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NITROGEN CONTENT AND UPTAKE BY SPRING WHEAT AND UNDERSOWN PERSIAN CLOVER DEPENDING ON PLANT DENSITY

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Abstract

In 2010-2012, a pot experiment was conducted in order to assess the impact of a sowing method and density of spring wheat and Persian clover on their uptake and content of nitrogen in different parts of the plants. The plants were grown in the following variants: a mixture, pure sowing, higher density (recommended in agricultural practice) and density reduced by 20%. Observations were performed in the following wheat development growth stages (BBCH): leaf development (12-14), tillering (21-23), stem elongation (31-32), inflorescence emergence (54-56) and ripening (87-89). The experiment included determinations of dry matter in different parts of plants (aerial organs, roots) and of the nitrogen content in dry matter. Based on the results, the total nitrogen content was determined in both species. The data were also used to calculate nitrogen translocation from the wheat vegetative mass to grain, and to calculate selected competition indicators. It was demonstrated that – regardless of the density of plants – the nitrogen uptake by spring wheat and Persian clover in the mixed sowing was lower than in the pure sowing treatment. Mixed sowing caused a more limited nitrogen uptake by the aerial parts of both species than by their roots. No impact was demonstrated of the plant density on the nitrogen uptake by different parts of spring wheat. Persian clover in pots with the lower sowing density absorbed less nitrogen than in pots with the recommended density. Nitrogen translocation from the vegetative parts to the grain of spring wheat between the inflorescence emergence and ripening stages was more effective in the mixture than in the pure sown plants. In mixed sowing, after nitrogen uptake, wheat proved to be a stronger competitor than Persian clover during the whole plant growing period.

Keywords: undersown, cover crop, growth stages, nitrogen uptake, aerial plant parts, roots, competition.

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INTRODUCTION

The beneficial influence of intercrops on the environment has made them a valuable element of organic and integrated farming. Depending on the natural, agrotechnical and management factors, different types of intercrops can be used (Jaskulski, Jaskulska 2006, Jaskulska, Gałęzewski 2009). One solution is to undersow one species into another, which is the main crop. Intercrop plants grow together with the main crop and, after the main crop is harvested in the autumn, they are most often ploughed in as green manure or harvested as fodder. Clover is a particularly valuable undersown crop owing to its positive impact on soil properties and its ability to fix atmospheric nitrogen, which it incorporates in its tissues, enriches the soil with and makes some of it available to cover crops (Känkänen et al. 2003). Clovers (like other legumes) use mostly atmospheric nitrogen, whereas cereals absorb mostly the nitrogen stored in the soil. While most information on this subject pertains to red clover (Känkänen, Eriksson 2007), little can be read about Persian clover, which – when supplied with adequate amounts of water – produces high yields of nitrogen-rich biomass. Its suitability as an undersown crop was confirmed by Płaza et al. (2013) and Zarea et al. (2010). Moreover, there are few reports on the nitrogen uptake by cereals and clover during a whole plant growing season and on its accumulation in different parts of plants of both species. Consequently, the following hypothesis was proposed: the nitrogen uptake by spring wheat and Persian clover growing in mixed sowing will be similar to the nitrogen uptake in pure sowing, and the process will not depend on the density of plants. This hypothesis was verified in an experiment whose objective was to evaluate the impact of Persian clover undersown into spring wheat, and of the density of plants on the uptake and content of nitrogen in different parts of plants of both species during the entire period of the growth of both plants.

MATERIAL AND METHODS

The research was based on three series of a pot experiment conducted in a greenhouse laboratory of the Faculty of Biology and Biotechnology of the Warmia and Mazury University in Olsztyn. The research was conducted in the following periods: 12 April – 19 July 2010, 24 March – 30 June 2011, and 16 March – 28 June 2012. Spring wheat (cv. Nawra variety) was pure sown or grown as a companion crop with Persian clover (cv. Gobry), which was undersown in two densities: recommended and reduced by 20%.

The experimental factors were:

1) the method of sowing spring wheat and Persian clover:
   - pure sowing (control),
   - mixed sowing.

2) the plant density:
  - recommended (according to good agricultural practice – *R*),
  - lower (reduced by 20% compared to the recommended density – *L*).

The research was conducted in 5 periods distinguished according to the development stages of spring wheat sown in pure sowing at the recommended density, i.e. at the BBCH stages of leaf development (12-14), tillering (21-23), stem elongation (31-32), inflorescence emergence (54-56) and ripening (87-89).

The experiment consisted of 120 pots (wheat in pure sowing and in mixed sowing with clover x 2 sowing densities x 5 development stages x 4 repetitions), measuring 22 cm in diameter and 25 cm in depth, in which grains were placed in soil equidistant from one another and to a depth of 3 cm (spring wheat) and 1 cm (Persian clover).

Nineteen seeds of spring wheat were sown in the pots with the recommended density, while the lower density meant 15 seeds per pot. In the mixture, 12 seeds of Persian clover in the pots with the recommended density and 9 seeds in the pots with the lower density were added to spring wheat seeds. This corresponded to the following plant densities per 1 m^2: spring wheat: recommended density – 500, lower density – 400; Persian clover: 300 and 240, respectively.

