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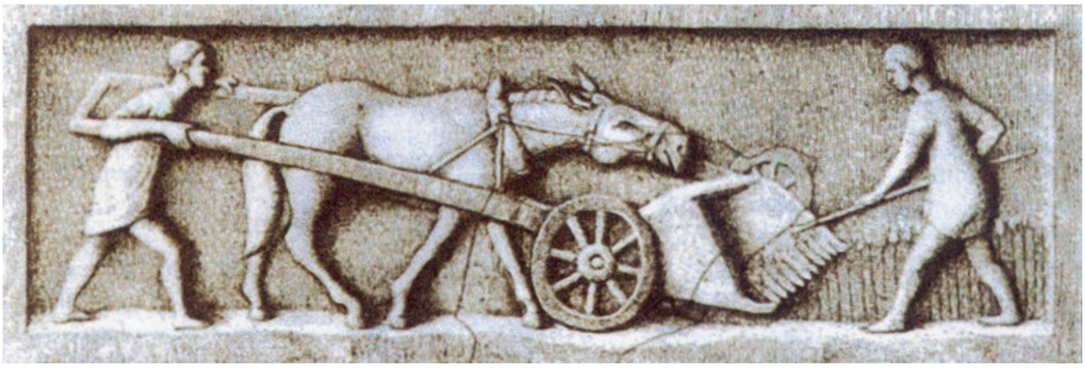

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Vegetative growth of apricot (*P. armeniaca* L.) cultivars and rootstocks

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Abstract: The continuous innovation in point of apricot cultivars and rootstocks requires comparative trials, which can be evaluated by precise measurements. An experiment is established, initiated from the recent trends. 15 scion cultivars were budded on 6 different rootstock cultivars. Apricot seedling, Montclar, Myrobalan 29C, Wavit, Rootpack R and Fehér besztercei were used as rootstock. Scions included traditional Hungarian cultivars (Gönci Magyar kajszi, Ceglédi óriás, Ceglédi szilárd, Pannónia), naturalized cultivars (Bergeron, Roxana) and modern cultivars too (Goldrich, Tardif de Valence, LadyCot, FlavorCot, PinkCot, Spring Blush). The experiment was settled at spring of 2018, with 3 × 5 m spacing. Sprouting was 98%, the deficiency was originated only from fawn damage. The main effect of the different rootstocks can be observed in the growth habit of scions, meanwhile the scion cultivar also has a moderate impact.

Keywords: Apricot, vegetative growth, rootstock, scion

Received 23 May 2020, Revised 6 October 2020, Accepted 10 October 2020

Introduction

The beneficial properties of a rootstock can predominate in the orchard in two different ways: On the one hand, when the rootstock cultivar (composing the root system and/or the trunk) provides its attributes to the complete engraftment. The rootage of the rootstock is responsible for the water- and nutrient uptake, and for the acclimatization to the different ecological conditions. Capability of growing a stable trunk with good fitness of the trunk-training rootstock pertains here. On the other hand, predomination manifests itself in the stock and scion interaction. The rootstock affects the vegetative and generative performance of the scion, like the productivity, time of fruiting, growth rate, the quality and storability of fruits (Hrotkó 1999; Darikova et al., 2011).

Advantage of the engraftment is securing the phenological phases, and the quicker reaching of productive status. Main benefit of the engraftment is the mass production at a moderate cost (Küppers 1978; Hrotkó 1999).

According to Szani et al. (2006), the most used rootstocks for apricot growing are apricot seedlings selected at Cegléd (C.1301, C1650 and C.1652). These are fully compatible with all of the scion varieties, but remain between the limits of the species.

At evaluation of monumental rootstock experiments, Southwick and Weis (1998) concluded, that using some myrobalan rootstocks increases the mortality of apricot trees with higher rates, than those from other species. It was considered as latent (incomplete) incompatibility. Though several researches were realized in case of apricot rootstocks, these results cannot be generalized (Milošević et al., 2014, Milatović et al., 2017). We have to find those scion-stock combinations which are suitable in our growing region (Oprita and Gavat, 2018).

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Materials and Methods

The trial was situated at the Research Station of Cegléd, Research Institute for Fruitgrowing and Ornamentals, National Agricultural Research and Innovation Center. The region has a temperate, continental climate with a semi-arid microclimate. This area is not optimal for apricot growing, however the one third of Hungarian apricot fields are situated in similar locations. The altitude is 96 meters above see-level. The surface is plain with some local differences (by 1-2 meters). The experimental orchard can be approached on concrete road, and has a fence around it.

The orchard was planted in spring of 2018 in 3*5 m spacing, living mulch between the rows were sod with grass at the same autumn. The irrigation is not resolved yet. Pruning is performed several times a year, such as foliar fertilization. Plant protection is outstandingly respected. Every scion-stock combination is pleaded by three trees per replicates, two replicates are settled. Replicates are situated at a totally random arrangement, none of the rootstocks, nor the scions have the same neighbor. In case of scions, traditional Hungarian cultivars, French, Spanish, Italian, Canadian and also afghani cultivars are represented. Used cultivars are summarized in Table 1.

Further back Fehér besztercei was used expansively in Hungary as a generatively propagated apricot rootstock, but it shows some incompatibility with the cultivars, therefor Apricot seedling is commonly used. In the last decade, Myrobalan 29C came into use, and Wavit is in raising amongst others. Used rootstock cultivars are summarized in Table 2. All of the 16 scion cultivars were grafted onto every mentioned rootstock cultivar.

Data collected at the autumn of the first growing season (2018) is represented in this study. Trunk perimeter and shoot length were measured by measuring-tape (mm accurately), crown diameter and total height were specified by measuring-rod (dm accurately). The angles of the shoots were calculated from the crown diameter and shoot length by the following equation:

$$\sin^{-1}\left(\frac{\text{crown diameter}}{2 \times \text{shoot length}}\right)$$

Results and Discussion

In this experiment the perimeter of trunks at 35 cm height, the length and angles of shoots, the diameter of crowns and the total height of the trees were investigated. Average of two replicates presented, each replicate contained three individuals. Figure 1. shows the average of trunk perimeter by scion and rootstock cultivars.

The averages of trunk perimeters were between 6.2 and 8.8 cm one year after plantation, their average was 7.1 cm. The highest value belonged to the Gönci Magyar kajszi (8.8 cm), followed by PinkCot and Bergarouge. Roxana, LilyCot and Spring Blush had the smaller perimeter, evenly 6.2 cm. In the aspect of rootstock cultivars, data of trunk perimeter ranges from 4.2 cm (Wavit) to 7.7 cm (Montclar), with a total average of 6.7 cm. Figure 2. shows the average shoot lengths.

In case of shoot lengths, higher differences were observable. At the first season Harogem (137 cm) Goldrich (133 cm) and Ceglédi óriás (118 cm) had the largest growth. The smallest enlargement belonged to Bergeron (79 cm), followed by PinkCot (81 cm) and Ceglédi szilárd (83 cm). Average shoot length was 100 cm. Per rootstocks, Wavit had the smallest growth (54 cm). Fehér besztercei (81 cm), Apricot seedling (94 cm), Myrobalan 29 C (95 cm), Montclar (108 cm) and Rootpack R (111 cm) was the ranking. Average shoot length of the rootstocks was 91 cm. Figure 3. shows the angles of the shoots.

To establish of a good income with a more intensive apricot orchard, it is essential to know the space demand of the different scion

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Table 1. Used scions and their state of origin

Scion cultivar	State of origin
Ceglédi óriás	Hungary
Ceglédi szilárd	Hungary
Gönci Magyar kajszi	Hungary
Pannónia	Hungary
FlavorCot	USA
Goldrich	USA
LillyCot	USA
Spring Blush	USA
TomCot	USA
Bergeron	France
Bergarouge	France
LadyCot	France
PinkCot	France
Tardif de Valence	Italy
Harogem	Canada
Roxana	Afghanistan

Table 2. Used rootstock cultivars, their species and propagation

Rootstock cultivar	Species	Propagation
Fehér besztercei (Fb)	*Prunus domestica* L.	generatively/vegetatively
Montclar (MC)	*Prunus persica* L.	vegetatively
Myrobalan 29C (My)	*Prunus cerasifera myrobalana* Ehrh.	vegetatively
Rootpack R (RR)	*P. cerasifera myr.* × *P. dulcis* Mill.	vegetatively
Apricot seedling (As)	*Prunus armeniaca* L.	vegetatively
Wavit (Wv)	*Prunus domestica* L.	vegetatively

and rootstock cultivars (Glišić et al., 2014). Highest angles were observed at Pannónia (52°), LilyCot (50°) and Goldrich (50°). Steepest shoots were grown on Bergarouge (35°), LadyCot (36°) and Bergeron (37°). Average angle of shoots was 43° to vertical after the first season. When data was observed by rootstock cultivars, major differences remarked. Regressive sequentially the following angles were calculated: Rootpack R (47°), Montclar (45°), Myrobalan 29 C (44°), Apricot seedling (44°), Fehér besztercei (32°), Wavit (22°). Average shoot-angle of the rootstock cultivars 39° to the vertical. Figure 4. shows the diameter of crowns of the combinations.

Crown diameters had a very large range after the first year, when the average of the scions was 87 cm. LilyCot (117 cm), TomCot (113 cm), Pannónia (112 cm) was the ranking. The narrowest cultivars were Bergeron (64 cm), Gönci Magyar kajszi (66 cm) and LadyCot (67 cm). In the aspect of rootstock cultivars, even larger differences were observable. The widest crown belonged to Rootpack R (102 cm), followed by Montclar

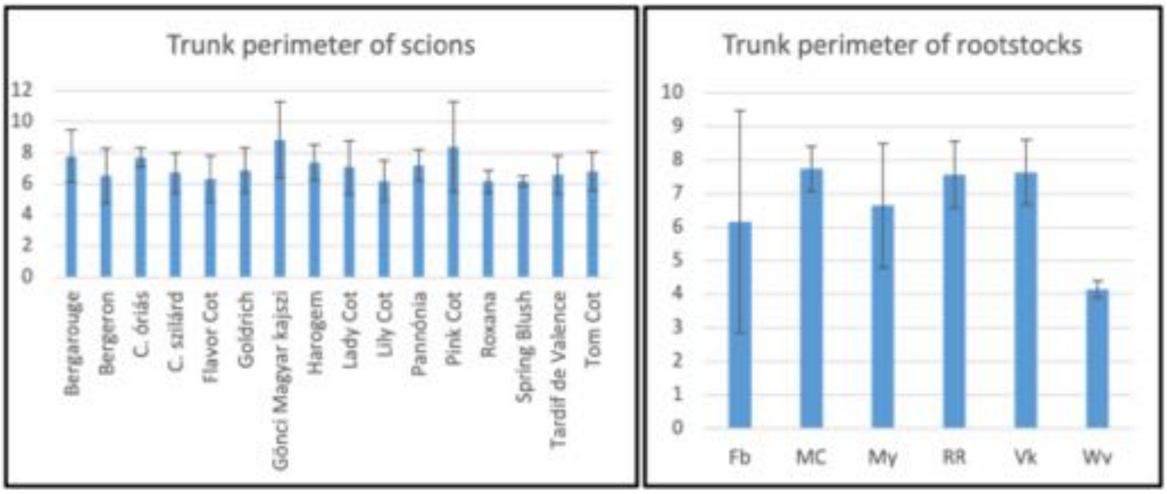

Figure 1. Average trunk perimeter by scion and rootstock cultivars at 35 cm height (cm).

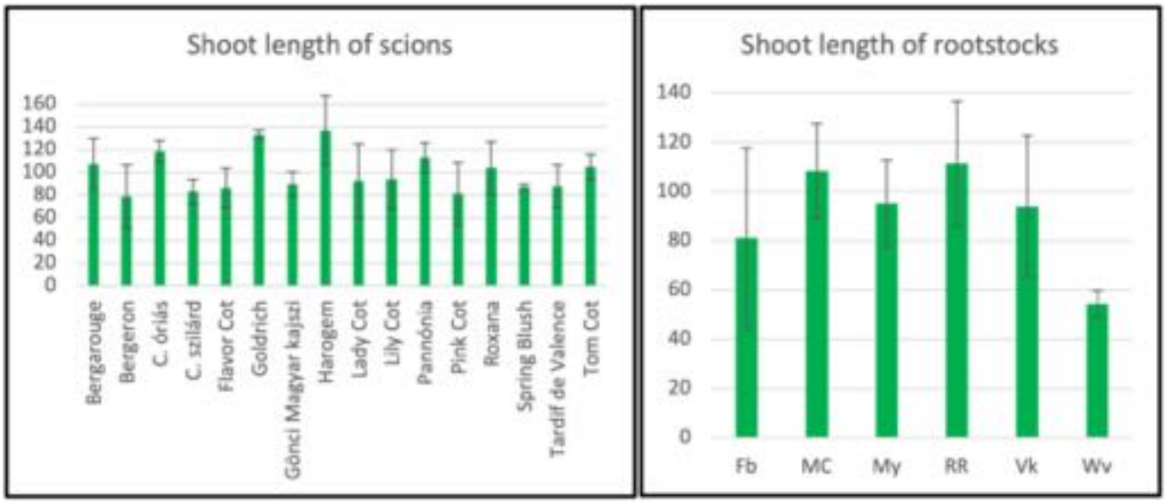

Figure 2. Average shoot lengths by scion and rootstock cultivars (cm).

(92 cm), and Myrobalan 29 C (88 cm). After this Apricot seedling came by 85 cm. Fehér besztercei (57 cm) and Wavit (34 cm) rootstocks had the two smallest crowns. Their average width was 77 cm. Figure 5. shows the total height, including the 80 cm of the trunk too.

