Bulgarian Journal of Agricultural Science, 21 (No 1) 2015, 78-88
Agricultural Academy

THE EFFECT OF WATER DEFICIT AND INTERSPECIFIC COMPETITION ON SELECTED PHYSIOLOGICAL PARAMETERS OF SPRING BARLEY AND ITALIAN RYEGRASS

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Abstract

JASTRZĘBSKA, M., M. K. KOSTRZEWSKA, M. WANIC and K. TREDER, 2015. The effect of water deficit and interspecific competition on selected physiological parameters of spring barley and Italian ryegrass. *Bulg. J. Agric. Sci.,* 21: 78–88

Stomatal conductance, transpiration and photosynthetic rates of spring barley and Italian ryegrass plants subjected and not subjected to interspecific competition at different water supply levels were compared in a pot experiment. The experimental factors were: water supply – optimal for the analyzed species and reduced by 50%, sowing regime – single species sowing (pure-sown barley, pure-sown ryegrass) and mixed species sowing (barley mixed-sown with ryegrass, ryegrass mixed-sown with barley). Gas exchange was analyzed during five BBCH growth stages for pure-sown barley under optimal soil moisture conditions: leaf development (10-13), tillering (22-25), stem elongation (33-37), heading (52-22) and ripening (87-91). The measurements for barley were completed at the heading stage. The photosynthesis/transpiration ratio was used to determine water use efficiency (*WUE*) in plants.

Water deficit generally decreased stomatal conductance, transpiration and photosynthetic rates of spring barley and Italian ryegrass. In water-deficient treatments, ryegrass was characterized by higher water use efficiency throughout the growing period, and spring barley – only during the tillering stage. Interspecific competition had little effect on stomatal conductance and transpiration rates of both species as well as the photosynthetic rate of barley, but it increased the photosynthetic rate of ryegrass. Under optimal soil moisture conditions, interspecific competition reduced stomatal conductance and increased photosynthetic rate in ryegrass. Water stress alone and in combination with interspecific competition produced similar results. A combination of water deficit and interspecific competition lowered the photosynthetic rate of spring barley, in particular at the tillering stage. In treatments with optimal water conditions, ryegrass mixed-sown with spring barley was characterized by higher *WUE* than pure-sown ryegrass. Water stress increased *WUE* of ryegrass in all sowing regimes. Spring barley from water-deficient treatments was generally characterized by higher *WUE* in pure-sown stands, in particular at the tillering stage. Competition from ryegrass did not induce significant changes in *WUE* of barley plants in water-deficient or optimal water treatments.

Key words: stomatal conductance; transpiration; photosynthesis; water use efficiency; undersowing

Abbreviations: GS –stomatal conductance; *E* – transpiration rate; *A* –CO_2 assimilation rate, photosynthetic rate;*WUE* –water use efficiency; HW – optimal water supply for the analyzed species (higher dose); LW – water supply reduced by 50% (lower dose); BP –pure-sown barley; BM –barley mixed-sown with ryegrass; RP –pure-sown ryegrass; RM –ryegrass mixed-sown with barley

Introduction

Intercropping and undersowing increase production and offer numerous economic and environmental benefits (Jaskulska and Gałęzewski, 2009). Those practices are used mainly in organic and integrated farming systems. The most popular companion crops are legume plants and grasses, and spring barley is believed to be the best cover crop on account of its early ripening and efficient water use (Kuraszkiewicz, 2004). In unsupportive habitats, undersown crops can compete with

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and lower the yield of the main crop species (Andrzejewska, 1999; Hauggaard-Nielsen et al., 2001).

In the Polish climate, water stress is one of the key factors inhibiting the growth of crop plants (Starck et al., 1995). Water is essential for plant growth and function, and water deficiency impairs development processes in plants. Plants grown in water-deficient environments are unable to manage water efficiently, which limits transpiration and leads to stomatal closure. The above reduces carbon dioxide uptake in plants and slows down photosynthesis (Akinci and Lösel, 2012). Mild water stress induces biochemical changes which enable plants to survive in an unsupportive environment. Severe water deficit can lead to functional and structural changes in the photosynthetic apparatus, thus impairing the growth and yield of plants (Lawlor and Tezara, 2009; Akinci and Lösel, 2012).

Plant responses to water stress are largely determined by their drought tolerance, which is a species-specific or even a variety-specific trait (Roohi et al., 2013), as well as environmental conditions (Galon et al., 2013). Barley is least sensitive to water deficit (Rudnicki, 1995), whereas ryegrass is a relatively sensitive species (Rumasz-Rudnicka, 2010). The presence of a competing species leads to changes in environmental conditions.

Competition among plants is a complex process. It is strongly influenced by individual and group traits of the competing populations as well as abiotic and biotic factors (Satorre and Snaydon, 1992; Concenço et al., 2012). Competition intensity can vary at different stages of plant development, and its effects may be difficult to predict (Wanic et al., 2013).

