volume, whereas grain sulphur status (sulphur concentration and N : S ratio) was more influential (Zhao et al., 1999c).

Sulphur deficiency decreases grain size and baking quality because of formation of disulfide bonds formed from the sulphydryl groups of cysteine. This effects the viscoelasticity of dough (Györi, 2005). The baking quality of wheat was improved by sulphur application, showing high correlation between loaf volume and the sulphur content of grain and thus improving rheological properties (extensibility) of dough (Singh, 2003). Sulphur deficit may result in harder grain; the dough made from such grain is usually stiff and is not elastic (Ryant & Hřivna, 2004).

Recent studies have also shown that breadmaking quality correlated more closely with grain sulphur concentration than with nitrogen concentration (Zhao et al., 1999b; McGrath, 2003).

A synergistic effect between the applied nitrogen and sulphur fertilizers appears to increase nitrogen and sulphur assimilation in wheat grain and may improve bread-making qualities. When nitrogen and sulphur fertilizers were applied simultaneously, flour protein content and dough strength, swelling and extensibility were increased (Tea et al., 2007).

The sulphur nutrition of wheat grain, measured as sulphur concentration and N : S ratio, is an important factor affecting the breadmaking quality of wheat. High grain

sulphur concentrations and low N : S ratios were associated with increasing concentrations of soluble glutenin in flour and reduced ratios of insoluble to soluble glutenin in flour. As a result, there were probably more cysteine residues available for the production of disulphide and other types of bonds, producing dough that was more extensible and pliable, ultimately producing bread loaves of better quality (e.g. higher loaf volume). According to these results, it is evident that the sulphur nutrition of grain, measured as total sulphur concentration and N : S ratio should be considered, in addition to the concentration of nitrogen, in the quality evaluation of wheat grain (Flaten, 2004). The trials conducted by the Estonian Research Institute of Agriculture at different sites during several years have shown that even in the Estonian conditions winter wheat starts suffering more and more from sulphur deficiency, and that sulphur fertilization during the growing season considerably enables an increase in the yielding ability (Järvan & Adamson, 2004; 2005).

As to wheat for human consumption, in addition to the usual quality indices (moisture, test weight, falling number) that first of all depend on the weather and post-harvest processing, the biological quality of protein is extremely important. The baking properties of wheat, its quality as bread-making cereal, depend on it as well. The protein content can be increased by nitrogen fertilization. But in this case, as a rule, the biological quality of protein decreases, as the proportion of low-value reserve proteins increases and that of proteins soluble in water and salts decreases.

The biological quality of protein improves with the increase of the content of non-replaceable amino acids, in particular of amino acids containing sulphur and participating actively in metabolism (Hagel, 1999). Sulphur-containing methionine and cysteine are very important from the point of view of wheat's baking properties.

The aim of this research was to identify the impact of sulphur fertilization on the yield of winter wheat, on some of the quality indices of yield and protein quality, including the content of non-replaceable amino acids, and on the baking properties of flour.

MATERIALS AND METHODS

The article is based on the quality analyses and contents of major amino acids of grain samples taken from trial variants of four field trials and two production trials, and analyses and test bakings of flours made from the production trials' grain.

Field trials were conducted in 2004, 2005 and 2007 in Saku in North-Estonia (59° 18' N, 24° 39' E) and in 2005 in Auksi in South-Estonia (58° 27' N, 25° 36' E). Trial soils and their agrochemical properties were as follows: in Saku break-stony soil – Calcaric Cambisol (FAO…1994), pH_{KCl} 6.6–7.1, humus 2.9–3.1%, P 90–116 mg kg^{-1} (DL method), K 168–206 mg kg^{-1} (DL method), Mg 52–87 mg kg^{-1} (AL method), and mobile S 8–10 mg kg^{-1} (ISO 11048); in Auksi pseudopodzolic soil – *Podzoluvisol* (FAO ..., 1994), pH_{KCl} 6.2, humus 2.8%, P 80, K 156, Mg 81 mg kg^{-1}, and mobile S 6 mg kg^{-1}.

