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Effect of sulphur and nitrogen fertilisation on winter wheat in *Calcaric Luvisol*

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Abstract

In 2019–2021, the experiment was carried out with the aim to investigate how sulphur (S) fertiliser rates under different nitrogen (N) fertiliser backgrounds influenced winter wheat grain yield, grain quality, and plant productivity indicators. The winter wheat (*Triticum aestivum* L.) cultivar 'Janne' was grown in four N background treatments with N_{60}, N_{120}, N_{180}, and N_{240} without S fertilisation and also fertilised with S_{15} (in all N fertilisation treatments), S_{30} (only with N_{120} and N_{240}), and S_{60} (only with N_{240}). The results showed that S fertilisation increased the wheat grain yield significantly in individual research years and only in the plots fertilised with higher N rates. N fertilisers increased the protein content, sedimentation, and gluten content of the grain, while S fertilisers only in one year and only in the N_{180} fertilisation treatment. The results of experiment showed that the N:S ratio for the winter wheat at the BBCH 30 growth stage was in the range of 8–16, and at the BBCH 65 it was in the range of 9–19 indicating that the winter wheat was optimally or even more than optimally supplied with S.

Keywords: nitrogen to sulphur ratio, mineral nitrogen, mineral sulphur, grain yield, grain quality.

Introduction

Sulphur (S) is one the most important plant nutrition elements after nitrogen (N), phosphorus (P), and potassium (K). In the absence of this element, the process of photosynthesis is disrupted in plants, protein synthesis slows down, and more non-protein N accumulates in cereal grain (Skwierawska et al., 2016). Optimum fertilisation with S improves plant growth and development, in addition also improving the crop uptake of other elements in the soil. The need for S correlates with the amount of N, since both substances play a key role in protein synthesis. Therefore, it is important to pay attention to the nitrogen to sulphur ratio (N:S) when investigating the plant uptake of S.

N:S is usually in the range of 7–15 for the aboveground part of the plant during its growth (Kanal et al., 2003). This N:S ratio in plants should be 15 (Jamal et al., 2010) with an optimum variation range of 14–17 (Reussi et al., 2012; Sedlár et al., 2019), and in wheat grain it should be 7.5 (Jamal et al., 2010).

Sulphur availability to plants is largely dependent on mobile S concentration in soil (Jamal et al., 2010), the majority of which is sulphate and, like nitrate, is poorly sorbed by soil fines, and most of which is present in the soil solution and, therefore, migrates rapidly through the soil profile (Eriksen, 2009). Studies in Lithuania have shown that mineral sulphur (S_{min}) is more abundant in heavy-textured soils with its higher concentration remaining in autumn after rapeseed and winter crops, and the lower one after spring crops, perennial grasses, and in pasture fields. S_{min} concentration varies considerably from year to year with 30–60 and 60–90 cm soil layers often containing more than those of 0–30 cm. Monitoring data shows that low to medium levels of S_{min} usually prevail in soil in spring; however, they usually increase in summer (Staugaitis et al., 2015).

In fertile soils, winter wheat is grown using intensive cultivation technologies and plants are fertilised with high N rates. Based on research by scientists, higher N rates in parallel require higher rates of S. Evidence from different researchers shows a varying efficiency of S fertilisers on agricultural crops (Aula et al., 2019) including winter wheat. In some experiments, S fertilisers, whether applied to soil in spring or as an additional fertilisation during the growing season, increased the yield of winter wheat grain, which contained more protein (Staugaitienė et al., 2013; Klikocka et al., 2016) and had better baking properties (Sedlár et al., 2019). However, contrary data is also available, where S fertilisation did not increase wheat yield (Dhillon et al., 2019) or increased in some years or in some areas only (Kulhánek et al., 2014).

The hypothesis of this study is that the optimal fertilisation with S improves the plant uptake of N fertilisers and increases the yield and quality of wheat.

The aim of the study was to determine how different rates of sulphur fertilisers in the presence of various backgrounds of nitrogen fertilisers, influence the grain yield of winter wheat, its quality, and plant productivity in the fertile *Calcaric Luvisol*.

Material and methods

Soil characterisation and scheme of the experiment. The experiment was performed at the Rumokai Experimental Station of the Institute of

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Agriculture of Lithuanian Research Centre for Agriculture and Forestry in 2019–2021. The experiment was conducted on the *Calcaric Luvisol* (WRB, 2015). Soil texture was silt loam on loam and heavy clay loam. The winter wheat (*Triticum aestivum* L.) cultivar 'Janne' was sown on 11 September in 2018, 18 September in 2019, and 21 September in 2021, and was harvested on 30 July in 2019, 10 August in 2020, and 26 July in 2021. Before sowing, the winter wheat was fertilised at a rate of $N_{15}P_{63}K_{108}$, and complex NPK 5-21-36 fertilisers were applied. In spring, the winter wheat was fertilised with nitrogen (N) and sulphur (S) fertilisers according to the scheme given in Table 1.

The fertilisers used during the growth of winter wheat were ammonium nitrate (34.1% N) and ammonium sulphate (24.0% S and 21.0% N). The plants were fertilised in spring after the resumption of vegetation on 2 April in 2019, 26 March in 2020, and 8 April in 2021; for the second time, they were fertilised on 26 April in 2019, 22 April in 2020, and 5 May in 2021; and for the third time, the plants were fertilised on 23 May in 2019, 23 June in 2020, and 9 June in 2021.

The experiment consisted of 12 treatments, each with four replications. The experimental plot size was 72 m² (12 × 6 m), and the harvested area was 36 m² (9 × 4 m). The randomised experimental design was applied.