The objective of this study was to compare stomatal conductance, transpiration and photosynthetic rates of spring barley and Italian ryegrass plants subjected and not subjected to interspecific competition at different water supply levels. An alternative hypothesis stating that water deficit and interspecific competition influence gas exchange and water use efficiency in plants was tested against the null hypothesis that the above factors do not affect the evaluated physiological parameters.

Materials and Methods

Experimental design

A pot experiment was carried out at the Greenhouse Laboratory of the Faculty of Biology and Biotechnology at the University of Warmia and Mazury in Olsztyn (20°30'E 53°47'N). The experiment was performed in three series in 2009-2011. The evaluated crop plants were spring barley cv. Rastik (hulless) and Italian ryegrass cv. Gaza.

The experimental factors were:

– water supply: optimal for the analyzed species (HW) and reduced by 50% (LW);

– sowing regime: single species sowing (pure-sown barley – BP, pure-sown ryegrass – RP) and mixed species sowing (barley mixed-sown with ryegrass – BM, ryegrass mixed-sown with barley – RM).

Soil material was obtained from brown topsoil developed from slightly loamy sand. It was characterized by slightly acidic pH, humus content of 1.22-1.91% and average content of phosphorus, potassium and magnesium. One week before sowing, each pot was filled with 8 kg of soil mixed with mineral fertilizers (g pot^{-1} in terms of pure ingredient): N – 0.5 (urea), P – 0.2 (monopotassium phosphate), K – 0.45 (potassium sulfate).

In treatments with optimal water conditions, water was supplied in the amount of 17000 cm^3 per pot during the growing season, and in treatments with reduced water supply – 8500 cm^3. The optimal water dose was determined in a trial experiment during which soil moisture content, evaporation, transpiration and water content of plants were measured. During the growing season, the amount of water supplied to plants differed subject to the growth stages of the analyzed species and soil moisture content.

The experiment had an additive design (Semere and Froud-Williams, 2001) with four replications. In each pot with pure-sown barley and pure-sown ryegrass, 18 germinating kernels of every species were planted. In mixed-sown treatments, the number of germinating kernels was doubled (18 + 18). Kernels were planted with the use of templates, with identical spacing, at a depth of 3 cm.

Physiological measurements

Greenhouse temperature was maintained at 20-22°C throughout the experiment. It was lowered to 6-8°C for nine days at full emergence to support vernalization.

Gas exchange was analyzed during five BBCH growth stages for pure-sown barley under optimal soil moisture conditions: leaf development (10-13), tillering (22-25), stem elongation (33-37), heading (52-22) and ripening (87-91). The measurements for barley were completed at the heading stage. Gas exchange was measured with the use of the EijkelkampLCi compact photosynthesis system. Stomatal conductance (*GS*), transpiration rate (*E*) and photosynthetic rate (*A*) were determined in the youngest fully-unfolded leaves of three shoots that were randomly sampled from each treatment. Ten measurements were performed in each leaf at five-second intervals. The photosynthesis/transpiration ratio was used to determine water use efficiency (*WUE*) in plants.

Statistical analysis

The results were processed by analysis of variance (ANOVA) for a completely randomized design, and differences between treatments were analyzed by Duncan's test. The probability of error was set at P=0.05. The mean values for the three experimental series were presented in table format.

Results

Spring barley

Water deficit (LW) lowered stomatal conductance (*GS*) in all growth stages of barley. It had the inhibitoriest effect at the tillering stage (when *GS* was reduced by more than 50% in comparison with optimal water treatments – HW) and the least inhibitory influence at the stem elongation stage (unproven difference – Table 1). Regardless of water stress, *GS* at the leaf development stage was significantly higher in barley plants mixed-sown with ryegrass (BM). The progressive increase in *GS* at the tillering and stem elongation stages leveled out significant differences between treatments with different sowing regimes. A considerable drop in *GS* at the heading stage contributed to differences between treatments: higher values of *GS* were observed in pure-sown barley (BP). No significant differences in the mean *GS* values of barley were observed throughout the entire growing season.

An interaction between the experimental factors influenced *GS* of barley already at the leaf development stage. BM-HW plants were characterized by significantly higher *GS* than BP-HW plants, whereas significantly lower and similar values of *GS* were observed in BP-LW and BM-LW plants. At the tillering stage, *GS* increased considerably in BP-HW and BM-HW plants, and the values noted in the above groups were highly similar (non-significant differences). A minor increase in *GS* was noted in BP-LW and BM-LW treatments, but significant differences were reported (BM-LW >BP-LW). At the stem elongation stage, *GS* increased in all treatments excluding BP-HW, and significant differences were observed only between BM-HW and BM-LW treatments. At the heading stage, *GS* was relatively low, but the influence of water stress was further exacerbated by interspecific competition. The values noted in treatments were divided into four homogenous groups.

Water-stressed barley (LW) was characterized by a lower transpiration rate (*E*) than plants with optimal water supply (HW, Table 2). The transpiration rate of barley mixed-sown with ryegrass (BM) was higher than that of pure-sown barley (BP) at the leaf development and tillering stages, whereas lower values of *E* were noted in BM plants at the stem elongation and heading stages.

