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GAS EXCHANGE INTENSITY OF SPRING WHEAT AND UNDERSOWN PERSIAN CLOVER UNDER CONDITIONS OF DIVERSIFIED DENSITY OF PLANTS

Maria Wanic, Monika Myśliwiec, Magdalena Jastrzębska, Marta K. Kostrzewska, Krzysztof Orzech

Chair of Agroecosystems
University of Warmia and Mazury in Olsztyn

Key words: stomatal conductance, photosynthesis, transpiration, water use efficiency, undersown crop.

Abstract

The influence of competitive interactions between spring wheat and the undersown Persian clover, as well as the diversified density of plants, on the stomatal conductance and intensity of the processes of photosynthesis and transpiration in both species was evaluated during a pot experiment conducted between 2010 and 2012. The spring wheat and Persian clover cultivation methods – pure sowing, cultivation in a mixture of the species and the density of plants – higher (consistent with recommendations of agricultural technology) and lower (decreased by 20% of the recommended density) were the factors of the experiment. The gas exchange processes were analysed during 5 periods determined by the spring wheat development rhythm (leaf development, tillering, stem elongation, inflorescence emergence, ripeninig). Based on the quotient of the photosynthesis intensity and transpiration intensity, the water use efficiency (WUE) index was computed. It was shown that wheat cultivated with the undersown Persian clover was characterised by lower stomatal conductance, CO_2 assimilation and transpiration. Water use efficiency in the process of photosynthesis did not change under the influence of the sowing method almost throughout most of the experimental period. In the mixture, the Persian clover photosynthesis intensity was lower than in the pure sowing during the stages of the cereal tillering and ripening. In this sowing method, the lower stomatal conductance, transpiration and water use efficiency were recorded during the generative development of the cereal.

Address: Maria Wanic, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-718 Olsztyn, Poland, phone: +48(89) 523-48-85, e-mail: maria.wanic@uwm.edu.pl
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INTENSYWNOŚĆ WYMIANY GAZOWEJ PSZENICY JAREJ I WSIEWKI KONICZYNY PERSKIEJ W WARUNKACH ZRÓŻNICOWANEGO ZAGĘSZCZENIA ROŚLIN

Maria Wanic, Monika Myśliwiec, Magdalena Jastrzębska, Marta K. Kostrzewska, Krzysztof Orzech

Katedra Agroekosystemów
Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: przewodność szparkowa, fotosynteza, transpiracja, wskaźnik efektywności wykorzystania wody, wsiewka.

Abstrakt

W doświadczeniu wazonowym, zrealizowanym w latach 2010–2012, oceniano wpływ oddziaływań konkurencyjnych między pszenicą jarą i wsiewką koniczyny perskiej oraz zróżnicowanego zagęszczenia roślin na przewodność szparkową oraz intensywność procesów fotosyntezy i transpiracji u obu gatunków. Czynnikami doświadczenia były: sposób uprawy pszenicy jarej i koniczyny perskiej – siew czysty, uprawa we wzajemnej mieszance; zagęszczenie roślin: większe (zgodne z zaleceniami agrotechniki), mniejsze (zmniejszone w stosunku do poprzedniego o 20%). Procesy wymiany gazowej analizowano w pięciu okresach wyznaczonych przez rytm rozwojowy pszenicy jarej (wschody, krzewienie, strzelanie w źdźbło, kłoszenie, dojrzałość). Na podstawie ilorazu intensywności fotosyntezy i transpiracji obliczono współczynnik wykorzystania wody (WUE). Wykazano, że pszenica uprawiana z wsiewką koniczyny odznaczała się mniejszą przewodnością szparkową, asymilacją CO_2 oraz transpiracją. Efektywność wykorzystania wody w procesie fotosyntezy prawie w całym badanym okresie nie zmieniała się pod wpływem sposobu siewu. W mieszance intensywność fotosyntezy koniczyny perskiej była mniejsza niż w siewie czystym w fazie krzewienia i dojrzałości zboża. W tym sposobie siewu mniejszą przewodność szparkową, transpirację oraz efektywność wykorzystania wody odnotowano w okresie rozwoju generatywnego zboża.

