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EFFECT OF COMPETITIVE INTERACTIONS AND WATER STRESS ON THE MORPHOLOGICAL CHARACTERISTICS OF RED CLOVER (*Trifolium pratense* L.) CULTIVATED WITH SPRING BARLEY (*Hordeum vulgare* L.)

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Abstract. On the basis of a pot experiment set up according to the additive design, the effect of competitive actions of spring barley on the morphological characteristics of red clover in the conditions of water stress was evaluated. Biometric analyses were carried out on five dates set by the growth rhythm of barley in pure sowing with a higher water dose. The study included: plant height, number of shoots and leaves per plant, and root length. On the basis of plant biomass, relative yield, relative yield total, and competitive balance index were determined. It was demonstrated that spring barley, from tillering to the end of growth, limited red clover plant height and root length. At the straw-shooting stage, negative effect of the cereal intensified and caused the formation of a smaller number of leaves and shoots than in clover in pure sowing. Water shortage caused plant shortening (from barley tillering to the end of growth) and reduction in the number of leaves (tillering – earing) and shoots (straw-shooting and ripening) of clover.

Key words: competitive balance index, developmental stages, plant interactions, relative yield, relative yield total, water deficiency

INTRODUCTION

High participation of cereals in the sowing structure (71.8% in 2014, Central Statistical Office of Poland data 2015) makes farmers search for solutions that limit the negative effect of cereal plant concentration in crop rotation. One of the possible solutions is the application of regenerative crop in the form of catch crop, including intercrop. It increases the biomass of post-harvest residue, which is a source of organic

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matter in the soil [Svenson *et al*. 1994, Thomsen and Christensen 2004], improve soil structure, as well as its physiochemical properties and biological activity [Thorup-Kristensen *et al.* 2003, Wojciechowski 2009], limit soil erosion, infestation, and the occurrence of disease and pests [Kolhoff and Simon 1985, Jensen 1991, Andrzejewska and Ignaczak 1996, Kuś and Jończyk 2000, Wanic *et al.* 2005, and Teasdale *et al.* 2007]. Even short-term introduction of another species into the growth results in the increase in biological diversity, as well as increases the complexity of agroecosystem, and potential interactions that occur there become more difficult to predict. Interactions between plants, including competition, are of great significance in the formation of plant community [Begon *et al.* 1990]. Intercrop may compete with cultivated plants for the limited environmental supplies, mainly water and nutrients, which may contribute to a decrease in yield [Wanic *et al.* 2006, Sobkowicz and Lejman 2011]. Water is one of the major factors that determine plant productivity, and therefore it ought to be expected that its shortage would increase competition intensity. However, some reports claim that in the conditions of strong abiotic stress (including water stress), competitive interactions lose strength, but they increase in the habitats rich in environmental growth factors [Grime 1977, Briones *et al.* 1998]. Therefore, there is a need to continue the research leading to the recognition of interactions that occur in the lowland meadow of plants grown in different environmental conditions.

Taking the above premises into account, research was initiated, the aim of which was to determine how joint cultivation with spring barley at diversified bedding humidity affects the formation of the morphological characteristics of red clover. Study hypothesis assumed that competitive interactions on the part of spring barley would limit the growth and development of red clover during cultivation.

MATERIAL AND METHODS

The basis of the research was a pot experiment carried out at the greenhouse laboratory at the Biology and Biotechnology Department of the University of Warmia and Mazury in Olsztyn in three cycles matching the successive growth periods (three years) of the length of, respectively, 102, 97, and 98 days (which fell in the months of April – July). The subject of the evaluation was red clover (cultivar Bona). The experimental factors were as follows:

1 – red clover sowing method: single-species (P) and mixed (growth with barley cultivar Rabel – M),
2 – bedding irrigation level: higher level, which met the plants' needs – H and lower level– dose lowered by 50% – L.

Bedding was composed of brown leached soil, formed from heavy clay of slightly acidic pH (pH in 1 M KCl from 5.6 to 6.2), in which organic matter amounted to 1.84%-2.52%. Soil was characterized by high richness in phosphorus (9.24-11.61 $mg \cdot 100\ g^{-1}$ soil) and magnesium (8.80-9.11 mg), and average in potassium (12.87-14.53 mg). Before filling the pots (in the amount of 8 kg/pot), the bedding was enriched with mineral fertilizers at the dose of pure component (g/pot): P – 0.2 g (monopotassium phosphate), K – 0.45 g (potassium sulphate) and N – 0.3 g (urea) – under the mixture and N – 0.125 g under clover.