Table 1. Nitrogen (N) and sulphur (S) fertilisation scheme for winter wheat

Treatment No.	Total N and S application rate kg ha^{-1}	N and S application rate kg ha^{-1} in autumn + next year's fertilisation I + fertilisation II + fertilisation III
1.	N_{60}	$N_{15} + N_{45} + N_0 + N_0$
2.	$N_{60}S_{15}$	$N_{15} + N_{45}S_{15} + N_0 + N_0$
3.	N_{120}	$N_{15} + N_{60} + N_{45} + N_0$
4.	$N_{120}S_{15}$	$N_{15} + N_{60}S_{15} + N_{45} + N_0$
5.	$N_{120}S_{30}$	$N_{15} + N_{60}S_{30} + N_{45} + N_0$
6.	N_{180}	$N_{15} + N_{90} + N_{45} + N_{30}$
7.	$N_{180}S_{15}$	$N_{15} + N_{90}S_{15} + N_{45} + N_{30}$
8.	$N_{180}S_{30}$	$N_{15} + N_{90}S_{30} + N_{45} + N_{30}$
9.	N_{240}	$N_{15} + N_{120} + N_{45} + N_{60}$
10.	$N_{240}S_{15}$	$N_{15} + N_{120}S_{15} + N_{45} + N_{60}$
11	$N_{240}S_{30}$	$N_{15} + N_{120}S_{30} + N_{45} + N_{60}$
12.	$N_{240}S_{60}$	$N_{15} + N_{120}S_{30} + N_{45}S_{30} + N_{60}$

Plant protection products against weeds, diseases, and pests were used according to the need.

Before harvesting, crop samples were taken from four spots of each plot within the 0.25 m² area for the determination of the biometric indicators: plant density, total number of productive stems, straw and ear length, and number of grains per ear. During harvesting, the grain collected from each experimental plot was weighed separately, grain moisture content and cleanness were determined, and samples were taken to determine 1000 grain weight and quality. Grain yield was expressed at 14% moisture and clean mass. The crude protein content in grain was determined by the Kjeldahl method; it was obtained by multiplying the total nitrogen (N_{tot}) by 6.25. Starch content was determined using a polarimeter, wet gluten by washing the dough using the Perten Instruments' Gluten Index method (Glutomatic System specified with CSN EN ISO 21415-1), sedimentation by the Zeleny test (ISO 5529), and mass per hectolitre using an HLM device compliant with ISO 7971-2.

To assess the uptake of N and S by plants, the N_{tot} and total sulphur (S_{tot}) concentration in the aboveground mass of growing winter wheat were determined at the BBCH 30 growth stage (GS) of winter wheat on 25 April in 2019, 17 April in 2020, and 28 April in 2021 and at the BBCH 65 GS on 03 June in 2019, 17 June in 2020, and 25 June in 2021. For this purpose, 10 plants were taken from replications 1 and 2 of each plot, in which N_{tot} and S_{tot} were determined by wet digestion with concentrated H_2SO_4, followed by the Kjeldahl method for N_{tot} and the turbidimetric method for S_{tot}.

Weather conditions (Table 2). 2018–2019 growing season. September 2018 was dry and warm with a daily mean temperature of 15°C, and a low total of 19.6 mm of precipitation fell during the month. Due to that fact, winter wheat germination was delayed. October and November were warmer than the normal, and the soil moisture was normal due to a sufficient rainfall in October. The plants were well rooted and vigorous in November. The winter was warm: the average daily temperature was 0.03°C in December, −4.1°C in January, 1.8°C in February, and 3.9°C in March. The winter wheat overwintered well. The mean air temperature in April and May was higher than the standard climate normal (SCN). During that period, the plants felt a lack of moisture due to the drought. At the end of April, the winter wheat was already at the BBCH 32 GS in terms of development. It rained profusely only in the second half of May. June was hot and not rainy, and the precipitation was only 25.7 mm. During that period, the plants felt a lack of moisture again. In the first 10-day period of July, it rained heavily, which was enough to restore moisture reserves in the soil before harvest.

2019–2020 growing season. In September–November 2019, it was 1–3°C warmer than usual, and the monthly precipitation rate reached 44–47 mm, only in November the rainfall was lower – 13.2 mm. The sown winter wheat germinated evenly, and the plants were 8–12 cm tall by December. The moisture of the soil plough layer ranged from normal to dryish; however, at that time the water evaporation was slow, and the moisture was sufficient for the plants. The winter was unusually mild: the mean daily temperature values in December–February were 2.8–2.9°C, and it was a little colder in March – 1.8°C. The winter wheat overwintered well, and its condition remained almost unchanged during the winter until the beginning of April, when its growth resumed. April and May were cool; however, the amount of precipitation varied significantly – only 2.1 mm in April and 93.8 mm in May. Dry March and almost rainless weather in April dried up the soil; therefore, the plants felt a lack of moisture and were less tillered in the second half of April. Rainy May restored soil moisture reserves, and even rainier June, when 129.4 mm of rain fell during the month, led to excess moisture in that month and facilitated the spread of *Septoria tritici* and *Drechslera tritici-repentis*. July and the first 10-day period of August were cool, slightly humid, and favourable for grain formation in ears.

