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ORIGINAL PAPER

MAGNESIUM CONTENT AND UPTAKE BY THE AERIAL BIOMASS OF *HORDEUM VULGARE* L. AND *LOLIUM MULTIFLORUM* LAM. IN PURE AND MIXED STANDS UNDER WATER DEFICIT*

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Abstract

Magnesium (Mg) is essential for proper bodily functions in living organisms. The content of this nutrient in plants is variable, and its uptake by plants is influenced by both abiotic and biotic factors. The aim of this study was to determine the effect of interactions between spring barley and Italian ryegrass in pure and mixed stands on the Mg content of the aerial biomass and uptake by both plant species under different soil moisture conditions. Three series of a pot experiment were conducted in greenhouse under controlled conditions. The following experimental factors were analyzed: (1) stand type (each plant species was sown in pure and mixed stands), (2) water supply (optimal and reduced by 50%). The Mg content of the aerial biomass of plants was determined in five growth stages (leaf development, tillering, stem elongation, heading, ripening) corresponding to the development of pure-sown barley under optimal water supply conditions. The Mg content was determined by atomic absorption spectrometry, and the Mg uptake was calculated based on the Mg content and dry matter accumulation in plants. Neither a stand type nor the water deficit had a significant effect on the Mg content in the aerial biomass of barley and Italian ryegrass during their growth and development. Both the stand type and water supply significantly affected the Mg uptake by the aerial parts of plants. From emergence to heading, the Mg uptake by spring barley was hindered by water deficit rather than by competition with Italian ryegrass. The responses of Italian ryegrass to water stress and competition with barley varied in the initial growth stages, whereas during later growth stages, the Mg uptake was limited by competition with barley rather than by water deficit.

Keywords: spring barley, Italian ryegrass, magnesium accumulation, growth stages, plant parts.

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INTRODUCTION

Magnesium (Mg) is essential for proper bodily functions in living organisms. The important role played by Mg during the growth and development of plants has been well documented in review articles (Shaul 2002, Maathuis 2009, Guo et al. 2016). Magnesium also plays a key role in photosynthesis, being the central ion in the porphyrin ring of chlorophyll that absorbs sunlight. Magnesium promotes enzyme-substrate interactions by forming chelate bonds, which speeds up enzymatic reactions. It also participates in the synthesis, transport and storage of carbohydrates, proteins and fats.

The Mg content of crop plants usually ranges from 0.1% to 1% on a dry matter basis, depending on species (Jācobsone et al. 2015, Rusinovci et al. 2016, Brodowska et al. 2017), variety (Martyniak 2009), growth stage (Slamka et al. 2011) and plant parts (Martyniak 2009, Tränkner et al. 2016, Wilczewski et al. 2016, Brodowska et al. 2017). The Mg requirement for the optimal plant growth is 0.15-0.35 g kg^{-1} DM of vegetative parts (Shaul 2002).

Nutrient uptake by plants is influenced by both abiotic and biotic factors. Water deficit inhibits macronutrient assimilation from soil (Suriyagoda et al. 2014), and may affect the macronutrient content of plants (Baligar et al. 2001, Gonzalez-Dugo 2012, Mahdavi et al. 2016, Brodowska et al. 2017). Potassium exerts an antagonistic effect on Mg uptake (Jaskulska et al. 2015). Macronutrient uptake from soil and macronutrient accumulation in different plant parts may vary when two or more plant species utilize the same environmental resources (Wanic, Michalska 2009). Under nutrient deficiency conditions, plants enter into competitive interactions, and the outcome of competition can be evaluated at the end of the growing season. Such relationships are observed between cover crops and undersown catch crops. The interactions between cereals and legumes have been extensively researched (Wanic, Michalska 2009, Sobkowicz, Lejman 2011, Mariotti et al. 2015), but non-legume companion plants are also increasingly investigated (Płaza et al. 2009).

The aim of this study was to determine the effect of interactions between barley and Italian ryegrass in pure and mixed stands on the Mg content of the aerial biomass and uptake by both plant species under different soil moisture conditions. The alternative hypothesis postulating that interspecific competition and water stress change the Mg content and uptake by plants was tested against the null hypothesis that the above factors do not affect the analyzed parameters.

