DOI: 10.5586/aa.1655

Publication history
Received: 2015-09-29
Accepted: 2016-02-19
Published: 2016-04-11

Handling editor
Piotr Sugier, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Poland

Authors' contributions
MW: idea of the research; MW, MJ, MMy, MMi: execution of studies; MW, MMy: analysis of results; MW, MMi: writing the paper

Funding
The paper was prepared based on funds allocated for statutory research of the University of Warmia and Mazury in Olsztyn (subject number: 1016-0819).

Competing interests
No competing interests have been declared.

Citation
Wanic M, Myśliwiec M, Jastrzębska M, Michalska M. Interactions between spring wheat (*Triticum aestivum* ssp. *vulgare* L.) and undersown Persian clover (*Trifolium resupinatum* L.) depending on growth stage and plant density. Acta Agrobot. 2016;69(1):1655. http://dx.doi.org/10.5586/aa.1655

ORIGINAL RESEARCH PAPER

Interactions between spring wheat (*Triticum aestivum* ssp. *vulgare* L.) and undersown Persian clover (*Trifolium resupinatum* L.) depending on growth stage and plant density

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Abstract

A pot experiment was conducted in the years 2010–2012. The competitive interactions between spring wheat and undersown Persian clover, depending on plant density have been established. The plants were grown in a mixture and in pure sowing at a higher density (according to the rules of proper agricultural practice) and at a density reduced by 20%. Based on measurements of dry matter in the aboveground parts and roots conducted at the wheat growth stages (BBCH) such as: leaf development (12–14), tillering (21–23), stem elongation (31–32), inflorescence emergence (54–56), and ripening (87–89), calculations of indicators such as: relative yield, relative yield total, competitive balance index and relative efficiency index, were performed. Competition between spring wheat and Persian clover continued from the wheat tillering stage until the end of vegetation. The strongest interactions were at the stem elongation stage (the plants competed for 95% of the growth factors), while at the end of vegetation the competition decreased slightly (it concerned 85% of the resources). The aboveground parts influenced one another with higher intensity than the roots. This was visible particularly well during the inflorescence emergence stage, during which the plants accumulated only 8% of the resources in the aboveground parts, while 89% was accumulated in the roots. Wheat proved to be the stronger competitor for the growth factors. It reduced by more than twice the increase in the biomass of Persian clover from tillering until the end of vegetation. In the mixture, the relative growth rate of the aboveground parts of clover was higher than in the case of wheat, while the growth rate of the roots was similar for both species. Plant density had no significant impact on the intensity of mutual interactions.

Keywords

spring wheat; Persian clover; growth stages; aboveground parts; roots; competition indicators

Introduction

The beneficial influence of intercrops on the soil environment and sanitary conditions of corn has made them a valuable element of the crop rotation system, which is recommended for growing in all systems of agricultural farming [1,2]. Their positive role is primarily related to the enrichment of soil with organic matter and minerals, the prevention of the movement of nitrogen into the deeper layers of the soil, the prevention of erosion, diversification of biological life in the soil, the increase of biodiversity of agroecosystems and their stability, and in many cases also to the control of weeds and reduction of diseases [2–6]. Sometimes, the impact of intercrops on the physical properties of soil (especially on its structure and moisture) is emphasised [7,8]. Their impact on determining yield of plants is smaller. One of the forms of intercrops is undersown crops. They grow together with the main crop and, after the main crop is harvested, their aboveground mass is collected for fodder or (which has been recently most popular) it is incorporated into the soil as green manure. The role of undersown crops in shaping the properties of the agroecosystem is diverse and depends largely on the species of the undersown crop and main crop as well as on abiotic and biotic environmental factors [9–13].

Spring wheat is considered a very good main crop, primarily because of its relatively weak tillering, low growth and poor foliage [14]. Clovers, thanks to their well-developed root system, high aboveground biomass, and especially the ability to fix atmospheric nitrogen, are considered precious undersown plants. Most information on this subject in the literature pertains to red clover and white clover, or their mixtures with grasses [6]. Relatively little information can be found on Persian clover. However, this plant, if supplied with adequate amounts of water, produces high yields of nitrogen-rich biomass, and its suitability as an undersown crop was confirmed by studies conducted by Płaza et al. [15] and Zarea et al. [16].

There is a variety of interactions between the main crop and undersown crop. Most frequently, competition for soil resources and light occur. However, species-to-species chemical interactions can also occur [17]. These interactions affect plant density, plant development rhythm, morphological features as well as productivity and fertility [18]. The intensity and direction of the above-mentioned changes depend on the choice of partner species (and varieties), the abundance of habitat, plant growth stage, and plant density [12,19]. A series of papers on such interactions between main crops and undersown crops have recently been published [11,13,20]. However, there is little information about this type of interaction between spring wheat and Persian clover throughout the period of their common vegetation. Furthermore, studies in this field are mainly focused on the aboveground parts, and there are few studies related to root systems.

Based on the above, a hypothesis was presented that competitive interactions can occur between spring wheat and Persian clover. This process would occur with varying intensity depending on the growth stage of plants and their density within the crop, and the aboveground parts and roots of both species will react differently to their combined cultivation. The hypothesis was verified in a pot experiment whose objective was to evaluate competitive interactions between spring wheat and Persian clover in the entire period of common vegetation, depending on their density within the crop.

Material and methods

The basis for the research was three series of a pot experiment conducted at the greenhouse laboratory of the Warmia and Mazury University in Olsztyn. The research was conducted in the following periods: series I – from 12 April to 19 July 2010; series II – from 24 March to 30 June 2011, and series III – from 16 March to 28 June 2012. Spring wheat ('Nawra' variety) was sole cropped and was also grown as a companion crop with Persian clover ('Gobry' variety), which was the undersown crop in two densities: recommended and reduced by 20%. The variety of spring wheat was 'Nawra', and the variety of Persian clover was 'Gobry'.