The experiment was set up according to the additive design [Semere and Froud-Williams 2001, Sobkowicz 2001], in which the number of plants in the mixture is their sum in pure sowing. In Kick-Brauckmann pots with the diameter of 22 cm and depth of 28 cm, eight clover seeds in pure sowing and eight clover seeds with 18 spring barley seeds in mixed sowing were sown. The established density was maintained until the end of the experiment. At the beginning, the experiment was made of 80 pots in total (four factor combinations × five developmental stages × four repetitions), in which the part designed for measurements at a given stage was discarded.

Plants were supplied with water with the application of two levels of soil irrigation: a higher dose and a dose lower by 50%. Higher dose was set basing on a trial experiment, in which measurements of soil humidity, evaporation, transpiration and water content in plants were taken. The supplied daily amounts of water were diversified depending on the degree of plant development during growth. In the successive cycles, higher dose amounted to, in total, 13400, 12300, and 10900 cm^3 of water per pot.

Competitive actions of barley in relation to clover were studied on five dates, set by the developmental rhythm of the cereal plant grown in pure sowing with a higher water dose, that is at the stages of (according to the BBCH scale): leaf development (10-13), tillering (22-25), straw-shooting (33-37), earing (52-55), and ripening (87-91). All the plants uptaken from the pots dedicated to the given stage were analyzed by evaluating: plant height (of the main shoot), shoot number per plant, leaf number per plant, and main root length. Then, the above- and below-ground plant parts were dried to air-dry mass and weighed. On the basis of the thus obtained data, the following parameters were calculated:

– relative yields (RY_B and RY_C):

$$RY_B = Y_{MB}/Y_{PB}$$
$$RY_C = Y_{MC}/Y_{PC}$$

– relative yield total (RYT):

$$RYT = RY_B + RY_C$$

– competitive balance index (CB):

$$CB = \ln [(Y_{MB}/Y_{MC})/(Y_{PB}/Y_{PC})]$$

where:

RY_B – spring barley relative yield,
RY_C – red clover relative yield,
Y_{PB} – spring barley yield in pure sowing,
Y_{PC} – red clover yield in pure sowing,
Y_{MB} – spring barley yield in a mixture with red clover,
Y_{MC} – red clover yield in a mixture with spring barley,
ln – natural logarithm.

In the additive design, RY<1 means competition, RY>1 positive interactions, and RY=1 no interactions [Austin *et al.* 1988]. In this design, RYT>1 may signify partial complementariness in resource winning by mixture components, positive interactions between the species, or incomplete resource winning by the species in pure sowing [Sackville Hamilton 1994]. The last phenomenon occurs always at the early developmental stages of plant in the additive design. Before competition or other interaction of the emerging plants occur, RYT in the additive design equals two because yield of every species is the same in a mixture and in pure sowing. Therefore, RYT in the additive design is also indirectly a measurer of interspecies competition intensity during plant growth. Competitive balance index demonstrates, on the other hand, which species is more competitive. When CB = 0, the species are characterized by the same competitiveness. In the experiment, CB is calculated as barley competitiveness in relation to clover.

The obtained results were statistically processed with the use of the analysis of variance and the Duncan's test for the evaluation of inter-pot differences. Error probability of P = 0.05 was assumed. In the tables, mean values from three study cycles are presented.

