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ORIGINAL PAPER

EFFECT OF LIQUID AMIDE NITROGEN FERTILIZER WITH MAGNESIUM AND SULPHUR ON SPRING WHEAT CHLOROPHYLL CONTENT, ACCUMULATION OF NITROGEN AND YIELD*

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Abstract

Field experiments with spring wheat (*Triticum aestivum* L.) were carried out at the Experimental Station of Vytautas Magnus University, Agriculture Academy in Lithuania. Spring wheat was dressed with $N_{90}P_{90}K_{116}$ at sowing and N_{60} was applied at the stem elongation stage. Liquid amide (NH_2) nitrogen fertilizer with magnesium and sulphur was applied either at the tillering stage or at the stem elongation stage. The experiment treatments (nutrient doses in kg ha^{-1}): 1) control; 2) 0.3 Mg + 0.4 S + 1.1 N (F1); 3) 0.6 Mg + 0.8 S + 2.2 N (F2); 4) 0.9 Mg + 1.2 S + 3.3 N (F3); 5) 1.2 Mg + 1.6 S + 4.4 N (F4); 6) 3.0 Mg + 4 S + 11.0 N (F5). Liquid fertilizer applied at the spring wheat's tillering stage in the soil with a moderate status of magnesium and sulphur did not significantly change the content of chlorophyll *a* in wheat leaves within 10 days after application. A significant effect of this fertilizer on the chlorophyll *a* content was identified when spring wheat at the stem elongation stage had been treated with F2 - F5 (2014) and F1 - F5 (2015) fertilizer doses. Significant effect on the carotenoid content was recorded in the plots applied F3 (2014) and F2 - F4 (2015) doses. A significant increase in the nitrogen content in wheat plants was observed in the treatments fertilized with F1 - F5 doses at the tillering stage and those applied F3 - F5 fertilizer doses at the stem elongation stage. The relationship between the nitrogen content in wheat plants and fertilizer doses was strong and significant. Significant grain yield increases were obtained in the treatments fertilized with F3 - F5 doses ates at tillering and those fertilized with F2 and F3 doses at the stem elongation stage. Statis-

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tically significant correlations between fertilizer doses and grain yield were ,,determined for both growth stages at which the fertilizer had been applied, i.e. at tillering and at stem elongation. Fertilizers tended to increase the grain protein content; however, a significant increase in the grain protein content was observed only in the treatment fertilized with F3 dose at stem elongation.

Keywords: spring wheat, fertilization, nitrogen, magnesium, sulphur, photosynthesis pigments, yield, protein.

INTRODUCTION

The supply of nitrogen, magnesium and sulphur as well as their interaction are considered the major factors influencing the vegetative growth of plants. Nitrogen is involved in the key processes responsible for solar energy capture, transformation and partitioning of the fixed assimilates among plant organs during the course of vegetative production and development (GRZEBISZ 2013).

Magnesium is a very important element for plant growth (STAUGAITIS, RUTKAUSKIENĖ 2012, GRANSEE, FÜHRS 2013). It is the central atom of an *a* chlorophyll molecule and plays a key role in photosynthetic activity, physiological and biochemical processes and protein synthesis, and in plant defence mechanisms in abiotic stress situations (MENGUTAY et al. 2013, VERBRUGGEN, HERMANS 2013, SENBAYRAM et al. 2015). Moreover, it is involved in carbohydrate transport from source-to-sink organs. SHAUL (2002) has reported that the photosynthetic activity of leaves, dry matter and nitrogen remobilization from vegetative plant parts and the rate of assimilate transportation to developing seeds depend on magnesium. This element is indispensable for the activity of many enzymes and has important roles in transport of photoassimilates into sink organs such as roots, shoots tips and seeds (CAKMAK 2013, SENBAYRAM et al. 2015). The availability of magnesium to plants depends on various factors, including soil texture, climatic and growing conditions, plant species, crop rotation, and fertilization (JASKULSKA et al. 2015).). Magnesium nutrition of crops also depends on the potassium to magnesium ratio, as potassium excess reduces magnesium availability (K has a strong antagonistic effect on the Mg uptake) to plants (SHAUL 2002, GRZEBISZ 2013).

Sulphur plays an important role in plant metabolism and affects the production and quality of wheats (JÄRVAN et al. 2008). Adequate sulphur nutrition improves photosynthesis and growth of plants, and it has regulatory interaction with nitrogen assimilation (SALVAGIOTTI et al. 2008). KULHANEK et al. (2014) studies indicated that the effect of applied sulphur on the wheat yield and grain quality is closely related to nitrogen supply. The effect of sulphur addition had relevance when nitrogen was not a limiting factor, showing a positive interaction between these two nutrients on crop growth, reflected in higher nitrogen use efficiency (SALVAGIOTTI, MIRALLES 2008).

