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EFFECTS OF LIGHT INTENSITY AND SPECTRAL COMPOSITION ON THE GROWTH AND METABOLISM OF SPINACH (*SPINACIA OLERACEA* L.)

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Abstract: Spinach rich in proteins, minerals (Fe, K) and antioxidants (vitamin C) is often cultivated in greenhouses under artificial light. In this study, the effects of light intensity and spectral composition provided by light emitting diodes (LEDs) was studied on the yield quality and quantity of spinach. Plants were grown under 3 different light intensities (100/200/300 μmol $m^{-2}s^{-1}$) and 3 spectral compositions (white/white completed with blue or far-red) for 4 weeks. Then plant weight, leaf number and area, photosynthetic activity and pigment composition of leaves were determined. The leaf quality was characterized by measurements of protein, starch, soluble sugar and ascorbate contents of leaves. Moreover, minerals (K, Fe and NO_3^-) were also determined. While the biomass production was mainly determined by the light intensity, the yield quality is influenced significantly by the spectral composition. When the white light was supplemented with blue, high protein and ascorbate content was achieved. When far-red was added to white light, elevated sugar and Fe accumulation was observed in the leaves, while the K content decreased. In order to reach the hight quality and quantity of food production, not only the light fluence, but also the spectral composition should be regulated.

Keywords: LED lighting, spinach, hydroponics, artificial light

INTRODUCTION

Light is one of the most important environmental factors that affect plant growth and development. Through photosynthesis and the operation of photoreceptors, both the light intensity and the spectral composition have broad regulatory effects on the morphogenesis, such as leaf growth and expansion, on many

physiological and metabolic processes in plants, which finally determines the main characteristics of plants: the appearance and quality.

With the use of artificial light in phytotrons, greenhouses and in modern plant factories, the light environment of plants inherently changes compared to the natural light situation. This influences multiple aspects of plant functioning, including photosynthesis, photomorphogenesis, water relations, nutritional quality, biomass and yield production and quality. In most cases, the metal halide, high-pressure sodium (HPS) lamps and/or fluorescent lamps are used either as supplementary or sole-source (Kim *et al.* 2004). They are often inefficient for plant cultivation due to their fix and rather different spectral distribution than the solar light (Morrow 2008; Darko *et al.* 2014). The plants grown under these artificial light conditions often shows many symptoms of suboptimal light condition, such as internode elongation, shift in flowering time, decrease of fertility and low quality and quantity of crop production. In addition, these lamps have high energy consumption and high operation temperature make them economically inefficient (Kim *et al.* 2004, Morrow 2008).

Light emitting diodes (LED) represent an innovative artificial lighting source in plant cultivation. LEDs have low energy consumption, long-lifetime and small size, high fluence and variable spectral characteristics, which make them better suited to crop production than other artificial lighting systems (Morrow 2008). Therefore, the application of LEDs in agricultural/horticultural practice is increasing continuously. Cultivation of plants under LED lighting in environmentally controlled rooms enables standard vegetable production regardless of the weather conditions. The aim is to achieve high productivity and/or high vegetable quality without excess energy consumption. Changes of spectral composition could provide a solution for improving the yield and quality of crops.

Spinach (*Spinacia oleracea)* plants are widely cultivated in greenhouses throughout the world. This leafy green is rich in nutrients such as protein, dietary fiber, vitamins and folate and minerals such as K, Ca, Mg, Mn, and Fe, which may play an important role in human nutrition and diet. Most studies on the effect of light intensity and spectral composition delivered by LEDs on the quality of leafy vegetables have focused on biomass

production, leaf area and branching, while less is known about how LEDs affect the mineral composition and nutritional quality (Lin *et al.* 2013). In addition, spinach is less characterized than lettuce (Burattini *et al.* 2017).

This paper aims to determine whether similar productivity and/or quality can be achieved even under limited light intensity by changing of spectral composition of light. Therefore, spinach plants were cultivated under white light at low (100 μmol $m^{-2}s^{-1}$), medium (200 μmol $m^{-2}s^{-1}$) or high (300 μmol $m^{-2}s^{-1}$) light intensities and at white light (200 μmol m^{-2} s^{-1}) with far-red or blue light supplementation in hydroponic growth conditions. The biomass production, leaf architecture will be compared together with the physiological response of plants , which was determined through monitoring the photosynthetic activity and chlorophyll contents of leaves. The leaf quality was estimated by determination of leaf protein, starch, and soluble sugar contents, by the measurements of nitrate, ascorbate, iron and potassium contents of plants grown under different light environment. We hope that this complex research brings a better understanding of the relationship of light conditions and yield quality and can provide further information for efficient indoor plant cultivation.

MATERIALS AND METHODS

Plant cultivation

Spinach (*Spinacia oleracea* L.) cv. Sparrow RZ F1 hybrid variety was used in the experiments. The seeds were germinated at 5°C for three days, then at 10°C for seven days and finally at 15°C for further 3 days. The plantlets were put into Jiffy coco plugs (Jiffy-7C, 50mm std, Jiffy Products S.L. Ltd, Sri Lanka) and placed in phytotron growth chamber equipped with LED light sources and automatic control system for circulation of nutrient solution in the Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences. The light characteristics used in the experiments are summarized in *Table 1*. Briefly, 3 different spectral compositions (White light, blue dominant white light and white light complemented with far-red radiation) at the same light intensity (200 μmol $m^{-2}s^{-1}$) and 3 different light intensities (low: 100 μmol $m^{-2}s^{-1}$, medium: 200 μmol $m^{-2}s^{-1}$ or high: 300 μmol $m^{-2}s^{-1}$)

were used for spinach cultivation in hydroponic growth system using diluted Hoagland solution described by Pál *et al.* (2005). The pH and the ionic strength of the nutrient solution was kept between 6.0-6.5 and EC 1.1-0.9 respectively. Plants (at least 15 plants of each light regimen) were grown for 4 weeks at 20/15°C day/night temperature and 75% of humidity. The growth and development of spinach plants were monitored twice a week, and the measurements were started in the fourth week.

Table 1. The light spectral composition used in the experiment.

	Light characteristics				
	White 300	**White 200**	**White 100**	**White + FR 200**	**White + Blue 200**
PAR (400-700nm) μmol m^{-2} s^{-1}	310	202	100	205	208
Ratio:					
Blue: Red	0.208	0.200	0.184	0.189	4.86
Red: Far-red	8.0	8.8	10.2	1.0	9.2
Blue: Far-red	1.7	1.7	1.8	0.18	44.7

12h/12h photoperiod were used.

Determination of the photosynthetic activity of leaves

The photosynthetic activity of plants was determined on intact leaves with a Ciras 3 Portable Photosynthesis System (Amesbury, USA) instrument using a narrow leaf area (4.5 cm^2) chamber equipped with a chlorophyll *a* fluorescence module. Since the Ciras 3 uses LED modules, the photosynthetic activity of leaves was measured under the same light conditions as was found in growth chambers. The net assimilation rate (A), stomatal conductance (gs), intracellular CO_2 concentration (Ci) and transpiration (E), as well as the effective quantum yiled of PS II [Y(II)] were determined at the steady state of photosynthesis by the use of 400 μL L-1 CO_2 level.

Determination of biomass production, leaf area and sample collection for the analytical investigations

At harvest, the plant weight, leaf number and leaf area were determined in order to characterize the biomass production of plants. 10 plants of each light regimens were measured. The leaf area was determined using an Area Meter 500 from ADC

Bioscientific Ltd. Then, samples for biochemical investigations were collected. Thus, 3 leaf discs (with 1.3 cm diameters) were collected from 5 plants (5 x 3 leaf discs) of each light regimen for determination pigment composition of leaves. Also, 5 x 0.2g samples were collected for determination of protein, starch, soluble carbohydrate and ascorbate content of leaves, respectively. In addition, samples were collected for determination of mineral (K, Fe and nitrate) content of leaves. The leaf samples were frozen immediately and stored at -80 °C until utilization.

Analytical investigations

Chlorophyll *a* and *b*, as well as the carotenoid contents of leaf discs, were determined using a Cary-100 UV-Vis spectrophotometer (Varian, Middelburg, Netherlands) after extraction of leaf discs in 80% acetone, according to the method of Lichtenthaler (1987).

The total protein content was determined according to the method of Bradford using bovine serum albumin as standard (Kruger 2009). Total soluble carbohydrates were extracted and measured following the method of Antron as described in Sinay and Karuwal (2014). The starch content was determined from twice-washed and dried pellet remaining after extraction of total soluble sugars according to the method of Thayumanavan and Sadasivam (1984).

Similar isolation protocol was used for determination of K, NO_3^-, Fe and ascorbic acid contents. All compounds were diluted with MQ water (1:10). The measurements were performed by the use of RQflex plus 10 (Merck KGaA 64271 Darmstadt, Germany) instrument according to the manufacturer's instructions for K: 1.17945.000; NO_3^-: 1.14761.0002; Fe: 1.14761.0002 and Ascorbic acid: 1.16981.000.

Statistical analyses

The experiments were repeated 3 times. In each experiments at least 15 plants were grown under each light regimens and the samples were collected from randomy selected plants. The measuerements were repeated at least in 5 biological replicates. The data were analysed by SPSS 16.0 statistical program and Tukey's *post hoc* test were used to determine differences between treatments. Different letters indicate significant differences at the $P < 0.05$ level.

RESULTS

Morphological responses of spinach cultivated under different light regimens

To characterize the plants grown under different light environment several morphological parameters, such as leaf number, leaf area, and leaf mass were determined (*Figure 1*). All leaf mass, leaf area and leaf number increased with increasing of white light intensity (*Figure 1A-C*). However, the specific leaf area, the ratio of leaf area to leaf mass, decreased with increasing light intensity (*Figure 1D*). It is due to the fact that the increase of light intensity increased leaf area of plants with relatively less extent (3.23 and 4.47 fold change) than the leaf mass (they was 4.91 and 8.61 fold change). When the spectral composition of light was compared in grown of spinach plants at 200 μmol m^{-2} s^{-1}, the supplementation of white light with FR resulted in a slightly higher expansion of leaves and longer stems (data not shown) than without far-red application. Inversely, the blue light decreased the leaf area and created a compact and short plant. While the effect of spectrum in leaf area and weight were not significant, the specific leaf area increased under far-red and decreased when blue light was applied (*Figure 1*).

Photosynthetic properties of spinach under different light environment

Evidently light regulates the photosynthetic processes. As the light intensity increased, so did the CO_2 assimilation rate (Pn), stomatal conductance (gs) and transpiration (E) in spinach leaves, meanwhile the intracellular CO_2 level decreased (*Figure 2*). On the other hand, not only the intensity of the light, but the modification of spectral composition also influenced the CO_2 assimilation processes: the blue light reduced and the FR light slightly (but not significantly) stimulated the CO_2 assimilation capacity of plants (*Figure 2A*). However, the stomatal conductance changed inversely, it increased to blue and slightly decreased to far-red, when they were compared to white light having the same light intensity (*Figure 2B*).

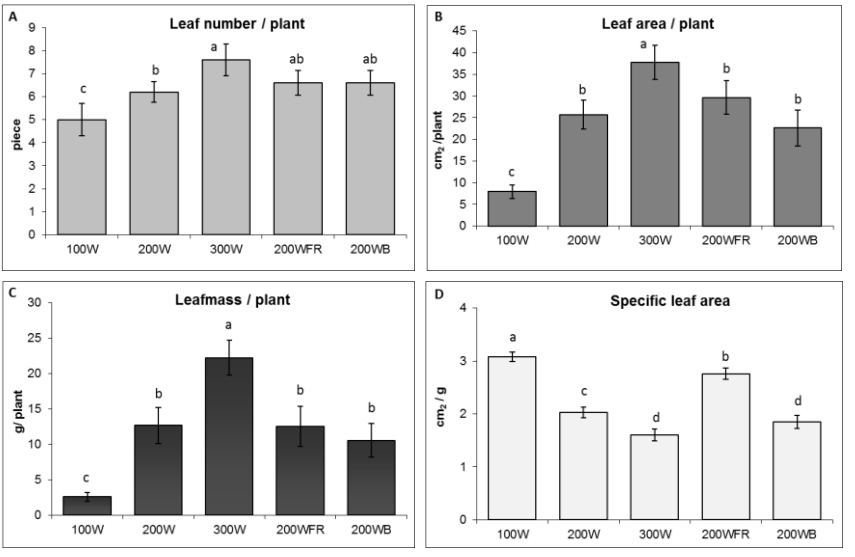

Figure 1. Effect of the light intensity and spectral composition on the growth parameters of spinach. A: Leaf number; B: Leaf area: C: Leaf mass; D: Specific leaf area. Values are the mean ± STD of 10 biological replicates per light treatment. The different letters indicate statistically significant differences at $P < 0.05$, using Tukey's *post hoc* test.

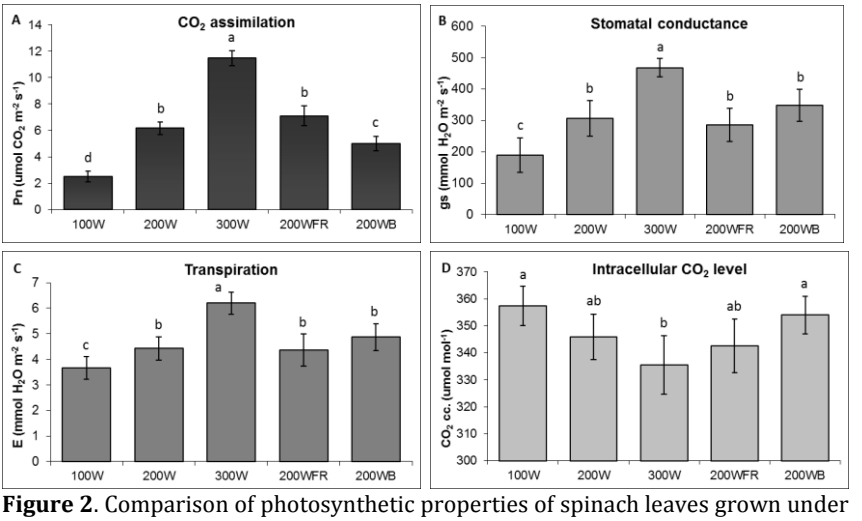

Figure 2. Comparison of photosynthetic properties of spinach leaves grown under different light regimens. A: Net CO_2 assimilation rate (Pn); B: Stomatal conductance (gs); C: Transpiration (E) and D: Intercellular CO_2 level. Values represent the means (± STD) of 5 independent measurements per light treatment. The different letters indicate statistically significant differences at $P < 0.05$, using Tukey's *post hoc* test.

The photosynthetic electron transport processes was also investigated. The effective quantum yield of PSII, Y(II), determined at steady of photosynthesis indicates the ratio of the number of photons utilized photochemically/total number of quanta absorbed. Typically, the conversion ratio of absorbed light energy to photochemistry in PS II decreased in parallel to the increase of light intensity in spinach leaves, which was indicated by the lower Y(II) values (*Figure 3*). Lower Y(II) was also measured in leaves grown under the blue light as compared to those plants grown under white light at the same light intensity with or without FR application. In fact, Y(II) values were similar in spinach leaves grown under blue light at 200 µmol $m^{-2}s^{-1}$ and under white light at 300 µmol $m^{-2}s^{-1}$.

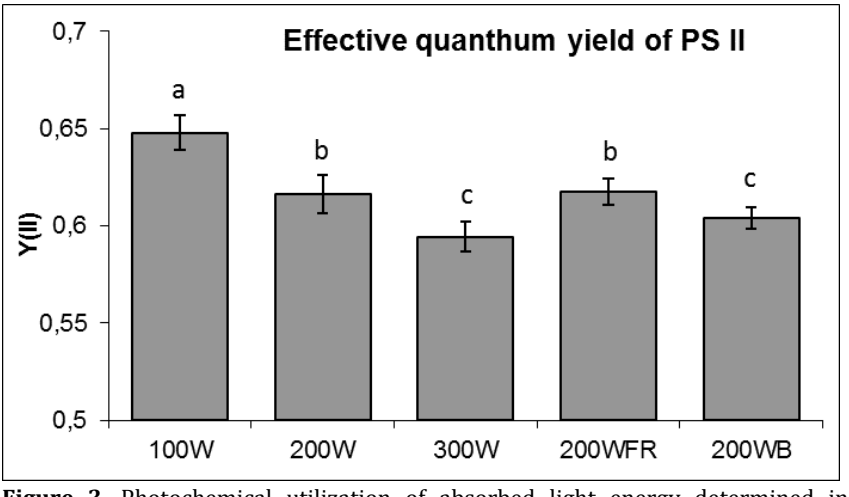

Figure 3. Photochemical utilization of absorbed light energy determined in spinach leaves grown under different light conditions at steady state level of photosynthesis. Y(II) – effective quantum yield of PSII photochemistry. Values represent the means (± STD) of 5 independent measurements per light treatment. The different letters indicate statistically significant differences at $P < 0.05$, using Tukey's *post hoc* test.

When the pigment composition of leaves grown under different light regimens were compared, two interesting tendency was observed: the pigment composition hardly varied in the function of light intensity, but changed according to the spectral composition (*Table 2*). The lowest chlorophyll *a* and b contents were detected in plants grown under blue light, followed by the highest light

intensity, while the highest chlorophylls were detected when FR was applied.

These results suggest that both the light fluence and spectral composition determines the photosynthetic properties of plants.

Table 2. Effect of the light intensity and spectral composition on chlorophyll (a+b) and carotenoid contents of leaves.

	Pigment composition				
µg/g FW	**White 300**	**White 200**	**White 100**	**White + FR 200**	**White + Blue 200**
Chl a+b	1498±126b	1853±189a	1760±186a	1855±205a	1390±215b
Carotenoids	332±47a	378±37a	334±32a	329±37a	300±42a
Chl a/b	2.97±0.33a	3.27±0.20a	3.44±0.22a	3.08±0.21a	3.41±0.20a
Chl/Car	4.51±0.38b	4.90±0.36b	5.27±0.34ab	5.63±0.28a	4.62±0.44b

Effect of light intensity and spectral composition on the yield quality of spinach leaves

The leaf quality was estimated by determination of leaf protein, starch, and soluble sugar contents and by the measurements of nitrate, ascorbate, iron and potassium contents of plants grown under different light environment (*Figure 4*).

The primary metabolites, such as the soluble sugars, starch and proteins increased with increasing light intensity in spinach leaves (*Figure 4A-C*). It was mainly due to the light-dependent increase of the photosynthetic activity. Blue light stimulated the protein accumulation, while decreased significantly the amount of soluble sugars and starch in spinach leaves as compared to white light used at the same light intensity. The supplementation of white light with far-red resulted in a significant accumulation of soluble sugars, while the proteins level decreased to the similar value found in leaves grown under low (100 µmol $m^{-2}s^{-1}$) light intensity (*Figure 4A*).

Spinach is an excellent source of vitamin C. The amount of ascorbate was hardly affected by light intensity and spectral composition (*Figure 4D*). Significant increase in ascorbate content was detected only when high proportion of blue light was applied.

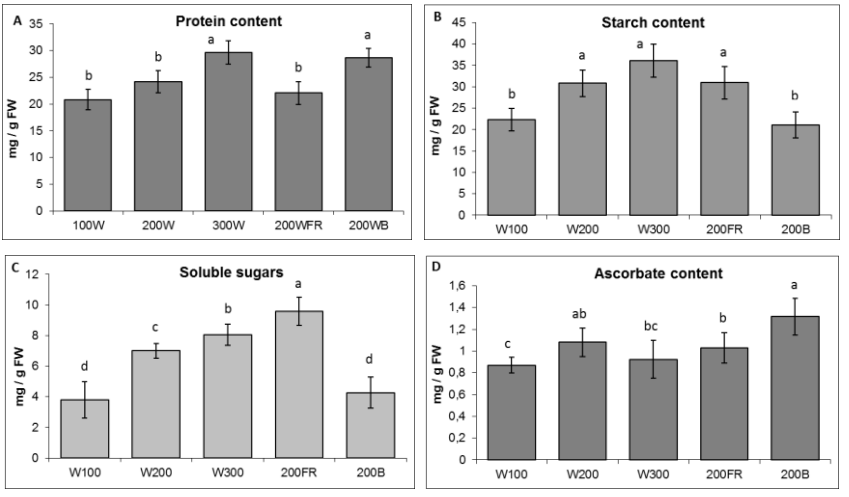

Figure 4. Production of primary and secondary metabolites in spinach leaves grown under different light regimens. A: protein content; B: starch content; C: soluble sugar content; D: ascorbate content. Values are the mean ± STD of 5 biological replicates per light treatment. The different letters indicate statistically significant differences at $P < 0.05$, using Tukey's *post hoc* test.

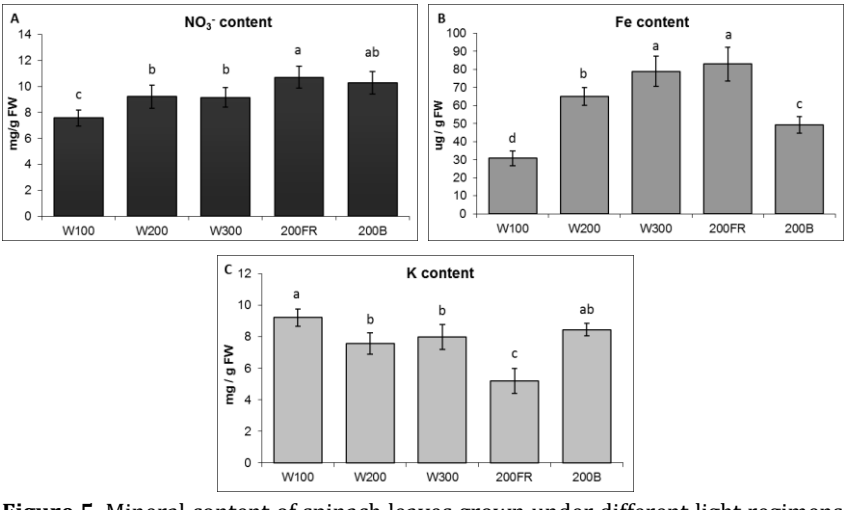

Figure 5. Mineral content of spinach leaves grown under different light regimens. A: nitrate content; B: iron content; C: potassium content; Values are the mean ± STD of 5 biological replicates per light treatment. The different letters indicate statistically significant differences at $P < 0.05$, using Tukey's *post hoc* test.

Among minerals, the K, Fe and NO_3^- content of leaves were determined (*Figure 5*). Surpisingly, only the accumulation of Fe showed ligh intensity dependent changes in spinach (*Figure 5B*). Neither the NO_3^- nor the K content showed similar tendency (*Figure A* and *C*). The spectral composition influenced both the iron and the potassium content of leaves. The blue light reduced the accumulation of Fe, while far-red decreased the K content of leaves (*Figure 5B* and *C*). All these modifications affects the yiled quality of spinach.

DISCUSSION

Cultivation of leafy plants under artificial lighting becomes more and more important in the future to produce high quality and quantity of foods throughout year. They are influenced by both light fluence and spectral composition. Providing variable fluence and spectral composition, LEDs give a unique possibility to optimize the growth conditions.

Through activation of photosynthetic processes, the increase of light intensity forces the biomass production which in case of leafy plants, manifests in the increase of leaf number, area and mass. In our experiments it seemed that the biomass production was mainly determined by the light intensity while the spectral composition was less influenced. However, the increase of photosynthetic activity induced leaf expansion less than producing assimilates, resulting in a decrease of sepcific leaf area. This phenomenon (e.g. sepcific leaf area decreased with increasing light intensity) was also observed by Xiao-Xue *et al.* (2013) and Urrestarazu *et al.* (2016). In the opinion of Gommers *et al.* (2013), this response to light could help plants to increase the efficiency of light capture and maximize carbon gain at low light intensity (Gommers *et al.* 2013). It can help plants under shade environment. Our results can support it, since the specific leaf area (cm^2/g) also increased when the white light was supplemented with far-red light. However, it should be mentioned that the application of far-red light resulted in weak and thin leaves (data not shown). Inversely blue light inhibited the leaf expansion thus decreased the specific leaf area and increased the leaf thickness (it was observed, but not measured), similarly as was found at higher light intensity by us and by Meziane and Shipley (1999).

Photosynthetic pigments absorb and convert the light energy into chemical energy via complex photosynthetic machinery. Blue and red light play an active role in photosynthesis and also stimulate chlorophyll and carotenoid biosynthesis (Xiao-Xue *et al.* 2013, Wang *et al.* 2015). In the present study, light intensity caused the largest effects on photosynthetic processes, which were manifested in elevated CO_2 assimilation capacities, induction of stomata opening and transpiration, and also in the decreased ratio in the conversion of absorbed light energy to photochemistry in PS II, as demonstrated by the low Y(II) value. However, not only the light intensity but also its spectral composition affects CO_2 assimilation processes. The blue light decreased the CO_2 assimilation, while stimulated the stomatal opening. Kim *et al.* (2004) also found that the high proportion of blue light decreased the CO_2 assimilate rate, in spite of the fact that blue light stimulated the stomatal opening. The blue light-induced stomatal movement is explained by (Kinoshita and Hayashi 2011), who found that the blue light-induced stomatal opening is mediated by phototropins (activate by blue light) through the activation of the plasma membrane H^+-ATPase in guard cells. When the effects of blue light were studied on cucumber plants grown under different combinations (0-100%) of red and blue light, Hogewoning *et al.* (2010) was found that the blue light provided 'sun-type' characteristics of leaves even at the relatively low growth irradiance. Blue light overexcited the PS II reaction centres resulting in a lower photochemical utilization of absorbed light energy at PS II, similarly as was found under higher light intensity. We also found the decrease of Y(II) when blue light was applied. A relative overexcitation of PSII can results in an imbalance of photosynthetic electron transport between PS II and PS I, which can decrease the efficiency of the photosynthetic processes. In addition, the blue light-induced 'sun-type' characteristics of leaves was manifested also in the decrease of chlorophylls in spinach leaves, similarly as was found by Zhang *et al.* (2016) and Monostori *et al.* (2018). To ensure the optimal growth a fine balance between the blue and red ratio is necessary to provide equilibrated and efficient utilization of absorbed light energy between the PS II and PS I.

While it is widely understood that light intensity can positively affect the accumulation of assimilates, the effects of light quality is

more complex. In this way, the higher light intensity was used, the more primary assimilates including proteins, starch and sugars were produced in spinach leaves. But, light quality also affected the production of primary and secondary metabolites in spinach leaves. Blue light significantly increased protein and ascorbate contents of leaves, while less sugar and starch were accumulated in these plants. In fact, when the plants were grown under white light supplemented with blue, as high protein and ascorbate content was obtained at 200 μmol $m^{-2}s^{-1}$ light intensity as plants achieved under higher 300 μmol $m^{-2}s^{-1}$ light intensity. On the other hand, when the light was completed with far-red light the spinach leaves contained more sugars and Fe than in any other cases. The blue-light induced shift in protein and ascorbate metabolism was also detected in lettuce (Chen *et al.* 2016). However, the reason of protein accumulation is controversial. As the nitrate provides main N source in proteins of plants, it was suppose that the high protein content is originated from the elevated nitrate uptake. However, when Zhang *et al.* (2018) compared the effect of different LEDs (white, red and blue in different proportion) on the nitrogen metabolism in lettuce, they found that both red and blue light promoted the N assimilation by improving the activity of the N-metabolism-related enzymes such as nitrate and nitrite reductases, in lettuce. In other studies, it was found that the nitrate reductase activity was stimulated by red light. Samuolienė *et al.* (2009) used high-intensity red LEDs treatment 3 days prior to harvets to reduce the nitrate content in lettuce. It can be useful, especially in case of leafy plants. However, our results can not prove these findings since the accumulation of nitrates did not change either by light intensity (except at low light, when the low transpiration reduced the nitrate and any kind of ion uptakes) or by spectral composition. Nevertheless, the physiological significance of LED-induced regulation of nitogen metabolism remained undercharacterized.

CONCLUSION

Although the mechanisms of the changes in spinach growth under LED lighting are not well understood yet, the results showed that LED light can be used to modify the plant growth and metabolism in spinach. Via the variable fluence and spectral composition, the LEDs provide a unique possibility to change both the light intensity

and the spectral composition during the lifetime of plants in order to ensure the best light combinations resulting in the highest nutritional values of leafy plants in indoor plant cultivation. On the bases of the results it seems that white light at adequately high intensity (300 μmol $m^{-2}s^{-1}$) can ensure the optimal growth for spinach to reach the harvest quality in 4 weeks.