The height of Ceglédi óriás exceeded of the scion cultivars with its average 232 cm It was followed by Bergarouge (191 cm), Pannónia (180 cm) and Gönci Magyar kajszi (179 cm). Lowest trees were in average of Roxana (124 cm), PinkCot (131 cm) and Lily-Cot (143 cm). Average of tree height was 162 cm in total. Amongst the rootstock cultivars Rootpack R was the highest (187 cm), followed by Montclar (186 cm) and Apricot seedling (179 cm). The sequence continued with Myrobalan 29 C (140 cm), Wavit (112 cm) and Fehér besztercei (105 cm). In division of rootstocks, the average of tree heights was 150 cm. Total height of trees is smaller in average than Ognjanov et al. (2018) found in Serbia due to more drained soil conditions.

Table 3. and Figure 6. show the dispersion of the previously described values. Trunk

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Table 3. Trunk perimeter, total height, crown diameter, angle of shoots and shoot length of scion cultivars

	Trunk perimeter (cm)	Total height (cm)	Crown diameter (cm)	Angle of shoots(deg.)	Shoot length(cm)
Bergarouge	7.8	191.3	71.4	35.2	107.5
Bergeron	6.5	163.3	63.5	37.2	79.1
Ceglédi óriás	7.7	232.2	89.6	46.1	118.5
Ceglédi szilárd	6.7	143.9	84.2	42.9	83.0
Flavor Cot	6.3	142.1	80.1	42.0	86.6
Goldrich	6.9	158.1	106.8	50.4	133.4
Gönci Magyar kajszi	8.8	179.0	66.4	37.7	89.9
Harogem	7.4	173.3	107.0	48.4	137.3
Lady Cot	7.1	172.1	67.3	36.2	92.6
Lily Cot	6.2	142.6	117.4	49.7	94.2
Pannónia	7.2	179.8	112.3	51.7	113.1
Pink Cot	8.4	131.3	83.3	43.2	81.3
Roxana	6.2	124.1	88.3	45.0	104.2
Spring Blush	6.2	144.6	70.4	37.4	86.9
Tardif de Valence	6.6	162.4	74.7	37.4	87.8
Tom Cot	6.8	149.1	113.3	45.7	104.9
Dispersion	0.8	25.7	18.0	5.4	17.5
Mean ± SE	7.1 ± 0.2	161.8 ± 1.3	87.3 ±1.1	42.9 ±0.6	100.0 ± 1.0
SD (5%)	1.0	29.9	19.6	5.8	20.1

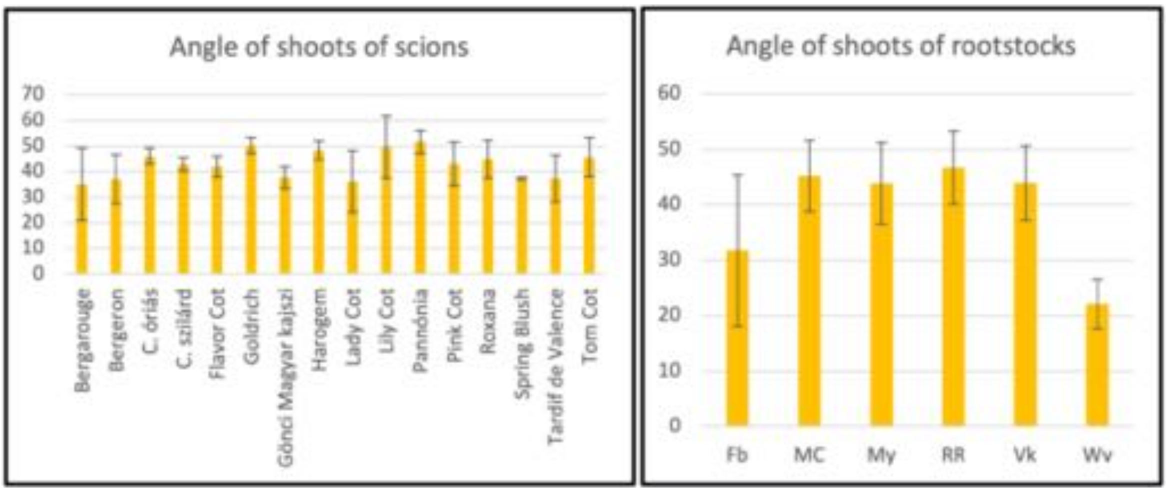

Figure 3. Included angle of shoots to vertical by scion and rootstock cultivars (deg.).

perimeter, total height, crown diameter, angle of shoots and shoot length of scion cultivars, their mean values, the dispersion, the SE and SD values can be observed there.

Evaluating the data shows, the higher value of an attribute, the higher is the disperse

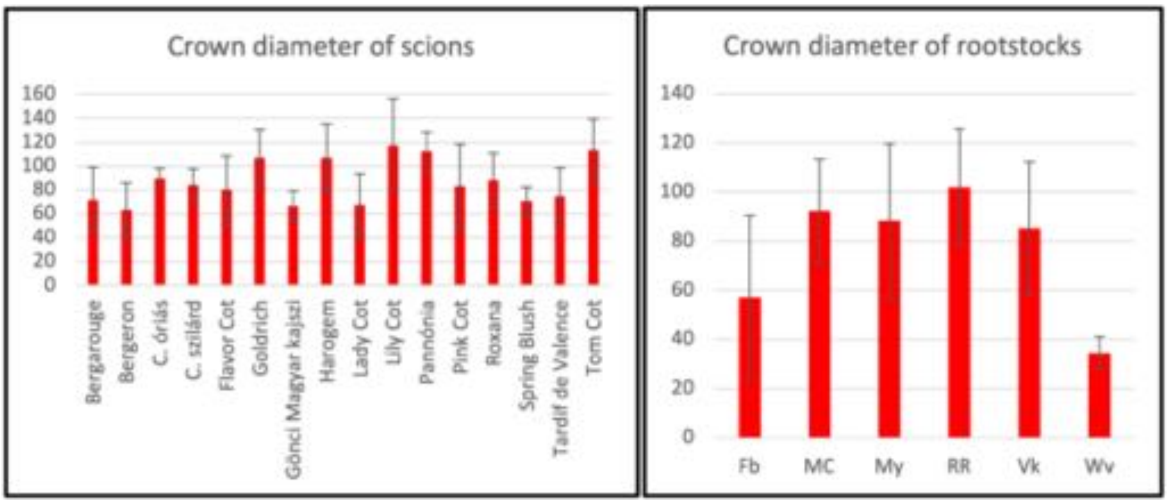

Figure 4. Average crown diameters by scion and rootstock cultivars (cm).

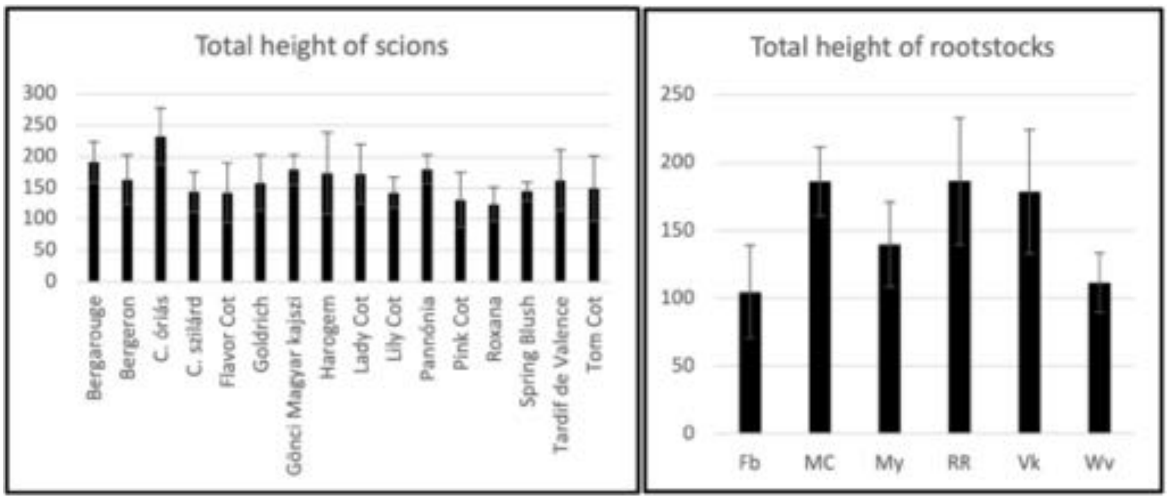

Figure 5. Average total height by scion and rootstock cultivars (cm).

of it. This property of the analysis comes from the recording of data, and is not considered as a scientific achievement in this experiment. However, it can be seen that in every measured attribute the dispersion between rootstock cultivars were higher than between scions. Percental fraction of distributional values of the rootstocks were established from these data. 63.2% of trunk perimeter, 58.6% of total height, 57.9% of crown diameter, 64% of angle of shoots, 53.7% of shoot length depends on the cultivar of rootstock.

Conclusions

1000 apricot trees were planted from 96 combination of 6 rootstock and 16 scion cultivars. Data were collected from 5 properties which describe well the vegetative growth of an engraftment. Scion cultivars represents the cultivar preferences in Hungary, while there are less used rootstock cultivars (Rootpack R, Montclar) in this trial too.

Highly vigorous rootstocks are Montclar and Rootpack R, these cultivars had the highest values in all cases. Apricot seedling and My-

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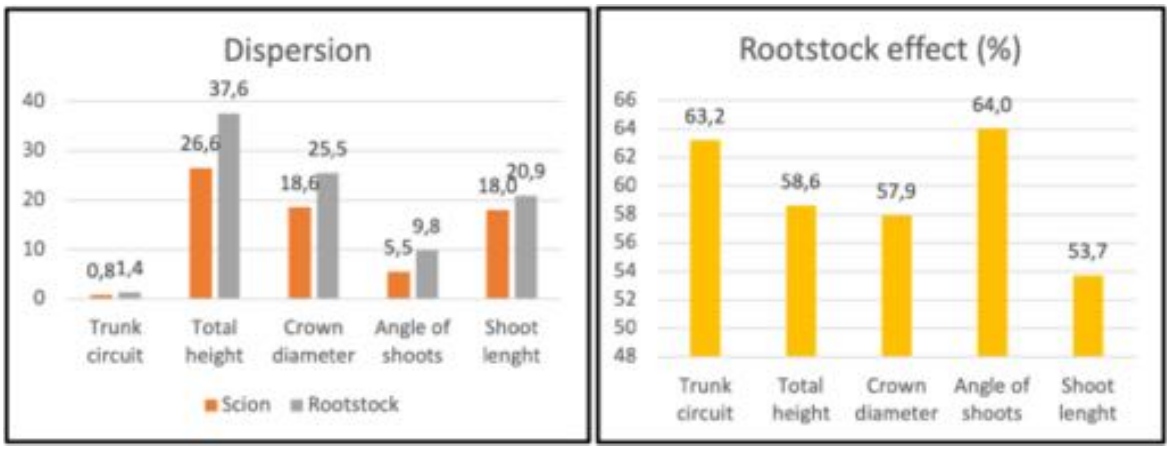

Figure 6. Dispersion of data divided by scion and rootstock cultivars, and fraction of rootstocks of dispersion (%).

robalan 29 C can be typified with moderate growth habit with very similar characteristics. Weak growth was observed at Fehér besztercei and Wavit rootstocks. Wavit rootstock cultivar had the lowest parameters in every case. Here, it had to be declared, that our non-irrigated circumstances are not ideal for this last two rootstocks (for they plum origin).

In case of scion cultivar, it is harder to make up discrete categories like previously. Some parameters are not in strong correlation with others, but differences can be observed here too. As a general principle vigorous growth typifies the Ceglédi óriás, Pannónia, Goldrich, Harogem, TomCot and Bergarouge cultivars. Roxana, PinkCot, Ceglédi szilárd and Spring Blush cultivars appeared with expressly weak growth. The other cultivars positioned between this two separate groups.

It appears unequivocally, that the rootstock cultivars give the bigger portion of the differences of growth habit in this experiment, therefor it has to be a very important element of the planning of a growing system. Vigorously growing rootstocks are suitable for replanting or refilling apricot trees, while weak rootstocks are only acceptable with proper irrigation and fertilization for ensuring the lower spacing in the orchard. Besides this, scion cultivars responsible for important differences (36-46%). Accordingly, if other factors permit, the vegetative growth could also be an aspect of choosing a cultivar.

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Different organic mulch materials affect the abundance of enchytraeids in an open-field experiment

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Abstract: Organic mulch may provide favourable soil conditions e.g. soil moisture or organic matter content, which may enhance the number of enchytraeids. However, there is no sufficient information about the relationship between plant-based mulch and this beneficial soil animal group. Therefore, an open-field experiment was conducted with tomato as a test plant to examine the effect of different types of organic mulch materials on the natural occurence of enchytraeids. Our study microplots received the following treatments: 1) yard-waste compost, 2) walnut leaf litter, 3) mixed leaf litter without walnut and 4) wheat straw. Control microplots were left unmulched. Randomized block design was used with eight replications to the treatments and four to the control. At the end of the growing season, three soil samples were taken from the root zone of each plant with a split soil corer, and enchytraeids were extracted by the wet funnel method. Living enchytraeids were counted under a dissecting microscope, and their density values were estimated. Worms were identified in five randomly selected samples of each treatment. In addition, soil moisture was determined by oven-drying as well. Mulch material types had significantly different effects on both Enchytraeid density and soil moisture. Under walnut leaf litter, mixed leaf litter and straw cover, higher numbers of individuals were found. In the case of soil moisture content, straw mulching had the highest value, while compost and uncovered surfaces the lowest. It appears that mulch materials serve as food source and provide favourable conditions for enchytraeid communities as well.
Keywords: Annelids, compost, leaf litter, potworm, straw
Received 14 May 2020, Revised 29 October 2020, Accepted 24 November 2020

Introduction

The use of organic, plant-based materials as surface mulch has several positive effects: controlling weeds, enhancing root growing, increasing soil humus content by the decomposition of materials (Goulet et al. 2004, Pupalienè et al. 2015, Stefanovits et al. 1999). Mulching in general, helps to preserve soil moisture and prevents soil degradation (Adekalu et al. 2007, Pinamonti 1998). In addition, it can reduce the fluctuation of soil temperature (Pinamonti 1998).