The factors of the experiment were as follows:

- spring wheat and Persian clover sowing method: (***i***) pure sowing (control); (***ii***) mixed sowing
- growth stages – BBCH (their determinant was wheat sown in pure sowing in a pot at the recommended density): (***i***) leaf development (12–14); (***ii***) tillering (21–23); (***iii***) stem elongation (31–32); (***iv***) inflorescence emergence (54–56); (***v***) ripening (87–89)

- plant density: (***i***) higher (according to the rules of proper agricultural practice), in this study referred to as "recommended"); (***ii***) lower (reduced by 20% compared to the previous one).

The experiment was set up according to the additive pattern, in which the number of plants in a mixture was the sum of their densities in pure sowing. This experimental design allowed for the study of interactions between spring wheat and Persian clover from the beginning of their vegetation and eliminated the influence of interspecific competition on this process [21].

The experiment consisted of 120 pots (two species in pure sowing and in mixed sowing × two sowing densities × 5 growth stages × 4 replicates) of the Kick-Brauckmann type with a diameter of 22 cm and a depth of 25 cm. In the pots, the caryopses and the grains were sown at the same distance from each other (thanks to templates) at a depth of 3 cm for spring wheat and 1 cm for Persian clover. In the pots with the recommended density, in both sowing methods 19 caryopses of spring wheat and 12 seeds of Persian clover were sown, and in the pots with the lower density 15 caryopses and 9 seeds were sown respectively. This corresponded to the following density of plants per 1 m^2: spring wheat: recommended density – 500, lower density – 400; Persian clover: 300 and 240, respectively.

The pots were filled with soil material in the form of Eutric Cambisol (Humic) soil with the content of the fraction below 0.02 mm equal to 64%, dust (0.1–0.02 mm) – 12%, and sand (>1 mm) – 24%. This soil was characterized by the content of organic carbon from 1.06% to 1.46%, a slightly acidic reaction (pH in 1 M KCl from 5.6 to 6.2), as well as the following nutrient availability (mg × 100 g^{-1} of soil): a high content of phosphorus (9.2–11.6) and magnesium (8.8–9.1), and a medium content of potassium (12.9–14.5). The soil was taken from a depth of 0–25 cm.

Mineral NPK fertilization was applied one week before sowing the plants. Aqueous solutions of urea, monopotassium phosphate, and potassium sulfate were prepared, added to the soil at an appropriate dose, carefully mixed, and the mixture was placed in pots. For all plants, the same fertilization with phosphorus and potassium was applied in the following amounts (g × pot^{-1}): P – 0.200 and K – 0.450. The nitrogen rate varied depending on the species and sowing method, and was as follows (in g pot^{-1}): 0.500 in the case of spring wheat sown in pure stand, 0.300 in the case of mixed sowing of spring wheat with Persian clover, and 0.125 in the case of Persian clover.

Soil moisture in the vegetation period was maintained at a constant level of 60% of the maximum water-holding capacity, and the missing moisture was supplemented daily whenever necessary. The air temperature in the laboratory was kept in the range of 20–22°C for nearly all the time. The experiment was conducted under natural light.

In the period when spring wheat reached the appropriate growth stage, all the plants were removed from the pots (those that were intended for the specific growth stage), and then the aboveground part was separated from the roots. The roots were thoroughly washed on sieves and gently separated from one another. The aboveground parts and roots were dried to air-dry mass and then weighed. In this way, their yield in the individual growth stages was obtained. Detailed results on the dry matter of plants were included in a separate paper [22].

Based on the values representing dry matter (of the aboveground parts and roots), calculations of relative yield (RY) [23], relative yield total (RYT) [23], competitive balance index (Cb) [24], and relative efficiency index of the species cultivated in a mixture [25] were calculated:

- relative yield: $RY_i = Y_{ij}/Y_{ii}$; $RY_j = Y_{ji}/Y_{jj}$
- relative yield total: $RYT = RY_i + RY_j$
- competitive balance index: $Cb = \ln[(Y_{ij}/Y_{ji})/(Y_{ii}/Y_{jj})]$
- relative efficiency index: $REI = RGR_{ij} - RGR_{ji}$ determined based on the following formula: $RGR = 1/w \times dw/dt = d(\ln w)/dt$

where: RY_i – relative yield of *i* species (spring wheat); RY_j – relative yield of *j* species (Persian clover); Y_{ii} – yield of species *i* (spring wheat) in pure sowing; Y_{jj} – yield of species *j* (Persian clover) in pure sowing; Y_{ij} – yield of species *i* (spring wheat) in mixed sowing with species *j* (Persian clover); Y_{ji} – yield of species *j* (Persian clover) in mixed sowing with species *i* (spring wheat); *w* – aboveground dry matter of the plant;

dw – increase of aboveground dry matter of the plant; *dt* – period of time in which the increase takes place (it was set at 1 day); *d* – delta; ln – natural logarithm.

The results presented in this study were averaged from three series of the experiments. They were processed statistically by way of variance analysis for factorial systems, with an error probability of $\alpha = 0.05$, using the Statistica software. Two factors were taken into account in statistical analysis: growth stage and plant density. To evaluate the differences between the experimental designs, Tukey's test was used, which served to determine uniform groups.

