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ORIGINAL PAPER

EFFECT OF A BIOSTIMULANT ON THE ACCUMULATION OF Mg IN WINTER OILSEED RAPE UNDER DIFFERENT MINERAL FERTILIZATION DOSES*

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Abstract

Increased fertilization doses stimulate biomass production and raise plants' demand for nutrients. Biostimulants may improve absorption of nutrients by plants and prevent nutrient deficiency resulting from their lower concentrations in biomass. The aim of this study was to evaluate Mg accumulation in winter oilseed rape at flowering, fruit development and ripening as affected by the application of a seaweed biostimulant, at different doses of NPK tested in two field experiments, with and without S fertilization. The field experiments were set up in Alfisol, on adjacent plots, in Poland (53°13′N. 17°51′E). In the first experiment, S was used in a dose of 36 kg ha^{-1}; in the second experiment there was no S application. In both experiments, different NPK doses were used: high 180 N, 70 P, 132 K (kg ha^{-1}) or low 144 N, 35 P, 66 K (kg ha^{-1}). In addition, the biostimulant Kelpak SL was applied (in doses 2 dm^3 ha^{-1} in autumn + 2 dm^3 ha^{-1} in spring) or there was no biostimulant application. It was found that the response of oilseed rape to the biostimulant and NPK fertilization level, expressed by the amount of dry matter production and Mg accumulation in whole plants, was more favourable in the experiment with the S application prior to sowing the crop. The biostimulant increased the dry matter production while maintaining a similar Mg concentration in plants as compared with the control. Moreover, in S-fertilized oilseed rape, an increase in the Mg accumulation in response to the biostimulant was the highest (22%) at the ripening stage, whereas in the plants not fertilized with sulphur (the second experiment), this increase was significant only at fruit development. In oilseed rape fertilized with S, higher NPK doses caused an increase in the dry matter production and a decrease in the Mg concentration in the aerial parts at the flowering and fruit development stages. At the ripening stage, with a similar response of dry matter, the Mg concentration in plant remained unchanged, hence the Mg accumulation in biomass was by as much as 26% higher than after the application of lower NPK doses.

Keywords: dry matter accumulation, growth stage, nitrogen, magnesium content, phosphorus, potassium, sulphur.

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INTRODUCTION

Preparations produced from marine algae are an important group of biostimulants. Beneficial effects of algal extracts indicate their usefulness in cultivation of horticultural and agricultural crops (Khan et al. 2009, Craigie 2011, Sharma et al. 2014). Application of biostimulants increases the resistance of plants to stress (Sharma et al. 2014, Stadnik, de Freitas 2014). It was also demonstrated that biostimulants can improve the uptake and accumulation of macro- and microelements (Jannin et al. 2012, Papenfus et al. 2013, Shah et al. 2013, Billard et al. 2014, Szczepanek et al. 2015), which may have a favourable impact on crop yields. However, the fundamental condition for obtaining high crop yields is to provide balanced fertilization (Szczepaniak 2015, Wang et al. 2015). Many researchers point to mutual relationships of nutrients affecting their accumulation, aerial and underground biomass production and seed yield (Giovahi, Saffari, 2006, Ngezimana, Agenbag 2014). The accumulation of nutrients in oilseed rape changes at successive developmental stages (Gaj 2011, Szczepaniak 2014, Wang et al. 2015). An increased fertilization level, especially with nitrogen, stimulates biomass production and increases plant demands for the other nutrients, which may result in their deficiency (Grant, Bailey 1993, Yasari, Patwardhan 2006, Brennan, Bolland 2009, Wang et al. 2015). By improving the uptake of nutrients, an application of biostimulants may prevent a decrease in their concentration in biomass (Billard et al. 2014). Magnesium is one of macroelements regarded to be essential for the growth and development of oilseed rape. Its deficiency may have an unfavourable effect on metabolism, reduce the effectiveness of other nutrients and lower the yield (Grzebisz et al. 2010, Szczepaniak et al. 2015).

The aim of this study was to assess the magnesium concentration and accumulation by the aerial parts and roots of winter rapeseed at successive stages of the generative development: flowering, fruit development and ripening, after an application of a biostimulant, under the condition of different NPK fertilization levels, tested in two field experiments: with and without fertilization with S.