Mulching has an effect not only on soil but on the fauna living within or on the surface of the soil. However, the efficacy of soil cover depends on mulch type and the casts of soil organisms. According to Addison et al. (2013), mulching may primarily increase the presence of omnivorous species. Straw and compost mulching may enhance the number of natural enemies and compost increased the abundance of soil macroinvertebrates (Thomson and Hoffman 2007). At the same time, fewer arthropod pests were found when straw, compost or woodchips mulch was used, compared to the unmulched control (Addison et al. 2013). Both leaf litter and hay mulch enhanced not only the total number, but the diversity of carabids (Dudás et al. 2016). Arthropods were found more diverse and abundant under black walnut leaves (Summers and Lussenhop 1976). Tea and flower compost enhanced the accumulation of free-living nematodes in mulched soil (Langat et al. 2008). Compared to the unmulched control, alfalfa hay mulch and vermicompost increased the density of bacterivore nematodes but decreased the presence of fungivore, predatory-omnivore and plant-parasitic nematodes (Forge et al. 2003, Xiao et al. 2016). Rye straw decreased the number of root-knot nematodes, *Meloidogyne graminicola* on wheat roots (Kandel et al. 2011). However, green and yard-waste composts did not affect plant-parasitic nematodes (McSorley and Gallaher 1995). Jodaugienė et al. (2010) found that the density of earthworms was higher under straw and grass mulch, but it was not different when collected from unmulched control plots or from under peat or sawdust mulch. Black walnut leaves also decreased earthworm abundance (MacDaniels and Pinnow 1976).

Enchytraeids are considered to be ecosystem engineers with ants, earthworms and termites (Brussaard et al. 2012). Similar to earthworms, they are able to provide favourable conditions for microbial activity (Kasprzak 1982). According to Domínguez and Bedano (2016), enchytraeids and earthworms complement each other: in the rice-growing system, the drainage period would be more appropriate for earthworms, while enchytraeids would be more efficient during the very moist or flooded conditions (John et al. 2019). The role of enchytraeids in agroecosystems is essential: they can influence the aeration and permeability of soil for roots by their burrowing activity and they can also stabilize soil structure by their mucilages and excrements (Didden 1991). According to Didden (1991), organic farming, with the application of organic manure, cover crops, and the use of shallow soil work, favour enchytraeids.

Enchytraeids consume, beside other food sources, bacteria, fungi, mineral particles and leaf litter (van Vliet and Hendrix 2012) and choose old and decomposed leaves more frequently than fresh ones. However, their food preferences are different among species (Dózsa-Farkas 1976).

The connection between enchytraeids and different organic matters is contradictory: while Ricci et al. (2015) did not observe any positive change in the abundance of enchytraeids to the effect of animal manure and composted sludge, Andrén and Lagerlöf (1983) found that the addition of sludge and farmyard manure addition enhanced their numbers. Beside other soil-dwelling animals, enchytraeids as well may respond differently to various organic matters. The most frequent mulchers are organic farmers, who apply organic or inorganic mulch materials on the soil surface. Common organic mulch materials are compost, straw and wood chips (Dezsény 2015). Furthermore, a large amount of mulch material in the form of leaf litter is produced at defoliation in every autumn in Hungary. However, the use of this valuable organic matter is not sufficiently prevalent either in agriculture, or in horticulture. We aimed to examine the effect of four types of organic mulching materials (standard yard-waste compost, walnut leaf litter, mixed leaf litter and wheat straw) on enchytraeid communities in an open-field long-term experiment.

Materials and Methods

Open-field experiment

Study plots were established on the Experimental Field of the Department of Integrated Plant Protection of the Szent István University (Gödöllő, Hungary). The experiment was conducted during the growing season of 2019 with the Hungarian landrace tomato 'Dány' (RCAT057829) as crop plant.

Before our study, a long-term mulching ex-

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periment has been going on between 2016 and 2018 with potato ('Hópehely' and 'Démon') with the same mulching treatments and setup (Fehér et al. 2017, Südiné Fehér et al. 2019) in the same place.

The dominant soil type of the plots was coarse sand. We erected pinewood frames to separate treatments on the soil surface. Thus, we had 36 microplots, each measuring 2 m × 2 m on a total area of 144 m^2. In one microplot, four tomato plants were planted, giving an isolation distance of 1 m between plants. Microplots received the following treatments: 1) standard yard-waste compost ('Zöld Híd Komposzt' 04.2/3245-2/2017 Nébih 2020), 2) walnut (*Juglans regia*) leaf litter, 3) mixed leaf litter (without walnut), 4) wheat straw. Control microplots were left unmulched. Leaf litters were collected in Gödöllő after the fall of leaves in autumn of 2018. Mixed leaf litter contained the leaves of *Acer* spp., *Aesculus hippocastanum*, *Castanea sativa*, *Corylus avellana*, *Platanus* × *acerifolia*, *Quercus* spp., *Salix* spp., *Sorbus* spp. and *Tilia* spp. at the ratio of approximately 1:1. Compost material was provided by Zöld Híd B.I.G.G. Nonprofit Kft. (Gödöllő, Hungary), and straw was collected by a local farmer of Gödöllő (József Babarczi). In the case of mulching treatments, eight replications were used. In addition, there were four replications for the unmulched control. Replications were set in randomized block design (Figure 1). Mulch materials were spread in a 15 cm-thick layer on 3 April 2019.

Plots has not received soil work since 2016. Tomatoes were planted into undisturbed soil on 28 May, and plots were hand-weeded or hoed every ten days during the growing season (Petrikovszki et al. 2020). After collecting soil samples, the experiment was terminated, and plants were removed.

Data collection

Enchytraeids were collected at the end of the growing season, 4 October 2019. Three soil samples were taken from the root zone of each plant with a split soil corer (diameter of 5 cm) to 10 cm depth (volume approximately 200 cm^3 per sample), mixed and then a single aliquot with the volume of 200 cm^3 was taken for worm extraction by wet funnel method (O'Connor 1962). Living enchytraeid individuals were counted under a dissecting microscope (Olympus SZ-60, ×10–×20). In five randomly selected samples of each treatment, worms were identified *in vivo* under a light microscope (Euromex Delphi-X, magnification between ×100 – ×400, mostly ×200) using the guides of Schmelz and Collado (2010, 2012).

Juvenile specimens were not appropriate for identification (except for *Buchholzia appendiculata* (Buchholz, 1862)). Therefore, they were excluded from species composition analysis, but were reckoned in total enchytraeid density.

To measure soil moisture, further three samples were taken from each microplot (volume approximately 100 cm^3). From each sample 40–60 g subsamples were taken to determine soil moisture by oven-drying (105°C for 48 hours).

Air temperature data were collected daily from the website of the Hungarian Meteorological Service (OMSZ). Precipitation was measured every day with a rain gauge (Figure 2).

Data analysis

The effects of the different mulch types on soil moisture and enchytraeids were investigated using linear mixed-effects models with Gaussian error structure (Faraway 2006). In the models, mulching treatment (four levels + control) was used as fixed factor, while plant individuals were treated as random term. Goodness-of-fit values of the models were measured by likelihood-ratio test-based coefficient of determination (R^2_{LR}; Bartoń 2020), the explanatory power of the treat-

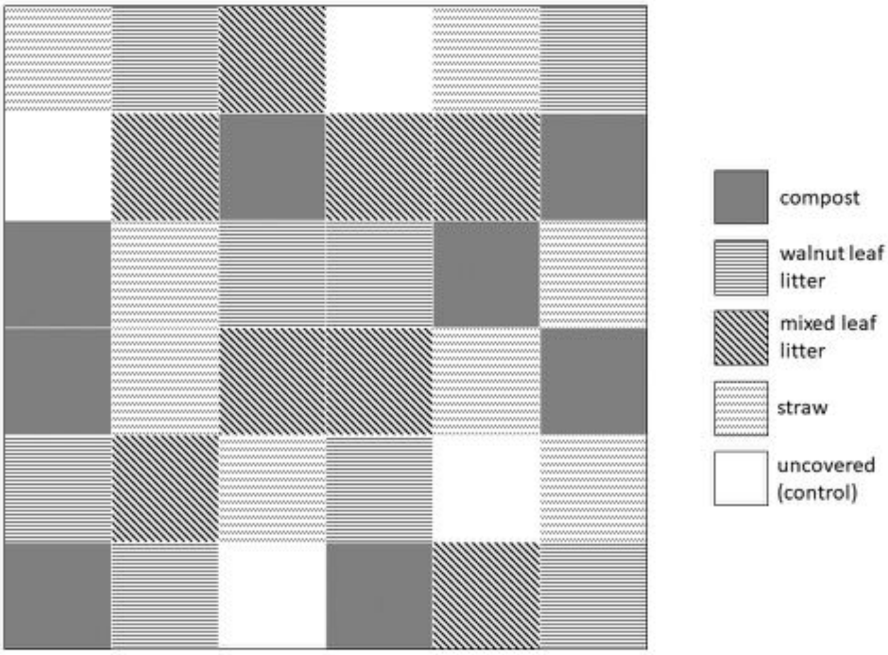

Figure 1. Arrangement of mulch treatments.

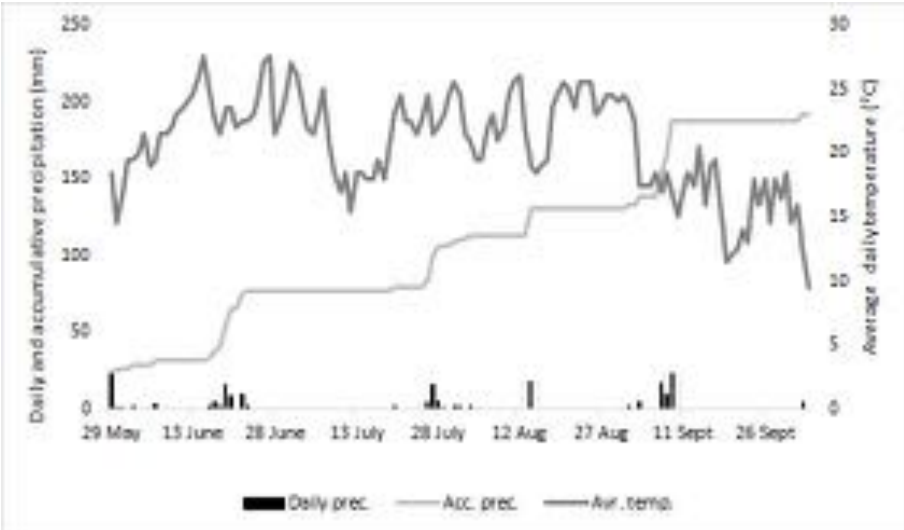

Figure 2. Average daily temperature, precipitation, and accumulative precipitation amount in the growing season of 2019.

ments was evaluated F-statistics (Faraway 2006). The differences between the treatment levels were tested using Tukey-type multiple comparisons procedure (using $p \leq 0.05$ significance level) for all of the pairwise comparisons by general linear hypothesis tests (Hothorn et al. 2008).

The effects of the treatments on species composition was explored by non-metric multidimensional scaling (NMDS) using Euclidean distance function and square root transformation of the abundance data (Borcard et al.

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2011). Moreover, permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) based on Bray-Curtis dissimilarity with 9999 permutations was applied to measure the separation between the treatment types. The relationship between enchytraeids density and soil moisture levels was tested by Pearson product-moment correlation coefficient.

All analyses were made in R version 3.4.1 (R core Team 2017). The GLMM of abundances was made by the "lme" function of "nlme" package (Pinheiro et al. 2017). The likelihood test based coefficient of determination was calculated by "r.squaredGLMM" function of "MuMIn" package (Barton 2016). Multiple comparisons of linear hypothesis test were made by "glht" function of "multcomp" package (Hothorn et al. 2008). NMDS were made by "metaMDS" function, PERMANOVA by the "adonis" function of "vegan" package (Oksanen et al. 2017).

Results

The total number of specimens, 236, belonged to six genera, 13 species:

Achaeta eiseni Vejdovský, 1878
Achaeta pannonica Graefe, 1989
Buchholzia appendiculata Buchholz, 1862
Enchytraeus buchholzi s.l. Vejdovský, 1879
Enchytraeus varithecatus Bouguenec & Giani, 1987
Enchytronia parva Nielsen & Christensen, 1959
Fridericia bisetosa Levinsen, 1884
Fridericia connata Bretscher, 1902
Fridericia galba Hoffmeister, 1843
Fridericia hegemon Vejdovský, 1878
Fridericia nemoralis Nurminen, 1970
Fridericia paroniana Issel, 1904
Marionina communis Nielsen & Christensen, 1959

The most frequent species was *E. buchholzi*, which was found in all treatments with a relative abundance of 4.5–27.3%. The second dominant species was *B. appendiculata* with 14.7–37.1% relative abundance; however, this species occurred only in walnut leaf litter, mixed leaf litter and straw microplots. *F. paroniana* had the highest abundance in walnut leaf litter samples (19.7%) and the third highest in total (10.5%), but in other samples, this species was subdominant. Only two specimens of *E. varithecatus* was found and only in straw-covered samples.

Walnut litter microplots contained 30.5% of the total number of mature specimens, straw-covered microplots 30%, mixed litter 17.5%, and compost mulch and uncovered microplots both 11-11%. The highest density was 19 400 individuals/m^2 (mixed leaf litter), while there were three samples (in compost and control microplots) with no specimens (Table 1).

According to our models, mulch types had significant effects on density ($R^2_{LR} = 0.25$, $F_{(4,67)} = 5.88, p = 0.0004$). Under walnut leaf litter, mixed leaf litter and straw cover, higher number of individuals was found than in the case of compost and uncovered microplots (Figure 3).