Results

At the stage of leaf development, Persian clover favorably influenced the increase in the aboveground mass of spring wheat, causing it to be greater by 9% in mixed cultivation than in pure sowing (Tab. 1). In the subsequent growth stages, a significant negative impact of the undersown crop on the main crop appeared, which lasted until the end of growth. At the stage of tillering, it was characterized by a 5% reduction of biomass, and in the subsequent periods it increased, reaching its peak values at the stages of stem elongation and ripening. In these periods, the aboveground biomass in the mixture was smaller than in pure sowing by 18% and 20%, respectively. Roots demonstrated another response to common cultivation. At the stage of inflorescence emergence, clover stimulated their development in wheat, resulting in their better formation by 33% on average, as compared to pure sowing. In other periods (which did not differ significantly from each other), a small (negative or positive) impact of clover on wheat was recorded. The different response of the aboveground parts and roots to mixed cultivation was reflected in the biomass of whole plants of this cereal. The relative yield at the stage of leaf development indicates a slight positive impact of clover on wheat. At the stage of tillering, a trend towards a negative effect on the

Tab. 1 Relative yield (RY) of spring wheat.

Plant density	Growth stage (BBCH)					
	leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	ripening (87–89)	average
Aboveground mass						
Recommended	1.02 b	0.92 cd	0.88 d	0.85 de	0.76 e	0.89 b
Lower	1.16 a	0.98 bc	0.76 e	0.91 cd	0.83 de	0.93 a
Average	1.09 a	0.95 b	0.82 cd	0.88 c	0.80 d	0.91
Roots						
Recommended	0.89 cd	1.02 b	0.83 d	1.47 a	0.97 c	1.04 a
Lower	1.06 ab	1.03 b	1.12 a	1.19 a	0.97 c	1.07 a
Average	0.98 b	1.03 b	0.98 b	1.33 a	0.97 b	1.06
Whole plant						
Recommended	0.98 a	0.95 a	0.87 bc	0.93 a	0.77 c	0.90 b
Lower	1.13 a	0.99 a	0.86 bc	0.95 a	0.84 bc	0.95 a
Average	1.06 a	0.97 ab	0.87 bc	0.94 b	0.81 c	0.93

The table presents the comparison of mean values calculated for the major factors (growth stages and plant densities) as well as for their interaction. a, b, c, d, e – values marked with the same letter do not differ significantly ($p \leq 0.05$), according to Tukey's test.

cereal was observed in clover. The negative effect of the undersown crop on the wheat crop persisted until the end of growth, reaching its peak size at the end of the growing season (a 19% reduction in biomass).

Relative yields also changed, depending on plant density. A significantly stronger response to mixed cultivation was demonstrated by the aboveground parts of wheat grown at the recommended density as compared to the lower one, with the exception of the stem elongation stage in which a contrary situation was reported. On average for the growth stages, the relative yield of roots for both plant density patterns was at a similar level. This indicated a small positive impact of clover on root development in wheat. Conversely, significant differences caused by sowing density occurred at the stages of leaf development and stem elongation. In these periods, in the experimental design with a lower density their mass in mixed sowing was greater than in pure sowing (by 6% and 12%, respectively), while for the recommended density it was smaller (by 11% and 17%). The analysis of the impact of sowing density on relative yields of whole wheat plants demonstrated that, on average for the growth stages, they reached significantly lower values in the experimental design with the recommended plant density, as compared to the lower one. The interaction of growth stages with sowing density turned out to be non-significant. For the mixture, only a trend to stimulate the growth of wheat was found in the experimental design with the recommended plant density, while for the lower one a trend towards its slight decrease. On the other hand, at the end of the growing season at both sites a negative impact of clover on wheat was recorded, with a clear trend to increase this impact under the conditions of higher sowing density.

During the entire period of mixed cultivation, spring wheat definitely limited the accumulation of dry matter in the aboveground parts of Persian clover (Tab. 2). As early as at the initial growth stage, in the mixture it was reduced by 58% compared to pure sowing. From leaf development till stem elongation, the intensity of this process increased, and then it gradually decreased in strength until the end of the growing season. In the period of the strongest interaction (stem elongation), the limiting

Tab. 2 Relative yield (RY) of Persian clover.

Plant density	Spring wheat growth stage (BBCH)					
	leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	ripening (87–89)	average
Aboveground mass						
Recommended	0.39 a	0.27 b	0.12 c	0.20 bc	0.36 a	0.27 a
Lower	0.44 a	0.28 bc	0.21 bc	0.19 bc	0.28 bc	0.28 a
Average	0.42 a	0.28 bc	0.17 d	0.20 cd	0.32 b	0.28
Roots						
Recommended	0.45 a	0.29 c	0.27 c	0.74 a	0.61 a	0.47 a
Lower	0.25 b	0.31 bc	0.44 b	0.37 b	0.75 a	0.42 b
Average	0.35 b	0.30 b	0.36 b	0.56 a	0.68 a	0.45
Whole plant						
Recommended	0.55 a	0.25 bc	0.13 c	0.23 bc	0.35 abc	0.30 a
Lower	0.52 a	0.28 bc	0.23 bc	0.19 bc	0.33 bc	0.31 a
Average	0.54 a	0.27 b	0.18 b	0.21 b	0.34 b	0.31

See Tab. 1 for explanations. a,b,c,d – values marked with the same letter do not differ significantly ($p \leq 0.05$), according to Tukey's test.

impact of wheat was demonstrated in the reduction of the accumulation of aboveground mass by Persian clover by as much as 83% compared to pure sowing. The roots of clover showed a very strong negative response to mixed cultivation from the beginning of the growing season till the stem elongation stage. During this period the reduction of their mass in the mixture, in relation to pure sowing, was from 70 to 75%. From the inflorescence emergence stage, a clear (significant) reduction in the negative impact of wheat was recorded, which lasted until the end of the growing season. At the inflorescence emergence stage, the mass of roots in the mixture was lower than in pure sowing by 44%, and at the ripening stage by 32%. The RY values calculated for whole plants of clover indicate that at the leaf development stage the presence of wheat led to a weaker development of clover (by 46% as compared to pure sowing). The negative impact of wheat on clover underwent a 2-fold (significant) intensification at the tillering stage, and it remained at a similar level till the end of the growing season. The average plant density for the growth stages significantly differentiated the value of the RY index calculated for roots. Its value for the experimental design with the recommended plant density was significantly higher, which indicates a stronger negative impact of the reduced plant density. The interaction of sowing density with growth stages demonstrated that only at the final stage of growth was the impact of wheat on the size of the aboveground mass of clover stronger at the site with the lower plant density. At the other growth stages, plant density did not differentiate the studied feature significantly. A clear trend towards a stronger negative response of the roots growing in pots with the recommended plant density was observed only at the stem elongation stage.