RESULTS

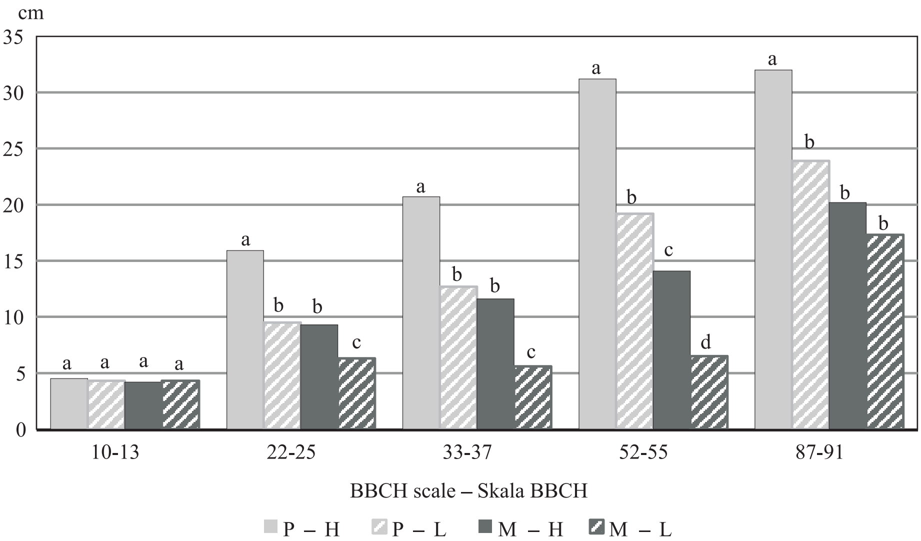
In the initial growth period, at the stage of spring barley leaf development, the sowing method and level of plant supply in water did not affect significantly red clover plant height (Table 1).

Table 1. Biometrical features of red clover
Tabela 1. Cechy biometryczne koniczyny czerwonej

Features Cechy	Factors Czynniki	Factor level* Poziom czynnika*	Development stages of spring barley Faza rozwojowa jęczmienia jarego (BBCH)				
			10-13	22-25	33-37	52-55	87-91
Plant height Wysokość roślin, cm	sowing type rodzaj siewu	P	4.4 a	12.7 a	16.7 a	25.2 a	28.0 a
		M	4.3 a	7.8 b	8.6 b	10.3 b	18.8 b
	irrigation level poziom nawadniania	H	4.4 a	12.6 a	16.2 a	22.7 a	26.1 a
		L	4.3 a	7.9 b	9.2 b	12.9 b	20.6 b
Shoot number per plant, no Liczba pędów na roślinie, szt.	sowing type rodzaj siewu	P	1.0 a	1.1 a	1.5 a	3.1 a	3.2 a
		M	1.0 a	1.0 a	1.0 b	1.1 b	1.1 b
	irrigation level poziom nawadniania	H	1.0 a	1.1 a	1.5 a	2.3 a	2.5 a
		L	1.0 a	1.0 a	1.0 b	1.9 a	1.8 b
Leaf number per plant, no Liczba liści na roślinie, szt.	sowing type rodzaj siewu	P	2.2 a	4.0 a	6.4 a	15.4 a	20.6 a
		M	2.1 a	2.7 a	3.1 b	4.3 b	6.1 b
	irrigation level poziom nawadniania	H	2.2 a	3.8 a	5.8 a	11.1 a	15.9 a
		L	2.1 a	3.0 b	3.8 b	8.6 b	10.7 a
Main root length Długość korzenia głównego, cm	sowing type rodzaj siewu	P	4.4 a	15.6 a	18.5 a	22.9 a	24.0 a
		M	4.2 a	9.9 b	11.7 b	12.5 b	16.8 b
	irrigation level poziom nawadniania	H	4.3 a	14.5 a	16.2 a	19.0 a	21.0 a
		L	4.3 a	11.0 a	14.0 a	16.5 a	19.8 a

* P – pure sowing – siew czysty, M – mixed sowing – siew mieszany, H – higher water dose – wyższa dawka wody, L – lower water dose – niższa dawka wody
a, b – homogenous groups, mean values marked with the same letters do not differ significantly at P = 0.05
a, b – grupy jednorodne, średnie wartości oznaczone tą samą literą nie różnią się istotnie na poziomie P = 0.05

From barley tillering to the end of growth, clover plants that grew in its vicinity were lower than in pure sowing. Water deficiency was also the cause of limited growth of clover shoots in that period. In relation to the pots with optimum bedding humidity, the differences oscillated between 4.7 (during tillering) and 9.8 cm (during earing). More favourable conditions for clover growth were noted in pure sowing with a higher water dose. At tillering and straw-shooting of the cereal, stress caused by competition on its part had the same effect on clover plant height as water deficiency (Fig. 1). Accumulation of two stress factors limited plant height even more. At earing, negative effect of competition was stronger than that of water shortage by itself, and joint action of both stress factors was felt by plants even more severely. During spring barley ripening, the height of red clover plants that competed with cereal and were subject to water deficiency was similar, and the effect of two factors at the same time was statistically the same as for each of them separately.



for explanation see Table 1 – objaśnienia pod tabelą 1

Fig. 1. Red clover height
Rys. 1. Wysokość koniczyny czerwonej

Mixed sowing resulted in the creation of a significantly lower shoot number by red clover from the straw-shooting stage to barley ripening (Table 1). Worse plant supply in water limited significantly their branching at straw-shooting and barley ripening.