At the early developmental stage the application of far-red can induce leaf expansion, while before harvest the increase of the proportion of blue light can stimulate the accumulation of proteins and ascorbate content, which can improve the leaf quality. However, the appliaction of red or far-red light can increase the sugar content, which imporve the sweetness of leaves. Anyway, although the LEDs are suitable for manipulation the growth and metabolism in plants, it is unlikely that only one spectral combination can ensure the optimal growth and yield quality throughout the life cycle.

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WILD PEARS OF ARMENIA: DIVERSITY, ENDEMICS AND CONSERVATION

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Abstract: The paper presents the wild pear diversity and distribution in Armenia with focus on endemism. As result of the fieldwork, literature and herbarium studies six main "hotspots" for pear diversity are identified within the country. The list of pear species for each site are given. The questions and challenges in research and conservation of *Pyrus* L. in Armenia are discussed - the main difficulties are linked with the proper evaluation of the morphological polymorphism in populations and ongoing hybridization processes within the genus. It is mentioned, that there is strong need for critical taxonomic review of *Pyrus* sp. in Armenia and so the taxonomic status of some endemic pear species need to be clarified.

Keywords: *Pyrus* sp. in Armenia, wild pears, flora of Armenia, endemic pears, *Pyrus gergerana, Pyrus daralagezi*

INTRODUCTION

Armenia is located in the Caucasus Ecoregion – one of the Planet's biodiversity hotspots. This small mountainous country is remarkable for its rich and diverse flora and vegetation. Flora of Armenia includes about 3800 vascular plant species, 144 of which are local endemics (Anonymus 2014). Rare species, genetic diversity within a number of taxa, including wild relatives of cultivated plants and habitats of regional and global conservation concern are of particular scientific interest and conservation importance. *Pyrus* L. is one of the most interesting genera in this context: there are 32 pear species in the flora of Armenia 12 of which are endemics of Armenia and 6 are endemics of the Southern

Transcaucasia. 18 from all the known pear species were described from Armenia (Akopian 2007).

Pyrus L. represents deciduous tree and shrub species, estimated number of which differs considerably, ranging from 20 to 80 species. They are distributed in temperate Eurasia, reaching the Atlas Mountains in North Africa as well as Japan and South China. The centers of diversity for *Pyrus* are in the mountainous regions of East Asia, the Mediterranean, and South-West Asia, including the Caucasus (Korotkova *et al.* 2018).

Transcaucasia is considered as one of the speciation and evolution centers for pear species (Gabrielyan 1988). Gladkova (1990) mentions the Caucasus to be the main center for diversity of wild pears. Wild pears in the Caucasus form two main ecological groups: mesophytic and xerophytic and, accordingly, found in arid open woodlands and in deciduous forests, where occur mixed with oak or form small groves on forest glades and by the forest edges. Wild pears actively reproduce on open grasslands left after felling as pear is one of the "pioneers" of the forest vegetation (Sokolov 1954). One can also see sparse pear communities by the lower limit of deciduous forests – result of the trees selective cutting.

Especially remarkable for the wild pear diversity are the southern provinces of Armenia: except the fact, that all 32 pear species of Armenian flora are found there, half of them are characteristic only to this part of Armenia. Particularly interesting is Vayots Dzor province with 25 pear species, 9 of which are national endemics.

In general, 10 pear species are listed in the Red Data Book of Armenia (Tamanyan *et al.* 2010) and all under threatened categories; 7 of them are Armenian endemics. 9 pear species from Armenian flora are included in the IUCN Red List (http://www.iucnredlist.org/) under threatened categories – all are national endemics.

Here is their list with the IUCN Red List status: *Pyrus browiczii* Mulk. (CR), *P. sosnovskyi* Fed. (EN), *P. tamamschjanae* Fed. (EN), *P. complexa* Rubtzov (VU), *P. theodorovii* Mulk. (EN), *P. hajastana* Mulk. (EN), *P. daralagezi* Mulk. (EN), *P. voronovii* Rubtzov (CR), *P. gergerana* Gladkova (CR).

Not only particular species, but wild pear communities of Armenia are of conservation importance (Asatryan and Fayvush 2013). Lack or absence of data on distribution, biology and threats

to these unique botanical objects as well as absence of any research on population level arouse difficulties for their effective conservation.

In 2016 and 2017 we carried out work with literature, herbarium studies and field research on some endemic pears of Armenia in order to clarify their distribution and to collect data on the threats to the species. With support from Fauna and Flora International (FFI) in the framework of the Global Trees Campaign (GTC) the following scoping grants have been implemented by "Nature Rights Protection" NGO: "Herher pear scoping project", "Scoping wild pears in southern Armenia" in 2016, "Identification of the pear species and their distribution in the Herher state sanctuary" in 2017–2018. *P. gergerana* (*Figure 1*) was chosen as the main target species, and two other rare endemic pear species *P. daralagezi* (*Figure 2*) and *P. voronovii* were involved too as their distribution areas partially overlap with the area of *P. gergerana*.

So, the main objectives of the studies were the following:

1) to check the presence of endemic species *P. gergerana, P. darlagezi* and *P. voronovii* in the locations, known for them from previous investigations;

2) to make an assessment of the pear diversity on the territory of Herher state sanctuary.

Only two trees of *P. gergerana* were found in the area around village Goghtanik, Vayots Dzor province and one – on the sanctuary's territory. Four trees of *P. daralagezi* were found on the territory of Herher state sanctuary and this was a new location for the species. The presence of *P. daralagezi* near Kechut reservoir (its locus classicus) was confirmed. As a result of our research we consider the taxonomic status of *P. voronovii* doubtful. None of the available herbarium specimens has rhomboid leaves as given in the original description of the species and seen on the type specimen. Also, we didn't find any individual which could be identified as *P. voronovii*.

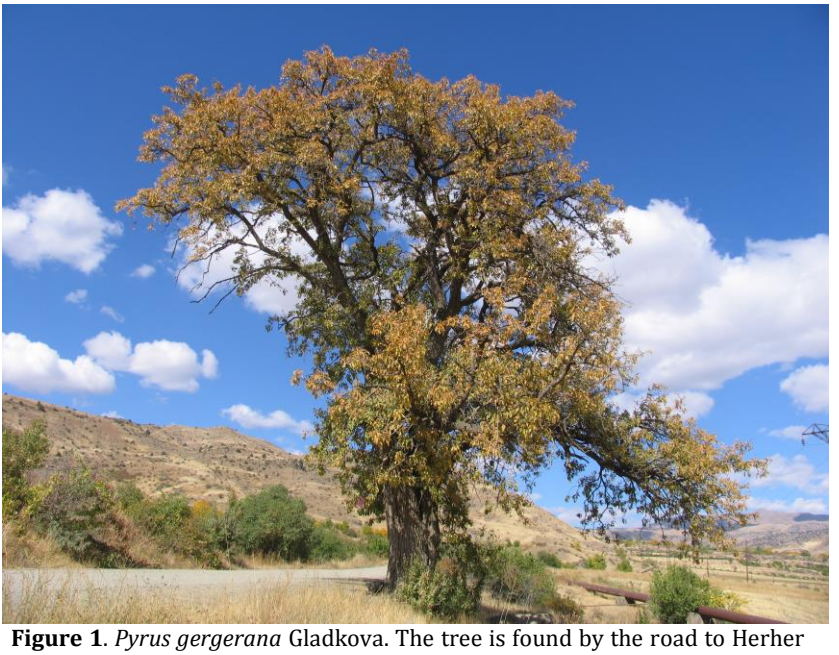

Figure 1. *Pyrus gergerana* Gladkova. The tree is found by the road to Herher village and the locals consider it a symbol of the village. This is the biggest known individual for the species (photo by A. Asatryan)

Figure 2. *Pyrus daralagezi* Mulk. (photo by A. Asatryan)

Except *P. gergerana* and *P. daralagezi*, the following pear species have been identified as occurring on the sanctuary's territory: *P. salicifolia, P. pseudosyriaca, P. nutans, P. caucasica, P. medvedevii,* endemics of Armenia *P. elata* and *P. hajastana*. Also, four hybrid forms, possibly between *P. salicifolia* and *P. oxyprion, P. pseudosyriaca* and *P. nutans, P. pseudosyriaca* and *P. daralagezi, P. pseudosyriaca* and *P. elata* were found (Asatryan 2018).

Sensible intrageneric and intraspecific variability of the taxonomically important traits such as, leaf (*Figure 3*) and fruit (*Figure 4*) shape, size, colour and texture often creates difficulties in identification and so, assessment of the species distribution.

Figure 3. Diversity of Armenia's wild pear leaves (photo by A. Asatryan)

The research let us to identify sites of 'concentration' of genetic diversity of wild pears in Armenia, to explore the distribution of some endemic species and to outline the difficulties in effective conservation of wild pears in Armenia.

Figure 4. Fruit diversity within *Pyrus pseudosyriaca* Gladkova (photo by A. Asatryan)

The main aim of the study was to process the collected data to identify the main 'hotspots' of the pear diversity in Armenia and give their descriptions. Some questions aroused during the implementation of the above mentioned projects are discussed as well.

MATERIALS AND METHODS

The research has been carried out in 2016–2018 and included field surveys in the south of Armenia (Vayots Dzor and Syunik provinces). The itinerary was designed in accordance with the target species' (*P. gergerana*, *P. daralagezi* and *P. voronovii)* distribution data, taken from the herbarium of the Institute of Botany after A.L. Takhtajyan of the National Academy of Sciences, Republic of Armenia (ERE).

Almost all pear trees along the roadsides have been studied along the trip (most of the known locations for the target species were by the roads), herbarium samples were taken for further

processing and identification. The specimens of special interest were marked with labels.

The literature on pear species and their habitats in Armenia was studied; special attention was paid to the original descriptions of the target species and other endemics in order to be prepared to distinguish them both in the field and during identification of the collected herbarium.

Herbarium material – about 150 sheets, on *Pyrus* sp. kept in the Institute of Botany NAS RA were studied. For the endemics we analysed the original descriptions of the species and the herbarium samples (including the type specimens) to understand the main diagnostic features for the target species and to make comparisons with the herbarium identifications made by previous researchers.

The research area covered roads to Herher village, then Yeghegis river gorge, way to Jermuk and its surroundings, including the forest near Kechut reservoir, the roads from Kapan to Vachagan and Srashen villages, Geghi river gorge, Tashtun pass, surroundings of Tashtun and Lichq villages. The field research covered also the area of Herher state sanctuary (6139 hectares) in Vayots Dzor province.

The pear diversity hotspots were identified by analysing the data on the species' distribution, taken from the herbarium of the Institute of Botany of the NAS RA (ERE) and from our field research.

Certain difficulties and problems became evident during the work. We had difficulties trying to identify some samples – they just did not match any of the described taxa. High level of polymorphism in populations, variability of diagnostic features of the species, big number of hybrid forms etc. made the identification process challenging. There is strong need of critical taxonomic review of the group, based on field observations, statistical data, DNA studies etc.

RESULTS

About 120 herbarium samples have been collected during our fieldtrips and processed later.

Six main diversity hotspots for *Pyrus* sp. in Armenia have been identified (*Figure 5*). The pear diversity for each of them is represented below – based on herbarium and literature studies and

data, collected during our fieldwork. The sites on the map are marked according to their numbers given here.

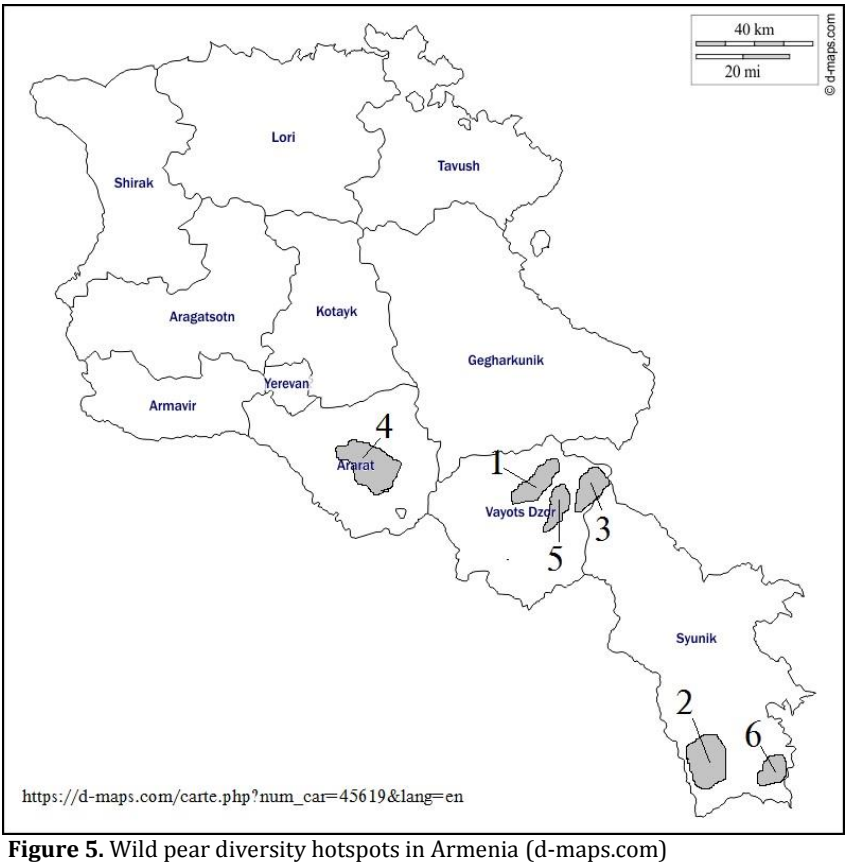

Figure 5. Wild pear diversity hotspots in Armenia (d-maps.com)

1. Yeghegis river gorge, Vayots Dzor province

The total number of pear species is 25, 8 of them are endemics of Armenia.

According to the herbarium data: *P. ketzkhovelii, P. vsevolodii, P. demetrii, P. hyrcana, P. turcomanica, P. caucasica, P. raddeana, P. acutiserrata, P. syriaca, P. medvedevii, P. fedorovii, P. georgica, P. takhtadzhianii, P. communis* and following endemics *P. browiczii, P. elata, P. complexa, P. daralagezi, P. sosnovskyi, P. hajastana, P. tamamschjanae;* we added to the list *P. salicifolia, P. oxyprion, P. pseudosyriaca* and the endemic *P. gergerana.*

2. Tashtun pass, surroundings of Tashtun and Lichq villages and part of Megri Pass, Syunik province

The total number of pear species is 22, 8 of them are endemics of Armenia. According to the herbarium data: *P. grossheimii, P. hyrcana, P. demetrii, P. raddeana, P. takhtadzhianii, P. acutiserrata, P. syriaca, P. saliciflia, P. medvedevii,* endemics *P. voronovii, P. daralagezi, P. complexa, P. gergerana, P. tamamschjanae, P. elata;* we added to this list *P. caucasica, P. zangezura, P. nutans, P. pseudosyriaca, P. georgica* and two endemics *P. megrica, P. hajastana.*

3. Surroundings of Jermuk town, Vayots Dzor province

The total number of pear species is 19, 5 of them are endemics of Armenia. According to the herbarium data: *P. nutans, P. fedorovii, P. takhtadzhianii, P. medvedevii, P. caucasica, P. ketzkhovelii, P. syriaca, P. pseudosyriaca, P. zangezura, P. salicifolia* endemics *P. sosnovskyi, P. hajastana, P. daralagezi, P. gergerana, P. megrica* we found *P. oxyprion, P. taochia, P. georgica, P. acutiserrata.*

4. "Khosrov Forest" state reserve, Ararat province

The total number of pear species is 15, 5 of them are endemics of Armenia. According to the herbarium data: *P. communis, P. caucasica, P. vsevolodii, P. turcomanica, P. syriaca, P. salicifolia, P. medvedevii, P. oxyprion, P. fedorovii, P. takhtadzhianii* and endemics *P. tamamschjanae, P. sosnovskyi, P. theodorovii, P. hajastana, P. chosrovica.*

5. Surroundings of Herher village and Herher state sanctuary, Vayots Dzor province

The total number of pear species is 14, 5 of them are endemics of Armenia. According to the herbarium data: *P. demetrii, P. fedorovii, P. takhtadzhianii, P. salicifolia* and endemics *P. gergerana* and *P. hajastana;* we added to the list *P. nutans, P. communis, P. pseuadosyriaca, P. caucasica, P. medvedevii* and endemics *P. sosnovskyi, P. daralagezi, P. elata*.

6. Shikahogh state reserve, Syunik province

The total number of pear species is 12, 3 of them are endemics of Armenia. According to the herbarium data: *P. communis, P. hyrcana, P. caucasica, P. zangezura, P. raddeana, P. syriaca, P. medvedevii*, *P.*

fedorovii, P. takhtadzhianii and endemics *P. tamamschjanae, P. megrica* and *P. gergerana*.

DISCUSSION

The main characteristics of the 'hotspots' for pear diversity in Armenia are the following: they located in deep gorges and valleys and include fragments of arid open forest where narrow leaved pear species occur (mainly *P. salicifolia* and *P. oxyprion*) and deciduous forest, where broad leaved mesophytic species occur (*P. caucasica*, *P. syriaca, P. pseudosyriaca, P. daralagezi* and others).

The pear diversity areas contain also old settlements (at least one village) and the roads. The 'intermediate' leaved pear species and hybrid forms are found mostly by the roadsides and on the glades in the deciduous forests. So, all the pear trees found in the area, including ones in orchards and village gardens have been involved in hybridization process. Certain questions appear in relation to the original descriptions of some rare endemic species, which have been described from just one tree with no data on population and distribution of the particular taxon. Clarifications of their taxonomic status need to be done.

According to Gladkova (1989) *P. sosnovskyi, P. demetrii, P. tamamschjanae, P. vsevolodii* are close and represent garden escapees on the different stages of transformation from *P. communis* group. Such a high level of polymorphism in *Pyrus*, according to her (Gladkova 1990) is caused by two groups of factors: one represents natural evolution, the other is linked with human activity. She thinks that it is here, in the Caucasus region, where the ways of evolution of two ecological groups of species, formed in different ecological conditions, linked. The first group is formed with more or less mesophile species, which ancestors have been part of ancient Tertiary forest flora, remnants of which are still found in relic refugiums in Eastern Asia and Transcaucasia. Our target *P. daralagezi* belongs to this group. The second group is formed with xerophyte species of *P. salicifolia* type, which have been formed in later ages – in arid conditions of the Mediterranean area. Intensive hybridisation processes between representatives of these two groups have been the causes of appearance of many more or less stabile forms carrying the intermediate features of

both groups. The other target species *P. gergerana* is from this group.

Gladkova (1990) writes, that morphologically similar forms appear in different spots of the distribution area as a result of hybridisation of the parental forms. Very often they occur by the roadsides and nearby villages. Many of these individual trees of hybrid origin became the only specimens, which have been considered in description of new species.

Armenia is located in the South-Western Asian – one of the Vavilov's world centers of origin of cultivated plants (Vavilov 1926) and is notable for great diversity of wild relatives of cultivated plants. Caucasus is known as one of the most ancient centres of agriculture and domestication of wild plants. Human activity has been another factor, promoting active hybridisation in populations of wild pears. Main centres for pear diversity are linked with ancient settlements – still existing or abandoned. During many centuries wild forms have been domesticated with further selection activities, at the same time the opposite process of escaping from gardens used to take place. One can still find many ancient pear sorts all over Armenia, which are close to wild forms.

Fruits of wild pears particularly, fruits of Caucasian pear (*P. caucasica*), which is more common in the north of the country, also *P. salicifolia* and *P. pseudosyriaca* in the southern Armenia are used widely in Armenia by local communities and companies to produce compote, vodka, vinegar. In the southern Armenia, where the diversity of species and forms is much higher locals distinguish particular trees by characteristics of the fruits such as the taste, juiciness and the time when they are perfect for eating: some pear fruits have astringent taste and become edible (soft and brown) some weeks after they fell from a tree. This data, which comes from ages-long observations and practice on site may be very valuable not just for promoting *in-situ* conservation, but for researchers who work on taxonomy of this group. Wild pear seedlings are used as rootstocks for grafting (Sokolov 1954).

Pyrus L. is the largest among the genera of Armenian flora, represented in the IUCN Red List. 9 of total 71 plant species of Armenian flora, listed there under threatened categories are pear species. As mentioned before, 10 pear species are included in the Red Data Book of Armenia, but only part of the populations of just 5

of them are located on the protected areas, others are not protected (Tamanyan *et al.* 2010).

Some of the wild pear species of Armenia are under *ex-situ* protection in the Institute of Botany of the NAS RA: seeds of 7 species (*P. caucasica, P. demetrii, P. fedorovii, P. georgica, P. medvedevii, P. salicifolia, P. syriaca*) are stored in the Seed bank of Armenian flora and 13 species are represented in the live collections of Yerevan Botanical Garden, which is a part of the institute; those are *P. caucasica, P. communis, P. daralagezi, P. fedorovii, P. gerogica, P. medvedevii, P. oxyprion, P. salicifolia, P. sosnovskyi, P. takhtadzhianii, P. tamamschjanae, P. zangezura* (Akopian 2015) and *P. gergerana.*

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BRYOFLORISTICAL DATA FROM THE GUTÂI MOUNTAINS (ROMANIAN EASTERN CARPATHIAN, TRANSYLVANIA)

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Abstract: The main aim of this study was to explore the bryophyte diversity and distribution patterns in the Gutâi Mountains. From our collections hitherto 52 bryophyte species were identified. The 12 Marchantiophyta and 40 Bryophyta species belong to 45 genera of 27 families. *Nardia scalaris* is new for the whole Gutâi Mountains. Among them the vulnerable *Grimmia muehlenbeckii* and the very rare *Riccardia palmata* are worth to be mentioned.

Keywords: bryoflora, rare species, Gutâi Mountains, Romania

Rezumat: Lucrarea prezintă distribuţia speciilor de briofite din arealul Munţilor Gutâi. Din colecţia recentă au fost identificate 52 specii de briofite. Cele 12 specii de Marchantiophyta şi 40 de specii de Bryophyta aparţin la 45 de genuri şi 27 de familii. *Nardia scalaris* este o semnalare nouă pentru Munţii Gutâi. *Grimmia muehlenbeckii* este o specie vulnerabilă, iar *Riccardia palmata* este rară, ambele meritând a fi menţionate.

Cuvinte cheie: brioflora, specii rare, munţii Gutâi, Gutin, România

INTRODUCTION

First bryological records of the Gutâi (Gutin) Mountains were published at the end of XIXth century (Juratzka 1882) and the investigations continues in the XXth century, which till now is far from complete (Pop 1942; Boros 1943, 1951; Boros and Vajda 1967; Raţiu and Moldovan 1972a, 1972b, 1974; Ştefureac 1974, 1976–1977; Mititelu and Dorca 1983; Coldea and Plămadă 1989). A very detailed floristical and vegetational study is given by Moldovan (1970) and one study was published on saxicolous

lichens from the Gutâi Mountains (Codoreanu 1972). Tamás Pócs with his wife visited and collected in the area during the summer of 1993.

Much less bryological investigations has been done in the past twenty years completing with additional floristical data (Jakab 1999, Ardelean *et al.* 2008). We started our work in 2018 and our aim is to continue bryological exploration of this area.

Study area

The Gutâi Mountains are a mountain range within the Vihorlat – Gutâi area of the inner Eastern Carpathians. Igniş and Gutâi mountains are situated at the western and southern limit of Maramureş Land, they are the oldest sector of the volcanic range in Eastern Carpathians. Separated by mountain passes from the neighbouring units (Huta 587 m, Gutâi 984 m, Neteda 1039 m) they are two separate units distinguished by geoforms originating from different types of volcanic activity: Ignis mountains as andesitic plateau, mostly stratified, with small depressions, an end cliff and residual forms, named rocks (Piatra Săpânţei, Piatra Goală, Piatra Rea etc); Gutâi Mountains with pyroxene andesite, mostly vertical columns with a controversial neck – Creasta Cocoşului and cone shaped summits (*Figure 1*). On the northern limit of the mountains, a piedmont range forms contact with the Maramureş lowland, often associated with the mountain range due to the position of the settlements around the massifs (Ilies *et al.* 2017). The Gutâi Mountains have several higher regions: Gutâiul Mare (1443 m), Creasta Cocoşului (1395 m), Trei Apostoli (1398 m), Gutâiul Doamnei (1426 m) and Secătura (1390 m). Creasta Cocoşului is a protected area of national interest and is included in the Gutâi-Creasta Cocoşului Natura-2000 site it is a ridge formation about 200 metres in length and located at an average altitude of 1200 metres, surrounded by mixed forests, large beechwood and spruce areas, the peat bog at Tăul Chendroaiei (Chendroaia's Pond), juniper areas and mountain pastures. The climate of the SE Carpathians is colder and more continental than that of the NW Carpathians (Hajdú-Moharos 1996). The Firiza Lake was established in 1964, when 52 m high dam gates were closed to stem the Firiza water tributary of the Sasar at Baia Mare. The lake has a length of 3 km and a width of 1 km. Built for the Baia Mare city water supply, now the Firiza Lake is used for recreational and

leisure and is one of the favorite places of population in Baia Mare, the landscape is particularly special, with coniferous and deciduous forest around.

Figure 1. View from the Creasta Cocoşului summit (Photo: Róbert Sass-Gyarmati).

MATERIAL AND METHODS

The byophytes enumerated below were collected from the Gutâi Mountains between 8-9 August 2018 by Andrea and Róbert Sass-Gyarmati and identified by Andrea Sass-Gyarmati and the species *Grimmia muehlenbeckii* identified by Peter Erzberger. The collection was made in various vegetation types: meadows, beech and spruce forests and subalpine belts. The Romanian distribution of mosses was established from Plămadă (1998) and Mohan (1998), while that of the liverworts from Ştefănuţ (2008). The nomenclature of liverworts follows Ştefănuţ (2008) modified by Söderström *et al*. (2016), nomenclature of mosses follows Hill *et al.* (2006), except *Racomitrium affine* which was recently included to *Bucklandiella* (F. Weber & D. Mohr) Bednarek-Ochyra & Ochyra (Ochyra *et al*. 2003). and *Racomitrium aquaticum* also recently included to *Codriophorus* (Brid. ex Schrad.) Bedn.-Ochyra & Ochyra,

Bednarek-Ochyra (2006). The classification of liverworts (Marchantiophyta) follows Söderström *et al.* (2016), while the classification of mosses (Bryophyta) follows Goffinet and Shaw (2009). The species in each family are arranged in alphabetical order. Species names are followed by the collecting site number, and by the substrate on which they were grown. The collected specimens are deposited in the Herbarium of Eger (EGR). The collecting sites are listed in the Appendix.

RESULTS

List of species

During the field study 52 bryophyte species were found in the investigated area. The 12 Marchantiophyta and 40 Bryophyta species belong to 45 genera of 27 families.

