

Wanic M., Myśliwiec M., Orzech K., Michalska M., Denert M. 2017. Competition for nitrogen, phosphorus, potassium and magnesium between spring wheat and Persian clover depending on the density of plants. J. Elem., 22(3): 1081-1093. DOI: 10.5601/jelem.2017.22.1.1307

#### **ORIGINAL PAPER**

# COMPETITION FOR NITROGEN, PHOSPHORUS, POTASSIUM AND MAGNESIUM BETWEEN SPRING WHEAT AND PERSIAN CLOVER DEPENDING ON THE DENSITY OF PLANTS\*

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#### Abstract

A pot experiment completed in 2010-2012 analysed the relationship between spring wheat and the undersown Persian clover in the competition for nitrogen, phosphorus, potassium and magnesium depending on the plant density during the spring wheat growth stages of inflorescence emergence (BBCH 54-56) and ripening (BBCH 87-89). A mixture was composed according to the additive pattern. The analyses covered the aerial parts and roots of both species. The species were cultivated in a mixture and pure sowing at higher density (consistent with agricultural technology recommendations) and a density 20% lower than recommended. Based on the measurements of dry mass of the aerial organs and roots, as well as the content of N, P, K and Mg in the plant parts, the uptake of these elements by both species was calculated. The above data were the basis for computing the relative yield (uptake) – RY, relative yield total (uptake) – RYT, index of competitive balance (Cb) and relative efficiency index (REI). It has been shown that spring wheat and Persian clover competed for the whole pool of nitrogen and potassium and almost the entire content of phosphorus and magnesium. Wheat was a stronger competitor in obtaining the nutrients than clover. It showed the strongest competition against undersown clover for magnesium and the weakest one for nitrogen. Wheat competed more intensively with Persian clover for phosphorus and potassium in the treatment where the density was lower than recommended during the inflorescence emergence stage and for nitrogen and magnesium during the ripening stage.

**Keywords**: main crop, undersown, inflorescence emergence stage, ripening stage, aerial parts, roots, competition indicators.

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<sup>\*</sup> This study was financed by funds allocated to the statutory research at the University of Warmia and Mazury in Olsztyn (subject numer 20.610.023-300).

# INTRODUCTION

The prevalence of cereals in cultivated fields in combination with the application of significant quantities of synthetic mineral fertilizers and plant protection media has resulted in numerous unfavourable changes in agricultural systems. These changes manifest themselves in decreased biodiversity, environment contamination, impoverishment of soil organic carbon, movement of elements (particularly nitrogen), into the deeper soil profile levels and ground waters and decreased activity of soil microorganisms combined with the impoverishment of their species composition. It is possible to limit negative consequences of such cultivation by natural solutions including enrichment of the composition of species in the agrocenosis by growing undersown crops (JASKULSKA, GALEZEWSKI 2009). In northern Europe, given the climate conditions, undersown crops are the most suitable for cultivation (BERGKVIST 2003). The best outcomes are obtained when species cultivated jointly are systematically distant. Phenological differences mean that they can use soil resources at different times from different soil layers and in different forms. Moreover, the differences in the architecture of stems allow the species to use space in the standing crop better, which in turn facilitaes a more effective light absorption (JASTRZEBSKA et al. 2015a). Clovers are good undersown plants because of their absorption of atmospheric nitrogen, which they build into their tissues providing some of it to the accompanying species. Furthermore, they enrich the soil with this element (KÄNKÄNEN, ERIKS-SON 2007).

Different interactions occur between the main crop plants and the undersown species. Competition, that is the negative influence that alters the development rhythm of plants, their structure and fertility as well as the intensity and rate of obtaining biogens, is one of the more important consequences. In extreme cases, it may lead to the elimination of some or all individuals from the stand (SOBKOWICZ 2003, WANIC et al. 2016a,b).

The research hypothesis was formulated assuming that competitive interactions with respect to biogens between spring wheat and Persian clover would occur and their intensity would depend on the density of plants. The validity of this assumption was verified in a pot experiment, whose aim was to investigate competition between spring wheat and undersown Persian clover for nitrogen, phosphorus, potassium and magnesium under conditions of different densities of both species.

