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Effect of sulphur and micronutrients fertilization on yield and fat content in winter rape seeds (*Brassica napus* L.)

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ABSTRACT

Three-year strict experiments with winter rapeseed were conducted in three experimental stations. In the research, winter rape fertilization with elemental sulphur (S) in the doses: 20, 40 and 60 kg S/ha, boron (B) – in the dose of 2 kg B/ha and copper (Cu) – 5 kg Cu/ha were applied. Microelements were introduced separately, in treatments B and Cu, as well as in combination B + Cu. The experimental soils featured low content of sulphate sulphur SO_4^{2-}-S and boron, medium and low copper content. Rapeseed fertilization with sulphur, boron and copper resulted in their increased concentration in plants. Sulphur at 40 and 60 kg S/ha doses affected the increase in oilseed rape grain yield by 11–12% compared to the not fertilized treatment. The dose of 20 kg S/ha did not show any significant influence on yield. The yield increased by more than 10% in the comparison to the treatment without B and Cu, was obtained due to boron, as well as combination of boron and copper fertilization. A significant increase in fat content, in relation to not fertilized treatment, ranking from 1.0–1.4% dry matter, was recorded after fertilization with the highest sulphur dose – 60 kg S/ha, as well as after application of boron and copper fertilization.

Keywords: macroelement; sulfur deficit; plant nutrition; yield-forming effect; canola

Sulphur (S) is one of four major macroelements, after nitrogen, phosphorus and potassium, which is considered as indispensable as far as appropriate plant growth and development are concerned (Anjum et al. 2012). In the regions of intensive rapeseed production, a significant negative balance of this component was observed. Plant malnutrition due to sulphur deficit has become one of the important problems in modern agriculture, especially in the Northern European countries and many other countries all over the world. The data by the Sulphur Institute, Washington, indicate that in 2000 global sulphur deficit reached 7.5 million tons per year (Scherer 2001). According to the Sulphur Institute (TSL), plant nutrient sulphur deficit in 2010 (in tons) was 5.8 mln in Asia, 1.5 mln in North America , 1.5 mln in Africa, 0.9 mln in Latin America and 1.0 mln in Europe. It is estimated that in 2015 the mentioned deficit will amount 12.5 million tons per year (Messick 2013). These forecasts result mainly from considerable reduction in sulphur compounds emission to the atmosphere, and therefore, in diminished sulphur deposition on the areas of agricultural production. Moreover, soil richness in microelements has been systematically decreasing. Worldwide limiting micronutrients (as well as in Poland) are boron (B) and copper (Cu). These micronutrients are essential for rapeseed, as they influence plant generative development, as well as the development of plant roots (Nadian et al. 2010). In Poland, boron deficit in soils is estimated at 90% of arable land whereas copper deficit at 40%. The mentioned components are indispensable for proper growth and development of rapeseed and they also participate in fat metabolism. The reports on boron deficit for plants in the world refer mainly to Asian countries, like China, India or Pakistan. Low boron content brings about a considerable decrease in wheat yield, in spite of the fact that this crop shows low sensitivity to this microelement deficit (Rerkasem and Jamjod 2004). Approximately 9 million ha of sandy and sandy loam soils in Australia characterizes copper deficit, while in Canada 1.5 million ha of

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arable land require fertilization with this nutrient (Karamanos and Goh 2005).

The objective of this study was to evaluate the influence of combined fertilization with different doses of sulphur, boron and copper on yield and fat content in seeds of winter oilseed rape.

MATERIAL AND METHODS

The research involved a three-year experiment (2010–2013), conducted in the Experimental Station (ES) Baborówko (52°35'1.6125''N, 16°38'16.3501''E), ES Osiny (51°27'35.4659''N, 22°2'51.0307''E) and ES Jelcz-Laskowice (51°1'48.6194''N, 17°21'36.3187''E). The plant crop subjected to investigation was winter rape and crop cultivars were proper to each region of cultivation. The experiments were established as two-factorial, in 4 replications. At the increasing sulphur doses: a_1 – 0 (control); a_2 – 20 kg S/ha; a_3 – 40 kg S/ha; a_4 – 60 kg S/ha, winter rape fertilization with optimal doses of boron and copper: b_1 – 0 (control); b_2 – 2 kg B/ha, and b_3 – 5 kg Cu/ha; b_4 – 2 kg B/ha + 5 kg Cu/ha was applied. Individual plot size was 40 m^2.

