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The physiological and biochemical reactions of selected grass species cultivated with the hydrogel amendment under salt stress (preliminary studies)

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Abstract: *The physiological and biochemical reactions of selected grass species cultivated with the hydrogel amendment under salt stress (preliminary studies).* The aim of the study was to assess the influence of hydrogel on the free proline accumulation, relative chlorophyll *a* content and relative water content (RWC) of red fescue (cv. 'Raisa'), smooth-meadow grass (cv. 'Jarotka') and perennial ryegrass (cv. 'Stadion') exposed to simulated salt stress. The experiment was conducted for four months in pots under greenhouse conditions. The hydrogel was used in the amount of 2 g per 1 dm^3 of the medium. Salt stress in pots with and without hydrogel amendment was simulated on one-month-old plants by adding three different concentrations of water and road-de-icing salt solutions. The salinity increased proline accumulation and reduced relative chlorophyll *a* content in all of the tested species. Salt stress did not influence RWC in smooth-meadow grass and red fescue, but decreased RWC in perennial ryegrass. Under saline conditions hydrogel had no effect on the proline accumulation and RWC of all of the tested species. The relative chlorophyll *a* content was increased by sorbent only in perennial ryegrass under medium salt stress. The results obtained supported the conclusion that whereas the level of soil salinity was the main factor that had influenced the parameters tested; the efficacy of hydrogel under saline conditions was poorly indicated. The results should, however, be confirmed in field studies and experiments using other physiological and biochemical indicators of salt stress and taking into consideration other cultivars and species of turf.

Key words: hydrogel, drought, soil salinity, lawns, *Festuca rubra*, *Poa pratensis*, *Lolium perenne*

INTRODUCTION

Hydrogels are substances that ensure water-storage and thus allow plants to mitigate drought [Trippei et al., 1991]. Soil super sorbents support the growth and development of many agricultural (cotton), horticultural (tomato, tobacco) or forestry (pine seedlings) species [Wallace and Wallace, 1986; Al-Humaid, 2005; Sarvás et al., 2007]. The results of a pilot study conducted by the authors showed that, under simulated drought stress, gel sorbent enhances the germination, growth and dry matter of several grass species [Hadam, 2010]. In spite of the high water requirements of lawns, the water sorbent meant the frequency of watering could fall from three times a week to once a week, which reduced irrigation costs by three and a half times.

Grasses are the most common greenery alongside traffic lanes, where they shape landscape and microclimate conditions [Wysocki and Stawicka, 2005]. Unfortunately, due to the high costs of

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irrigation these plants are neglected and therefore some innovative cultivation, such as the application of gels, is needed.

Despite the above, hydrogels can also absorb cations. Akhter et al. [2004] observed that this ability decreases the resistance of gel sorbents to drought. On the other hand, numerous authors have shown that the application of hydrogel under saline conditions gives some benefits to crops. It has been observed that it increases the tolerance of wheat [Saleh and Hussein, 1987], maize [El-Sayed and Kirkwood, 1992] and bean [Aydin et al., 2011] to salt stress.

In Poland the problem of soil salinity relates mainly to plants, including grasses, on green belts near to roads. There, the use of salts, especially sodium chloride, for de-icing is frequent and high [Borowski, 2008]. Nevertheless, the effect of hydrogel on turf species growing under such conditions remains relatively unexplored.

Perennial ryegrass and red fescue are the most common grass species found alongside Polish traffic lanes [Wysocki, 1994; Harkot et al., 1998; Harkot et al., 1999]. Borowski [2008] observed that *Lolium perenne* is the most tolerant species to NaCl salinity whereas *Festuca rubra* is less tolerant. It was found that smooth-meadow grass (*Poa pratensis*), which is present near the roadside grass in the lowest amount, is the most sensitive species to salt stress. Hanslin and Eggen [2005] have indicated, however, that such differences in the tolerance of grass also concerns species and cultivars.