Introduction

In caring for the balance of agricultural systems, increasing attention is paid to environmentally friendly farming methods. Increasing the diversity of crops by, among other things, application of undersown crops, plays an important role in those systems (JASKULSKA and GAŁĘZEWSKI 2009). The undersown crops vegetate in the field together with the main crop until its harvest and then continue their development in the autumn and are subsequently harvested for feed or (which has become the most frequent practice recently) they are ploughed down as so-called *green manure*. Undersown crops cultivation has a positive influence on the sanitary status of the field by limiting the occurrence of weeds and pathogens. It prevents water erosion, when reduce leaching of mineral compounds (mainly nitrates) to the deeper layers of the soil. It increases the content of humus and mineral components in the soil, stimulates biological life of the soil and supports its biological balance (HOLLAND 2004, BLACKSHAW 2005, KÄNKANEN and ERIKSSON 2007, JASKULSKA and GAŁĘZEWSKI 2009, GAUDIN et al. 2013). Several studies highlight, the positive influence of undersown crops on the physical characteristics of the soil (particularly its structure and humidity) (RAIMBAULT and VYN 1991, UNGER and MERLE 1998). The importance of the undersown crops in the main crop yield formation is smaller and unclear. The role of the undersown crop in that aspect differs depending on the main crop and the undersown crops species, level of agricultural technology applied, and weather and soil conditions, as well as the yield accomplished by those crops (MICHALSKA et al. 2008, TREDER et al. 2008, SOBKOWICZ and PODGÓRSKA-LESIAK 2009, PICARD et al. 2010, WANIC et al. 2013, WANIC et al. 2016a).

Spring wheat is classified as a very good plant for cultivation with undersown crops because of poor tillering, small height and no excessively abundant foliage (ZAJĄC 2007). In addition, clovers are considered valuable undersown crop plants mainly because of their well-developed root system, extensive above-ground biomass and symbiotic fixation of atmospheric nitrogen. The most extensive collection of studies available concerns the red and the white clover as well as their mixtures with grasses (KÄNKÄNEN and ERIKSSON 2007). Little information however can be found concerning the Persian clover although it is a plant, which under favourable habitat conditions (particularly humidity conditions), generates high yields of biomass abundant with nitrogen. Its suitability as the undersown crop was confirmed by PŁAZA et al. (2013).

In mixed crop stand, diversified interactions occur between the main crop and the undersown crop. In most cases, those interactions assume the form of competition for environmental resources (water, light, nutrients and space); however, that competition may also take place by means of various chemical compounds excreted into the environment. Such competition results in a reduction of the plants' population, change of the development rhythm, morphological characteristics and fertility (SHEAFFER et al. 2002, SOBKOWICZ 2003, SOBKOWICZ and PODGÓRSKA-LESIAK 2009). This results in obtaining yields different from those expected. The interactions between plants may also influence their physiological processes. The intensity and direction of competition depend on the choice of component species (and cultivars), their development period, density of plants and abundance of resources in the habitat (THORSTED et al. 2006, MICHALSKA et al. 2008, TREDER et al. 2008).

The literature offers little information on the influence of competition on CO_2 assimilation, transpiration and activity of the stomata of both species. Given the above, the research hypothesis was formulated assuming that competitive interactions would occur between the spring wheat and Persian clover and that they would influence the progress of the above-indicated processes while their intensity would depend on the development stage and density of the plants.

Evaluation of the influence of spring wheat cultivation with Persian clover and of the plant density on the stomatal conductance, CO_2 assimilation and transpiration during the entire period of common vegetation of both species was the aim of the studies.

Material and Methods

The studies were based on a pot experiment conducted in three series in the greenhouse laboratory of the University of Warmia and Mazury in Olsztyn. The experiments were conducted during the following periods: series I: from 12 April until 19 July 2010, series II – from 24 March until 30 June 2011, and series III – from 26 March until 28 June 2012. Spring wheat (cultivar Nawra) and Persian clover (cultivar Gobry) were cultivated in pure and mixture stands in two density variants: the recommended density and density decreased by 20% from the recommended values.

The factors of the experiment were:

I. cultivation method of spring wheat and Persian clover:
   – pure sowing,
   – mixed sowing,

II. plant density:
   – higher (according to the recommendations of agricultural technology) referred herein as the "recommended density",
   – lower (decreased as compared to the recommended by 20%).

The experiment was established according to the additive pattern whereby the number of plants in the mixture was the sum of their numbers in pure sowing. This pattern allowed the study of the competition between the spring wheat and Persian clover from the very beginning of the vegetation and levelled the influence of intraspecific competition on the development of that process (SEMERE and FROUD-WILIAMS 2001).