The examined fertilization was introduced to the soil as a pre-sowing one; sulphur fertilization – in the form of granulated fertilizer WIGOR S (90% S), fertilization with microelements in the form copper sulphate $CuSO_4 \cdot 5 H_2O$ and boric acid H_3BO_3. Because of low doses of microelements, soil spraying with water solutions of reagents was introduced in order to obtain their uniform spreading.

The experiments were established on light soils (loamy sand), which were of a slightly acid reaction and featured optimal supply with plant basic nutrients – P_{Egner}, K_{Egner} and $Mg_{Schachstschabel}$. The soils in all the experiments, according to the requirements, contained low content of sulphate sulphur SO_4^{2-}-S and boron, as well as medium content of copper (Table 1).

During the growing season, in order to determine the state of plant nutrition regarding their S, B and Cu requirements, indicator parts of winter rape – young, fully developed leaves at 30–50 cm of plant height, were subjected to analysis. The assessment of plants supply with sulphur was done on the basis of classes of S total content elaborated by Schnug and Haneklaus (1994). The content of boron and copper was determined as related to the optimal range, developed by Bergmann (1986).

The content of SO_4^{2-}-S in the soils was determined according to the method of plasma emission after extraction in ammonium acetate, boron – due to the method of plasma emission and copper – after extraction in 1 mol/L HCl, using the method of atomic absorption spectrometry. In winter rape plants and grain the content of total sulphur, boron and copper was determined due to the method of plasma emission spectrometry. Raw crude fat content in seeds was determined using the gravimetric method by Soxhlet after extraction with ethyl ether.

The yield and fat content in rapeseed were worked out statistically using ANOVA. The significance of cross-object differences in the analysis of variance were evaluated with the Tukey's test ($P \leq 0.05$).

Table 1. pH indicator and the content of easily available forms of sulphur (S), boron (B) and copper (Cu) in experimental soils

	Consecutive years of the experiments	$pH_{1 mol/L KCl}$	SO_4^{2-}-S	B	Cu
			(mg/kg)		
	I	6.98	8.81	0.42	4.63
Baborówko	II	6.16	6.72	0.45	5.32
	III	6.30	5.80	0.65	3.20
	I	6.02	9.24	1.05	1.09
Jelcz-Laskowice	II	5.60	5.78	0.92	2.26
	III	5.80	3.32	1.20	2.30
	I	5.98	4.30	0.82	2.22
Osiny	II	5.63	8.58	1.10	1.50
	III	5.80	9.30	1.20	3.59
Optimal content			10–15	1.3–4.3	1.6–4.9

Optimal content of SO_4^{2-}-S by Lipiński et al. (2003), content of B and Cu by Bergmann (1986)

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RESULTS AND DISCUSSION

Mean contents of sulphur, boron and copper in plants. They were statistically comparable within particular sites. In the leaves of rapeseed from control treatments, not fertilized with sulphur, low concentrations of total sulphur in comparison to optimal range 0.55–0.65% dry matter (DM) were recorded (Figure 1). Those contents indicated insufficient plant nutrition, regarding this nutrient, in all the experimental stations. In the leaves from the treatments fertilized with sulphur in the doses of 20, 40 and 60 kg S/ha it was possible to state optimal and more than optimal contents of this element. The increase in sulphur content in plants in relation to not fertilized objects was not always statistically significant. As it results from research, sulphur, supplied in the elementary form in the autumn, was not washed out and was well used by plants in the spring, when rapeseed requirement for this component is the highest. The plants had in their disposal a large resource of this nutrient which was gradually released from the fertilizer.

In the experiments, in rapeseed leaves samples from control objects, low content of boron (< 30 mg/kg) and copper (< 5 mg/kg) in relation to plant nutrition requirements was recorded. In the objects fertilized with these components separately (objects B and Cu) and combined (B + Cu) in all the experiments plant nutrition was optimal in the case of boron (Figure 2). The application of copper did beneficially affect on its increased concentration in the plants only in ES Baborówko and ES Osiny (Figure 3). In ES Jelcz-Laskowice, introduction of copper did not cause its expected, increased concentration in the plants.

Rapeseed yields. Sulphur efficiency is especially evident at the deficit of this element in the soil. Mean values of oilseed rape grain yield from three-year experiments, obtained in particular stations, showed significant diversity according to the introduced fertilization (Table 2). The interaction between the experimental factors – fertilization with sulphur, boron and copper applied separately, as well as in combination, was statistically insignificant. The yield-forming effect of micronutrients was not dependent on sulphur fertilization. The dose of sulphur fertilization lower than 20 kg S/ha did not affect the increase in rapeseed yield in any of the experiments. Sulphur fertilization with the dose higher than 40 kg S/ha caused a significant increase in grain yield – by 11–12% in relation to control objects, ranging between 0.35–0.61 t/ha. The use of sulphur dose higher than 60 kg S/ha resulted in similar yield-forming effect.