At the leaf development and tillering stages, BM-HW plants were characterized by higher values of *E* than BP-HW plants. In the above growth stages, the values of *E* were generally lower in LW than in HW treatments, they were identical in BP-LW and BM-LW treatments upon seedling emergence, whereas lower values of *E* were noted in BP-LW than in BM-LW plants at the tillering stage. A drop in transpiration rate was observed in BM-LW plants at the stem elongation rate. The values of *E* decreased considerably in all barley plants during the heading stage (relative to the stem elongation stage), and the highest values were reported in BP-HW plants. Significantly lower transpiration rates were noted in BP-LW and BM-HW treatments, and a further decrease in *E* was reported in BM-LW plants.

In comparison with optimal water conditions (HW), water deficit (LW) lowered the rate of CO_2 assimilation (*A*) in barley only at the leaf development stage, and no differences in the values of *A* were noted between the remaining growth

Table 1
Stomatal conductance (GS) of spring barley (mol H_2O $m^{-2}s^{-1}$)

Source of variation (experimental factor)	Treatment	Growth stages of spring barley				
		leaf development	tillering	stem elongation	heading	average
Water supply	HW	0.052 a	0.149 a	0.165 a	0.034 a	0.100 a
	LW	0.029 b	0.066 b	0.143 a	0.022 b	0.065 b
Sowing regime	BP	0.038 b	0.101 a	0.150 a	0.040 a	0.082 a
	BM	0.044 a	0.114 a	0.158 a	0.017 b	0.083 a
Interaction of experimental factors	BP – HW	0.046 b	0.148 a	0.145 ab	0.049 a	0.097 a
	BM – HW	0.058 a	0.150 a	0.186 a	0.020 c	0.104 a
	BP – LW	0.029 c	0.054 c	0.156 ab	0.030 b	0.067 b
	BM – LW	0.029 c	0.078 b	0.130 b	0.014 d	0.063 b

HW – optimal water supply for the analyzed species (higher dose), LW – water supply reduced by 50% (lower dose); BP – pure-sown barley, BM – barley mixed-sown with ryegrass; a, b, c, d – homogeneous groups: values denoted by the same letters within experimental factors and the interactions between factors are not significantly different at P = 0.05.

stages (Table 3). At the leaf development stage, BM plants had significantly higher values of *A* than BP plants. At the tillering and stem elongation stages, no significant differences were found between BP and BM treatments, whereas the photosynthetic rate of BM plants was lower than that of BP plants at the heading stage.

The combined effect of the analyzed experimental factors was manifested at the leaf development, tillering and heading stages. During leaf development, BM-HW plants where characterized by higher values of *A* than BP-HW plants, whereas generally lower and similar values of *A* were noted in BP-LW and BM-LW treatments. The photosynthetic rate generally increased during tillering, and significantly lower values of *A* were observed only in BM-LW plants. No significant differences in *A* values were found between treatments at the stem elongation stage. The values of *A* decreased in all treatments at the heading stage, and they were characterized by the following pattern: BP-HW > BP-LW > BM-HW ≅ BM-LW.

Water supply differentiated water use efficiency (*WUE*) of barley plants at the leaf development and tillering stages, and the observed trends varied between the two stages (Table 4). During leaf development, LW plants were characterized by significantly lower *WUE* than HW plants. At the tillering stage, *WUE* values in HW treatments decreased significantly in comparison with the values reported in LW plants. At the stem elongation and heading stages, *WUE* values were relatively leveled out in HW and LW treatments. No significant differences in the *WUE* values of BP and BM plants were observed at the leaf development stage. A significant drop in *WUE* values was observed in BM relative to BP plants at the

Table 2
Transpiration rate (*E*) of spring barley (mmol H_2O m^{-2} s^{-1})

Source of variation (experimental factor)	Treatment	Growth stages of spring barley				
		leaf development	tillering	stem elongation	heading	average
Water supply	HW	1.27 a	2.32 a	1.84 a	0.76 a	1.55 a
	LW	0.90 b	1.26 b	1.75 a	0.55 b	1.12 b
Sowing regime	BP	1.00 b	1.56 b	1.91 a	0.82 a	1.32 a
	BM	1.17 a	2.02 a	1.67 b	0.52 b	1.35 a
Interaction of experimental factors	BP – HW	1.12 b	2.05 b	1.78 a	0.96 a	1.48 a
	BM – HW	1.41 a	2.59 a	1.90 a	0.58 b	1.62 a
	BP – LW	0.88 c	1.07 d	2.05 a	0.67 b	1.17 b
	BM – LW	0.91 c	1.45 c	1.42 b	0.45 c	1.06 b

HW – optimal water supply for the analyzed species (higher dose), LW – water supply reduced by 50% (lower dose); BP – pure-sown barley, BM – barley mixed-sown with ryegrass; a, b, c, d – homogeneous groups: values denoted by the same letters within experimental factors and the interactions between factors are not significantly different at P = 0.05.