Järvan et al. (2008) indicated that sulphur fertilization against nitrogen background N_{100} increased the winter wheat yield by 7.7–45.5% depending on the weather and soil conditions. According to Erdem et al. (2016), sulphur deficiency occurring during the very early growth stages of winter cereals caused an irreversible reduction of generative yield components, and the grain yield was significantly reduced if no sulphur was applied. Therefore, balanced sulphur fertilization ensures abundant and high-quality yield.

The study is aimed to estimate the complex effects of amide nitrogen, magnesium and sulphur on the photosynthetic pigments of spring wheat, nitrogen content, grain yield and protein content in grain as influenced by different fertilizer doses in the soil with a moderate rich magnesium (151-200 mg kg^{-1}) and sulphur (15-30 mg kg^{-1}) status.

MATERIAL AND METHODS

A field experiment was set up at Vytautas Magnus University, Agriculture Academy's Experimental Station in 2015 and 2016, in Lithuania (54°53′3.26″, 23°50′33.25″). The experimental plots were laid out in a randomized block design with four replications on a limnoglacional silty loam on moraine clay loam *Cal(ca)ri-Epihypogleyic Luvisol* with a pH_{KCl} of 6.8-7.2, phosphorus (P) content of 100.3-116.9 mg kg^{-1}, potassium (K) content of 126.2-139.4 mg kg^{-1}, magnesium (Mg) content of 95.3-109.8 mg kg^{-1}, sulphur (S) content of 7.6-9.2 mg kg^{-1} and organic carbon content of 11.7-12.1 g kg^{-1}. A spring wheat variety called Tybalt C_2 was grown in the experiment.

Complex fertilizer NPK 14–14–18 ($N_{90}P_{90}K_{116}$) was applied at sowing, while ammonium nitrate (N_{60}) was applied at the stem elongation (BBCH 32-35) stage. Additionally, liquid amide nitrogen fertilizer with magnesium and sulphur was foliar applied either at the spring wheat tillering stage (BBCH 25-27) or at the stem elongation stage (BBCH 32-35). The experiment included the following additional fertilization treatments (nutrients doses in kg ha^{-1}): 1) control; 2) 0.3 Mg + 0.4 S + 1.1 N-NH_2 (F1); 3) 0.6 Mg + 0.8 S + + 2.2 N-NH_2 (F2); 4) 0.9 Mg + 1.2 S + 3.3 N-NH_2 (F3); 5) 1.2 Mg + 1.6 S + 4.4 N-NH_2 (F4); 6) 3.0 Mg + 4.0 S + 11.0 N-NH_2 (F5). The following doses of fertilizer in commercial weight were applied in treatments F2 - F5: 5.1; 10.3; 15.5; 20.5; 51.4 l ha^{-1}. The composition of the liquid amide nitrogen fertilizer with magnesium and sulphur was as follows (g dm^{-3}): 4.2 Mg, 5.6 S, 15 N-NH_2. The total volume of the solution was 200 l ha^{-1}.

Experimental and analytical methods

The soil was analyzed for pH_{KCl} measured in 1M KCl extraction by the potentiometric method, organic carbon (C) was determined by the wet oxidation method, available phosphorus (P) and available potassium (K) by the Egner-Riehm-Domingo (A-L) method, available magnesium (Mg) by the atomic absorption spectrometric method (SVP D-06), sulphur by the turbidimetric method, the total nitrogen by the Kjeldahl method. Ten days after foliar application, plant samples (10 per plot) were analyzed for the chlorophyll *a* and *b* and carotenoid content in leaves (according to D. Wettstein). Nitrogen content in plants was measured by the Kjeldahl method. Dry matter was estimated by oven drying at 105°C for 16 h. Protein content was calculated by multiplying the nitrogen content by a coefficient of 5.7.

All experimental data were statistically processed by the analysis of variance (ANOVA) using a software package Selekcija (Raudonius 2017). The significance of data was determined by Fisher's criterion with a significance level of $p \leq 0.01$ and 0.05. The correlation coefficients and relationships between the indicators tested were determined using the software Statistica 7 (Hill, Levicki 2005).