In studies conducted by the authors on perennial ryegrass (cv. 'Stadion', 'Naki', 'Accent'), red fescue (cv. 'Aretа', 'Raisa') and smooth-meadow grass (cv. 'Jarotka', 'Limousine') cultivated with hydrogel under simulated salinity, the presence of road-de-icing salt (97% NaCl) was the main factor that influenced the growth and fresh matter of all of the plants. Nevertheless, under low and medium salinity, the gel was beneficial to the condition of all the cultivars of red fescue and smooth-meadow grass, as well as to the cv. 'Stadion' perennial ryegrass. No influence was observed on the cv. 'Naki' and 'Accent' perennial ryegrass [Hadam et al., 2011a and 2011b; unpublished studies]. Studies of the influence of hydrogels on selected biochemical and physiological processes in grasses exposed to simulated salt stress are needed to find a better explanation of the results obtained.

One of the most commonly used biochemical indicators of plant tolerance to salinity is the accumulation of proline [Chen et al. 2001; Turan and Aydin, 2005]. An increased amount of this amino acid has also been observed in the leaves of several grass species cultivated under saline conditions [Huimin et al., 2001; Borowski, 2008]. As salt stress causes osmotic stress in plants, the water capability in their cells decreases, which is evidenced by turgor loss and a reduction in relative water content [Morant-Manceau et al., 2004]. Under saline conditions the intensity of photosynthesis may also decrease. A simple and quick indicator of such a reaction is the assessment of relative chlorophyll *a* content by means of a chlorophyll content meter [Jaleel et al., 2008].

The purpose of the present study was therefore to establish whether hydrogel influences proline accumula-

tion, relative water content and relative chlorophyll *a* content in the leaves of perennial ryegrass, smooth-meadow grass and red fescue exposed to simulated salt stress.

MATERIAL AND METHODS

Experimental design

The study was conducted over four months in the greenhouse of the Faculty of Horticulture and Landscape Architecture at Warsaw University of Life Sciences – SGGW.

An experimental growth medium, which consisted of sand, peat and horticultural soil (1 : 1 : 1), was mixed with hydrogel in the amount recommended by the manufacturer (2 $g \cdot dm^{-3}$ of the medium). The substrates prepared in this way were put into $0.8 \cdot dm^{-3}$ plastic pots (12 cm). Pots with and without hydrogel amendment were sown with (1) perennial ryegrass (*Lolium perenne*) 'Stadion' (0.123 g of seeds per pot; equivalent to ca. 10.8 g/m^2), (2) smooth meadow-grass (*Poa pratensis*) 'Jarotka' (0.074 g of seed per pot; equivalent to ca. 6.5 $g \cdot m^{-2}$) and (3) red fescue (*Festuca rubra*) 'Raisa' (0.086 g of seeds per pot; equivalent to ca. 7.6 $g \cdot m^{-2}$). The number of seeds planted per pot was estimated based on seedling emergence, seedling purity and the sowing standard for each tested species [Rutkowska and Pawluśkiewicz, 1996].

Simulation of salinity stress

After a month of sowing, the plants were treated with a water solution of Kłodawska road-de-icing salt (containing 97% NaCl) in the amount of 80 ml per pot and in a dose of: 0, 5 or 10 $g \cdot dm^{-3}$. Based on the salinity curve for the experimental substrate used, the electricity conductance obtained was low (1.30 $mS \cdot cm^{-1}$), medium (5.45 $mS \cdot cm^{-1}$) or high (9.91 $mS \cdot cm^{-1}$) [Kreeb, 1979].

The experiment was conducted in four replications, where one replication was a single pot with one of each species cultivated (with or without hydrogel amendments) at each salinity level.

Three months after the salt treatment, the grasses cultivated with and without the hydrogel additive were evaluated for several biochemical and physiological parameters used commonly in the assessment of salinity effects on plants [Poontariga et al., 2000; Khan et al., 2007; Dhanapackiam and Muhammad Ilyas, 2010]: free proline accumulation – by the Bates method [1973], the relative chlorophyll *a* content measured with a CCM-200 (chlorophyll content meter; Opti-Science, USA) and relative water content.