The experiment consisted of 120 pots (two species in pure and mixture x two sowing densities x 5 development stages x 4 replicates). Kick-Brauckmann type pots 22 cm in diameter and 25 cm deep were used for the experiment. The seeds were sown in the pots at equal distance from one another (thanks to patterns) and placed in the soil at the depth of: 3 cm (spring wheat) and 1 cm (Persian clover). In the pots with the recommended density, for both sowing methods, 19 grains of spring wheat and 12 grains of Persian clover were planted. In the lower density pots, those numbers were 15 and 9 respectively. This corresponded to the numbers 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 substrate composed of Eutric Cambisol (Humic),which had the following percentage of the fractions: 64% of grains less than 0.02 mm (clay), 12% of silt (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.

The mineral NPK fertilisation was applied once, one week before the sowing date. Water solutions of urea, monosodium phosphate and potassium sulphate were prepared. They were applied in the appropriate doses to the soil, mixed well and transferred to the pots. Identical fertilisation with phosphorus and potassium was applied to all the plants [g pot^{-1}]: P – 0.200 and K – 0.450. The dose of nitrogen was diversified depending on the species and sowing method and it was [g pot^{-1}]: for spring wheat in pure sowing – 0.500, for the mixture of spring wheat with Persian clover – 0.300 and for Persian clover as pure crop – 0.125.

During the plants' vegetation, the greenhouse temperature was maintained within 20–22°C. Only during the leaves formation stage it was decreased to 6–8°C to allow the wheat to undergo the process of vernalisation. Soil humidity during vegetation was maintained at a constant level of 60% of the maximum water capacity of the soil and the shortages were replenished daily as necessary. The vegetation of plants took place under conditions of natural illumination.

The gas exchange measurements were conducted during 5 periods determined by the development rhythm of spring wheat cultivated in pure crop in those objects with the recommended density of plants, that is the stages (BBCH) of: leaves development (12–14), tillering (21–23), stem elongation (31–32), inflorescence emergence (54–56) and ripening (87–89). Measurements on the spring wheat plants were started at the leaf development stage and concluded at the stage of inflorescence emergence. Measurement of Persian clover plants started at the tillering stage and during the inflorescence emergence stage of the spring wheat (an earlier time was impossible because of the leaves being too small for analysis) and ended at the stage of cereal ripening. The gas exchange was measured using the compact photosynthesis testing system Eijkelkampl LCi. Stomatal conductance, CO_2 assimilation and transpiration were measured on three random selected stems during each measurement period. The measurement was taken on the youngest fully developed leaf in ten replicates. The results obtained served computation of the water use efficiency – WUE index: assimilation/transpiration.

The experiment results are presented in the form of average values for three series of tests.

They were processed statistically by means of variance analysis at the significance level of $p = 0.05$. The Tukey's test (HSD) was used for evaluation of differences between objects. The computations were conducted using the *Statistica* computer software.

Results

The stomatal conductance in the leaves of wheat cultivated in the mixture with Persian clover was lower than in case of pure crop cultivation throughout the entire vegetation period (Table 1). The largest differences between the mixture and pure sowing were recorded during the stem elongation stage (more than 50%), and the smallest during the inflorescence emergence (almost 30%). No significant influence of plant density on the activity of stomata was found from the leaf development stage until the stem elongation stage. During the inflorescence emergence stage, higher conductance characterised leaves of wheat in objects with the recommended density (by 23.1%). During the leaf development stage, mixture limited conductance in the leaves of wheat cultivated at the recommended density more than in the case of cultivation at the lower density. During the inflorescence emergence, no significant differences between the mixture and the pure stand were found in the objects with the recommended density. On the other hand, significantly lower activity of stomata was recorded in case of mixture. During the other periods, the sowing method differentiated the studied characteristics in a similar way in objects of both densities.

Table 1

Stomatal conductance of spring wheat [mol H_2O m^{-2} s^{-1}]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)
A_1	B_1	0.29^a	0.27^a	0.18^a	0.34^a
	B_2	0.15^c	0.18^b	0.09^b	0.29^a
Average for A_1		0.22^a	0.23^a	0.14^a	0.32^a
A_2	B_1	0.33^a	0.32^a	0.17^b	0.20^a
	B_2	0.08^b	0.33^a	0.19^c	0.21^b
Average for A_2		0.27^a	0.25^a	0.14^a	0.26^b
Average for A	B_1	0.31^a	0.30^a	0.19^a	0.34^a
	B_2	0.18^b	0.18^b	0.09^b	0.24^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with Persian clover
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

Persian clover cultivated in the mixture with spring wheat was characterised by significantly lower stomatal conductance during the stages of wheat tillering and inflorescence emergence by 58.0 and 40.6% respectively (Table 2).