There was a significant separation among the treatments based on the performed PERMANOVA ($R^2 = 0.28$, $F = 1.96$, $p = 0.0046$). The NMDS (stress = 0.134) revealed that the microplots of compost and uncovered had homogenous and slightly separated groups, while the treatments of walnut leaf litter, mixed leaf litter and straw were more heterogeneous as it is observable on the ordination diagram (Figure 4).

A strong treatment effect was observed on the soil moisture ($R^2_{LR} = 0.68$, $F_{(4,67)} = 37.97, p < 0.0001$). Straw mulching had the highest soil moisture, in walnut and mixed leaf litter, soil water content was intermediate, while compost and uncovered surface induced the driest soil conditions (Figure 5).

Density and soil moisture were significantly correlated according to the Pearson correla-

Table 1. Density and soil moisture (mean ± SD) values in different treatments

Treatment	density (individual/m^2) mean ± SD	soil moisture (m/m%) mean ± SD
compost	2710 ± 2150	3.33 ± 0.85
walnut leaf litter	6380 ± 3980	3.56 ± 0.70
mixed leaf litter	6480 ± 5290	2.55 ± 0.82
straw	7780 ± 3390	4.56 ± 1.08
uncovered (control)	2110 ± 2160	2.52 ± 0.75

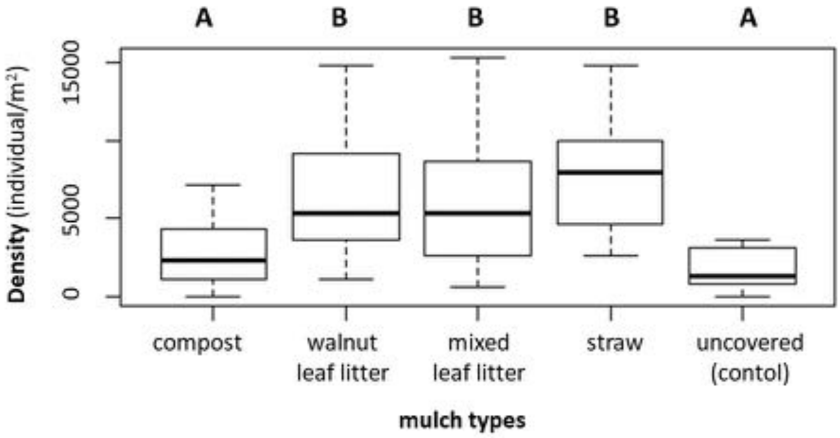

Figure 3. Boxplot of the enchytraeid density under different mulch types. Different letters indicate significant difference between means ($p \leq 0.05$).

tion analysis (r = 0.38, p-value = 0.0009). In general, the density of enchytraeids increased by the higher values of soil moisture values of different treatments (Figure 6). However, 6480 individuals were counted at 2.55% soil moisture content under mixed leaf litter (Table 1).

Discussion

The enchytraeid density of microplots was found in fair accordance with soil moisture. The highest numbers of enchytraeids and the highest soil moisture were also observed under straw mulch. Since mycelial web in these microplots were observed and according to studies, enchytraeid communities contain 80% microbivorous and 20% saprovorous feeders (Didden 1993), so not only soil moisture but mycelia as a food source can increase their number under straw mulching. Walnut- and mixed leaf litter-covered plots also had a significantly humid soil compared to control. Leaf litter also is considered to provide nutrients for enchytraeids, and the larger body size *Fridericia*

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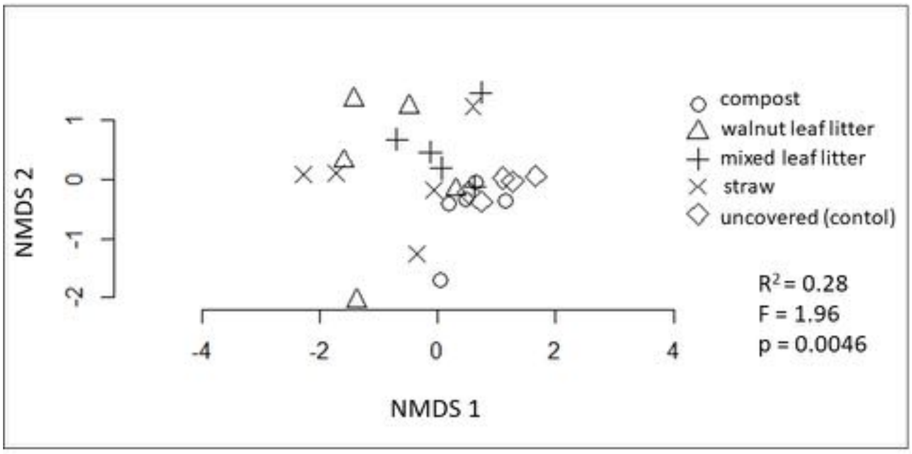

Figure 4. Non-metric multidimensional scaling ordination of the different mulch types based on the density of enchytraeid species. The compositional difference between treatments expressed as the results of the PERMANOVA (R^2 – coefficient of determination, F and p values) are portrayed on the panel.

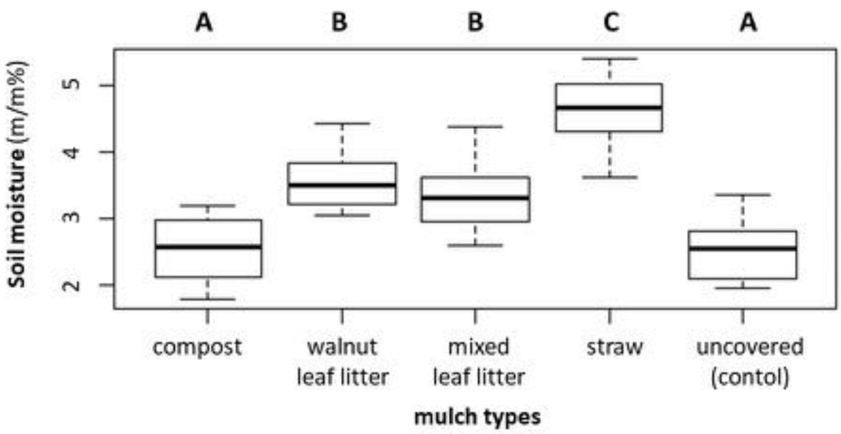

Figure 5. Boxplot of soil moisture under different mulch types. Different letters indicate significant difference between means ($p \leq 0.05$).

species (e.g. *F. hegemon*, *F. galba*) prefer to consume this kind of organic matter (Dózsa-Farkas 1976), which can be an explanation for their high density.

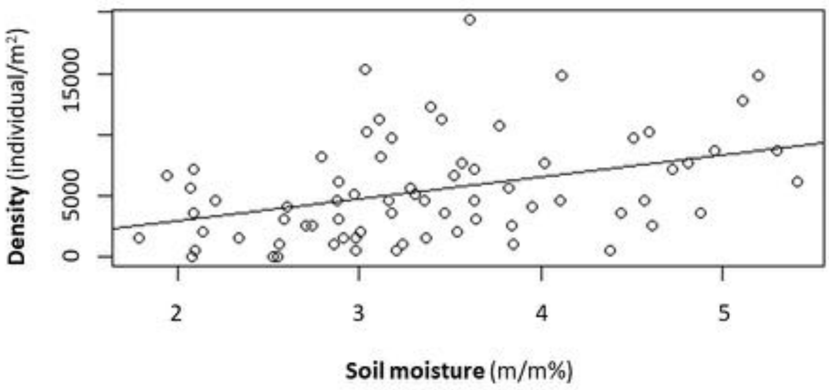

Figure 6. Correlation test (Pearson) between soil moisture and the density of enchytraeids in the soil samples.

The lowest number of enchytraeids was found in the control (uncovered) and compost-covered microplots. These treatments also had the lowest soil moisture content. Generally, compost is a drier mulch type than litter or straw. Furthermore, its black colour promotes a more intense and quicker warm-up. Our findings are in accordance with the study of Dózsa-Farkas (1977), who established that individuals of *Fridericia galba* perish more rapidly on higher temperatures correlated to lower soil moisture content than on lower temperatures.

Our soil moisture values under different mulch materials were quite low (between 2.52% and 4.56%), which is considered a very dry soil condition. Among soil organisms, enchytraeids are sensitive to desiccation (Didden 1993). However, Maraldo and Holmstrup (2009) examined the effect of different drought events on enchytraeid populations. One of their treatments, artificial drought with only 5% soil water content was achieved on sandy soil, a condition, which was similar to our open-field conditions before sampling. While they did not find any Enchytraeid individual in May and June, however, between August and October, enchytraeid densities were higher than 10000 individuals per m^2 in each month. They concluded that not only the moisture conditions at the time of sampling, but also moisture conditions in the past could have influenced the population of enchytraeids (Maraldo and Holmstrup 2009). This can explain the relatively high densities we found in dry conditions, since only 50 mm precipitation fell between 7 and 9 September, one month before sampling.

Besides mulching, avoidance of disturbance can also increase the number of enchytraeids. The abundance and biodiversity of soil macrofauna, in general, were significantly higher under no-till with mulch system than under conventional tillage (Brévault et al. 2007), while enchytraeid densities and biomass in the no-till system were significantly higher under no-till conditions (Parmelee et al. 1990, Fijuta and Fujiyama 2000). In general, mulch keeps the

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soil cooler and moist, which is an advantage not only for plants but for soil fauna as well. Mulch materials also contain nutrients for soil fauna, and consequently, enchytraeid communities prefer mulch-covered soil. Their presence has several beneficial effects on soil properties including structure, porosity, or organic matter content. Due to these coherent effects, mulching is beneficial in agricultural and horticultural production as well.

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Comparative study of flowering phenology of selected plant life forms in urban and rural environments. Preliminary results

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Abstract: Global climate change has unforeseeable ramifications for the ecosystem of our home planet. In Europe, more than half of the vascular plant flora may become endangered by the year 2080 as a result of climatic changes. Urban climate conditions are considered similar to the changing global climate conditions. The concept of our study is based on the Space for Time Substitution method, utilizing its advantage of saving time and resources compared to long-term monitoring. To find out how excess heat in urban environments affects the phenological flowering patterns of species we planted specimens representing 6 different life-forms of the Raunkiær system (phanerophytes, chamaephytes, hemicryptophytes, geophytes, hemitherophytes, therophytes). Each category was represented by at least 5 species and each species by 5-5 specimens in Budapest, Füvészkert and in the MATE Botanical Garden of Gödöllő. All the species in the experiment averaged at 7.62 days earlier flowering onset in Budapest. The peak of the flowering had 12.94 days of difference, while the end of flowering had 2.9 days of difference, with the earlier being Budapest. There is a strong significant difference ($P < 0.001$) in the onset of the flowering of *Globularia cordifolia* between the locations, regarding the peaks of flowering there is a strong significance ($P < 0.001$) for *Inula ensifolia*, regarding the end of flowering there is a strong significance ($P < 0.001$) for *Polygonatum multiflorum.* To clarify other driving forces and the role of abiotic parameters in the flowering phenology patterns, further study is required.
Keywords: Reproductive phenology, plant life forms, climate change, heat island, botanical garden

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Introduction

United Nations 1992 Framework Convention on Climate Change (UNFCCC) – referencing prior research results – clearly states that the mean temperature of the planet Earth is rising (http1). By today the theory of global warming is widely supported by multi-disciplinary research, including the 2007 IPCC report (IPCC 2007). According to the World Meteorological Organisation's study, compared to the average mean temperature between 1961 and 1990 by 2015 the difference reached 1 °C, and by 2020 Global Mean Surface Temperature was around 1.2°C warmer than the pre-industrial baseline (1850–1900) (http1, http2). The last decade (2011–2020), is the warmest on record (http2). Another 2–4°C rise is expected during the next century (MEA 2005). This change has unforeseeable ramifications for the ecosystem of our home planet. In Europe, more than half of the vascular plant flora may become endangered by the year 2080 as a result of climatic changes (Thuiller et al. 2005).

Unfortunately, this significant environmental crisis is unsolved to this day (IPCC 2007, Hufnagel & Sipkay 2012). Based on current research it seems that climate change can no longer be stopped. Therefore, it is crucial to investigate possible adaptations (Li et al. 2019).

Urban climate conditions are considered similar to the changing global climate conditions; therefore, many researchers study urbanized areas as small-scale experiments, or models, of global climate change (Ziska et al., 2003). Thus the urban environment is suitable for the application of the Space for Time substitution method. Which method encompasses analyses in which contemporary spatial phenomena are used to understand and model temporal processes that are otherwise unobservable, most notably past and future events. This method is used to predict the effects of climate change on biodiversity, identifying general trends, therefore its application saves time and money compared to long-term studies. (Pickett 1989, Blous et al. 2013)

Thus, it is key to examine the patterns and shifts in the patterns of flowering phenology in urban areas compared with rural ones. Cities are strongly affected by climate change.

Fifty years of data from the Copernicus Program on more than 100 000 European municipalities confirm that the continent is heating up at every latitude. In a third of these municipalities, the average temperature has risen by more than 2°C between the 1960s and the last decade (http3, http4). The survey compared the mean temperatures of the municipalities of the period between 1961–1970 with the period between 2009–2018. According to the report of this program mean temperature of Budapest increased from 8.04°C to 12.04°C, and in Gödöllő from 7.56°C to 10.76°C. According to this change (4°C and 3.2°C), both cities belong to the top third of the data. The increase in the capital city Budapest was 0,8°C bigger than that of Gödöllő, which is a small town with more green areas. According to the report, it is clear, that capitals and their suburbs are affected most significantly, especially in central-eastern Europe. Riga and Budapest are the two European capitals that have warmed most of all.

Phenology is the study of the timing of recurrent biological events, the causes of their timing with regards to biotic and abiotic forces, and the interrelation among phases of the same or different species (Lieth 1974). Many research shows that temperature change significantly affects the life cycle of plants (e.g. Scheifinger et al. 2003, Kunkel et al. 2004, Hansen et al. 2006, Lehoczky et al. 2016).