MATERIALS AND METHODS

Three experimental series were conducted at the Greenhouse Lab of the Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn. The tested plant species were spring barley (hulless cv. Rastik) and Italian ryegrass (cv. Gaza).

The experimental factors were:

1) stand type: barley pure stand, Italian ryegrass pure stand, barley-Italian ryegrass mixture;
2) water supply: optimal and reduced by 50%.

In the treatment with optimal water supply, water was supplied in the amount of 17,000 cm^3 pot^{-1} during the growing season, and in the treatment with reduced water supply – in the amount of 8,500 cm^3. The optimal amount of water had been established based on a preliminary experiment, in which the soil moisture content, water evaporation from soil, transpiration from plants and water content in plants had been measured. During the growing season, the amount of water supplied to plants varied subject to the growth stage of the analyzed species and soil moisture content.

Soil samples were collected from the uppermost layer of typical brown soil developed from slightly loamy silty sand. The soil had slightly acidic reaction and the organic carbon content of 0.71% - 1.11%, and it was moderately rich in phosphorus, potassium and magnesium. One week before sowing, each pot was filled with 8 kg of soil which had been thoroughly mixed with mineral fertilizers (g pot^{-1}, on a pure ingredient basis): N – 0.5 (urea), P – 0.2 (monopotassium phosphate), K – 0.45 (potassium sulfate).

The experiment had an additive design (Semere, Froud-Williams 2001), according to which the number of plants in the mixture was equal to the sum of crop densities in pure stands. In the pure sowing variant, 18 barley or Italian ryegrass seeds able to germinate were sown in each pot, and in the mixed sowing variant, there were 18 barley seeds and 18 Italian ryegrass seeds sown together. Seeds were spaced on the substrate surface at equal distances from each other, which was achieved with templates, and they were placed at a depth of 3 cm. Each experimental series was set up according to a completely randomized design in four replication.

Throughout the experiment, ambient temp. in the laboratory was maintained at 20-22°C. The temperature was lowered to 6-8°C for 9 days at full emergence to support vernalization.

The Mg content of the aerial biomass of plants was determined in five growth stages (BBCH-scale): leaf development (10-13), tillering (22-25), stem elongation (33-37), heading (52-55) and ripening (87-91), corresponding to the development of pure-sown barley under optimal water supply conditions. When barley had reached each of the above growth stages, all plants were removed from pots (used in a given growth stage) and the aerial parts were separated from the roots. At successive growth stages, the aerial parts of barley and ryegrass were divided into leaves, stems and spikes. Plant material was air-dried, weighed and assayed for Mg content. The analyses were performed with the atomic absorption spectrometry method at the Chemical and Agricultural Station in Olsztyn. Magnesium uptake was calculated based on dry matter accumulation (Wanic et al. 2013) and the Mg content of aerial biomass.

The results in the tables are means for the three series of the experiment. The results from the experiment underwent statistical processing by analysis of variance (ANOVA) for two-factor experiments in fully randomized configuration. Homogeneous groups were identified by the Duncan's test. The probability of error was set at $p < 0.05$.

RESULTS AND DISCUSSION

Magnesium concentrations in the aerial biomass of barley varied throughout plant growth and development (Table 1). In the leaf development and tillering stages, the Mg content of shoots was similar. From the stem elongation stage until the end of the growing season, more Mg was accumulated in leaves than in stems. From the heading stage, the Mg content was

Table 1

Magnesium content of the aerial biomass of barley (g kg^{-1} d.m.)

Growth stage of barley (BBCH)	Plant part	Stand type		Water supply		Stand type			
						P		M	
						water supply			
		P	M	O	L	O	L	O	L
10-13*	leaves	1.9^a	1.9^a	1.9^a	1.8^a	1.9^a	1.9^a	2.0^a	1.8^a
22-25	leaves	1.8^a	1.8^a	1.7^a	1.9^a	1.8^a	1.9^a	1.7^a	1.9^a
33-37	leaves	1.9^a	1.8^a	1.8^a	1.8^a	1.9^a	1.8^a	1.8^a	1.8^a
	stems	1.6^a	1.6^a	1.5^a	1.6^a	1.5^a	1.6^a	1.5^a	1.6^a
52-55	leaves	1.8^a	1.6^a	1.7^a	1.7^a	1.8^a	1.7^a	1.6^a	1.6^a
	stems	1.0^a	1.0^a	1.0^a	1.1^a	0.9^a	1.0^a	0.9^a	1.0^a
	spikes	1.3^a	1.3^a	1.3^a	1.3^a	1.3^a	1.3^a	1.4^a	1.3^a
87-91	leaves	1.6^a	1.7^a	1.7^a	1.6^a	1.6^a	1.6^a	1.7^a	1.6^a
	stems	0.7^a	0.8^a	0.7^a	0.8^a	0.6^a	0.7^a	0.7^a	0.8^a
	spikes	1.7^a	1.6^a	1.7^a	1.6^a	1.8^a	1.6^a	1.7^a	1.5^a