During the whole experiment the plants were irrigated to the optimal level of the capillary water capacity of the tested medium and measurements were conducted by soil moisture meter (ECHO--EC5) every day. The grasses were also mown once a week to a height of 5 cm. The temperature and air humidity in the greenhouse, which fluctuated from 22.9 to 35.0°C and from 32.5 to 42.2% respectively, were measured daily by thermo-hygrometer EPI 8703.

Statistical analysis

The data were analysed using a two-factor analysis of variance (ANOVA) from the Statgraphics 4.1 Plus software. The

significance of the differences between the combinations was examined with Duncan's test at $\alpha = 0.05$. The results show the mean values.

RESULTS

The effect on free proline accumulation

Salt additive resulted in an increase in the proline accumulation in the leaves of the perennial ryegrass (Fig. 1a). The higher concentration of salt was in the pots in which significantly higher proline content was determined. Such a reaction was independent of the hydrogel amendment.

It was also observed that in both media (with and without the sorbent) the smooth-meadow grass accumulated significantly greater amount of proline, when it was treated with the medium salt concentration by comparison with the pots, where plants were cultivated at a low salinity level (Fig. 1b). Proline accumulation under high salt stress was significantly greater than that observed under low and medium salt concentration in the pots with the hydrogel amendment. In the pots without the sorbent the amount of accumulated amino acid showed no significant difference compared to that determined in the pots with and without gel amendment under medium saline conditions and compared to the pots with gel under highly saline conditions.

The free proline content in red fescue increased significantly under medium salt stress (Fig. 1c) independently of the hydrogel amendment. Under high salt concentration the amount of proline in pots with and without gel amendment decreased significantly, however plants cultivated with the hydrogel accumulated significantly more amino acid than those without the gel.

The effect on the relative chlorophyll *a* content

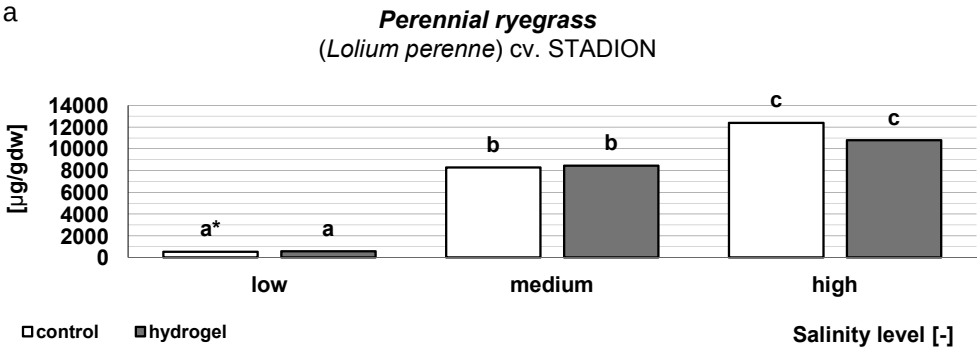
Under low salinity the relative chlorophyll *a* content in leaves of perennial ryegrass was not dependent on the hydrogel amendment: the values determined in pots with and without gel additive showed no significant difference (Fig. 2a). The influence of sorbent was observed, however under medium salt stress, where the amount of the dye in plants cultivated with gel was significantly higher than in the pots without it. In addition, the relative chlorophyll *a* content in plants cultivated with hydrogel under medium salinity was comparable to that observed at a low salinity level.

The hydrogel did not affect the relative chlorophyll *a* content in leaves of smooth-meadow grass (Fig. 2b). Under low salinity it was even significantly lower than in the control pots (those without gel). It was observed that in both sets of pots (with and without hydrogel amendment) the amount of dew decreased significantly as salinity increased.