The pots were filled with substrate composed of EutricCambisol (Humic), which had the following percentage of the fractions: 64% of grains less than 0.02 mm, 12% of dust (0.1-0.02 mm) and 24% of sand (> 1 mm) The soil was slightly acidic (pH in 1 M KCl from 5.6 to 6.2), and had the content of organic carbon from 13.2 to 14.4 g kg^{-1}, the content of nitrogen from 0.69 to 0.74 g kg^{-1}, a high content of phosphorus (9.2-11.6 mg 100 g^{-1} of soil) and magnesium (8.8 - 9.1 mg 100 g^{-1} of soil), and a medium content of potassium (12.9-14.5 mg 100 g^{-1}). The soil was taken from the depth of 0-25 cm.

Mineral fertilization (PK) was the same, regardless of the sowing method of spring wheat or the plant density, and equalled (g pot^{-1}): P – 0.200 and K – 0.450. The N dose was (g pot^{-1}): 0.500 in pure sown spring wheat, 0.300 in mixed sowing with Persian clover, and 0.125 in Persian clover treatments. The fertilizers were applied one week before the plant sowing, in the form of urea, monopotassium phosphate and potassium sulphate water solutions.

The soil moisture was maintained at a constant level of 60% of the maximum water capacity, and any shortage of the soil moisture level was replenished daily. The air temperature in the laboratory was kept in the range of 20-22^{o}C for nearly all the time. The experiment was conducted under natural light.

The dates of plant harvest for analysis were indicated by the spring wheat growth stages noted in the pure sowing treatment with the recommended sowing density. When spring wheat reached a specified development stage, all the plants were removed from the pots designed to be kept until that development stage. Afterwards, the aerial organs was separated from the roots. The roots were thoroughly washed on sieves and gently separated from each other. As spring wheat developed, leaves, stems and ears were separated. The identified parts of the plants were dried to air-dry mass and their content of total nitrogen was determined. The analyses were performed in the Chemical-Agricultural Station in Olsztyn (standard PB 05). The analysed samples came from 3 series and 4 replications. Based on the results and the yields of dry plant mass, the nitrogen uptake by the aerial parts and roots of both plants was determined. The data concerning plant biomass were published earlier (WANIC, MYŚLIWIEC 2014). The translocation of nitrogen in the aerial parts of wheat between its inflorescence emergence and ripening was calculated using the following formulas (DORDAS 2012 *a*, *b*):

1. N translocation (kg ha^{-1}) = N content in the shoots in the inflorescence emergence stage – N content in the ripening stage (shoots - grain);
2. Translocation efficiency (%) = (N translocation/N content in the inflorescence emergence stage) x 100;
3. Contribution of the N content in the inflorescence emergence stage to the grain in the ripening stage (%) = (N translocation/N content in the grain in the ripening stage) x 100.

Taking into account the nitrogen uptake (nitrogen yield), calculations were performed of the following indicators showing competitive interactions between the two species (DE WIT, VAN DEN BERGH 1965):

1) relative yield total – $RYT = RYi + RYj$,

2) competitive balance index – $C_b = \ln[(Yij/Yji) / (Yii/Yjj)]$,

where:

RYi – relative yield of wheat determined from the formula:
$RYi = Yij / Yii$

RYj – relative yield of clover determined from the formula:
$RYj = Yji / Yjj$,

where:

Yii – yield of species *i* (spring wheat) in pure sowing;

Yjj – yield of species *j* (Persian clover) in pure sowing;

Yij – yield of species *i* (spring wheat) in mixed sowing with species *j* (Persian clover);

Yji – yield of species *j* (Persian clover) in mixed sowing with species *i* (spring wheat).

The results were averaged from three series of the experiments. They were processed statistically by variance analysis, in accordance with the model appropriate for a fully random system at an error probability of $p = 0.005$, and homogenous groups were determined based on the Tukey's test. The calculations were performed using the *Statistica* software.

RESULTS

The total nitrogen content in spring wheat growing with undersown Persian clover was lower than the content in pure sown wheat, from the stem elongation to the ripening stage (Table 1). The negative impact of clover on the nitrogen concentration in roots was also visible during the tillering stage. The undersown clover had the strongest negative effect on the concentration of nitrogen in leaves, and the weakest one – in roots and heads. The lower plant density had a positive impact on the nitrogen concentration in roots during the leaf development stage and in stems during the inflorescence

Table 1

Nitrogen content in spring wheat plants (g kg^{-1})