The trials were performed on the nitrogen background of N_{100} that was applied as solid top dressing divided into two portions N_{60} + N_{40} kg ha^{-1} at the beginning and end of tillering. In the control variant ammonium nitrate (N_{100} S_0, variant B) was used; in the second variant (variant C) the same nitrogen rate was applied with the fertilizer

Axan (containing 27% N and 2.7% S) or Axan Super (containing 27% N and 3.7% S). In the field trials in Saku there was also a variant D, in which Axan or Axan Super was applied in the first top dressing and ammonium nitrate in the second. The trials included also a non-fertilized variant A – a so-called field background.

The field trials were performed on 25 m² trial plots in four replications. Yields were harvested with a combine harvester, dried and sorted and calculated to 14% moisture. Grain samples were taken from all four replications of the trial variants; preliminary quality indices (falling number, contents of protein and wet gluten and gluten index) were determined from each of them. For the determination of amino acids an average grain sample was made for each variant and these samples were analysed in the laboratory in three replications. The results were processed using dispersion analysis.

The production trials were conducted in Viljandi County in Auksi in two winter wheat fields of Lapi Farm. In trial 1 (Auksi-Otsa), summer wheat was used as a preceding crop; the soil's pH_{KCl} was 6.2, P 81, K 140, Mg 113 mg kg^{-1}. In trial 2 (Auksi-Oru), field grass fallow was a preceding crop, soil's pH_{KCl} being 6.0, P 211, K 214, Mg 128 mg kg^{-1}. Prior to sowing in autumn N_{12} P_{26} K_{75} S_9 kg ha^{-1} was applied with a complex fertilizer. In the top dressing of winter wheat the effect of ammonium nitrate (variant B) and that of Axan Super (variant C) were compared. In two top dressings nitrogen was applied in total 75 kg ha^{-1}. A third fertilization that was planned for the stage of stem elongation to improve the quality of yield was not performed. Due to extraordinary long drought the effectiveness of solid fertilizers applied at such a late stage would probably have remained very low. The production trials were harvested with a combine. In the production fields yields were calculated for both trial variants from areas of 2 ha after drying and sorting of grain. From the trial variants' yields samples were taken to determine the quality indices of wheat and to make test bakings.

The quality analyses and test bakings were performed in the plant production laboratory of the Agricultural Research Centre according to the following methods: moisture content – ICC 110/1:1976; wet gluten content – ICC 155:1994; protein content – ICC 105/2:1994; determination of falling number – ISO 3003:2004; farinographic analysis of flour – ICC 115/1:1992; determination of amino acids – 98/64EMÜ HPLC UV; nitrogen and sulphur elemental analysis – ISO 15178:2000; baking trial – PMK TMAL manual no 11, reference to baking trial manual of Helsinki Kasvintuotannon Tarkastuskeskus Viljalaboratorio and GOST 27669.

RESULTS AND DISCUSSION

Sulphur fertilization affected the yielding ability of winter wheat 'Lars' and the quality indices of yield differently in different years (Table 1). Two topdressings with Axan (variant C, total N_{100} S_{10} kg ha^{-1}), in comparison with the fertilization with ammonium nitrate (variant B, N_{100} kg ha^{-1}), increased the wheat yield in Saku on break-stony soil under the weather conditions of 2004 by 43.0% and in 2005 by 15.7%, and in Auksi on pseudopodzolic soil in 2005 by 7.7%. When Axan was applied in the first fertilization round and ammonium nitrate in the second (variant D), the wheat yield on break-stony soil in 2004 was 31.4%, in 2005 12.8% and in 2007 9.0% higher than in the case of fertilizing only with ammonium nitrate.

Table 1. The effect of fertilization on the yield and biological quality of proteins on winter wheat (var. Lars) in field trials.