RESULTS AND DISCUSSION

Balanced nutrition of plants and the proper ratio of nitrogen, sulphur and magnesium in plants promote the biosynthesis of photosynthetic pigments and improve the activity of the photosynthetic apparatus (Yong et al. 2010, Mengutay et al. 2013). Studies showed that the plant chlorophyll content was positively correlated with the nitrogen and magnesium content (Schlichting et al. 2015). The value of the leaf chlorophyll content can help to understand the nutritional status of the plant, and scientifically guide the fertilization management to ensure good crop quality and yield (Menesatti et al. 2010).

The data obtained during the two experimental years suggest that spring wheat fertilized with the above mentioned fertilizer at the tillering stage accumulated insignificantly higher chlorophyll *a* content in leaves compared with the control treatment (Table 1). The chlorophyll *a* content in plants at the tillering stage tended to increase in response to fertilization with liquid amide nitrogen fertilizer with magnesium and sulphur; however, no significant changes were determined. The data of the correlation regression analysis indicate that the changes in chlorophyll *a* depending on amide nitrogen fertilizer with magnesium and sulphur application doses varied according to the quadratic equation: $y = 1.482 + 0.0097x - 0.0002x^2$. Fertilizers were responsible for 41.4% ($R^2 = 0.414$) of the change in the chlorophyll *a* content. Staugaitis et al. (2012) have reported that magnesium fertilizers are effective only in soil low in the carbonate content, i.e., in soil with a low Mg content.

Table 1

The content of chlorophyll *a* in spring wheat leaves 10 days after fertilizer application at tillering and at stem elongation stages

Fertilization treatments (nutrients doses in kg ha^{-1})	Chlorophyll *a* content (mg g^{-1})			
	tillering stage		stem elongation stage	
	2014	2015	2014	2015
Control (without fertilizer)	1.471*a*	1.520*a*	1.880*a*	1.821*a*
0.3 Mg + 0.4 S + 1.1 N (F1)	1.471*a*	1.522*a*	1.810*a*	2.103*b*
0.6 Mg + 0.8 S + 2.2 N (F2)	1.490*a*	1.541*a*	2.051*b*	2.090*b*
0.9 Mg + 1.2 S + 3.3 N (F3)	1.592*a*	1.643*a*	2.202*c*	2.292*c*
1.2 Mg + 1.6 S + 4.4 N (F4)	1.573*a*	1.534*a*	2.154*bc*	2,014*b*
3.0 Mg + 4 S + 11 kg N (F5)	1.552*a*	1.533*a*	2.137*bc*	2.137*bc*

Values followed by the same letters in columns are not significantly different ($p < 0.05$).

The effect of amide nitrogen fertilizer with magnesium and sulphur applied at the spring wheat stem elongation stage on the chlorophyll *a* content in leaves was significant (Table 1). This can be explained by the fact that the content of magnesium in cereals decreases at the beginning of stem elongation and this depends little on the content of this element in the soil (Cakmak, Kirkby 2008, Grzebisz 2013), while the plant's need for magnesium is high at this stage. In 2014, the chlorophyll *a* content was significantly increased by F2 - F5 fertilizer doses compared with the control treatment. The highest chlorophyll *a* content was determined in the treatment fertilized with F3 compared with F4 and F5 doses; however, no significant differences were identified. In 2015, significant effect on the changes in chlorophyll *a* was exerted by F1 - F5 fertilizer doses. The highest chlorophyll *a* content was determined in spring wheat plants fertilized with F3 dose, and this increase was significant compared with F1, F2 and F4 fertilizer doses. The importance of magnesium for changes in the chlorophyll content has been also documented by Laing et al. (2000).

The data averaged over the two experimental years suggest that liquid amide nitrogen fertilizer with magnesium and sulphur applied at F2 - F5 doses at spring wheat's stem elongation stage increased the chlorophyll *a* content in plants by 8.5-13 mg g^{-1}. Skudra and Ruza (2017) have found that the highest chlorophyll content in leaves, stems and ears was obtained by using additional sulphur in two trial years. Nitrogen fertilization significantly affected the chlorophyll content in leaves and stems in one trial year. The relationship between chlorophyll *a* and fertilizer doses applied at the stem elongation stage was corroborated by a strong and significant correlation ($R^2 = 0.539$, $p < 0.05$). The effect of different doses of liquid amide nitrogen fertilizer with magnesium and sulphur on the accumulation of chlorophyll *a* in plants was best described by the quadratic equation: $y = 1.87 + 0.0318x - 0.0007x^2$.