Table 3
Photosynthetic rate (*A*) of spring barley (μmol CO_2 $m^{-2}s^{-1}$)

Source of variation (experimental factor)	Treatment	Growth stages of spring barley				
		leaf development	tillering	stem elongation	heading	average
Water supply	HW	3.23 a	3.83 a	2.79 a	1.20 a	2.76 a
	LW	2.47 b	3.45 a	2.98 a	1.07 a	2.49 a
Sowing regime	BP	2.70 b	3.82 a	2.85 a	1.48 a	2.71 a
	BM	3.01 a	3.45 a	2.91 a	0.82 b	2.55 a
Interaction of experimental factors	BP – HW	2.97 b	3.77 a	2.65 a	1.65 a	2.76 a
	BM – HW	3.46 a	3.88 a	2.92 a	0.77 c	2.76 a
	BP – LW	2.43 c	3.87 a	3.05 a	1.30 b	2.66 a
	BM – LW	2.52 c	3.03 b	2.90 a	0.86 c	2.33 b

HW – optimal water supply for the analyzed species (higher dose), LW – water supply reduced by 50% (lower dose); BP – pure-sown barley, BM – barley mixed-sown with ryegrass; a, b, c, d – homogeneous groups: values denoted by the same letters within experimental factors and the interactions between factors are not significantly different at P = 0.05.

tillering stage. *WUE* values of BP decreased at the stem elongation stage and then *WUE* remained at a similar level in the above treatments, and a further drop in WUE was reported in BM plants during heading.

An interaction between the experimental factors influenced the *WUE* values of barley at the tillering stage. BP-LW plants were characterized by significantly higher *WUE* than BP-HW plants as well as BM-HW and MB-LW plants which had similar *WUE* values. At the stem elongation stage, *WUE* was reduced by 50% in the BP-LW treatment, and it was significantly lower in comparison with the remaining treatments where it had risen inconsiderably. At the heading stage, *WUE* of BM-HW plants was significantly lower (compared with the previous stage) than in BP-HW and BP-LW plants.

Italian ryegrass

In comparison with HW treatments, water deficit (LW) lowered stomatal conductance (*GS*) of Italian ryegrass nearly throughout the entire growing season (Table 5). The only exception was the heading stage of barley when *GS* (in general at low level) was relatively higher in water-deficient ryegrass plants (LW). During the leaf development and tillering stages of barley, stomatal conductance of ryegrass was not affected by the sowing regime, despite an increase in *GS* values during tillering. At the stem elongation stage, stomatal conductance increased only in pure-sown ryegrass (RP), which contributed to significant differences between RP and RM treatments. The *GS* values of ryegrass decreased considerably and were leveled out at the heading stage of barley, regardless of

Table 4
Water use efficiency (*WUE*) of spring barley (µmol CO_2 mmol H_2O^{-1})

Source of variation (experimental factor)	Treatment	Growth stages of spring barley				
		leaf development	tillering	stem elongation	heading	average
Water supply	HW	3.18 a	1.92 b	2.20 a	1.87 a	2.29 a
	LW	2.85 b	2.82 a	1.92 a	2.11 a	2.43 a
Sowing regime	BP	3.02 a	2.85 a	1.94 a	2.16 a	2.49 a
	BM	3.02 a	1.89 b	2.19 a	1.83 b	2.23 b
Interaction of experimental factors	BP – HW	3.10 a	2.11 b	2.21 a	2.12 a	2.39 b
	BM – HW	3.25 a	1.73 b	2.19 a	1.64 b	2.20 b
	BP – LW	2.93 b	3.59 a	1.68 b	2.20 a	2.60 a
	BM – LW	2.76 b	2.05 b	2.19 a	2.03 ab	2.26 b

HW – optimal water supply for the analyzed species (higher dose), LW – water supply reduced by 50% (lower dose); BP – pure-sown barley, BM – barley mixed-sown with ryegrass; a, b, c, d – homogeneous groups: values denoted by the same letters within experimental factors and the interactions between factors are not significantly different at P = 0.05.

Table 5
Stomatal conductance (*GS*) of Italian ryegrass (mol H_2O $m^{-2}s^{-1}$)

Source of variation (experimental factor)	Treatment	Growth stages of spring barley					
		leaf development	tillering	stem elongation	heading	ripening	average
Water supply	HW	0.086 a	0.142 a	0.196 a	0.037 b	0.089 a	0.110 a
	LW	0.052 b	0.110 b	0.112 b	0.043 a	0.051 b	0.074 b
Sowing regime	RP	0.071 a	0.128 a	0.178 a	0.042 a	0.051 b	0.094 a
	RM	0.066 a	0.124 a	0.131 b	0.039 a	0.089 a	0.090 a
Interaction of experimental factors	RP – HW	0.090 a	0.149 a	0.251 a	0.042 a	0.049 b	0.116 a
	RM – HW	0.081 a	0.134 a	0.142 b	0.032 b	0.130 a	0.104 b
	RP – LW	0.053 b	0.107 b	0.105 b	0.041 a	0.053 b	0.072 c
	RM – LW	0.051 b	0.113 b	0.119 b	0.046 a	0.049 b	0.076 c

HW – optimal water supply for the analyzed species (higher dose), LW – water supply reduced by 50% (lower dose); RP – pure-sown ryegrass, RM – ryegrass mixed-sown with barley; a, b, c, d – homogeneous groups: values denoted by the same letters within experimental factors and the interactions between factors are not significantly different at P = 0.05

the sowing regime, whereas significantly higher *GS* was reported in RM than in RP plants at the ripening stage.