Table 2

Stomatal conductance of Persian clover [mol H_2O m^{-2} s^{-1}]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	rripening (87–89)
A_1	B_1	1.12^a	0.98^a	2.55^a	0.18^{bc}
	B_2	0.38^b	0.72^b	1.22^b	0.41^a
Average for A_1		0.75^a	0.85^b	1.89^a	0.30^a
A_2	B_1	0.87^a	1.17^a	2.13^a	0.12^c
	B_2	0.46^b	0.92^{ab}	1.56^b	0.23^b
Average for A_2		0.67^a	1.05^a	1.85^a	0.18^b
Average for A	B_1	1.00^a	1.08^a	2.34^a	0.15^b
	B_2	0.42^b	0.82^a	1.39^b	0.32^a

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with spring wheat
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

The opposite situation was recorded at the end of the vegetation period. Higher stomatal activity (by more than twofold) was recorded in the mixture than in the pure sowing. Sowing density had significant influence on the studied characteristic during the stem elongation and ripening stages. During the first of those periods, the leaves of clover growing at the lower density were characterised by higher activity than those of the clover cultivated at the higher density. During the latter period, the situation was the opposite. Plant density had similar influence on the magnitude of the differences between the sowing methods during the stages of tillering, inflorescence emergence and ripening. During the stem elongation period, the stomata of plants in pure sowing were characterised by significantly higher activity than in the plants in mixture solely for those objects with the recommended density of plants.

Addition of Persian clover limited CO_2 assimilation by spring wheat from the leaf development stage until the inflorescence emergence stage (Table 3).

The negative influence of clover on carbon dioxide assimilation was the most pronounced during the leaf development and stem elongation stages. In the mixture, it was lower than in pure sowing by 36.8 and 27.4% respectively. That addition had the smallest limiting influence on the process of photosynthesis by wheat during the inflorescence emergence stage (the difference between the pure and the mixture was 6.6%). Lower density of plants influenced that process negatively during the leaf development stage (by 25.5%) and tillering (by 14.0%). The influence was positive during the stem elongation stage (by 17.9%) and the inflorescence emergence stage (by 6.6%). Poorer CO_2 assimilation by the wheat plants in the mixture than in the pure crop was found in both objects with different densities of plants.

Persian clover in the mixture assimilated significantly less CO_2 than in the pure sowing during the tillering stage (by 21.9%) and the ripening stage (by 41.2%) – Table 4.

Table 3

Photosynthetic rate of spring wheat [µm CO_2 m^{-2} s^{-1}]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)
A_1	B_1	6.72^a	3.94^a	3.92^{ab}	3.81^{ab}
	B_2	4.73^b	3.07^b	2.78^c	3.50^b
Average for A_1		5.73^a	3.51^a	3.35^b	3.66^b
A_2	B_1	5.52^b	3.25^b	4.53^a	4.00^a
	B_2	3.01^c	2.78^c	3.36^c	3.79^b
Average for A_2		4.27^b	3.02^b	3.95^a	3.90^a
Average for A	B_1	6.12^a	3.60^a	4.23^a	3.91^a
	B_2	3.87^b	2.93^b	3.07^b	3.65^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with Persian clover
a, b, c – value marked with the same letter do not differ significantly ($p \le 0.05$)

Table 4

Photosynthetic rate of Persian clover [µm CO_2 m^{-2} s^{-1}]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	rripening (87–89)
A_1	B_1	5.69^b	6.25^a	6.99^a	6.70^a
	B_2	4.44^c	5.61^a	6.16^{ab}	3.39^b
Average for A_1		5.07^b	5.93^a	6.58^a	5.05^a
A_2	B_1	7.80^a	6.66^a	5.66^b	4.42^b
	B_2	6.09^b	5.56^a	5.39^b	3.14^b
Average for A_2		6.95^a	6.11^a	5.53^b	3.78^b
Average for A	B_1	6.75^a	6.46^a	6.33^a	5.56^a
	B_2	5.27^b	5.59^a	5.78^a	3.27^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with spring wheat
a, b, c – value marked with the same letter do not differ significantly ($p \le 0.05$)

During the stages of stem elongation and inflorescence emergence, the differences between the sowing methods were smaller and assumed the character of a trend. In those objects with lower plant density, photosynthesis progressed more intensively than in the objects with the higher plant density during the tillering stage (by 37.1%). During the stem elongation stage, photosynthesis was similar at both objects (no significant differences). Then during the stages of inflorescence emergence and ripening of the wheat, it showed lower values (by 16.0 and 25.1% respectively). The negative influence of wheat on assimilation was visible in both levels of plant density during the tillering stage, while during the ripening stage it was visible in the objects with the recommended density.