Table 2

The content of chlorophyll *b* in spring wheat leaves 10 days after fertilizer application at tillering and at stem elongation stages

Fertilization treatments (nutrients doses in kg ha^{-1})	Chlorophyll *b* content (mg g^{-1})			
	tillering stage		stem elongation stage	
	2014	2015	2014	2015
Control (without fertilizer)	0.421*bc*	0.440*ab*	0.483*a*	0.490*a*
0.3 Mg + 0.4 S + 1.1 N (F1)	0.374*ab*	0.422*ab*	0.693*bc*	0.573*b*
0.6 Mg + 0.8 S + 2.2 N (F2)	0.356*a*	0.401*ab*	0.641*b*	0.541*b*
0.9 Mg + 1.2 S + 3.3 N (F3)	0.451*c*	0.464*b*	0.724*c*	0.590*bc*
1.2 Mg + 1.6 S + 4.4 N (F4)	0.371*ab*	0.390*ab*	0.652*bc*	0.615*bc*
3.0 Mg + 4 S + 11 kg N (F5)	0.390*ab*	0.381*a*	0.633*b*	0.652*c*

Values followed by the same letters in columns are not significantly different ($p < 0.05$).

The highest chlorophyll *b* content (2014) was determined in the treatment fertilized with F3 at the tillering stage, compared with the control – no significant differences were identified (Table 2). Spring wheat fertilized with F2 dose (2014) of liquid amide nitrogen fertilizer with magnesium and sulphur at this stage accumulated significantly lower chlorophyll *b* content in leaves, compared with the control treatment. The data from the experimental year 2015 suggest that the highest chlorophyll *b* content was accumulated by spring wheat fertilized with F3 dose at the tillering stage. No correlation was established between the fertilizer doses and chlorophyll *b* content.

A marked fertilizer effect on the changes in the chlorophyll *b* content was determined when spring wheat had been fertilized at the stem elongation stage. At this stage, the chlorophyll *b* content distinctly increased when fertilized with F1 - F5 doses, compared with the control. In 2014, the highest chlorophyll *b* content was identified in the leaves of spring wheat fertilized with F3 dose and it was significantly higher compared with fertilization with F2 and F5 doses. In 2015, the highest chlorophyll *b* content was found having fertilized the crop with F5 dose; however, it was not significantly higher compared with F3 and F4 doses. Zhian and Rezheen (2013) have reported that foliar Mg application significantly increased chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids in the leaves. The analysis of the correlation regression data indicates that the chlorophyll *b* content, depending on a fertilizer dose, varied according to the quadratic equation ($y = 0.5236 + 0.0134x - 0.0003x^2$) curve. The correlation coefficient was moderately strong and statistically significant ($R^2 = 0.428$, $p < 0.05$).

The content of carotenoids varied inconsistently in response to spring wheat fertilization with amide nitrogen fertilizer with magnesium and sulphur at both the tillering and stem elongation stages (Table 3). Yoo et al. (2003) have ascertained that biosynthesis of carotenoids in plants is a genetic characteristic, but environmental conditions also play an essential role.

Table 3

The content of carotenoids in spring wheat leaves 10 days after fertilizer application at tillering and at stem elongation stages

Fertilization treatments (nutrients doses in kg ha^{-1})	Carotenoid content (mg g^{-1})			
	tillering stage		stem elongation stage	
	2014	2015	2014	2015
Control (without fertilizer)	0.613*a*	0.651*a*	0.660*ab*	0.601*a*
0.3 Mg + 0.4 S + 1.1 N (F1)	0.571*a*	0.723*ab*	0.704*b*	0.630*ab*
0.6 Mg + 0.8 S + 2.2 N (F2)	0.550*a*	0.724*ab*	0.706*b*	0.652*b*
0.9 Mg + 1.2 S + 3.3 N (F3)	0.612*a*	0.652*a*	0.790*c*	0.724*c*
1.2 Mg + 1.6 S + 4.4 N (F4)	0.550*a*	0.755*b*	0.641*a*	0.640*b*
3.0 Mg + 4 S + 11 kg N (F5)	0.574*a*	0.701*ab*	0.692*b*	0.613*ab*

Values followed by the same letters in columns are not significantly different ($p < 0.05$).