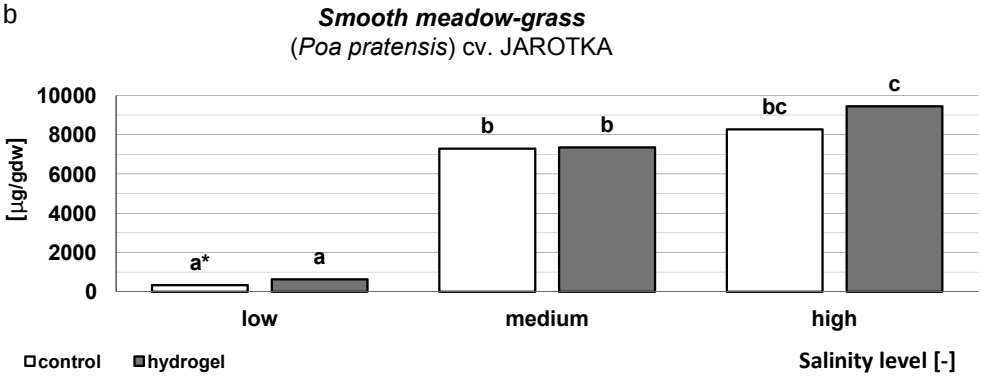
There was no significant difference in red fescue under low salt stress between pots with and without hydrogel (Fig. 2c).

The medium and high salinity measurements of relative chlorophyll *a* content for red fescue could not be conducted as the leaf width was insuficient. Under high salinity the smooth-meadow grass and perennial ryegrass were completely dried, meaning that, in this case too, measurements could not be made.