During the leaf development stage, taking the average for plant density, the sowing method did not significantly diversify the transpiration from the spring wheat leaves (Table 5). During the further development stages, that cereal transpired less water with the presence of the undersown crop than in pure sowing. The highest influence of the undersown crop on the development of that process was visible during the stem elongation stage (when the difference between the mixture and pure sowing was 46.1%). No clear influence of plant density on water transpiration from wheat plants was established. During the leaf development stage, it was similar in objects of both densities. During tillering and stem elongation, transpiration was more intensive amongst the objects with lower density (by 25.8 and 46.3% respectively). During the inflorescence emergence, the situation was the opposite and higher transpiration was recorded for those objects with the recommended density (by 25.0%). The interaction between the sowing method and plant density showed that

Table 5

Transpiration rate of spring wheat (mmol H_2O m^{-2} s^{-1})

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)
A_1	B_1	2.91^c	1.79^{ab}	2.13^b	2.98^b
	B_2	3.65^{ab}	1.47^b	1.06^c	2.52^a
Average for A_1		3.28^a	1.63^b	1.60^b	2.75^a
A_2	B_1	4.10^a	2.18^a	2.99^a	2.25^{bc}
	B_2	3.10^{bc}	1.92^a	1.69^b	2.14^c
Average for A_2		3.60^a	2.05^a	2.34^a	2.20^b
Average for A	B_1	3.51^a	1.99^a	2.56^a	2.62^a
	B_2	3.38^a	1.70^b	1.38^b	2.33^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with Persian clover
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

during the leaf development stage, plants with the recommended density and mixture transpired more water than in case of pure sowing. In the case of the lower density, the situation was the opposite and they transpired less water. During tillering and stem elongation, the sowing method at both densities changed that characteristic in a similar way and during the inflorescence emergence stage, larger differences between the mixture and the pure sowing occurred with the objects with the recommended plant density.

Transpiration from the Persian clover leaves during the stages of wheat tillering, inflorescence emergence and ripening was more intensive in the pure sowing than in the mixture by 26.3, 34.1 and 65.3% respectively (Table 6). During the tillering stage, this process was 11.7% greater in the objects with the lower than recommended plant density. During stem elongation, the process developed similarly with both density objects. During the period of generative development of the cereal, more water transpired plants in pots with the recommended density (during the inflorescence emergence stage by 15.7% and during the ripening stage by 50.3%). Interaction of the experimental factors showed that during the tillering stage, mixture limited transpiration in a similar way in objects of both densities. During the stem elongation stage, mixture did not diversify its intensity depending on the experimental factors, while during the inflorescence emergence and ripening stages it limited water transpiration from the plants in objects with lower density more than pure sowing.

Table 6

Transpiration rate of Persian clover [mmol H_2O m^{-2} s^{-1}]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	rripening (87–89)
A_1	B_1	7.20^a	6.29^a	7.81^a	6.24^a
	B_2	6.11^b	6.26^a	6.48^b	4.88^a
Average for A_1		6.66^b	6.28^a	7.15^a	5.56^a
A_2	B_1	8.53^a	6.54^a	7.45^a	5.30^a
	B_2	6.35^b	5.86^a	4.90^c	2.10^c
Average for A_2		7.44^a	6.20^a	6.18^b	3.70^b
Average for A	B_1	7.87^a	6.42^a	7.63^a	5.77^a
	B_2	6.23^b	6.06^a	5.69^b	3.49^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with spring wheat
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

The index values of water use efficiency for the spring wheat photosynthesis process provide information that during the leaf development stage it managed water better in case of pure sowing than in mixture (by 60.5%) and during the stem elongation – worse (by 35.2%) – Table 7. During the tillering and inflorescence emergence stages, no significant differences between the sowing methods were found. Wheat managed water more economically in the objects with the recommended density than those with lower density from the leaf development stage until the stem elongation stage. The role of sowing density in development of water usage economy during the analysed period decreased systematically, however, with the passage of time. The result was that during the inflorescence emergence stage the wheat managed water in a more economical way in the objects with the lower density. During the entire period studied, the sowing method differentiated the values of water usage in a similar way in objects of both plant densities.