Location, year, treatment	Fertilizer rate kg ha^{-1}	Yield t ha^{-1}	Protein %	Wet gluten %	Gluten index %	Amino acids in grain, g kg^{-1}				Amino acids in protein, g kg^{-1}			
						CYS	THREO	METH	LYS	CYS	THREO	METH	LYS
Saku, 2004													
A	0	3.15	10.6	23.0	77	2.60	3.17	2.04	4.88	24.5	29.9	19.2	46.0
B	N_{100}	3.44	14.1	31.9	45	2.34	3.73	1.86	5.24	16.6	26.7	13.2	37.2
C	N_{100} S_{10}	4.92	11.6	25.1	74	2.53	3.75	2.14	4.66	21.8	32.3	18.4	40.2
D	N_{100} S_6	4.52	12.1	26.6	68	-	-	-	-	-	-	-	-
LSD_{05}		0.27	0.7	1.8	7	0.07	0.28	0.04	0.05				
Saku, 2005													
A	0	4.58	11.1	21.9	79	2.35	2.82	1.77	3.28	21.2	25.4	15.9	29.5
B	N_{100}	5.08	13.9	31.5	32	2.23	2.74	1.26	3.13	16.0	19.7	9.1	22.5
C	N_{100} S_{10}	5.88	13.4	30.2	51	2.91	3.40	1.98	3.65	21.7	25.4	14.8	27.2
D	N_{100} S_6	5.73	13.2	30.3	49	-	-	-	-	-	-	-	-
LSD_{05}		0.32	0.4	0.9	11	0.31	0.16	0.19	0.23				
Auksi, 2005													
A	0	3.52	11.5	20.8	86	2.42	2.95	1.82	4.55	21.0	25.6	15.8	39.6
B	N_{100}	6.11	13.2	27.6	61	2.75	3.63	2.11	5.11	20.8	27.5	16.0	38.7
C	N_{100} S_{10}	6.58	13.7	29.3	82	3.10	3.53	2.29	5.30	22.6	25.8	16.7	38.7
LSD_{05}		0.45	0.5	2.2	16	0.15	0.22	0.07	0.12				
Saku, 2007													
A	0	3.47	7.9	11.2	98	1.47	3.09	1.12	2.56	18.6	39.1	14.2	32.4
B	N_{100}	5.66	10.6	22.8	75	1.74	4.03	1.34	2.77	16.4	38.0	12.6	26.1
C	N_{100} S_{14}	5.92	10.1	21.0	90	1.76	3.90	1.40	2.53	17.4	38.6	13.9	25.0
D	N_{100} S_7	6.17	10.0	20.7	92	1.71	3.85	1.33	2.49	17.1	38.5	13.3	24.9
LSD_{05}		0.36	0.4	0.6	5	0.08	0.23	0.07	0.15				

A — field background; B — ammonium nitrate; C — Axan; D — Axan + ammonium nitrate

The difference in effectiveness of sulphur fertilization in different years was first and foremost caused by different weather conditions during the growth periods, but probably also by differences in fertilization during autumn sowing. In all field trials early red clover seed field was a preceding crop; it was ploughed prior to winter wheat sowing. In Saku in the trials of 2004 and 2005 no fertilizer was applied to wheat in autumn; in 2007 N_{12} P_{26} K_{50} S_{15} kg ha^{-1} was applied with a complex fertilizer during sowing. In the trial in Auksi wheat received during sowing N_{12} P_{26} K_{75} S_9 kg ha^{-1}.

In the spring of 2005 winter wheat plants did not show as serious sulphur deficiency symptoms as in the cold and dry spring of 2004; probably a sufficient amount of sulphur was released evenly in the soil. Therefore, in the conditions of 2005 the effectiveness of sulphur applied in topdressing remained relatively low (Adamson & Järvan, 2006). Even when a considerable amount of sulphur has been applied to soil in autumn, the effectiveness of sulphur fertilization in spring may remain relatively low. The trials that were conducted in several years on different soils with many trial variants indicated that sulphur should be applied to winter wheat in the first topdressing (Adamson & Järvan, 2006). The effect on the yielding ability is considerably lower with late sulphur application. The investigations by Luo et al. (2006) showed that late sulphur application was not necessary for optimising most bread-making quality parameters, either.

The results of field trials indicated that although on break-stony soil sulphur fertilization of winter wheat reduced the contents of protein and wet gluten, sulphur increased the biological value of proteins. The first indication was the increase of gluten index. In the case of NS-fertilizer the gluten index was 22.7–64.4% higher than with N-fertilizer. A possible decrease in the protein content after sulphur fertilization has been observed also by other scientists. Protein content in grain may decrease when crop yield responds to sulphur, likely due to a dilution of nitrogen in the grain (Ron & Loewy, 2007).