Marchantiophyta

Conocephalaceae

Conocephalum conicum (L.) Dumort. – 4: on irrigated rocks

Marchantiaceae

Marchantia polymorpha L. – 4: on irrigated rocks

Aneuraceae

Riccardia palmata (Hedw.) Carruth – 4: on decaying wood

Lophoziaceae

Lophozia ventricosa (Dicks.) Dum. – on decaying wood

Scapaniaceae

Diplophyllum albicans (L.) Dumort. – 5: on soil covered rocks
Scapania undulata (L.) Dumort. – 4: on irrigated volcanic rocks

Gymnomitriaceae

Nardia scalaris Gray – 5: on soil. It was collected also by S. & T. Pócs in 1993 (unpublished).
Marsupella emarginata (Ehrh.) Dumort. – 5: on soil

Radulaceae

Radula complanata (L.) Dumort. – 1, 4: bark of *Fagus*

Lophocoleaceae

Chiloscyphus polyanthos (L.) Corda – 4: on irrigated volcanic rocks

Lophocolea heterophylla (Schrad.) Dumort. – 1: on decaying wood

Plagiochilaceae

Plagiochila porelloides (Torrey. ex Nees) Lindenb. – 4: on soil

Bryophyta

Andreaceae

Andreaea rupestris Hedw. – 6: on volcanic rocks

Tetraphidaceae

Tetraphis pellucida Hedw. – 6: on decaying wood

Polytrichaceae

Atrichum undulatum (Hedw.) P. Beauv. – 1, 4: on soil

Oligotrichum hercynicum (Hedw.) Lam. & DC. – 3: on soil

Pogonatum urnigerum (Hedw.) P. Beauv. – 3: on soil

Polytrichastrum alpinum (Hedw.) G. L. Sm. – 5: on soil

Polytrichastrum formosum (Hedw.) G. L. Sm. – 2, 4: on soil

Polytrichum juniperinum Hedw. – 6: on rocks

Encalyptaceae

Encalypta streptocarpa Hedw. – 5: on soil

Grimmiaceae

Grimmia muehlenbeckii Schimp. – 6: on rocks

Codriophorus aquaticus (Brid.) Bednarek-Ochyra & Ochyra. Syn.: *Racomitrium aquaticum* (Hedw.) Brid. – 4: on rocks

Bucklandiella affinis (F. Weber & D. Mohr) Bednarek-Ochyra & Ochyra. Syn.: *Racomitrium affine* (F. Weber et D. Mohr) Lindb. – 6: on soil

Ditrichaceae

Ceratodon purpureus (Hedw.) – 1: on disturbed soil

Dicranaceae

Dicranella heteromalla (Hedw.) Schimp. – 2, 5: on decaying wood

Dicranoweisia crispula (Hedw.) Milde – 4, 6: on volcanic rocks

Dicranum flagellare Hedw. – 4: base of *Fagus*
Dicranum scoparium Hedw. – 1: base of *Carpinus*
Paraleucobryum longifolium (Hedw.) Loeske – 4: on soil covered rocks, 6: on rocks

Pottiaceae

Bryoerythrophyllum recurvirostrum (Hedw.) P. C. Chen – 5: on soil covered rocks
Didymodon fallax (Hedw.) R. H. Zander – 5: on soil
Gymnostomum calcareum Nees & Hornsch. – 6: on vertical cliff

Bryaceae

Bryum pseudotriquetrum (Hedw.) P. Gaertn. – 3: on irrigated rocks

Mniaceae

Plagiomnium undulatum (Hedw.) T. J. Kop. – 4: on soil covered rocks
Rhizomnium punctatum (Hedw.) T. J. Kop. – 2, 4: on soil

Leskeaceae

Leskea polycarpa Hedw. – 4: on bark
Pseudoleskeella nervosa (Brid.) Nyholm – 4: on bark

Amblystegiaceae

Amblystegium serpens (Hedw.) Schimp. – 2, 4: on tree base
Amblystegium subtile (Hedw.) Schimp. – 2: on tree base
Sanionia uncinata (Hedw.) Loeske – 4: on tree base

Hylocomiaceae

Pleurozium schreberi (Willd. ex Brid.) Mitt. – 13: on soil

Pterigynandraceae

Pterigynandrum filiforme Hedw. – 4: on *Fagus* bark

Thuidiaceae

Abietinella abietina (Hedw.) M. Fleisch. – 1: on soil

Brachytheciaceae

Brachythecium rutabulum (Hedw.) Schimp. – 1: on soil
Brachythecium rivulare Schimp. – 2: on wet soil

Brachythecium salebrosum (Hoffm. ex F. Weber et D. Mohr.) Schimp. – 1,4: on soil

Plagiotheciaceae

Plagiothecium denticulatum (Hedw.) Schimp. – 1: on tree base
Plagiothecium laetum Schimp. – 4: on tree base

Hypnaceae

Ctenidium molluscum (Hedw.) Mitt. – 4, 5: on rocks
Hypnum cupressiforme Hedw. – 1: on rocks

Lembophyllaceae

Isothecium myosuroides Brid. – 1: on tree base

DISCUSSION

The results of this study contributes to the knowledge of the biodiversity in Gutâi Mountains. The main reason for relatively high biodiversity is the variety of habitat types that can be found in this area.

Nardia scalaris Gray – circumboreal, mountain taxon it is not known from the Gutâi Mountains. Based on Mohan checklist occurs in Maramureşului Mountains: Vl. Jâjla, Turcul and several localities from the romanian Carpathians: Iezer Păpuşa Mountains, Bihor Mountains, Bucegi Mountains, Retezat Mountains, Cibinului Mountains and Mlaştina turbăria Cristişor.

Riccardia palmata (Hedw.) Carruth. – circumboreal, mountain species, it is reported only from one locality from Gutâi Mountains: Cheile Tătaru at Mara (Boros and Vajda 1967). Other reports from surroundings are from Borşa, Secului Valley, Sighet, Poiana Şarampoiului Forest, Mara, Runc Valley, Puzdra Mountain, (Boros and Vajda, 1967); between Tocila Valley and Băiuţ (Jakab 1999), well distributed in the Romanian Carpathians (Mohan 1998).

Grimmia muehlenbeckii Schimp. – is treated as vulnerable (VU) in Romania (Ştefănuţ and Goia 2012), it is known just from few localities in the country: jud. Alba: Vl. Galbina, Mtele Găina; jud. Gorj: Mţii Parâng: pasul Surduc; jud. Harghita: Munţii Hargita; jud. Hunedoara: Deva; jud. Maramureş: Muntele Pietrosul Rodnei; jud.

Suceava: Mtele Ceardac. (Mohan 1998). These findings should enhance the knowdledge of bryoflora, the results emphasizes the importance of further research in this highly valuable area.

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APPENDIX

List of collecting sites from the Gutâi Mts:

1. Munţii Gutâi (Gutin hegység), Maramureş County. Firiza Lake above Firiza village in acidophyllous *Fagus-Carpinus* forest at 5-600 m alt. 47°43'30.95"N, 23°35'54.45"E. Date: 08. Aug. 2018. Coll.: A. & R. Sass-Gyarmati No. 1801
2. Munţii Gutâi (Gutin hegység), Maramureş County. Gutin Pas (Pasul Gutâi). Acidophyllous beech forest (Luzulo-Fagetum) at 980 m alt. N47°42'0.02", E23°47'33.77". Date: 09. Aug. 2018. Coll.: A. & R. Sass-Gyarmati No. 1802
3. Munţii Gutâi (Gutin hegység), Maramureş County. Spring bogs Poiana Boului (Ökörmező), NE from Baia Sprie (Felsőbánya), at 1055 m alt. N47°41'49.37", E23°48'13.03". Date: 09. Aug. 2018. Coll.: A. & R. Sass-Gyarmati No. 1803
4. Munţii Gutâi (Gutin hegység), Maramureş County. Subalpine beech forest below the forest line along the path to Creasta Cocoşului Peak summit between 1100-1200 m alt. N 47°42'14.42", E 23°50'28.66". Date: 09. Aug. 2018. Coll.: A. & R. Sass-Gyarmati No. 1804
5. Munţii Gutâi (Gutin hegység), Maramureş County. Subalpine *Vaccinium* dwarf bush on the Creasta Cocoşului (Kakastaréj) summit at 1400-1420 m alt. N47°42'14.22", E 23°50'30.55". Date: 09. Aug. 2018. Coll.: A. & R. Sass-Gyarmati No. 1805
6. Munţii Gutâi (Gutin hegység), Maramureş County. Volcanic rocks above forest line near Creasta Cocoşului (Kakastaréj) crest at 1400 m alt. N47°42'14.55", E23°50'30.53" Date: 09. Aug. 2018. Coll.: A. & R. Sass-Gyarmati No. 1806

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ONNIA TRIQUETRA (PERS.) IMAZEKI, A PINE ASSOCIATED POLYPORE SPECIES REPORTED FOR THE FIRST TIME FROM HUNGARY

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Abstract: *Onnia triquetra*, a *Pinus* specialist polypore species in Europe, is reported for the first time in Hungary. Historical and recent collections of almost 60 years were examined. The macro- and micromorphological characteristics are given, along with ecological inferences of the species and its hosts in Hungary.

Keywords: Hymenochaetaceae, Europe, native, new record

INTRODUCTION

Hungarian forests are composed mainly of deciduous tree species, covering 89.5% of the forest area. The most common species are oaks, poplars, beech and hornbeam, among aliens the black locust. Conifers represent only 10.5% of the forests, with a significant decline in their actual area in the last two decades (from 243 500 hectares in 2000 to 195 000 hectares in 2016). The most widespread conifer species are Scots pine (*Pinus sylvestris* L.) and Austrian pine (*Pinus nigra* J.F.Arnold), covering an area of 6.2% and 3.2% respectively (Komarek 2018). Scots pine (*Pinus sylvestris* L.) is considered to be native, while Austrian pine (*P. nigra* J.F.Arnold) was introduced to the Pannonian basin, with its first plantations established in the 19th century (Borhidi 2003; Tamás 2003; Bölöni *et al.* 2008).

The effects of such forest plantations on local biodiversity depend on the sensibility and ecological specificity of the site (e.g. the surrounding landscape), in which they are found. Thus, planted

forests – both native and exotic – may have positive, as well as negative effects on biodiversity at the population, stand or landscape level (Humphrey *et al.* 2000; Mack *et al.* 2000; Carnus *et al.* 2006; Cseresnyés and Tamás 2014). Due to the wide ecological tolerance of Scots pine, it is hard to describe the effects of its semi-natural stands on local biodiversity. In this regard, indicator species for these semi-natural stands should be recognized on the local level (Kelly and Conolly 2000). In case of Austrian pine, it is widely accepted that its plantations had detrimental effects on the Hungarian sites where they were planted in the past. Many local species disappeared from the native flora and fauna of such plantations (Török and Tóth 1996; Bartha *et al.* 2004; Cseresnyés and Tamás 2014).

On the other hand, vast diversity of fungi can be seen in plantations of exotic conifers (Newton and Haigh 1998; Humphrey *et al.* 2000). These planted stands could serve as potential substrata for native lignicolous fungal assemblages, which could include rare and threatened species (Humphrey *et al.* 2000; Ryvarden and Melo 2014).

The *Onnia* P. Karst. genus (Hymenochetaceae) contains nine white rot polypore species, from which seven are growing on gymnosperms. *Onnia tomentosa* (Fr.) P. Karst. and *O. leporina* (Fr.) H. Jahn grow mainly on *Picea*, but also can be found on *Pinus*, *Abies* and *Larix*, while *O. triquetra* (Pers.) Imazeki, *O. subtriquetra* Vlasák & Y.C. Dai, *O. microspora* Y.C. Dai & L.W. Zhou, *O. kesiya* Zhou & F.Wu and *O. tibetica* Y.C. Dai & S.H. He occurs mainly on *Pinus* (Dai 2010, 2012; Ryvarden and Melo 2014; Ji *et al.* 2017). These fungi are characterized by annual, sessile or stipitate basidiocarps; pileal surface from yellowish brown to dark brown and velutinate to rough; pore surface from yellowish brown to dark brown; duplex context. Main microscopic characteristics are: monomitic hyphal system with generative hypha bearing simple septa; presence of mostly hooked hymenial setae; and hyaline, thin-walled, smooth, nonamyloid, nondextrinoid, and acyanophilous basidiospores (Dai 2010; Ji *et al.* 2017). The genus is widespread; its distribution extends from boreal to subtropical climates, being present throughout the Northern Hemisphere (Dai 2012; Ryvarden and Melo 2014; Lockman and Kearns 2016; Ji *et al.* 2017).

In Europe, *Onnia tomentosa*, *O. leporina* and *O. triquetra* are considered to be native. In contrast to the first two circumboreal

species, *O. triquetra* is geographically restricted to Europe, growing mainly on *Pinus* (Ryvarden and Gilbertson 1993; Dai 2012; Ji *et al.* 2017). The possible occurrence of *O. triquetra* in Hungary was implied first by Zoltán Igmándy in the 1980's (Igmándy 1989), but since then it was never found.

In Igmándy's own collection, among deposited unidentified specimens, we have found a sample collected from a *P. sylvestris* trunk near Szilvágy (Zala County, Western Hungary) from 1959, which we identified as *O. triquetra*. Almost 60 years later, a recently collected specimen from *P. nigra* in the Búbánat-völgy (Komárom-Esztergom County, North Hungary) was identified to be the same species.

MATERIALS AND METHODS

The historical specimen (Z. Igmándy 1131) collected by L. Haracsi is available at the Institute of Silviculture and Forest Protection, University of Sopron (Sopron, Hungary); the new specimen (Borsicki 100916) is deposited at the personal fungarium of the last author (PV). Macromorphological descriptions are based on field notes. The micromorphological data were obtained using a light microscope following the methods by Papp and Dima (2018). Microscopic characters, measurements and drawings were made from slide preparations stained with Melzer's reagent. The sections were studied at 1000× magnification using a Zeiss Axio Imager A2 light microscope (Zeiss, Göttingen, Germany) attached with an AxioCam HRc camera (Zeiss, Göttingen, Gemany). Drawings of the micromorphological characteristics of the basidiocarps were made using a drawing tube. Spores were measured from sections cut from the tubes. For the measurements, we used the Axio Vison Release 4.8. software. The following abbreviations were used in the description of the basidiospores: IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, L = mean spore length, W = mean spore width, Q = variation in the L/W ratios, n = number of spores measured.

TAXONOMY

Onnia triquetra (Pers.) Imazeki [as 'triqueter'], Mycol. Fl. Japan, Basidiomycetes 2(4): 386 (1955) – (*Figure 1* and *2*)
Basionym: *Boletus triqueter* Pers., Observ. mycol. (Lipsiae) 1: 86 (1796)
Synonyms: *Polyporus triqueter* (Pers.) Pers., Mycol. eur. (Erlanga) 2: 57 (1825); *Inoderma triquetrum* (Pers.) P. Karst. [as 'triqvetrum'], Meddn Soc. Fauna Flora fenn. 5: 39 (1879); *Ochroporus triqueter* (Pers.) J. Schröt., in Cohn, Krypt.-Fl. Schlesien (Breslau) 3.1(25–32): 485 (1888) [1889]; *Mucronoporus tomentosus* var. *triqueter* (Pers.) Domański, Orloś & Skirg., Flora Polska. Grzyby, II: 321 (1967); *Mucronoporus circinatus* var. *triqueter* (Pers.) Domański, Orloś & Skirg., Fungi, Polyporaceae 2, Mucronoporaceae 2, Revised transl. Ed. (Warsaw): 289 (1973)

Specimens examined: Hungary, Zala County, Szilvágy, on *Pinus sylvestris* trunk, 22 Jan 1959, leg. L. Haracsi, herb. Z. Igmándy 1131. Komárom-Esztergom County, Esztergom, Búbánat-völgy, on *Pinus nigra* trunk, 10 Sept 2016, leg. I. Borsicki, herb. Borsicki 100916.

Basidiocarps annual, sessile or laterally stipitate/substipitate, soft corky or corky. Pileus dimidiate, with 3–4 cm diameter and 2–3 cm thickness at centre. Pileal surface golden brown to rust-brown, tomentose to velutinate, concentric zones indistinct; margin sharp to blunt. Pore surface yellowish brown to rusty brown; sterile margin pale yellowish; pores angular, 3–4 per mm, later more angular to semi labyrinthine and up to 1–2 mm wide in places; dissepiments thin, slightly lacerate. Context duplex; upper layer rust-brown, spongy; lower layer umber-brown, corky, demarcation zone indistinct between the two layers; entire context up to 1 cm thick. Tubes pale umber-brown, slightly paler than context and pore surface, hard corky, up to 5 mm long. Stipe rust-brown, hirsute to velutinate; 1–2 cm long, 0.5–1 mm in diameter; pores decurrent on stipe.

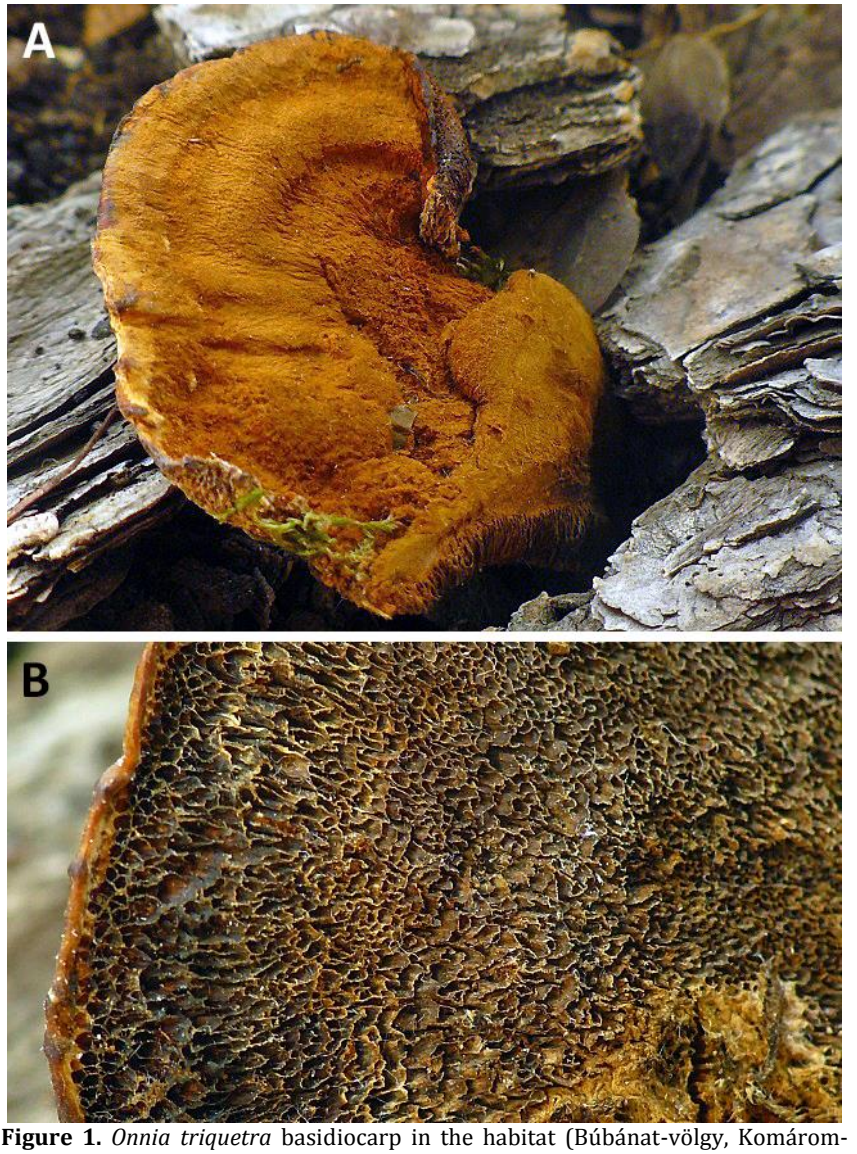

Figure 1. *Onnia triquetra* basidiocarp in the habitat (Búbánat-völgy, Komárom-Esztergom County, Hungary) **A.** pileal surface **B.** pore surface. Photographs by I. Borsicki.

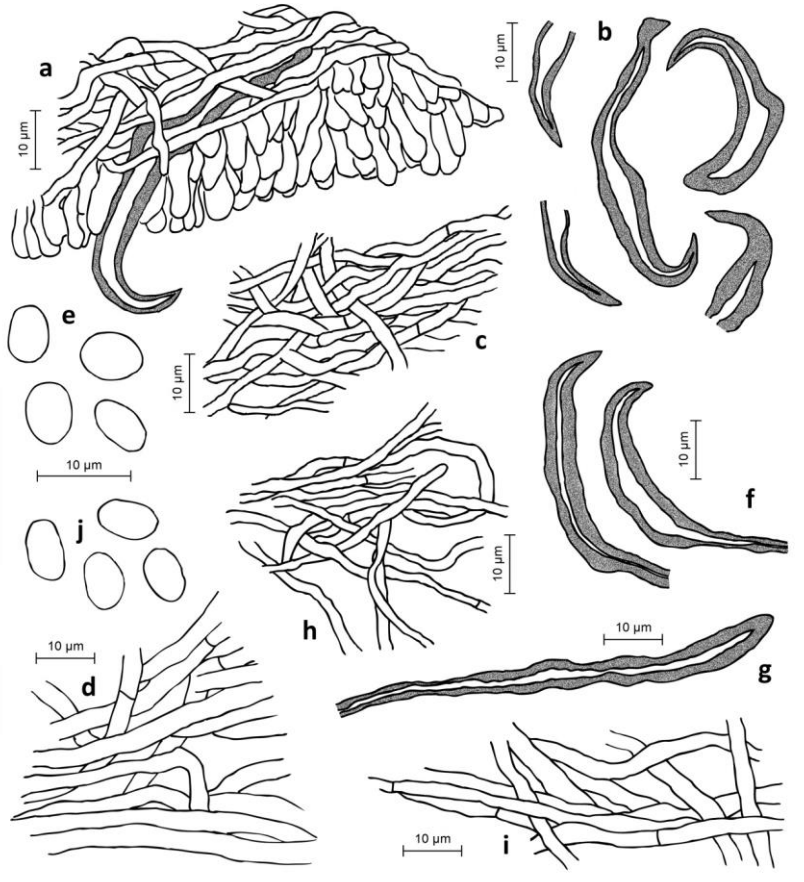

Figure 2. Micromorphological structures of *Onnia triquetra*, collected from *Pinus sylvestis* (a-e) and *Pinus nigra* (f-j) **a.** section of trama **b.** setae **c.** hyphae from trama **d.** hyphae from context **e.** basidiospore **f.** setae **g.** narrow setae **h.** hyphae from trama **i.** hyphae from context **j.** basidiospores.

Hyphal system monomitic; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH. Tramal hyphae hyaline to pale yellowish, thin- to slightly thick-walled, occasionally branched and septate, agglutinated, 2.5–6 µm in diam. Contextual hyphae yellowish to golden brown, thin- to slightly thick-walled, rarely branched, with frequent simple septa, regularly arranged, agglutinated, 5–8 µm in diameter. Contextual hyphae at upper

spongy layer pale yellowish, thin-walled, unbranched or rarely branched, with frequent simple septa, interwoven, 3–6 µm in diam; hyphae of lower solid layer pale yellowish, septate, rarely branched, 3–5 µm in diameter. Hyphae from stipe similar to those in context. Setae abundant, mostly hooked, sometimes narrow, sharp-pointed, dark brown, thick-walled, deep-rooting, 28–68 × 8–13 µm. Basidia clavate, with four sterigmata, simple septate at the base, 9–17 × 4.8–6 µm. Basidiospores cylindric-ellipsoid, hyaline, thin-walled, smooth, mostly glued together in tetrads, IKI–, (5.84–) 6.15–6.61(–7.03) × (4.12–)4.24–4.68(–5.02) µm, L=6.4 µm, W= 4.5 µm, Q=1.35–1.50, Q_{av}=1.43 (n=50).

DISCUSSION

The concept of whether a planted and/or non-native forest is beneficial or detrimental for local biodiversity has been much debated. It is often assumed, that tree plantations impoverish local flora and fauna; however, proper forest management can improve the balance between sustainable biodiversity and timber production (Hartley 2002). In case of non-indigenous gymnosperm plantations, both negative (Török and Tóth 1996; Mack *et al.* 2000; Magura *et al.* 2000; Brockerhoff *et al.* 2003; Cseresnyés and Tamás 2014) and positive (Fisher and Goldney 1998; Brockerhoff *et al.* 2003) impacts on natural biodiversity have been observed, related to different ecological and management qualities of the stands.

Plantations of native and non-native gymnosperms have considerable impact on fungal biodiversity. Exotic *Pinus radiata* plantations were found to be less rich in lignicolous saprotrophs and ectomycorrhizal fungi, compared to native oak forests in the Basque Country (Sarrionandia *et al.* 2015). Although the study recorded numerous host specific fungi within the *P. radiata* stands, the overall fungal community consisted mainly of generalist species (i.e. non habitat specialists), presumably because the stands did not reach maturity due to local forest management practices. The authors concluded that exotic plantations in their older, mature stages, with patches of native trees are more capable of contributing to a higher macrofungal diversity. Humphrey *et al.* (2000) came to a similar conclusion in terms of exotic *Picea sitchensis* and native *Pinus sylvestris* plantations in Great Britain. They have met unexpectedly high incidences of rare and

threatened fungi species in stands near to the extant native pinewoods (acting as sources of inoculum). They also emphasized the importance of understory diversity and the volume of fallen deadwood, as well as maintaining the over-mature growth stage of the stands through some form of continuous cover management system. Dejene *et al.* (2017) also confirmed the role of older stands of alien gymnosperms in shaping fungal diversity in case of *Pinus patula* in Ethiopia. Johnston (2010) pointed out, that though introduced *Pinus* species in New Zealand could serve as hosts for indigenous non-lichenised fungi, exotic plants are usually associated with exotic fungi. A similar observation was made by Campi *et al.* (2015) in case of *P. taeda*. Functional composition of wood-decaying assemblages may also differ amongst native and non-native conifers: white rotting or brown rotting fungi could be more or less dominant on the non-indigenous substrata, depending on host species and geographical region (Edman and Fällström 2013).

In Europe, the main distribution area of Scots pine is Central and Northern Europe, while more to the south it is usually restricted to higher elevations (Boratyński 1991). Due to its wide tolerance to climatic and edaphic conditions, this species sustains on many different habitat types (Kelly and Conolly 2000; Köbölkuti *et al.* 2017). In Hungary, Scots pine occurs mainly in the westernmost part of the country (Vendvidék, Őrség, Kemeneshát) in acidophilus coniferous forests. Small stands are also present in the xerophilous forest communities in Central- and Western Transdanubia, on calcareous, sandy substrates, like the habitat in Fenyőfő (Kelly and Conolly 2000; Borhidi 2003; Bölöni *et al.* 2008; Köbölkuti 2018). It has been stated that original Scots pine stands in Hungary are of small size, they do not cover large territories, some being considered relict stands. However, Scots pine forests are preserved by land use that has a crucial role in their survival. Populations in the present are mostly considered to be of planted origin (Völgyi 1955; Mason and Alía 2000; Bölöni *et al.* 2008).

Another important species in Hungary from the *Pinus* genera is Austrian pine. Being an Alpine-Mediterranean tree, Austrian pine is alien to the Pannonian Basin. It was first introduced to Hungary in the 19th century for soil preservation and landscape protection purposes. Trees were planted on hillsides, dolomite slopes and on sandy substrates in the lowlands (Tamás 2003). Today, larger

plantations are found mainly in the Great Hungarian Plain, but there are also plantations in the Transdanubian- and in the Northern Hungarian Mountains (Cseresnyés and Tamás 2014). The establishment of these plantations had a harmful effect on the native vegetation of dolomite slopes, warm tolerant oak forests and sandy grasslands. The above mentioned habitats contain numerous endemic species; the monodominant structure of the plantations resulted in closed canopies, impoverished understory and reduced diversity of the local plant and animal communities (Török and Tóth 1996; Bartha *et al.* 2004; Bíró 2008; Cseresnyés and Tamás 2014). Moreover, the accumulating litter under the canopy of Austrian pine plantations contributed to elevated fire risks. In this regard, both needles, cones and fallen branches could serve as focal points for forest fires due to the relatively slow decomposition rate (Cseresnyés *et al.* 2006).

Both Scots pine and Austrian pine are serving as substrata for numerous native polypore fungi in Europe. Many of these species are found in East- and Central Europe, including Hungary (Ryvarden and Gilbertson 1993, 1994). Conifer-dwelling macrofungi in Hungary were already being studied more than a half century ago. Throughout these decades, numerous poroid species were recorded on hosts from the *Pinus* genera (Igmándy 1954; Völgyi 1955; Pagony 1977). Amongst these, species inhabiting both gymnosperm and angiosperm trees are represented in the highest amount (e.g. *Bjerkandera adusta* (Willd.) P. Karst., *Fomitopsis pinicola* (Sw.) P. Karst., *Pappia fissilis* (Berk. & M.A. Curtis) Zmitr., etc.). The angiosperm hosts include species from natural forest communities, e.g. *Fagus, Quercus, Carpinus, Populus* (Szabó 2012; Siller *et al.* 2013). Thus, polypores inhabiting both native and non-native gymnosperms and angiosperms may be originally considered indigenous to Hungary, with a preference for a broad range of hosts.