Table 7

Water use efficiency (WUE) of spring wheat [μm CO_2 mmol H_2O]

Plant density (*A*)	Sowing method (*B*)	Growth stages of spring wheat (BBCH)			
		leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)
A_1	B_1	2.31^a	2.20^a	1.84^b	1.28^c
	B_2	1.30^c	2.09^a	2.62^a	1.39^c
Average for A_1		1.81^a	2.15^a	2.23^a	1.34^b
A_2	B_1	1.35^c	1.49^c	1.52^b	1.78^b
	B_2	0.97^d	1.45^c	1.99^a	1.77^b
Average for A_2		1.16^b	1.47^b	1.76^b	1.78^a
Average for *A*	B_1	1.83^a	1.85^a	1.68^b	1.53^{ab}
	B_2	1.14^b	1.77^a	2.31^a	1.58^a

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with Persian clover
a, b, c – value marked with the same letter do not differ significantly ($p \le 0.05$)

In case of the Persian clover, the sowing method's influence on the water use efficiency in photosynthesis was manifested at the inflorescence emergence stage (Table 8). The clover managed water better in the mixture than in the pure sowing (by 24.1%). During the tillering stage, clover used water more effectively in the objects with the lower density. During the other periods studied, the WUE index experienced no significant changes influenced by the density of plants. Interaction of the experimental factors showed that during the inflorescence emergence stage, Persian clover used water in more economical way in the mixture than in the pure stand at the object with lower plants density. During the ripening stage, water use in the photosynthesis process was more effective in the object with the recommended plant density in pure sowing than in the object with lower density in the mixture.

Table 8

Water use efficiency (WUE) of Persian clover (μm CO_2 mmol H_2O)

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	rripening (87–89)
A_1	B_1 B_2	0.79^b 0.73^b	0.99^a 0.90^a	0.90^a 0.95^a	1.07^b 0.69^c
Average for A_1		0.76^b	0.95^a	0.93^a	0.88^a
A_2	B_1 B_2	0.91^a 0.96^a	1.02^a 0.95^a	0.76^c 1.10^a	0.83^{bc} 1.50^a
Average for A_2		0.94^a	0.99^a	0.93^a	1.17^a
Average for A	B_1 B_2	0.85^a 0.85^a	1.01^a 0.93^a	0.83^b 1.03^a	0.96^a 1.10^a

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with spring wheat
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

Discussion

The response of plants to common cultivation was manifested through a change in their development rhythm, morphological characteristics, productivity and yield. This was the consequence of changes in the physiology of plants (YIN et al. 2009), including stomatal conductance, photosynthesis and transpiration. In the analysed experiment, strong competition between spring wheat and Persian clover in the mixture was documented (WANIC et al. 2016 b). Competition for the limited growth factors may decrease the intensity of photosynthesis, which was confirmed by the studies by JASTRZĘBSKA et al. (2015) as well as by our own studies. The studies showed that CO_2 assimilation by spring wheat and the undersown of Persian clover in mixture was lower than in the case of the pure sowing. In the case of wheat, the negative influence of the undersown on the process was most clearly visible during the leaf development and stem elongation stages. In Persian clover, the influence was the most pronounced during the stages of the cereal tillering and ripening. During the leaf development stage, poorer CO_2 assimilation by the wheat did not result from development of leaves with smaller area (it was even larger than in the case of pure sowing) and darkening by the partner in the mixture (the plants were small) but from other influences that disrupted that process (maybe allelopathy) (HARKOT and LIPIŃSKA 1995). This is also confirmed by lower activity of the wheat stomata during that period. Starting with the tillering stage, the wheat in the mixture with clover absorbed less nitrogen than in the pure sowing, which influenced the photosynthesis process negatively because the photosynthetic capacity of the leaves is correlated closely with their nitrogen (EVERS et al. 2010). OLSZEWSKA (2008) obtained different results. She recorded positive influence of white clover on the development of photosynthesis in *Festulolium braunii*, resulting from the absorption of atmospheric nitrogen by the clover and making a part of it available to the grass. Moreover, in our own studies, in the presence of the clover undersown the wheat developed the leaves with a smaller surface area and lower stomata activity (particularly during the stem elongation stage). ZHOU and PENG (2012) also reported restriction of the photosynthesis resulting from limitation of the stomatal conductance. Lower assimilation of CO_2 by the wheat did not result, however, from limiting light access to the leaves of the cereal because during the entire vegetation period its plants were higher than the clover plants. Persian clover in the mixture was subject to strong wheat pressure from the very beginning of vegetation. According to NIELS et al. (2001), in the mixture the dominant species uses the light more effectively in photosynthesis than the subordinated one but only under conditions of good nitrogen supplies. Shortage of nitrogen in the leaves (see our earlier work – WANIC et al. 2016b) decreased photosynthesis. Similar conclusions can be found in the work by ŻUK-GOŁASZEWSKA (2008). In the analysed experiment, absorption of nitrogen by both species was lower in the pure sowing, which meant that the effective use of sunlight by both species was relatively lower. Smothered by the cereal, which was taller, of greater mass and better equipped with the leaves, the clover's assimilation apparatus were less developed than in the case of pure sowing. Already during the tillering stage, the mass of its stems was lower by more than a half than in pure sowing (WANIC and MYŚLIWIEC 2014). In addition, LÜSCHER et al. (2001) showed that competition reduced the mass of stems and carbon content in them significantly. During the entire period of common cultivation, the clover was effectively shaded by the cereal, which limited its access to the sun. This, in combination with lower absorption of nitrogen than in pure sowing and limitation of stomatal activity during certain periods, disrupted photosynthesis.