Towards the end of the last century, phenological observations were recognized for their capability to visually and quantitatively assess climate change effects on ecosystems. Several studies demonstrate significant shifts in phenological phases of plants across Europe (e.g., Menzel and Fabian 1999; Menzel 2000, Chmielewski and Roetzer 2001, Schleip et al. 2009). Shifts in phenological events in the Carpathian Basin are particularly poorly documented, with a few exceptions coming from the works of Keresztes (1984), Walkovszky (1998), Schieber et al. (2009), Eppich et al. (2009), Molnár et al. (2012), Varga et al. (2012), Lehoczky et al. (2016), and Szabó et al. (2016). Keresztes (1984), Walkovszky (1998), and Varga et al. (2012) are focusing on their study on *Robinia pseudoacacia*, a non-native species for the Carpathian Basin, however, there is a lack of studies with emphasis on native species' phenology in Hungary. There is less available phenological data on wild-growing plants, then from cultivated species (Walkovszky 1998, Hunkár 2012). Szabó et al. (2016) showed in their study based on the long-term data from the Hungarian Meteorological Service recorded between 1952 and 2000, that native plant species advanced their flowering time (1952–2000) by 1.9–4.4 days per decade.

In the last few years, more and more researches use remote sensing to detect changes in phenological patterns between urban and rural areas (Yao et al. 2017, Luo et al. 2020, Jia et al. 2021). In Northeast China, the start of the growing season in old urban areas had become earlier and the differences of the start of the growing season between urbanized areas and the ru-

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ral area changed greatly during 2001–2015 (-0.79 days/year, $P < 0.01$). Meanwhile, the length of the growing season in urban areas had become increasingly longer than in rural areas (0.92 days/year, $P < 0.01$) (Yao et al. 2017). In China, the time of the start of the growing season of inland cities of Liaoning Province had negative correlations with urban size. Specifically, when the urban size increased 10-fold, the start of the growing season advanced by 10.03 days (Luo et al. 2020). Other research, based on MODIS data, claims that overall in China, the phenology shifted earlier by 8.6 ± 0.54 days for the start of the growing season in urban core areas compared with their rural counterparts. (Jia et al. 2021). A comprehensive understanding of species phenological responses to global warming requires observations that are both long-term and spatially extensive. Long-term data series deriving from the same place are rare (Hunkár et al. 2012). It is also very important to choose the correct methodology for the research. Primack (1985) described the methodology of collecting flowering phenology data. For the observation of individual plants, he suggests counting every flower open on the entire plant on every day that the plant is in bloom, to note the date of first flowering, the date of the last flowering, and the date on which the most flowers are open (date of maximum flowering). According to him the duration of flowering may be an appropriate statistic for comparative purposes for species that begin and stop flowering abruptly and have about the same number of flowers open per day.

Previous research on the Carpathian Basin (Keresztes 1984, Walkovszky 1998, Schieber et al. 2009, Eppich et al. 2009, Molnár et al. 2012, Varga et al. 2012, Lehoczky et al. 2016, and Szabó et al. 2016) did not follow Primack's method but still provided important data. Several previous studies (e.g., Menzel 2000, Roetzer et al. 2000, Walkovszky 1998, Eppich 2009) confirmed that flowering phases advanced during the 20th century, which is connected with the increasing temperature. Szabó et al. (2016) examined flowering phenological records for six species (*Convallaria majalis*, *Taraxacum officinale*, *Syringa vulgaris*, *Sambucus nigra*, *Robinia pseudoacacia*, *Tilia cordata*) based on phenological observations from the Hungarian Meteorological Service recorded between 1952 and 2000. Altogether, four from the six examined plant species showed a significant advancement in flowering onset with an average rate of 1.9–4.4 days per decade. The examined species showed a difference where flowering occurred on the Great Hungarian Plain before West-Hungary. Using a long-term data series of 144 years (1851–1994) Walkovszky (1998) showed 3–8 days advancement in the flowering date of *Robinia pseudoacacia*, relating the event to the mean temperature of March–May. Templ et al. (2017) examined the flowering phenology of plant species a 3000-km-long, North-South transect from northern to eastern Central Europe over the period 1970–2010 to identify the spatio-temporal patterns biogeographical regions of Europe. The results show that Continental, Alpine and Boreal regions have a greater shift in flowering phenology (2.2–9.6 days per decades) than in Pannonian and Mediterranean regions.

Strategy for Plant Conservation (GSPC; Secretariat of the CBD 2002, Convention of Biological Diversity 2010) define the role of botanic gardens, so they have responsibilities in research, teaching, and public education in the field of botany and in conservation both in *ex situ* conservation and in *in situ* conservation serving resources for restoration projects. Botanic gardens are contributing to climate change-related research. The project of International Phenological Gardens (IPG) started in 1957 and now encompasses 50 botanical gardens across Europe and collected 65 000 observations of

23 plant species during the decades (Primack & Miller-Rushing 2009). This project used clonal plant material to reduce the amount of genetic variation, so that variation in phenology reflects the influence of environmental factors rather than genetic differences among individuals (Primack & Miller-Rushing 2009).

This study aims to find out how excess heat in urban environments affect the phenological flowering patterns of species belonging to different life forms, and how different the effect is in colder rural mesoclimactic environments. To observe this, we planted plants representing six different life forms in two distinct locations. One was in downtown Budapest in ELTE Botanical Garden (Füvészkert), and the other in MATE Botanical Garden of Gödöllő. The flowering of the plants was recorded between the 1st of March and 25th of December in 2020. We hypothesized locations with higher mean temperatures would result (i) in an earlier onset and (ii) and an earlier end of flowering, compared to colder locations.

The following abbreviations were used: Phanerophytes (Ph), Chamaephytes (Ch), Geophytes (Ge), Hemicryptophytes (He), Hemitherophytes (HT), Therophytes (Th).

Materials and Methods

Study area

Examination covering the entire vegetation cycle of species have been implemented in the Experimental Site of Hungarian University of Agriculture and Life Sciences Gödöllő Botanical Garden (47°35’36.2"N 19°22’06.2"E, 250 m elevation, mean annual temperature is 9.7°C; the average amount of precipitation is 560 mm) (Dövényi et al. 2008, Szirmai et al. 2014) and the Eötvös Loránd University Botanical Garden Füvészkert (Budapest 47°29’05.6"N 19°05’05.7"E, 114 m elevation, mean annual temperature is 10.4°C; the average amount of precipitation is 514 mm) (Dövényi et al. 2008, Orlóci et al. 2019). Within a radius of 250 m around the two botanical gardens, the following local climate zones (LCZ) are present. In Budapest: LCZ 5 - Open mid-rise 60%, LCZ 6 - Open low rise 20% LCZ 2 - Compact mid-rise 20%. In Gödöllő: LCZ A - dense trees 40%, LCZ D - low plants 50%, LCZ 6 - Open low-rise 10% (Stewart & Oke 2012, http5). In the text, we refer to the experiment locations as Gödöllő and Budapest.

For the 34 species, 34 homogenous row shaped patches have been created within the two selected areas with 5 repetitions by species and by location.

Ex situ phenological experiment

We planted specimens representing 6 different life-forms of the Raunkiær system (phanerophytes, chamaephytes, hemicryptophytes, geophytes, hemitherophytes, therophytes). Each category was represented by at least 5 species (Table 1) and each species by 5–5 specimens in both locations.

The observation units were put in standard-sized flowerpots with a diameter of 27 cm for phanerophytes, and 14 cm for the rest, containing at least one specimen from the given species. We tried to maximize genetic conformity in each species. This was achieved by using clones in phanerophytes, obtained from the Hungarian University of Agriculture and Life Sciences Soroksári Botanical Garden. In the case of seed-sown species, the propagating material was collected from one specimen per species in the Gödöllő Botanical Garden and the Eötvös Loránd University Botanical Garden Füvészkert, while in the case of other species we obtained specimens propagated from the horticulture of Beretvás és Társai Kft. We collected and planted the specimens to the experimental patches during December of 2019.

To minimize external factors, we used a standard soil mix and we put the plants in the patches of similar characteristics and fol-

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Table 1. The species included in the experiment grouped by life-forms

Ph	Ch	He
Cornus sanguinea	*Dianthus plumarius*	*Euphorbia polychroma*
Prunus spinosa	*Sedum album*	*Ajuga reptans*
Ligustrum vulgare	*Vinca minor*	*Inula ensifolia*
Prunus fruticosa	*Thymus vulgaris*	*Sedum acre*
Cotinus coggygria	*Cerastium tomentosum*	*Briza media*
Prunus tenella	*Globularia cordiflora*	
Rosa spinosissima		
Ge	HT	Th
Iris pumila	*Daucus carota***	*Hibiscus trionum*
Polygonatum multiflorum	*Dipsacus pilosus***	*Solanum nigrum*
Convallaria majalis	*Dipsacus lacinatus***	*Silene latifolia*
*Galanthus nivalis**	*Capsella bursa-pastoris*	*Portulaca oleracea*
*Eranthis hyemalis**	*Malva sylvestris***	*Consolida regalis*
		Papaver rhoeas

* the species were introduced after the start of the growing season, therefore were not included in the record
** resulting from their life-form, flowering in the first year is not expected (Krumbiegel, 2008)

lowed the same protocol during their observation. In practice, this meant frequent weeding and regular irrigation during the summer months. Observations were realized weekly on the same day of the week from March to December of 2020, on both locations, collecting flowering phenological data. For the study of the flowering phenological data, we used Primack's (1985) method. The timing of the start, peak, and end of flowering was recorded in an Excel sheet. We recorded the end of flowering when there was no more flower on any of the specimens. The peak was the first day from all the dates when the specimen had the maximum number of flowers noticed. In case of solitary flower we recorded the number of flowers, while in case of *Briza media (He), Capsella bursa-pastoris (HT), Sedum album (Ch)* and *Thymus vulgaris (Ch)* we recorded the number of inflorescences for each observation unit. During the growing season specimens of *Cornus sanguiena (Ph), Ligustrum vulgare (Ph), Prunus tenella (Ph), Rosa spinosissima (Ph), Dianthus plumarius (Ch), Sedum album (Ch), Vinca minor (Ch), Thymus vulgaris (Ch), Cerastium tomentosum (Ch), Ajuga reptans (He), Inula ensifolia (He), Sedum acre (He), Briza media (He), Iris pumila (Ge), Polygonatum multiflorum (Ge), Capsella bursa-pastoris (HT), Hibiscus trionum (Th), Solanum nigrum (Th), Silene latifolia (Th), Portulaca oleracea (Th), Consolida regalis (Th), Papaver rhoeas (Th)* flowered. In case of *Portulaca oleracea (Th)* we could only infer the flowering from the fruiting because there was no flower detected at the times of observation. The flowering of the *Papaver rhoeas (Th)* could only be detected on one of the locations, on the other a short flowering period could be inferred from the buds and fruits. Therefore, these two species were excluded from the evaluation of the flowering data. On both observation locations only *Prunus tenella (Ph), Dianthus plumarius (Ch), Se-*

dum album (Ch), Vinca minor (Ch), Thymus vulgaris (Ch), Cerastium tomentosum (Ch), Ajuga reptans (He), Inula ensifolia (He), Sedum acre (He), Briza media (He), Iris pumila (Ge), Polygonatum multiflorum (Ge), Capsella bursa-pastoris (HT), Hibiscus trionum (Th), Solanum nigrum (Th), Silene latifolia (Th) flowered. All species – except *Iris pumila (Ge)* – produced enough flowers for statistical analysis of the data.

Statistical analysis
Figures were created with Sigma Plot 12.0. For statistical analysis, we used Windows Excel 2016, while we calculated the two-tailed t-test to show the difference between locations. The results shown in the figures are the averages and standard deviations for each species in both locations.

Results

From the 16 species flowering on both locations 12 species *Globularia cordiflolia (Ch)* (Figure 1), *Briza media (He)* (Figure 2), *Hibiscus trionum (Th)* (Figure 3), *Silene latifolia subsp. alba (Th), Inula ensifolia (He), Polygonatum multiflorum (Ge), Dianthus plumarius (Ch), Sedum album (Ch), Vinca minor (Ch), Thymus vulgaris (Ch), Cerastium tomentosum (Ch)* and *Capsella bursa-pastoris (HT)* confirmed our hypothesis, by flowering earlier in Budapest than in Gödöllő. From the listed species the average difference in the onset of flowering was 10.66 days. *Solanum nigrum (Th), Sedum acre (He)* started the flowering on the same week on both locations. *Prunus tenella (Ph), Ajuga reptans (He)* and *Iris pumila (Ge)* started to flower earlier in Gödöllő, contradicting our hypothesis. Species with the earlier onset of flowering happening in Gödöllő had an average difference of 2.2 days. All the species in the experiment averaged at 7.62 days earlier flowering onset in Budapest. The peak of the flowering had 12.94 days of difference, while the end of flowering had 2.9 days of difference, with the earlier being Budapest.

Globularia cordifolia (Ch), Inula ensifolia (He), Polygonatum multiflorum (Ge), Dianthus plumarius (Ch), Thymus vulgaris (Ch), Cerastium tomentosum (Ch) and *Capsella bursa-pastoris (HT)* had an earlier onset as well as an earlier end of flowering in Budapest compared to Gödöllőn. The flowering of *Sedum album (Ch)* had an earlier onset in Budapest and a simultaneous end on both locations. The flowering of *Hibiscus trionum (Th)* and *Vinca minor (Ch)* started earlier and ended later in Budapest. The flowering of *Ajuga reptans (He)* and *Iris pumila (Ge)* started later and ended later in Budapest. The flowering of *Prunus tenella (Ph)* started later in Budapest and ended at the same time. The flowering of *Solanum nigrum (Th)* and *Sedum album (Ch)* started in the same week and ended earlier in Budapest. The flowering of *Silene latifolia subsp. alba (Th)* started in the same week in both locations, but in contrast to the former two ended later in Budapest. The average duration of the flowering of all species was 4.72 days longer in Budapest compared to Gödöllő. Flowering duration of *Hibiscus trionum (Th), Silene latifolia subsp. alba (Th), Globularia cordifolia (Ch), Briza media (He), Sedum album (Ch), Vinca minor (Ch), Capsella bursa-pastoris (HT), Ajuga reptans (He)* and *Iris pumila (Ge)* was longer in Budapest, compared to Gödöllő.