During initial stages of ryegrass development (up to the tillering stage of barley), no interaction was observed between the experimental factors, and homogenous groups were identified based on the water supply criterion. At the stem elongation stage, *GS* of RP-HW plants was relatively high in comparison with RP-LW, RM-HW and RM-LW treatments. At the heading stage of barley, the *GS* values of ryegrass were lowered, and a significant decrease in *GS* was reported in RM-HW plants. A significant increase in *GS* values was noted in the above treatment at the ripening stage of barley, whereas *GS* of RP-HW, RP-LW and RM-LW plants remained at a low and similar level.

Water-deficient ryegrass plants (LW) were generally characterized by lower *E*, excluding at the heading stage of barley when the reverse was reported (Table 6). The sowing regime (RP or RM) had no effect on the values of *E* at the leaf development, tillering and stem elongation stages of spring barley. Contrary to the ripening stage, RM plants were characterized by significantly lower *E* than RP plants at the heading stage.

In early stages of ryegrass development, which corresponded to the leaf development and tillering stages of barley, no interaction was observed between the experimental factors, and homogeneous groups were identified based on the water supply criterion. The experimental factors exerted a combined effect on the values of *E* from the beginning of stem elongation to the end of ripening, but the nature of the observed changes differed across growth stages. At the stem elongation stage of barley, RM-HW plants were characterized by lower *E* than RP-HW plants. Water stress significantly inhibited transpiration, and more profound changes were noted in RP-LW than in RM-LW treatments. Transpiration was generally slowed down at the heading stage, and the values of *E* were stabilized in RP-HW, RP-LW and RM-LW treatments, whereas a clear drop in *E* was noted in RM-HW plants. The transpiration rate of RM-HW plants increased at the ripening stage, whereas lower and generally stable levels of *E* were maintained in the remaining treatments.

The photosynthetic rate (*A*) of water-stressed (LW) ryegrass plants increased at the leaf development stage in comparison with HW treatments (Table 7). At the tillering stage, similar values of *A* were noted in HW and LW ryegrass plants, whereas a marked decrease in *A* was observed in LW treatments at the stem elongation stage. The values of *A* decreased and were leveled out in HW and LW ryegrass plants at the heading stage of barley. At the end of the barley growing season, ryegrass plants from HW treatments demonstrated significantly higher values of *A* than LW plants. Interspecific competition from barley lowered *A* in ryegrass plants at the leaf development and heading stages, whereas an increase in the analyzed parameter was observed during the tillering, stem elongation and ripening stages of barley.

During leaf development, competition from barley had no effect on the photosynthetic rate of HW ryegrass plants (RP-HW ≅ RM-HW), but it decreased the value of *A* in LW plants (RP-LW > RM-LW). An increase in *A* was noted in RM-HW and RM-LW treatments at the barley tillering stage. During stem elongation, the presence of a competing species did not influence *A* in HW ryegrass plants (RP-HW ≅ RM-HW), whereas in the group of water-stressed plants (LW), interspecific competition had a more inhibitory effect on *A* in RP-LW than in RM-LW treatments. At the heading stage, the photosynthetic rate of ryegrass was substantially lower than at the

Table 6
Transpiration rate (*E*) of Italian ryegrass (mmol H_2O $m^{-2}s^{-1}$)

Source of variation (experimental factor)	Treatment	Growth stages of spring barley					
		leaf development	tillering	stem elongation	heading	ripening	average
Water supply	HW	2.18 a	2.96 a	3.06 a	1.21 b	1.60 a	2.20 a
	LW	1.52 b	2.00 b	1.70 b	1.52 a	1.00 b	1.55 b
Sowing regime	RP	1.85 a	2.41 a	2.37 a	1.45 a	1.06 b	1.83 a
	RM	1.85 a	2.58 a	2.41 a	1.29 b	1.52 a	1.93 a
Interaction of experimental factors	RP – HW	2.18 a	2.93 a	3.31 a	1.36 a	1.04 b	2.16 a
	RM – HW	2.18 a	2.99 a	2.80 b	1.06 b	2.17 a	2.24 a
	RP – LW	1.53 b	1.90 b	1.41 d	1.54 a	1.09 b	1.49 b
	RM – LW	1.51 b	2.12 b	2.00 c	1.51 a	0.88 b	1.60 b

HW – optimal water supply for the analyzed species (higher dose), LW – water supply reduced by 50% (lower dose);
RP – pure-sown ryegrass, RM – ryegrass mixed-sown with barley; a, b, c, d – homogeneous groups: values denoted by the same letters within experimental factors and the interactions between factors are not significantly different at P = 0.05.

previous stage, and relatively higher values of *A* were noted in RP-HW than in RM-HW ryegrass plants. Under water deficit (LW), the presence of barley had no effect on parameter *A* (RP-LW ≅ RM-LW). At the ripening stage of barley, mixed-sown ryegrass was characterized by higher values of *A*, in particular under optimal water conditions (RM-HW).