A bit smaller amount of *Pinus*-inhabiting polypores in Hungary are restricted only to gymnosperms: e.g. *Neoantrodia serialis* (Fr.) Audet, *Climacocystis borealis* (Fr.) Kotl. & Pouzar, *Gloeophyllum sepiarium* (Wulfen) P. Karst., *Gloeoporus taxicola* (Pers.) Gilb. & Ryvarden, *Osmoporus odoratus* (Wulfen) Singer, *Skeletocutis amorpha* (Fr.) Kotl. & Pouzar. Some species are found on *Pinus* exclusively: e.g. *Antrodia ramentacea* (Berk. & Broome) Donk, *Dichomitus squalens* (P. Karst.) D.A. Reid, *Diplomitoporus flavescens*

(Bres.) Domański, *Porodaedalea pini* (Brot.) Murrill. According to Igmándy (1981), a small number of these species associated with Scots pine were also found on Austrian pine (e.g. *Climacocystis borealis*, *Postia fragilis* (Fr.) Jülich), while some other species were only present on the former host (e.g. *Dichomitus squalens, Fuscoporia viticola* (Schwein.) Murrill).

Taken these into consideration, it seems that naturalized and non-native *Pinus* plantations have an important role in conserving indigenous polypore diversity. Igmándy's collection of *Onnia triqetra* from 1959 shows, that even a wider spectrum of poroid species were most probably present since a long time ago in Hungarian semi-natural Scots pine forests. The ongoing shrinkage of these forests (KSH 2013; Komarek 2018) and the recent occurrence of *O. triquetra* on Austrian pine highlights the importance of planted, non-native stands of conifers in preserving fungal biodiversity. These stands could serve as possible surrogate habitats for indigenous fungi, as it was presented in many other cases (Humphrey *et al.* 2000; Johnston 2010; Edman and Fällström 2013; Sarrionandia *et al.* 2015; Dejene *et al.* 2017).

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EFFECTS OF WATER DEFICIT AND SALT STRESS ON SOME PHOTOSYNTHESIS PARAMETERS IN WHEAT AND *AEGILOPS COMOSA* LINES

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Abstract: Photosynthetic responses of *Aegilops comosa* genotypes were compared to those of wheat Mv9kr1 and Chinese spring in order to verify whether *Ae. comosa* TA2760 and MvGB1039 genotypes are potentially suitable gene sources for improving the drought and salt tolerance of bread wheat. Although there are some differences between the non-stressed plants and the measure of the decrease of the net-photosynthesis (P_N), it was strongly inhibited by water deficit. Salt stress had similar effect on P_N but at the highest (300 mM) NaCl concentration P_N of the genotypes showed some activity. Severe drought induced a strong decrease in the effective quantum yield of PS II (ϕ_{PSII}) in the genotypes, while it was moderate in the case of salt treatment. Moreover, ϕ_{PSII} was unaffected by the increase of NaCl concentration in wheat lines. Parallel with the decrease in ϕ_{PSII}, the photoprotective mechanisms were enhanced in the wheat and wild wheat genotypes during water deficit. These results suggest that the *Ae. comosa* genotypes seem to respond to these stress factors with similar photosynthetic activity to the wheat lines. Thus, based on the above-mentioned facts, the examined *Ae. comosa* lines are not particularly good candidates for improving drought tolerance of wheat.

Keywords: wheat, *Aegilops comosa*, photosynthesis, water deficit, salt stress

INTRODUCTION

Abiotic environmental factors due to the forecasted effects of global climate change can considerably endanger the productivity of cultivated plants. An important problem is the rhapsodic rainfall

pattern (Trenberth *et al.* 2007), which often results agricultural damages by drought and/or salt stress. Although drought is the most common abiotic stress factor (Araus *et al*. 2002) but also the agricultural lands are often affected by salt stress (Munns 2005). In field conditions, a positive relationship of photosynthetic capability and crop production has been well documented. At the same time, photosynthesis is particularly sensitive to drought and salinity (Ashraf and Harris 2013, Dulai *et al*. 2014, Szopkó *et al*. 2017a, Szopkó and Dulai 2018).

Under stress conditions the limitation of photosynthetic capacity takes place in two stages: (i) limitation associated with decreased stomatal conductance, known as stomatal limitation (L_s, Centritto *et al.* 2003, Dulai *et al.* 2014); (ii) limitation due to nonstomatal processes (L_{ns}) mainly at severe drought and higher salt concentrations or longer salt stress (James *et al.* 2002, Centritto *et al.* 2003, Munns *et al.* 2006, Szopkó *et al.* 2017b). Under moderate water deficit or in the first stage of salt stress, which also has osmotic effect, the reduction in net photosynthesis (P_N) mainly due to the stomatal closure (Chaves 1991; Medrano *et al*. 2002). In this case the CO_2 diffusion into the leaves is also restricted resulting in a decrease in the intercellular CO_2 concentration (C_i) (Cornic 2000) and CO_2 carboxylation (Flexas *et al*. 2004). At the same time, the reduced stomatal conductance (g_s) may contribute to maintaining water content through a decreased transpiration rate, which could be favourable for minimizing Na^+ transport towards the shoots (Tester and Davenport 2003). Thus, closed stomata have both positive and negative effects on photosynthesis (Szopkó *et al*. 2017b).

When the stress turns severe, photosynthesis is also limited by factors other than stomatal closure. The drought induced non-stomatal limitation of P_N may be caused by the restricted mesophyll conductance (Loreto *et al*. 2003) or by metabolic factors (Medrano *et al*. 1997, Centritto *et al*. 2003, Chaves *et al*. 2003). Similar to water deficit, salt stress also has many consequences for non-stoma-dependent processes as well. Salt-induced non-stomatal inhibition (L_{ns}) can be observed when the CO_2 assimilation is disturbed by the presence of toxic ions. This limitation may be associated with limited Rubisco activity a reduced amount of Rubisco protein or week efficiency of PSII in the second stage of salt stress (Muranaka *et al*. 2002, Kalaji *et al.* 2011), when a high

concentration of toxic Na^+ and Cl^- ions evolves in the leaves (Munns and Tester 2008).

During drought and salt stress photosynthesis is often hindered by the secondary effect of disturbed water and ion homeostasis. The increasingly severe limitation of photosynthesis leads to the plant absorbing more light energy than that can be used by CO_2 fixation (Smirnoff 1993). Although the excess light can be partially dissipated as heat, it has the potential to cause the over-reduction of the linear electron transport chain, leading to oxidative damage (Smirnoff 1993, Flexas *et al*. 2004). Under these circumstances the down-regulation of photosynthesis by non-radiative energy dissipation (Demmig-Adams *et al*. 1996) and/or photorespiration represent an efficient defence mechanism in C_3 plants. Thus, the facility to maintain promising photosynthesis and consequently achieve adequate growth and production are based on these intensive protecting/regulating mechanisms.

Interspecific hybridization of wheat with wild relatives is an appropriate breeding strategy to improve the stress tolerance (Colmer *et al.* 2006, Schneider *et al*. 2008, Pradhan *et al*. 2012). *Aegilops* species are widely used as genetic resources in the breeding of bread wheat. These plants are native in the Mediterranean coastal areas characterised by hot, dry vegetation periods often with high salinity (Molnár *et al*. 2004, Dulai *et al*. 2006). Relating to this, *Aegilops* species might adapt to the unfavourable environmental conditions thus their ability to tolerate some abiotic stresses has already been partly described (Zaharieva *et al.* 2001, Molnár *et al.* 2004, Dulai *et al*. 2006).

However, the drought and salt tolerance of some *Aegilops comosa* genotypes is unclear. The aim of the present study was to clarify the drought and salt tolerance of the *Ae. comosa* TA2760 and MvGB1039 lines. For this purpose these plants were exposed to drought and salt stress and the photosynthetic responses of these genotypes were compared to those of wheat Mv9kr1 and Chinese spring. It is revealed that whether *Ae. comosa* TA2760 and MvGB1039 genotypes are potentially suitable gene sources for improving the drought and salt tolerance of bread wheat.

MATERIALS AND METHODS

Plant materials

In our experiments *Ae. comosa* TA2760*,* MvGB1039, Mv9kr1 and Chinese spring wheat genotypes were investigated. The seeds of lines were provided by Márta Molnár-Láng and István Molnár, Agricultural Institute of the Hungarian Academy of Science (Martonvásár, Hungary). The seeds were germinated in laboratory conditions on filter paper moistened with distilled water than they were grown in half-strength modified Hoagland nutrient solution (Nagy and Galiba 1995) in 1500 ml pots or were planted in soil (5 seeds/pot). Plants grow at 25/20°C in a growth chamber with a photosynthetic photon flux density of 200 μE m^{-2} s^{-1} and 14/10 hours of light/dark illumination. Salt stress was induced by applying 150 and 300 mM concentration of NaCl (Sigma, St. Louis, USA) in seven-day cycles. After reaching the highest salt concentration, the salt was eliminated from the medium. Measurements were made before the treatment (5-week old plant), after each seven-day treatment and after two and seven days of regeneration without NaCl. The watering of the *Aegilops* lines and wheat lines grown in soil was abolished after the age of 5 weeks. In the case of water-deficient plants, the measurements were performed on the 4th, 7th and 10th day of water shortage. The regeneration ability was investigated by the total humidification of the soil.

Gas exchange measurements

The CO_2 assimilation of intact leaves was measured with an infrared gas analyser (GFS-3000FL, Walz, Effeltrich, Germany). The net assimilation rate (P_N) was calculated in the light-saturated state of photosynthesis (1000 μmol m^{-2} s^{-1}) using the equations reported by von Caemmerer and Farquhar (1981). The gas exchange chamber parameters were 25°C, 20% relative humidity. The CO_2 concentration of the reference air was 360 ppm.

Chlorophyll fluorescence measurements

The *in vivo* chlorophyll *a* fluorescence was measured in dark-adapted intact leaves using a dual channel P700 and chlorophyll fluorescence measuring system (Dual PAM-100, Walz, Effeltrich, Germany). The initial level of fluorescence (F_0) was excited by a

weak 460-nm light beam after 15 min dark adaptation. The maximal fluorescence level of the dark- (F_m) and light- (F_m') adapted leaves were determined by applying saturating flashes (15000 μmol m^{-2} s^{-1} PAR) with 0.8 s duration. Photosynthesis was induced by continuous illumination of the leaf at 1000 μmol m^{-2} s^{-1} (650 nm, actinic light) for 10 min. The fluorescence parameters were calculated as described by van Kooten and Snel (1990) on the basis of the following equations: maximal quantum yield of PSII, $F_v/F_m=(F_m-F_0)/F_m$; effective quantum yield of PSII, $\Phi_{PSII} = (F_m' - F_s)/F_m'$; non-photochemical quenching, NPQ= $(F_m - F_m')/ F_m'$.

Statistical analysis

Five measurements were performed on each genotypes and treatment for chlorophyll fluorescence and for CO_2 gas exchange analyses. The results are presented as the means ± standard deviations (SD) of five independent experiments. Differences between treatments or genotypes within each treatment were determined by means of Tukey's post hoc test ($p \leq 0.05$) using the SPSS 16.0 software.

RESULTS

Water deprivation, similar to increase of salt concentration, resulted in a gradual decrease in stomatal conductance. In the water stressed lines almost total closure of the stomata was observed at the 10-day water stress while it was not fully complete at the highest salt concentration (data not shown). Although there are some differences between the non-stressed plants and the measure of the decrease of the CO_2 assimilation rate, P_N was strongly inhibited by water deficit at the seventh day of treating (*Figure 1A*). When the drought stress was more severe (at the tenth day of the treatment) P_N was almost fully inhibited in all of the examined genotypes. Salt stress had similar effect on P_N but at the highest (300 mM) NaCl concentration, P_N of the genotypes showed some residual activity. However, the measure of this inhibition was more or less proportional to that of the water-deficient plants. During the regeneration period P_N was restored swiftly for the drought treated plants: the recovery was complete on the second day of the relaxation period. In the case of salt stress the

restoration was slower but also complete by the 7th day of the regeneration period.

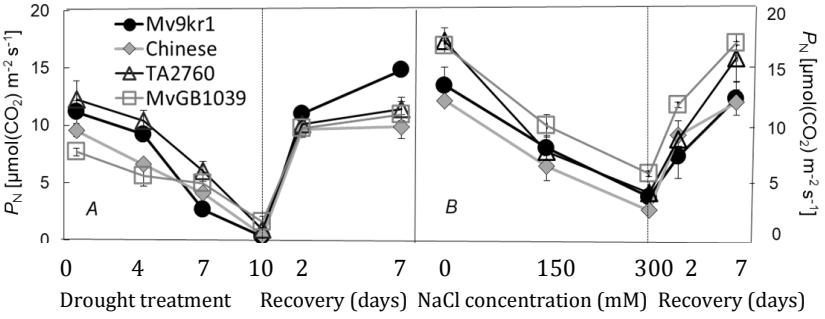

Figure 1. Effects of water deficit, NaCl concentration and recovery on net photosynthesis (P_N) in wheat (Mv9kr1, Chinese spring) cultivars and *Aegilops comosa* (TA2760, MvGB1039) genotypes.

Chlorophyll fluorescence measurements provide a fast and adequate method for analysing the functioning of the photosynthetic apparatus. Although there was a slight decrease in the optimal quantum yield of PS II (F_v/F_m) by the second day of the regeneration period in the case of the salt treatment, both water and salt stresses did not result a significant decrease in this parameter in any plants (*Figure 2C, F*). In untreated plants and under mild (four day drought treatment and 150 mM NaCl) stress conditions, ϕ_{PSII} was similar in the genotypes (*Figure 2A, D*). The severe drought induced a strong decrease in the effective quantum yield of PS II (ϕ_{PSII}) in the genotypes, while it was moderate in the case of salt treatment. Moreover, ϕ_{PSII} was unaffected by the increase of NaCl concentration in wheat Mv9kr1. Parallel with the decrease in ϕ_{PSII}, the non-photochemical quenching (NPQ), reflecting the regulated heat dissipation, increased especially during the drought treatment, and slowly recovered to the original level after the stress treatments (*Figure 2B*). By contrast, NPQ showed a slight increase in Mv9kr1 and Chinese spring wheat cultivars during salt treatment. At the same time, this parameter rose sharply in *Ae. comosa* TA2760 and MvGB1039 and did not recovered fully during the seven days relaxation period (*Figure 2E*). Thus, the 10-day water deprivation induced NPQ in all lines with very similar extent, but the increase of photo-protective

mechanisms were detected only in *Ae. comosa* genotypes when salt stress was developed.

DISCUSSION

Under water deficit and/or salt stress, the stomata closure is a well-known phenomenon (Molnár *et al*. 2004, Dulai *et al*. 2014, Szopkó *et al*. 2017b). The decrease in stomatal conductance (g_s) affects not only the regulation of water loss through the transpiration, but also inhibits the photosynthetic CO_2 fixation by limiting CO_2 diffusion into the leaves (Chaves 1991, Cornic 2000, Flexas and Medrano 2002). As mentioned above, the water deprivation caused an almost total closure of the stomata at the 10-day water stress (data not shown). In our experiments, the CO_2 assimilation rate modified likewise as g_s: P_N values were substantially decreased not only in wheat but also in wild wheat lines. Although the better tolerance of several *Aegilops* species to drought is well documented (Molnár *et al*. 2004, Dulai *et al*. 2006), these results indicate that drought tolerance of the examined wild wheat lines according to CO_2 assimilation processes is similar to those in wheat genotypes. Thus, based on the overall photosynthetic capacity, the examined *Ae. comosa* lines are not particularly good candidates for improving drought tolerance of wheat.

Similar to drought, photosynthetic processes are also modified during salt stress (Szopkó *et al*. 2017b). Prior to the accumulation of toxic ions, salt treatment also causes osmotic stress, influencing the water status, stomatal conductance and net carbon fixation capacity of plants (Munns 2002, Munns and Tester 2008). P_N was a little bit higher in *Ae. comosa* MvGB1039 than this was in wheat lines at all levels of salt treatment ($P \leq 0.05$). Apart from this, it decreased considerably even at a moderate stress level in all lines.

These results show that the examined lines were not able to maintain their photosynthesis at a promising level during salt stress. Compared to the drought treatment, at 300 mM salt concentration P_N of the genotypes showed a little bit higher activity than the 10-day water deprivation. This is probably due to the less strong closure of stomata.

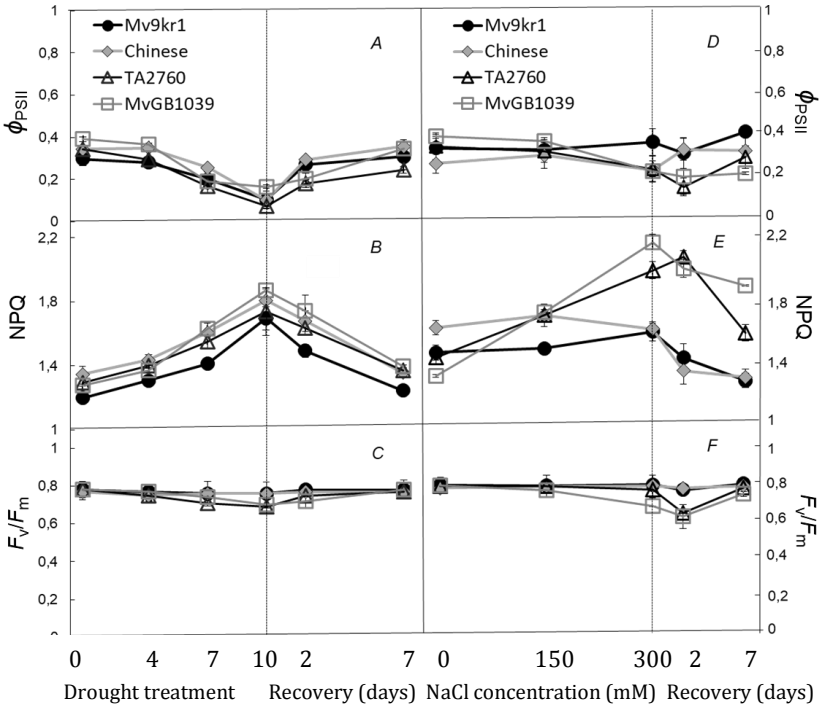

Figure 2. Effects of water deficit, NaCl concentration and recovery on effective quantum yield (A, D), non-photochemical quenching (B, E) and optimal quantum yield of PS II (C, F) in wheat (Mv9kr1, Chinese spring) cultivars and *Aegilops comosa* (TA2760, MvGB1039) genotypes.

When drought or salt stress become severe and CO_2 assimilation is strongly inhibited, the role of non-stomatal factors in the limitation of photosynthesis usually becomes more pronounced (Brugnoli and Lauteri 1991, Qin *et al.* 2010). One of the non-stomatal factors affecting CO_2 fixation during water deficit or salt stress is the inhibition of the photochemical and electron transport processes (Keck and Boyer 1974, Giardi *et al*. 1996, Szopkó *et al*. 2017b). At the same time, the contribution of these processes to the limitation of P_N usually depends on the duration/intensity of the treatment (Kalaji *et al.* 2011). In the present experiments, the optimal quantum yield (F_v/F_m) were practically unaffected by the treatments. Consequently, our results show that the applied water deficit and salt treatment has only a marginal effect on the capacity of primary charge separation. Thus, no PSII damage observed, as

also reported by previous studies (Ben *et al*. 1987, Grieu *et al*. 1995, Dulai *et al*. 2006, 2014, Stiller *et al*. 2008, Szopkó *et al.* 2017b).

The different performance of effective quantum yield of PSII (ϕ_{PSII}), indicated that the electron transport processes were influenced distinctly by drought and salt stress in the given lines. Drought has significant effect on ϕ_{PSII} in all lines: the values of this parameter gradually decreased during the treatment indicating that electron transport processes were partly down-regulated in these genotypes. Parallel with the decrease of ϕ_{PSII}, the photoprotective mechanisms were intensely accelerated in the wheat and wild wheat genotypes during water deficit, as indicated by the higher values of the non-photochemical quenching (NPQ). These processes compete with primary photochemistry for the absorbed excitation energy, leading to a decrease in ϕ_{PSII} (Genty *et al.* 1989) and an increase in non-radiative energy dissipation in the light-harvesting complexes (Horton and Ruban 2005, Chaves *et al.* 2009). In spite of the drought treatment, ϕ_{PSII} was less sensitive to the applied range of salt stress in most of the lines, and even in wheat genotypes, it has not decreased in parallel with the treatment. It seems unlikely that the down-regulation of the PSII-driven electron transport is responsible for the limitation of photosynthesis, because the decrease in ϕ_{PSII} was relatively moderate and the relaxation of photosynthetic CO_2 fixation in wheat genotypes was faster than the recovery of ϕ_{PSII}. It has also been shown by several authors (Apostolova *et al*. 2006, Dulai *et al*. 2014, Szopkó *et al*. 2017b) that PSII is usually more sensitive to stress factors (drought, salt, heat, etc.) than PSI. Moreover, PSI activity may even be enhanced during salt stress (Sudhir *et al.* 2005). Consequently, there is a possibility that electrons may also follow a cyclic route, around the PSI and known as cyclic electron flow (CEF), which generate ΔpH across the thylakoid membranes leading to the formation of ATP but not NADPH, thus preventing the over-reduction of the acceptor side of PSI. Consequently, CEF helps to prevent the subsequent oxidative damage when carbon fixation is limited by water deficit or salt stress (Golding and Johnson 2003, Dulai *et al.* 2014, Szopkó *et al*. 2017b). Based on the higher values of NPQ in all lines under severe drought and in *Aegilops* genotypes under severe salt stress might make it possible that CEF may help to prevent the over-reduction of the electron

transport chain and subsequent oxidative damage (Dulai *et al.* 2014, Szopkó *et al.* 2017b).

In conclusion, the results proved that the examined *Aegilops comosa* lines were not able to maintain their photosynthesis at a promising activity under drought and salt stress. These lines seem to respond to these stress factors with similar photosynthetic activity to the wheat lines. Thus, based on the above-mentioned facts, the examined *Ae. comosa* lines are not particularly good candidates for improving drought and/or salt tolerance of wheat.

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UNDERSTORY CONDITION IN AN OAK FOREST AFTER 4 DECADES FOR OAK DECLINE IN HUNGARY

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Abstract: At the study area serious oak decline was detected from 1979-80 in a mixed oak forest, an area covered by a sessile oak–Turkey oak forest (*Quercetum petraeae-cerridis*). The shrub community was divided into low (lower than 1.0 m in height) and high shrub layer (≥ 1.0 m). The goals of this study were to determine the conditions of shrub layer and analyse the possible changes in the shrub layer after 4 decades of the serious oak decline. In 2017, 17 shrub species were continuously observed in the understory. The density of shrub layer was 25,103 specimens ha^{-1}. The significant part of shrubs lived (91.6%) in the low shrub layer, with only a small part of them (8.4%) forming the high shrub layer. The most common species of the shrub community was *Euonymus verrucosus* with 1989 shoots in the monitoring plot. The mean height and mean diameter of the high shrub species changed between 1.29-8.74 m and between 0.81-9.61 cm. The mean cover of the high shrub species changed between 0.56 m^2 and 12.67 m^2. Our results suggest that three woody species, *Acer campestre*, *Acer tataricum* and *Cornus mas* responded successfully to the oak decline.

Keywords: shrub layer, Síkfőkút Project, field maple, mean size, foliage cover

INTRODUCTION

Oak decline has been described as a widespread and complex phenomenon in many countries (Tomiczek 1993, Sonesson and Drobyshev 2010). An increase in the death of oak species has been observed in many regions of Hungary since 1978 (Igmándy *et al.* 1987). In the Síkfőkút research stand (*Quercetum petraeae-cerridis* Soó 1963) species composition of the canopy was stable until 1979 and the healthy *Quercus petraea* Matt. L. (sessile oak) and *Quercus cerris* L. (Turkey oak) also remained constant. Serious oak decline

was first reported in 1979–80 and by 2017, 62.9% of the oaks had died.

Relatively few studies deal with shrub communities and shrub layer dynamics after oak death and the relationship between the trees and shrubs (Légaré *et al.* 2002). Understory and overstory relationships are complex and mutual but are dominated by the canopy structure and condition (Burrascano *et al*. 2011, Burton *et al*. 2011, Cutini *et al*. 2015). Shrub layers of forest ecosystems change dynamically and respond sensitively to the environmental changes (Chipman and Johnson 2002, Rees and Juday 2002). They are strongly related to the composition and structure of the overstory (Klinka *et al.* 1996, Palik and Engstrom 1999). Shrub species play a major role in the cycles of some essential nutrients, including the dynamics of nitrogen, potassium and carbon (Gilliam 2007). The shrub layers are directly contributes to forest biodiversity (Kerns and Ohmann 2004, Aubin *et al.* 2009), including compositional and structural diversity, enhancing the aesthetics of forest ecosystems and helping to protect watersheds from erosion (Alaback and Herman 1988, Halpern and Spies 1995, Muir *et al.* 2002). Shrubs provide food and habitat, among others, for song-birds, forest ungulates and arthropoda (González-Hernández *et al.* 1998, Yanai *et al.* 1998), can mitigate forest decline and influence forest regeneration through affecting light availability (Kunstler *et al.* 2006).

Misik *et al*. (2013) described the possible responses of parameters of understory shrub layer to the remarkable changes in stand density on the study site. Misik *et al*. (2014) reported the dynamics behind the increase in the sizes of woody species and the structure of the new subcanopy layer below the canopy.

The aim of the study was to investigate the composition, size condition, foliage cover and diversity of understory shrub layer in the oak forest and analysed how shrub layer changed after four decades of serious oak decline.

MATERIALS AND METHODS

Study site

The reserve research site (Síkfőkút Project) was established in 1972 by Jakucs (1985) and is located in the Bükk Mountains (47°552 N, 20°462 E) in the north-eastern part of Hungary at an

altitude of 320-340 m a.s.l. and 6 km from the city of Eger (*Figure 1A*). Mean annual temperature is 9.9°C and mean annual precipitation ranges typically from 500 to 600 mm. Descriptions of the geographic, climatic, and soil conditions, and vegetation of the forest were reported in detail by Jakucs (1985, 1988), Tóth *et al.* (2013) and Fekete *et al.* (2014). The *Quercetum petraeae-cerridis* community with a dominant canopy of *Q. petraea* and *Q. cerris* deciduous tree species structure is presented in the works of Mázsa *et al*. (2005), Kotroczó *et al*. (2007) and Fekete *et al*. (2017); the long-term dynamics of understory shrub layer and oak seedling dynamics are described among others in works of Misik and Kárász (2010) and Misik *et al*. (2013, 2017). The plots under study were made up of evenly aged temperate, mixed species deciduous forest that was at least 110 years old and had not been harvested for more than 55 years.

Sampling and data analysis

The structural condition of the shrub layer was monitored on an "A" plot at the research site, 48 m × 48 m in size; the plot was divided into 144 permanent subplots of 4 m × 4 m (*Figure 1B*).

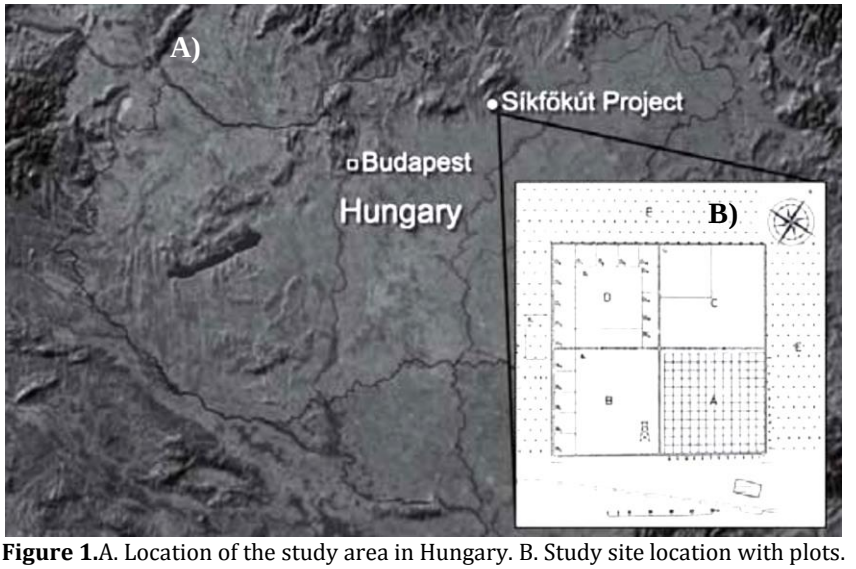

Figure 1.A. Location of the study area in Hungary. B. Study site location with plots.