Mean yields obtained in the experiments confirmed yield-forming effectiveness and efficiency of rapeseed fertilization with boron and copper. The study conducted in ES Baborówko proved the increase in grain yield by 11% after boron fertilization and by 12% as a result of combined fertilization with boron and copper (B + Cu) in comparison to control objects. In ES Osiny, a significant field-forming effect was recorded after the combined use of both microelements (B + Cu), since the yield was higher by 12% (Table 2).

Fat content in rapeseed. Both sulphur deficit and its excessive amounts adversely affect fat content in rapeseed grain. The research also proved that oil content in rapeseed grain could be increased by boron and copper fertilization. In the experiments discussed above, applied fertilization with S, B

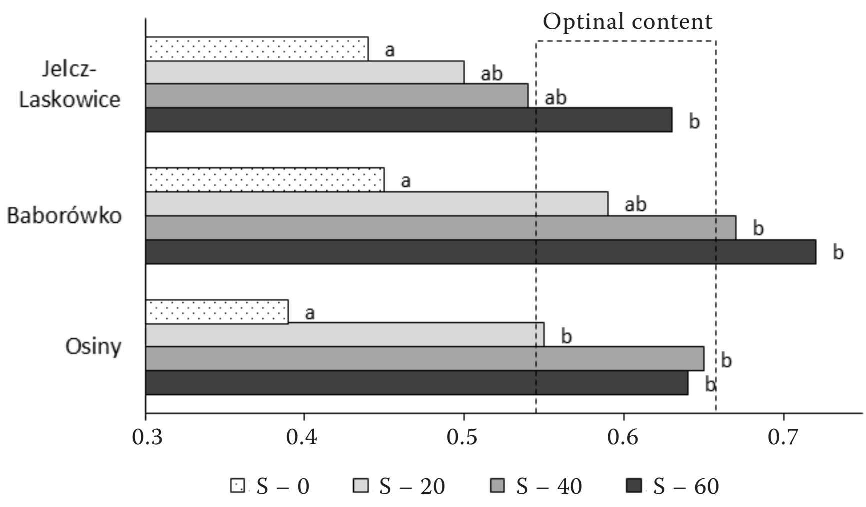

Figure 1. Mean sulphur content in rape leaves (% dry matter). Values marked with the same letter are not significantly different according to the Tukey's test. Sulphur doses: 0, 20, 40 and 60 kg S/ha

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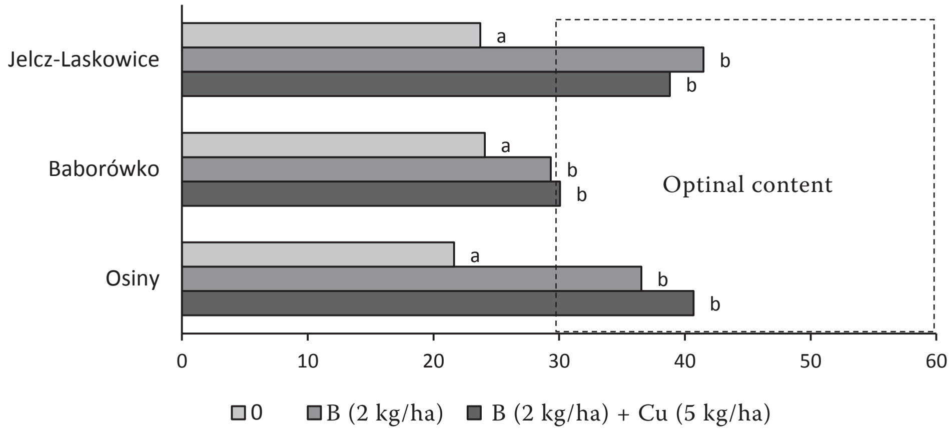

Figure 2. Mean boron (B) content in rape leaves (mg/kg dry matter)

and Cu influenced the increased fat concentration in oilseed rape grains in relation to control objects. In ES Jelcz-Laskowice and ES Osiny, a significant increase in fat content – more than 1% in comparison to control, was obtained due to fertilization with higher doses of sulphur – 40 and 60 kg S/ha (Table 3).