In 2014, the fertilizer applied at the tillering stage did not have a significant effect on the carotenoid content. In 2015, only F4 dose stood out with a positive significant effect on the carotenoid content, compared with the control. Regarding fertilization at the stem elongation stage, the carotenoid content in 2014 significantly increased having used F3 dose, while in 2015 having used F2 - F4 doses. A comparison of plants fertilized with different fertilizer doses evidenced that significant changes in the carotenoid content were obtained having fertilized spring wheat with F3 dose. Bojović et al. (2005) have reported that the carotenoid content showed little variation as a function of mineral nutrition. Zhian and Rezheen (2013) have found that the carotenoid content in plants also depends on the magnesium content.

Nitrogen content significantly increased in plants under the effect of fertilizer when it had been applied at F1 - F5 doses at tillering and at F3 - F5 doses at stem elongation (Table 4). Tea et al. (2007) have reported

Table 4

The content of nitrogen in spring wheat leaves 10 days after fertilizer application at tillering and at stem elongation stages

Fertilization treatments (nutrients rates kg ha^{-1})	Nitrogen content (g kg^{-1} DM)			
	tillering stage		stem elongation stage	
	2014	2015	2014	2015
Control (without fertilizer)	24.0*a*	24.8*a*	19.0*a*	20.0*a*
0.5 kg MgO + 1.0 kg SO_3 + 1.1 kg N–NH_2 (F1)	26.2*b*	25.8*b*	22.0*a*	22.7*ba*
1.0 kg MgO + 2.0 kg SO_3 + 2.2 kg N–NH_2 (F2)	27.0*bc*	26.0*b*	22.2*a*	25.0*c*
1.5 kg MgO + 3.0 kg SO_3 + 3.3 kg N–NH_2 (F3)	28.0*c*	27.5*c*	23.0*b*	25.6*c*
2.0 kg MgO + 4.0 kg SO_3 + 4.4 kg N–NH_2 (F4)	32.0*d*	31.0*e*	26.1*c*	24.2*b*
5.0 kg MgO + 10.0 kg SO_3 + 11.0 kg N–NH_2 (F5)	27.1*bc*	30.0*d*	23.3*b*	24.4*bc*

Values followed by the same letters in columns are not significantly different ($p < 0.05$).

that a synergistic effect between the applied nitrogen and sulphur fertilizers appears to increase nitrogen and sulphur assimilation in wheat plant and grain. Grzebisz (2013) have reported that magnesium is involved in simultaneously controlling processes responsible for photosynthesis, assimilate production and partitioning among plant parts, and it seems to be a major player in nitrogen uptake and its utilisation.

The statistical relationship between the nitrogen content in the aerial part of spring wheat and fertilizer doses was described by the quadratic equations presented in Figure 1. The correlation between the nitrogen con-

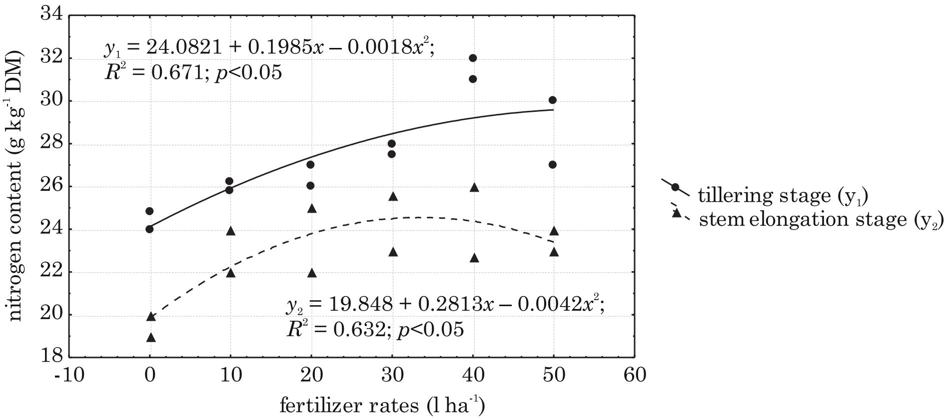

Fig. 1. The relationship between nitrogen content (y, g kg^{-1}) in plants and fertilizer doses (x, l ha^{-1}) of liquid amide nitrogen fertilizer with magnesium and sulphur

tent in plants and fertilizer doses was strong and significant when fertilized at tillering (R^2 = 0.671, $p < 0.05$) and at stem elongation (R^2 = 0.632, $p < 0.05$).

When fertilizing at the tillering stage, the dry matter content in the aerial part of spring wheat increased marginally under the influence of liquid amide nitrogen fertilizer with magnesium and sulphur (Table 5). In the soil moderately rich in magnesium and sulphur, F1 - F5 fertilizer doses ensured similar effect. The correlation regression analysis showed a weak relationship (R^2 = 0.244, $p < 0.05$) between the dry matter content and fertilizer doses (Figure 2).