Growth stage (BBCH)	Plant part	Plant density				Average for plant density		Average for sowing method	
		recommended (*R*)		lower (*L*)					
		sowing method				density		sowing method	
		*P**	*M***	*P*	*M*	*R*	*L*	*P*	*M*
1. Leaf development (12-14)	leaves	47.4 *a*	47.0 *a*	46.8 *a*	46.3 *a*	47.2 *a*	46.6 *a*	47.1 *a*	46.7 *a*
	roots	16.2 *ab*	16.2 *ab*	22.4 *a*	14.7 *b*	16.2 *b*	18.6 *a*	19.3 *a*	15.5 *a*
	average	37.6 *a*	38.7 *a*	41.1 *a*	38.7 *a*	38.2 *a*	39.9 *a*	39.4 *a*	38.7 *a*
2. Tillering (21-23)	leaves	34.8 *a*	28.3 *b*	35.9 *a*	34.2 *a*	31.6 *a*	35.1 *a*	35.4 *a*	31.3 *a*
	roots	11.6 *a*	10.4 *ab*	11.8 *a*	9.3 *b*	11.0 *a*	10.6 *a*	11.7 *a*	9.9 *b*
	average	28.2 *a*	29.0 *a*	30.4 *a*	26.6 *a*	28.6 *a*	28.5 *a*	29.3 *a*	27.8 *a*
3. Stem elongation (31-32)	stems	31.7 *a*	24.4 *b*	32.7 *a*	23.7 *b*	28.1 *a*	28.2 *a*	32.2 *a*	24.1 *b*
	leaves	20.0 *a*	14.8 *b*	21.1 *a*	14.8 *b*	17.4 *a*	18.0 *a*	20.6 *a*	14.8 *b*
	average abovegroud	25.8 *a*	19.4 *b*	26.3 *a*	18.7 *b*	22.6 *a*	22.8 *a*	26.1 *a*	19. 1*b*
	roots	10.8 *a*	10.2 a	11.2 *a*	9.5 *a*	10.5 *a*	10.4 *a*	11.0 *a*	9.9 *a*
	average	21.8 *a*	17.1 b	22.3 *a*	16.8 *b*	19.5 *a*	19.6 *a*	22.1 *a*	17.0 *b*
5. Inflorescence emergence (54-56)	stems	17.0 *b*	12.6 c	22.0 *a*	13.9 *c*	14.8 *b*	18.0 *a*	19.5 *a*	13.3 *b*
	leaves	13.9 *a*	9.5 b	15.0 *a*	8.6 *b*	11.7 *a*	11.8 *a*	14.5 *a*	9.1 *b*
	heads	22.7 *a*	20.0 a	19.6 *a*	21.4 *a*	21.4 *a*	20.5 *a*	21.2 *a*	20.7 *a*
	average abovegroud	18.8 *a*	15.4 b	19.1 *a*	16.0 *b*	17.1*a*	17.6 *a*	19.0 *a*	15.7 *b*
	roots	12.1 *a*	7.9 b	8.7 *b*	7.4 *b*	10.0 *a*	8.1 *b*	10.4 *a*	7.7 *b*
	average	18.0 *a*	13.9 c	18.3 *a*	14.4 *b*	16.0 *a*	16.4 *a*	18.2 *a*	14.2 *b*
8. Ripening (87-89)	stems	10.4 *b*	7.1 c	11.0 *a*	7.6 *c*	8.8 *b*	9.30 *a*	10.7 *a*	7.4 *b*
	leaves	9.50 *a*	4.7 b	11.4 *a*	4.3 *b*	7.1 *a*	7.9 *a*	10.5 *a*	4.5 *b*
	heads: husks grains	 10.7 *a* 23.0 *a*	 7.7 a 20.4 a	 10.5 *a* 22.0 *a*	 7.5 *a* 21.3 *a*	 9.2 *a* 21.7 *a*	 9.00 *a* 21.7 *a*	 10.5 *a* 22.5 *a*	 7.60 *a* 20.9 *a*
	average abovegroud	17.3 *a*	13.2 b	17.2 *a*	14.1 *b*	15.3 *a*	15.7 *a*	17.3 *a*	13.7 *b*
	roots	9.0 *ab*	8.2 ab	9.8 *a*	7.4 *b*	8.6 *a*	8.6 *a*	9.40 *a*	7.8 *b*
	average	16.6 *a*	12.7 c	15.4 *b*	13.4 *c*	14.7 *a*	14.4 *a*	16.0 *a*	13.1 *b*

a, *b*, *c* – values marked with the same letter do not differ significantly ($p \leq 0.05$)
* *P* – pure crop; ** *M* – cultivation in a mixture with Persian clover

emergence and ripening stages. A reverse relationship was noted in roots during inflorescence emergence. In the remaining time period, no significant differences relative to the sowing density were observed in the analysed parts of plants. The interaction of the experimental factors demonstrated that the nitrogen concentration was lower in a mixed plant stand than in pure-sown plants in the following stages and plant parts: leaf development – in roots from the lower density treatment; tillering - in leaves from the higher density; inflorescence emergence – in all aerial parts (particularly in stems) from plants grown in the lower density.

Persian clover responded negatively to the presence of spring wheat by decreasing its absorption of nitrogen from the onset of growth to the cereal inflorescence emergence stage (Table 2). This response was more pronounced in roots than in aerial parts, where significant differences between the mixed and pure sowing were found only in the tillering stage. It is worth noting that the differences between the mixed and the pure sown clover plants were smaller than those in wheat, except the nitrogen content in roots of clover during the leaf development stage. The nitrogen content in roots of Persian

Table 2

Nitrogen content in Persian clover plants (g kg^{-1})