MATERIAL AND METHODS

The study was based on field experiments located next to each other, in the Kuyavian-Pomeranian region, Poland, 53°13′N. 17°51′E. The subject of the study was winter rapeseed, cv. Chagall, cultivated in the successive years 2011-2013, after winter wheat. These experiments were conducted in Alfisol (Soil Survey Staff 2010), where the topsoil was characterized by a medium content of available P (64.0 mg kg^{-1}) and high of K (126.0 mg kg^{-1}), both determined with the Egner-Riehm method, a medium content of available Mg (32-43 mg kg^{-1} of soil), determined with the Schachtschabel method, and a medium content of available sulphur (13 S mg kg^{-1}), determined with the sulphate turbidimetric method. The soil had slightly acidic reaction (pH in 1M KCl 5.7-6.1). The content of organic carbon 7.55-7.80 g kg^{-1} and total nitrogen 0.69- 0.75 g kg^{-1} in the soil was relatively low.

In the first experiment, S fertilization was applied in a dose of 36 kg S ha^{-1}; in the second experiment, there was no S application. In both experiments, different NPK fertilization levels were used: high 180 N, 70 P, 132 K (kg ha^{-1}) or low 144 N, 35 P, 66 K (kg ha^{-1}). The seaweed biostimulant Kelpak SL was applied (2 dm^3 ha^{-1} in autumn after the formation of 4-5 leaves and 2 dm^3 ha^{-1} in spring, 10-15 days after the onset of growth). Alternatively, there was no biostimulant application. Doses commonly used in farming were assumed to represent the low fertilization level. The high fertilization level was determined taking into consideration the soil abundance, unit uptake and expected yield. Mineral fertilization into soil with phosphorus (triple superphosphate 40%, granular), potassium (potash salt 60%, granular) and sulphur (elemental sulphur 36%, granular) was applied pre-sowing in autumn, whereas nitrogen (ammonium nitrate 34%) was supplied in spring, at the begiining of the growth (100 kg ha^{-1}, granular), while the other part was given after 3 weeks. The biostimulant Kelpak SL applied in the experiment is obtained from marine macroalgae (*Ecklonia maxima* Osbeck), which belongs to the division of brown algae (Phaeophyta) and was collected on the coast of Africa. This biostimulant contains phytohormones: auxins and cytokinins (11 and 0.031 mg dm^{-3}, respectively), alginians, amino acids, as well as small amounts of macro- and microelements (Stirk at al. 2004, 2014).

The experiments were established in a randomized split-block design, in four replications, and the plot area was 13 m^2. Plant samples (roots and shoots i.e. stems, leaves and pods) were collected from each plot three times, every 30 days, at the following developmental stages: flowering (BBCH 65-67), development of fruit (BBCH 74-78) and ripening (BBCH 86-87), from an area of 0.5 m^2. The content of magnesium was determined using the colorimetric method with titanium yellow in each plant sample (in four replications for each experimental treatment).

The results were analysed statistically using the statistical program Analysis of Variance for Orthogonal Experiments, developed by the UTP University of Science and Technology in Bydgoszcz, Poland. The differences between the values were verified with the Tukey's test.

RESULTS AND DISCUSSION

Barłóg and Grzebisz (2000) report that winter oilseed rape in the period from the spring start of growth to the technical maturity is characterized by a changing dynamics of dry matter accumulation, and point to a very high increase between the flowering and the fruit development stages. Similarly, in the present study, the dry matter accumulation rate of winter oilseed rape in the analyzed period of the generative growth was the highest. The total dry matter accumulation in the aerial and underground parts of oilseed rape was almost 2.3-fold higher at fruit formation than at flowering (Figure 1). An

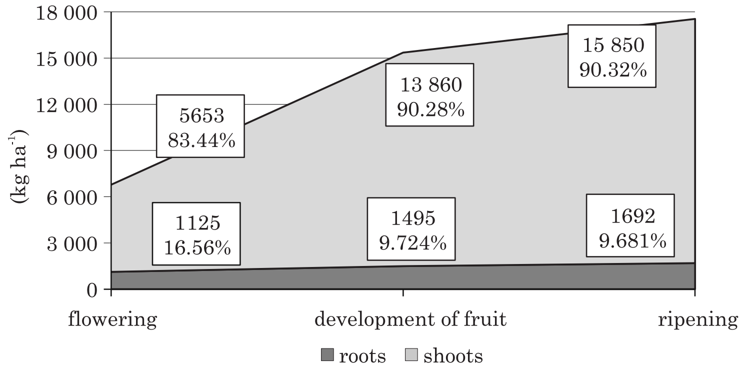

Fig. 1. Amount and participation of root and shoot dry matter weight of oilseed rape in generative development stages, means for 2011-2013

increase in plant dry matter between the fruit development stage and ripening was smaller: 14.2%. Moreover, a distinct increase in the proportion of dry matter of shoots and an accompanying decrease in the proportion of dry matter of roots in the total plant mass occurred between the flowering and fruit development stages.