2020–2021 growing season. September 2020 was dry and warm, the mean daily temperature was 15.4°C (SCN – 12.8°C), and the precipitation rate was

Table 2. Weather conditions during the winter wheat growing season (data of Kybartai Automatic Meteorological Station)

Growing season	Month											
	09	10	11	12	01	02	03	04	05	06	07	08
Mean daily temperature °C												
2018–2019	15.0	9.4	3.2	0.03	−4.1	1.8	3.9	8.4	12.5	20.4	17.1	18.4
2019–2020	13.6	9.8	5.5	0.9	2.8	2.8	4.0	7.1	10.5	18.6	17.6	18.9
2020–2021	15.4	10.7	5.8	1.1	−3.0	−4.2	2.2	6.0	11.5	19.2	22.1	16.6
SCN	12.8	7.9	2.5	−1.4	−2.7	−2.4	1.2	7.3	13	15.7	18	17.4
Monthly precipitation rate mm												
2018–2019	19.6	47.8	22.8	61.1	56.7	31.1	33.2	0.2	54.3	25.7	93.3	51.3
2019–2020	44.5	46.6	13.2	32.8	53.9	40.5	26.2	2.1	93.8	129.4	33.6	79.5
2020–2021	18	58.9	21.8	22.1	38.4	9.5	21.8	47.1	93.3	25.6	66.9	118.5
SCN	72	51	47	44	42	43	33	36	32	50	72	81

18 mm (SCN – 72 mm). There was a lack of moisture for winter wheat germination. October was warmer than usual, and the amount of precipitation was slightly higher than the SCN. The warm and humid weather of that month facilitated the final germination and further development of the wheat. November and December were slightly warmer than usual, and the amount of precipitation accounted for around half of the SCN. January and February were colder than the SCN; however, the sufficient snow cover provided good protection for the wheat against frost. Cold and rainy weather prevailed in April and May. June was hot and dry, and the precipitation amount was only 25.6 mm. The weather in July was also hot, but wetter. Due to the heat during those two months the grain maturity in ears was rapid, the crop was harvested early, and the grain was smaller.

Soil chemical analysis. Soil samples for agrochemical analyses were taken after the plant growth resumption in early spring on 2 April and 18 March in 2020 and on 7 April in 2021. Soil acidity (pH), concentrations of plant available phosphorus (P_2O_5) and potassium (K_2O) as well as humus were determined in the 0–20 cm layer. The concentrations of mineral nitrogen (N_{min}) and mineral sulphur (S_{min}) were determined in the 0–30 and 30–60 cm layers. In the soil, the S_{min} concentration was also detected at the BBCH 65 GS (on 3 June in 2019, 17 June in 2020, and 16 June in 2021) and during harvesting on 30 July in 2019, 10 August in 2020, and 27 July in 2021. Soil samples were collected from replications 1, 2, and 3 of the experimental area, where one composite sample from the 0–20 cm layer consisted of 20 subsamples and composite samples from the 0–30 and 30–60 cm layers consisted of 9 subsamples. Soil pH_{KCl} was determined in 1 M KCl extraction using the potentiometric method (ISO 10390), ratio 1:5, available P_2O_5, and available K_2O were measured by the Egner-Riehm-Domingo (A-L) method, and humus was evaluated by using a carbon analyser after the dry combustion compliant according to ISO 10694, where the organic carbon concentration was multiplied by 1.724. N_{min} concentration was determined in 1 M KCl extraction in the air-dry samples (ratio 1:5) using the spectrometry (FIA) method compliant according to ISO 14265-2, and S_{min} in the same samples and the same extraction by the turbidimetric method. The soil pH_{KCl} value in the 0–20 cm soil layer was 5.9–6.4, humus – 1.91–2.33%, P_2O_5 148–165 mg kg^{-1}, and K_2O – 200–216 mg kg^{-1}.

In spring, the N_{min} concentration was distributed across the soil layers as follows: in the 0–30 cm layer, it was 9.4 ± 1.58 mg kg^{-1} in 2019, 6.2 ± 3.45 mg kg^{-1} in 2020, and 16.3 ± 7.79 mg kg^{-1} in 2021; in the 30–60 cm layer, it was 5.4 ± 0.62, 3.1 ± 2.04, and 2.3 ± 0.61 mg kg^{-1}, respectively. N_{min} concentration converted to kg ha^{-1} was obtained as follows: in 2019, in the 0–30 cm layer, it was 42.5 ± 7.09 kg ha^{-1}; in the 30–60 cm layer, it was 24.2 ± 2.77 kg ha^{-1} with the total level in 0–60 cm of 66.7 ± 9.75 kg ha^{-1}. In 2020, the N_{min} concentration in those layers was 28.0 ± 15.53, 14.0 ± 9.16, and 42.0 ± 24.69 kg ha^{-1}, respectively; in 2021, it was 73.4 ± 35.1 and 10.3 ± 2.74 with the total level of 83.7 ± 37.84 kg ha^{-1}. According to the assessment valid in Lithuania (Staugaitis, Vaišvila, 2019), the N_{min} concentration in the 0–60 cm soil layer was low in 2019 and 2020, and it was moderate in 2021.