The analysis of the RYT index demonstrates that as early as at the stage of leaf development the species had too few growth factors to satisfy their combined needs (Tab. 3). Therefore, a process of competition for them started, which lasted until the end of the growing season. At the leaf development stage, the species competed for 41% of resources and used 59% in a complementary manner. However, this applies only to clover, as wheat used them fully (as demonstrated by the RY values discussed

Tab. 3 Relative yield total (RYT).

Plant density	Spring wheat growth stage (BBCH)					
	leaf development (12–14)	**tillering (21–23)**	**stem elongation (31–32)**	**inflorescence emergence (54–56)**	**ripening (87–89)**	**average**
Aboveground mass						
Recommended	1.41 ab	1.19 bc	1.00 c	1.05 c	1.12 bc	1.15 a
Lower	1.60 a	1.26 bc	0.97 c	1.10 c	1.11 c	1.21 a
Average	1.51 a	1.23 b	0.99 b	1.08 b	1.12 b	1.18
Roots						
Recommended	1.34 b	1.31 bc	1.10 b	2.21 a	1.58 b	1.51 a
Lower	1.31 bc	1.34 bc	1.56 a	1.56 b	1.72 b	1.50 a
Average	1.33 b	1.33 b	1.33 b	1.89 a	1.65 a	1.51
Whole plant						
Recommended	1.53 a	1.20 b	1.00 c	1.16 bc	1.12 c	1.20 a
Lower	1.65 a	1.27 b	1.09 c	1.14 c	1.17 bc	1.26 a
Average	1.59 a	1.24 b	1.05 b	1.15 b	1.15 b	1.23

See Tab. 1 for explanations. a,b,c – values marked with the same letter do not differ significantly ($p \leq 0.05$), according to Tukey's test.

previously). During this period, the limited resources of the plant accumulated in a larger amount in the aboveground parts than in the roots. The process of competition intensified at the tillering stage (the plants competed for 76% of growth factors), it increased even more at the stem elongation stage (only 5% of the resources were used by wheat and clover in a complementary manner), and then in the further period its intensity was slightly weaker. At the end of the growing season, growth factors were enough to cover 15% of the needs of both species and most of them were used by wheat. From the tillering stage till the end of the growing season, the competition limited the accumulation of biomass in the aboveground parts more than in the roots. A particularly big difference in the distribution of biomass between the roots and the aboveground parts occurred at the inflorescence emergence stage.

Limited resources were to a much greater extent used to increase the biomass of the roots than that of the aboveground parts. The plants accumulated in their aboveground parts only 8% of the resources, while 89% were accumulated in their roots. Plant density did not affect the value of the RYT index in the entire growing season. Differences in its value calculated for the roots were recorded only at the stem elongation and inflorescence emergence stages. In the first of these periods, competition limited the uptake of growth factors at the site with the recommended plant density more (by as much as 90%) than with the reduced one (by 44%); at the latter stage an opposite situation was observed. In the mixture, the roots at the site with the recommended plant density absorbed more resources than for pure sowing (by 21%), while in the case of the lower one the absorption of resources was smaller (by 44%). In the mixture throughout the growing season, spring wheat dominated over Persian clover, as indicated by the positive values of the competitive balance index (Tab. 4). The intensity of the competitive effects of wheat on clover had a variable nature in the course of its development. This cereal affected clover in a significantly more intensive way from tillering till the inflorescence emergence stage, whereas this impact was smaller at the beginning (leaf development) and at the end (ripening) of mixed cultivation. The strongest influence became apparent at the stem elongation stage. At

Tab. 4 Competition balance index (Cb).

Plant density	Spring wheat growth stage (BBCH)					
	leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	ripening (87–89)	average
Aboveground mass						
Recommended	0.47 d	1.24 bc	1.99 a	1.44 abc	0.72 d	1.17 a
Lower	0.96 c	1.25 bc	1.31 abc	1.54 ab	1.01 c	1.21 a
Average	0.72 d	1.25 b	1.65 a	1.49 a	0.87 c	1.19
Roots						
Recommended	0.67 cd	1.28 a	1.13 b	0.68 cd	0.45 d	0.84 b
Lower	1.44 a	1.17 b	0.93 c	1.18 b	0.25 e	0.99 a
Average	1.06 b	1.23 a	1.03 b	0.93 b	0.35 c	0.92
Whole plant						
Recommended	0.53 d	1.26 a	1.84 a	1.36 a	0.73 cd	1.14 a
Lower	1.13 ab	1.24 ab	1.33 a	1.53 a	1.03 abc	1.25 a
Average	0.83 b	1.25 a	1.59 a	1.45 a	0.88 b	1.20

See Tab. 1 for explanations. a,b,c,d – values marked with the same letter do not differ significantly ($p \leq 0.05$), according to Tukey's test.

the leaf development stage, the competitive advantage of wheat over clover was much more pronounced for the accumulation of biomass in the roots than in the aboveground parts.