Response of the plant dry matter accumulation to the biostimulant application and to NPK doses was more frequent in the first experiment with pre-sowing S fertilization (Tables 1, 2). In this case, the increased NPK doses and the application of the biostimulant stimulated the production of oilseed rape dry matter in each of the analyzed growth stages. In the second experiment, where sulphur was not applied, a significant increase in dry matter

Table 1

Total dry matter of oilseed rape – results of ANOVA analysis, means for 2011-2013

Growth stage	Experiment[#]					
	I – S_{36}			II – S_0		
	NPK[‡]	B[†]	NPK x B	NPK	B	NPK x B
Flowering	**	*	*	ns	*	ns
Development of fruit	**	*	ns	ns	ns	ns
Ripening	**	*	ns	*	ns	ns

[#] Experiment: I – 36 S; II – 0 S (kg ha^{-1}),
[‡] NPK: 180 N, 70 P, 132 K or 144 N, 35 P, 66 K (kg ha^{-1}),
[†] B: biostimulant Kelpak SL 4 dm^3 ha^{-1},
* $P \leq 0.05$; ** $P \leq 0.01$; ns – non-significant.

Table 2

Total dry matter of oilseed rape depending on the biostimulant and mineral fertilization doses, means for 2011-2013 (kg ha^{-1})

Treatment	Experiment					
	I – S_{36}			II – S_0		
	$N_{180}P_{70}K_{132}$	$N_{144}P_{35}K_{66}$	mean	$N_{180}P_{70}K_{132}$	$N_{144}P_{35}K_{66}$	mean
Flowering						
Biostimulant	8573	6701	7637	6788	6742	6765
Control	6695	6429	6562	6696	5592	6144
Mean	7634	6565	7099	6742	6167	6455
Development of fruit						
Biostimulant	18 686	14 992	16 839	15 617	14 589	15 103
Control	16 152	14 239	15 196	14 548	14 025	14 287
Mean	17 419	14 616	16 017	15 082	14 307	14 695
Ripening						
Biostimulant	22 278	17 815	20 047	17 448	16 635	17 041
Control	18 010	15 755	16 883	17 723	14 661	16 192
Mean	20 144	16 785	18 465	17 585	15 648	16 617

under the influence of the biostimulant was shown at flowering, and a favourable effect of the increased NPK doses was observed only at the ripening stage.

Oilseed rape has high requirements with regard to S and it may show signs of deficiency of this nutrient as early as at the bud and flowering stages (Grant, Bailey 1993). According to Vong at al. (2007), S uptake by plant rape cultivated for 28, 42 and 63 days was 2.3, 9 and 11.8 mg S in calcareous soils and 1.5, 5 and 10 mg in acid brown soil. In other studies, it was shown that S application had a positive effect on the dry matter accumulation (Giovahi, Saffari 2006), seed yield and yield components of oilseed rape (Amanullah et al. 2011). Moreover, it was indicated that fertilization with S stimulates the effectiveness of other nutrients. Yasari and Patwardhan (2006) report that dry matter accumulation at the fruit development and ripening of oilseed rape was significantly higher after the application of NPKS fertilization as compared with NPK. Sulphur addition affects N metabolism (Sarda et al. 2014) and improves N-use efficiency (Fismes et al. 2000). The study by Grant and Bailey (1993) indicates that the deficiency of S may severely retard oilseed rape growth, particularly if high amounts of N are applied.

The favourable effect of seaweed extract application on the accumulation of oilseed rape dry matter demonstrated in the present study may have resulted from the content of phytohormones responsible for the plant growth (Tarakhovskaya et al. 2007, Kurepin et al. 2014). Billard et al. (2014) attribute the significant increase in the total dry matter of oilseed rape after an application of seaweed extract to an elevated expression of genes encoding nutrient (particularly N and S) transporters.

The content of Mg in oilseed rape depends on the plant organ and is mostly higher in leaves than in the stem or pods (Barłóg, Grzebisz 2000). The study by Szczepaniak et al. (2015) indicates that NPK fertilization increases the Mg concentration in leaves of oilseed rape at the flowering stage as compared with the control (without fertilization) and an additional application of S did not have an effect on the concentration of this nutrient as compared with NPK fertilization alone. In the present study, the Mg content in the aerial parts was dependent on the NPK and S fertilization level and the growing stage of plants (Tables 3, 4). At the fruit development stage,

Table 3

Content of Mg in plant - results of ANOVA analysis, means for 2011-2013 (kg ha^{-1})

Part of plant	Growth stage	Experiment#					
		I – S_{36}			II – S_0		
		NPK‡	B†	NPK x B	NPK	B	NPK x B
Shoot	flowering	*	ns	*	ns	ns	ns
	development of fruit	**	ns	ns	**	ns	ns
	ripening	ns	ns	ns	*	ns	ns
Root	flowering	ns	ns	ns	ns	ns	ns
	development of fruit	*	ns	ns	*	ns	ns
	ripening	ns	ns	ns	ns	ns	ns

Explanations see Table 1.