Spring wheat fertilized at the stem elongation stage accumulated significantly more dry matter in the aerial plant part when F3 - F5 doses had been used, compared with the control (Table 5). Compared with the spring wheat fertilized with F1 - F3 doses, significantly higher dry matter content in each experimental year was obtained having fertilized spring wheat with F4 and F5 doses. The relationship between the dry matter content and fertilizer doses was moderately strong (R^2 = 0.482; $p < 0.05$) – Figure 2.

In the soil moderately rich in magnesium and sulphur, liquid amide nitrogen fertilizer with magnesium and sulphur had a positive impact

Table 5

The content of dry matter in spring wheat leaves 10 days after fertilizer application at tillering and at stem elongation stages

Fertilization treatments (nutrients rates kg ha^{-1})	Dry matter content (%)			
	tillering stage		stem elongation stage	
	2014	2015	2014	2015
Control (without fertilizer)	18.50*a*	18.20*a*	20.60*a*	19.20*a*
0.5 kg MgO + 1.0 kg SO_3 + 1.1 kg N–NH_2 (F1)	18.50*a*	18.44*a*	21.59*ab*	20.00*ab*
1.0 kg MgO + 2.0 kg SO_3 + 2.2 kg N–NH_2 (F2)	18.52*a*	18.62*a*	21.47*ab*	19.50*a*
1.5 kg MgO + 3.0 kg SO_3 + 3.3 kg N–NH_2 (F3)	18.72*a*	19.00*a*	22.72*bc*	20.50*b*
2.0 kg MgO + 4.0 kg SO_3 + 4.4 kg N–NH_2 (F4)	17.60*a*	18.90*a*	24.70*dc*	23.00*c*
5.0 kg MgO + 10.0 kg SO_3 + 11.0 kg N–NH_2 (F5)	18.76*a*	18.00*a*	24.00*c*	22.50*c*

Values followed by the same letters in columns are not significantly different ($p < 0.05$).

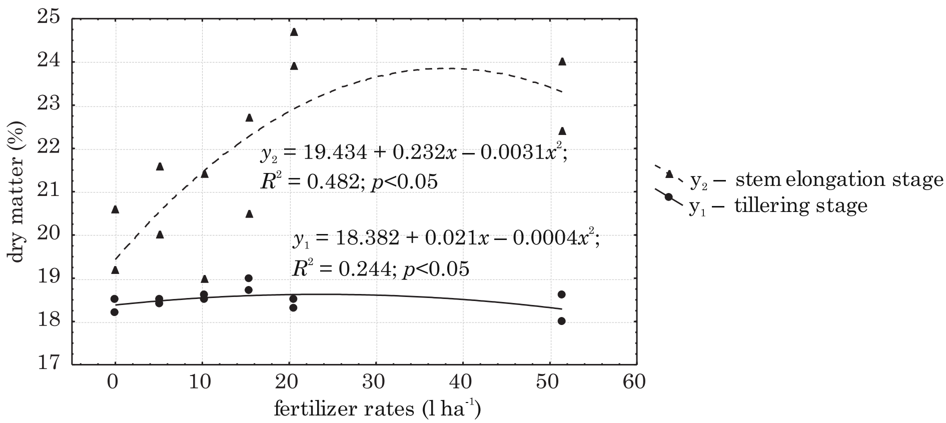

Fig. 2. The relationship between dry matter content (*y*) in plants and fertilizer doses (*x*, l ha^{-1}) of liquid amide nitrogen fertilizer with magnesium and sulphur

on grain yield. Grzebisz (2013) have found that magnesium application in cereals resulted in a higher number of ears and/or thousand grain weight, stressing the magnesium-sensitive stages of yield formation. The optimal yield forming effect of fertilizer magnesium can generally occur under conditions of a relatively low nitrogen supply, but a high supply of magnesium.