a



b



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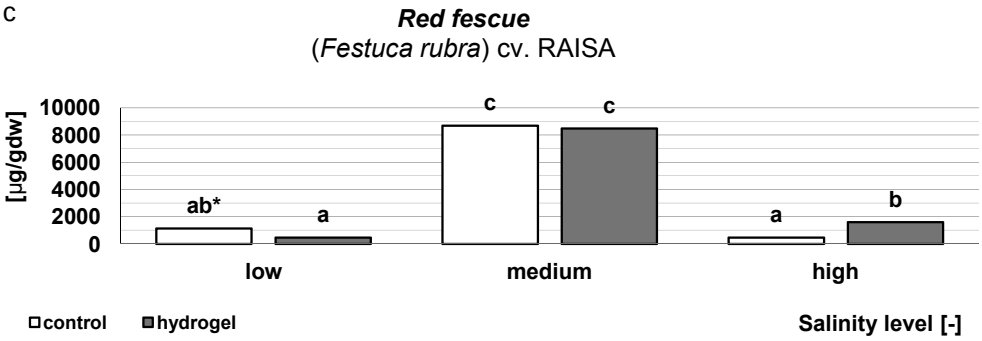
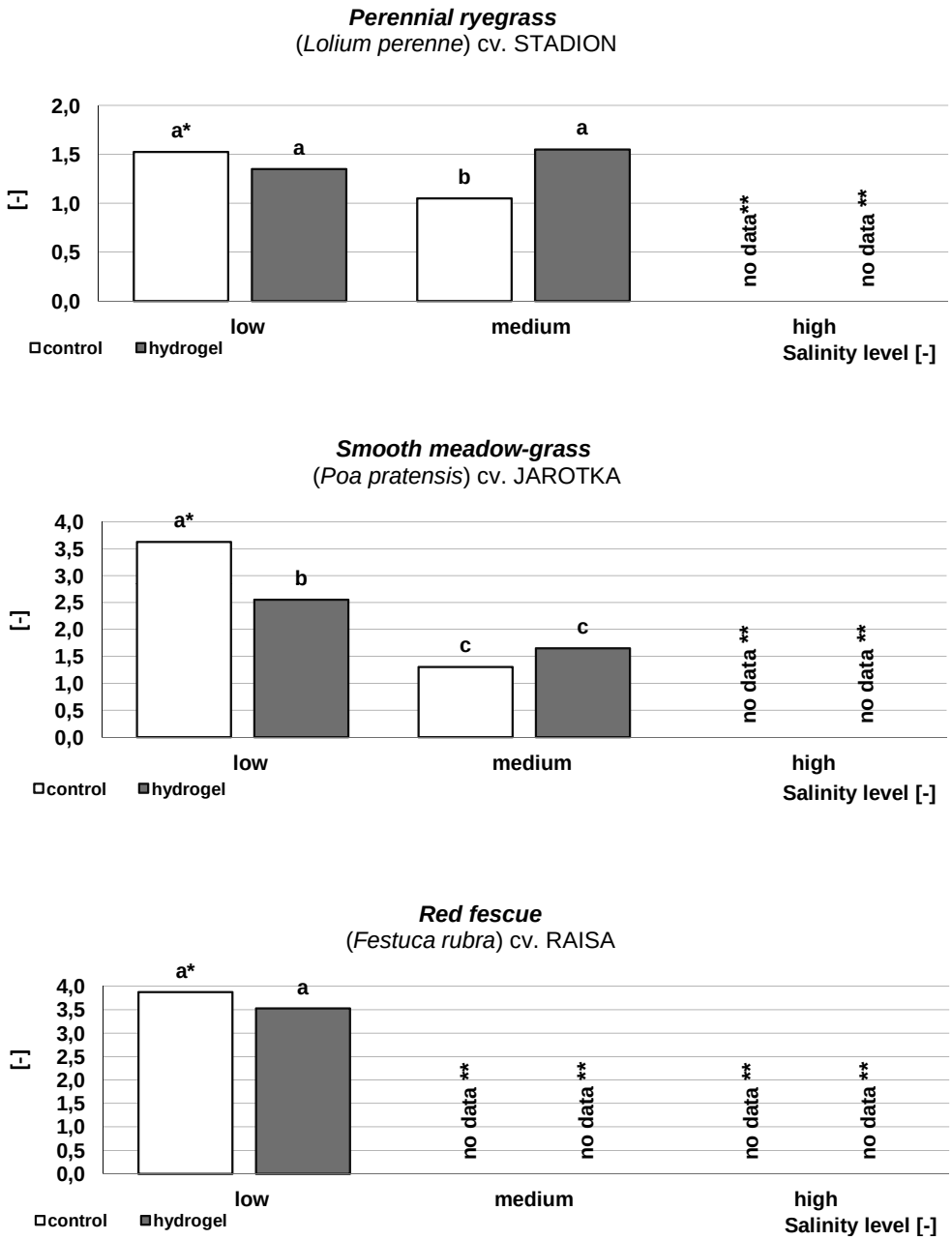


*Where the letters are different there is a significant difference between means.

FIGURE 1. Free proline accumulation in the leaves of the plants (a) perennial ryegrass (*Lolium perenne*) 'Stadion', (b) smooth-meadow grass (*Poa pratensis*) 'Jarotka' and (c) red fescue (*Festuca rubra*) 'Raisa' cultivated under saline conditions with and without hydrogel amendment



*Where the letters are different there is a significant difference between means.
**Measurements could not be conducted due to the weak condition of the plants.

FIGURE 2. Relative chlorophyll *a* content of the plants (a) perennial ryegrass (*Lolium perenne*) 'Stadion', (b) smooth-meadow grass (*Poa pratensis*) 'Jarotka' and (c) red fescue (*Festuca rubra*) 'Raisa' cultivated under saline conditions with and without hydrogel amendment

The effect on relative water content

The relative water content in leaves of perennial ryegrass cultivated under medium saline conditions was significantly smaller than in plants growing in low saline soil. This reaction was independent of the hydrogel amendments (Fig. 3a).

Salinity and hydrogel had no significant effect on relative water content in the leaves of smooth-meadow grass (Fig. 3b) and of red fescue (Fig. 3c).

The measurements for relative water content were not conducted under high salinity due to the weak condition of all of the plants.