Table 4

Content of Mg in shoots of oilseed rape depending on the biostimulant and mineral fertilization doses, means for 2011-2013 (kg ha^{-1})

Treatment	Experiment					
	I – S_{36}			II – S_0		
	$N_{180}P_{70}K_{132}$	$N_{144}P_{35}K_{66}$	mean	$N_{180}P_{70}K_{132}$	$N_{144}P_{35}K_{66}$	mean
Flowering						
Biostimulant	3.06	3.40	3.23	3.24	3.22	3.23
Control	3.14	3.17	3.16	3.09	3.29	3.19
Mean	3.10	3.29	3.20	3.17	3.25	3.21
Development of fruit						
Biostimulant	2.58	2.73	2.65	2.51	2.70	2.60
Control	2.41	2.73	2.57	2.43	2.64	2.53
Mean	2.50	2.73	2.61	2.47	2.67	2.57
Ripening						
Biostimulant	2.33	2.12	2.23	2.12	2.36	2.24
Control	2.18	2.24	2.21	2.20	2.30	2.25
Mean	2.25	2.18	2.22	2.16	2.33	2.25

the content of Mg in shoots decreased under the influence of the higher NPK fertilization both in oilseed rape fertilized and not fertilized with sulphur. A similar response was observed at the flowering stage in plants treated with sulphur, as well as at the ripening stage in plants not fertilized with this nutrient. Almost invariably, where a decrease in the Mg content in shoots under the influence of higher NPK doses occurred, there was also an increase in dry matter accumulation (Table 2). Thus, it is likely that the so-called dilution of the nutrient content, connected with an increased biomass production, appeared.

The present study indicated a slight, gradual decrease in the Mg concentration along with the progressing generative growth (Table 4). A similar trend of changes in the content of this element is presented by Barraclough (1998). The biostimulant application makes it possible to maintain a relatively constant Mg concentration in the shoots of winter oilseed rape, irrespective of the amount of produced biomass (Table 2). No significant decrease in the Mg concentration was recorded at any development stage in plants treated with the biostimulant, despite the increase in dry matter production, particularly in oilseed rape fertilized with sulphur (Table 4). In other laboratory analyses (Billard et al. 2014) the Mg concentration in oilseed rape plants increased after the application of a seaweed extract, even though the dry matter production increased as well. The content of Mg in the roots decreased at the successive development stages and, on average for the NPK and S fertilization level and biostimulant application, it reached 3.21 mg kg^{-1} at the flowering stage, 2.57 mg kg^{-1} at fruit development, and 2.25 mg kg^{-1} at ripening (data nor presented). No effect of the factors studied on the Mg concentration in roots was shown (Table 3).

The total accumulation of Mg in aerial and underground parts of oilseed rape increased between flowering and fruit development from 21.4 to 37.5 kg ha^{-1} (by 75%) and to 39.3 kg ha^{-1} at the stage of ripening (by an additional 4.8%) – Figure 2. In the study by Barłóg and Grzebisz (2000), the maximum Mg accumulation in the aerial parts of oilseed rape was lower than in the pre-

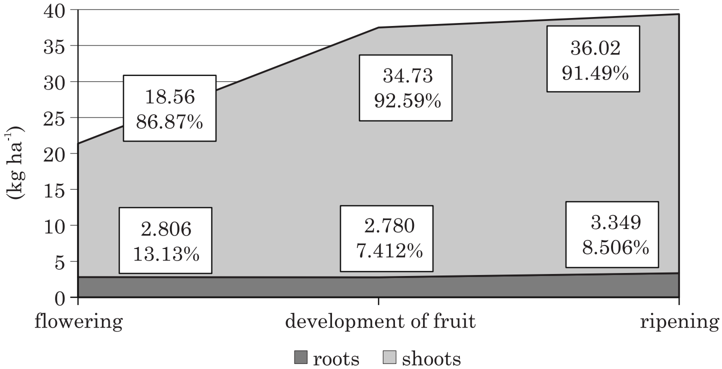

Fig 2. Amount and participation of Mg accumulation in roots and shoots of oilseed rape in generative development stages, means for 2011-2013

sent study, but the accumulation dynamics of this element at particular generative growth stages was similar. In the research by Barraclough (1989), an intensive increase in the Mg accumulation in the aerial organs was recorded between flowering and fruit development, after which there was an evident decrease at harvest, possibly because not all of the leaf litter was collected. In the present study, between the flowering and fruit development stages, as in the case of dry matter accumulation (Figure 1), a clear increase in Mg accumulation in the shoots was recorded, as well as a reduced accumulation of this element in the roots (Figure 2).