At the tillering stage, its extent was similar for the aboveground parts and roots, while from the stem elongation stage till the end of the growing season it was most clearly visible in the aboveground parts. On average, for the growth stages no significant impact was recorded of plant density on the value of the analyzed index, calculated both for whole plants and their aboveground parts. The increased competitive advantage of wheat roots over clover roots occurred for the experimental design with the lower density. Plant density also contributed to the differentiation of the values of the competitive balance index at the analyzed growth stages. The lower plant density stimulated wheat plants to a stronger competition with the undersown crop at the beginning (leaf development) and at the end (ripening) of the growing season more than the recommended one. At the other growth stages, the analyzed index did not change significantly depending on sowing density. A comparison of the intensity of the interactions of the aboveground parts of wheat demonstrates that they were stronger for the recommended density than for the lower one at the leaf development and ripening stages. In turn, the root system of this cereal exerted a stronger influence on clover at the recommended sowing density at the tillering, stem elongation and ripening stages, whereas this influence was weaker at the leaf development and inflorescence emergence stages, as compared to the sites with the lower plant density.

In the mixture, the relative growth rate of spring wheat was lower in comparison to Persian clover for the average of plant density and growth stages (Tab. 5). Only in the early growing season (from sowing till leaf development) was an advantage of wheat over the undersown crop observed in terms of the growth rate of the aboveground mass. During this period, the relative growth rate of the roots of both species was the same. Starting from the leaf development stage till the end of mixed growing, clover exhibited a higher growth rate than wheat. As regards the increase in aboveground mass, it was demonstrated most clearly between inflorescence emergence and ripening, while for the roots between stem elongation and inflorescence emergence.

The values of the relative efficiency index, on average for the growth stages, did not show differences depending on sowing density. However, their significant impact

Tab. 5 Relative efficiency index (REI).

Plant density	Spring wheat growth stage (BBCH)					
	leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	ripening (87–89)	average
Aboveground mass						
Recommended	0.08	0.03	−0.03	−0.08	−0.20	−0.04
Lower	0.05	−0.19	−0.06	−0.03	−0.05	−0.06
Average	0.07	−0.08	−0.05	−0.06	−0.13	−0.05
Roots						
Recommended	0.00	0.06	−0.16	−0.07	−0.01	−0.04
Lower	0.00	−0.01	−0.11	−0.02	0.03	−0.02
Average	0.00	0.03	−0.14	−0.05	0.01	−0.03
Whole plant						
Recommended	0.08	0.09	−0.19	−0.15	−0.21	−0.08
Lower	0.05	−0.20	−0.17	−0.05	−0.02	−0.08
Average	0.07	−0.06	−0.18	−0.10	−0.12	−0.08

was visible at the growth stages studied. In the interphase between leaf development and tillering at the site with the recommended density, the growth rate of wheat was higher than of clover (especially for the aboveground parts) in comparison to the lower density. From stem elongation till the end of the growing season, a more distinct advantage of clover over wheat in terms of the increase in aboveground biomass and roots occurred at the site with the lower density. Plant density had no significant impact on the relationships concerning the growth rate of both species in the mixture between tillering and stem elongation.

Discussion

In the present study, at the early stage of the growing season a small positive effect of undersown Persian clover on the development of the aboveground parts of spring wheat and its lack in terms of the increase in root mass was demonstrated. This was reflected by the development of plants with a larger weight and assimilation surface by this cereal as compared to pure sowing, which gave it a competitive advantage over clover at the later growth stages [22,26]. However, Kraska [27] showed that undersown red clover does not significantly influence germination, the length of the first leaf, or the length and number of roots of spring wheat. During this period, the cereal negatively affected the initial development of both the aboveground parts (RY = 0.42) and the roots of Persian clover (RY = 0.35). The effect of the above was the formation of smaller and less leafy shoots, and roots of reduced length and weight [22] by clover. However, the negative impact of wheat on clover did not result from the competitive interactions for nutrients, space and light (the plants were small, their life needs were inconsiderable, and they did not shade one another), but from allopathic interactions. This made clover develop smaller roots and shoots than in pure sowing. The results are partly confirmed in the work by Księżak [28] who demonstrated that the root secretions of spring wheat have an inhibitory effect on the initial development of pea and spring vetch. In a mixture, as early as in the initial vegetation period, some species grow faster than others, and this leads to their dominance at later growth stages [29,30]. In our own research, the development of plants characterized by greater weight, more abundant foliage and increased length and root weight by wheat as compared to clover determined its dominance in the crop [22,26]. Therefore, it could successfully compete with clover for water, nutrients and light. It assimilated resources at levels even higher than in pure sowing. However, the inhibitory impact of wheat on clover resulted in the effect that both species together used less than 60% of the available resources.

In the analyzed experiment, there was a deficiency of growth factors for both species, starting from the tillering stage. Competition between them began, and it lasted till the end of the growing season. Its highest intensity was manifested at the stem elongation and inflorescence emergence stages, in which the plants competed for the whole pool of growth factors. The intensity at the end of the growing season was slightly weakened. Also, Carof et al. [20] demonstrated that the most favorable period for competition is stem elongation and/or inflorescence emergence and flowering. These are periods of rapid growth of both aboveground parts and roots, and thus a greater demand of plants for nutrients, water and light, and under deficiency conditions of these factors, of a strong competition for them. Except for the initial period of development, shoots interacted more strongly than roots. Therefore, in the present experiment the competition for light turned out to be stronger than the competition for soil resources. Bergkvist [31] suggests that in a mixture the mutual shading of plants limits their growth more than the deficiency of nitrogen and water. In turn, Thorsted et al. [19] demonstrated a stronger influence of underground competition on the development of spring wheat, and of aboveground competition on the development of white clover. Faget et al. [32] also indicate a significant role of competition between plant roots in mixed sowing. The relationships between the root systems of plants cultivated in a combination are very complex and not fully understood. Much interesting information on their interactions in a mixture will be delivered by a detailed study using specialized equipment [32,33].