Water-deficient ryegrass plants (LW) were characterized by higher *WUE* than HW plants throughout nearly the entire experiment (Table 8). The only exception was the heading stage of barley, when differences in *WUE* between the above treatments were insignificant. At the initial stages of ryegrass development (until the tillering stage of barley), *WUE* values in RM plants were significantly higher than in RP treatments. The reverse was reported at the stem elongation and heading stages. Towards the end of the barley growing season, RM ryegrass plants used water significantly more efficiently than plants not exposed to interspecific competition (RP).

The experimental factors exerted a combined effect on *WUE* values already at the initial stages of ryegrass development when *WUE* was lower in RP-HW than in RP-LW plants, and *WUE* values in RM-HW and RM-LW treatments were identical and similar to those noted in RP-LW plants. A general drop in *WUE* was reported at the tillering stage, and new homogeneous groups emerged mainly in response to a profound drop in *WUE* in RM-HW plants and a somewhat smaller decrease in *WUE* in the RM-LW treatment. At the stem elongation stage, a further drop in *WUE* was observed in the above treatments, and *WUE* values in the RM-HW treatment reached those noted in RP-HW plants. A considerable drop in *WUE* was reported in the RP-LW treatment and a

Table 7
Photosynthetic rate (*A*) of Italian ryegrass (µmol CO_2 $m^{-2}s^{-1}$)

Source of variation (experimental factor)	Treatment	Growth stages of spring barley					
		leaf development	tillering	stem elongation	heading	ripening	average
Water supply	HW	3.02 b	4.36 a	3.96 a	1.65 a	2.32 a	3.06 a
	LW	3.71 a	4.13 a	3.07 b	1.67 a	1.91 b	2.90 b
Sowing regime	RP	3.56 a	3.75 b	3.19 b	1.87 a	1.44 b	2.76 b
	RM	3.17 b	4.76 a	3.86 a	1.45 b	2.78 a	3.20 a
Interaction of experimental factors	RP – HW	3.54 a	3.77 b	3.72 ab	1.95 a	1.27 c	2.85 b
	RM – HW	3.89 a	4.94 a	4.19 a	1.36 c	3.38 a	3.55 a
	RP – LW	3.58 a	3.73 b	2.65 c	1.80 ab	1.60 c	2.67 b
	RM – LW	2.46 b	4.56 a	3.52 b	1.54 bc	2.21 b	2.86 b

HW – optimal water supply for the analyzed species (higher dose), LW – water supply reduced by 50% (lower dose);
RP – pure-sown ryegrass, RM – ryegrass mixed-sown with barley; a, b, c, d – homogeneous groups: values denoted by the same letters within experimental factors and the interactions between factors are not significantly different at P = 0.05.

Table 8
Water use efficiency (*WUE*) of Italian ryegrass (µmol CO_2 mmol H_2O^{-1})

Source of variation (experimental factor)	Treatment	Growth stages of spring barley					
		leaf development	tillering	stem elongation	heading	ripening	average
Water supply	HW	2.06 b	1.66 b	1.41 b	1.37 a	1.59 b	1.62 b
	LW	2.43 a	2.15 a	2.17 a	1.29 a	2.19 a	2.05 a
Sowing regime	RP	2.04 b	1.85 b	1.87 a	1.40 a	1.40 b	1.71 b
	RM	2.45 a	1.95 a	1.70 b	1.26 b	2.38 a	1.95 a
Interaction of experimental factors	RP – HW	1.70 b	1.47 c	1.30 c	1.39 ab	1.29 c	1.43 c
	RM – HW	2.42 a	1.85 b	1.53 c	1.35 ab	1.89 b	1.81 b
	RP – LW	2.38 a	2.24 a	2.45 a	1.41 a	1.51 bc	2.00 a
	RM – LW	2.49 a	2.06 ab	1.88 b	1.17 b	2.89 a	2.10 a

HW – optimal water supply for the analyzed species (higher dose), LW – water supply reduced by 50% (lower dose);
RP – pure-sown ryegrass, RM – ryegrass mixed-sown with barley; a, b, c, d – homogeneous groups: values denoted by the same letters within experimental factors and the interactions between factors are not significantly different at P = 0.05.

less profound decrease was reported in RM-LM plants at the heading stage. During that time, RP-LW plants were characterized by significantly higher *WUE* than RM-LW plants. At the ripening stage, the values of *WUE* increased in RM-HW and RM-LW plants, and the noted increase significantly differentiated RM-LW plants from the remaining treatments.

Discussion

In response to environmental stress, changes in gas exchange take place earlier than biomass allocation (Yin et al., 2009). The effect of water stress on the physiology of plants, including crop plants, has been well documented, but the effects of interspecific competition on physiological processes remain insufficiently investigated (Deen et al., 2003).