Sulphur fertilization increased the content of sulphur-containing amino acids in winter wheat. On the average of three trials conducted in Saku on break-stony soil the cysteine content increased 14.3% and the methionine content 23.7%. Investigating changes in the contents of major amino acids (cysteine, treonine, methionine and lysine) in the protein of winter wheat, it became evident that fertilization affected their proportions. In the case of applying only nitrogen (variant B) the content of amino acids was, as a rule, lower than in the non-fertilized variant (variant A); sulphur application improved the protein quality. On the average of three trials in Saku, NS-fertilization (variant C) in comparison with N-fertilization (variant B) increased the amino acid content in the protein of winter wheat as follows: cysteine – 4.0 g kg^{-1}, i.e. 24.5%, treonine – 4.0 g kg^{-1}, i.e. 14.4%, methionine – 4.1 g kg^{-1}, i.e. 35.3% and lysine – 2.2 g kg^{-1}, i.e. 7.7%.

The investigations by Timms et al. (2006) showed that increasing protein content correlated with a lower proportion of the sulphur amino acids cysteine and methionine. Applied sulphur positively affects the quality of wheat flour by increasing gluten and protein concentration. Furthermore, sulphur positively affects the development, stability, softening, and quality of dough as well as bread volume. Wheat fertilization with sulphur and nitrogen affects sulphur content and N : S ratio in grain (Podlesna & Cacak, 2008).

Concentrations of sulphur (S) in wheat grain below 1.2 mg g^{-1} are below the critical value. For breadmaking wheat the N : S ratio in grain must be lower than the critical value of 17 (Zhao et al., 1995; Sahota, 2006). In the three trials carried out in Saku on break-stony soil the sulphur content of wheat grain was in variant B (N_{100} kg ha^{-1}) 1.04–1.16 mg g^{-1} and in variant C (N_{100} S_{10} kg ha^{-1}) 1.46–1.55 mg g^{-1}. The nitrogen and sulphur ratio (N : S) in wheat grain was in variant B 17.0–20.8 and in variant C 13.0–15.2. Thus, winter wheat that was fertilized only with nitrogen did not meet the required parameters. When sulphur was applied in addition to nitrogen, the above-mentioned baking parameters improved.

The positive effect of sulphur fertilization was particularly evident in the production trials conducted in South-Estonia on pseudopodzolic soil in 2006 (Table 2). In the comparison with ammonium nitrate (variant B, two applications in total N_{75} kg ha^{-1}), fertilization with Axan (variant C, N_{75} S_{10} kg ha^{-1}) increased the yield of winter wheat by 39.8–45.5%.

Table 2. The effect of fertilization on the yield and biological quality of proteins of winter wheat (var. Lars) in production trials in 2006.

Location Treatment	Rate kg ha^{-1}	Yield t ha^{-1}	Protein %	Wet gluten %	Gluten index %	Amino acids in grain, g kg^{-1}			
						CYS	THRE	METH	LYS
Trial No 1 Auksi-Otsa									
B	N_{75}	3.39	10.6	20.6	97	2.03	3.75	1.48	2.75
C	N_{75} S_{10}	4.74	12.0	24.7	88	2.56	4.33	1.65	2.88
$LSD_{0.95}$						0.12	0.28	0.19	0.17
Trial No 2 Auksi-Oru									
B	N_{75}	5.36	9.9	17.4	99	1.93	3.53	1.32	2.48
C	N_{75} S_{10}	7.80	13.0	26.1	96	2.53	4.68	1.66	3.02
$LSD_{0.95}$						0.12	0.15	0.10	0.07

B — ammonium nitrate; C — Axan Super

The below normal protein and wet gluten content levels in these trials were probably caused, on one hand, by the low level of nitrogen fertilizers and, on the other hand, by a very warm and precipitation-poor summer.

Time, as expressed through seed-fill duration, is an important component of the productivity and quality of grain crops. Seed-fill duration decreases as temperature increases over 30°C. Water stress during seed filling accelerates leaf senescence and shortens the seed-filling period (Egli, 2004). It is well known that climate influences baking quality by altering yield and/or the protein content of the wheat. The weather conditions during the grain ripening period had the greatest effect on the winter wheat grain protein and wet gluten content, gluten index, sedimentation, and falling number (Mašauskiene & Cesevičiene, 2006). Different temperatures, especially during the grain filling period, induced different baking qualities of the wheat (Hagel, 2005).