At the straw-shooting stage, water stress limited the number of clover shoots to the level noted in mixed sowing, while during earing only the sowing method reduced the number of shoots (Fig. 2). Also towards the end of growth, a significant increase in the interaction of the study factors on this characteristic was noted. The number of clover shoots in pure sowing with a lower water dose was significantly lower (by 1.4) in comparison with the pots with the same sowing method and a higher dose, whereas the next homogenous group was made of plants growing in the presence of competition at both irrigation levels.

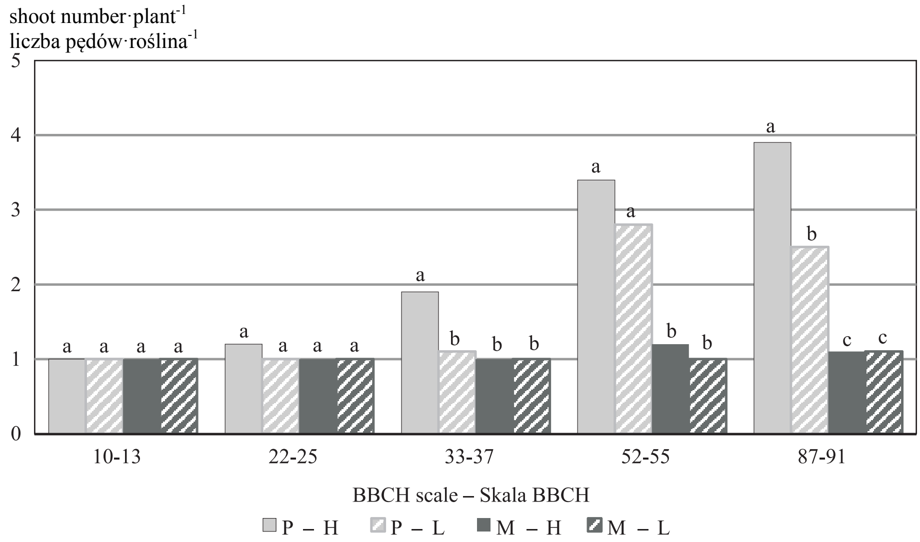


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Fig. 2. Shoot number in red clover
Rys. 2. Liczba pędów koniczyny czerwonej

Vicinity of cereal in the initial growth period (leaf development – tillering) did not have a significant effect on the number of red clover leaves (Table 1). The situation changed from the straw-shooting stage of barley, when its presence caused a decrease in the number of assimilation clover organs in relation to pure sowing. This relation lasted until the end of growth, although the differences deepened in time (from 3.3 to 14.5 fewer leaves from the plant). Limited access to water supplies resulted in a statistically significant reduction in the number of leaves formed by the legume from tillering to earing of barley in comparison with the pots irrigated more abundantly. Factor interaction in the initial growth period did not demonstrate a significant effect on the analyzed characteristic (Fig. 3). It showed during barley tillering and marked itself the most strongly at the straw-shooting stage. In pure sowing, lower level of bedding humidity resulted in the formation by clover of a significantly lower number of leaves in relation to plants watered more abundantly. However, no significant effect of water doses on the values of the discussed characteristic was noted in plants sown in a mixture, which generally formed fewer leaves than the plants in pure sowing. At the earing stage, the number of plant leaves in pure sowing was higher by 11.1 than in mixed sowing, and a lowered water dose resulted in their reduction by 2.5 leaves from the plant in relation to a higher dose. During ripening, the effect of cereal on the number of leaves deepened; clover in pure sowing formed 14.5 more of them than in a mixture. On the other hand, plant supply in water at that time had no effect on the discussed characteristic. Factor interaction demonstrated a significant negative effect of the cereal on the number of assimilation organs, in particular in the conditions of lower bedding humidity.