According to the statistical analysis, there is a strong significant difference ($P < 0.001$) in the onset of the flowering of *Globularia cordifolia (Ch)* between the locations. *Polygonatum multiflorum (Ge), Cerastium tomentosum (Ch)* and *Capsella bursa-pastoris (HT)* showed a medium significance ($P < 0.01$). There was a still significant but weaker connection ($P < 0.05$) for the onset of flowering of *Thymus vulgaris (Ch)* between the two locations.

Regarding the peaks of flowering there was

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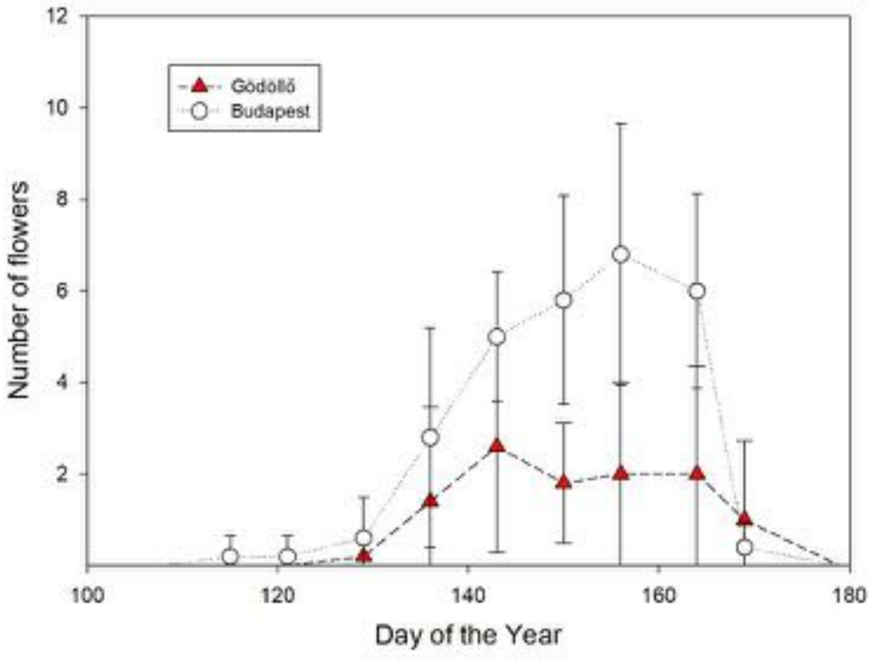

Figure 1. The flowering period and pattern of *Globularia cordifolia*.

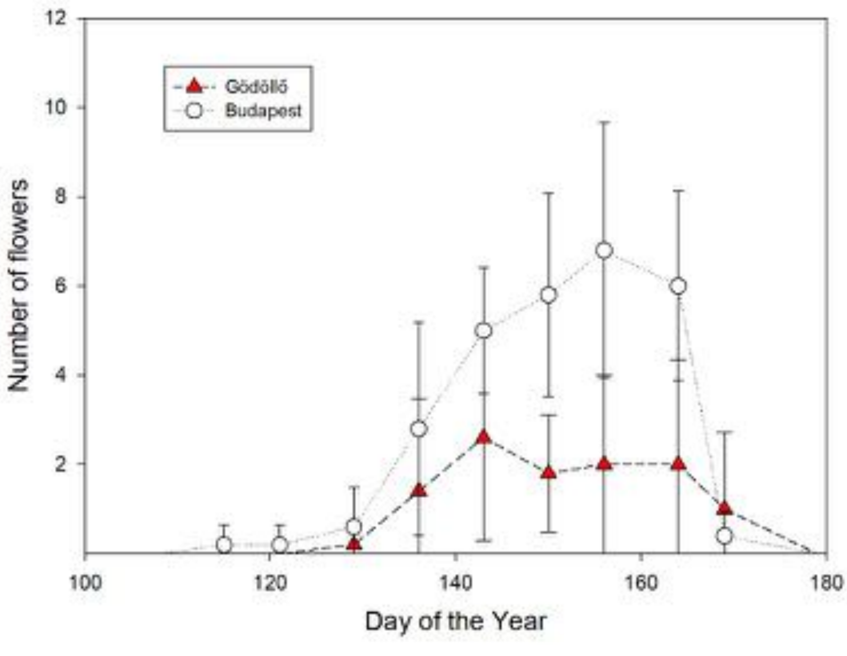

Figure 2. The flowering period and pattern of *Briza media*.

a strong significance ($P < 0.001$) for *Inula ensifolia (He)* medium significance ($P < 0.01$) for *Globularia cordifolia (Ch), Dianthus plumarius (Ch), Thymus vulgaris (Ch), Cerastium tomentosum (Ch), Capsella bursa-pastoris (HT)* and a weaker significance ($P < 0.05$) for *Polygonatum multiflorum (Ge)*.

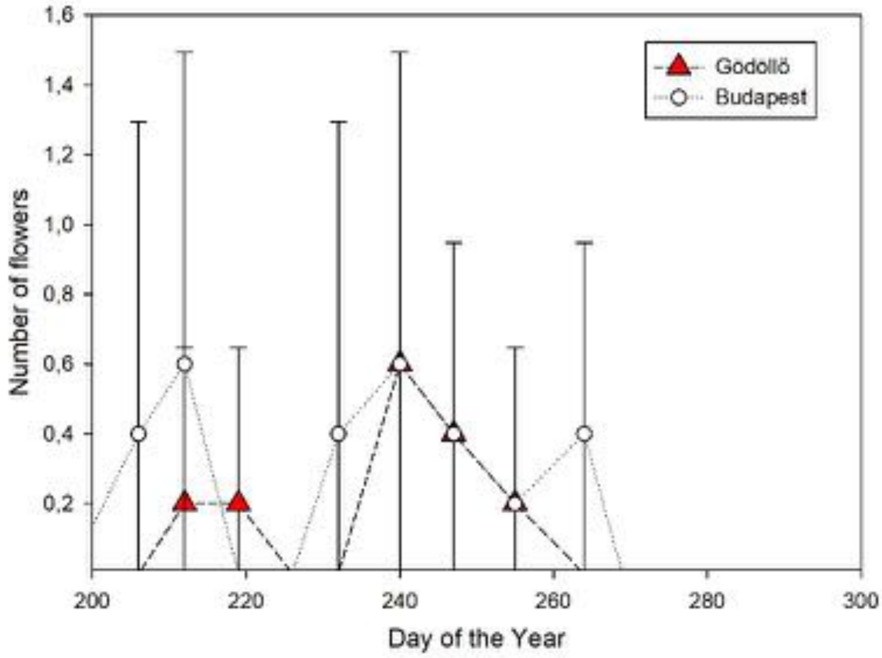

Figure 3. The flowering period and pattern of *Hibiscus trionum*.

Regarding the end of flowering there is a strong significance ($P < 0.001$) for *Polygonatum multiflorum (Ge)*, medium siginificance ($P < 0.01$) for *Globularia cordifolia (Ch), Thymus vulgaris (Ch)* and a weaker significance ($P < 0.05$) for *Sedum acre (He), Dianthus plumarius (Ch)* and *Cerastium tomentosum (Ch)*.

Discussion

Our results show that the onset and end of flowering occurred earlier in the capital city with a higher mean temperature than in the lower mean temperature rural area. However, the flowering period lasted longer in Budapest than the rural area with a 3°C lower mean temperature. Only a few studies (e.g., Roetzer et al. 2000, Ziska et al. 2003, Neil & Wu 2006, Jochner et al. 2012) focused on the difference in phenological patterns between urban and rural areas. Roetzer et al. (2000) analyzed data for four spring-blooming plants from 10 central European observation stations between 1951 and 1995. At almost all stations, the plants tended to bloom earlier in urban areas than in surrounding rural areas by about 2–4 days. Jochner et al. (2012) investigated the role of elevation and urbanization in the shift of flowering phases of tree species, and an advance of 2.6–7.6 days was observed between an entirely rural area and an entirely urban one. Roetzer et al. (2000) showed, that in European cities with strong urban climate effects e.g., Munich, Vienna and Hamburg, phenophases are beginning 3–16 days earlier. His results are in agreement with our results as the plants grown in Budapest have on average 7.62 days earlier onset of flowering compared to Gödöllő. This result could mean that downtown Budapest is strongly affected by the urban climate effect. Researchers attribute advanced flowering in urban environments to the Heat Island Effect (Neil & Wu 2006), which is clearly noticeable in our results as well.

Extension of growing periods in urban habitats has also been reported in the northern

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hemisphere (e.g., Zhang et al. 2004, Neil and Wu 2006) while possible protracted flowering periods within urban landscapes are poorly studied, especially in Europe. Davis et al. (2016) examined eucalyptus trees in streets in Sidney, Australia, and observed, that 3 tree species from the family Myrtaceae showed a significantly longer flowering period on the streets of the city than in their natural habitats. Our results are consistent with this study, as the species participating in the experiment had a 4.72 days longer flowering period in Budapest compared to Gödöllő.

Compared to the onset of flowering there are significantly fewer studies regarding the end of flowering and the possible advancement of it. Masetti et al. (2015) studied *Tilia* × *europea* in Florence, Italy, and concluded that both the start and end of flowering advanced by 1.4 days in urban areas. In our experiment *Globularia cordifolia (Ch), Inula ensifolia (He), Polygonatum multiflorum (Ge), Dianthus plumarius (Ch), Thymus vulgaris (Ch), Cerastium tomentosum (Ch)*, and *Capsella bursa-pastoris (HT)* started to flower earlier in Budapest compared to Gödöllő. All the species in the experiment had an average of 2.9 days advancement at the end of flowering in Budapest compared to Gödöllő, which is in agreement with the observation of Masetti et al. (2015).

Nail and Wu (2006) observed the phenomenon that early spring bloomers in these environments tend to be more sensitive than mid- or late-spring bloomers. For the future investigation of this conclusion, we will introduce 2 early spring bloomers *Eranthis hyemalis (Ge)* and *Galantus nivalis (Ge)* from 2021.

Is there a significant difference between the years? Exactly with which abiotic parameters do the flowering phenology correllate? These are questions to be addressed in future studies.

Based on our result, not all of these questions can be answered. Peñuelas and Filella (2001) showed that the advancements of phenological events were significantly correlated, with temperature increase over the 30 years before then, however, the significance of other factors is unclear. According to Rathcke and Lacey (1985) and Neil and Wu (2006) photoperiod, temperature, and soil moisture have been recognized as the main environmental triggers for leafing and flowering. Zhang et al. (2004) claim that it is the interaction between temperature and photoperiod that is responsible for initiating flowering Szabó et al. (2016) claim that the flowering phenophase shows the strongest correlation with the average air temperature of a few months preceding the event, while Eppich et al. (2009) came to the conclusion that the average of the daily temperature fluctuation in the given period and the number of frosty days are the most important triggers for flowering phenology. To clarify the driving forces of flowering phenology patterns we installed micrometeorological equipment to continuously measure abiotic parameters on both locations.

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Seasonal variation of antioxidant enzymatic responses in the desiccation-tolerant bryophyte *Syntrichia ruralis* (Hedw.) Web. & Mohr.

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Abstract: Bryophytes are poikilohydric organisms that can be used as model plants to study desiccation tolerance mechanisms. The main objective of this study was to examine the activities of the antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT) and guaiacol peroxidase (POD) in the rehydrated and desiccated states in *Syntrichia ruralis* (Hedw.) Web. & Mohr. from two slopes, one North-east (NE) and one South-west (SW) facing and collected in different seasons. Our results showed seasonal variation in the enzymatic activities of APX, CAT and POD between the slopes in both the rehydrated and desiccated states. The mean value of all the activities of APX, CAT and POD and MDA contents (a measure of lipid peroxidation) tended to be higher in moss cushions collected from the NE compared to the SW facing slopes except in summer season. The mean values of all enzymatic activities were higher in desiccated states as compared with rehydrated states. Protein content has lower values in summer and winter season. Differences in the antioxidant activities of the mosses growing on the two slopes may reflect adaptations to desiccation stress.

Keywords: Poikilohydric, catalase, ascorbate peroxidase, guaiacol peroxidase, lipid peroxidation

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Introduction

The soil surface in arid areas is frequently covered by cryptobiotic crusts (CBCs) comprising communities. These formations were previously often referred to as algal crusts (Komáromi 1979; 1980; 1983), microbiotic soil crusts (Eldridge and Greene 1994), biological soil crusts (Evans and Johansen 1999, Belnap et al. 2001), or cryptogamic crusts (Strandling et al. 2002). Nowadays, however, the "cryptobiotic crust, CBC" has become generally accepted (Pócs 2006. 2008). Arid and semi-arid areas are subjected to frequent drought, and as a result are often rich in desiccation-tolerant species. These species make a significant contribution to grassland diversity and facilitate fundamental ecosystem functioning such as carbon storage in nutrient-poor environments, increase water retention capacity and interacting with vascular plants by seedling establishment (Lindo and Gonzalez, 2010). Furthermore, bryophytes in grasslands could be used as indicators to track nutrient fluxes, pollutants, or climate change in grasslands (Müller et al. 2012). However, global climate change is affecting CBCs, and therefore their species composition may be affected (Rodriguez-Caballero et al. 2018). Therefore, investigations to study the seasonal variations on desiccation tolerant species can provide important information related to the aspects of desertification. Some poikilohydric mosses (desiccation tolerants) can survive during dry conditions and can fully recover on rehydration (Alpert and Oliver 2002). In the Hungarian steppe, desiccation tolerant mosses such as *Syntrichia ruralis* (Hedw.) Web. & Mohr. have been reported to be abundant between the scattered tufts of dominant grasses *Festucetum vaginatae danubiale* association (Csintalan et al. 2000). More generally, *S. ruralis* has a worldwide distribution, and is an important component of many biological soil crusts (Belnap et al. 2016).