Water deficit slows down photosynthesis and transpiration by reducing stomatal conductance for CO_2 and water vapor (Lawlor and Tezara, 2009; Motzo et al., 2013). The results reported for spring barley and Italian ryegrass throughout the entire growing season in this experiment corroborate the findings of other authors. The relative drop in the photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (*GS*) of barley and ryegrass was comparable. In a study of several lines and varieties of triticale, wheat and barley, Roohi et al. (2013) observed a drop in the gas exchange parameters of all genotypes subjected to water stress, and the noted decrease differed across the analyzed species and varieties. Olszewska et al. (2010) noted that water stress induced different changes in the photosynthetic and transpiration rates of various grass species (*Lolium perenne* L.*, Dactylis glomerata* L.*, Festuca pratensis* Huds.*, Phleum pratense* L.*, Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl.)

Instantaneous *WUE*, expressed by the *A/E* ratio, is an indicator of the amount of CO_2 assimilated by photosynthesis per unit of transpired water. It indicates whether plant leaves are capable of optimizing their carbon dioxide uptake relative to water loss in a changing environment (Swarthout et al., 2009). The majority of plants increase their water use efficiency when the soil moisture content decreases during drought because *A* is lower than *E* during a given reduction in *GS* (Jones, 1993; Earl, 2002). In our experiment, the above correlation was observed in ryegrass and in barley at the tillering stage. Olszewska et al. (2010) reported an increase in *WUE* of all analyzed grass species subjected to water stress. In the work of Lucero et al. (2000), progressive water stress did not alter *WUE* of white clover, but it contributed to an increase in *WUE* of perennial ryegrass which was higher than in white clover. Olszewska et al. (2010), Lucero et al. (2000) and Turner et al. (2012) observed that ryegrass is characterized by relatively high water use efficiency. The results reported for barley in this experiment are consistent with the findings of Wall et al. (2011), where *WUE* of spring barley did not change in response to water stress, as well as the results reported by Thameur et al. (2012) where the decrease in the photosynthetic rate of water-stressed barley plants was more profound than the drop in their transpiration rate. According to Farquhar et al. (1989), photosynthesis and transpiration decrease at a similar rate during progressive water deficit. Turner et al. (2012) noted a negative correlation between *WUE* of the analyzed grasses (*Lolium perenne, Dactylis glomerata, Festuca arundinacea* Schreb.) and the applied watering regime. In plants exposed to high levels of water stress, the decrease in *A* was more profound than the drop in *E*, which led to a decrease in *WUE* (Swarthout et al., 2009). Prolonged water deficit can damage the leaf's photosynthetic apparatus (Lawlor and Tezara, 2009; Akinci and Lösel, 2012).

Physiological growth parameters generally change when plants are subjected to severe interspecific competition. Iqbal and Wright (1999) demonstrated that strong competition from densely growing weeds lowered the net photosynthetic rate of spring wheat. In a study by Niu et al. (2006), interspecific competition lowered the photosynthetic rate in C3 grass (*Leymus chinensis* (Trin.) Tzvelev), but it did not influence the above parameter in C4 grass (*Chloris virgate* Sw.). Wang et al. (2005) demonstrated that enhanced interspecific competition lowered the values of *GS*, *A* and *E* in *Atriplex prostrata* Boucher ex DC. Jensen et al. (2011) observed a decrease in the transpiration rate of oak seedlings exposed to competition from shrubs. In a study by Aspiazú et al. (2010), cassava plants accompanied by *Commelina benghalensis* L. were characterized by higher *A* than plants grown with other weed species (*Biden spilosa* L.*, Brachiaria plantaginea* (Link) Hitchc.) or pure-sown cassava plants which were characterized by similar values of *A*. Galon et al. (2013) reported that increased competition (higher density) from *Brachiria brizantha* (Hochst.) Stapf. led to a decrease in *GS* and *A* in one and a drop in *E* in two out of the three analyzed sugar cane varieties, whereas the above parameters were relatively stable in the remaining treatments.

In the present study, the interactions between barley and ryegrass had different impacts on gas exchange parameters of the studied species. In general, interspecific competition had a minor effect on *GS* and *E* in both species and *A* in barley, but it contributed to an increase in *A* in ryegrass over the growing season.

The variations in physiological parameters induced by interspecific competition can be attributed to differences in the ability of plants to efficiently use the existing resources, in particular water which affects the availability of CO_2 in the mesophyll, leaf temperature and, consequently, photosynthetic rate. Competition for water can also affect the absorption of nutrients from soil, and it forces plants to compete for light and nutrients (Concenço et al., 2012). Water availability is not synonymous with competition for water. Severe water deficit is not always associated with increased competition for water (Casper and Jackson, 1997). Researchers are only beginning to make headway on the correlations between plant physiology and competition mechanisms (Concenço et al., 2012).

In the current study, the presence of a competing species reduced *GS* and enhanced *A* in ryegrass plants grown under optimal water conditions. Water stress alone and in combination with competition had similar effects. The combination of biotic and abiotic stressors lowered *A* in barley, in particular at the tillering stage.