The subplots were established in 1972; the understory data collected at subplots measured during the 2017 field season on site. Woody shoots of the understory were classified as subcanopy trees when between 8.0–13.0 m in height. The shrub specimens of the vegetation lower than 1.0 m in height were categorized as low understory; higher specimens between 1.0 m and 8.0 m were categorized as high understory. Oak stems < 50.0 cm and between 50.0 and 100.0 cm in height were inventoried and categorized as oak seedling and oak saplings. The term "dominant woody" is used to refer to certain species that play a key role in a shrub layer based on the high densities and largest mean sizes. The following measurements were carried out for understory shrub species in each subplot: species composition, frequency (occurrence % in subplots of the monitoring plot), species density, height and diameter, foliage cover of species and of shrub layer and finally diversity indices. The shrub specimen's density was extrapolated for one hectare. It was recorded the specimen height with a scaled pole and the diameter at a height of 5 cm above the soil surface with a digital calliper. The size condition of each high shrub specimen was measured; in the low shrub layer, a number of specimens were randomly selected in proportion to the species density to determine the size parameters. Effective foliage, duplex- and multiplex (shrub canopy overlapped other shrubs) and finally the simplified cover on the basis of all the shrub specimens foliage of the shrub layer were recorded in the quarter hectare plot. The following diversity indices were used: Shannon-index (H) and Evenness (E).

$$H' = -\Sigma (p_i \times \ln p_i)$$
$$E = H'/H_{max} = H'/\ln S$$

where: p_i – proportion of specimens found in the i^{th} species, S – total number of species in the shrub layer. Evenness was calculated as the ratio of observed diversity (H) to maximum diversity (H_{max}) (Magurran 1988).

RESULTS

Composition and density

Seventeen native woody species were identified across the entire study area in 2017. In the high shrub layer (composed 13 species) was not lived *Q. cerris*, *Quercus pubescens* Willd. (downy oak),

Rhamnus cathartica L. (buckthorn) and *Rosa canina* L. (dog rose). Three native woody species were detected in the subcanopy layer; *Acer campestre* L. (field maple), *Cornus mas* L. (European cornel) and *Acer tataricum* L. (Tatar maple) (*Table 1*).

The density of shrub layer per hectare was 25,103 specimens. The most common low shrub species were *Euonymus verrucosus* Scop. (spindle tree) and *Q. petraea*; the most common high shrubs were *A. campestre* and *E. verrucosus*. The significant part of shrubs lived (91.6%) in the low shrub layer, with only a small part of them (8.4%) forming the high shrub layer. The most common species of the shrub community was *E. verrucosus* with 1989 shoots in the study site; followed them *Q. petraea* and *A. campestre* (*Table 1*). Many *A. campestre* specimens (almost 28% of these woody species) and some *A. tataricum* (almost 13%) and *C. mas* (8% of these species) were present as subcanopy species in the sample site below the canopy between 8.0-13.0 m in 2017.

Table 1. Species composition and density condition of the understory shrub layer on the Síkfőkút mixed oak forest in 2017.

species	low shrub layer			high shrub layer			total density ind.
	density ind.	density ind. ha^{-1}	rate %	density ind.	density ind. ha^{-1}	rate %	
A. campestre	553	2400	10.44	125	543	25.80	678
A. tataricum	59	256	1.11	20	87	4.13	79
C. mas	35	152	0.66	86	373	17.72	121
C. sanguinea	191	829	3.60	46	199	9.45	237
Cr. monogyna	91	395	1.72	53	230	10.93	144
E. europaeus	429	1862	8.10	12	52	2.47	441
E. verrucosus	1866	8098	35.21	123	534	25.37	1989
J. regia	14	61	0.26	2	9	0.43	16
L. vulgare	330	1432	6.23	12	52	2.47	342
Lo. xylosteum	14	61	0.26	2	9	0.43	16
Q. cerris	146	634	2.76	0	0	0.00	146
Q. petraea	965	4188	18.21	1	4	0.19	966
Q. pubescens	480	2083	9.06	0	0	0.00	480
P. avium	109	473	2.06	2	9	0.43	111
Rh. cathartica	3	13	0.05^{-2}	0	0	0.00	3
R. canina	11	48	0.21	0	0	0.00	11
T. cordata	3	13	0.05^{-2}	1	4	0.19	4
total	5299	22998	100	485	2105	100	5784

Size condition

The mean height of the shrub species changed between 1.29 m and 8.74 m in the high shrub layer [except of the characteristically tree size *Prunus avium* L. (wild cherry) and *Tilia cordata* Mill. (small-leaved lime) species]. It was measured 5.26-8.74 m mean height by the dominant woody species of the shrub community. It was recorded between 0.81 cm and 9.61 cm mean diameter values of the high shrub species in 2017. The biggest species was the *A. campestre* with 8.74 m mean height and 9.61 cm mean diameter.

Table 2. Height and diameter condition (means ± standard deviation) of the understory shrub layer on the Síkfőkút mixed oak forest in 2017.

species	low shrub layer			high shrub layer	
	mean height (cm±S.D.)	mean diameter (mm±S.D.)	measured shoots number	mean height (m±S.D.)	mean diameter (cm±S.D.)
A. campestre	16.67±8.92	3.02±2.03	76	8.74±5.86	9.61±5.42
A. tataricum	24.30±17.28	3.85±2.33	18	5.31±1.88	6.08±3.00
C. mas	39.93±26.86	4.20±2.07	19	5.26±1.99	6.95±2.91
C. sanguinea	39.78±22.55	4.25±1.75	27	1.81±0.80	1.37±0.94
Cr. monogyna	36.98±22.88	6.15±3.20	24	2.62±1.43	3.11±1.60
E. europaeus	18.53±12.99	3.01±1.55	55	3.43±0.80	3.88±1.71
E. verrucosus	29.22±17.99	4.17±2.18	202	1.84±0.81	2.12±1.63
J. regia	33.74±11.51	4.15±1.24	11	1.30±0.21	1.07±0.16
L. vulgare	28.78±16.21	3.68±1.81	75	1.29±0.21	0.81±0.21
Lo. xylosteum	41.15±28.26	5.10±3.80	6	1.43±0.27	1.82±0.42
Q. cerris	14.50±5.81	2.03±1.22	13	-	-
Q. petraea	13.69±6.66	2.03±1.57	148	1.16±0.00	1.79±0.00
Q. pubescens	15.53±7.18	2.35±1.51	35	-	-
P. avium	21.43±10.50	3.55±2.53	41	10.08±12.47	15.08±18.34
Rh. cathartica	12.98±3.39	2.93±1.80	3	-	-
R. canina	12.20±3.76	1.53±0.93	5	-	-
T. cordata	22.02±4.90	20.58±10.82	3	10.46±0.00	9.87±0.00

The biggest height and diameter values were detected for a single *A. campestre* with 23.40 m and for a single *P. avium* with 28.04 cm under the canopy. The mean height of the other shrubs (except of the tree size *P. avium* and *T. cordata* species) was 1.86 m. It was recorded 2.00 cm mean shoot diameter of these species in the high shrub layer. The mean values changed between 12.20 and 41.15 cm in height and between 1.53 and 6.15 mm in diameter (except of some *T. cordata*) in the low shrub layer (*Table 2*). The highest species was observed as *Lo. xylosteum* and the thickest as *Cr. monogyna* among the low shrubs (*Table 2*).

Table 3. Foliage cover condition (means ± standard deviation) of the understory shrub species on the Síkfőkút mixed oak forest in 2017.

species	low shrub layer		high shrub layer	
	mean cover (cm^2±S.D.)	measured shoots number	mean cover (m^2±S.D.)	total cover (m^2)
A. campestre	419.98±291.06	150	12.34±12.16	1542.09
A. tataricum	500.13±418.86	31	9.97±7.08	199.39
C. mas	1561.17±1469.36	6	12.67±11.28	1089.64
C. sanguinea	462.71±410.46	21	0.92±0.98	42.39
Cr. monogyna	788.35±743.71	20	2.28±2.73	121.02
E. europaeus	681.31±680.82	70	3.93±2.74	47.16
E. verrucosus	316.83±246.02	212	1.75±1.93	215.60
J. regia	1226.14±650.22	7	0.56±0.22	1.13
L. vulgare	679.43±775.65	45	0.74±0.80	8.86
Lo. xylosteum	2630.57±1377.09	7	0.90±0.74	1.80
Q. cerris	275.65±400.05	17	-	-
Q. petraea	271.44±409.36	124	3.08±0.00	3.08
Q. pubescens	395.68±858.85	22	-	-
P. avium	554.94±584.76	17	18.00±24.01	34.19
Rh. cathartica	194.67±125.12	3	-	-
R. canina	274.29±454.98	7	-	-
T. cordata	53.00±46.70	3	13.27±0.00	13.27

Foliage cover

The mean cover of the shrub species changed between 0.56 m^2 and 12.67 m^2 in the high shrub layer. It was measured 9.97-12.67 m^2 mean foliage cover by the dominant woody species of the shrub community. The mean cover of other shrubs in the high shrub layer changed between 0.56-3.93 m^2. The highest cover values were detected for a two *P. avium* and single *T. cordata* tree species with 18.00 m^2 and with 13.27 m^2 under the investigation. In the low shrub layer the mean foliage cover was detected between 195 and 2631 cm^2 (except of the three *T. cordata* specimens because they have lost of own remarkable foliage) (*Table 3*). In 2017 was measured a relatively high size of the foliage condition. The effective cover was 91.26%, the duplex- and multiplex cover was 44.77% and was measured 144.08% total foliage cover in the high shrub layer (*Table 4*).

Table 4. Foliage cover condition (rate in % and size in square meter) of the understory high shrub layer on the Síkfőkút mixed oak forest in 2017.

foliage cover	effective cover	duplex and multiplex cover	simplificalt cover
rate (%)	91.26	44.77	144.08
size (m^2)	2102.61	1031.54	3319.62

Diversity indices

Shannon-Wiener index varied between 1.87 and 2.07 in the understory shrub layer in 2017. The highest index was recorded in the total shrub layer; followed them tightly the low shrubs. In the low shrub layer was measured only 1.39 Shannon index value without oak seedlings and saplings. Evenness index varied between 0.71 and 0.73 in the understory. It was detected a low difference between the different shrub layers. In the low shrub layer was measured 0.53 Evenness value without oak seedlings and saplings (*Table 5*).

Table 5. Shannon and Evenness indices of the understory shrub layer on the Síkfőkút mixed oak forest in 2017.

layers / index	low shrub layer	low shrubs without oaks	high shrub layer	shrub community
Shannon	2.0118	1.3900	1.8770	2.0719
Evenness	0.7100	0.5267	0.7318	0.7313

DISCUSSION

The consequences of tree decline cause notable changes in the light and stand thermal conditions which led to structural changes of the shrub layer (Chapman *et al.* 2006). Our results suggest and confirm that the decreasing tree density in canopy led to the remarkably structural changes of the shrub community. In the past 4 decades despite the heavy oak decline; there is no new shrub species established in the study site of Síkfőkút. Only one woody species, a single *T. cordata* shoot was established in the forest as new species. Similarly to our site, in the Vár-hegy forest reserve of Hungary the species composition of understorey (herb and shrub layer) did not change after serious oak decline in the 1970s and 1980s (Horváth 2012). The total density of shrub community decreased considerably, from 97,201 to 25,103 specimen's ha^{-1} on Síkfőkút site (*Table 1*). Refutation, Chapman *et al*. (2006) described that in

the upland oak forest of the USA the total shoot density in the understorey were substantially higher in 2002 than in 1934, increasing from 240 to 688 trees ha^{-1}, while the density of most oak and shortleaf pine species in the canopy decreased appreciably over time.

On site the cover percentage of canopy layer decreased remarkably until 1998 after the large-scale oak decline. Consequently, heterogeneous sizes of canopy gaps were formed in the studied forest. A similar situation could be detected in Vár-hegy forest, where 20–50% of canopy gaps were formed as the consequences of oak decline in the 1980s (Horváth 2012). The autochthonous species *Q. petraea* and *Q. cerris* formed a nearly monolayer canopy (Čermák *et al*. 2008), therefore they could not fully compensate the significantly reduced foliage cover of canopy after the tree decline. In our forest stand the late seral species, as *A. campestre* and *A. tataricum* are generally shade tolerant and respond positively to canopy gaps. Our results confirm this thesis, because the mean sizes of these species increased considerably in the last 4 decades. Before the oak decline the largest specimen of the shrub layer was one *A. campestre* shoot with 4.9 m height. In 2017 as the largest shrub specimen was recorded also one *A. campestre* with 23.4 m height. In addition, the mean height and mean diameter values of the three dominant woody species in 2017 exceeded the biggest sizes before the oak decline (*Table 2*). The rate of the shrub layer's foliage (effective and duplex and multiplex) cover was 64.4% and 13.8% before the oak decline. These values increased to 91.3% and 44.8% after 4 decades (*Table 4*).

Shannon index and Evenness varied between 1.53-2.22 and 0.66-0.77 before the oak decline. These values changed only slightly after 4 decades in the shrub layer of the mixed oak forest. The highest negative difference was detected in the low shrub layer (*Table 5*). Onaindia *et al*. (2004) results suggested to use Shannon diversity and Evenness indices to evaluate the effects of disturbances in temperate forest stands. In the study of De Grandpré *et al*. (2011) the Shannon index increased significantly ($P < 0.001$) with time since treatment application in Canada; along a canopy gap severity gradient in old-growth and mature forest communities.

CONCLUSIONS

Seventeen native woody species were identified across the long-term study area in 2017. The high shrub layer composed only 13 woody species. The density of shrub layer was 25,103 specimens ha^{-1} in 2017. The most common low and high shrub species was *E. verrucosus* and *A. campestre*. The mean height of the shrub species changed between 1.29 m and 8.74 m in the high shrub layer. It was recorded between 0.81 cm and 9.61 cm mean diameter values of the high shrubs. The mean values changed between 12.20 and 41.15 cm in height and between 1.53 and 6.15 mm in diameter in the low shrub species. The mean cover values changed between 0.56 m^2 and 12.67 m^2 in the high shrub layer. In the low shrub layer the mean foliage cover was detected between 195 and 2631 cm^2. Shannon-Wiener index varied between 1.87 and 2.07 in the understory shrub layer of the forest stand. Evenness index varied between 0.71 and 0.73 in the understory. Our results from 2017 suggest that the shrub layer responded positively to the oak decline; this is especially true to the mean sizes of dominant woody species.

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COMPARATIVE ECOPHYSIOLOGICAL STUDY OF THE SEASONALLY DEPENDENT NON-STRUCTURAL CARBOHYDRATE POOL OF THE FRUCTAN-ACCUMULATING *HELIANTHUS TUBEROSUS*, *CICHORIUM INTYBUS* AND *DACTYLIS GLOMERATA*

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Abstract: Our experimental results show that fructans play a major role in the physiological processes of the three species. The two species (*Helianthus tuberosus, Cichorium intybus*) belonging to the *Asteraceae* tend to accumulate low molecular weight oligosaccharides and the species (*Dactylis glomerata*) belonging to the *Poaceae* accumulates medium and high molecular weight oligo- and polysaccharides to increase physiological performance, stress tolerance. Taking into account the climatic and weather conditions, we concluded that the fructan production of the plants belonging to the *Asteraceae* investigated in North Hungary is mainly influenced by the cold, while the species belonging to the *Poaceae* is influenced by temperature factors and also the amount of precipitation.

Keywords: non-structural carbohydrates, fructans, seasonality, *Helianthus tuberosus*, *Cichorium intybus, Dactylis glomerata*

INTRODUCTION

In addition to starch and sucrose, the main products of photosynthesis, a third type of short- and long-term carbohydrate and energy storage component, fructan, is produced in a small fraction (15%) of the flora (Van der Meer *et al.* 1994). Fructans have been researched for over 210 years. In 1993, Suzuki and Chatterton published a complete book on the subject. Fructans were isolated not only from higher plants, but also from bryophytes (Maas and Craigie 1964, Sulieman *et al*. 1979, Marschall 1998, Marschall 2010) and from a number of fungi, algae and bacteria (Lewis 1984). Plant carbohydrates can be divided into two groups

based on their function: structural and non-structural carbohydrates (NSC). The former includes cell wall constituents such as cellulose, pectin, etc., while the latter includes components involved in metabolic processes such as glucose, starch and fructans. Non-structural carbohydrates are the primary products of photosynthesis, key regulators of adaptation to environmental stress, and provide substrates for growth and metabolism, and thus a plant's NSC level provides an important indicator of its carbon source and sink capacity (Liu *et al.* 2018). The role of fructans as storage carbohydrates and their function in desiccation tolerance and low temperature stress is well emphasized in higher plants (Marschall 2010). Fructans also act as antioxidants, scavenging reactive oxygen species and preventing cell damage under abiotic stress conditions (Peshev *et al*. 2013). Fructans are water-soluble carbohydrate chains that are usually formed by the attachment of several fructose units (a few hundred or even thousands) to a glucose unit on a single end-chain sucrose molecule (Chatterton *et al.* 2006), but this terminal and the glucose unit may also be missing. In each case, their basic unit is a trisaccharide monomer consisting of a glucose and 2 fructose molecules in 3 possible modes of linking (G-F-F in two ways, F-G-F). There are three main types of fructans: linear inulins containing mainly β (2-1) bonds, e.g. 1-kestose, levans (or fleins) containing β (2-6) bonds, e.g. 6-kestose and the graminans containing branched bonds e.g. kestopentaose 6,6 & 1 (Van den Ende *et al.* 2011) and studies have reported two other major groups, the inulin neoseries and the levan neoseries (Vijn and Smeekens 1999). In the latter, glucose is not located terminally but within the molecule and is bound to the fructoses at sites 2-1 and 2-6. Their economic importance is that they improve the quality of forage crops and are suitable for human consumption.

About 15% of the Angiosperms, about 45,000 species, are capable of synthesizing fructans, fructose-based oligo- and sucrose-based polysaccharides (Van den Ende *et al*. 2011). The most studied species come from the order of the *Asterales*, the *Poales*, the *Liliaes*, the *Dipsacales* and the *Boraginales* (Van den Ende *et al*. 2011). The plant part in which fructan accumulates (root, tuber, stem, leaf) is a characteristic feature of the order (Van der Meer *et al.* 1994). Fructans are stored in wheat and barley in stems and leaves, tulips and onions in their onions, and in the root/tuber of

chicory and Jerusalem artichoke. They can accumulate up to 20-50% of the dry weight in the above mentioned different plant parts, but their content can even reach 70% of the dry weight in bulbs (Marschall 2010). Fructan-rich species accumulate only traces (~1%) of starch. This also proves that fructan is a real alternative to starch. At cellular level, fructans accumulate in the vacuole (Wagner and Wiemken 1986), where they play an important role in turgor regulation (Pontis 1989), and because of the size of the vacuole is relatively large compared to other cellular organelles, it allows accumulation of high concentrations of fructan (de Moraes *et al.* 2016). More molecules mean that these cells are more resistant to osmotic pressure or even cold, so these plants are better adapted to sudden climate changes. The size of fructan polymers can be altered quickly; this could be an explanation for their role in osmotic adjustment. Polimerization or breakdown of fructan will alter vacuolar osmotic potential, and hence may alter turgor pressure (Marschall 2010). The DP (degree of polymerization) appears to closely track changes in the external environment.

Fructans were later also discovered in the apoplast (Livingston and Hanson 1998, Van den Ende *et al.* 2005). To explain this, it has been hypothesized that fructan molecules are transported to the outside of the plasma membrane by exocytosis via vesicles (Valluru and Van den Ende 2008). Cytoskeletal microtubules, which have a wide variety of functions, are very sensitive to temperature drop, which can cause their depolymerization (Kerr and Carter 1990). It is likely that fructans protect plants from various environmental stresses such as frost and drought (Valluru and Van den Ende 2008) by stabilizing membranes. It was found that fructans inserted between the headgroups of different kinds of phospholipids with some preference for phosphatidylethanolamine (Vereyken *et al.* 2001, Hincha *et al.* 2002, 2003). Pollock's (1986) study shows that starch synthesis decreases dramatically when the temperature drops below 10°C, but photosynthetic processes and fructan production are much less sensitive to low temperatures, suggesting that fructan production benefits those plants that actively photosynthesize during the winter and early spring. The protection of the photosynthetic apparatus as temperature rises, and mobilization of carbohydrates stored in fructans for rapid growth are strong influencing factors in the evolution of fructan

production (Vijn and Smeekens 1999). As well as being the main storage carbohydrate, vacuolar fructans with their synthesis can regulate the concentration of sucrose in plant cells, thereby preventing the inhibition of photosynthetic sugar-induced feedback (Pollock 1986). Global distribution shows that the temperate climate is particularly rich in fructan-producing plants, whereas in the tropical regions they are virtually absent (Hendry and Wallace 1993). Drought, high irradiance or/and low temperature favours fructan accumulation in Angiosperms, so their relevance is linked to desiccation and freezing resistance or emphasized in response to cold and dry seasons (Marschall 2010).

Plant fructans may differ in their molecular structure and weight, in the degree of polymerization (DP), and in the coupling pattern between fructosyl groups (Abeynayake *et al*. 2015). The degree of polymerization (DP) is determined by the number of glucose-fructose units. Fructan oligosaccharides are typically characterized by DP <10. Inulin-type chains are generally between DP 2-60 (Sissons and Fellows 2014), while levan types are slightly longer (DP <200) (Avigad and Dey 1997). These values vary with the current weather conditions and the physiological requirements of the plant. Low polymerization, short oligosaccharides belong to the low molecular weight group (LMW), and long polysaccharides belong to the high molecular weight group (HMW). The simplest, inulin-type fructans occur mostly in dicotyledons, while the levan and graminan types occur in monocotyledons (Van den Ende *et al*. 2011). Although there are many different structural forms of fructans, their appearance in each species is species-specific, ranging from simple inulin homologous in Jerusalem artichoke tubers to complex branched bonds in the leaves of grasses (Cairns and Ashton 1993). A study of Cairns and Ashton (1993) reveals that this species specificity does not depend on the particular plant tissue/cell but on the enzymes that synthesize it in the species.

One enzyme responsible for the synthesis of inulin-type fructan molecules is fructan: fructan fructosyltransferase (FFT), F-type (uses fructose as a donor) fructosyltransferase (FT), and another enzyme which is S-type (uses sucrose as a donor) FT (Van den Ende *et al.* 2011), sucrose: sucrose fructosyltransferase (SST), which binds a β-D-fructose moiety of a sucrose to another D-glucose moiety of sucrose, a trisaccharide, kestose (GFF), and then added further fructose units in the chain extension process

(Marschall *et al.* 1998). Generally, a primary sucrose acts as a fructose acceptor at the start of chain extension, leaving a glucose moiety at one end of the polymer (Franken *et al.* 2013). Whereas in monocotyledons, where the other two fructan types occur, a much more complex mixture of FTs is required, depending on the species (Yoshida *et al.* 2007).

Changes in plant fructan content to various environmental influences

Depending on environmental conditions and plant ontogeny, fructans may be stored where they are synthesized or at their site of use (Van der Meer *et al.* 1994). The accumulation and reduction of fructans in plants is mainly dependent on the temperature, i.e. the season and the amount of precipitation. Chatterton and coworkers (1989) found that in grasses growing in cold seasons, in addition to being a reserve carbohydrate, fructans accumulate in the vacuole, allowing photosynthesis to continue at low temperatures when other carbohydrate reserves would saturate it. Puebla and coworkers (1997) compared the fructan production of two *Bromus* species (*Poaceae*) in the context of temperature and precipitation differences. Cold-adapted species synthesized fructan permanently, whereas species adapted to warmer climates accumulated it only under cold stress. In a study by Pollock and Jones (1979) fructan levels and mean molecular weights were measured throughout the year in leaves and stems of four forage grasses (*Poaceae*). In contrast to other studies, the major period of fructan synthesis in all varieties was autumn and winter, with maximal values in December. Extensive hydrolysis, accompanied by a decline in mean molecular weight, occurred between January and April. Fructan accumulation was maximal in periods when growth was limited. In low temperature wheat (*Triticum aestivum*), as the carbohydrate is produced in excess, the plant stores fructan in the stem (Bancal and Triboi 1993). Ernst and coworkers (1996) concluded that the inulin reserves of *Asteraceae* species were highest in the autumn months before the plants start to break it down due to the low temperature. In the case of woody plants, the exact opposite is true, at least in a study by Furze and coworkers (2019). Their research, which included deciduous and evergreen species, reveals that the amount of non-structural carbohydrates (including fructans) was highest during the growth period. Liu and

coworkers (2018) experimented with 20 evergreen woody plants derived from a subtropical monsoon evergreen forest. They found that starch and NSC concentrations were higher in the dry season in that region, while soluble sugar concentration and the sugar/starch ratio were higher in the wet season. Several studies have been carried out with transgenic plants that otherwise do not produce fructans (Van der Meer *et al.* 1994, Pilon-Smits *et al.* 1995, Pilon-Smits *et al.* 1999, Li *et al.* 2007, Kawakami *et al.* 2008). Kawakami and coworkers (2008) introduced the genes of two fructan synthase enzymes into the genome of a frost-sensitive rice (*Oryza sativa* L.) and concluded that the plant became more resistant to cold after producing fructan molecules.

The aim of this paper is 1) to compare the non-structural carbohydrate pool and its alterations of the fructan-accumulating *Helianthus tuberosus*, *Cichorium intybus* and *Dactylis glomerata* depending on sesonality; 2) to investigate the ratio of high and low molecular weight of fructans of plant organs exposed to stress (temperature and precipitation changes) as a function of seasonality; 3) to obtain new information about the physiological role of soluble carbohydrates, especially fructans in Angiosperms, and their metabolism under various environmental conditions.

MATERIALS AND METHODS

Plant material

Helianthus tuberosus L., or Jerusalem artichoke, is a 1.5-3 m high, invasive, aggressive species that survives on many soils due to its broad tolerance. Tubers are also suitable for human consumption and contain inulin-type of fructan, which consists of β-D-fructosyl units. It is a popular model plant for fructan researchers. In most cases, tubers of the plant have been used for these studies, but similar regulatory mechanisms exist in the stem, although the accumulation of high-polymerisation fructans in tubers is faster (Soja *et al*. 1989). The inulin content and degree of polymerization depend on the variety, the growth conditions, the time of harvest and the maturity of the tubers (Bach *et al.* 2015). Soja and coworkers (1989) also found that, before flowering, the stem is the potential fructan storage organ, and after flowering (at the end of autumn), carbohydrates as sucrose units are translocated into tubers.

Jerusalem artichoke plants used for the experiments were collected in Füzesabony, from a constantly maintained garden in 2018 and 2019 in three seasons, summer, autumn and winter. Laboratory measurements were carried out on tubers only.

Cichorium intybus L. is an 80-100 cm tall native species of Hungary. It belongs to the *Asteraceae* family, like the Jerusalem artichoke, and it also contains inulin-type of fructans (Bonnett *et al*. 1994). It is also a frequently used species in studying fructan-metabolism. The leaves of chicory contain low concentrations of fructans which are most abundant in the basal region of the petiole (Ernst *et al.* 1995). Concentration of fructans with a high degree of polymerisation (DP) increased significantly during the July-September period, and a decrease of the glucose concentration was found at the same time (Van den Ende *et al.* 1996). In early October, important changes occurred over a very short period. Concentration of fructans with a low degree of polymerisation (DP) increased in parallel with a significant increase in fructose and sucrose concentrations, but the number of fructans with a high DP decreased. Cichory used for the experiments was collected from the EKU Almagyar Campus in Eger, in 2018 and 2019 in three seasons, summer, autumn and spring. Laboratory measurements were carried out on the root of the plant only.

Dactylis glomerata L. is a 60-120 cm high, monocot perennial grass that is native to Hungary. There is another type of fructan in the leaves and stems of the plant, levan. The longest fructan chain (DP = 314) described in *Dactylis glomerata* (Yamamoto and Mino 1985). This carbohydrate consists of linear (2-6) linked β-D-fructosyl units and is also found in other grasses (Bonnett *et al.* 1997). Other species from the *Poaceae* such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and rye (*Secale cereale*) are also widely used as model plants in fructan research. They are also capable of synthesizing Graminin-type fructans. Volaire and coworkers (1998) reported that the reason why *Dactylis* can survive extreme drought, even in Mediterranean areas, is related to the plant's high content of fructans with a high degree of polymerization. Sanada and coworkers (2007) in a comprehensive European survey measured the soluble carbohydrate and fructan content of the plant. They concluded that the highest fructan concentrations in Central Europe occured in autumn, which was also due to the fact that the plant was preparing for the winter cold.