Drought and heat are two of the main stresses that limit the survival of moss biocrusts in arid areas (Chongfeng et al. 2017). These stresses increase reactive oxygen species (ROS) production inside the plant cell (Cruz de Carvalho et al. 2012). ROS include superoxide radicals (O^{2-}), hydroxyl radicals (OH·), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). They can oxidize cellular components such as lipids (membranes), proteins (enzymes), DNA and RNA, which can eventually lead to cell death (Leprince et al. 2000; Mittler 2002; Cruz de Carvalho 2008). Plants, including mosses, have an antioxidant system for the detoxification of excess ROS using enzymatic and non-enzymatic antioxidants. Enzymes that remove ROS include catalase (CAT), superoxide dismutase (SOD) and guaiacol peroxidase (POD) while non-enzymatic antioxidants include glutathione (GSH), vitamin C, and carotenoids. Furthermore, some enzymes regenerate oxidized ROS scavenging molecules such as glutathione reductase (GR) (Zhang et al. 2017). Stress has often been shown to increase the activity of these enzymes, for example SOD activity and catalase in drought tolerant *Syntrichia ruralis* reached maximum levels after slow drying for 5h (Dhindsa and Mattowe 1981) and SOD activity was greatly increased during desiccation in the moss *Atrichum androgynum* (Mayaba and Beckett 2003). Similarly, Onele et al. (2018) showed that slow drying induced POD in *Dicranum scoparium*.

Stress tolerance varies greatly between bryophytes, but species growing on open exposed dry habitats, rock surfaces can tolerate fast, prolonged desiccation and frequent dry and wet cycles (Bewley, 1972; Proctor et al. 2007a). The desiccation tolerant moss *S. ruralis* has been used as an experimental model plant to understand how plants respond to environmental stress (Oliver et al. 2000b; Dinakar et al. 2012). It has both constitutive and inducible mechanisms that can reduce and repair cellular damage and enable it to regain its normal metabolism within minutes on rehydration (Péli et al. 2005). Studies have been carried out at the molecular level on *S. ruralis* (Scott and Oliver 1994; Wood and Oliver, 1999; Zeng et al. 2002; Oliver et al. 2004) and also at the physiological level (Tuba et al. 1996; Csintalan et al. 1999, 2000; Kalapos and Mázsa 2001; Proctor et al. 2007; Barón et al. 2009). However, further research is needed to clarify and understand the metabolism at the enzymatic level and to study the mechanism behind the species distribution in open grasslands. Therefore, activities of some antioxidant enzymes were examined in this study and could be helpful in finding the role of these enzymes in bryophytes. The main objective of this study was to observe the seasonal variation in the antioxidant enzymes CAT, APX, POD along with protein and MDA contents during rehydration and desiccation in *S. ruralis* to understand the response of these antioxidant enzymes to stress. We hypothesized that there can be seasonal variation in antioxidant enzymatic activities under different degrees of environmental stresses such as drought, heat, and variation in extreme temperatures.

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Materials and Methods

Plant Material

Syntrichia ruralis (Hedw.) Web. & Mohr (synonym: *Tortula ruralis*) (Pottiaceae) is also known as sandhill screw moss. This species grows as extensive mats on open exposed areas of sandy dunes in semi-arid grassland and plays an important role in CSCs by binding sand particles. Moss cushions of *S. ruralis* were collected in an air-dried state from semi-arid sandy grassland near Bócsa-Bugac in the Kiskunság region (central Hungary 46° 53' 29" N, 19° 26' 35.6" E) in late winter (March 2018), spring (May 2018), summer (July 2018) and autumn (October 2018). Samples were selected from two different slopes, a north-east (NE) and a south-west (SW). Average annual temperature and average annual precipitation along with monthly changes in meteorological parameters such as photosynthetically active radiation, temperature, precipitation, and relative humidity were also recorded during investigated year (2018) at the sample site. These climatic conditions were presented in Ruchika et al. 2020.

Experimental set-up

Air-dried field samples were transported back to the laboratory in paper envelopes and kept at room temperature for two days in opened paper envelopes. Later, samples were cleaned by removing sand particles. For the rehydration treatment, five replicates of moss cushions were transferred on wet filter paper trays placed in transparent plastic boxes partially filled with water. They were sprayed with distilled water, and maintained constantly hydrated for 72 h. For the desiccation treatment, samples were slowly desiccated by placing them in petri dishes for 48 h. Samples (0.3 g) were divided in two different treatments: rehydrated (Rehy) and desiccated (Desic) to determine the activities of antioxidant enzymes and the protein content. 0.2 g was used to determine lipid peroxidation products (MDA content). A similar experiment set-up was followed in each season. Rehydrated and desiccated shoot tips of *S. ruralis* are shown in Figure 1 (A and B, respectively).

Water content (WC%) were measured and calculated by using the fresh (FW) and oven-dried (DW) weight of the samples after small intervals of rehydration (2h, 6h, 12h, 24 h, 72 h) and drying out at 80 °C, respectively; $WC = [(FW - DW) / DW] \times 100$ (Péli et al. 2011). Figure 2 shows changes in water content during rehydration-dehydration cycle.

Extraction of plant material and antioxidant enzyme assays

Moss shoots (0.3 g) from both slopes and in rehydrated and desiccated states were ground to a fine powder in liquid nitrogen and homogenized in 2 mL of potassium phosphate extraction buffer (125 mM, pH =7.8) using a pre-chilled mortar and pestle. The extract was centrifuged at 4 °C for 10 min at $15000 \times g$ (RCF) in a cooling centrifuge. The supernatant was used to determine the assay of catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POD) according to (Dazy et al. 2009) with some modifications. The molar extinction coefficient (ε) was used to calculate the enzymatic activities and expressed as Units mg^{-1} protein content.

Ascorbate peroxidase (APX, EC 1.11.1.11)

The APX reaction mixture consisted of 125 mM potassium phosphate buffer (pH= 7.0), 5 mM Na-ascorbate, 1 mM Na_2-EDTA, 100 mM H_2O_2 and 0.1 mL plant enzyme extract. The decrease of ascorbate concentrations was measured for 100 sec at 25 °C ($\varepsilon_{290} = 2.8\ mM^{-1}cm^{-1}$).

Catalase (CAT, EC 1.11.1.6)

Catalase activity was determined by measuring the decrease in the H_2O_2 concentration. The CAT reaction mixture (1 mL) contained

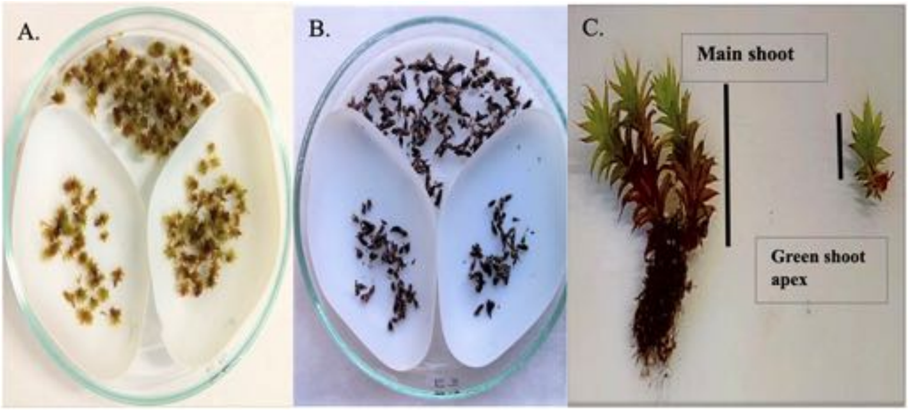

Figure 1. Shoots of the moss *Syntrichia ruralis* in the (A.) rehydrated state and (B.) desiccated state (slow drying) and two parts of the green shoot (C.).

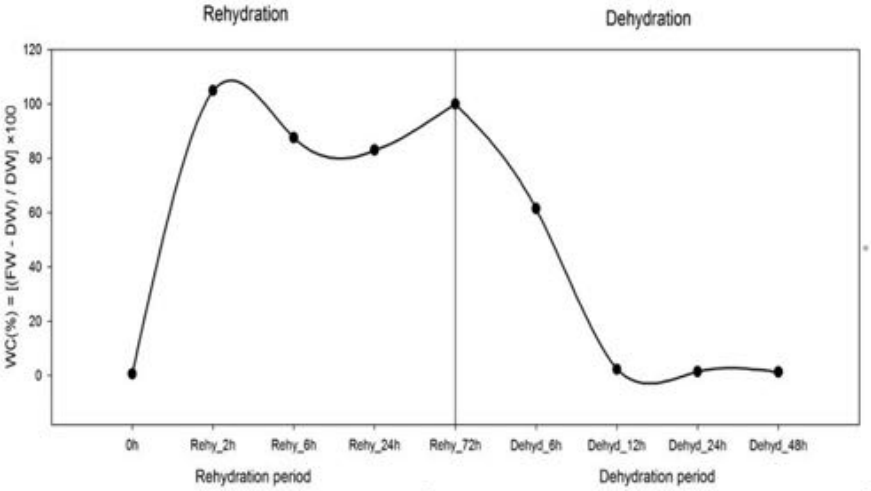

Figure 2. Graphical representation of water content percentage (WC%) during rehydration period (left) and dehydration period (right).

125 mM potassium phosphate buffer (pH= 7.0), 100 mM H_2O_2, and 0.1 mL plant enzyme extract. The decrease in the H_2O_2 concentration in a reaction mixture was measured for 340 sec at 25 °C (ε_{240} = 36.6 $mM^{-1}cm^{-1}$).

Guaiacol peroxidase (POD, EC 1.11.1.7)

The POD reaction mixture (1 mL) contained 125 mM potassium phosphate buffer (pH= 7.0), 34 mM guaiacol, 100 mM H_2O_2, and 0.1 mL plant enzyme extract. The increase in tetra guaiacol concentration in a reaction mixture was measured for 150 sec, 25 °C (ε_{470} = 26.6 $mM^{-1}cm^{-1}$).

Protein determination

The concentration of protein was determined according to the (Bradford,1976) with some modification. Coomassie blue dye-binding assay was used for the quantification of soluble protein content. Bovine serum albumin (BSA) was used for the preparation of the standard curve. Enzyme extracts of samples from both slopes were measured spectrophotometrically at 595 nm. Protein content were

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calculated later using the standard curve and expressed in mg.

Lipid peroxidation

Lipid peroxidation was measured as the MDA content determined by the thiobarbituric acid (TBA) reaction according to Heath and Packer (1968) with some modifications. Moss shoots (0.2 g) were homogenized in 2 mL of 0.1% TCA extraction buffer under cold conditions. The suspension was centrifuged at 15000 × g (RCF) for 10 min at 4 °C and supernatant was collected. Samples consisted of 200 μL of the supernatant, to which 1800 μL of TCA (20%) containing TBA (0.5%) buffer was added. The assay mixture was heated at 95 °C for 30 min. The content was cooled to stop the reaction for 5-10 min on ice and re-centrifuged at 10000 × g (RCF) for 10 min at 4 °C. Each sample from the slopes comprised of five replicates. MDA concentration (mM) was calculated as (A_{532} – A_{600} /155) expressed as nmol g^{-1} dry weight.

Statistical analysis

All the variables were tested for normality and equal variance using the Shapiro-Wilk test and Levene's test, respectively. ANOVA post-hoc (Tukey's test) was performed on the experimental data comparing the different antioxidant enzymatic activity between the north-east and south-west slopes with respect to different seasons in rehydrated and dehydrated states. Differences are significant at a level ($p \leq 0.05$). Statistical analyses were performed using the statistical software R programming language version 3.5.3 for Windows (R development Core Team, Auckland, New Zealand).

Results

Activity of APX, CAT, POD in mosses collected from the NE and SW slopes in the rehydrated and desiccated states

Antioxidant enzymatic activity results were represented in two different states, i.e., rehydrated (rehy) and desiccated (desic) between north-east (NE) and south-west (SW) slopes with respect to different period of collection (Figure 3). The activities of APX, CAT and POD observed to be higher in material from the NE while compared to the SW facing slopes in all seasons except opposite trend was seen in summer season. All the activities tended to be higher in desiccated states than in rehydrated material for both slopes. All the activities were followed similar trend upon rehydration and desiccation, these activities increased first from spring to summer season and then declined in autumn season. Again, it was increased in the colder winter season. In both rehydrated and desiccated states, all activities were higher in summer and winter season and lower in spring and autumn. APX (Figure A-B) and POD (Figure E-F) activities were showed variations throughout the year in both rehydrated and desiccated states between both NE and SW slopes. CAT activities did not vary much throughout the year in both rehydrated and desiccated states for the material from the NE and SW facing slopes (Figure C-D).