Balík et al. (2009), in their field experiments, applied combined rapeseed fertilization with nitrogen and sulphur (50 kg S/ha) in the form of ANS (ammonium nitrate – sulphate). The fertilization had a positive impact on mineral S content in the soil and in the plants. The concentration of organic S in rapeseed above-ground biomass increased from 0.33% fresh matter (FM) to optimal concentration 0.79% FM. The highest sulphur concentration – 1.76% S occurred in the upper leaves. After ANS form application, increased Cu concentration in rapeseed leaves and inflorescences was noted. The contents of Cu ranged from 1.56–8.75 mg/kg FM, depending on the plant part and the growth stage. The highest Cu uptake was recorded for ANS combination.

In other experiments by the author, the state of rapeseed nutrition with copper was efficiently affected by copper fertilization in the dose of 12 kg Cu/ha. The fertilization with lower copper doses, 4 and 8 kg Cu/ha was not effective (Sienkiewicz-Cholewa 2008a). However, copper is a component which very quickly becomes retarded in the soil and, therefore, cannot be easily available for plants. It accumulates mainly in roots and, under unfavorable weather conditions, its transport can be more difficult (Stanisławska-Glubiak et al. 2007). In a long-term experiment by Pageau et al. (1999), boron concentration in rapeseed leaves during plant flowering was lower than 15 mg B/kg. After introduction of boron in the doses of 0.5, 1.0 and 2.0 kg/ha, boron concentration in plants increased according to increased doses, up to its optimal content.

Advantageous effect of sulphur fertilization on grain yield was reported by some authors. Significantly increased grain yield were obtained by Jankowski et al. (2008) and Wielebski (2008), after the introduction of 10–30 kg S/ha fertilization. Malarz et al. (2011) reported 12% increase in grain yield of crossbred rape cultivar after the spring application of 60 kg S/ha in the form of ammonium sulphate. Podleśna (2003) used a higher dose of

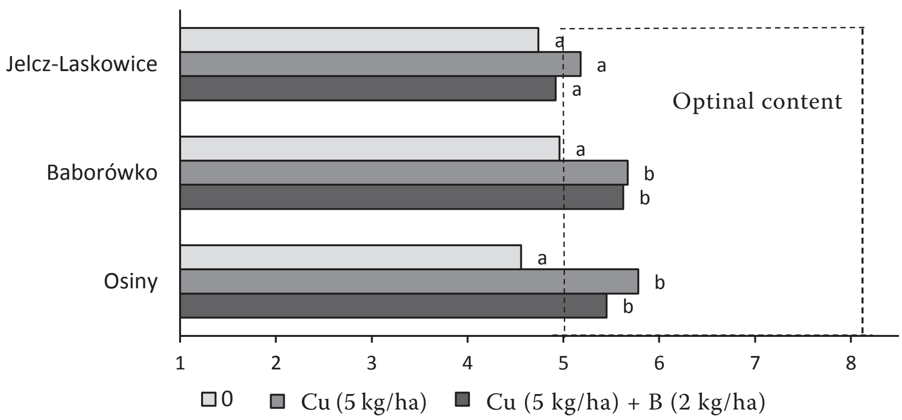

Figure 3. Mean copper (Cu) content in rape leaves (mg/kg dry matter)

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Table 2. Average rape seed yields in separate trials (t/ha)

Experimental station	Sulphur fertilization (kg/ha)				$LSD_{0.05}$
	0	20	40	60	
Baborówko	3.657[a]	3.833[a]	4.011[b]	4.283[b]	0.375
Jelcz-Laskowice	3.315[a]	3.682[ab]	3.926[b]	3.847[ab]	0.501
Osiny	3.857[a]	4.066[ab]	4.259[b]	4.265[b]	0.356
	Boron and cooper fertilization (2 kg B and 5 kg Cu/ha)				$LSD_{0.05}$
	0	B	Cu	B + Cu	
Baborówko	3.508[a]	3.900[b]	3.788[ab]	3.947[b]	0.381
Jelcz-Laskowice	3.112	3.560	3.300	3.510	ns
Osiny	3.920[a]	4.110[ab]	4.304[ab]	4.393[b]	0.411

Values marked with the same letter are not significantly different within location; ns – not significant

sulfur – 80 kg S/ha and obtained a significant increase in oilseed rape grain, even by 0.81 t/ha as a result of optimal plant nutrition.