Then, by the beginning of the growing season, when the plant had to mobilize its carbohydrate reserves, fructan concentration had declined as the plant produced new shoots and leaves and was ready to flower. *Dactylis glomerata* used for the experiments was collected from the EKU Almagyar Campus in Eger, in two seasons, in the summer of 2018 and in the spring of 2019. Stems, internodes and leaves of the plant were used in laboratory measurements. The fructan content was generally the highest in the nodules. The plant materials of the 3 fructan-accumulating Angiosperms (*Helianthus tuberosus*, *Cichorium intybus* and *Dactylis glomerata)* were collected on the same day of the season, e.g. summer, winter and spring (August, February, April), and within 1 month in autumn (October-November). What was important for the experiment was the weather conditions that characterized the sampling and pre-sampling periods.

Preparation of plant extracts

Samples for carbohydrate analysis were taken in the middle of the photoperiod. Plant tissue was extracted with 80% ethanol followed by hot water (Marschall *et al.* 1998). Supernatant and sediment extracts were also used for assays. 0.5/0.1 g of fresh plant tissue (root, tuber, stem, leaves) was homogenized with 2 ml of 80% ethanol, then was centrifugated for 10 minutes (10.000G). To the sediment remaining in the tube 1 ml of hot distilled water was added for further extraction and after shaking it was incubated at 70°C for 2 hours. During incubation time, the sample was shaken every 30 minutes. Then the tube was centrifuged for 10 minutes at 10.000G and the supernatant (= sediment extract) was kept for assays. Supernatants and sediment extracts were used for fructan, total soluble carbohydrate assay, osmotic potential measurement and thin layer chromatography.

Determination of total soluble sugar and fructan content, and osmotic potential

Total soluble sugars were detected in plant extracts by the Dubois method (1956). Fructans were quantified using a ketose-specific method with resorcinol (Farrar 1993, Marschall *et al*. 1998). Extracts were deionised with a mixed bed of Dowex2-1 and Dowex2-50 ion exchange resins before TLC. Fructans were separated by TLC on silica gel plates (Cairns and Pollock 1988) and

detected with urea-phosphoric acid stain (Wise *et al.* 1995). Plant extracts (50 µl) were used for osmotic potential determination with a freezing point osmometer (OSMOMAT 010). Values are expressed in MPa according to Bajji *et al.* 2001.

RESULTS

The soluble carbohydrate content of the Jerusalem artichoke tuber, which also includes fructans, was high in summer due to the high metabolic activity (*Figure 1*). The plant saved its excess carbohydrate reserves during the spring/summer period so that it could be mobilized from here to the above-ground parts with the onset of cold. This is also evidenced by the high content of the high molecular weight of non-structural sugars, 16100 µM g^{-1} d.w., which represents 60% of the total amount. By the end of fall, the total soluble carbohydrate content decreased to about one-fifth. This can be explained by the fact that the plant redistributed the energy reserves from the tubers to the stems and leaves, further operating the photosynthetic apparatus at low temperatures and low hours of sunshine. Content of high molecular weight (HMW) sugars was reduced to 30% of the total amount, and at the expense of long molecule chain degradation, the resulting mono- and oligosaccharides entered the metabolic pathways. In line with this, the proportion of the low molecular weight carbohydrates (LMW) increased from 40% to 70% in summer, proving the previous statement. By the end of the winter, the total soluble carbohydrate content began to increase compared to the previous season. This is because, as spring approached, the plant slowly began to accumulate carbohydrates in the tuber again. This finding also explains that LMW carbohydrate levels increased by nearly 75% during the 4 months between the two samplings. However, HMW was further reduced to 10%, therefore almost all carbohydrates were present in LMW sugars.

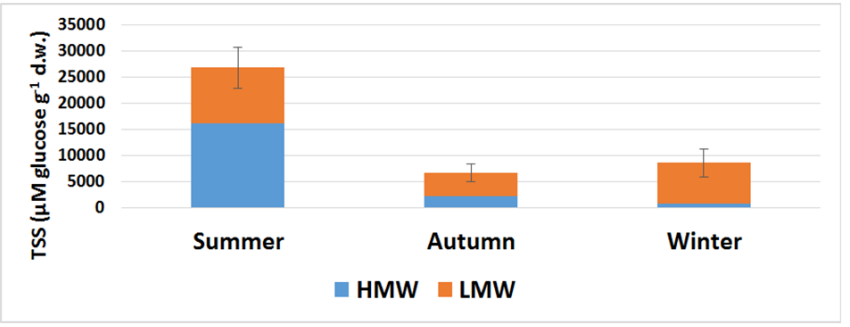

Figure 1. Total soluble sugar content (µM glucose g^{-1} d.w.) of *Helianthus tuberosus* tuber depending on seasonality. HMW (blue column): high molecular weight of soluble carbohydrates; LMW (orange column): low molecular weight of soluble carbohydrates; Error bars are STDs, which concern the sum of HMW + LMW fructans (total fructan), where n=5.

The total fructan content of *H. tuberosus* in summer (in August) was low (*Figure 2*), consistent with the results of Ernst *et al.* 1996 and De Carvalho *et al*. 1997. So, most of the carbohydrates were present in other types of sugars in the tuber. The content of HMW fructans were higher than LMW's, with a ratio of 58: 42%. This means that the plant stored other sugar molecules rather than fructans. In autumn, even if the fructan content of the tubers was reduced to a minimum, but also the HMW: LMW ratio of its, the plant started to use fructans in metabolic processes as the temperature decreased. This is consistent with the change in HMW: LMW ratio of total soluble carbohydrates over the same period. In winter there was a significant change in the values, the amount of fructan oligosaccharides being extremely high. Because of the frosty soil and low temperatures, the plant had accumulated enormous amounts of fructan molecules by the end of winter. This finding is consistent with the hypothesis of Dias-Tagliacozzo *et al*. 1999, that *V. herbacea* (*Asteraceae*), which they investigated, began to intensively transfer its unused photo-assimilates from above-ground organs to its roots as a result of low temperatures. What is striking is that the amount of HMW was less than 1%, so the plant actively used HMW fructans in its metabolism.

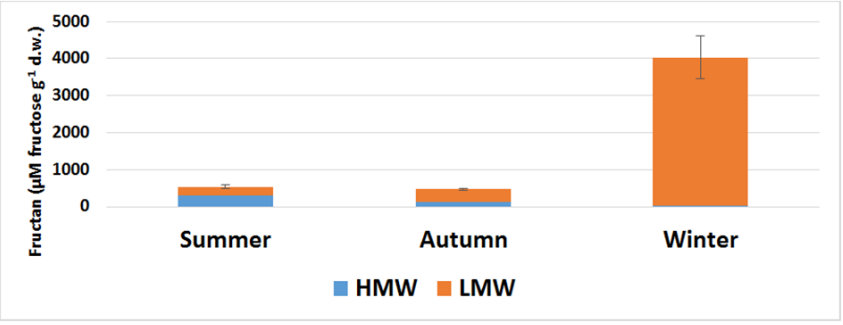

Figure 2. Fructan content (µM fructose g^{-1} d.w.) of *Helianthus tuberosus* tuber depending on seasonality. HMW (blue column): high molecular weight of fructans; LMW (orange column): low molecular weight of fructans; Error bars are STDs, which concern the sum of HMW + LMW fructans (total fructan), where n=5.

In summer fructans were found (*Figure 2*) to be 540 µM g^{-1} d.w. of the total soluble carbohydrate content of 26600 µM g^{-1} d.w. (*Figure 1*), barely 2%. In the autumn, this distribution changed, with fructan molecules accounting for almost one tenth of the soluble carbohydrates. This result also demonstrates that, in colder weather, the Jerusalem artichoke started intensive fructan synthesis. In winter, there was a radical change in the total soluble sugar: total fructan distribution. The proportion of fructans increased to nearly 50%. From this result, it is clear that this species switched from synthesizing other sugars to fructan accumulation under stress (*Figure 3*).

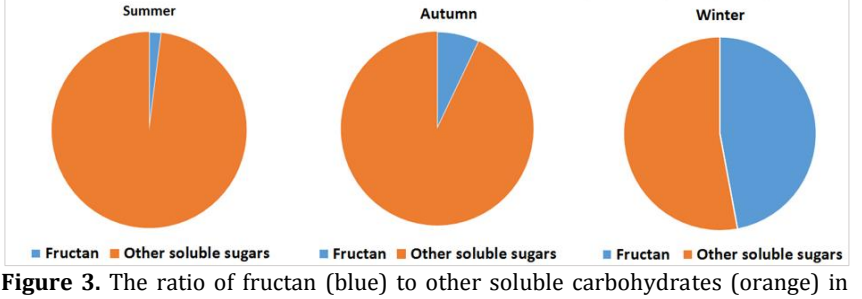

Figure 3. The ratio of fructan (blue) to other soluble carbohydrates (orange) in *Helianthus tuberosus* tuber depending on seasonality. The plant had total soluble carbohydrate reserves of about 26750 µM g^{-1} d.w. in summer, 6613 µM g^{-1} d.w. in autumn and 8571 µM g^{-1} d.w. in winter.

The soluble carbohydrate content (TSS) of the root of *Chicorium intybus* was low in summer (*Figure 4*). This is in line with Limami

and Fiala's (1993) results measured in France. The value is due to the fact that during the growth period (March-August), the plant devoted its carbohydrate reserves to the development of the above-ground organs, mainly the leaves, the flowers and the crop, and there were no significant physiological processes in the root. The HMW: LMW ratio was 38: 62%. During the summer period, there there was a small amount of high molecular weight soluble carbohydrates in the root of the plant, but a significant change occurred in the subsequent period. In the autumn the TSS content of the root increased significantly, its value increased sixfold. A similar result was obtained by the two researchers mentioned above, although they did not observe the same degree of change. Due to the low temperatures, as the stems and leaves began to degrade, the plant shifted the main metabolic processes to the root system. It can be seen that the amount of HMW carbohydrates increased threefold compared to the end of summer, but the amount of LMW carbohydrates increased more than eight times during the same period. The distribution of the two carbohydrates relative to each other – the amount of LMW carbohydrates being more than 4.5 times that of HMW carbohydrates – shows that root metabolism became very active as winter approached. These data are consistent with the results of Van den Ende and coworkers (1996). According to their measurements, the levels of free fructose and sucrose peaked around October-November, while that of glucose decreased. This latter event was probably due to the fact that the plant incorporated glucose into fructans. We couldn't sample during the winter period because of the weather conditions, and therefore we do not have data for this season. In the spring, the amount of TSS decreased to less than half of the level measured only half a year earlier. By April, the plant was already in a period of growth, developing the above-ground organs. Thus, following the likely winter peak, the plant mainly mobilized the fructose and sucrose molecules in the stems and leaves. The proportion of high: low molecular weight carbohydrates was 36: 64%, so the proportion of HMW increased compared to the autumn value, but this was only because most of the oligosaccharides were removed from the root system.

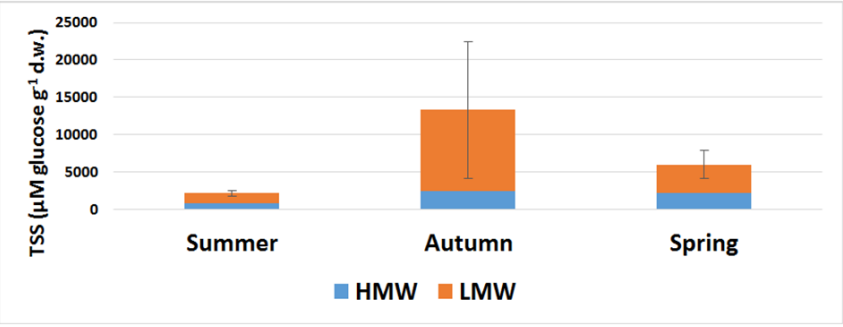

Figure 4. Total soluble sugar content (µM glucose g^{-1} d.w.) of *Cichorium intybus* root depending on seasonality. HMW (blue column): high molecular weight of soluble carbohydrates; LMW (orange column): low molecular weight of soluble carbohydrates; Error bars are STDs, which concern the sum of HMW + LMW fructans (total fructan), where n=5.

The summer fructan content of the chicory root, as in papers of Ernst and coworkers (1996) and Van den Ende and coworkers (1996), was very low (76 µM g^{-1}d.w.) (*Figure 5*). This piece of data also demonstrates that below-ground plant parts play an important role during this period, not in storage but in mobilizing the components involved in energy metabolism. In the autumn, the amount of fructan in the root system increased to 409 µM g^{-1} d.w., more than five times higher than the value at the end of summer. As is the case with other species in the *Asteraceae* family, with the persistence of low temperatures and the degradation of the above-ground organs, the plant began to accumulate fructan reserves in the roots. This is also evidenced by the high molecular weight fructans polymerization, the HMW: LMW distribution was 56: 44%. This is also supported by the fact that the proportion of HMW fructans in the proportion of HMW soluble carbohydrates increased threefold compared to summer data. By mid-spring, there was a significant change in the amount of total fructan and HMW-LMW fructans also. Total fructan levels increased above 3000 µM g^{-1} d.w., more than seven times the value five months earlier. However, the most important change occurred in the relative amounts of HMW-LMW fructans, their proportion in April being 3: 97%. As the temperature increased with the onset of spring, degradation of the high molecular weight fructans occured, which resulted in the reintroduction of the stored fructose and glucose molecules into metabolic processes. The following numbers

show this well: HMW fructans decreased by almost a third, while LMW fructans increased from 180 to 2980 µM g^{-1} d.w., in just 5 months, an increase of 1600%. There is a striking difference between total summer and spring fructan content. Due to the development of new vegetative and reproductive shoots (stem, leaves, flowers), the plant uses up all the storage carbohydrates. Thus, during the warmer months, it almost completely empties the fructan reserves of the roots. We found that the fraction of fructan molecules: TSS was very low, barely 4%, approaching the end of the growth phase (*Figure 4, 5*). During this period, other soluble carbohydrates (mainly sucrose) were accumulated in the roots. This ratio decreased further in the autumn, despite the fact that the plant had begun active fructan synthesis. This decrease is well explained by the results of Van den Ende and coworkers (1996). In spring, with the onset of the growth phase, more than half of the soluble carbohydrates in the roots were present in fructans. HMW fructans accounted for only 4% of the total soluble HMW carbohydrates, while nearly 78% of the soluble LMW carbohydrates were fructans (*Figure 5*). This proves that during the colder months, the plant stored large amounts of fructose and glucose molecules in its roots in polysaccharides, which were degraded to oligosaccharides at the start of the developmental phase (*Figure 6*), and started an intensive photosynthetic carbon gain by for example the rosette leaves at the beginning of the vegetative period.

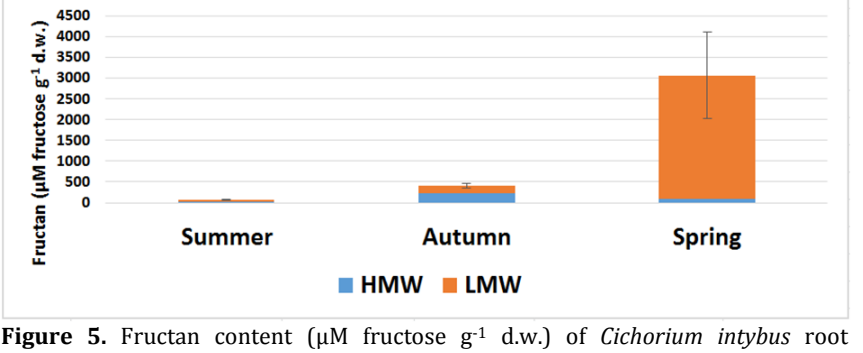

Figure 5. Fructan content (µM fructose g^{-1} d.w.) of *Cichorium intybus* root depending on seasonality. HMW (blue column): high molecular weight of fructans; LMW (orange column): low molecular weight of fructans; Error bars are STDs, which concern the sum of HMW + LMW fructans (total fructan), where n=5.

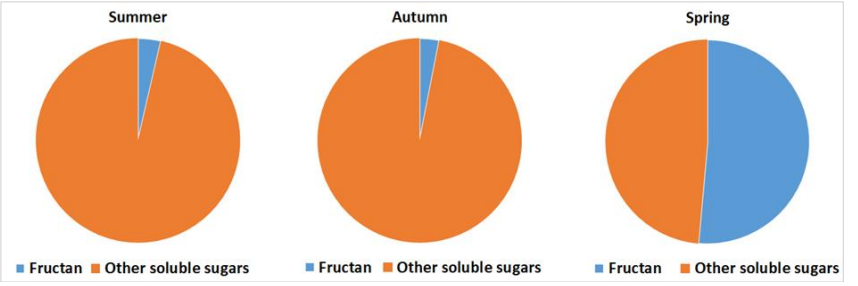

Figure 6. The ratio of fructan (blue) to other soluble carbohydrates (orange) in *Cichorium intybus* root depending on seasonality. The plant had total soluble carbohydrate reserves of about 2114 µM g^{-1} d.w. in summer, 13273 µM g^{-1} d.w. in autumn and 5957 µM g^{-1} d.w. in spring.

The total soluble carbohydrate content of the stem of *Dactylis glomerata* was lower at the end of summer (870 µM g^{-1} d.w.) than in spring (*Figure 7*). By this time of the year, the plant had already used up most of its sugar reserves. This result is consistent with the results of Belesky and coworkers (2006). They conducted investigations with *Dactylis* in the northeastern United States during the same periods, under conditions similar to those prevailing in our country. The amount of HMW carbohydrates measured in August was more than 560 µM g^{-1} d.w., which is almost 65% of the total amount. By this time, the metabolic activity of the plant was not as high as in the previous period. In the spring of the following year, there was a significant change in TSS values. The spring value was 4.5 times the summer value (*Figure 7*). This change in the total soluble sugar content is also characteristic of forage plants and the *Poales*. By the end of April, the main task for the plant was to produce new leaves and flowers. The HMW: LMW carbohydrate ratio also changed, with LMW carbohydrates accounting for 64% of the total. HMW carbohydrate increased to 2.5 times the value of the previous summer. Due to more intensive metabolic processes, the plant was able to accumulate more reserves in its stem. This is the plant's strategy for surviving the summer drought.

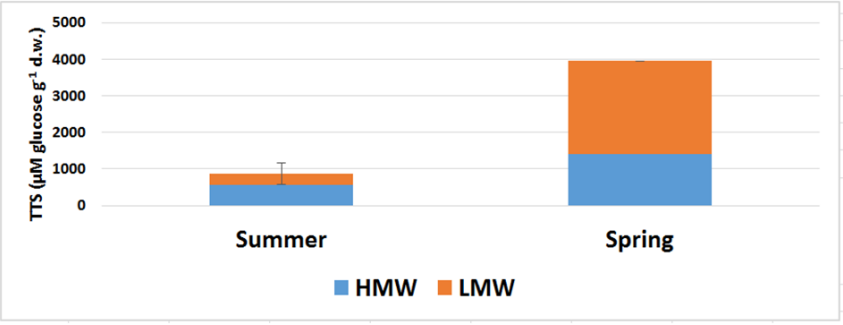

Figure 7. Total soluble sugar content (µM glucose g^{-1} d.w.) of *Dactylis glomerata* stem depending on seasonality. HMW (blue column): high molecular weight of soluble carbohydrates; LMW (orange column): low molecular weight of soluble carbohydrates; Error bars are STDs, which concern the sum of HMW + LMW fructans (total fructan), where n=5.

By the end of summer, as in some other species belonging to *Poaceae*, a large amount of fructan had accumulated in the stem (*Figure 9*). This result is consistent with the measurements of Sanada and coworkers (2007), who measured an autumn peak. The HMW: LMW ratios were 64: 36% by the end of summer. This means that most of the fructan molecules were present in the highly polymerized state, and with this reserve the plant prepared for the fall/winter period. By mid-spring, there was a significant decrease in both total fructan and HMW-LMW fructans. The amount of TSS fell to almost a seventh. In the growth period the plant virtually completely depleted its fructan polymer reserve. In this season the HMW fructans were only 6% of the value of HMW fructans at the end of the summer. The change in LMW fructans was not so drastic, falling to 30% in comparison with the August value. HMW: LMW fructan contents relative to each other showed a distribution of 29: 71%. The few fructan molecules that were present in the stem were largely in oligosaccharides.

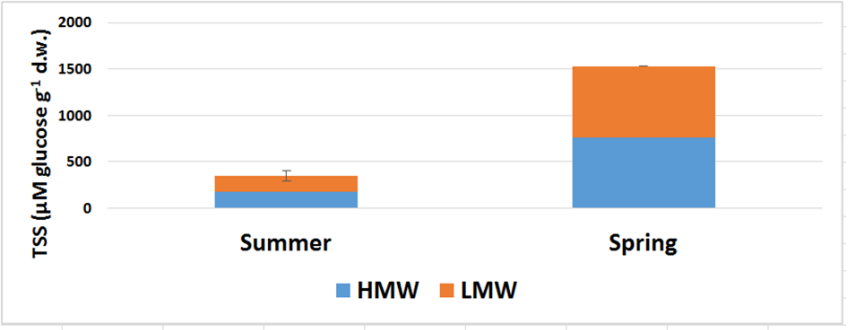

Figure 8. Total soluble sugar content (µM glucose g^{-1} d.w.) of *Dactylis glomerata* leaf depending on seasonality. HMW (blue column): high molecular weight of soluble carbohydrates; LMW (orange column): low molecular weight of soluble carbohydrates; Error bars are STDs, which concern the sum of HMW + LMW fructans (total fructan), where n=5.

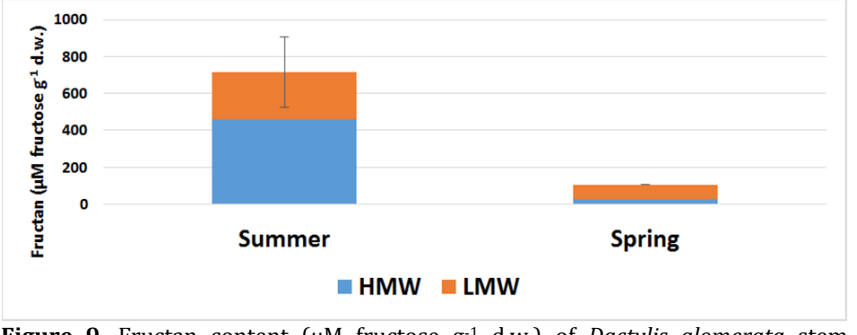

Figure 9. Fructan content (µM fructose g^{-1} d.w.) of *Dactylis glomerata* stem depending on seasonality. HMW (blue column): high molecular weight of fructans; LMW (orange column): low molecular weight of fructans; Error bars are STDs, which concern the sum of HMW + LMW fructans (total fructan), where n=5.

Fructans were present in an astounding proportion of the total soluble sugars at the end of the summer, more than 82% of the TSS (*Figure 11*). This is one of the reasons why the forage crops are harvested in the summer, as fructans improve their quality. In the spring, the opposite was true. Fructans accounted for less than 3% of the total soluble carbohydrates. This piece of data also proves that the plant needed the monosaccharides stored in the fructans and released them (*Figure 9*). The soluble carbohydrate content measured in the leaf of *D. glomerata*, as in the case of the stem, was generally lower at the end of summer than in spring, less than 350 µM g^{-1} d.w. (*Figure 8*). At this time of the year, the degradation in

the above-ground parts of the plant had already begun, mainly in the leaves. This result is in line with the findings of Belesky and coworkers (2006). The amounts of the high and the low molecular weight carbohydrates measured in August were the same (*Figure 8*). This means that the leaves had a well-balanced storage and active use of soluble sugars. By the end of April, there was a significant change in TSS content. It increased more than fourfold compared to the summer value, which is also consistent with the results of Belesky and coworkers (2006). In spring time intensive photosynthesis resulted in high carbohydrate production in the renewed leaves. It is interesting, however, that the HMW: LMW ratio was 50: 50%, as in the summer. It is likely that the distribution of soluble poly-, oligo- and monosaccharides in the leaves is constantly balanced.

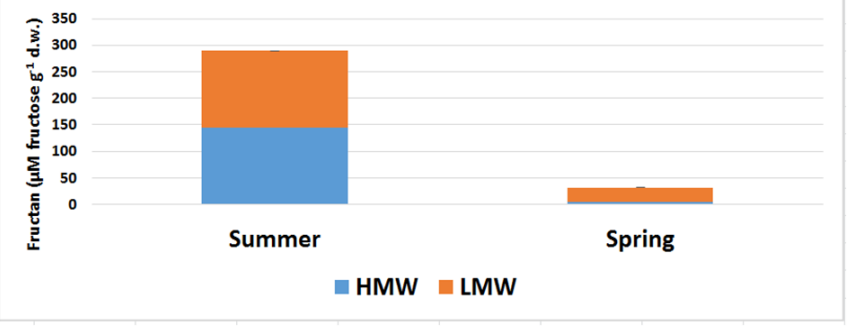

Figure 10. Fructan content (µM fructose g^{-1} d.w.) of *Dactylis glomerata* leaf depending on seasonality. HMW (blue column): high molecular weight of fructans; LMW (orange column): low molecular weight of fructans; Error bars are STDs, which concern the sum of HMW + LMW fructans (total fructan), where n=5.

The fructan content of the leaf of *D. glomerata* in August, as in the case of the stem, was higher than in spring, and was ~ 290 µM g^{-1} d.w. (*Figure 10*). By the end of the growing season, the plant leaf had accumulated relatively high levels of fructans as in forage crops. Interestingly, the distribution of HMW: LMW was 50-50%. It is likely that in the following months, the plant began to gradually utilize the energy stored in fructans, which primarily meant the depolymerization of high molecular weight fructans, which would explain the change in the spring. In April, fructan content was only 33 µM g^{-1} d.w.. This represents a reduction of nearly 90% over the last 7 months (*Figure 10*). The proportion of HMW: LMW also

changed, their proportion being 16: 84%. The fructan polymers were practically completely degraded, although not much of the smaller molecules remained in the leaves. The number of short-chain fructans in the leaf decreased to nearly one-fifth, while only 3% of the summer amount remained in HMW sugars. Results show (*Figure 11*) that at the end of summer, as in the stem, fructans accounted for the majority of soluble carbohydrates in the leaf, representing 83%. In spring, this ratio was reversed, with only 2% of the total soluble carbohydrate content being fructan molecules. The fresh shoots mobilized the monosaccharides from the fructan reserve. Comparing the stem and leaf values, TSS in the stem was 2.5-fold higher in the summer and 2.6-fold higher in the spring. Total fructan content was 2.5-fold higher in the summer and 3.3-fold higher in the stem than in the leaves. As Waite and Boyd (1953) and Belesky and coworkers (2006) described, regardless of the season, soluble sugars, including fructans, are much more present in the above-ground parts of the shoot, especially in the stem. This feature is also typical of the *Poales* order. Therefore, for feeding purposes the stem is considered to be more valuable than the leaf parts. However, we found great similarities in the change of the amount of soluble carbohydrate and fructan, and thus in their relative distribution, between the two seasons. The amount of TSS decreased in the same proportion as the fructan did between the two sampling periods. Although less efficient in the autumn and winter period, photosynthesis occurred, but the plant consumed most of its energy reserves, so storage sugars were only present in small amounts in the spring. The ability of fructan accumulation in this plant has developed not only to withstand frost but also to survive the summer heat and drought, so it is important for the plant to synthesize it in all parts and to increase its amount until warmer temperatures arrive.