* explanations in Material and Methods, P – barley pure-sown, M – barley mixed-sown with Italian ryegrass, O – optimal water supply, L – water supply reduced by 50%;
a – homogeneous groups (values followed by the same letters within experimental factors and their interactions are not significantly different at $p < 0.05$).

higher in spikes than in stems. During the ripening stage, Mg concentrations in spikes and leaves were similar. Tränkner et al. (2016) demonstrated that due to its mobile nature, Mg can be relocated from older to younger leaves, which enables the plant to enter the generative phase.

In the present study, neither a stand type nor the water supply affected the Mg content of the aerial parts (stems, leaves and spikes) of barley throughout the growing season (Table 1). No differences in Mg concentrations in barley plants exposed to water stress were observed by Krizek and Foy (1988), either. Martyniak (2009) found that the soil moisture content had no significant effect on Mg accumulation in the postharvest residues and roots of spring wheat, which only tended to decrease with increasing humidification. In a study by Slamka et al. (2011), Mg concentrations were significantly higher in the aerial biomass of spring barley under optimal water supply than under drought stress. The cited authors also found that higher doses of nitrogen fertilization under water stress increased Mg concentrations in the aerial biomass of barley. According to Brodowska et al. (2017), the Mg content of spring barley grain and straw decreases in growing seasons with low precipitation levels.

Magnesium uptake in the dry biomass of barley was significantly affected by the experimental factors already in early growth stages. The presence of Italian ryegrass in a stand limited the accumulation of Mg in the biomass components of barley throughout its growth and development (Table 2).

Table 2

Magnesium uptake by barley (mg pot^{-1})

Growth stage of barley (BBCH)	Plant part	Stand type		Water supply		Stand type			
						P		M	
		P	M	O	L	water supply			
						O	L	O	L
10-13*	leaves	1.56^{a}	1.32^{b}	1.62^{a}	1.26^{b}	1.70^{a}	1.41^{c}	1.54^{b}	1.11^{d}
22-25	leaves	7.13^{a}	6.56^{b}	8.86^{a}	4.83^{b}	9.16^{a}	5.10^{c}	8.56^{b}	4.56^{d}
33-37	leaves	8.43^{a}	6.43^{b}	10.29^{a}	4.57^{b}	11.70^{a}	5.17^{c}	8.88^{b}	3.98^{d}
	stems	8.32^{a}	7.35^{b}	11.69^{a}	3.98^{b}	12.29^{a}	4.35^{c}	11.09^{b}	3.61^{d}
52-55	leaves	9.73^{a}	6.65^{b}	10.15^{a}	6.22^{b}	12.53^{a}	6.92^{c}	7.77^{b}	5.53^{d}
	stems	6.29^{a}	5.23^{b}	7.69^{a}	3.83^{b}	8.34^{a}	4.26^{c}	7.05^{b}	3.40^{d}
	spikes	2.46^{a}	1.87^{b}	3.40^{a}	0.93^{b}	3.71^{a}	1.20^{c}	3.09^{b}	0.66^{d}
87-91	leaves	7.25^{a}	6.85^{b}	8.38^{a}	5.71^{b}	8.46^{a}	6.03^{b}	8.31^{a}	5.38^{c}
	stems	4.25^{a}	4.00^{b}	4.52^{a}	3.73^{b}	4.46^{a}	4.04^{b}	4.59^{a}	3.42^{c}
	spikes	3.87^{a}	2.50^{b}	4.51^{a}	1.86^{b}	5.62^{a}	2.11^{c}	3.39^{b}	1.60^{d}

* explanations in Material and Methods, P – barley pure-sown, M – barley mixed-sown with Italian ryegrass, O – optimal water supply, L – water supply reduced by 50%;
a, *b*, *c*, *d* – homogeneous groups (values followed by the same letters within experimental factors and their interactions are not significantly different at $p < 0.05$)

At the stem elongation and heading stages, the differences in Mg accumulation effected by the sowing regime were greater in leaves than in stems. These differences somewhat decreased at ripening, while remaining significant. Italian ryegrass had the most significant effect on Mg accumulation in barley spikes during ripening.