Joint growth with barley resulted in a significant shortening of red clover roots in comparison with single-species pots almost throughout the entire growth year (except leaf development, Table 1). However, no significant effect of the applied water doses on the length of the legume root system was noted. Interaction of factors demonstrated that in the period from tillering to straw-shooting, clover roots in pure sowing with a higher water dose were significantly longer than in the pots with barley and water deficiency (Fig. 4). At the successive stages, overlapping of the experimental factors confirmed a significant negative effect of the cereal on the main root length of the legume in relation to single-species pots regardless of the water dose.

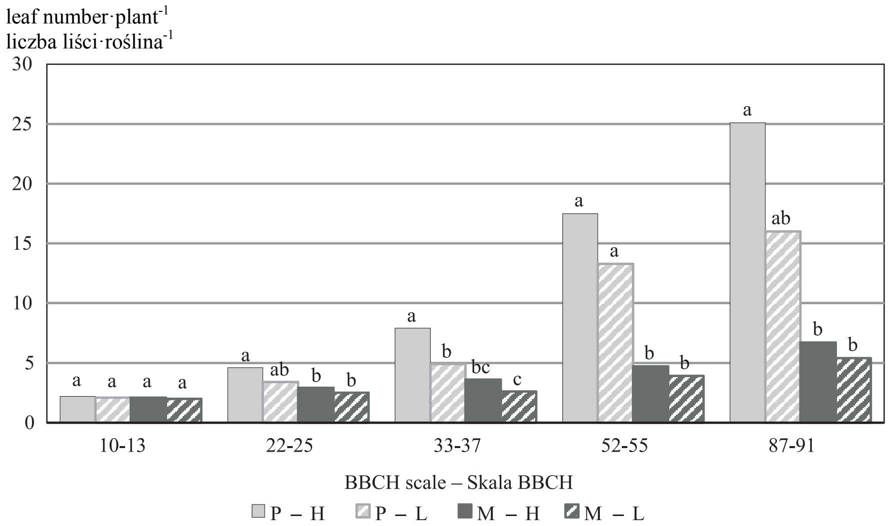

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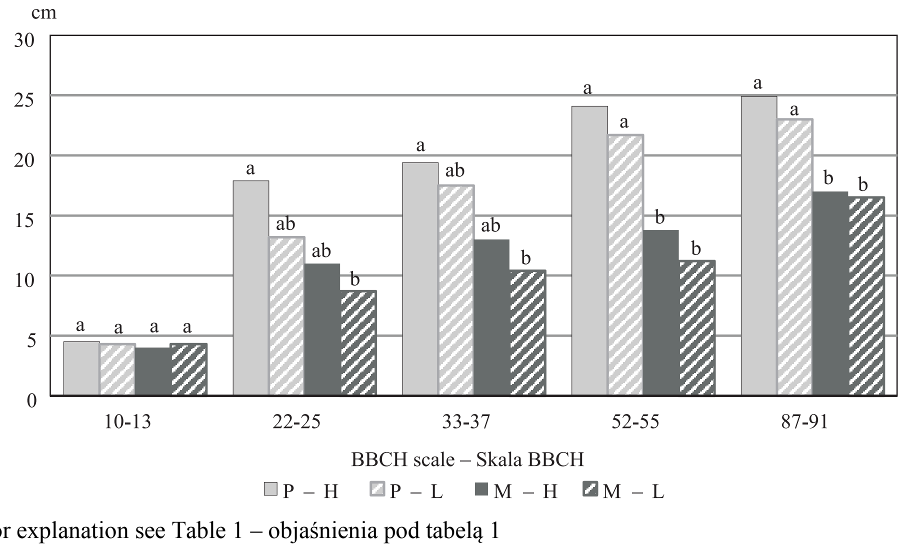
Fig. 3. Leaf number in red clover
Rys. 3. Liczba liści koniczyny czerwonej



for explanation see Table 1 – objaśnienia pod tabelą 1

Fig. 4. Root length in red clover
Rys. 4. Długość korzenia koniczyny czerwonej

Competition between spring barley and red clover in the pot with a lower water dose started at leaf development, and with a higher water dose at cereal tillering (Table 2). On the bedding with higher humidity, it increased until barley ripeness, and on the bedding with poorer water supply it disappeared at tillering but returned at a later stage of growth and increased its effect. In both pots, the process occurred with the greatest intensity at earing and ripeness. In the mixture, regardless of the degree of bedding humidity, until the stage of generative development, barley assimilated more resources than in pure sowing, and at further developmental stage equally or less. In the pot with more abundant watering, starting from leaf development, clover in the presence of barley obtained fewer resources than in pure sowing. This was clearly marked already at cereal tillering but reached the greatest scope during earing and ripening. In the conditions of drought, poorer resource obtainment in a mixture than in pure sowing, except tillering, lasted throughout the entire growth period and was the most visible at barley earing and ripening. In pots with both types of water supply, spring barley was a definitely stronger competitor. Its negative effect on clover was the most visibly marked at earing and then, towards the end of growth, lessened (especially in the more dry pot).