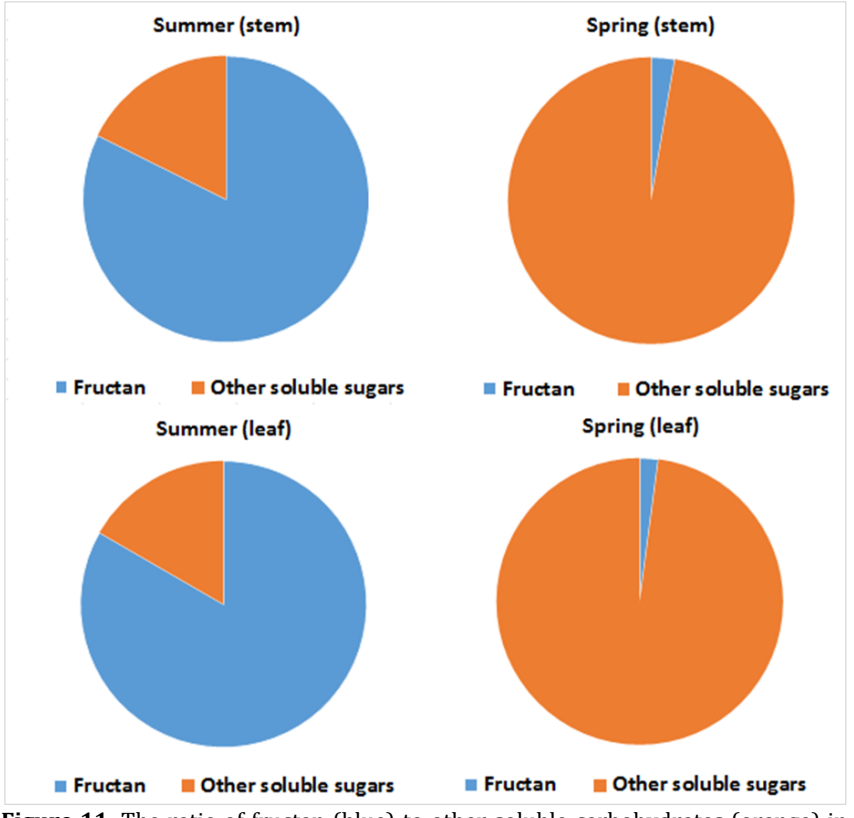

Figure 11. The ratio of fructan (blue) to other soluble carbohydrates (orange) in *Dactylis glomerata* stem and leaf depending on seasonality. The plant had total soluble carbohydrate reserves of about 870 μM g^{-1} d.w. in the stem and 347 μM g^{-1} d.w. in the leaf in summer, 3944 μM g^{-1} d.w. in the stem and 1527 μM g^{-1} d.w. in the leaf in spring.

Comparison of the TSS content of the two plants shows *H. tuberosus* peaks in summer and *C. intybus* in autumn. This is because the former blooms from late summer to late autumn, the latter from June to November. As a result, the Jerusalem artichoke needs to use less storage carbohydrate to develop inflorescence and thus it can accumulate. Another influencing factor is that due to the Hungarian macroclimatic conditions, it does not produce any fruit, while the *C. intybus* does. This is also a carbohydrate consuming process. *D. glomerata* shows the species- and order-specific differences to the other two plants. The low summer TSS content suggests that most of it is incorporated into structural

components and used up for growth. It is important to note that while in the case of *H. tuberosus* and *C. intybus* below-ground storage organs were examined, in *D. glomerata* we examined above-ground organs. We also found a large difference in fructan content between the plants of the two orders. In the below-ground organs (tuber, root) of the *Asteraceae* species following the low-activity summer/autumn synthesis, the amount of fructan oligosaccharides, mainly LMW, is likely to have increased dramatically in winter in the case of the Jerusalem artichoke, and also in chicory (based on spring data only). In the case of *D. glomerata*, based on only the analysis of the the above-ground organs (stem end leaves), the amount of fructans peaked in the fall in order for the plant to survive winter frosts. We could draw the same conclusions as Yoshida and Tamura (2011). During the physiological process that is called 'hardening', carbohydrates are accumulated in their tissues, therefore plants can acclimate to cold months by increasing their tolerance to freezing.

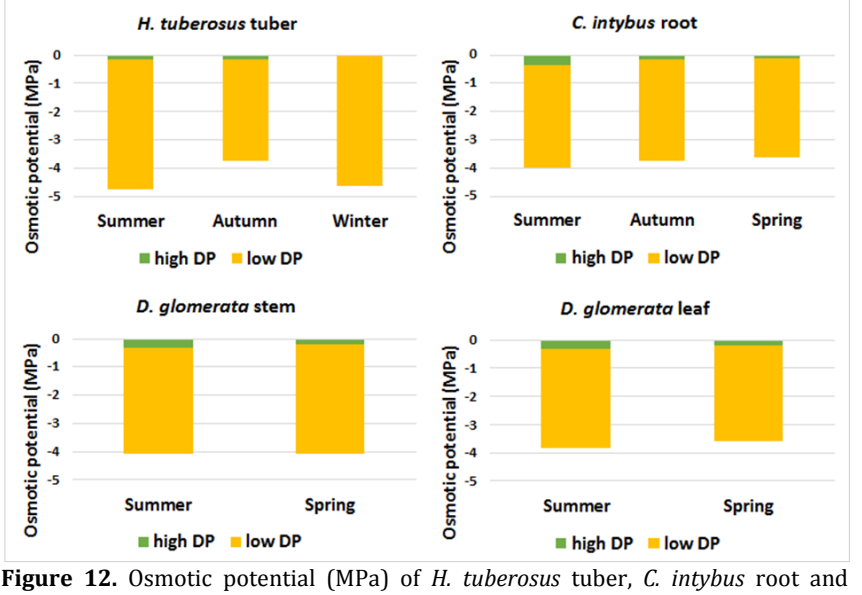

Figure 12. Osmotic potential (MPa) of *H. tuberosus* tuber, *C. intybus* root and *Dactylis glomerata* stem and leaf depending on seasonality. Osmotic potential values of the plant extract containing soluble carbohydrates with a high degree of polimerization (DP) (green), and a low degree of polimerization (DP) (yellow).

Osmotic potential, as a component of water potential, results from inorganic and organic molecules within plant extracts. As the concentration of molecules increases, the osmotic potential of the tissue is reduced. The more molecules present, the more negative the osmotic, or solute, potential is. Our hypothesis was that the supernatant of the fructan-accumulating plant extract contained more fructan molecules with a low degree of polymerization, and the sediment less molecules but with a high degree of polymerization. The soluble carbohydrates are an important but not exclusive component of the osmotic (solute) potential. HMW fructans and other carbohydrates in the sediment contribute little to the osmotic potential. LMW fructans and other carbohydrates in the supernatant accounted for a significant proportion of the osmotic potential.The values of osmotic potentials (*Figure 13*) prove that the amount of soluble carbohydrates correlates with them. As the amount of enzymes, other proteins, amino acids, polyols, quaternary ammonium derivatives and dissolved minerals – as other possible osmoregulators – were not measured, their influence levels could not be determined. The tuber of the Jerusalem artichoke had the highest osmotic potential, partly because it had the largest amounts of TSS and, on the other hand, the function of the tuber was different from other organs and other physiological processes were involved, mainly water and nutrient storage. Although the values of the *C. intybus* were lower, they did not correlate so well with the given TSS contents. The physiological processes of the root are also different: uptake of water and minerals, lateral root formation, and hormone synthesis, which all have a significant effect on the osmotic potential. We measured higher values for the *D. glomerata* stem than for the *C. intybus*, regardless of the difference in TSS content. In addition to transporting many substances in the stem – nutrients, hormones, water, ions – photosynthesis, the components used and produced by have a significant effect on the osmotic potential. Although the leaf is the main organ of photosynthesis, the production and use of nutrients is more balanced than that in the stem, so the osmotic potential values of the leaf are slightly more positive. In the case of the Jerusalem artichoke tuber, the actual amount of TSS, since it is not a photosynthetic organ, was more closely related to the change in osmotic potential than in the other two plants. This relationship may have been due to the circumstance described above for the

Jerusalem artichoke, namely that it blooms relatively shortly and yields no fruit, unlike *C. intybus* and *D. glomerata* (*Figure 13*). Due to the biological processes required for these, significant protein, carbohydrate and ion metabolism occurs in the plant, so these factors are thought to 'have distorted' or modified the degree of relationship between the two values (change in TSS amount vs osmotic potential). As also contributed to this the fact that in the case of *D. glomerata* an above-ground organ was investigated, which was also influenced the values of osmotic potentials.

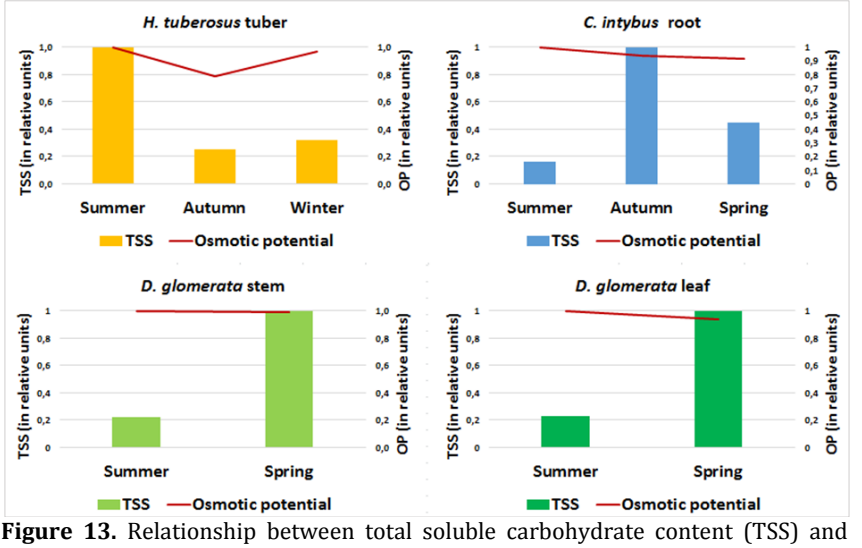

Figure 13. Relationship between total soluble carbohydrate content (TSS) and osmotic potential (OP) (both in relative units) of *H. tuberosus* tuber, *C. intybus* root and *Dactylis glomerata* stem and leaf depending on seasonality.

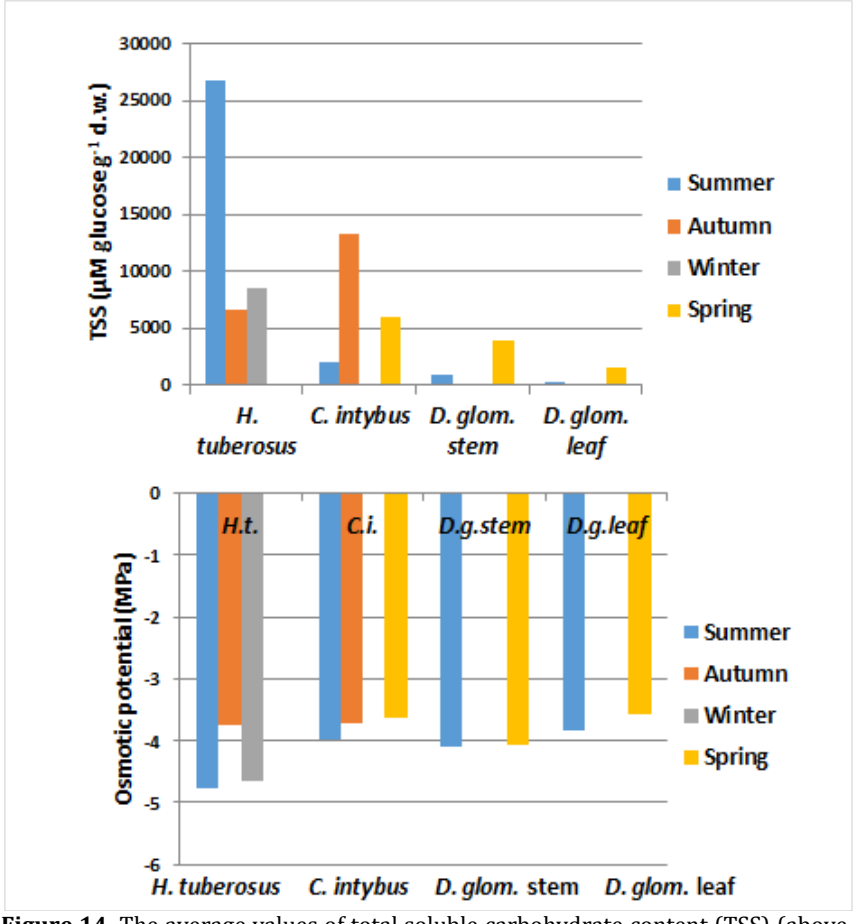

Figure 14. The average values of total soluble carbohydrate content (TSS) (above graph) and osmotic potential (OP) (below graph) (both in absolute values) of *H. tuberosus* tuber, *C. intybus* root and *Dactylis glomerata* stem and leaf depending on seasonality.

Thin layer chromatography (TLC) was used to determine the quality of the soluble carbohydrates and to measure their degree of polymerization. We found differences between the TSS of the three plants and between the supernatant and sediment extracts from that time of the year. There is a clear difference between the chromatograms of the supernatant and the sediment samples, since the former contains the oligosaccharides with lower DP, which can be separated by this technique, and the latter the high molecular weight ones. The aforementioned difference was most evident in

the *Asteraceae*, since higher amounts of fructans were present in their supernatants (with lower DP), whereas running *D. glomerata* samples confirmed the earlier finding that their fructans are mainly stored with higher DP. The DP of fructans in *D. glomerata*‘s supernatants is also different from that in the studied *Asteraceae* plants. Molecules with a higher degree of polymerization are 'stuck' near the starting point of the TLC. In the case of the Jerusalem artichoke (*Figure 15*), the chromatogram of the summer supernatant sample is the palest compared to its autumn and winter samples. This confirms the values obtained when quantifying the fructan content, namely that fructans were present in the summer supernatant in the smallest amounts (*Figure 5*). The differences in sediment chromatograms may be due to the fact that the fructans continued to undergo the most intensive polymerisation in the autumn, so that the oligosaccharides were detectable even from the sediment at that time, whereas it appears that the sediment was practically emptied of fructans in winter. Interestingly, although much higher amounts of fructans were present in winter, the chromatogram of the autumn supernatant sample appears to be more contrasted. The difference between the data of the two seasons was that while in the autumn enzymatic processes for accumulation or storage were in progress, by the end of winter they were already oriented towards usage. In terms of degree of polymerization, the presence of DP 3-12 fructans was well detectable in all seasons. The major soluble carbohydrates in *H. tuberosus* are sucrose and a homologous series of fructans including the trisaccharide 1- kestose, which is characteristic of inulin-type fructans (Marschall *et al.* 1998). Glucose and fructose were present at considerably lower concentration. We used sucrose (DP = 2) and the extract *H. tuberosus* tuber (DP = 3-12) as standards, no trisaccharides (neokestose, isokestose, cestose; DP = 3). For inulin reference the well-known chromatographic data of *H. tuberosus* tuber extract were used (Cairns and Pollock 1988, Smouter and Simpson 1991).

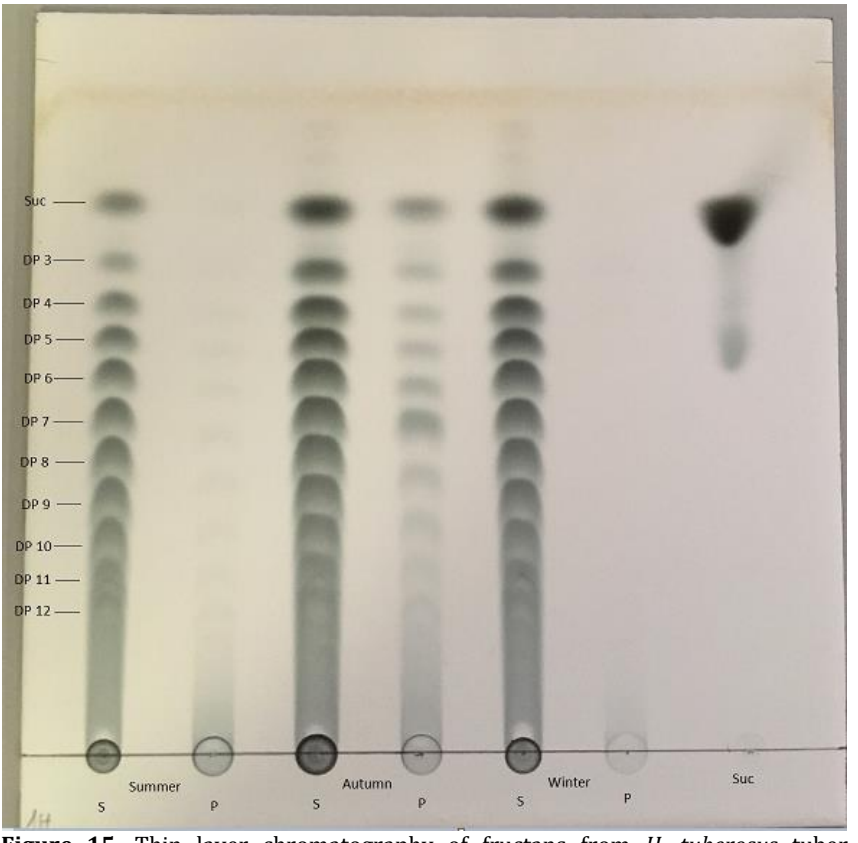

Figure 15. Thin layer chromatography of fructans from *H. tuberosus* tuber depending on seasonality (summer, autumn, winter). Plant tissue was extracted with 80% ethanol followed by hot water (Marschall *et al.* 1998). 25 µl of supernatant (S) and also pellet (P) extracts were used for TLC. SUC, 10 µl of 10 mM sucrose; DP 3-DP 12, fructans of degree of polymerization 3-12.

The appearance of the TL chromatograms in the case of chicory (*Figure 16*) is not as marked as in the case of the Jerusalem artichoke. The quantitative determination revealed that smaller fructan amounts were measured in this species. There is a large difference between the chromatograms of the autumn and spring supernatants. This can be explained by the fact that, as in the case of the Jerusalem artichoke, the fructans are polymerized in the fall and stored until mid-spring. Both summer and autumn samples showed the presence of DP 3-12 fructans. TLC showed that fructans form a homologous series of increasing DP in a similar manner to

fructans in *H. tuberosus* and contain the isokestose-type trisaccharide.

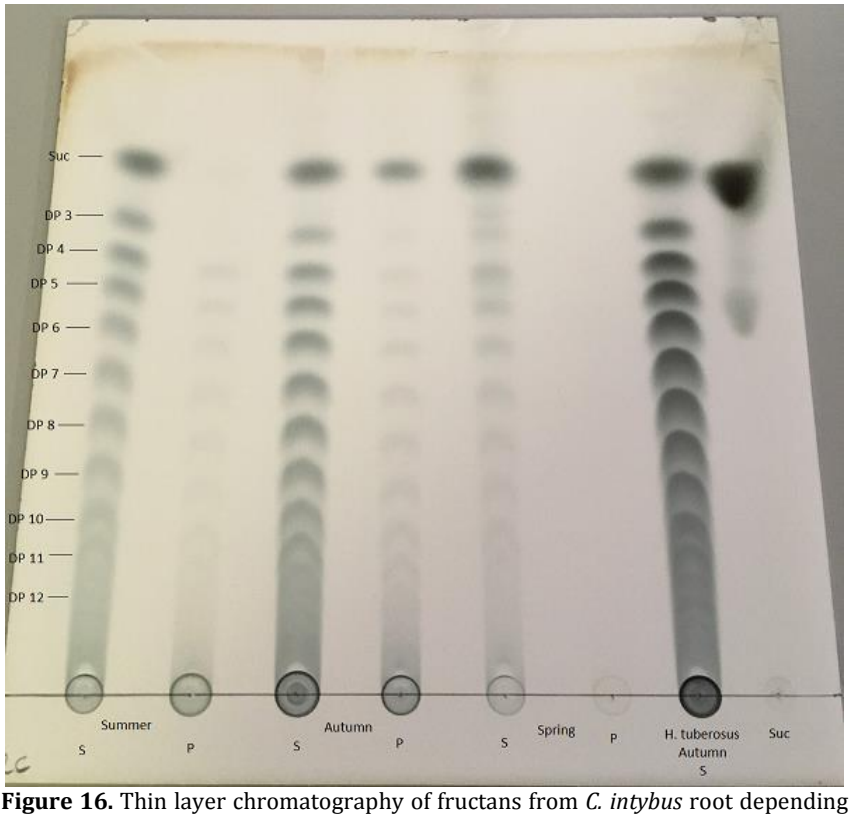

Figure 16. Thin layer chromatography of fructans from *C. intybus* root depending on seasonality (summer, autumn, spring). Plant tissue was extracted with 80% ethanol followed by hot water (Marschall *et al.* 1998). 25 µl of supernatant (S) and also pellet (P) extracts were used for TLC. SUC, 10 µl of 10 mM sucrose; DP 3-DP 12, fructans of degree of polymerization 3-12. We used sucrose (DP = 2) and the extract *H. tuberosus* tuber (DP = 3-20) as standards, no trisaccharides (neokestose, isokestose, cestose; DP = 3).

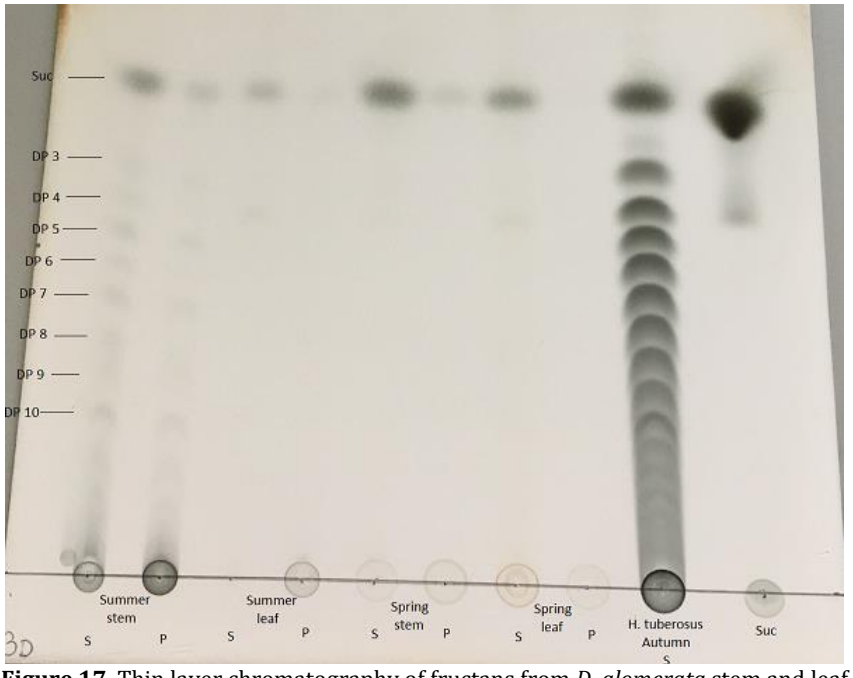

Figure 17. Thin layer chromatography of fructans from *D. glomerata* stem and leaf depending on seasonality (summer, spring). Plant tissue was extracted with 80% ethanol followed by hot water (Marschall *et al.* 1998). 25 µl of supernatant (S) and also pellet (P) extracts were used for TLC. SUC, 10 µl of 10 mM sucrose; DP 3-DP 10, fructans of degree of polymerization 3-10. We used sucrose (DP = 2) and the extract *H. tuberosus* tuber (DP = 3-20) as standards, no trisaccharides (neokestose, isokestose, cestose; DP = 3).

The separation of the fructans of *D. glomerata* by TLC (*Figure 17*) gave a completely different result, and its chromatograms were faint. We could detect a homolog series of fructan oligosaccharides with DP 3-10 only from the supernatant of the summer stem sample, but this is negligible compared to the Jerusalem artichoke's autumn supernatant. In the spring samples, the homologous series of fructans between DP 3 and 10 was not visible with the consistently applied loading sample amount. Interestingly, the presence of sucrose was most detectable in the case of both stems and leaves in spring, which could mean more intensive depolymerization processes. Since fructan levels in the summer supernatant samples were measured at a few hundred µM g^{-1} d.w., it is likely that their degree of polymerization is greater than ten. By this method, carbohydrates with DP >10 are difficult to detect. The

trisacharide kestose mobility was different from the chicory inulin-type isokestose, which would prove that the *D. glomerata* contains other types of fructans. It contains levan-type fructans (Suzuki 1989, Bonnet *et al.* 1997).

CONCLUSION

Our experimental results show that fructans play a major role in the physiological processes of the three species. The two species belonging to the *Asteraceae* tend to accumulate low molecular weight oligosaccharides and the species belonging to the *Poaceae* accumulates medium and high molecular weight oligo- and polysaccharides to increase physiological performance, stress tolerance. Taking into account the climatic and weather conditions, we concluded that the fructan production of the plants belonging to the *Asteraceae* investigated in North Hungary is mainly influenced by the cold, while the species belonging to the *Poaceae* is influenced by the temperature factors and also by the amount of precipitation. The tuber of *H. tuberosus* is the main storage organ for protection against stress caused by low temperature. Our results confirm that this species developed its fructan synthesis ability largely through defense. The molecules of free glucose, fructose and sucrose in the soluble carbohydrate pool gradually used to synthesize fructans. The species increased the synthesis of fructans as the temperature decreased. While only a little amount of inulin-type fructans was stored in the tubers during the summer, the plant began to intensify their synthesis and polymerization of fructan chains in the autumn. In winter, when average daily temperatures were at their lowest, the Jerusalem artichoke met the conditions for survival by depolymerization of fructan molecules and by maintaining its oligosaccharides in extensive metabolic processes. A related *Asteraceae* species, *C. intybus*, showed similar characteristics in its root, but there were also differences. These arose because this species blooms longer than the Jerusalem artichoke and usually yields fruit as well. Therefore the time available for fructan accumulation is shorter and starts later when the temperature is already lower. But like *H. tuberosus*, this plant also produced mono- and disaccharides from WSC and used it for their synthesis. In the summer, fructans were only minimally detected in the samples, but by the end of the autumn, significant fructan synthesis had taken

place. Data from mid-spring showed that fructan molecules were still present in large amounts in the root, and the energy and carbon stored in them could be used for growing new shoots, leaves and flowers. This assumption is confirmed by the large decrease in fructan content of the root measured at the end of summer.

The carbohydrate and, mostly, the fructan metabolism of *D. glomerata* differs from that of the previous two plants. This species produces levan-type fructans not only against cold stress but also against summer drought. Examining its stem and leaf, we found great similarity in the dynamics of fructan amount alteration. However, by the end of the summer, the amount of fructans in the stem was twice as high as in the leaf. The reason for this is that the stem acts as a central distribution channel and as a partly winter-resistant storage organ, so this ratio is similar at any time of the year. With the passing of winter and the coming of the growing season, *D. glomerata* released the glucose and fructose stored in the fructans to produce new shoots and leaves, and then flowers and seeds.

It is important to know that the amount of non-structural carbohydrates, i.e. fructans, decreases with increasing precipitation. Fodder plant producers and pet nutritionists can draw important conclusions from the results of these studies, given the importance of the amount of carbohydrate a pet consumes in a day.

Two other areas, the food and pharmaceutical industries, can also benefit from the research results. Inulin-type fructans are a popular dietary supplement, but can be a good substitute for beet sugar and other sweeteners. Because the human body does not produce the enzyme needed to break it down, it cannot be absorbed. This fact is very useful in diabetics because, although sweet, it does not raise blood sugar levels. This allows diabetics to consume tubers (e.g. the Jerusalem artichoke) or onions containing inulin.

The three fructan-accumulating species we examined are well-established model plants in this research area. To the best of our knowledge, no studies have been conducted to date on how the total soluble carbohydrate (TSS) content, quantitative and qualitative fructan content and osmotic potential change under the

same environmental conditions in the 3 plant species, collected at the same time, as a function of seasonality.

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THE BRYOPHYTE FLORA OF THE ERDŐTELEK ARBORETUM IN HUNGARY

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Abstract: According to the present investigations 54 bryophyte species were collected in the Arboretum of Erdőtelek, including 3 liverworts and 51 mosses. Most of this species are common in Hungary, one of them is vulnerable (*Orthotrichum patens*) and three species are listed as near threatened in the Hungarian Red Data List: *Brachythecium glareosum*, *Cirriphyllum piliferum* and *Orthotrichum obtusifolium*. In the recent paper a comparison of the number of bryophytes recorded in Hungarian botanical gardens and arboretums is presented.

Keywords: bryophyte diversity, NE-Hungary, comparison, red-list status, size of territories

INTRODUCTION

There are only a few publications on the bryophyte flora of the arboretums and botanical gardens of central and eastern european countries, for example Czech Republic (Hradílek 2012; Soldán 1999; Wallnerová 2015) Slovakia (Godovičová 2017) Romania (Ştefureac and Lungu 1961; Plămadă 1963), Poland (Wolski *et al*. 2012) and Ukraine (Mamchur *et al*. 2018).
The first significant description of the bryophyte flora of the hungarian botanical gardens were from Vácrátót (Vajda 1954) and Szigliget (Vajda 1968). Since then several new investigations were published in succession on mostly unexplored botanical gardens, arboretums, and parks: Tata (Agostyán) (Szűcs 2009), Zirc (Galambos 1992, Szűcs 2013), Martonvásár (Nagy *et al.* 2016), Soroksár (Németh and Papp 2016), Eger (Szűcs *et al.* 2017),

Gyöngyös (Mátraháza) (Szűcs *et al.* 2018), Budapest (Rigó *et al.* 2019) and Göd (Fintha *et al.* in press).