DISCUSSION

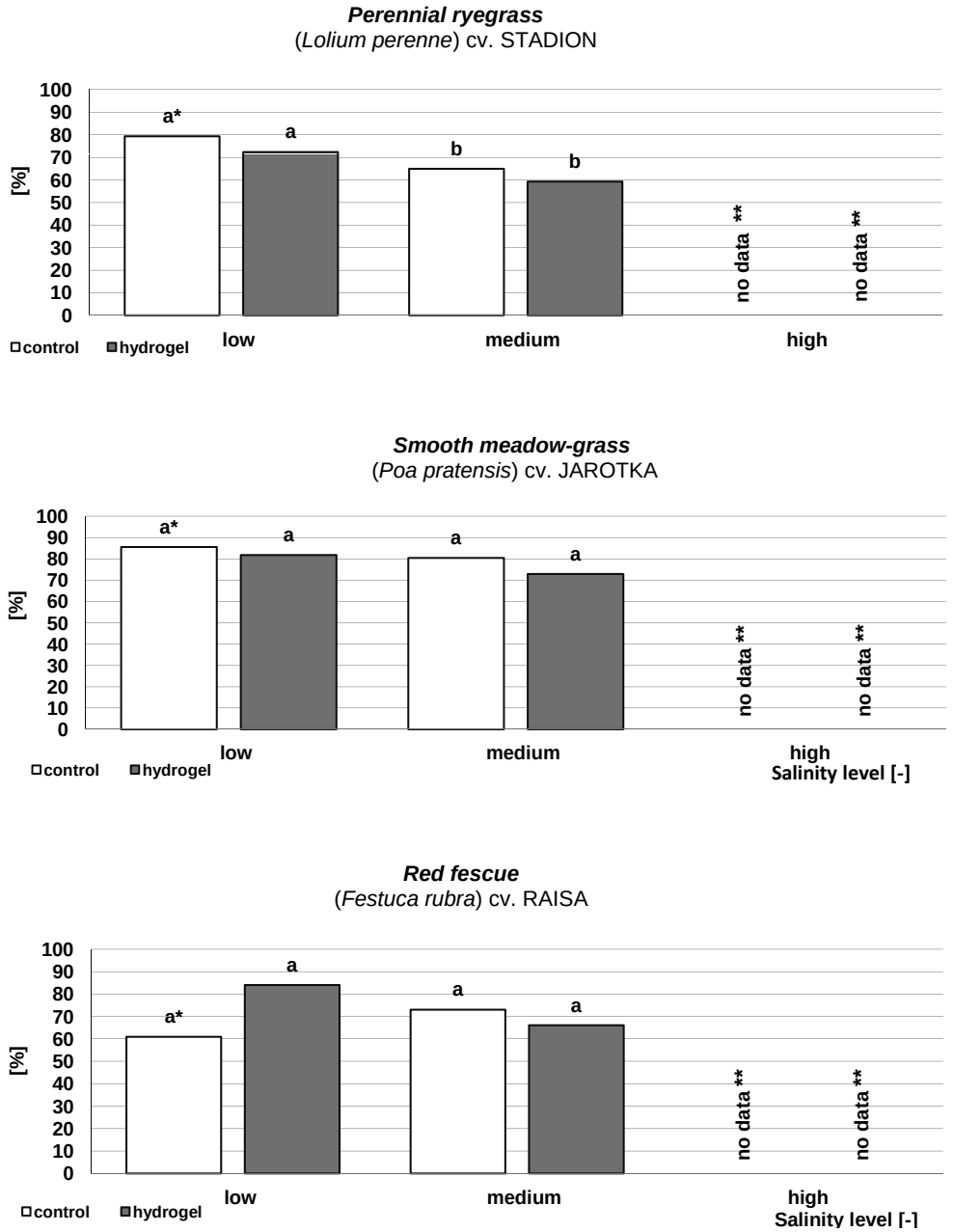
Proline accumulation is a protective mechanism observed in plants subjected to stress factors [Phang et al., 2008]. Under unfavourable conditions, such as salinity, their amount in plants grows [Mattioli et al., 2009], which was also observed in this study. The salt additive resulted in an increased proline accumulation in the leaves of perennial ryegrass and smooth-meadow grass. Similar results have been observed in other studies that investigated the impact of salinity on turfs [Huimin et al. 2001; Borowski, 2008] and different plant species [Mansour et al., 2005; Turan and Aydin, 2005].