Under the optimal water supply, compared with water supply reduced by 50%, barley plants accumulated significantly more Mg in dry biomass. Slamka et al. (2011) also noted significantly lower Mg uptake by the aerial biomass of spring barley exposed to drought stress. In the current study, differences in Mg accumulation in barley biomass were observed already during leaf development, and they were more pronounced during subsequent growth stages until stem elongation. Variation in Mg accumulation in vegetative organs (leaves and stems) decreased in the heading and ripening stages, but they remained significant. In these growth stages, Mg uptake in barley spikes were also significantly lower under water deficit relative to the optimal water supply.

An analysis of the effect exerted by the interaction between a stand type and water supply on Mg accumulation in barley biomass revealed that in all growth stages of barley, Mg uptake by dry biomass was the highest in the pure stand and under the optimal water supply. In comparison with the above treatment, the inhibitory effect exerted by Italian ryegrass on Mg accumulation in barley biomass was weaker than that of water stress from the beginning of the growing season until the heading stage. During ripening, the above relationship was observed only in barley spikes, whereas the stand type had no influence on Mg uptake in leaves and stems under optimal water supply. The lowest Mg accumulation in barley biomass was noted in the mixed stand under water deficit. The observed Mg concentrations in the aerial parts of barley in the analyzed growth stages can be attributed to the lower biomass of barley grown in the mixed stand with Italian ryegrass and under water stress (Wanic et al. 2013).

The Mg content of the aerial parts of Italian ryegrass varied throughout the growing season (Table 3). Magnesium concentrations were usually higher in leaves than in stems, and the differences became more pronounced during the growth and development of plants. Neither a stand type nor the water supply affected the Mg content. According to Staugaitis and Rutkauskiene (2012), the Mg content of ryegrass dry biomass increases in years characterized by high temperatures and high precipitation levels. Płaza et al. (2009) demonstrated that ryegrass, compared with legumes (red clover, white clover) undersown in spring barley, accumulated significantly less Mg as well as N, P, K and Ca in biomass. In this study, the Mg content of the aerial parts of Italian ryegrass grown in the mixed stand with barley, determined at the end of the growing season, was comparable with that reported by Płaza et al. (2009).

Magnesium uptake by Italian ryegrass was significantly affected by the

Table 3

Magnesium content of the aerial biomass of Italian ryegrass (g kg^{-1} d.m.)

Growth stage of barley (BBCH)	Plant part	Stand type		Water supply		Stand type			
						P		M	
						water supply			
		P	M	O	L	O	L	O	L
10-13*	leaves	2.8^{a}	2.9^{a}	2.8^{a}	2.9^{a}	2.7^{a}	2.9^{a}	2.9^{a}	2.9^{a}
22-25	leaves	3.0^{a}	2.7^{a}	2.8^{a}	2.8^{a}	2.9^{a}	3.0^{a}	2.7^{a}	2.6^{a}
33-37	leaves	3.0^{a}	2.7^{a}	2.9^{a}	2.8^{a}	3.0^{a}	3.0^{a}	2.8^{a}	2.6^{a}
	stems	2.8^{a}	2.6^{a}	2.8^{a}	2.6^{a}	2.8^{a}	2.8^{a}	2.8^{a}	2.4^{a}
52-55	leaves	3.4^{a}	3.2^{a}	3.6^{a}	2.9^{a}	3.6^{a}	3.1^{a}	3.6^{a}	2.8^{a}
	stems	2.4^{a}	2.4^{a}	2.5^{a}	2.3^{a}	2.4^{a}	2.3^{a}	2.5^{a}	2.2^{a}
87-91	leaves	2.9^{a}	3.0^{a}	3.1^{a}	2.8^{a}	3.2^{a}	2.7^{a}	3.1^{a}	2.9^{a}
	stems	1.7^{a}	1.8^{a}	1.7^{a}	1.8^{a}	1.7^{a}	1.7^{a}	1.7^{a}	1.9^{a}