The previous author's study on copper fertilization of rapeseed stated that rapeseed nutrition with copper was efficiently affected by this microelement fertilization in the dose of 12 kg Cu/ha. The fertilization with lower copper doses, 4 and 8 kg Cu/ha was not effective (Sienkiewicz-Cholewa 2008b). In field experiment, Yang et al. (2009) obtained a significant increase in grain yield of rapeseed, cultivated on sandy soil, after its fertilization with microelements. Combined application of B + Mo or B + Cu resulted in higher grain efficiency in comparison to the use of B, Mo and Cu fertilization separately. The yield increased after the combined application of microelements by 68.1%.

Beneficial effect of sulphur fertilization, in the dose of 60 kg S/ha, on fat content was reported by Malarz et al. (2011). Fat concentration in the treatments fertilized with gypsum, as well as elemental sulphur in Wigor (90% S) fertilizer, increased by 0.5% in relation to the treatment without sulphur fertilization. Ahmad et al. (2007) also recorded the lowest values of fat content in the treatment where sulphur had not been applied. The concentration of oil increased from 41.8–42.8% after 20 kg S/ha had already been applied. The increase in sulphur dose to 30 kg S/ha did not have a significant impact on the content of oil. Also, McGrath and Zhao (1996), Jackson (2000), Ahmad et al. (2005), as well as Malhi et al. (2007) in their investigation proved an increased oil concentration in the objects fertilized up to the dose of 40 kg S/ha. As it resulted from the research conducted, the increase in sulphur fertilization dose occurred to be inefficient (Podleśna 2003, Jankowski et al. 2008, Podleśna and Strobel 2009).

Lošák et al. (2011) studied the effect of nitrogen and sulphur fertilization on yield and fat content in *Camelina sativa* (flase-flax). A pot experiment was carried out with nitrogen applied at three in-

Table 3. Average fat content in rapeseed in separate trials (% seed dry matter)

Experimental station	Sulfur fertilization (kg/ha)				$LSD_{0.05}$
	0	20	40	60	
Baborówko	40.53	40.56	40.40	41.00	ns
Jelcz-Laskowice	41.91[a]	42.31[ab]	42.40[ab]	42.92[b]	0.831
Osiny	45.80[a]	46.60[ab]	46.50[ab]	46.83[b]	0.990
	Boron and cooper fertilization (2 kg B and 5 kg Cu/ha)				$LSD_{0.05}$
	0	B	Cu	B + Cu	
Baborówko	40.51[a]	41.03[ab]	40.80[ab]	41.94[b]	0.760
Jelcz-Laskowice	41.91	42.00	42.42	42.31	ns
Osiny	44.80[ab]	44.80[a]	45.10[ab]	45.80[b]	0.986

Values marked with the same letter are not significantly different within location; ns – not significant

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creasing rates in a range from 63 up to 127 kg/ha and sulphur additions applied as $(NH_4)_2SO_4$ to achieve a soil sulphate in a range 75 and 135 kg S/ha. Camelina seed yield increased with increasing nitrogen doses at the same low sulphur level. The increase in nitrogen fertilization reduced seed oil content from 39.8–37.1%. Differences in crop parameters between the sulphur treatments were not statistically significant, although the higher sulphur treatment tended to increase seed yield as well as oil content compared with the low sulphur treatment. Similar results were obtained in other studies by Šípalová et al. (2011).

A rarely conducted research involved rapeseed fertilization, which indicates their positive influence effect on the fat content. Yang et al. (2009) in their study, proved that boron fertilization in the dose 7.5 kg/ha increased fat content by 3%. Lääniste et al. (2004) obtained a significant increase in the examined parameter, by 1.1% after foliar application of boron fertilizer. In another research, dealing with soil-applied boron fertilization in the dose of 1 kg B/ha, increased oil content in oilseed rape grain on average by 4.4% (Pageau et al. 1999). Spychaj-Fabisiak et al. (2011) reported a considerable increase in fat concentration in grain – by 1.3%, after combined foliar application of magnesium sulphate and boron in microelement fertilizer Solubor. A long-term research by the author proved, apart from the yield-forming role of copper for rapeseed, a statistically confirmed influence of this microelement on the content of oil in grain. In the conditions of copper deficit in the soil, fertilization of different rapeseed cultivars in the doses of 8 and 12 kg Cu/ha caused increase in fat content, even by 2.2% (Sienkiewicz-Cholewa 2008a, 2010). It was concluded that winter rape fertilization with elemental sulphur, boron and copper resulted in increased concentration of these components in the plants. Sulphur fertilization, both in the dose of 40 and 60 kg S/ha, increased the yield of oilseed rape grain.

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