S_{min} concentration in the 0–30 cm soil layer in spring varied within 1.7–2.3 mg kg^{-1} in all years, while the concentration in the 30–60 cm layer was significantly higher – 4.0–6.3 mg kg^{-1} (Table 3). Across both layers, the S_{min} concentration averaged 33.8 kg ha^{-1} in 2019, 36.0 kg ha^{-1} in 2020, and 28.4 kg ha^{-1} in 2021, and according to the assessment valid in Lithuania (Staugaitis, Vaišvila, 2019), S_{min} in the soil was high in 2019 and 2020, and moderate in 2021. S_{min} concentration in the summer before the winter wheat harvest was found to be even higher with an average level of 49.1 kg ha^{-1} in the 0–60 cm layer in the summer at the BBCH 65 GS in 2019, 59.4 kg ha^{-1} in 2020, and 51.3 kg ha^{-1} in 2021. At harvesting, it was 43.7, 61.2, and 53.6 kg ha^{-1},

Table 3. Mineral sulphur (S_{min}) concentration in soil during the winter wheat growth, mg kg^{-1}

Soil layer cm	After resumption of vegetation in spring	In summer at BBCH 65 GS	At harvesting
2019			
	2 April	3 June	30 July
0–30	2.0 ± 0.61	4.4 ± 2.18	5.3 ± 4.20
30–60	5.5 ± 1.21	7.6 ± 2.39	5.9 ± 1.94
2020			
	03.18	06.17	08.10
0–30	1.7 ± 0.06	5.2 ± 1.63	6.1 ± 1.35
30–60	6.3 ± 1.84	8.2 ± 1.84	7.9 ± 2.06
2021			
	7 April	25 June	25 July
0–30	2.3 ± 0.62	4.1 ± 1.60	3.5 ± 0.76
30–60	4.0 ± 2.16	4.6 ± 1.29	3.9 ± 0.81

Note. Data are provided as mean ± standard deviation.

respectively.

Depending on the rates of S fertilisers applied, the S_{min} concentration in the 0–60 cm soil layer in the summer at the BBCH 65 GS was obtained as follows: at S_0 – 10.7 ± 1.70, S_{15} –11.35 ± 4.25, S_{30} – 13.85 ± 6.01, and S_{60} – 15.00 ± 3.25 mg kg^{-1} in 2019. In 2020, it was found as follows: at S_0 –13.3 ± 1.22, S_{15} – 12.7 ± 1.67, S_{30} – 15.0 ± 7.15, and S_{60} – 12.5 ± 1.77 mg kg^{-1}, respectively; and in 2021: at S_0 – 7.9 ± 0.96, S_{15} – 8.0 ± 1.01, S_{30} – 7.8 ± 1.24, and S_{60} – 16.8 ± 1.98 mg kg^{-1}, respectively. This shows that the S_{min} concentration in the soil varied considerably, and it was difficult to identify the patterns of variation with S fertiliser rates; however, in 2019 and 2021, S_{60} increased its concentration compared to that of the plots unfertilised with S.

Statistical analysis. The trial data were statistically processed using Microsoft Office Excel and the analysis of variance (ANOVA) (Raudonius, 2017). The means were compared by using the least significant difference (LSD) calculated at the 0.05 probability level (Fisher's LSD test).

Results and discussion

Winter wheat grain yield. The average data of three-year experiment showed that the N fertilisers without S increased the winter wheat grain yield up to the N_{180} rate, which was the optimal (Table 4). In contrast, the higher rate of N_{240} did not increase yield any longer in a significant way, and even yield decreasing trends were observed in 2019 and 2020.

Regarding the effect of S fertilisers on the grain yield of winter wheat, the data obtained by individual researchers are very different. Some argue that S fertilisers do not affect grain yield (Dhillon et al., 2019). Others report that S fertilisers promote N uptake and increase grain yield at higher N rates (Klikocka et al., 2017; Rossini et al., 2018).

In our experiment, the application of N fertilisers at a rate of S_{15} in combination with the S fertilisers in all four N fertilisation treatments analysed: N_{60}, N_{120}, N_{180}, and N_{240}, did not significantly increase the wheat grain yield in any year. According to the average data of three years, a significant increase in yield after increasing the S fertiliser rate to S_{30} was obtained in the two N fertilisation treatments: N_{120} and N_{180}. Meanwhile, according to the data of individual years, a significant increase in the yield was obtained only in 2020 in the N_{180} fertilisation treatment. In 2019, increasing the S fertiliser rate to S_{60} when fertilising it in the N_{240} fertilisation treatment reliably increased the grain yield. In 2021, after fertilising with S_{60}, a significant decrease in yield was obtained compared to the fields fertilised only with the N fertilisers. This could have been influenced by the lodging of wheat in the fields fertilised with $N_{240}S_{60}$. Higher rates of S increased the plant height. In 2021, on the second 10-day period of June, a heavy rain and wind have lodged taller plants.

Table 4. Influence of sulphur (S) fertilisation on the grain yield (t ha^{-1}) of winter wheat

Treatment	2019	2020	2021	2019–2021	Increase %
N_{60}	6.40	5.90	4.29	5.53	–
$N_{60}S_{15}$	6.22	5.96	4.26	5.48	−0.9
N_{120}	6.97	7.31	5.99	6.76	–
$N_{120}S_{15}$	7.13	7.42	5.99	6.85	1.3
$N_{120}S_{30}$	7.16	7.37	6.15	6.89	1.9
N_{180}	7.59	7.73	6.84	7.39	–
$N_{180}S_{15}$	7.45	7.76	6.89	7.37	−0.3
$N_{180}S_{30}$	7.56	7.98	7.03	7.52	1.8
N_{240}	7.27	7.58	7.09	7.31	–
$N_{240}S_{15}$	7.30	7.60	7.27	7.39	1.1
$N_{240}S_{30}$	7.45	7.68	7.39	7.51	2.7
$N_{240}S_{60}$	7.98	7.61	6.61	7.40	1.2
LSD_{05}	*0.370*	*0.196*	*0.355*	*0.122*	

Note. The means were compared by using the LSD calculated at the 0.05 probability level.