EVERS et al. (2010) also link the decrease in the photosynthesis rate with increased shading by the leaves of the neighbouring plant, the decrease of nitrogen content and the resulting lower photosynthetic capacity in the leaves. In a situation where the plants compete for light, reduction in the surface area of their leaves decreases light absorption and simultaneously increases light absorption by the neighbour (HIKOSAKA et al. 2012). This is confirmed by our own studies as well as LÜSCHER et al. (2001), who showed reduction of photosynthesis in clover leaves in the lower parts of the stand where the light availability was less than for ryegrass leaves due to the clover being shaded by the grass.

Competition limited the volume and quality of the light penetrating into the stand and consequently worsened the functioning of the clover leaves (LÜSCHER et al. 2001). Their mass was lower by more than 70% from that in pure sowing (WANIC and MYŚLIWIEC 2014). According to LÜSCHER et al. (2001) the response of clover to shading in the dense stands manifests through elongation of stems and leaf petioles. This growth utilises a large proportion of assimilates at the expense of the leaves. This causes slowdown in the clover growth rate resulting from a decrease in the surface area of the leaves and consequently lower photosynthesis intensity. This was not confirmed by TREDER et al. (2016), who recorded reduction in the height of red clover plants in a stand of spring barley. In the analysed experiment, during the cereal ripening stage, the decrease in CO_2 assimilation by the clover resulted less from shading by the wheat and more from the shortage of biogens, particularly the poor binding of nitrogen.

In our own studies, wheat cultivated in the mixture transpired less water than in the case of pure sowing from the tillering stage until the end of the inflorescence emergence stage, while for the Persian clover this lasted almost throughout the entire vegetation period. JASTRZĘBSKA et al. (2015) also recorded lower water transpiration from spring barley leaves during the stages of stem elongation and inflorescence emergence. OLSZEWSKA (2008) presented different results. She showed that grasses in the mixture with white clover transpired more water than in pure sowing. In the analysed experiment, poorer water evaporation from plants in the mixture was associated with decreased stomatal activity in that object. The stomata allow plants regulation of water circulation and CO_2 assimilation as well as adjustment of those processes to environmental changes (YAN et al. 2012, CORDOBA et al. 2015). They react to changes in environmental conditions by altering their activity and adjusting assimilation and transpiration accordingly. In the analysed experiment, plants in the mixture growing at twice the higher density than in the pure sowing had less growth factors available to them (consequently they were under stress conditions). The reaction to this situation was a decrease in the surface and density of stomata and their activity (VARONE et al. 2012). BLAIKE et al. (1988) report that white clover, in response to the stress resulting from water shortage, reacted by closing the stomata, thus limiting transpiration on the one hand and CO_2 assimilation on the other. Lower water transpiration from the Persian clover leaves also resulted from shading by wheat that was higher and had better foliage as well as a formation of the leaves with much smaller mass and surface than in case of the pure sowing (MYŚLIWIEC et al. 2014). Decreased transpiration, however, may be favourable to the plant because it improves its water balance (RABHI et al. 2012).