The proline content in red fescue also increased under medium salt stress. Under high salinity this species was probably unable to defend itself against the stress, which meant that the proline accumulation under these conditions was significantly lower and comparable to that observed at a low level of salinity.

It was observed that hydrogel had no effect on proline accumulation in the saline conditions in all of the tested species. At all levels of salinity, the amount of amino acid in perennial ryegrass and smooth-meadow grass cultivated with the sorbent was comparable to the amount of amino acid in the plants grown in the substrate without hydrogel. The gel sorbent influenced red fescue only at high salinity. In these conditions the proline content with the hydrogel amendment was significantly greater than in the control group. The sorbent had no impact, though, under low and medium saline conditions.

The data on proline accumulation do not explain the results from the previous studies, which indicated that hydrogel enhanced the fresh matter of perennial ryegrass, red fescue and smooth-meadow grass in conditions of low and medium salinity [Hadam et al., 2011a and 2011b; unpublished studies]. It can therefore be suggested that proline accumulation is not affected by the hydrogel in the grasses. Biomass accumulation is, however, strongly associated with the intensity of photosynthesis, which, in turn, is dependent, among other factors, on the amount of chlorophyll [Santos, 2004]. On the other hand, exposure of plants to salt stress may reduce the content of this dye [Stępień and Kłobuś, 2006].

The results presented demonstrate that the influence of hydrogel on relative chlorophyll *a* content under saline conditions depends on the species tested. In the leaves of smooth-meadow grass the amount of photosynthetic dye decreased as salinity increased in all pots – with or without the gel amendment (Fig. 2b).



*Where the letters are different there is a significant difference between means.
** Measurements could not be conducted due to the weak condition of the plants.

FIGURE 3. The relative water content of the plants (a) perennial ryegrass (*Lolium perenne*) 'Stadion', (b) smooth-meadow grass (*Poa pratensis*) 'Jarotka' and (c) red fescue (*Festuca rubra*) 'Raisa' cultivated under saline conditions with and without hydrogel amendment

In contrast, while in the subsoil without the hydrogel the amount of relative chlorophyll *a* content in the leaves of perennial ryegrass decreased as salinity increased, the amount of the dye in this species cultivated with the gel amendment was stable up to medium salinity (Fig. 2a). This may suggest that under medium salt stress hydrogel increased the fresh matter production of perennial ryegrass by creating the appropriate conditions for photosynthesis.

The photosynthetic activity of perennial ryegrass enhanced by the gel amendment may also explain the decreasing relative water content observed in this species (Fig. 3a). The stomatal apparatus is opened during photosynthesis, which results in the assimilation of CO_2 and increased transpiration [Stępień and Kłobuś, 2006]. On the other hand, the decreasing RWC in the leaves of perennial ryegrass cultivated without sorbent under medium saline conditions could be the effect of another mechanism. The water available to *Lolium perenne* could have been reduced due to a high concentration of salt in the medium. A phenomenon such as this is often expressed in turgor loss and a decreased relative water content [Cicęk and Cakirlar, 2002]. The results presented are also consistent with other studies that have observed that changes in relative water content under saline conditions depend on the species [Abt-el Baki et al., 2000; Morant-Manceau et al., 2004]. Whereas salinity had an impact on relative water content in the leaves of perennial ryegrass, it did not affect this parameter in the smooth-meadow grass (Fig. 3b). As hydrogel had no influence on the RWC of this species it is possible that *Poa pratensis* had activated a protective mechanism against the stress prompted by stomatal closure [Brugnoli and Lauteri, 1990].