According to the data of our research, the trend of increasing the grain yield of winter wheat was determined by increasing the rate of S fertilisers, but no significant differences were found. This is probably due to the S_{min} concentration in the soil, which was high both in spring and at the BBCH 65 GS as well as during harvesting. No significant correlation between the amount of S fertilisers applied (kg ha^{-1}) and the grain yield (t ha^{-1}) was obtained in any of the experimental years: $r = 0.539$ in 2019, $r = 0.303$ in 2020, and $r = 0.255$ in 2021. Neither significant correlation was found between the S_{min} concentration (mg kg^{-1}) in the 0–30 and 0–60 cm soil layers at the BBCH 65 GS and the grain yield (t ha^{-1}) with the correlation coefficients $r = 0.229$ and $r = 0.240$, respectively.

In experiments of other researchers, where S increased the yield of wheat, in one experiment, the concentration of S_{min} in the 0–30 cm soil layer was found to be 1.83, in the 30–60 cm one it was 0.50 mg kg^{-1}, and in another experiment 0.5 and 0.5 mg kg, respectively (Staugaitienė et al., 2013). This shows that in the experiments of these researchers, there was little and very little S_{min} concentration in the soil. Therefore, it can be stated that before fertilising with S it is necessary to test the concentration of S_{min} in the soil and only then decide whether it is necessary to fertilise with S. The fact that S fertilisers do not influence winter wheat is also suggested by other researchers (Kulhánek et al., 2014).

Productivity of winter wheat. The number of productive stems in the plots varied within the range of 492–669 m^{-2} during the experimental years with the lowest number of stems being obtained in all years when fertilised at N_{60} (Table 5). The three-year average data showed that the S fertilisers increased the number of productive stems. It was 4.7% higher at the S_{15} rate with the N_{60}, 3.1% higher at the S30 rate with the N_{120}, 6.2% higher at the S_{30} rate with N_{180}, and 3.3% higher at S_{60} with N_{240} fertilisation treatments. Those rates of the S fertilisers increased the number of productive stems in the same treatments in 2021 with increasing trends in productive stems in 2019 and 2020.

The straw length of winter wheat varied between 63.4 and 67.7 cm in 2019, 65.9–74.0 cm in 2020, and 56.5–67.3 cm in 2021; it was influenced by the N fertilisers. During the experimental years, the straw length increased with increasing N fertiliser rates from N_{60} to N_{180}. Regarding all N fertilisation treatments, any

Table 5. Influence of sulphur (S) fertilisation on the productivity indicators of winter wheat (2019–2021)

Treatment	Number of productive stems per m^{-2}	Straw length cm	Ear length cm	Number of grains per ear	1000 grain weight g
N_{60}	530	62.9	8.0	41.6	37.19
$N_{60}S_{15}$	555	62.1	8.0	42.3	37.16
N_{120}	581	66.5	8.6	43.2	37.35
$N_{120}S_{15}$	563	66.0	8.3	42.0	36.64
$N_{120}S_{30}$	599	66.4	8.4	43.1	36.73
N_{180}	614	67.9	8.7	44.9	37.61
$N_{180}S_{15}$	622	67.5	8.5	43.6	36.74
$N_{180}S_{30}$	652	68.3	8.8	45.1	37.46
N_{240}	611	67.5	8.5	42.2	36.55
$N_{240}S_{15}$	623	66.6	8.6	44.9	37.34
$N_{240}S_{30}$	618	67.0	8.8	46.1	37.40
$N_{240}S_{60}$	631	68.1	8.7	46.7	37.64
LSD_{05}	*17.7*	*1.40*	*0.28*	*1.21*	*0.894*

Note. The means were compared by using the LSD calculated at the 0.05 probability level.

regular influence of the S fertilisers on the straw length in any year was not found.

During the experimental years, ear length varied between 7.8 and 9.3 cm with the lowest average length of 8.0 cm obtained with N_{60} and $N_{60}S_{15}$. In terms of all N fertilisation treatments, any patterned influence of the S fertilisers on the ear length was not found. Sulphur fertilisers had no influence on 1000 grain weight either. Meanwhile, in terms of the number of grains per ear during all experimental years, a regular effect of the sulphur fertilisers (S_{15}, S_{30}, and S_{60}) was observed in the N_{240} fertilisation treatment with an increase in the average number of grains per ear of 2.7, 3.9, and 4.5, respectively.

The weight per hectolitre of grain varied from year to year: 80.2–82.6 kg hl in 2019, 76.1–79.1 kg hl in 2020, and 76.9–79.8 kg hl in 2021. Nitrogen fertilisation increased the hectolitre weight; however, any regular influence by S fertilisers was not found (Table 6).

Table 6. Influence of sulphur (S) fertilisation on the grain quality of winter wheat (2019–2021)

Treatment	Weight per hectolitre kg hl^{-1}	Starch	Crude protein	Sedimentation	Gluten
		%			
N_{60}	76.98	69.17	9.18	20.44	16.33
$N_{60}S_{15}$	76.90	69.14	9.21	20.49	16.23
N_{120}	78.20	68.97	11.23	33.96	21.19
$N_{120}S_{15}$	78.39	69.01	11.14	32.42	20.61
$N_{120}S_{30}$	77.68	68.97	11.09	31.88	20.21
N_{180}	79.34	68.16	12.94	44.56	25.70
$N_{180}S_{15}$	79.36	67.69	13.47	47.87	26.83
$N_{180}S_{30}$	79.58	68.01	13.31	46.87	26.53
N_{240}	79.20	67.66	13.71	49.86	27.56
$N_{240}S_{15}$	79.37	67.42	13.76	50.74	27.70
$N_{240}S_{30}$	79.22	67.31	13.67	49.61	27.32
$N_{240}S_{60}$	79.82	67.33	13.68	50.44	27.56
LSD_{05}	*0.710*	*0.307*	*0.304*	*2.173*	*1.086*

Note. The means were compared by using the LSD calculated at the 0.05 probability level.