The different fertilizer doses applied at spring wheat's tillering and stem elongation stages increased grain yield, but the increase was not always significant (Figure 3). Significant grain yield increases were obtained when plants had been fertilized at the tillering stage with F3 - F5 doses (2014-2015) and at stem elongation stage with F2 and F3 doses in 2014 and with F2 - F4 doses in 2015 (Table 6). These findings agree with those obtained by other researchers. Klikocka et al. (2016) have reported that nitrogen fertilizer

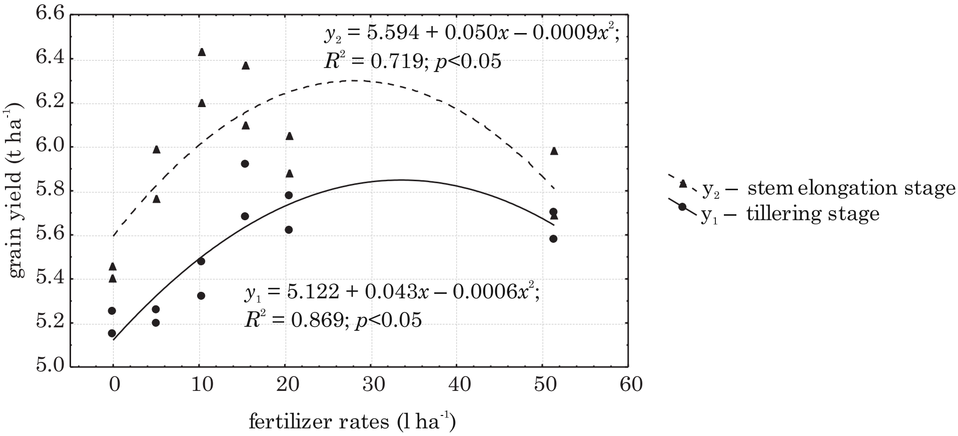

Fig. 3. The relationship between spring wheat grain yield (y, t ha^{-1}) and doses (x, l ha^{-1}) of liquid amide nitrogen fertilizer with magnesium and sulphur

Table 6

The effect of liquid amide nitrogen fertilizer with magnesium and sulphur on spring wheat grain yield

Fertilization treatments (nutrients rates kg ha^{-1})	Grain yield (t ha^{-1})			
	tillering stage		stem elongation stage	
	2014	2015	2014	2015
Control (without fertilizer)	5.25*a*	5.15*a*	5.46*a*	5.40*a*
0.5 kg MgO + 1.0 kg SO_3 + 1.1 kg N–NH_2 (F1)	5.26*a*	5.20*a*	5.84*a*	5.76*a*
1.0 kg MgO + 2.0 kg SO_3 + 2.2 kg N–NH_2 (F2)	5.48*a*	5.32*b*	6.43*c*	6.20*b*
1.5 kg MgO + 3.0 kg SO_3 + 3.3 kg N–NH_2 (F3)	5.92*c*	5.68*c*	6.37*c*	6.10*b*
2.0 kg MgO + 4.0 kg SO_3 + 4.4 kg N–NH_2 (F4)	5.78*bc*	5.62*c*	5.88*a*	6.05*b*
5.0 kg MgO + 10.0 kg SO_3 + 11.0 kg N–NH_2 (F5)	5.70*b*	5.58*c*	5.98*a*	5.69*a*

Values followed by the same letters in columns are not significantly different ($p < 0.05$).

in combination with sulphur and magnesium increased cereal grain yield. Tea et al. (2007) suggest that without adequate magnesium and sulphur, crops cannot reach their full potential in terms of yield, quality or protein content; nor can they make efficient use of the applied nitrogen. Grzebisz (2013) has linked grain yield changes to magnesium deficiency in plants, which determines formation of yield structural elements and at the same time the yield. Järvan et al. (2012) indicated that the effect of applied sulphur on wheat yield is closely related to nitrogen supply. The application of sulphur containing fertilizers on break-stony soil increased grain yield by 21%. However, the findings of Staugaitis et al. (2017) show that foliar fertilization of spring wheat with nitrogen, sulphur and micronutrients increased the grain yield only in two years out of five, and the average yield increase resulting from the foliar fertilization during all five years was −0.38 – +2.11%.

The correlation regression analysis showed that when spring wheat had been fertilized at the tillering stage a very strong and significant ($R^2 = 0.869$, $p < 0.05$) correlation was established between grain yield and fertilizer doses, described by a quadratic equation (Figure 3). When spring wheat had been fertilized at the stem elongation stage, the relationship between these indicators was strong and significant ($R^2 = 0.719$, $p < 0.05$).

Many researchers have stressed the importance of nitrogen, magnesium and sulphur on grain quality indicators. Magnesium activates a large number of enzymes in plants, and its simultaneous supply increases the rate of mineral nitrogen transformation into proteins. Nitrogen and sulphur are both important constituents of protein and play an important part in the formation of the gluten quality of flour (Jankowski et al. 2016, Klikocka et al. 2016).