The WUE expressed by the ratio of assimilation to transpiration is an important indicator, which informs about adjustment of the plants to stressful situations in the environment. During drought, the plants generally close the stomata, which prevents water loss, decreases photosynthesis and leads to a general increase in water use efficiency (XING and WU 2012). Thus, this indicator tells us whether under changing environmental conditions the plant leaves optimise the CO_2 assimilation rate relative to the water loss (SWARTHOUT et al. 2009). HAFID et al (1997) highlighted strong positive correlation of the WUE index with CO_2 assimilation, transpiration and osmotic regulation. In own studies, the WUE index reached higher values in the wheat than in the clover, which indicates more effective water management by that cereal (LUCERO et al. 2000, JASTRZĘBSKA et al. 2015). It showed that in the mixture, the wheat and the clover managed water in a similar way as in the pure sowing throughout almost the entire vegetation period. Lower stomatal conductance limited to a similar degree the CO_2 assimilation and transpiration, which did not change the water balance of the plant. LUCERO et al. (2000) also did not find any influence on the WUE by mutual interactions of white clover and Italian ryegrass. According to FARQUHAR et al. (1989), during a slow build-up of stress (in the case of those authors, the water stress) photosynthesis and transpiration decrease at a similar pace and hence, the WUE is subject to no evident changes.

In own studies, the influence of plant density on gas exchange differed depending on the species and development stage. Generally, in case of the wheat, the decrease in the CO_2 assimilation was observed in those objects with the higher density during the stages of stem elongation and inflorescence emergence. The decrease in transpiration was observed during the stages of tillering and stem elongation. Higher evaporation of water from that species was found, however, during the tillering stage in the objects with the higher plant density. In the clover, higher density influenced the assimilation increase during the stages of generative development of the cereal while the rate of transpiration showed no recordable related with the density of plants. Dense sowing may lead to shading of plants as the photosynthetic system shows high sensitivity to changing characteristics of the environment, particularly the light intensity (BRESTIC and OLSOVSKA 2001, PAYNTER et al. 2001). On the other hand, excessively sparse sowing may increase ventilation of the standing crop and thus increase transpiration. THORSTED et al. (2006), based on the studies concerning winter wheat and white clover, claim that in a stand of lower density the competition between species (mainly for the light) is weakened, which has positive influence on the assimilation process. In the studies by GALON et al. (2013), it was shown that with the increasing competition (resulting from an increase in plant density) from *Brachiria* *brizantha,* reduction in assimilation and stomatal conductance in sugar cane leaves was recorded. This matches our own studies. The referenced authors, however, obtained different results in the case of transpiration.

Conclusions

1. The stomatal conductance of spring wheat in the mixture with Persian clover was lower than in the pure sowing throughout the entire vegetation period. The Persian clover was characterised by lower stomatal activity in the mixture during wheat tillering and inflorescence emergence and higher activity during wheat ripening stage.
2. The higher plant density increased spring wheat stomatal conductance during its inflorence emergence only. In case of the Persian clover no clear influence of the plant density on that characteristic was recorded.
3. The spring wheat in the presence of the Persian clover undersown assimilated less CO_2 than in pure sowing. The CO_2 assimilation by the Persian clover in the mixture with wheat progressed less efficiently than in the pure sowing during the stages of tillering and ripening of that cereal.
4. The intensity of spring wheat photosynthesis in the objects with lower density was higher during the stages of stem elongation and inflorescence emergence. During the stages of leaf development and tillering, it was lower. In case of the Persian clover, the process progressed more efficiently under conditions of higher plant density during the stages of inflorescence emergence and ripening of the cereal.
5. In the mixture, the spring wheat transpired less water than in pure sowing from the stage of tillering until inflorescence emergence. The Persian clover transpired less water during the stages of the cereal tillering, inflorescence emergence and ripening.
6. In the objects with the lower plant density, spring wheat transpired more water during the stages of tillering and stem elongation and less water during the inflorescence emergence stage. Transpiration from the leaves of Persian clover was more intensive in the objects with the recommended density during the stages of inflorescence emergence and ripening of wheat.
7. Wheat managed water more effectively in pure sowing than in the mixture during the leaf development stage while Persian clover was more effective in mixture during the inflorescence emergence stage. Until the stem elongation stage, wheat used water better in the objects with the recommended plant density and worse during the inflorescence emergence stage. Persian clover used water more effectively in the object with the density

lower than recommended during the tillering stage only. During the remaining vegetation period the density of plants had no influence on the examined characteristic.

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