Laboratory analyses carried out by Billard et al. (2014) showed that the application of a biostimulant from algae caused a significant increase in the amount of Mg in oilseed rape treated with the seaweed extract. In the present study, the response of oilseed rape in terms of the combined Mg accumulation in aerial and underground parts was dependent on fertilization with sulphur (Table 5). After the pre-sowing application of sulphur, the biostimulant application increased the Mg accumulation at each development stage, irrespective of the NPK fertilization level; if sulphur was not applied, this increase was significant only at the fruit development stage (Table 6). While maintaining a relatively constant Mg concentration in plants treated with the biostimulant, an increase in the Mg accumulation in plants, as in the case of the response to the NPK fertilization level, resulted from the volume of dry matter production (Tables 2, 4, 6).

Table 5

Total accumulation of Mg in oilseed rape - results of ANOVA analysis, means for 2011-2013

Growth stage	Treatments‡					
	I – S_{36}			II – S_0		
	NPK‡	B†	NPK x B	NPK	B	NPK x B
Flowering	ns	*	ns	ns	ns	ns
Development of fruit	*	*	ns	ns	*	ns
Ripening	**	*	ns	ns	ns	ns

Explanations see Table 1.

The NPK fertilization level had a significant effect on the total Mg accumulation in plants in the first experiment with pre-sowing sulphur fertilization (Table 5). At the fruit development and ripening stages, oilseed rape fertilized with higher NPK doses accumulated more Mg than in the case of lower doses (Table 6). At the fruit development stage, an increased Mg accumulation was recorded regardless of the significantly smaller Mg concentration in the shoots (Tables 4, 6). Demonstration of such relationships substantiates the conclusion that the Mg accumulation at these plant development stages depends primarily on the production of dry matter (Table 2). Similarly, Billard et al. (2014) conclude that the amount Mg per plant after seaweed application was consistent with the increase of whole-plant biomass.

Table 6

Total accumulation of Mg in oilseed rape depending on the biostimulant and mineral fertilization doses, means for 2011-2013 (kg ha^{-1})

Treatment	Experiment					
	I – S_{36}			II – S_0		
	$N_{180}P_{70}K_{132}$	$N_{144}P_{35}K_{66}$	mean	$N_{180}P_{70}K_{132}$	$N_{144}P_{35}K_{66}$	mean
	Flowering					
Biostimulant	26.17	21.72	23.95	21.66	21.25	21.45
Control	20.78	20.56	20.67	20.52	18.30	19.41
Mean	23.47	21.14	22.31	21.09	19.78	20.43
	Development of fruit					
Biostimulant	45.30	38.42	41.86	37.48	36.42	36.95
Control	37.32	36.41	36.86	33.86	34.84	34.35
Mean	41.31	37.42	39.36	35.67	35.63	35.65
	Ripening					
Biostimulant	52.85	38.13	45.49	37.26	38.82	38.04
Control	39.57	35.12	37.34	39.36	33.86	36.61
Mean	46.21	36.62	41.42	38.31	36.34	37.33

CONCLUSIONS

1. Based on the analysis of dry matter production and Mg accumulation in winter oilseed rape plants in the period of their generative growth, it can be concluded that this plant responds more favourably to the application of a biostimulant or increased level of NPK fertilization if it is additionally fertilized with sulphur.

2. In oilseed rape nourished with sulphur, increased NPK doses cause an increase in the dry matter production by plants, but also a decrease in the Mg content in shoots during flowering and fruit development. The biostimulant increases the amount of plant dry matter in the period from flowering to ripening, while preventing a decrease in the Mg concentration in aerial and underground parts of oilseed rape.

3. Magnesium accumulation in aerial and underground parts of winter oilseed rape depends mostly on the amount of dry matter produced. In oilseed rape fertilized with sulphur, an application of higher NPK doses causes an increase in the Mg accumulation in plants at the stages of fruit development and ripening. A similar effect was caused by an application of the biostimulant, which increased the Mg accumulation as early as the flowering stage.

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