* Explanations see Table 1

experimental factors. Italian ryegrass grown in the mixed stand with barley accumulated less Mg throughout the growing season, and the noted differences were significant from the tillering stage of barley (Table 4). Water deficit also limited Mg uptake. The effect exerted by water supply on Mg accumulation in leaves was not significant only in the stem elongation stage in the pure and mixed stands. An interaction between the experimental factors was also noted in this stage. Water stress significantly decreased Mg accumulation in ryegrass stems in pure stands, whereas its effect was not significant in mixed stands. From the heading stage, the highest Mg concentrations were found in the aerial biomass of Italian ryegrass grown in the

Table 4

Magnesium uptake by Italian ryegrass (mg pot^{-1})

Growth stage of barley (BBCH)	Plant part	Stand type		Water supply		Stand type			
						P		M	
						water supply			
		P	M	O	L	O	L	O	L
10-13*	leaves	0.36^{a}	0.34^{a}	0.40^{a}	0.31^{b}	0.42^{a}	0.30^{b}	0.38^{a}	0.31^{b}
22-25	leaves	5.25^{a}	2.16^{b}	5.35^{a}	2.06^{b}	7.44^{a}	3.07^{b}	3.27^{b}	1.05^{c}
33-37	leaves	10.30^{a}	4.05^{b}	8.96^{a}	5.40^{a}	12.03^{a}	8.58^{ab}	5.89^{bc}	2.21^{c}
	stems	7.71^{a}	1.58^{b}	6.80^{a}	2.50^{b}	11.41^{a}	4.01^{b}	2.18^{b}	0.98^{b}
52-55	leaves	34.11^{a}	10.80^{b}	31.46^{a}	13.45^{b}	46.97^{a}	21.26^{b}	15.96^{c}	5.64^{d}
	stems	13.84^{a}	3.83^{b}	12.65^{a}	5.02^{b}	19.67^{a}	8.01^{b}	5.62^{c}	2.04^{d}
87-91	leaves	34.86^{a}	13.25^{b}	31.70^{a}	16.41^{b}	46.08^{a}	23.64^{b}	17.33^{c}	9.18^{d}
	stems	9.77^{a}	3.34^{b}	8.93^{a}	4.18^{b}	13.66^{a}	5.88^{b}	4.20^{c}	2.49^{d}

* Explanations see Table 2

pure stand under optimal water supply. In comparison with the above treatment, the presence of barley in a stand exerted a stronger inhibitory effect on Mg accumulation in ryegrass biomass than water stress did. The interaction between both factors contributed to even greater differences in Mg accumulation in ryegrass biomass.

The significant differences in Mg uptake, observed in this study, resulted from the competitive interactions between the analyzed species, which influenced biomass accumulation. The aerial biomass of Italian ryegrass was smaller in mixed stands with barley than in pure stands (Wanic et al. 2013). Biomass yield is also significantly affected by water supply. Under the optimal water supply, *L. multiflorum* produces longer leaves and shoots (Simić et al. 2009, Kostrzewska et al. 2013), and biomass yield increases (Simić et al. 2009, Wanic et al. 2013). Soil moisture stress significantly reduces the dry matter yield of grasses, and the drought resistance of *L. multiflorum* is significantly lower than that of *Dactylis glomerata* and *Festuca pratensis* (Staniak 2016). Older plants are more sensitive to drought than younger plants, which can be attributed to higher temperatures in summer and autumn than in spring (Fariaszewska, Staniak 2015).

CONCLUSIONS

Neither a stand type nor the water deficit had a significant effect on Mg concentrations, but both factors significantly affected the Mg uptake by the aerial parts of plants. The accumulation of Mg in the aerial biomass of both plant species was lower in mixed stands than in pure stands, and under water deficit than under the optimal water supply. From emergence to heading, Mg accumulation in the aerial biomass of spring barley was hindered by water deficit rather than by competition with Italian ryegrass. The responses of Italian ryegrass to water stress and competition with barley varied in the initial growth stages, whereas during later growth stages, Mg uptake was limited by competition with barley rather than by water deficit.

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