Table 2. Relative yield (RY) and relative yield total (RYT) of red clover and spring barley and competitive balance index (CB)
Tabela 2. Plon względny (RY) i całkowity plon względny (RYT) koniczyny czerwonej i jęczmienia jarego oraz wskaźnik równowagi konkurencyjnej (CB)

Development stages of barley Fazy rozwojowe jęczmienia (BBCH)	RY red clover koniczyna czerwona		RY spring barley jęczmień jary		RYT		CB	
	H*	L	H	L	H	L	H	L
10-13	0.91a	0.87a	1.14a	1.01a	2.05a†	1.88a†	0.24a	0.14a
22-25	0.29b	0.99a	1.15a	1.06a	1.44b†	2.05a†	1.39a†	0.07b†
33-37	0.32b	0.43a	1.05a	1.06a	1.37b†	1.49a†	1.17a†	0.91b†
52-55	0.07a	0.08a	0.97a	0.99a	1.04a	1.06a	2.59a†	2.52a†
87-91	0.09b	0.16a	0.81b	0.94a	0.90b†	1.10a†	2.21a†	1.77b†

* H – higher water dose – wyższa dawka wody, L – lower water dose – niższa dawka wody
a, b – homogenous groups, mean values marked with the same letters do not differ significantly at P = 0.05
a, b – grupy jednorodne, średnie wartości oznaczone tą samą literą nie różnią się istotnie na poziomie P = 0.05
† – RYT ≠ 1, Cb ≠ 0

Competition between both species took place with a significantly greater force in the pot with better water supply at barley tillering, straw-shooting, and ripeness. Humidity conditions significantly diversified resource uptake by barley only at ripeness; it was more effective in the more dry pot. On the other hand, for clover in a mixture, better conditions occurred in the pot with lower humidity at tillering, straw-shooting, and ripeness. In the same periods, barley affected clover with significantly greater force in pots with more abundant watering.

Effects of clover and water deficiency on the morphological characteristics of barley were described by Treder *et al.* [2015]. In short, joint cultivation of spring barley with red clover had no effect on the morphological characteristics of barley throughout the growth period. Water deficiency, however, caused a reduction in blade length, leaf number, limited total tillering, and decreased spike length and grain number per spike at earing. Overlapping of stresses caused by the competitive effect of red clover and water deficiency resulted in the reduction in the number of barley production shoots.

DISCUSSION

In the conducted experiment, strong negative effect of barley competitive actions on the morphological characteristics of clover was found. It demonstrated itself in the shortening of plant height and limiting the numbers of shoots and leaves in the period of dynamic plant development. Sobkowicz and Lejman [2011], while analyzing the morphological characteristics of Persian clover, found no effect of joint growth with barley and with barley and seradella on its height and branch number. On the other hand, in the experiment by Lucero *et al.* [1999], white clover on plots with root and shoot competition of ryegrass formed longer shoots than with its lack, whereas the effect of only grass shoots contributed to their significant shortening in the legume. It results from the present study that clover response to water deficiency in the soil was equally strong as to the effects of barley. This demonstrated itself with the changes in plant sort, as the plants were lower, poorly layered, and formed a lower number of assimilation organs. According to Thomas [1984], white clover cultivation in the conditions of drought and competition on the part of ryegrass leads to the formation of shortened shoots. Those authors, as a major factor that limits the possibility of competing with grass, indicate poorly developed and short root system of legumes in the initial growth period. Belaygue *et al.* [1996] demonstrated that short drought caused a reduction in white clover in the shoot number by 20%-30% but it did not affect the number of leaves per shoot, whereas longer water deficiency also resulted in poorer tillering and reduction in leaf number per shoot. Shoot shortening and a decrease in the number of leaves of white clover under the effect of water deficiency was also observed by Lucero *et al.* [1999].