Winter wheat grain quality. Sulphur deficiency has been reported by some researchers to reduce N absorption resulting in reduced protein synthesis in winter wheat grains and reduced cooking properties (Tao et al., 2018; Yu et al., 2018; Filipek-Mazur et al., 2019). Additional fertilisation with S improves the quality of wheat grains (Geng et al., 2016). According to our research data, the fertilisation with S significantly improved the grain quality in only one research year and only in the N_{180} fertilisation treatment (Table 6). In our opinion, this was due to the meteorological conditions. In 2019 and 2020, after fertilising the wheat with N and S, the weather was dry for a whole month: the precipitation rate in 2019 April was 0.2 mm, and in 2020 it was 2.1 mm. Plants could not absorb nutrients. In 2021 April, the precipitation was 47.1 mm. The moisture regime was adequate and even throughout the month, favourable for the uptake of nutrients. Other researchers also point out to the influence of environmental conditions on the efficiency of N and S fertilisers in wheat crops (Salvagiotti et al., 2009; Matějková et al., 2010; Wang et al., 2011). In our opinion, the moisture regime has a similar effect on the S uptake. The variations in grain sedimentation and gluten were similar to those for crude protein with an increase in only one year and only in the case of N_{180} fertilisation treatment.

In addition, no qualitative analysis of grain protein was performed in our study. According to the literature (Benin et al., 2012; Kato, 2012; Dostálová et al., 2015), the total protein content of cereals is most affected by N, and S improves the qualitative protein composition. The amount of starch in grains decreased with increasing fertilisation rates.

Concentration of N and S in winter wheat during the growing season. Concentration of N_{tot} in the aboveground part of the plant depended on the conditions of a year, the stage of wheat growth, and the rate of N fertilisers applied (Table 7). The highest N concentration was observed at the BBCH 30 GS in 2021 and at the BBCH 65 GS in 2019. Nitrogen fertilisers increased the N concentration in the plants. That was observed at both growth stages; however, while the maximum N concentration at the BBCH 65 GS in all experimental years was found in the plants fertilised with N_{240}, at the

Table 7. Influence of nitrogen (N) and sulphur (S) fertilisation on the N_{tot} and S_{tot} concentration in winter wheat plants, % DM

Treatment	N			S		
	2019	2020	2021	2019	2020	2021
			BBCH 30 GS			
N_{60}	3.14 ± 0.28	2.59 ± 0.03	3.89 ± 0.25	0.23 ± 0.01	0.19 ± 0.01	0.35 ± 0.02
$N_{60}S_{15}$	3.04 ± 0.13	2.81 ± 0.01	3.76 ± 0.25	0.22 ± 0.02	0.20 ± 0.01	0.39 ± 0.01
N_{120}	3.37 ± 0.25	2.65 ± 0.08	4.49 ± 0.13	0.24 ± 0.03	0.20 ± 0.01	0.39 ± 0.03
$N_{120}S_{15}$	3.56 ± 0.22	2.85 ± 0.13	4.31 ± 0.29	0.24 ± 0.00	0.21 ± 0.01	0.49 ± 0.02
$N_{120}S_{30}$	3.49 ± 0.25	2.85 ± 0.06	4.38 ± 0.14	0.25 ± 0.04	0.22 ± 0.00	0.53 ± 0.01
N_{180}	3.45 ± 0.19	3.09 ± 0.18	4.27 ± 0.40	0.25 ± 0.02	0.21 ± 0.01	0.37 ± 0.00
$N_{180}S_{15}$	3.23 ± 0.04	2.99 ± 0.10	4.90 ± 0.04	0.24 ± 0.01	0.20 ± 0.00	0.54 ± 0.00
$N_{180}S_{30}$	2.96 ± 0.17	2.97 ± 0.40	4.65 ± 0.35	0.25 ± 0.05	0.19 ± 0.01	0.55 ± 0.01
N_{240}	3.19 ± 0.14	2.52 ± 0.16	4.73 ± 0.04	0.25 ± 0.00	0.17 ± 0.02	0.40 ± 0.05
$N_{240}S_{15}$	2.88 ± 0.12	2.91 ± 0.49	5.13 ± 0.21	0.24 ± 0.04	0.18 ± 0.03	0.56 ± 0.06
$N_{240}S_{30}$	3.07 ± 0.14	2.47 ± 0.01	4.91 ± 0.15	0.27 ± 0.01	0.19 ± 0.01	0.55 ± 0.03
$N_{240}S_{60}$	3.40 ± 0.02	2.72 ± 0.21	4.78 ± 0.52	0.28 ± 0.04	0.19 ± 0.00	0.59 ± 0.04
			BBCH 65 GS			
N_{60}	1.51 ± 0.20	0.84 ± 0.07	0.98 ± 0.06	0.13 ± 0.01	0.06 ± 0.00	0.09 ± 0.02
$N_{60}S_{15}$	1.73 ± 0.07	1.15 ± 0.03	1.14 ± 0.01	0.15 ± 0.02	0.08 ± 0.00	0.11 ± 0.02
N_{120}	1.74 ± 0.18	1.25 ± 0.13	1.34 ± 0.04	0.15 ± 0.01	0.09 ± 0.00	0.10 ± 0.01
$N_{120}S_{15}$	1.60 ± 0.40	1.33 ± 0.12	1.59 ± 0.16	0.14 ± 0.03	0.10 ± 0.00	0.11 ± 0.00
$N_{120}S_{30}$	1.96 ± 0.11	1.23 ± 0.02	1.29 ± 0.11	0.19 ± 0.01	0.10 ± 0.02	0.09 ± 0.01
N_{180}	1.85 ± 0.05	1.26 ± 0.10	1.53 ± 0.04	0.16 ± 0.02	0.09 ± 0.01	0.09 ± 0.01
$N_{180}S_{15}$	1.80 ± 0.14	1.43 ± 0.30	1.67 ± 0.07	0.16 ± 0.00	0.10 ± 0.00	0.09 ± 0.00
$N_{180}S_{30}$	1.89 ± 0.10	1.46 ± 0.01	1.51 ± 0.11	0.19 ± 0.00	0.09 ± 0.01	0.16 ± 0.01
N_{240}	1.91 ± 0.03	1.54 ± 0.18	1.78 ± 0.11	0.17 ± 0.03	0.09 ± 0.01	0.13 ± 0.02
$N_{240}S_{15}$	1.95 ± 0.14	1.60 ± 0.04	1.68 ± 0.07	0.16 ± 0.01	0.09 ± 0.01	0.10 ± 0.02
$N_{240}S_{30}$	1.91 ± 0.26	1.44 ± 0.28	1.77 ± 0.14	0.16 ± 0.05	0.10 ± 0.02	0.13 ± 0.01
$N_{240}S_{60}$	2.03 ± 0.11	1.54 ± 0.12	1.82 ± 0.02	0.19 ± 0.01	0.11 ± 0.02	0.13 ± 0.01