## MATERIALS AND METHODS

The research was conducted is three series of a pot experiment situated in a greenhouse laboratory at the University of Warmia and Mazury in Olsztyn. The experiments were conducted during the following periods: series I from 12. 04. to 19. 07. 2010, II – from 24. 03. to 30. 06. 2011 and III – from 26. 03. to 28. 06. 2012. Spring wheat (cv. Nawra) and Persian clover (cv. Gobry) in pure sowing and in a mixture were cultivated at two densities: recommended (in line with agricultural technology) and lower (decreased by 20% compared to the recommended one).

The experimental factors were:

1) sowing pattern of spring wheat and Persian clover:

- pure sowing (control),
- cultivation in a mixture;
- 2) density of plants:
  - recommended,
  - lower.

Tests were conducted during the following growth stages of wheat: inflorescence emergence (BBCH 54-56) and ripening (BBCH 87-89).

The experiment consisted of 48 pots (wheat and clover in pure sowing and in a mixture x two sowing densities x 2 growth stages x 4 repetitions) 22 cm in diameter and 25 cm in depth.

In the pots with the recommended density, for both sowing methods, 19 seeds of wheat and 12 seeds of clover were sown, and 15 and 9 seeds were sown for the lower density. Following germination, if fewer than the planned number of plants were present in the pots, the shortage was compensated by planting missing plants (from additional pots).

Pots were filled with Eutric Cambisol (Humic) soil containing 64% of the <0.02 mm fraction, 12% of dust (0.1-0.02 mm) and 24% of sand (>1 mm). It was characterised by slightly acid reaction (pH in 1 M KCl from 5.6 to 6.2), organic carbon content of 13.2-14.4 g kg<sup>-1</sup>, nitrogen content of 0.69-0.74 g kg<sup>-1</sup> and high abundance (g kg<sup>-1</sup> of soil) of phosphorus (0.09-0.12) and magnesium (0.08-0.09), as well as an average abundance of potassium (0.13-0.15). The soil was obtained from the depth of 0 - 25 cm.

Mineral PK fertilisation was not differentiated between the species, sowing method or density of plants. It consisted of P - 0.200 and K - 0.450 (g pot<sup>-1</sup>) as pure component. The dose of N (g pot<sup>-1</sup>) was 0.500 for wheat in pure sowing, 0.125 for clover in pure sowing, and 0.300 for the mixture. The fertilisers were applied a week before sowing in the form of water solutions made from urea, monopotassium phosphate and potassium sulphate.

Soil humidity during the plant growing period was maintained at a constant level of 60% of the maximum water capacity.

The time for collecting plants for analyses was determined by the growth of spring wheat in the pure sowing and recommended density. When wheat reached the growth stage of inflorescence emergence and ripening, all plants were removed from the pots (intended for the given growth stage) and next aerial organs were separated from roots. The roots were washed in sieves and separated from one another. The separated aerial parts of plants and roots were dried to air-dry mass and the content of total nitrogen, phosphorus, potassium and magnesium was determined. Analyses were conducted at the Chemical Agricultural Station in Olsztyn. The analyses were made with the following methods: nitrogen – potentiometric titration with sodium hypobromite, phosphorus – spectrometric method, potassium – flame photometry method, magnesium – flame atomic absorption spectroscopy. Samples for analyses originated from 3 series and 4 replications. Based on the results of chemical analyses and measurements of dry mass of plants, the uptake of nitrogen, phosphorus, potassium and magnesium by wheat and clover was calculated. Data on the biomass of plants and detailed results concerning absorption of nitrogen by wheat and clover have been presented in earlier works (WANIC, MYŚLIWIEC 2014, WANIC et al. 2016*a*).

Taking into account the nitrogen, phosphorus, potassium and magnesium uptake (yield), the indicators defining the competitive interactions between spring wheat and Persian clover were computed (DE WIT, VAN DEN BERGH 1965):

- 1) relative yield (uptake) RY*i* and RY*j*;
- 2) total yield relative (uptake)– RYT = RYi + RYj;
- 3) competitive balance index  $C_{h} = \ln[(Yij/Yji) / (Yii/Yjj)];$
- index of relative effectiveness of species sown in the mixture REI = RGR*ij* – RGR*ji*;

$$RGR = 1/w \cdot dw/dt$$
,

where:

- RYi = relative yield (uptake) of wheat determined according to the formula: <math>RYi = Yij / Yii,
- $RY_j$  = relative yield (uptake) of clover determined according to the formula:  $RY_j = Y_{ji} / Y_{jj}$ , in which:
- Yii yield (uptake) of the species i (wheat) in pure sowing,
- Yjj yield (uptake) of the species j (clover) in pure sowing,
- Yij yield (uptake) of the species i (wheat) in the mixture with species j (clover),
- Yji yield (uptake) of the species j (clover) in the mixture with species i (wheat),
- $\operatorname{RGR} ij$  relative rate of increase of elements accumulation for species i in the mixture with species j,
- RGRji relative rate of increase of elements accumulation for species*j*in the mixture with species*i*,
- w mass of element uptaken with dry matter yield of a single plant,
- dw increase in the mass of elements,
- dt time interval at which the increase was determined (one day).