Analysis of the competitive indexes in the present study demonstrated that barley had a negative effect on clover, which deepened during growth. On the other hand, clover affected barley until cereal earing to a small degree (mostly positive). This resulted from faster initial growth of barley in a mixture, which gave it a competitive advantage over the legume. Also a negative allelopathic effect of barley on clover cannot be excluded. This is indirectly confirmed by the studies of Księżak [2010], who noted a harmful effect of root secretion of this cereal on the development of roots and shoots of common vetch. In that period, similarly to further developmental stages, barley formed a greater above-ground and root mass than clover. Moreover, its roots in a mixture, until earing, were better-developed than in pure sowing, which increased their competitive abilities of obtaining water and biogenes. It may have, therefore, effectively competed with clover under the ground and more skilfully obtained water and nutrients, as well as thanks to bigger, more massive and more abundantly leaved plants used more light. Also Ofori and Stern [1987] and Hauggaard-Nielsen *et al.* [2001] consider cereals to be stronger competitors than legumes, mostly due to faster initial rate of growth and better-developed roots. Känkänen and Eriksson [2007] demonstrated that clover intercrop poorly competes with barley, and therefore does not change significantly its mass, which was confirmed by the present study. Throughout a greater part of growth, competition on the part of barley was stronger in the pot with a higher water dose. Different results were presented by Lucero *et al.* [2000], who while studying competition between *Trifolium repens* and *Lolium perenne* demonstrated that the process intensifies in the conditions of water deficiency. Also Cousens *et al.* [2003] are of the opinion that better water conditions increase the competitive effect of the legume on the cereal, which, however, was not confirmed in the present experiment.

CONCLUSIONS

1. Red clover responded to the vicinity of spring barley with the shortening of the above- and below-ground plant parts from tillering to the end of growth, and also by forming a lower number of leaves and shoots from the straw-shooting stage to ripeness.
2. Water deficiency in the bedding resulted in the shortening of red clover plants from barley tillering to the end of growth, a decrease in leaf number from tillering to earing and in shoot number at the straw-shooting stage of barley and its ripening.
3. Competitive actions between spring barley and red clover in a habitat rich in water started at tillering, strengthened with every successive stage, and lasted until the end of growth. Water stress caused competition to manifest itself in the initial growth period, and its disappearance during tillering and reappearance from the straw-shooting stage to the end of growth.
4. Spring barley dominance over red clover in a more humid habitat started at tillering, increased gradually until earing, and lessened during ripeness. This advantage in the conditions of water stress was lower and started later (at the straw-shooting stage) and maintained the same dynamics of increase and decrease at the successive stages.

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WPŁYW ODDZIAŁYWAŃ KONKURENCYJNYCH I STRESU WODNEGO NA CECHY MORFOLOGICZNE KONICZYNY CZERWONEJ (*Trifolium pratense* L.) UPRAWIANEJ Z JĘCZMIENIEM JARYM (*Hordeum vulgare* L.)

Streszczenie. Na postawie doświadczenia wazonowego założonego według schematu addytywnego oceniano wpływ odziaływań konkurencyjnych jęczmienia jarego na cechy morfologiczne koniczyny czerwonej w warunkach stresu wodnego. Analizy biometryczne wykonywano w pięciu terminach wyznaczonych przez rytm wzrostu jęczmienia w siewie czystym z wyższą dawką wody. Badania obejmowały: wysokość roślin, liczbę pędów i liści na roślinie, długości korzeni. Na podstawie biomasy roślin określono plony względne, całkowity plon względny i wskaźnik równowagi konkurencyjnej. Wykazano, że jęczmień jary od fazy krzewienia do końca wegetacji ograniczał wysokość roślin i długość korzenia koniczyny czerwonej. Podczas strzelania w źdźbło negatywny wpływ zboża pogłębił się, powodując wykształcenie mniejszej liczby liści i pędów niż u koniczyny w siewie czystym. Niedobór wody spowodował skrócenie roślin (od fazy krzewienia jęczmienia do końca wegetacji) oraz redukcję liczby liści (krzewienie – kłoszenie) i pędów (strzelanie w źdźbło i dojrzewanie) koniczyny.

Słowa kluczowe: całkowity plon względny, deficyt wody, fazy rozwojowe, plony względne, wskaźnik równowagi konkurencyjnej, wzajemne oddziaływania roślin

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