Note. Data are provided as mean ± standard deviation.

BBCH 30 GS it was with N_{180} in one year and with N_{240} in another.

Winter wheat fertilisation with S did not increase the N concentration of plants in most years; however, in some years and in some treatments, it increased because of S fertilisation. For example, at the BBCH 30 GS, that was obtained with $N_{240}S_{60}$ in 2019, with $N_{60}S_{15}$, $N_{120}S_{15}$, and $N_{120}S_{30}$ in 2020, and with $N_{180}S_{15}$ and $N_{240}S_{15}$ in 2021. At the BBCH 65 GS, that was even more frequent, and at $N_{60}S_{15}$ it was achieved for all three years. For winter wheat, S fertilisers are likely to influence the intensification of protein synthesis (Skwierawska et al., 2016), especially in the treatments, where N application was lower, and this was more evident at a later BBCH 65 GS. However, this hypothesis without a more detailed distribution of N compounds in plants does not fully answer why the N concentration in S-fertilised wheat remained stable in some cases and increased in others.

Sulphur availability to winter wheat is indicated by the S_{tot} concentration in plants, which should be at least 0.3% for winter wheat during the booting stage (Breuer et al., 2003), and 0.55% for winter wheat according to other authors (Sedlár et al., 2019). However, the S_{tot} concentration is not a sufficiently suitable indicator for assessing S availability, as it varies significantly during plant growth depending on annual meteorological conditions and the plant mass volume (Reussi et al., 2012; Kulhánek et al., 2014). In our experiment, at the BBCH 30 GS, the S concentration varied between 0.22% and 0.28% in 2019 and between 0.17% and 0.22% in 2020, and in 2021 it was between 0.35% and 0.59%, i.e., twice as high as in the previous years. Due to such large difference, the S_{tot} analyses were repeated in 2021 and made in other laboratories; however, the results were the same. In our opinion, such high S concentration in plants may have been affected by lower plant densities in 2021 – it was 24.9–20.8% lower than in 2019 and 2020. No such differences in the S concentration were observed at a later stage, as rarer plants grew heavier (cold and rainy April, and the beginning of May 2021 was particularly favourable for tillering), and the number of stems did not differ significantly between the years.

At the BBCH 30 GS in 2020, the N and S fertilisers had no effect on the S_{tot} concentration in crops. In contrast, in 2019, only the lowest N_{60}, $N_{60}S_{15}$ and the highest $N_{240}S_{30}$, $N_{240}S_{60}$ rates of N and S fertilisers showed significant concentration differences in crops. The same results were also obtained in 2021. However, the influence of S fertilisers on the increase in S concentration was obtained with all N fertilisation treatments in that year. Thus, the influence of S fertilisers on the S concentration in crops varied considerably from year to year.

At the BBCH 65 GS, the S_{tot} concentration in plants was significantly lower than at the BBCH 30 GS ranging from 0.13% to 0.19% in 2019, from 0.06% to 0.11% in 2020, and from 0.09% to 0.16% in 2021. The conditions of individual years had a significant influence there as well, and the S concentration in winter wheat in 2020 was significantly lower compared to that of other experimental years. This may have been influenced by a heavy rainfall three days before sampling. The maximum N_{240} rate used in the experiment increased the S_{tot} concentration in crops in all three years compared to the minimum N_{60} rate. In addition, in 2019, there was a significant increase in S in the crops when fertilised with the $N_{120}S_{30}$, N_{180} S_{30}, and $N_{240}S_{60}$ rates, and in 2020 with the $N_{60}S_{15}$ rate, while the crops fertilised with other S fertiliser rates only showed an upward trend. In 2021, the S fertilisers did not increase the S concentration in crops, except for the $N_{180}S_{30}$ rate. Thus, the influence of S fertiliser rates on the S_{tot} concentration in the winter wheat varied considerably from year to year.