Growth stage (BBCH)	Plant part	Plant density				Average for plant density		Average for sowing method	
		recommended (*R*)		lower (*L*)					
		sowing method				density		sowing method	
		*P**	*M***	*P*	*M*	*R*	*L*	*P*	*M*
1. Leaf development (12-14)	aerial	47.6 *a*	36.6 *b*	41.4 *b*	44.6 *b*	42.1 *a*	43.0 *a*	44.5 *a*	40.6 *a*
	roots	24.7 *a*	11.2 *c*	16.8 *b*	8.9 *d*	18.0 *a*	12.9 *b*	20.8 *a*	10.1 *b*
	average	38.9 *a*	29.9 *c*	35.9 *b*	34.5 *b*	34.9 *a*	35.2 *a*	37.9 *a*	32.2 *b*
2. Tillering (21-23)	aerial	37.7 *a*	33.0 *a*	40.1 *a*	34.9 *a*	35.4 *a*	37.5 *a*	38.9 *a*	34.0 *b*
	roots	17.5 *b*	11.7 *c*	17.0 *b*	19.9 *a*	14.6 *b*	18.5 *a*	17.3 *a*	15.8 *b*
	average	33.0 *b*	26.9 *d*	34.7 *a*	31.1 *c*	30.0 *b*	32.9 *a*	33.9 *a*	29.0 *b*
3. Stem elongation (31-32)	aerial	31.5 *a*	28.4 *b*	30.8 *a*	30.7 *a*	30.0 *a*	30.8 *a*	31.2 *a*	29.6 *a*
	roots	17.7 *a*	10.5 *b*	18.7 *a*	17.7 *a*	14.1 *b*	33.6 *a*	18.2 *a*	14.1 *b*
	average	29.4 *a*	20.8 *b*	29.1 *a*	27.1 *a*	25.1 *b*	28.1 *a*	29.3 *a*	24.0 *b*
5. Inflorescence emergence (54-56)	aerial	24.7 *bc*	22.9 *c*	26.0 *a*	25.2 *ab*	23.8 *b*	25.6 *a*	25.4 *a*	24.1 *a*
	roots	17.6 *b*	13.2 *d*	19.2 *a*	15.0 *c*	15.4 *b*	17.1 *a*	18.4 *a*	14.1 *b*
	average	24.0 *a*	20.4 *b*	25.5 *a*	23.8 *a*	22.2 *b*	24.7 *a*	24.8 *a*	22.1 *b*
8. Ripening (87-89)	aerial	22.3 *a*	22.6 *a*	20.7 *a*	21.7 *a*	22.5 *a*	21.2 *b*	21.5 *a*	22.2 *a*
	roots	13.1 *b*	13.0 *b*	15.8 *a*	17.3 *a*	13.1 *b*	16.6 *a*	14.5 *a*	15.2 *a*
	average	21.8 *a*	21.7 *a*	20.6 *b*	21.2 *ab*	21.8 *a*	20.9 *a*	21.2 *a*	21.5 *a*

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

* *P* – pure crop; ** *M* – cultivation in a mixture with spring wheat

clover sown in the recommended plant density was higher than that in the lower density treatment only during the leaf development stage. In all the other stages, the opposite was observed: a higher content of this microelement was found in pots with the lower sowing density. The plant density did not result in any differences in the nitrogen content in the aerial parts of clover, except during the wheat inflorescence emergence stage, when a higher content was observed at the lower plant density, and during the ripening stage, when the opposite was true. Also, the interaction between the sowing method and the plant density turned out to be significant. In the leaf development stage, significant differences were found at the recommended density between the different sowing methods (with worse results achieved in the treatment with the undersown crop) in complete plants. This was particularly pronounced regarding the content of this nutrient in roots. At the tillering stage, mixed sowing reduced the concentration of nitrogen in whole plants to a similar extent in both treatments. During the stem elongation and the inflorescence emergence periods, a significant negative response to mixed sowing was demonstrated only by the plants growing in the pots with the recommended density. On the other hand, during the ripening period, mixed sowing did not result in significant differences in the content of nitrogen in either the pots with the higher density or the ones with the lower density,.

The nitrogen uptake by wheat during the leaf development stage was similar in the plants sown with either of the methods (Table 3). Starting from tillering, the undersown clover reduced the absorption of nitrogen by the cereal. The nitrogen uptake was lower than in the pure sown wheat by 9.4% during the tillering stage, by 34.1% in the stem elongation stage, by 23.5% in the inflorescence emergence stage and by 37.7% in the ripening stage. The nitrogen uptake by the different parts of wheat was also dependent on the sowing method. From the tillering stage until the end of the plant growing period, the undersown clover limited nitrogen accumulation in the entire aerial part of the plant (with the exception of husks in the ripening stage). However, it had no effect on the total content of nitrogen in the roots. During the whole plant growth period, the density of the plants did not affect the nitrogen uptake by wheat, although nitrogen accumulation was significantly higher in the heads during inflorescence emergence of wheat plants grown at the recommended density. The interaction of the factors demonstrated that clover as an undersown crop similarly reduced the nitrogen uptake in both plant densities from the stem elongation stage to the end of the concurrent growth. During the tillering stage, in the pots with the recommended density, the nitrogen uptake by wheat sown using both methods was identical. Meanwhile, at the lower density, the accumulation of this nutrient in wheat mixed with clover was significantly lower than in pure sown wheat (which was due to its accumulation in the leaves). In the inflorescence emergence stage, the interaction of the factors affected the accumulation of nitrogen in some parts of the plants. Mixed sowing had a stronger limiting effect

Table 3

Nitrogen uptake by spring wheat (g pot^{-1})