Wheat and clover assimilate resources proportionally to their size [34]. This was confirmed by the results of the present study. In the analyzed experiment, the competition proved to be asymmetric. Wheat assimilated a disproportionally higher amount of the limited resources than clover. As early as at the first stage of development, the higher growth rate of wheat as compared to clover gave it a competitive advantage, which persisted until the end of the growing season. Thanks to a better developed root system and aboveground parts (especially foliage), it could assimilate water and nutrients more efficiently and make better use of light [22,26]. However, from the tillering stage a negative impact of clover on wheat was recorded. Carof et al. [20] argue that during this period (tillering) the competition from clover has a significant impact on ear formation, so it is of great importance for determining the grain yield. However, the negative impact of the undersown crop on the cereal was not as large as in the opposite situation. Initially, it was expressed in the form of a tendency towards a slight (3%) reduction in biomass, it increased at the stem elongation stage (to 13%), decreased its intensity during inflorescence emergence (to 6%), and at the end of the growing season was strongest (the reduction in biomass by 19%). The presence of clover in the crop limited the accumulation of biomass only in the aboveground parts of wheat. The roots of this cereal reached a similar weight as in pure sowing, and at the inflorescence emergence stage even a higher one. Thus, under the conditions of deficiency of growth factors, wheat used them to a greater extent. Throughout the entire growing season, wheat plants were taller, had better foliage, and were characterized by a larger assimilation surface, while their roots were longer and more developed in comparison to clover. Therefore, their uptake of water was more efficient (even from the deeper layers) and they were not shaded by clover. Carof et al. [20] demonstrated that deep-reaching roots give an advantage to wheat over clover. This is confirmed by the studies of Thorsted et al. [19] who showed that wheat is a stronger competitor for soil resources and gains more of these resources than clover. Ofori and Stern [35] consider cereals as stronger competitors than legumes, mainly because of their faster growth rate and higher root density, as evidenced in the present study. Känkänen and Eriksson [6] proved that undersown clover poorly competes with barley and therefore does not change its weight. However, in our own research the undersown crop had the most limiting influence on grain yield, which was significantly lower than in pure sowing [22]. A reduction in the grain yield of winter wheat growing in combination with white clover was also noted in the study of Thorsted et al. [19]. Bergkvist [31] is of the opinion that a higher yield of wheat should not be expected when it grows in combination with while clover, because of the fact that it cannot assimilate growth factors (mainly N) from the soil as efficiently as in pure sowing. According to Thorsted et al. [19], the reduction in the grain yield of wheat was probably caused by competition for light and N during the growing season, and for water during grain filling. Bergkvist [31], and Schmidt and Curry [36] indicate the movement of nitrogen from clover plants to cereals. In our own research, it led to an increase in N content in wheat [37], which improved its condition and caused the reduction in the vegetative aboveground biomass not to be high, while in the roots this was not found or its positive effects were reported.

Clovers are considered to be undersown crops which poorly compete with cereals [6]. The present study confirmed this argument. In combination, Persian clover was dominated by wheat for the entire growing season. As early as at the tillering stage, the cereal reached a greater height, was leafier, and was characterized by longer and better developed roots. Thus, it used the limited growth factors more efficiently. In combination with a slower growth rate, its biomass in the mixture constituted less than 30% of the biomass obtained in pure sowing. The poor competitive ability of clover was also a result of the quantitative dominance of the cereal in the mixture. The pressure of wheat on clover was strongest at the stem elongation stage. This was reflected most clearly in the shoot weight of this plant (RY = 0.17). According to Thorsted et al. [19], competition for light plays an important role in interspecific relationships, especially within a crop of above 20 cm in height where clover is strongly shaded by the cereal. Probably, the deficiency of light has a greater impact on clover than on wheat and slows down its development. The results of the present study demonstrated a reduction in the negative impact of wheat on clover in the final period of the growing season. This is partly consistent with the data provided by other authors [12,13,17,38].

When wheat entered the ripening stage, its leaves dried up and fell off, its roots were reduced in weight, and a transfer of assimilates from the vegetative parts to the ear took place [22,26,37]. Accordingly, its vital needs became small. In this period, clover continued its growth, and as it was less shaded by the cereal and had a great pool of resources to use, it developed more abundantly than in the earlier periods. In the interphase between inflorescence emergence and ripening, its growth rate in the mixture was significantly higher as compared to wheat.

Competition between wheat and clover can change the intensity of interactions depending on plant density [19]. In the present study, the plants competed for a similar amount of growth factors in the case of both plant densities. They were slightly more efficiently used by wheat to build the aboveground parts at the site with the lower density. In this case, the competition of clover was smaller. Thorsted et al. [19] are of the opinion that increased spacing between rows reduces competition for light between plants, which translates into an increase in the aboveground biomass of wheat. In the present experiment, the number of leaves of spring wheat was higher for the lower plant density. These leaves were also characterized by a larger surface area [26]. Therefore, they could make better use of light for building their biomass. Moreover, the lower plant density, and thus a greater living space for individual wheat plants, meant that they could use more of the other growth factors, which led to a reduction in intraspecific competition between them [19,26]. Reduction of mutual shading allowed for greater access of light into the crop, and thus for the formation of more abundant foliage.

Conclusions

- Competition between spring wheat and Persian clover lasted throughout the growing season and was the strongest at the stem elongation stage of wheat.
- Persian clover had a negative influence on the increase in spring wheat biomass from the tillering stage (RY = 0.97) until the full ripeness stage (RY = 0.81), while wheat showed this influence on clover during the entire vegetation period [RY: from 0.54 (leaf development stage) to 0.18 – tillering].
- Competition was more limiting on biomass accumulation in aboveground parts than in roots from the stem elongation stage until the end of vegetation (in wheat by 16–55%, and in clover by 19–36%).
- Wheat proved to be the stronger competitor in obtaining growth factors. Its negative influence on clover was most pronounced during the stem elongation stage (Cb = 1.59).
- In combination, the relative growth rate of the Persian clover crop was higher than that of spring wheat, and the growth rates of roots were the same.
- The intensification of competitive interactions did not depend on plant density.