The N:S ratio in winter wheat plant mass at the BBCH 30 GS of different N- and N-fertilised plots varied between 11.5 and 15.0 in 2019, between 12.7 and 16.4 in 2021, and the lowest one, 8.1–11.7, was obtained in 2021 (Table 8). It was found that in those years or treatments, where the S_{tot} concentration in crops was the highest, the N:S ratio was the lowest. That was the case in 2021 for all treatments and in 2019, where $N_{240}S_{30}$ and $N_{240}S_{60}$ had been applied. When the given optimal N:S range of 14–17 (Reussi et al., 2012) was used for our wheat, this ratio was in most cases less than 14 or in some cases reached 15. Thus, in our experiment, at the BBCH 30 GS, the winter wheat was optimally or even more than optimally supplied with S.

Table 8. Influence of nitrogen (N) and sulphur (S) fertilisation on the N:S ratio in winter wheat plants

Treatment	2019	2020	2021	2019	2020	2021
	BBCH 30 GS			BBCH 65 GS		
N_{60}	13.7	13.8	11.0	12.1	15.9	11.2
$N_{60}S_{15}$	13.8	13.7	9.5	11.8	14.8	10.6
N_{120}	13.8	13.0	11.4	11.8	14.9	13.5
$N_{120}S_{15}$	15.0	13.8	8.8	11.4	12.6	14.9
$N_{120}S_{30}$	13.9	12.7	8.3	10.3	12.4	14.1
N_{180}	13.9	15.0	11.7	11.7	15.6	16.8
$N_{180}S_{15}$	13.3	14.9	9.1	11.3	17.0	18.1
$N_{180}S_{30}$	12.0	15.6	8.5	10.0	16.0	9.5
N_{240}	13.0	14.8	11.8	11.6	19.4	14.0
$N_{240}S_{15}$	11.9	16.4	9.1	11.9	17.3	16.2
$N_{240}S_{30}$	11.5	13.1	8.9	12.0	17.1	13.1
$N_{240}S_{60}$	12.1	14.0	8.1	10.7	14.4	14.0

At the BBCH 65 GS, the N:S ratio in plants ranged from 12.1 to 15.0 in 2019, from 12.4 to 19.4 in 2021, and from 9.5 to 18.1 in 2021. This indicates that the plants were also optimally supplied with S during that period and even more than optimally in 2019. Hence, the patterns are the same: in 2019, the S_{tot} concentration in plants was higher resulting in a lower N:S ratio, while in 2020, the plants had the lowest S_{tot} concentration resulting in the highest N:S ratio.

Conclusions

1. In the fertile *Calcaric Luvisol*, the sulphur (S) fertilisation in four nitrogen (N) fertilisation treatments. N_{60}, N_{120}, N_{180}, and N_{240}, significantly increased the wheat grain yield in individual research years and only at higher N rate. There was no correlation between the amount of S fertilisers applied and the grain yield.
2. The S fertilisers increased the number of productive stems in all N fertilisation treatments, while with the N_{240} the number of grains per ear and the 1000 grain weight increased. The N fertilisers increased the protein content, sedimentation, and gluten content in the winter wheat grain, whereas the S fertilisers increased the protein content only in one year and only with the N_{180} fertilisation treatment.
3. The methods of S diagnosis used do not fully reflect the values of existing S concentration in the soil and in plants vary widely from year to year, even in the same year, making it difficult to apply such diagnostics under production conditions.

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Sieros ir azoto trąšų įtaka žieminiams kviečiams trąšiame išplautžemyje

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Santrauka

2019–2021 m. atlikti tyrimai siekiant nustatyti, kaip sieros trąšų normos, esant įvairiems tręšimo azoto trąšomis fonams, lemia žieminių kviečių grūdų derlių, jo kokybę ir augalų produktyvumo rodiklius. Auginti veislės ‘Janne’ žieminiai kviečiai, kurie keturiuose tręšimo azoto trąšomis fonuose – N_{60}, N_{120}, N_{180}, ir N_{240} buvo siera netręšti, taip pat tręšti S_{15} (visuose fonuose), S_{30} (antrame–ketvirtame) ir S_{60} (tik ketvirtame). Tyrimo duomenimis, tręšimas siera kviečių grūdų derlių esmingai padidino tik atskirais tyrimų metais ir tik didesnėmis normomis azoto trąšų tręštuose laukeliuose. Azoto trąšos grūduose didino baltymų koncentraciją, sedimentaciją ir glitimą, o sieros trąšos – tik vienais metais ir tik N_{180} fone. Sieros trąšos žieminiams kviečiams neturėjo įtakos dėl didelio mineralinio sieros (S_{min}) kiekio dirvožemio 0–60 cm sluoksnyje – pavasarį jo nustatyta 42–84 kg ha^{-1}, o vasarą šis kiekis padidėdavo dar 1,4–1,7 karto. Tyrimo metu nustatyta, kad žieminių kviečių BBCH 30 tarpsniu augale N:S santykis svyravo 8–16, o BBCH 65 – 9–19 ribose, todėl žieminiai kviečiai siera buvo apsirūpinę optimaliai arba net daugiau optimumo.

Reikšminiai žodžiai: azoto ir sieros santykis, mineralinis azotas, mineralinė siera, grūdų derlius, grūdų kokybė.