Growth stage (BBCH)	Plant part	Plant density				Average for plant density		Average for sowing method	
		recommended (*R*)		lower (*L*)					
		sowing method				density		sowing method	
		*P**	*M***	*P*	*M*	*R*	*L*	*P*	*M*
1. Leaf development (12-14)	leaves	0.09 *a*	0.09 *a*	0.07 *a*	0.08 *a*	0.09 *a*	0.08 *a*	0.08 *a*	0.09 *a*
	roots	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*
	total	0.10 *a*	0.10 *a*	0.08 *a*	0.09 *a*	0.10 *a*	0.08 *a*	0.09 *a*	0.10 *a*
2. Tillering (21-23)	leaves	0.26 *b*	0.25 *b*	0.29 *a*	0.27 *b*	0.26 *a*	0.28 *a*	0.28 *a*	0.26 *b*
	roots	0.03 *a*	0.04 *a*	0.04 *a*	0.03 *a*	0.03 *a*	0.04 *a*	0.04 *a*	0.03 *a*
	total	0.29 *b*	0.29 *b*	0.33 *a*	0.30 *b*	0.29 *a*	0.31 *a*	0.32 *a*	0.29 *b*
3. Stem elongation (31-32)	stems	0.23 *a*	0.16 *b*	0.19 *a*	0.10 *b*	0.19 *a*	0.15 *a*	0.21 *a*	0.13 *b*
	leaves	0.14 *a*	0.09 *b*	0.15 *a*	0.08 *b*	0.12 *a*	0.12 *a*	0.15 *a*	0.09 *b*
	total	0.37 *a*	0.25 *b*	0.34 *a*	0.18 *b*	0.31 *a*	0.27 *a*	0.36 *a*	0.22 *b*
	roots	0.05 *a*	0.04 *a*	0.05 *a*	0.05 *a*	0.05 *a*	0.05 *a*	0.05*a*	0.05 *a*
	total	0.42 *a*	0.29 *b*	0.39 *a*	0.23 *b*	0.36 *a*	0.32 *a*	0.41 *a*	0.27 *b*
5. Inflorescence emergence (54-56)	stems	0.12 *a*	0.08 *b*	0.16 *a*	0.09 *b*	0.10 *a*	0.13 *a*	0.14 *a*	0.09 *b*
	leaves	0.09 *b*	0.05 *c*	0.10 *a*	0.05 *c*	0.07 *a*	0.08 *a*	0.10 *a*	0.05 *b*
	heads	0.26 *a*	0.20 *b*	0.20 *b*	0.22 *b*	0.23 *a*	0.21 *b*	0.23 *a*	0.21 *a*
	total	0.47 *a*	0.33 *c*	0.46 *a*	0.36 *b*	0.40 *a*	0.41 *a*	0.47 *a*	0.35 *a*
	roots	0.04 *a*	0.04 *a*	0.03 *a*	0.03 *a*	0.04 *a*	0.03 *a*	0.04 *a*	0.04 *a*
	total	0.51 *a*	0.37 *b*	0.49 *a*	0.39 *b*	0.44 *a*	0.43 *a*	0.51 *a*	0.39 *b*
8. Ripening (87-89)	stems	0.06 *a*	0.03 *b*	0.07 *a*	0.04 *b*	0.05 *a*	0.05 *a*	0.07 *a*	0.04 *b*
	leaves	0.06 *a*	0.02 *b*	0.07 *a*	0.02 *b*	0.04 *a*	0.04 *a*	0.06 *a*	0.02 *b*
	heads:	0.38 *a*	0.24 *b*	0.36 *b*	0.27 *b*	0.31 *a*	0.32 *a*	0.37 *a*	0.25 *b*
	husks	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*	0.01 *a*
	grains	0.37 *a*	0.23 *b*	0.35 *ab*	0.26 *b*	0.30 *a*	0.31 *a*	0.36 *a*	0.24 *b*
	total	0.50 *a*	0.29 *b*	0.50 *a*	0.33 *b*	0.40 *a*	0.41 *a*	0.50 *a*	0.31 *b*
	roots	0.02 *a*	0.02 *a*	0.03 *a*	0.02 *a*	0.02 *a*	0.02 *a*	0.03 *a*	0.02 *a*
	total	0.52 *a*	0.31 *b*	0.53 *a*	0.35 *b*	0.42 *a*	0.43 *a*	0.53 *a*	0.33 *b*

a, *b*, *c* – values marked with the same letter do not differ significantly ($p \leq 0.05$)
* *P* – pure sowing, ** *M* – cultivation in a mixture with Persian clover

on the nitrogen accumulation in the aerial mass in the treatment with the recommended plant density, and only in the leaves of plants grown at the lower density.

From the onset of the plant growth, spring wheat reduced the absorption of nitrogen by Persian clover (Table 4). In the leaf development stage, the nitrogen uptake was 54.5% lower than in the pure sown clover. The tendency grew stronger during the cereal tillering stage (clover mixed with wheat absorbed 80.9% less nitrogen than pure sown clover) and remained on a similar level until the inflorescence emergence stage. Subsequently, in the final stage of plant growth, it declined (to 67.1%). The accumulation of nitrogen in the leaves during the first period of growth of the mixture was significantly lo-

Table 4

Nitrogen uptake by Persian clover (g pot^{-1})

Growth stage (BBCH)	Plant part	Plant density				Average for plant density		Average for sowing method	
		recommended (*R*)		lower (*L*)					
		sowing method				density		sowing method	
		*P**	*M***	*P*	*M*	*R*	*L*	*P*	*M*
1. Leaf development (12-14)	aerial	0.011*a*	0.005*b*	0.007*b*	0.003*b*	0.008*a*	0.005*b*	0.009*a*	0.004*b*
	roots	0.002*a*	0.001*a*	0.001*a*	0.001*a*	0.002*a*	0.001*a*	0.002*a*	0.001*a*
	total	0.013*a*	0.006*b*	0.008*b*	0.004*c*	0.010*a*	0.006*b*	0.011*a*	0.005*b*
2. Tillering (21-23)	aerial	0.086*a*	0.017*b*	0.073*a*	0.012*b*	0.051*a*	0.043*b*	0.080*a*	0.015*b*
	roots	0.010*a*	0.002*b*	0.008*a*	0.003*b*	0.006*a*	0.006*a*	0.009 *a*	0.002 *b*
	total	0.096*a*	0.019*b*	0.081*a*	0.015*b*	0.058*a*	0.048*b*	0.089*a*	0.017*b*
3. Stem elongation (31-32)	aerial	0.189*a*	0.018*c*	0.156*b*	0.030*c*	0.103*a*	0.093*a*	0.173*a*	0.024*b*
	roots	0.016*a*	0.005*c*	0.011*b*	0.005*c*	0.011*a*	0.008*b*	0.013*a*	0.005*b*
	total	0.205*a*	0.023*c*	0.167*b*	0.035*c*	0.114*a*	0.101*b*	0.186*a*	0.029*b*
5. Inflorescence emergence (54-56)	aerial	0.356*a*	0.070*c*	0.300*b*	0.066*c*	0.213*a*	0.183*b*	0.328*a*	0.068*b*
	roots	0.018*a*	0.010*b*	0.017*a*	0.005*c*	0.014*a*	0.011*b*	0.017*a*	0.008*b*
	total	0.374*a*	0.080*c*	0.317*b*	0.071*c*	0.227*a*	0.194*b*	0.346*a*	0.076*b*
8. Ripening (87-89)	aerial	0.428*a*	0.151*b*	0.392*a*	0.110*b*	0.290*a*	0.251*b*	0.410*a*	0.130*b*
	roots	0.019*a*	0.010*c*	0.013*bc*	0.010*c*	0.014*a*	0.011*b*	0.016*a*	0.010*b*
	total	0.447*a*	0.161*b*	0.405*a*	0.120*b*	0.304*a*	0.263*b*	0.426*a*	0.140*b*