As a rule, on break-stony soil sulphur fertilization reduced the protein and wet gluten contents of winter wheat (Table 1); in the production trials on pseudopodzolic

soil these indices on the contrary increased (Table 3). Several scientists (Ryant, 2002; Honermeier & Simioniuc, 2004) claim that sulphur has a relatively low and unstable effect on the wheat's protein content; however, it increases the biological quality of protein. In the production trials sulphur increased the protein and wet gluten contents of wheat grain. The wet gluten quality improved considerably, because the content of amino acids increased as follows: cysteine – 26.1–31.1%, treonine – 15.5–32.6%, methionine – 11.5–25.8% and lysine – 4.7–21.8%.

Table 3. The effect of sulphur fertilization on baking properties of winter wheat grown on production fields in 2006 weather conditions.

Properties	Location and treatment, kg ha^{-1}				Standard flour T-550
	Auksi – Otsa		Auksi - Oru		
	N_{75}	N_{75} S_{10}	N_{75}	N_{75} S_{10}	
FLOUR					
protein,%	9.5	11.0	8.6	11.9	11.9
falling number, sec	372	377	317	380	349
wet gluten, %	21.9	26.8	19.6	29.1	28.1
gluten index, %	97	93	97	97	99
DOUGH					
water absorption, %	58.3	55.8	55.5	60.6	57.1
development time, min	1.8	1.7	1.5	2.2	1.7
stability, min	2.9	6.3	2.4	4.2	11.6
degree of softening	97	76	106	87	59
quality number	31	40	24	34	38
BREAD					
volume, cm^3	1252	1551	1095	1465	1640
specific volume, cm^3 g^{-1}	3.34	4.23	2.98	3.92	4.49
specific volume : protein	0.35	0.39	0.35	0.33	0.39
height : diameter	0.41	0.54	0.47	0.64	0.42
porosity 1:10	4	6	7	6	7
porosity, %	79	79	76	77	82

Our earlier studies (Järvan et al., 2006) indicated that the baking properties of winter wheat, which had been fertilized with nitrogen but grown under sulphur deficiency, became poor as the stability and quality of dough, the loaf volume and height-to-diameter ratio decreased. Similar results have been obtained by other scientists. Deficient sulphur nutrition gave a lower loaf volume and texture score (Honermeier & Simioniuc, 2004; Hagel, 2005; Timms et al., 2006).

Baking properties of winter wheat were determined and test bakings made from grain of production trials. At the stages of flour, dough and bakings, a total of 15 parameters were determined and are presented in Table 3.

Due to sulphur fertilization all major parameters of winter wheat's baking quality improved: stability and quality number of dough, loaf volume and specific volume and round loaf's height-to-diameter ratio. This year it was not possible to make attractive bakings of normal volume using wheat of the sulphur-less variant. The volumes of loaves made from wheat that had been fertilized only with nitrogen were 23.9–33.8% lower than those of products made from wheat fertilized with nitrogen and sulphur.

Decreasing sulphur contents lead to ever firmer dough and low baking volumes, whereas sulphur fertilization and increasing sulphur content of the wheat grain induces less tough dough and higher baking volumes (Hagel, 2005).

CONCLUSIONS

1. Sulphur fertilization (S_{10} kg ha^{-1}) on nitrogen background (N_{100} kg ha^{-1}) increased the winter wheat yield, depending on weather and soil conditions, 7.7–45.5%.
2. The effect of sulphur on the protein and wet gluten contents of wheat grain was not always one-directional, but in all trials the gluten index increased and the quality of protein improved under the influence of sulphur. Sulphur fertilization increased the content of amino acids in the protein of winter wheat in field trials on average as follows: cysteine – 24.5%, methionine – 35.3%, threonine – 14.4% and lysine 7.7%.
3. Due to sulphur fertilization all major parameters of winter wheat's baking quality improved: stability and quality number of dough, loaf volume and specific volume and round loaf's height-to-diameter ratio.
4. As to the baking properties of wheat, not only the protein and wet gluten contents are important parameters of baking quality, but also the quality of protein, including the proportion of sulphur-containing amino acids. Thus, in the production of bread-making wheat sulphur deficiency should be avoided to the extent possible.

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