The results were presented as averages from the three experimental series. Data concerning the relative yields (uptake), i.e. RY and RYT, were processed statistically by variance analysis according to the model appropriate to a completely randomized design at the error probability of p = 0.05, determining the homogenous groups based on the Tukey's test. The *t*-Student test was applied to verify whether the value RYT differs from 2, RY from 1, and the values Cb and REI from 0. Computations were performed using the Statistica 12.5 software package.

#### RESULTS

Statistical analysis of the RYT index showed that it had significantly lower values than 2, at the density representing in the mixture the sum of densities of both species from pure sowing (except for K in the roots in both plant density variants and Mg in the roots in the variant with the recommended density and on average for the plant density), which indicates competition between spring wheat and Persian clover for the analysed elements (Table 1). During the wheat inflorescence emergence and ripening stages, on average for density, plants competed for the whole pool of N and K and almost the entire pool of P and Mg. The complementary use concerned 28% of the P and Mg resources during inflorescence emergence, whereas during the ripening stage the respective figures were 13% and 6% of these elements only. The species competed for a similar pool of N and K during the inflorescence emergence and ripening stages. The competition for P and Mg concerned larger pools of the elements during the ripening stage than during inflorescence emergence stage. In the mixture, a stronger negative reaction to the deficiency of N, P, K and Mg was manifested by the aerial parts than by the roots. The density of plants did not significantly change the RYT index value computed for P and K during inflorescence emergence and ripening (except for P in the roots during the inflorescence emergence stage, where it achieved a significantly higher value in the treatment with the recommended density than in the one with the lower density) and for N during ripening. Competition for N during inflorescence emergence was more intensive in the treatment with the recommended density (except for the aerial mass). In the case of Mg, an opposite trend appeared. During the inflorescence emergence stage, the plants competed for a larger pool of this element in the pots with the lower density than in the ones where the plant density was higher. The density of plants did not change the RYT index value for Mg (except in the roots).

The value of the index of relative wheat yields (uptake), RY1, did not differ significantly from 1 for the N determined in roots during inflorescence emergence (on average for the plant density) and in roots in the treatment with the recommended density during ripening. The RY1 for P was not diffe-