References

1. Blaser BC, Gibson LR, Singer JW, Jannink JL. Optimizing seeding rates for winter cereal grains and frost-seeded red clover intercrops. Agron J. 2006;98:1041–1049. http:/dx.doi.org/10.2134/agronj2005.0340
2. Jaskulska I, Gałęzewski L. Aktualna rola międzyplonów w produkcji roślinnej i środowisku. Fragmenta Agronomica. 2009;26(3):48–57.
3. Blackshaw EE. Nitrogen fertilizer, manure and compost effects on weed growth and competition with spring wheat. Agron J. 2005;97:1612–1621. http://dx.doi.org/10.2134/agronj2005.0155
4. Gaudin ACM, Westra S, Loucks CES, Janovicek K, Martin RC, Deen W. Improving resilience of northern field crop systems using inter-seeded red clover: a review. Agronomy. 2013;3:148–180. http://dx.doi.org/10.3390/agronomy3010148

5. Holland JM. The environmental consequences of asopting conservation tillage in Europe: reviewing the evidence. Agric Ecosyst Environ. 2004;103:1–25. http://dx.doi.org/10.1016/j.agee.2003.12.018
6. Känkänen H, Eriksson C. Effects of undersown crops on soil mineral N and grain yield of spring barley. Eur J Agron. 2007;27:25–34. http:/dx.doi.org/10.016/j.eja.2007.01.010
7. Raimbault BA, Vyn TJ. Crop rotation and tillage effects on corn growth and soil structural stability. Agron J. 1991;83:979–985. http://dx.doi.org/10.2134/agronj1991.00021962008300060011x
8. Unger PW, Merle F. Cover crop effects on soil water relationships. Journal of Soil and Water Conservation. 1998;53(3):200–207.
9. Michalska M, Wanic M, Jastrzębska M. Konkurencja pomiędzy jęczmieniem jarym a grochem siewnym w zróżnicowanych warunkach glebowych. Cz. II. Intensywność oddziaływań konkurencyjnych. Acta Scientiarum Polonorum Agricultura. 2008;2:87–99.
10. Sobkowicz P, Podgórska-Lesiak M. Ocena oddziaływania jęczmienia uprawianego w mieszance z pszenżytem lub grochem w zależności od dawki nawożenia azotem. Fragmenta Agronomica. 2009;26(1):115–126.
11. Picard D, Ghiloufi M, Saulas P, de Touronnet S. Does undersowing winter wheat with a cover crop increase competition for resources and is it compatibile with high yield? Field Crops Res. 2010;115:9–18. http://dx.doi.org/10.1016/j.fcr.2009.09.017
12. Treder K, Wanic M, Nowicki J. The intensity of competitive interactions between spring wheat (*Triticum aestivum* L. Emend. Fiori et. Paol) and spring barley (*Hordeum vulgare* L.) under different fertilization conditions. Acta Agrobot. 2008;61(2):195–203. http://dx.doi.org/10.5586/aa.2008.048
13. Wanic M, Jastrzębska M, Kostrzewska MK, Treder K. Competition between spring barley (*Hordeum vulgare* L.) and Italian ryegrass (*Lolium multiflorum* LAM.) under different water supply conditions. Acta Agrobot. 2013;66(3):73–80. http://dx.doi.org/10.5586/aa.2013.040
14. Zając T. Porównanie wybranych cech morfologicznych i produkcyjności gatunków lucerny w zależności od doboru roślin ochronnych. Zeszyty Problemowe Postępów Nauk Rolniczych. 2007;516:291–301.
15. Płaza A, Gąsiorowska B, Makarewicz A, Królikowska M. Plonowanie ziemniaka nawożonego wsiewkami międzyplonowymi w integrowanym i ekologicznym systemie produkcji. Biuletyn IHAR. 2013;267:71–78.
16. Zarea M, Ghalavad A, Goltapeh EM, Rejali F. Effect of clovers intercropping and earthworm activity on weed growth. J Plant Prot Res. 2010;50(4):463–469. http://dx.doi.org/10.2478/v10045-010-0077-2
17. Sobkowicz P. Konkurencja międzygatunkowa w jarych mieszankach zbożowych. Wrocław: Wydawnictwo Akademii Rolniczej; 2003. (Zeszyty Naukowe Akademii Rolniczej we Wrocławiu. Rozprawy; vol 458).
18. Sheaffer CC, Gunsolus JL, Jewett JG, Lee SH. Annual Medicago as a smother crop in soybean. Journal of Agronomy and Crop Science. 2002;188(6):408–416. http://dx.doi.org/10.1046/j.1439-037X.2002.00590.x
19. Thorsted MD, Olesen JE, Weiner J. Width of clover strips and wheat rows influence grain yield in winter wheat/white clover intercropping. Field Crops Res. 2006;95:280–290. http://dx.doi.org/10.1016/j.fcr.2005.04.001
20. Carof M, Tourdonnet S, Saulas P, Le Floch D, Roger-Estrade J. Undersowing wheat with different living mulches in a no-till system (I): yield analysis. Agronomy for Sustainable Development. 2007;27:347–356. http://dx.doi.org/10.1051/agro:2007016
21. Semere T, Froud-Williams RJ. The effect of pea cultivar and water stress on root and shoot competition between vegetative plants of maize and pea. J Appl Ecol. 2001;38:137–145. http://dx.doi.org/10.1046/j.1365-2664.2001.00570.x
22. Wanic M, Myśliwiec M. Changes in spring wheat (*Triticum aestivum* ssp. *vulgare* L.) and Persian clover (*Trifolium resupinatum* L.) biomass under the influence of plant competition and density. Acta Agrobot. 2014;67(4):125–134. http://dx.doi.org/10.5586/aa.2014.050
23. Snaydon RW. Replacement or additive designs for competition studies? J Appl Ecol. 1991;28:930–946. http://dx.doi.org/10.2307/2404218
24. Wilson JB. Shoot competition and root competition. J Appl Ecol. 1988;25:279–296. http://dx.doi.org/10.2307/2403626
25. Pietkiewicz S. Wskaźnikowa analiza wzrostu. Wiad Bot. 1985;29(1):29–42.