a, *b*, *c* – values marked with the same letter do not differ significantly ($p \leq 0.05$)
* *P* – pure crop; ** *M* – cultivation in a mixture with spring wheat

wer than in the pure grown plants, without significant differences in the roots resulting from the sowing methods. In the tillering stage, the reduction was on a similar level in the aerial parts and in the roots, while being much lower in the roots than in the aerial parts during the remaining period. Clover growing in the pots with the lower plant density absorbed less nitrogen than clover growing in the pots with the recommended density during the whole plant growth. In this treatment, the nitrogen accumulation was significantly lower in the shoots during the leaf development and tillering stages, in the roots during the stem elongation stage, and in both parts of the plants during the other stages. It must be noted that the lower plant density had a more distinctly negative impact on the concentration of nitrogen in the aerial parts of plants than in the roots. Mixed sowing had a similar negative effect on the nitrogen absorption by clover in both plant densities during the leaf development, tillering and ripening stages. In the stem elongation and inflorescence emergence stages, the negative impact of undersown clover was more evident in the pots with the recommended density than in those with the lower density. In the stem elongation stage, this was manifested by lower nitrogen accumulation in all parts of the plants, but during the inflorescence emergence stage – only in the aerial mass.

No significant impact of the sowing method was demonstrated on the mass of translocated nitrogen from the spring wheat vegetative parts to grain between inflorescence emergence and ripening (Table 5). A tendency was observed for a decreasing value of this indicator in the pots with mixed wheat and clover sown at the recommended density. However, the efficiency of nitrogen translocation and the transport of the assimilates from the vegetative parts to grain during the analysed period was much higher in the pots with mixed wheat and clover. The plant density had no significant impact on the value of the analysed indicators.

Table 5

Nitrogen translocation in the aerial parts of the spring wheat between inflorescence emergence and ripening

Ratio	Plant density				Average for plant density		Average for sowing method	
	recommended (*R*)		lower (*L*)					
	sowing method				density		sowing method	
	*P**	*M***	*P*	*M*	*R*	*L*	*P*	*M*
Translocation (g pot^{-1})	0.34*a*	0.27*a*	0.31*a*	0.29*a*	0.31*a*	0.30*a*	0.33*a*	0.28*a*
Translocation efficiency (%)	72.3*b*	81.1*a*	67.4*b*	80.6*a*	76.7*a*	74.0*a*	69.9*b*	80.9*a*
Translocation to grain (%)	91.9*b*	117.4*a*	88.6*b*	115.4*a*	104.7*a*	102.0*a*	90.3*b*	116.4*a*

a, *b* – values marked with the same letter do not differ significantly ($p \leq 0.05$)
* *P* – pure crop; ** *M* – cultivation in a mixture with Persian clover

The competition for nitrogen between spring wheat and Persian clover started during the leaf development stage and continued until the end of plant growth (Table 6). It became more intensive at the stem elongation stage, and subsided during the inflorescence emergence and ripening stages. At leaf development, the plants competed for 43% of the nitrogen resources (they used the remaining part in a complementary manner), and at tillering – for as much as 90%. During the other stages, the plants competed for the entire nitrogen resource. In the leaf development and tillering stages, the plants competed to a similar extent with their aerial parts and with the roots. Starting from the stem elongation stage, the aerial parts competed more intensively. The density of plants significantly modified the interactions between the two species with their root systems during the leaf development, tillering and inflorescence emergence stages. At leaf development, the competition was observed only in the pots with the recommended density (it affected 50% of nitrogen resources), while during the tillering and the inflorescence emergence stages, it was more intensive in the pots with the lower density. During the whole plant growing period, wheat was a stronger competitor, which was indicated by values of the *Cb* index greater than zero.