Table 1

	Index	Growth stages (BBCH), plant parts									
Plant density		inflorescence emergence (54-56)			ripening (87-89)						
		aerial plant part	roots	total	aerial plant part	roots	total				
N											
Recommended	RY1	0.71a	1.02a	0.73 <i>a</i>	0.58a	$1.05a^{*}$	0.59a				
	RY2	0.20b	0.56b	0.21b	0.35b	0.52b	0.36b				
	RYT	0.91 <i>a</i>	1.58a	0.94a	0.93a	1.57a	0.95a				
Lower	RY1	0.78a	0.90a	0.80a	0.66a	0.71b	0.67a				
	RY2	0.22b	0.29b	0.22b	0.28b	0.77a	0.30b				
	RYT	1.00 <i>a</i>	1.19b	1.02b	0.94 <i>a</i>	1.48a	0.97a				
	RY1	0.75a	$0.96a^{*}$	0.77a	0.62a	0.88a	0.63a				
Average	RY2	0.21b	0.43b	0.22b	0.32b	0.65b	0.33 <i>b</i>				
	RYT	0.96	1.39	0.98	0.94	1.53	0.96				
P											
	RY1	$0.93a^{*}$	1.10 <i>a</i> *	$0.96a^{*}$	$0.78a^{*}$	$1.00a^{*}$	0.82a*				
Recommended	RY2	0.24b	0.75b	0.28b	0.35b	0.33c	0.35b				
	RYT	1.17a	1.85a	1.24a	1.13a	1.33a	1.17a				
	RY1	1.23a*	$0.90b^{*}$	1.16a*	0.80a*	1,00a*	0.81 <i>a</i> *				
Lower	RY2	0.15b	0.33c	0.16b	0.25b	0.67b	0.26b				
	RYT	1.38a	1.23b	1.32a	1.05a	1.67a	1.07a				
Average	RY1	1.08a*	1.00 <i>a</i> *	$1.06a^{*}$	$0.79a^{*}$	1,00a*	$0.82a^{*}$				
	RY2	0.20b	0.54b	0.22b	0.30b	0.50b	0.31 <i>b</i>				
	RYT	1.28	1.54	1.28	1.09	1.50	1.13				
				K							
	RY1	0.73a	1.42a*	$0.78a^{*}$	0.67a	0.69a	0.67a				
Recommended	RY2	0.18b	0.44b	0.19b	0.25b	0.43b	0.25b				
	RYT	0.91 <i>a</i>	1.86a**	0.97a	0.92a	1.12a	0.92a				
Lower	RY1	0.78a	1.39a*	0.81 <i>a</i> *	0.68a	0.71a	0.68a				
	RY2	0.14b	0.45b	0.14b	0.19b	$0.67b^{*}$	0.20b				
	RYT	0.92a	1.84a**	0.95a	0.87a	1.38a	0.88a				
Average	RY1	0.76a	1.41a*	0.80a*	0.68a	0.70a	0.68a				
	RY2	0.16b	0.45b	0.17b	0.22b	0.55b	0.23b				
	RYT	0.92	1.86	0.97	0.90	1.25	0.91				
Mg											
Recommended	RY1	1.14a*	$1.60a^{*}$	1.23a*	0.81a	0.80a	0.81 <i>a</i>				
	RY2	0.17b	0.67b	0.20b	0.24b	0.50b	0.27b				
	RYT	1.31 <i>a</i>	2.27a**	1.43 <i>a</i>	1.05a	1.30b	1.08a				
Lower	RY1	0.94 <i>a</i>	1.00a	0.95a	0.86a	0.60ab	0.81 <i>a</i>				
	RY2	0.14b	0.50b	0.17b	0.18b	1.00 <i>a</i>	0.23b				
	RYT	1.08b	1.50b	1.12b	1.04 <i>a</i>	1.60 <i>a</i>	1.04a				
Average	RY1	1.04a*	1.30 <i>a</i> *	1.09a*	0.84 <i>a</i>	0.70a	0.81 <i>a</i>				
	RY2	0.16b	0.59b	0.19b	0.21b	0.75b	0.25b				
	RYT	1.20	1.89**	1.28	1.05	1.45	1.06				

# Relative yield (uptake) of spring wheat (RY1) and Persian clover (RY2) and relative yield (uptake) total (RYT)

a,b,c – values marked with the same letter do not differ significantly ( $p \le 0.05$ )

\* – values RY do not differ significantly from 1

\*\* – values RYT do not differ significantly from 2

rent from 1 in roots, shoots and whole plant at both growth stages and in both densities. This indicator determined for K in the roots and total plants did not differ significantly from 1 in both plants density variants during the inflorescence emergence stage and during the same period for Mg in the roots and total plants in the treatments with the recommended density and on average for the plants density. In other cases, they showed to be significantly different from 1. The RY2 values for clover on the other hand differed significantly from 1 and were lower than RY1 for wheat (with the exception of K – roots, ripening stage, lower density), which indicates the negative influence of wheat on clover. Limited N, P, K and Mg resources were definitely used better by wheat than clover. The relative yield (uptake) of the cereal was significantly higher than the relative yield (uptake) of clover. During the inflorescence emergence stage, on average for the plant density in the mixture, wheat uptook 24% less nitrogen than in pure sowing, while clover absorbed 78% less of this element. During the ripening stage, these values were lower by 37 and 67%, respectively. During inflorescence emergence, the wheat's uptake of phosphorus in the mixture was 6% higher than in pure sowing, while that by clover was lower by 78%. During the further plant growth in the mixture, the efficiency of wheat to uptake this biogenic nutrient weakened, while clover became more efficient. During the inflorescence emergence stage, competition by clover was manifested by a 24% lower accumulation of K in the aerial parts of wheat and in clover by reduction as high as 84% compared to the pure stand. On the other hand, in the mixture, the cereal accumulated in the roots 40% more K than in pure sowing while the accumulation of K by clover was 55% lower. During ripening, the advantage of wheat in the competition for K decreased while the influence of clover increased. During the inflorescence emergence stage in the mixture, a favourable influence of clover on the uptake of Mg by wheat (particularly roots) was noted. However, at the end of the plant growing season the competitive influence of clover on wheat manifested itself in a lower (by 19%) Mg uptake by the cereal. During that time, the negative influence of wheat on clover manifested itself during inflorescence emergence and ripening by reducing the content of Mg by 81% and 75%, respectively. At inflorescence emergence, roots competed for it less strongly than the aerial parts. During ripening, higher efficiency of clover roots than stems in accumulating Mg was recorded, conversely to wheat, where the stems were more efficient. The combined influence of the experimental factors showed that the RY1 indicator values computed for wheat were significantly higher than the RY2 values for clover in the case of both plant densities (with the exception of the competition for N and P between roots during the ripening stage in the variant with the lower density).