The results of other studies also point to the complex nature of interactions between physiological parameters and interspecific competition. Iqbal and Wright (1998) analyzed the effects of water stress and competition between spring wheat and two weed species (*Phalaris minor* Retz., *Chenopodium album* L.) on their photosynthetic rates. The cited authors observed that water deficit significantly lowered the net photosynthetic rate of all species, and a greater decrease was noted in wheat and *P. minor* than in *C. album*. The observed drop resulted mainly from a decrease in stomatal conductance (*GS*). When the plants were repeatedly supplied with water, the photosynthetic rate was restored in wheat and *C. album*, but not in *P. minor*. In a study of *C. album*, interspecific competition increased the net photosynthetic rate of wheat and decreased the analyzed parameter in weed plants. The above was observed in both water supply regimes. According to Tworkowski (2000), *Prunus persica* (L.) Batsch) trees growing without competition from grasses (*Lolium perenne, Festuca arundinacea*) were taller than those growing in a competitive environment, and *GS* and *A* of water-stressed trees decreased faster and earlier in taller specimens.

In the present experiment, *WUE* decreased in spring barley and increased in Italian ryegrass plants exposed to competition. Lucero et al. (2000) did not observe any correlations between interspecific competition and *WUE* of white clover and perennial ryegrass. In the work of Galon et al. (2013), an increase in the density of *Brachiria brizantha* plants was accompanied by a reduction in *WUE* of one sugar cane variety, whereas no changes in the analyzed parameter were observed in the remaining two varieties. According to the cited authors, the former variety was capable of preserving normal rates of photosynthesis under exposure to water stress and interspecific competition, whereas in the remaining sugar cane varieties, the reduction in photosynthetic rate was similar to that noted in the rate of transpiration. In the work of Lucero et al. (2000), progressive water deficit did not affect *WUE* of white clover, but it increased the above parameter in perennial ryegrass. The above findings are partially consistent with the results of our experiment where *WUE* was higher in mixed-sown than in pure-sown ryegrass plants grown under optimal water conditions. Water deficit increased and stabilized *WUE* of ryegrass regardless of the sowing regime. In barley, *WUE* values were generally higher in water-stressed and pure-sown plants, in particular at the tillering stage. No significant changes in *WUE* were observed in mixed-sown barley plants subjected to water stress and supplied with optimal amounts of water.

In the current experiment, spring barley and Italian ryegrass were characterized by a similar pattern of seasonal variations in *GS*, *A* and *E*. The above parameters were generally lower during leaf development, they increased during tillering and stem elongation and decreased at the heading stage. At the ripening stage of barley, *GS*, *A* and *E* of ryegrass mixed-sown with barley increased considerably in treatments supplied with optimal amounts of water. Seasonal variations in physiological parameters were also observed by Wall et al. (2011) and Thameur et al. (2012) in barley and by Niu et al. (2006) in grasses and, similarly to this study, they were attributed to water availability (Wall et al., 2011; Thameur et al., 2012) and interspecific competition (Niu et al., 2006).

In the present study, *WUE* of barley and ryegrass decreased between leaf development and heading, but it increased in mixed-sown ryegrass at the ripening stage of barley, in particular in water-deficient treatments. Piotrowska et al. (2003) noted a clear drop in *WUE* across different growth stages of oats, regardless of the analyzed variety and the applied nitrogen fertilization rates.

Conclusions

Water deficit generally decreased stomatal conductance, transpiration and photosynthetic rates of spring barley and Italian ryegrass. In water-deficient treatments, water use efficiency in Italian ryegrass was generally higher throughout the entire growing season, and in spring barley – only at the tillering stage. Interspecific interactions between spring barley and Italian ryegrass had varied effects on gas exchange parameters in the evaluated species. In general, competition exerted little influence on stomatal conductance and transpiration rates of both species as well as on the photosynthetic rate of barley, but it increased the photosynthetic rate of ryegrass. Under optimal water conditions, interspecific competition decreased stomatal conductance and increased the photosynthetic rate of ryegrass. Water stress alone and in combination with interspecific competition produced similar results. Water deficit combined with interspecific competition lowered the photosynthetic rate of spring barley, in particular at the tillering stage. Under optimal water conditions, Italian ryegrass mixed-sown with spring barley was characterized by higher water use efficiency than pure-sown ryegrass. Water deficit increased *WUE* of both pure-sown and mixed-sown ryegrass. In water-stressed treatments; spring barley was characterized by higher *WUE* in pure-sown treatments, in particular at the tillering stage. No significant changes in *WUE* were observed between mixed-sown barley plants subjected to water stress and supplied with optimal amounts of water. Spring barley and Italian ryegrass were characterized by a similar pattern of seasonal variations in *GS*, *A* and *E*. A general increase in the above parameters was noted during the tillering and stems elongation stages of barley. At the ripening stage of barley, *GS*, *A* and *E* of ryegrass mixed-sown with barley increased considerably in treatments supplied with optimal amounts of water. *WUE* of barley and ryegrass decreased between seedling emergence and heading, but it increased in mixed-sown ryegrass at the ripening stage of barley, in particular in water-deficient treatments.

Acknowledgments

The study was supported financially by the Ministry of Science and Higher Education in 2009-2012 (grant No. N N310 082836).

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Received March, 26, 2014; accepted for printing December, 2, 2014.