26. Myśliwiec M, Wanic M, Michalska M. Response of spring wheat to the growth with undersown of Persian clover under controlled conditions. Acta Scientiarum Polonorum Agricultura. 2014;13(3):29–44.

27. Kraska P. The effect of soil extracts from a monoculture of spring wheat (*Triticum aestivum* L.) grown under different tillage systems on the germination of seeds. Acta Agrobot. 2011;64(1):79–90. http://dx.doi.org/10.5586/aa.2011.010

28. Księżak J. Effect of root excretions from spring cereal seedlings on seed germination of field pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.). Acta Scientiarum Polonorum Agricultura. 2010;9(2):7–17.

29. Andersen MK, Hauggaard-Nielsen H, Ambus P, Jansen ES. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. Plant Soil. 2004; 266: 273–287. http://dx.doi.org/10.1007/s11104-005-0997-1

30. Fukai S, Trenbath BR. Processes determining intercrop productivity and yields of component crops. Field Crops Res. 1993;34:247–271. http://dx.doi.org/10.1016/0378-4290(93)90117-4

31. Bergkvist G. Effect of white clover and nitrogen availability on the grain yield of winter wheat in three-season intercropping system. Acta Agric Scand B Soil Plant Sci. 2003;53(3):97–109. http://dx.doi.org/10.1080/09064710310011953

32. Faget M, Nagel K, Walter A, Herrera JM, Jahnke S, Schurr U, et al. Root-root interactions: extending our perspective to be more inclusive of the range of theories in ecology and agriculture using in-vivo analysis. Ann Bot. 2013;112(2):253–266. http://dx.doi.org/10.1093/aob/mcs296

33. de Kroon H. Ecology: how do roots interact? Science. 2007;318:1562–1563. http://dx.doi.org/10.1126/science.1150726

34. Bergkvist G, Stenberg M, Wetterlind J, Båth B, Elfstrand S. Clover cover crops under-sown in winter wheat increase yield of subsequent spring barley – effect of N dose and undersown grass. Field Crops Res. 2011;120:292–298. http://dx.doi.org/10.1016/j.fcr.2010.11.001

35. Ofori F, Stern WR. Cereal-legume intercropping systems. Advances in Agronomy. 1987;41:41–90. http://dx.doi.org/10.1016/S0065-2113(08)60802-0

36. Schmidt O, Curry JP. Effects of earthworms on biomass production, nitrogen allocation and nitrogen transfer in wheat-clover intercropping model systems. Plant Soil. 1999;214:187–198. http://dx.doi.org/10.1023/A:1004723914623

37. Wanic M, Myśliwiec M, Orzech K, Michalska M. Nitrogen content and uptake by spring wheat and undersown Persian clover depending on plant density. J Elem. 2016;21(1):231–246. http://dx.doi.org/10.5601/jelem.2015.20.2.892

38. Bulson HAJ, Snaydon RW, Stopes CE. Effects of plant density on intercropped wheat and field in an organic farming system. J Agric Sci. 1997;128:59–71. http://dx.doi.org/10.1017/S0021859696003759

Oddziaływania pomiędzy pszenicą jarą (*Triticum aestivum* ssp. *vulgare*) i wsiewką koniczyny perskiej (*Trifolum resupinatum*) w zależności od fazy rozwojowej i zagęszczenia

Streszczenie

W doświadczeniu wazonowym, prowadzonym w latach 2010–2012 oceniano oddziaływania konkurencyjne pszenicy jarej i wsiewki koniczyny perskiej, w zależności od zagęszczenia roślin. Badaniami objęto części nadziemne i korzenie obu gatunków. Gatunki uprawiano w mieszance i siewie czystym w zagęszczeniu większym (zgodnym z zaleceniami agrotechniki) i zmniejszonym w stosunku do niego o 20%. Na podstawie pomiarów suchej masy części nadziemnych i korzeni przeprowadzonych w fazach rozwojowych pszenicy: rozwój liści (12–14), krzewienie (21–23), strzelanie w źdźbło (31–32), kłoszenie (54–56) i dojrzewanie (87–89), wykonano obliczenia wskaźników: plonów względnych, całkowitego plonu względnego, wskaźnika równowagi konkurencyjnej oraz indeksu efektywności względnej. Wykazano, że konkurencja pomiędzy pszenicą jarą i koniczyną perską trwała od fazy krzewienia pszenicy do końca wegetacji. Z największą siłą zaznaczyła się ona w fazie strzelania w źdźbło (rośliny konkurowały aż o 95% czynników wzrostu), a pod koniec wegetacji uległa niewielkiemu osłabieniu (dotyczyła 85% zasobów). Z większym nasileniem oddziaływały na siebie części nadziemne niż korzenie. Oddziaływanie szczególnie wyraźnie uwidoczniło się to w fazie kłoszenia, w której rośliny akumulowały w częściach nadziemnych tylko 8% zasobów, a w korzeniach aż 89%. Silniejszym konkurentem o czynniki wzrostu okazała się pszenica. Ponad 2-krotnie ograniczyła ona przyrost biomasy koniczyny od fazy krzewienia do końca wegetacji. W mieszance względna

szybkość wzrostu części nadziemnych koniczyny była większa niż pszenicy, a korzeni u obu gatunków podobna. Zagęszczenie roślin pozostało bez wyraźnego wpływu na intensywność oddziaływań roślin na siebie.