The competitive balance index (Cb) shows the strength of mutual influence of the plants on each other (Figure 1). The Cb index had values significantly different and higher from 0 (except the K and Mg in roots in pots with the lower density during the ripening stage), which indicates that both spe-







cies influenced each other with different intensity. Thus, competition proved asymmetric. Wheat dominated over clover in obtaining the limited pool of elements. The exception here consists of the values describing the competition of the roots for N, K and Mg in the variant with the lower density during the ripening stage, where the wheat roots were a weaker competitor than clover roots in obtaining N and Mg, while their competitive potential in obtaining K was similar. The domination of the cereal over the undersown clover was more pronounced during inflorescence emergence than in the ripening stage (except the competition for N and P between roots in the treatment with the recommended density). The competitive advantage of wheat was more clearly visible in the aerial parts than in the roots. During the inflorescence emergence stage, the influence of wheat on clover in competing for P and K (except K in roots) was the most intensive in the treatment with the lower density. During that period, the Cb index determined for N in the aerial parts showed no differentiation under the influence of the different plant densities. This was in contrast to wheat roots, which had a much stronger influence on clover in the pots with the lower density. The intensity of competition for Mg changed slightly under the influence of different plant densities during the inflorescence emergence stage. At ripening, the lower density stimulated wheat plants to intensify competition for the analysed elements (with the exception of their roots). Consequently, the accumulation of these elements was higher in the aerial parts.

The values of the relative efficiency index (REI) differed significantly from 0 and were negative, which indicates that during the period between inflorescence emergence and ripening, the relative rate of the accumulation of elements in one clover plant was higher than that in one wheat plant (Table 2). The strongest dominance of clover over wheat was found in terms of the accumulation of phosphorus in aerial parts and accumulation of potassium in the roots. In the mixture, the dominance of clover over wheat in the

Table 2

Plant density	Plant parts	N	Р	К	Mg
Recommended	aerial	-0.03	-0.05	-0.03	-0.02
	roots	-0.04	-0.01	-0.14	-0.04
	total	-0.03	-0.03	-0.09	-0.03
Lower	aerial	-0.02	-0.07	-0.03	-0.04
	roots	-0.04	-0.03	-0.13	-0.06
	total	-0.02	-0.05	-0.08	-0.05
Average	aerial	-0.03	-0.06	-0.03	-0.03
	roots	-0.04	-0.02	-0.13	-0.05
	total	-0.03	-0.04	-0.09	-0.04

Relative efficiency index (REI)\*

\* all values significantly different from 0

accumulation of elements did not change distinctly under the influence of the tested plant densities.

### DISCUSSION

Spring wheat and Persian clover competed with each other for the analysed elements, which indicated that the nutrients were present in insufficient quantities to cover the total demands of both plants. The competion was stronger for N and K and weaker for P and Mg. During both periods analysed, the two species competed for N and K with similar force, whereas competition for P and Mg was weaker during the ripening stage than during inflorescence emergence. CAROF et al. (2007) and SOBKOWICZ (2003, 2006) reported about the strength of mutual influence of species during the period preceding grain filling. In the case of cereals, this is the period of intensive growth of both roots and aerial parts as well as a time when the head components are formed, and therefore there is a high demand for biogenic nutrients, for which the two plants compete whenever there is a shortage of any of them.