The result obtained above was consistent with studies of the powerful effects of salinity on plants [Neelam and Ajah, 2005]. Salt stress was the main factor affecting the physiological and biochemical processes found in the grass species tested. Under conditions of low and medium salinity it was the perennial ryegrass and smooth-meadow grass especially that responded to stress with a number of protection mechanisms.

Under saline conditions support for the influence of hydrogel on the biochemical and physiological reactions presented was, however, weak. The sorbent only enhanced relative chlorophyll *a* content in the leaves of perennial ryegrass under medium salt stress. This reaction was not found in the case of the other species tested. The proline accumulation and relative water content were not affected by the sorbent. It may therefore be concluded that an assessment of other biochemical and physiological indicators for the evaluation of hydrogel impact on turfs under saline conditions, with consideration given to larger amounts of perennial ryegrass, smooth-meadow grass and red fescue cultivars, is very necessary. The studies should also be confirmed by field trials.

CONCLUSIONS

1. Salt stress influenced all of the selected physiological and biochemical processes in the grass species tested – independently of the hydrogel amendment.

2. As salinity rose in the case of perennial ryegrass and smooth-meadow grass, so a higher amount of protective protein/proline was accumulated.
3. Proline accumulation in red fescue increased only under medium salinity. High salinity was already too great a stress for this species and it reduced osmoprotectant production.
4. The relative water content in the leaves of smooth-meadow grass and red fescue was stable under low and medium salinity.
5. A high salinity level had a particularly negative effect on all of the species tested, which meant that their relative water content and relative chlorophyll *a* content could not be measured.
6. Hydrogel had almost no effect on the proline accumulation of the grass species tested and cultivated under saline conditions. It did increase the relative chlorophyll *a* content of perennial ryegrass under medium salt stress, but had no influence on the amount of the dye in the leaves of smooth-meadow grass and red fescue. The sorbent did not affect the relative water content of any of the species tested.
7. The results did not confirm that hydrogel is an effective soil conditioner on lawn grass areas, that will improve their tolerance to saline conditions. The data presented should be tested in studies of other biochemical and physiological indices with consideration given to additional cultivars of perennial ryegrass, smooth-meadow grass and red fescue. The results should also be confirmed by field trials.

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Streszczenie: *Fizjologiczne oraz biochemiczne reakcje na zasolenie u wybranych gatunków traw uprawianych z dodatkiem hydrożelu (badania wstępne).* Celem badań była ocena wpływu hydrożelu na akumulację wolnej proliny, zawartość chlorofilu *a* i względną zawartość wody w liściach (RWC – ang. relative water content) traw narażonych na działanie stresu solnego. Doświadczenie wazonowe prowadzono przez cztery miesiące w warunkach szklarniowych. Wazony z hydrożelem oraz wazony kontrolne (bez dodatku sorbentu) obsiewano w siewach czystych jednym z trzech gatunków testowych życicą trwałą, wiechliną łąkową i kostrzewą czerwoną. Stres solny symulowano po mięsiącu od wysiewu, traktując rośliny trzema różnymi stężeniami soli do odladzania ulic. Zasolenie wpłynęło na wzrost akumulacji proliny i spadek względnej zawartości chlorofilu *a* u wszystkich testowanych gatunków. Stres solny nie spowodował obniżenia RWC w liściach wiechliny łąkowej i kostrzewy czerwonej, u życicy trwałej zaobserwowano jednak znaczący spadek RWC. Hydrożel nie wpłynął na większość oznaczanych parametrów, z wyjątkiem względnej zawartości chlorofilu *a* u życicy twałej utrzymywanej podłożu o silnym zasoleniu. Uzyskane wyniki pozwoliły stwierdzić, że zasolenie było głównym czynnikiem determinującym oznaczane parametry. Wpływ hydrożelu na fizjologiczno-biochemiczne reakcje traw był natomiast nieznaczny. Uzyskane wyniki należy potwierdzić w badaniach terenowych, a także w doświadczeniach z wykorzystaniem innych parametrów fizjologiczno-biochemicznych z uwzględnieniem większej liczby odmian badanych gatunków.