Table 6

Relative yield total (RYT) and competition balance index (Cb)

Growth stage BBCH	Plant part	RYT			Cb		
		*R**	*L***	average	*R*	*L*	average
1. Leaf development (12-14)	aerial	1.45 *a*	1.57 *a*	1.57	0.79 *b*	0.98 *a*	0.93
	roots	1.50 *b*	2.00 *a*	1.50	0.69 *a*	0.00 *b*	0.69
	total plant	1.46 *b*	1.63 *a*	1.57	0.77 *a*	0.81 *a*	0.89
2. Tillering (21-23)	aerial	1.16 *a*	1.10 *a*	1.12	1.58 *a*	1.73 *a*	1.60
	roots	1.53 *a*	1.13 *b*	0.97	1.90 *a*	0.69 *b*	1.22
	total plant	1.20 *a*	1.09 *a*	1.10	1.62 *a*	1.59 *a*	1.56
3. Stem elongation (31-32)	aerial	0.77 *a*	0.72 *a*	0.75	1.96 *a*	1.01 *b*	1.48
	roots	1.11 *a*	1.45 *a*	1.38	0.94 *a*	0.79 *b*	0.96
	total plant	0.80 *a*	0.80 *a*	0.81	1.82 *a*	1.03 *b*	1.44
5. Inflorescence emergence (54-56)	aerial	0.90 *a*	1.00 *a*	0.95	1.27 *a*	1.27 *a*	1.28
	roots	1.56 *a*	1.29 *b*	1.47	0.59 *b*	1.22 *a*	0.75
	total plant	0.94 *a*	1.02 *a*	0.98	1.22 *a*	1.27 *a*	1.25
8. Ripeninig (87-89)	aerial	0.93 *a*	0.94 *a*	0.94	0.50 *b*	0.86 *a*	0.67
	roots	1.53 *a*	1.44 *a*	1.29	0.64 *a*	-0.14 *b*	0.06
	total plant	0.96 *a*	0.96 *a*	0.95	0.50 *a*	0.80 *a*	0.64

a, *b* –values marked with the same letter do not differ significantly ($p \leq 0.05$)
* *R* – recommended density, ** *L* – lower density

Wheat's domination over clover was particularly evident during the tillering stage. No clear impact of the plant density on plant competion for nitrogen was demonstrated. In the pots with the recommended plant density, during the stem elongation stage the and during the leaf development, wheat dominated clover more distinctly, with respect to both shoots and roots, than in the pots with the lower density,. During the tillering and ripening stages it dominated clover with respect to roots. In the pots with the density lower than recommended, a stronger impact of the aerial parts was observed compared to the roots during the leaf development and ripening stages.

DISCUSSION

Our research has demonstrated that the nitrogen uptake by spring wheat in a mixture was the same as in pure sowing during the leaf development stage, but the N uptake by undersown Persian clover was lower, which manifested itself by clover's lower plant biomass and lower nitrogen concentration in biomass (Wanic, Myśliwiec 2014). This, however, was not due to any shortage of nitrogen in soil because the plants were small and their demand was low. It was most likely caused by the negative alleopathic effect between wheat and clover. Consequently, wheat had shorter and thinner roots as well as smaller shoots and smaller leaves compared to pure sown wheat (Wanic, Myśliwiec 2014). The results are partly confirmed by Księżak (2010), who demonstrated that root secretions of spring wheat could inhibit the initial development of pea and spring vetch. Also, the research conducted by Andersen et al. (2004) proved that pea grown in a mixture with barley absorbed less soil nitrogen than pure sown pea. Fukai and Trenbath (1993) claimed that in the initial plant growing period some species in a mixture grew faster than others, which gradually led to their domination. In the current experiment, the initially weaker growth of clover sown in a mixture gave wheat a competitive advantage, which grew stronger in the subsequent growth stages. Similar results were obtained by Andersen et al. (2004) in mixed sowing of spring barley with pea.

Starting from the tillering stage, nitrogen absorption by both species in the mixture was lower than its absorption by pure sown species, which was reflected by lower concentrations of this nutrient in the plants (from the stem elongation stage in wheat) and by its total content. During the tillering stage, wheat in the mixture was taller, had a larger mass of aerial parts as well as longer and better formed roots; consequently, it was more effective in absorbing water and nutrients (including soil nitrogen) than Persian clover (Myśliwiec et al. 2014, Wanic et al. 2014). The higher competitiveness of roots of cereals compared to that of legumes was implicated by Mariotti et al. (2009) and by Michalska and Wanic (2008). The process of atmospheric nitrogen absorption started by clover in the presence of the dominant cereal was most probably not as effective as by pure sown clover, mostly due to the the shading and the living space reduced by the cereal. Moreover, less nitrogen absorbed by clover was also a consequence of more numerous cereal plants than clover plants. Wheat in the mixture also absorbed less nitrogen than pure sown wheat, but the difference was not as pronounced as in clover. Bergkvist (2003) suggested that the impact of competition for light between clover and wheat before stem elongation was of key importance to the yield of this cereal. To achieve high cereal yield, competition from clover should be limited in this period (Thorsted et al. 2006*a*).

In the authors' research, the negative impact of mixed sowing on the nitrogen uptake by wheat was the strongest during stem elongation and ripening, but in the case of Persian clover it grew stronger until inflorescence emergence, after which it slightly decreased. The results correspond to the data obtained by Wanic et al. (2012) in their research on mixtures of spring barley and pea, and by Thorsted et al. (2006*a*), who studied mixtures of spring wheat and white clover. Thus, as the plant growth progressed, the soil nitrogen resources were increasingly depleted (as indicated by the RYT value). Combined with its limited assimilation by clover, the N total content turned out to be insufficient to satisfy the demands of both species. As a result of its dynamic growth during stem elongation, wheat was much taller than clover and therefore received more sunlight, while limiting its access to clover, which inhibited the legume's growth. Consequently, clover was suppressed by the taller, larger and more leafy wheat, and therefore absorbed less atmospheric nitrogen than pure sown clover. This was reflected by the smaller weight of this element accumulated in clover. The main reason was the poorer development of clover in the mixture compared to pure sown clover, because – compared to pure sown clover – the nitrogen concentration was much lower only in the roots and identical in the aerial parts. SOBKOWICZ (2006) claimed that the competition between triticale and horse bean was most pronounced in the period before grain filling, while MOLLA and SHARAIHA (2010) concluded that this type of interaction between wheat and barley was most intensive during the generative growth, which is partly confirmed by this research.