Results of our studies as well as those by BERGKVIST et al. (2011) showed that wheat and clover assimilate resources proportionally to their size, meaning that wheat's demand for nutrients is higher than that of clover. Wheat uses the majority of the limited pool of nutrients. THORSTED et al. (2006a) also inform about the higher competitive power of wheat compared to clover concerning soil resources of elements. In a mixture, already during the initial period of growing together, some species grow faster than others, which gives them the advantage in using the resources during the later growth stages (Andersen et al. 2004, WANIC, Myśliwiec 2014). In our study, as early as at the stage of the development of leaves and tillering, wheat plants had a better-developed system of roots and the aerial parts than clover (WANIC, MYŚLIWIEC 2014). Thus, wheat could be more efficient in the uptake of water with nutrients dissolved in it. In consequence, during the further growing period wheat developed higher plants, with more abundant foliage and a well-developed assimilation system and roots that were more efficient in using the soil resources, giving it an advantage over lower clover plants with more modest foliage and root systems (THORSTED et al. 2006b, Myśliwiec et al. 2014). This advantage continued until the end of the plant growing season. Clover, shaded by larger and more massive cereal, assimilated less CO<sub>2</sub> and atmospheric N. It also made limited use of biogenic nutrients from the soil. OFORI and STERN (1987) also found out that assimilation of atmospheric nitrogen by pea grown in a mixture with cereal was lower due to its being shaded by cereal. LI et al. (2001) report the high capability of wheat to obtain nutrients. Thus, competition was asymmetric. It was more favourable in terms of nutrient uptake to the dominating species than to the subdued species (ZHANG, LI 2003). It is hard to determine which part of nitrogen accumulated in clover plants originated from the atmosphere and which came from the soil resource. In the case of a low demand for N, plants absorb more N from the atmosphere (THORSTED et al. 2006b). It can only be assumed that in our research clover absorbed less atmospheric nitrogen (shaded by the cereal) and (probably) used the soil N to a lesser extent. Wheat competed with clover more strongly during the inflorescence emergence stage than during ripening. During the ripening stage, its influence on the undersown clover decreased by more than 40% in the competition for N and P and by more than 30% in the competition for K and Mg. On the other hand, the influence of clover on wheat increased. During the period between inflorescence emergence and ripening, clover's absorption of N, P, K and Mg increased by almost twofold in the mixture.

A stronger negative reaction to intercropped cultivation was manifested by the aerial parts than the roots, which is consistent with the research by MARIOTTI et al. (2009). Thus, competition for light was stronger than for soil resources. The above resulted in a larger decrease in the accumulation of the analysed elements in the stems of both species. JASTRZĘBSKA et al. (2015*b*) obtained similar research results.

In the mixture, the density of plants was not without influence on wheat's uptake of N, K and Mg. In the treatment with the lower density, cereal in the mixture absorbed more P than in pure sowing at inflorescence emergence and less P during the ripening stage. In the mixture, no clear influence of a plant density on N, P, K and Mg absorbed by clover was shown. Both species competed more intensively for the analysed elements in the variant with the lower density. Opinions on the subject in the literature are divided. Atts et al. (2012) did not find any influence of sowing density on significant changes in values of the competition balance index (Cb). KÄNKÄNEN and ERIKSSON (2007) did not record any clear influence of sowing density on barley grain yield and the content of nitrogen in this cereal, while ZUK-GOLASZEWSKA (2010) did not record such influence on the biomass of red clover. Also, studies by COUSENS and O'NEILL (1993) showed that the domination of a species in the mixed stand did not change relative to the density of plants. On the other hand, THORSTED et al. (2006b) showed that lowering the density by increasing the distance between rows led to higher yields of grain and accumulation of nitrogen in it, mainly as the result of limiting the competition for light and nitrogen. According to GRIST (1999), a larger area occupied by one species offers it a greater advantage in competition for light, which might lead to limiting the growth or even total elimination of the other species. In our study, decreasing the density of both species resulted in the situation where wheat, possessing a larger living space, influenced clover to an even greater degree.

#### CONCLUSIONS

1. Spring wheat and Persian clover competed for the whole pool of nitrogen and potassium and almost the entire content of phosphorus and magnesium.

2. Spring wheat was a stronger competitor in obtaining nutrients than Persian clover. The cereal was more dominant over the undersown clover during inflorescence emergence than ripening.

3. In the treatment with the density lower than recommended, wheat competed more strongly with clover for phosphorus and potassium during inflorescence emergence and for nitrogen and magnesium during the ripening stage.

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