Our assessment of the effects of liquid amide nitrogen fertilizer with magnesium and sulphur revealed a general trend indicating that the fertilizer insignificantly increased spring wheat's grain protein content when applied at the tillering stage (Table 7). It is likely that at this growth stage

Table 7

The effect of liquid amide nitrogen fertilizer with magnesium and sulphur on spring wheat grain protein content

Fertilization treatments (nutrients doses in kg ha^{-1})	Grain protein content (g kg^{-1} DM)			
	tillering stage		stem elongation stage	
	2014	2015	2014	2015
Control (without fertilizer)	103.8*a*	104.1*a*	103.2*a*	101.0*a*
0.3 Mg + 0.4 S + 1.1 N (F1)	107.7*a*	109.0*a*	111.0*ab*	112.0*b*
0.6 Mg + 0.8 S + 2.2 N (F2)	106.1*a*	109.0*a*	105.9*ab*	113.2*b*
0.9 Mg + 1.2 S + 3.3 N (F3)	104.4*a*	104.4*a*	112.0*b*	114.0*b*
1.2 Mg + 1.6 S + 4.4 N (F4)	105.6*a*	103.5*a*	105.2*ab*	103.6*a*
3.0 Mg + 4 S + 11 kg N (F5)	110.0*a*	104.0*a*	104.3*ab*	104.2*a*

Values followed by the same letters in columns are not significantly different ($p < 0.05$).

the plants contained sufficient concentrations of the tested nutrients, therefore no significant differences were identified. Gerendás, Führs (2013) have indicated that it is efficient to fertilize with magnesium when there is a shortage of this element. In this case, increasing Mg supply on Mg-deficient sites tends to increase the quality of agricultural crops, particularly when the formation of quality traits is dependent on Mg-driven photosynthesis and assimilate translocation within the plant.

In 2014, spring wheat fertilized with F3 dose at the stem elongation stage produced grain with significantly higher protein content, compared with the control. In 2015, a significant protein content increase resulted from F1 - F3 doses (Table 3). The relationship between fertilizer doses and grain protein content was strong ($R^2 = 0.614$, $p < 0.05$) in 2014 and moderate ($R^2 = 0.307$, $p < 0.05$) in 2015.

The importance of magnesium and sulphur for grain quality indicators has been pointed out by other researchers. Järvan et al. (2008) argue that the effect of sulphur on the protein and wet gluten content 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. They indicate that the quality parameter most benefited by sulphur was the gluten-index and especially the sedimentation value.

CONCLUSIONS

1. In the soil moderately rich in magnesium and sulphur, liquid amide nitrogen fertilizer with magnesium and sulphur did not significantly change the content of chlorophyll *a* in the spring wheat leaves in the treatments fertilized at the tillering stage. A significant effect of the fertilizer on the content of chlorophyll *a* was established when spring wheat had been fertilized at the stem elongation stage with F2 - F5 doses in 2014 and F1 - F5 doses in 2015. A significant effect on the content of carotenoid appeared when spring wheat had been fertilized with F3 dose in 2014 and F2 - F4 doses in 2015.

2. The dry matter content in spring wheat did not significantly change in response to fertilization at the tillering stage, while fertilization at the stem elongation stage resulted in significantly higher dry matter content in the aerial part of spring wheat plants fertilized with F3 - F5 doses. The relationship between the dry matter content and fertilizer doses ates was moderately strong ($R^2 = 0.482$; $p < 0.05$).

3. Nitrogen content in spring wheat plants significantly increased after fertilization with liquid amide nitrogen fertilizer with magnesium and sulphur at F1 - F5 doses at the tillering and at F3 - F5 doses at stem elongation stage. The relationship between the nitrogen content in plants and fertilizer doses was strong and significant $R^2_{\text{tillering stage}} = 0.671$, $R^2_{\text{stem elongation stage}} = 0.632$, $p < 0.05$.

4. Significant grain yield increases were obtained when spring wheat had been fertilized at the tillering stage with F3 - F5 doses and at the stem elongation stage with F2 and F3 doses. Statistically significant ($p < 0.05$) correlations were determined between fertilizer doses ates and grain yield when spring wheat had been fertilized at both the tillering ($R^2 = 0.869$) and stem elongation ($R^2 = 0.719$) stages.

5. Liquid amide nitrogen fertilizer with magnesium and sulphur tended

to increase the grain protein content; however, a significant increase in the protein content was observed only in the treatments fertilized at the stem elongation stage with F3 dose in 2014 and with F1 - F3 doses in 2015.

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