In both sowing methods, spring wheat increased its nitrogen uptake until the inflorescence emergence stage, and then, in the ripening stage, its total content remained at a stable level in pure sown wheat but decreased in the mixture. In the mixture, due to the shortage of growth factors, the wheat terminated vegetation sooner and dried up, significantly many leaves fell off and the roots shrunk. The reduced shading and more living space enabled the clover to absorb more nitrogen, thus reducing the differences between the two sowing methods. BERGKVIST et al. (2011) demonstrated that spring wheat, red clover and white clover in a mixture absorb resources in proportion to their size. However, if a dose of nitrogen is insufficient to ensure the growth of both species, clover more strongly responds to its shortage. The research conducted by ANDERSEN et al. (2004), supported by the work of OFORI and STERN (1987), also confirmed the reduced uptake of atmospheric nitrogen by pea growing in a mixture with barley. According to ANDERSEN et al. (2004), the above observation may indicate that growing cereals with legumes could be less beneficial than expected, due to the strong domination of the cereals in the mixture. It is true that wheat accumulated much more nitrogen than clover, but – compared to pure sowing – the reduction of the total nitrogen content in the wheat in the mixture was due to both the smaller biomass of the plants and to the lower concentration of nitrogen in wheat. SOBKOWICZ (2009) also demonstrated that the competitive impact of Persian clover on spring barley resulted in a reduced nitrogen uptake by the cereal. BERGKVIST (2003) suggested that its was difficult to achieve a higher yield of wheat growing in a mixture with white clover compared to pure sown wheat. Wheat growing in a mixture is unable to absorb nitrogen from the soil as efficiently as pure sown wheat. The author claims that this may limit the growth of wheat in a mixture, thus leaving more resources to clover. However, in our experiment, no such effect was observed because clover was more affected by the shortage of nitrogen. On the other hand, THORSTED et al. (2006*b*) are of the opinion that competition for nitrogen between wheat and clover may be limited by the clover's absorption of this element from the atmosphere. BERG-

KVIST (2003) and SCHMIDT and CURRY (1999) reported on the translocation of nitrogen from clover plants to cereals and grasses. In the analysed experiment, this could have resulted in an increased nitrogen content in wheat, thus reducing the differences between the sowing methods. This opinion is not shared by ATIS et al. (2012), who observed higher nitrogen content in mixed wheat and vetch than in pure sowings of those plants. On the other hand, in another report, BERGKVIST et al. (2011) indicated that undersown crops of white and red clover significantly increased the content of nitrogen in wheat.

In wheat, between inflorescence emergence and ripening, nitrogen was transferred from the vegetative part to the grain, and the efficiency of this process was higher in mixed sown plants than in pure sown plants. In the presence of clover, wheat plants ended their vegetation earlier than pure sown plants. Ageing and reduced intensity of photosynthesis caused the improved efficiency of the transfer (DORDAS, SIOULAS 2009). Moreover, the reduced translocation of nitrogen from the vegetative parts of a plant to the grain, which was observed in pure sown plants, may be due to the fact that more assimilates reaching the grain originated from photosynthesis during grain filling than from the material accumulated before inflorescence emergence, as reported by WARDLAW and PORTER (1967). In both sowing methods, the lower nitrogen content in the vegetative parts of plants during the ripening stage, compared to that during inflorescence emergence, also indicates the remobilization of this element (DORDAS2012a).

The competition between clover and wheat may be controlled by the spatial architecture of a plant stand (the width of rows, the sowing density) (THORSTED et al. 2006*b*). In the current experiment, the plant density did not affect the nitrogen uptake by wheat, as opposed to the uptake by Persian clover, which absorbed less nitrogen in the lower density treatment than in the one with the recommended density. With respect to clover, this was due to the smaller biomass of the plant rather than to its nitrogen concentration, which was larger in the pots with the lower density than in the ones with the recommended density during an almost whole vegetation period. The results obtained by ŻUK-GOŁASZEWSKA et al. (2010) did not demonstrate any impact of sowing density on the yield of green fodder or red clover. In both species, no impact was found of the density of plants on differences between the sowing methods. DORDAS (2012*a*) did not find any impact of sowing density on nitrogen concentration in barley grains either. At the same time, THORSTED et al. (2006b) observed a higher yield of and a higher nitrogen content in wheat grain in treatments with lower plant density, which, in their opinion, was due to increased inter-species and decreased intra-species competition for light and nitrogen during the vegetation.

CONCLUSIONS

4. Nitrogen uptake by spring wheat and Persian clover in the mixture was lower than in the pure sown plants in both treatments with different plant density. The concurrent growth of both species had a stronger limiting impact on nitrogen accumulation in their aerial parts than in the roots.

5. The density of the plants did not cause any differences in nitrogen uptake of the winter wheat, unlike in Persian clover, which absorbed less nitrogen in the pots with the lower density than in the pots with the recommended density.

6. Nitrogen translocation from the vegetative parts to the grain of spring wheat between inflorescence emergence and ripening was more effective in the mixture than in the pure sown plants.

7. In the mixture, spring wheat was more effective in absorbing nitrogen than Persian clover in the entire vegetative period.

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