This paper introduces the bryophyte flora of the Arboretum of Erdőtelek, based on the investigations conducted in 2016 and 2019. Results were also compared with the bryopyhte diversity of other Hungarian botanical gardens or arboretums.

MATERIALS AND METHODS

The nomenclature follows Söderström *et al.* (2016) for liverworts, Hill *et al.* (2006) for mosses. To establish the indicator and conservation status of taxa the Hungarian Red List was used (Papp *et al.* 2010). Site detail descriptions (in the *Appendix*) include data in the following order: habitats, GPS-coordinates, and date of collection. The designation of the quadrates according to the Central European Flora Mapping System were indicated in square brackets (Király *et al.* 2003). We used the Sørensen index (1948) for the comparison of the species composition of different localities. Collected specimens are deposited at the Cryptogamic Herbarium of the Department of Botany and Plant Physiology at the Eszterházy Károly University, Eger (EGR).

Study area

As a part of the Heves Plains (Hevesi sík) microregion, the present research area is located on the alluvium of the Laskó and Eger streams, at an altitude of 107-118 m. The area's topography has low lying ground, floodless, sligthly undulating plain surface. It's climate is moderately warm and dry with an avarege annual temperature of 10-10.2 °C and annual precipitation is approximately 520-560 mm. The microregion, due to the low water flow, is typically a dry, water-scarce area with a mosaic like soil formation. In the study area the most characteristic are loess materials, covering river and swamp clay on which brown Chernozem forest soils developed. On the western part of the microregion the formerly sandy vegetation has disappeared, but near Erdőtelek, by the spring of Hanyi-rill, there is a remnant of an alder swamp (Dövényi 2010).

The arboretum of Erdőtelek is located on the outskirts of the Great Plain (Alföld) in Heves County, which total area is 25.5 hectares, of which only 6 hectares can be visited by public. The

garden was established and transformed by József Kovács from the castle park into a rich dendrological collection. His important merit was to create an arboretum rich in evergreens on one of the dry, warm and low rainfall areas of the Great Plain. After World War II, the garden was almost destroyed due to damage caused by incompetented workers. It was a declared as a nature reserve in 1950. Occasionally, the tree trunks and the soil surface are covered by *Hedera helix.* Currently, the arboretum is a unit belonging to the Eszterházy Károly University. Maintenance consists of seasonal and local lawn mowing and leaf litter collection. There is an intensive horticulture activity in its the north-western part.

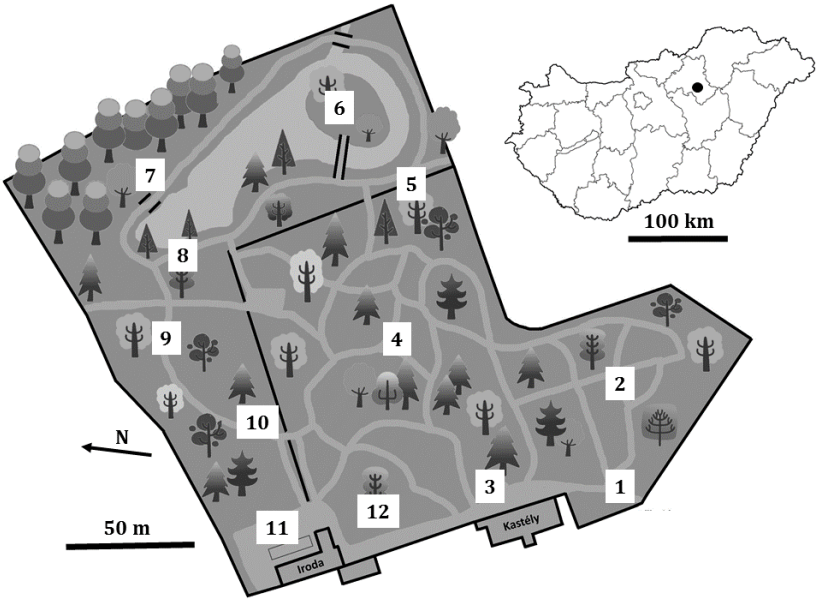

Figure 1. The collecting points in the Arboretum of Erdőtelek (map designed by Jana Táborská).

RESULTS AND DISCUSSION

Altogether 54 bryophyte species were identified from the Arboretum of Erdőtelek, including 3 liverworts and 51 mosses. Besides the common and frequent taxa, mosses which are still not threatened, but need attention (LC-att) according to the Hungarian Bryophyte Red List (Papp *et al.* 2010): *Brachythecium albicans*,

Orthotrichum speciosum, Orthotrichum striatum, Tortula lanceola, *Tortula papillosa* and *Tortula virescens*.

Near threatened (NT) species were: *Brachythecium glareosum*, *Cirriphyllum piliferum* and *Orthotrichum obtusifolium*. *Orthotrichum patens* belong to vulnerable (VU) category according to the red list.
Indicator bryophytes which by their mere presence denote the higher level of conservation value of the habitat, also occur in the arboretum are *Cirriphyllum piliferum*, *Orthotrichum speciosum*, *Orthotrichum striatum*, *Tortula lanceola* and *Tortula papillosa*.

Some common species of the most measured Hungarian botanic gardens and arboretums, includes: *Amblystegium serpens*, *Barbula unguiculata*, *Brachythecium rutabulum*, *Bryum argenteum*, *Ceratodon purpureus*, *Hypnum cupressiforme*, *Leskea polycarpa*, *Orthotrichum anomalum*, *Orthotrichum diaphanum*, *Oxyrrhynchium hians*, *Radula complanata*, *Syntrichia ruralis* and *Tortula muralis* occur also in the Arboretum of Erdőtelek.
The low number of liverworts in the territory is similar to the majority of other Hungarian botanic gardens, arboretums and parks (Szűcs 2017).

Table 1 shows a comparison between the species composition of the Erdőtelek Arboretum with other previously bryologically explored man made habitats (Botanical Garden of Eger, Mátrai Sanatorium park, Balaton village) species in the region calculated by Sørensen index. The greatest similarity was found in the Botanical Garden of Eger (0.7), but not far behind the value of Balaton village (0.67). The biggest difference was found in comparison with the Mátrai Sanatorium park (0.53).

Table 1. Comparison the territory, the distcance of localities, the altitude, the number of taxa and calculated Sørensen index of other territories with Arborétum of Erdőtelek.

Name of locality	territory (hectare)	distance from Arb. of Erdőtelek (km)	alt (meter)	number of taxa	Sørensen index
Bot. garden of Eger (Szűcs *et al*. 2017)	1	25	230	46	0.7
Mátrai Sanatorium, Mátraháza (Szűcs *et al.* 2018)	14	36	650-700	65	0.53
Balaton village (Zsólyom & Szűcs 2018)	82	45	290-320	61	0.67

Compared to the above mentioned gardens, the following taxa occur only in Erdőtelek: *Anomodon viticulosus*, *Brachythecium albicans*, *Leptobryum pyriforme*, *Orthotrichum patens*, *Plagiomnium rostratum*, *Porella platyhylla*, *Pseudocrossidium hornschuchianum*. *Figure 2* indicates the number of bryophytes identified in Hungarian botanical gardens, arboretums and parks compared to the size of these collection gardens.

It can be stated that most gardens have a larger area with higher species numbers. The arboretum of Erdőtelek, with its 6 hectares and 54 species, also reinforces this tendency and has almost the same value as the Huzella Garden in Göd (Fintha *et al.* in press).
The difference is remarkable compared to Soroksár and Martonvásár. The different value of Tata (Agostyán) is also due to the fact that the complete bryophyte flora of the arboretum has not been investigated yet (Szűcs 2009).

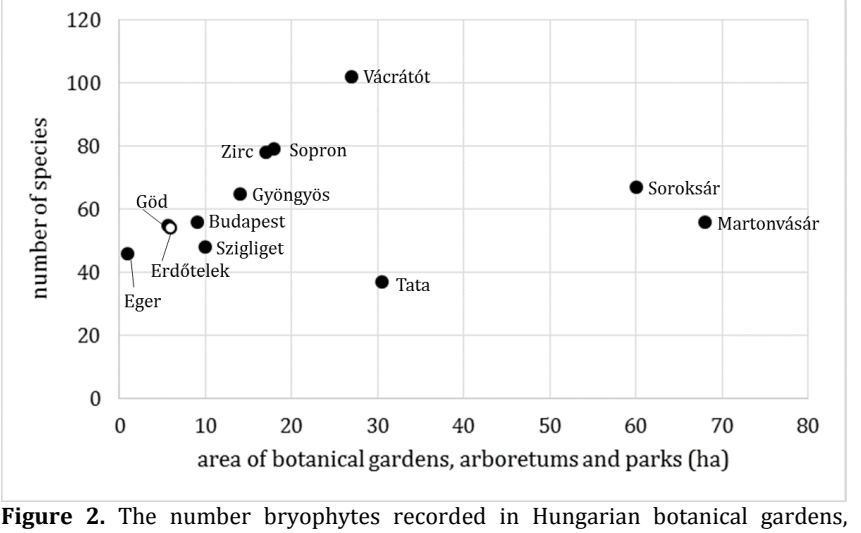

Figure 2. The number bryophytes recorded in Hungarian botanical gardens, arboretums and parks, in comparison to their sizes.
The data were obtained from the following sources: Tata (Agostyán) (Szűcs 2009), Budapest (Rigó *et al*. 2019), Eger, botanical garden (Szűcs *et al*. 2017), Erdőtelek (present work), Göd (Fintha *et al*. in press), Gyöngyös (Mátraháza) (Szűcs *et al*. 2018), Martonvásár (Nagy *et al*. 2016), Sopron (Szűcs 2017), Soroksár (Németh and Papp 2016), Szigliget (Vajda 1968), Vácrátót (Vajda 1954), Zirc (Galambos 1992, Szűcs 2013).

List of species

Numbers refer to sites (*Figure 1.*) listed in *Appendix*. The substrates given after a semicolon refer to all listed sites.

Marchantiophyta

Marchantia polymorpha L. – 11: soil in flower pots

Porella platyphylla (L.) Pfeiff. – 4: bark of old *Fraxinus*

Radula complanata (L.) Dumort. – 4: bark of old *Fraxinus* and *Quercus robur;* 7: bark of *Alnus glutinosa;* 10: bark of *Magnolia obovata*

Bryophyta

Amblystegium serpens (Hedw.) Schimp. – 1: decayed stump, tar paper; bark of *Castanea sativa* and *Padus cerasus*; 2: plaster; 4: bark of old *Fraxinus, Acer cappadocicum, Aesculus hippocastanum, Berberis vulgaris; Fraxinus excelsior, Tilia miranda, Tilia platyphyllos,* and *Malus halliana;* 5: tree base of *Betula pendula*; 8: bark of *Liriodendron tulipifera*

Anomodon viticulosus (Hedw.) Hook. & Taylor – 4: bark of not identified tree; 8: bark of *Liriodendron tulipifera*

Barbula unguiculata Hedw. – 2, 10, 12: soil; 11: soil in flower pots

Brachytheciastrum velutinum (Hedw.) Ignatov & Huttunen – 4: bark of *Quercus robur*; 5: soil

Brachythecium albicans (Hedw.) Schimp. – 5: tree base of *Betula pendula*

Brachythecium rutabulum (Hedw.) Schimp. 1, 4, 5: soil; 8: bark of *Liriodendron tulipifera*

Brachythecium glareosum (Bruch ex Spruce) Schimp. – 6: concrete

Bryum argenteum Hedw. – 12: disturbed and bare soil

Bryum caespiticium Hedw. – 11: soil in flower pots; 12: disturbed and bare soil

Bryum moravicum Podp. – 1: bark of *Castanea sativa*, tar paper, bark of old *Quercus robur;* 4: bark of *Quercus robur, Acer negundo,* and *Fagus sylvatica;* 8: bark of *Liriodendron tulipifera*

Calliergonella cuspidata (Hedw.) Loeske – 1, 2, 3, 4, 5, 10: soil

Campyliadelphus chrysophyllus (Brid.) R.S.Chopra – 11: soil in flower pots

Ceratodon purpureus (Hedw.) Brid. –11: soil in flower pots

Cirriphyllum piliferum (Hedw.) Grout – 1, 2, 3, 4, 5, 10: soil

Cirriphyllum crassinervinum (Taylor) Loeske & M.Fleisch. – 4: bark of *Prunus;* 12: soil

Fissidens taxifolius Hedw. – 4, 5: shaded soil

Funaria hygrometrica Hedw. – 11: soil in flower pots; 12: disturbed soil

Grimmia pulvinata (Hedw.) Sm. – 1: artifical rock, bark of *Padus cerasus*

Homalothecium lutescens (Hedw.) H.Rob. – 1, 4: soil, bark of *Prunus*

Homalothecium philippeanum (Spruce) Schimp. – 5: tree base of *Betula pendula*

Hypnum cupressiforme Hedw. – 1: bark of *Castanea sativa*, *Padus cerasus* and *Quercus robur;* 4: bark of *Prunus serrulata*, *Celtis occidentalis, Crataegus oxyacantha, Acer cappadocicum, Acer negundo, Aesculus hippocastanum, Berberis vulgaris; Fraxinus excelsior, Quercus robur, Tilia miranda, Tilia platyphyllos,* and *Malus halliana;* 7: bark of *Alnus glutinosa;* 10: bark of *Hibiscus syriacus*; 12: bark of *Acer pseudoplatanus*

Isothecium alopecuroides (Lam. ex Dubois) Isov. – 4: tree base of *Quercus robur*

Leptobryum pyriforme (Hedw.) Wilson – 12: disturbed soil

Leptodictyum riparium (Hedw.) Warnst. – 10: tree base of *Magnolia obovata*

Leskea polycarpa Hedw. – 1: decayed stump; on bark of *Castanea sativa* and old *Quercus robur*, and *Padus cerasus;* 3, 12: bark of *Acer pseudoplatanus;* 4: bark of *Celtis occidentalis,, Acer cappadocicum, Acer negundo, Aesculus hippocastanum, Berberis vulgaris, Fagus sylvatica, Fraxinus excelsior, Lonicera maackii, Tilia miranda, Tilia platyphyllos,* and *Malus halliana;* 8: bark of *Liriodendron tulipifera*;10: bark of *Hibiscus syriacus*

Orthotrichum affine Schrad. ex Brid. – 1: bark of *Berberis vulgaris*; 5: bark of *Morus alba*; 10: bark of *Hibiscus syriacus*

Orthotrichum anomalum Hedw. – 1: tar paper; artifical rock

Orthotrichum diaphanum Schrad. ex Brid. – 4: bark of *Lonicera maackii;* 7: bark of *Alnus glutinosa;* 10: bark of *Hibiscus syriacus*

Orthotrichum obtusifolium Brid. – 1: bark of *Padus cerasus* and *Berberis vulgaris*; 4: bark of *Lonicera maackii;* 8: bark of *Liriodendron tulipifera*; 10: bark of *Hibiscus syriacus*

Orthotrichum pallens Bruch ex Brid. – 5: bark of *Morus alba*
Orthotrichum patens Bruch ex Brid. – 5: bark of *Morus alba*; 10: bark of *Magnolia obovata*
Orthotrichum speciosum Nees – 4: bark of *Prunus serrulata*; 10: bark of *Hibiscus syriacus*
Orthotrichum stramineum Hornsch. ex Brid. – 1: bark of *Padus cerasus*
Orthotrichum striatum Hedw. – 5: bark of *Morus alba*
Oxyrrhynchium hians (Hedw.) Loeske – 1, 2, 3, 4, 6, 7, 9: soil
Phascum cuspidatum Hedw. – 9: bare soil; 12: disturbed soil
Physcomitrium pyriforme (Hedw.) Bruch & Schimp. – 11: soil in flower pots
Plagiomnium cuspidatum (Hedw.) T.J.Kop. – 1: bark of old *Quercus robur*
Plagiomnium rostratum (Schrad.) T.J.Kop. – 12: bare soil
Plagiomnium undulatum (Hedw.) T.J.Kop. – 1, 2, 3, 4, 5, 10: wet soil
Pseudocrossidium hornschuchianum (Schultz) R.H.Zander – 10: soil with gravel
Pseudoscleropodium purum (Hedw.) M.Fleisch. – 1, 4, 8, 10: wet soil
Pylaisia polyantha (Hedw.) Schimp. – 1: bark of *Padus cerasus*; 4: bark of *Prunus serrulata* and *Berberis vulgaris*; 7: bark of *Alnus glutinosa;* 8: bark of *Liriodendron tulipifera;* 10: bark of *Hibiscus syriacus*
Rhytidiadelpus squarrosus (Hedw.) Warnst. – 3, 8, 9, 10: wet soil
Schistidium crassipilum H.H.Blom 1: artifical rock
Syntrichia ruralis (Hedw.) F.Weber & D.Mohr – 1: bark of *Padus cerasus*; 4: bark of *Berberis vulgaris*; 11: artifical rock
Syntrichia papillosa (Wilson) Jur. – 1: bark of old *Quercus robur* and *Padus cerasus*; 4: bark of *Celtis occidentalis* and *Lonicera maackii* 12: bark of *Acer pseudoplatanus*
Syntrichia virescens (De Not.) Ochyra – 1: tar paper, bark of old *Quercus robur* and *Padus cerasus*; 4: bark of *Celtis occidentalis*, *Fraxinus excelsior,* and *Lonicera maackii*; 7: bark of *Alnus glutinosa;* 12: bark of *Acer pseudoplatanus*
Thuidium assimile (Mitt.) A.Jaeger – 3, 4, 5, 9, 10: soil
Tortula lanceola R.H.Zander – 12: disturbed and bare soil
Tortula muralis Hedw. – 1: artifical rock; 5: plaster and brick; 7: concrete

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APPENDIX

Site details

Collection of the specimens was carried out in Heves County, in the territory of the local administrative unit of Erdőtelek village. Each collection point belongs to 8387.2 quadrant.

1. dendrological collection, woody vegetation, roadside, pagoda, stone fence; N°47.688352, E°20.312575 (06.07.2016, 01.05.2019, 16.11.2019)
2. dendrological collection, woody vegetation, roadside, bare soil surface; N°47.688446, E°20.313391 (06.07.2016, 01.05.2019, 16.11.2019)
3. mown lawn, bare soil surface, abandoned building, N°47.688792, E°20.312468 (06.07.2016, 01.05.2019, 16.11.2019)
4. dendrological collection, woody vegetation, roadside, bare and shaded soil surface, mown lawn N°47.689319, E°20.312998 (06.07.2016, 01.05.2019, 16.11.2019)
5. dendrological collection, woody vegetation, roadside, lakeshore, mown lawn, bare soil; N°47.689316, E°20.314538 (06.07.2016, 01.05.2019, 16.11.2019)
6. island, mown lawn, concrete; N°47.689590, E°20.315104 (06.07.2016, 01.05.2019, 16.11.2019)
7. *Alnus glutinosa* vegetation, stone bridge, lakeshore, roadside; N°47.690168, E°20.314798 (01.05.2019, P., 16.11.2019)
8. dendrological collection, woody vegetation, roadside, mown lawn; N°47.689945, E°20.314516 (06.07.2016, 01.05.2019, 16.11.2019)
9. dendrological collection, woody vegetation, roadside, mown lawn; N°47.689994, E°20.313759 (06.07.2016, 01.05.2019, 16.11.2019)
10. dendrological collection, woody vegetation, roadside, mown lawn; N°47.689957, E°20.313290 (06.07.2016, 01.05.2019, 16.11.2019)
11. horticulture, outbuildings, roadside; N°47.689807, E°20.312183 (01.05.2019, 16.11.2019)
12. horticulture, foil tent, woody vegetation, roadside, bare soil; N°47.689599, E°20.312385 (06.07.2016, 01.05.2019, 16.11.2019)

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NEW MACROFUNGIAL RECORD IN HUNGARY: *ENTONAEMA CINNABARINUM* (COOKE & MASSEE) LLOYD

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Hungary, Pest County, Ócsa, Selyemrét Nature Trail, on *Fraxinus angustifolia* subsp*. pannonica*, leg.: Gabriella Fintha, det.: Lajos Benedek; 47°16'06"N 19°13'50"E, (EGR), 05.07.2019.

Entonaema cinnabarinum (Cooke & Massee) Lloyd is a remarkable fungus in the Xylariaceae (Xylariales, Ascomycota). In spite of its brigthly coloured immature stromata, it is rarely reported from Europe as southern France (Stadler *et al.* 2004), Bulgaria (Læssoe 1997) and southern Russia (Fedosova 2012). This species has a wide distribution in the world, reported from Africa, Australia, Costa Rica, New Caledonia, Sri Lanka (Rogers 1981), Japan, Philippines (Stadler *et al.* 2004). *E. cinnabarinum* was recognized as a tropical species, but recently, we are getting more European data. This species has not been published in Hungary, but one locality was known near Kaposvár, in Tókaj forest park (det.: P. Finy on 07.10.2018.). We report here a new locality found in the swamp forest near Ócsa, on May 2019 (*Figure 1*). In all cases, we founded specimens on bark of decaying *Fraxinus angustifolia* subs*. pannonica*. After finding the first site, we found more than 10 specimens the area, while searching for decaying *Fraxinus* that characterizes the species' specific habitat (*Table 1, Figure 2*). On several occasions, we discovered large colonies with specimens of different ages and sizes. We also collected mature and immature stromatas and in late autumn, we collected desiccated specimens as well (det.: G. Fintha on 29.10.2019.). Significant morphological

differences can be observed between young and old specimens. Young ones have a distinctive appearance with a light reddish-yellow or orange color. Stromata of *E. cinnabarinum* is turgid and resilient when fresh and the colored ectostromata surrounds the perithecia with the liquid-filled cavity, later becoming more reddish brown and darkening and at maturity forms even blackening toward senescence. When liquid is released from stromata, they collapse and start to dry. Dried specimens are similar to *Daldinia decipiens,* which is a common species in the study area. *Entonaema* differs from *Daldinia* by having stromata with a hollow interior and by the perithecia that are arranged in a single layer beneath a thin crust and are easily flattened when being touched (Srutka *et al.* 2017). In this protected area, the mass occurrence of the species provides an opportunity for the monitoring of morphological changes of this species.

Figure 1. *Entonaema cinnabarinum* in Selyemrét Nature Trail; 47°15’56”N 19°13’50”E; on bark of decaying *Fraxinus angustifolia* subs*. pannonica* (photo: G. Fintha).

Table 1. The GPS coordinates and substrates data for the sampling points.

number of sampling site	GPS coordinates	substrate
1	47°16'06"N 19°13'50"E	*Fraxinus angustifolia* subs. *pannonica*
2	47°15'56"N 19°13'50"E	*Fraxinus angustifolia* subs. *pannonica*
3	47°15'37"N 19°14'19"E	*Fraxinus angustifolia* subs. *pannonica*
4	47°15'29"N 19°15'18"E	*Fraxinus angustifolia* subs. *pannonica*
5	47°15'19"N 19°16'21"E	*Fraxinus angustifolia* subs. *pannonica*

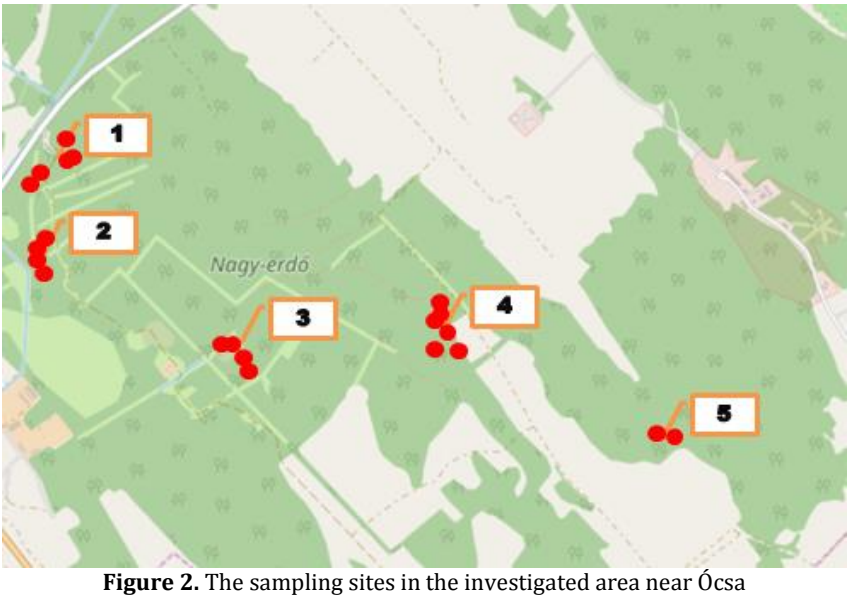

Figure 2. The sampling sites in the investigated area near Ócsa (©OpenStreetMap contributors).

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A NEW HUNGARIAN OCCURRENCE OF *ENTODON CONCINNUS* (DE NOT.) PARIS FROM WESTERN HUNGARY

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Hungary, Vas County, Apátistvánfalva village, Fő street 51, parking of Apát Hotel, on the top of embankment, in mown lawn, on soil (leg. Gabriella Fintha, 09.11.2019.; det. Péter Szűcs & Gabriella Fintha [EGR]; conf. Peter Erzberger) alt. 290 m, N°46.893611, E°16.260833; associated bryophtyes: *Climacium dendroides, Hylocomium splendens, Pseudoscleropodium purum, Rhytidiadelphus squarrosus, R. triquetrus*.

The *Entodon concinnus* has circumpolar distribution, and occurs basically on alkaline, calcareous, arid, grassy areas (Dierßen 2001). This taxon is common in the western parts of Europe and is not endangered (Hodgetts *et al.* 2019). In Hungary for a long time only one occurrence was known from the Botanical Garden of Vácrátót (Pócs *et al.* 2008), therefore, according to the Hungarian Red List (Papp *et al.* 2010) it is critically endangered in our country. In recent years, new populations of the taxon were detected (Király *et al.* 2019, Fintha *et al*. in press) from calcareous sandy soil, which are geographically close to the Vácrátót locality (*Figure 1*). Considering bryophyte species the Őrség region is well-explored (Pócs *et al.* 1958, Papp and Rajczy 1996, Ódor *et al.* 2002, Szűcs 2009), data of *E. concinnus* is new for this, and with greater outlook, for the whole Transdanubian region. The new data presented here support the hypothesis that the distribution of the taxon in Hungary is not limited to the calcareous sandy soils of the Pest sedimentary plain. It can occur throughout the country, even on acidic grounds, in regions with climatic conditions appropriate to the species' requirements.

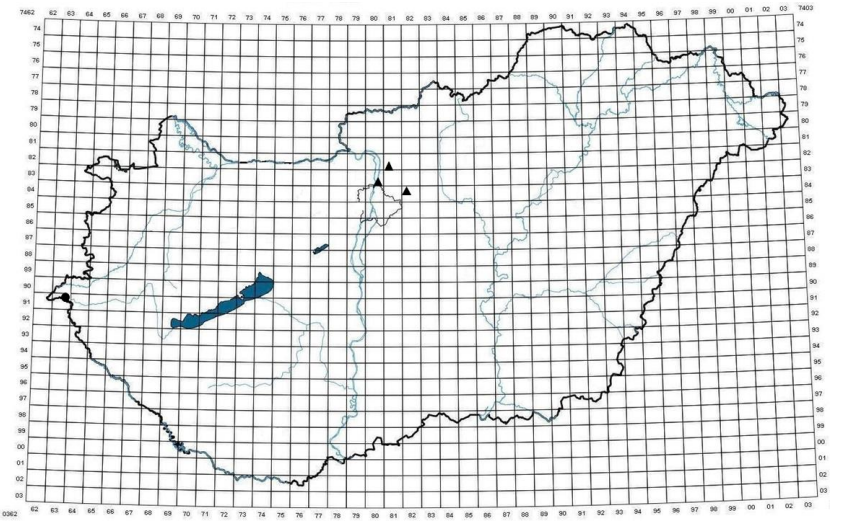

Figure 1. The distribution map of *Entodon concinnus* in Hungary; ● new occurrence, ▲ published and known occurrences (based on Pócs *et al*. 2008; Király *et al.* 2019, Fintha *et al*. in press).

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