Variation in protein determination (protein content) between the slopes (NE and SW) in seasons in the rehydrated and desiccated states

Protein content were represented in two different states, i.e., rehydrated (rehy) and desiccated (desic) between north-east (NE) and south-west (SW) slopes with respect to different period of collection (Figure 4A-D). On rehydration, the protein content was observed increased and desiccation resulted in a decrease level of the protein synthesis in all seasons in both NE and SW slopes. Overall, in spring and autumn season, protein content was found increased whereas in summer and winter season it become decreased. Based on slope-wise, protein content was not sig-

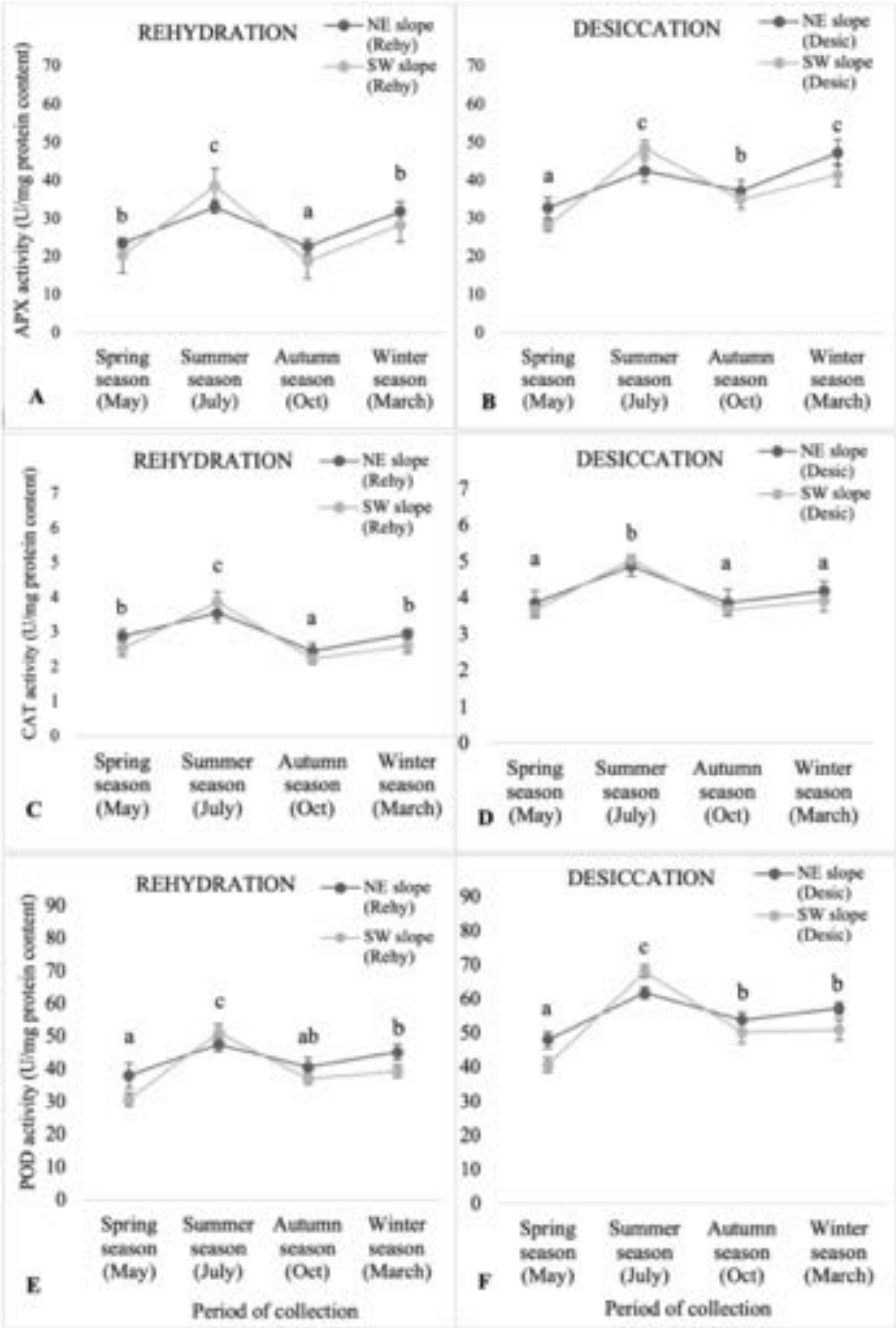

Figure 3. Effect of activity of antioxidant enzymes in *S. ruralis*: (A-B) APX ; (C-D) CAT; (E-F) POD in rehydrated (Rehy) and desiccated (Desic) states between north-east (NE) and south-west (SW) slopes with respect to different period of collection (Spring, Summer, Autumn, Winter season). The mean values ($n = 5$) $\pm$ SD with different alphabetical letters is significantly different at $p \leq 0.05$ using ANOVA post-hoc (Tukey's test).

nificantly different in rehydrated states ($p \geq 0.05$) while significant different in desiccated states. Based on season-wise, protein content was significantly different ($p \leq 0.05$).

Variation in lipid peroxidation (MDA content) between the slopes (NE and SW) in seasons in the rehydrated and desiccated states

MDA content differed significantly between

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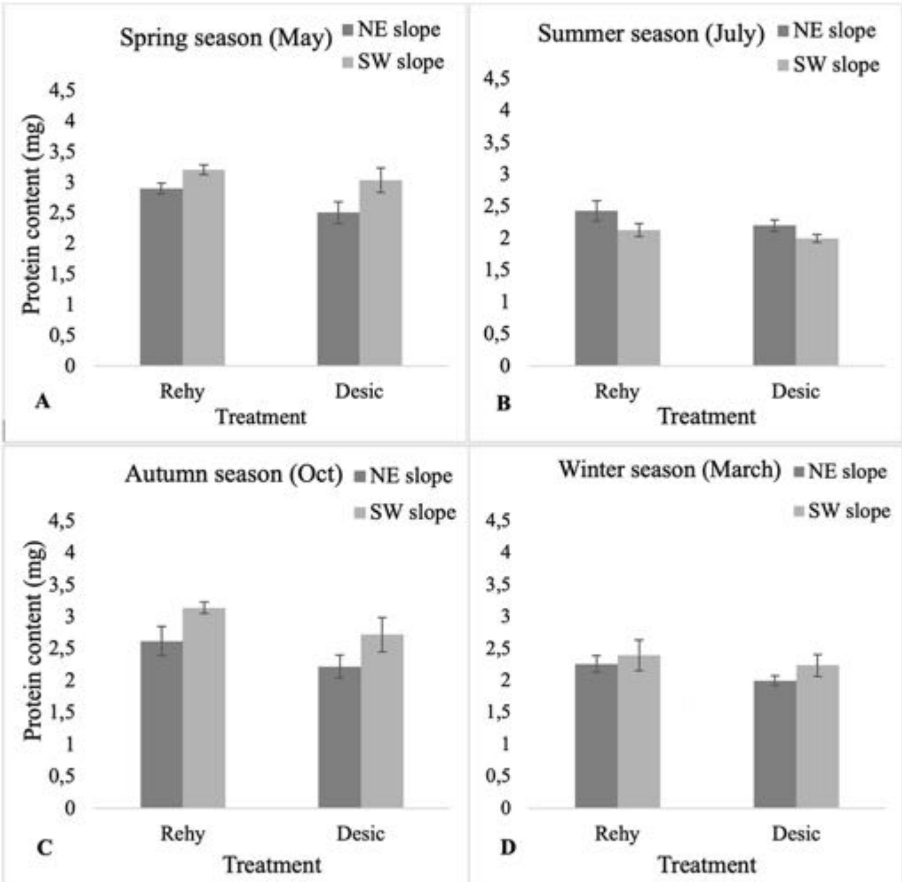

Figure 4. Protein content in *S. ruralis* (A-D) in rehydrated (Rehy) and desiccated (desic) states between north-east (NE) and south-west (SW) slope with respect to different period of collection (Spring, Summer, Autumn, Winter season). The mean values ($n = 5$) $\pm$ SD are represented using ANOVA.

each season in rehydrated states and desiccated states. It was not significantly different between the slopes ($p \geq 0.05$) although there was significant difference between seasons ($p \leq 0.05$). The concentration of the oxidized lipid MDA tended to be higher in desiccated material than rehydrated material (Figure 5A-B). In all seasons, MDA content was found higher in NE slope except summer season as compared to SW slope.

Discussion

In this present study, the activities of the antioxidative enzymes APX, CAT and POD were compared between the mosses growing on NE and SW slopes of semi-arid sandy grassland collected at different period of the year. Our results showed that mosses growing on the NE slope have higher enzymatic activities in both the rehydrated and desiccated states as compared to the SW slope except in summer season. It seems likely that the differences in the enzyme activities in the mosses growing on the two slopes are a consequence of the more stressful conditions on the NE facing slopes. Conditions on the SW slope are more optimal (e.g., favourable light conditions, better availability of water) for moss growth. This is suggested by a recent study on the photosynthetic efficiencies of mosses sampled from the two slopes

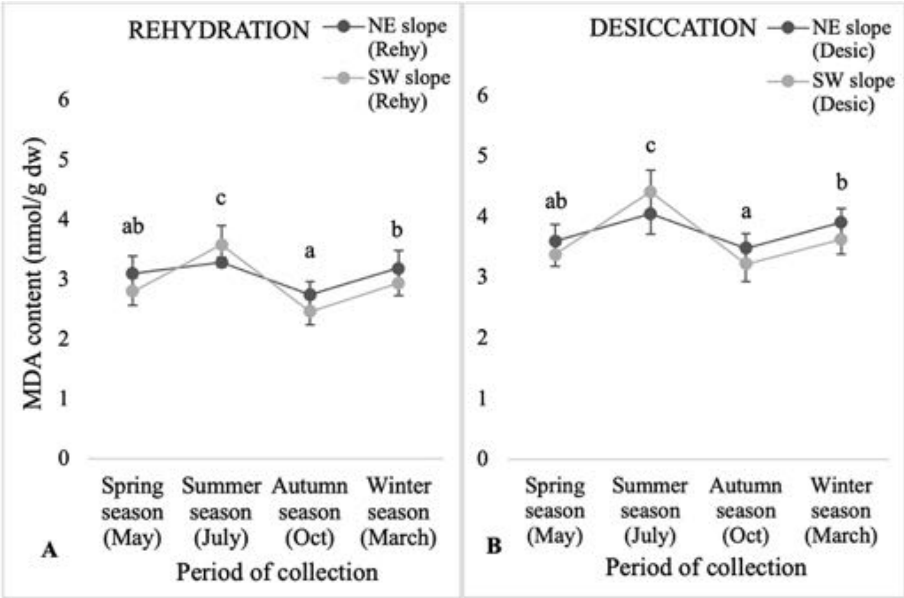

Figure 5. MDA content in *S. ruralis* in rehydrated (Rehy) and desiccated (desic) states between north-east (NE) and south-west (SW) slope with respect to different period of collection (Spring, Summer, Autumn, Winter season). The mean values ($n = 5$) $\pm$ SD with different alphabetical letters is significantly different at $p \leq 0.05$ using ANOVA post-hoc (Tukey's test).

(Ruchika et al. 2020). Similarly, higher activities of antioxidative enzymes suggests than mosses growing on the NE slope might be experiencing greater stress. In summer and winter season, qN and NPQ values were reported higher that may indicate the stressful environmental conditions (high light exposure and temperature variations) in these two seasons. In this study, it also showed higher activities in summer and winter season.

In plants, the production of antioxidant enzymes is one of the strategies to defend themselves from ROS injury during desiccation (Seel et al. 1991; Oliver and Bewley 1997). These mosses were collected from exposed areas in semi-arid sandy grassland that showed increased antioxidant enzymatic activities. However, similar results were also reported in the moss *S. caninervis* Mitt. collected from exposed areas that showed the highest antioxidant enzyme activity (Yin and Zang 2016).

Ascorbate peroxidase (APX) enzyme plays a key role in eliminating H_2O_2 and therefore it is an important component of the antioxidant system (Najami et al. 2008). APX activity was lower following rehydration (Figure 3A), presumably because of the reduction in oxidative stress. Bansal and Srivastava (2017) also reported a reduction in APX activities during rehydration in the moss *Brachythecium procumbens*. Catalase enzyme (CAT) is an important antioxidant enzyme that breaks down H_2O_2 to form water and oxygen (Zhang et al. 2017). In rehydrated mosses, CAT activities were observed similar in NE and SW slope (Figure 3C). Results are generally consistent with studies on other mosses that have found that CAT activity does not vary greatly during wetting /drying cycles, suggesting that CAT is probably a largely constitutive defence against oxidative stress (Mayaba and Beckett 2003). Guaiacol peroxidase (POD) activity, which will also remove H_2O_2, increased during slow desiccation in all moss

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samples as compared to rehydrated states (Figure 3F). Similar increases in POD activity have been observed during desiccation in *Brachythecium velutinum* (Paciolla and Tommasi 2003), *B. procumbens* (Bansal and Srivastava 2017), *Octoblepharum albidum* (Lubaina et al. 2013) and *Dicanum scoparium* (Onele et al. 2018).

During dehydration, plants deal with the water-deficit condition which causes lower water potential and declines the primary metabolism in bryophytes (Dinaker et al. 2002). In the desiccated state, accumulation of ROS increases the damage to proteins and lipids in the chloroplast also in mitochondria, peroxisomes, and plasma membrane (Scheibe and Beck 2011). However, there is a down-regulation of the synthesis of proteins during drying conditions (Cruz de Carvalho et al. 2014). Similarly, in this present study, results observed lower protein values during desiccation (Figure 4 A-D) which may indicate the damage of proteins. Higher values in antioxidant enzymatic activities might be indicated higher water deficit condition and imbalance of ROS production in NE slope. In the previous report, protein synthesis induced during rehydration (Oliver et al. 2004). Similarly, it may indicate the higher protein content values in the rehydrated state in both NE and SW slopes.

Lipid peroxidation (MDA content) is used as to indicate the degree of oxidative damage in plants (Liu et al. 2013). Increased stress is probably the reason for the higher MDA levels in mosses growing on the NE slope compared with those growing on the SW slope. Similar results were observed that MDA content increased during desiccation while comparing with rehydration (Figure 5). The lower level of lipid peroxidation in moss shoots suggests that this moss might be better protected from oxidative damage during rehydration. However, in contrast, Zhang et al. (2017) reported that in species *Bryum argenteum* Hedw. and *Barbula fallax*, Hedw. MDA content increased first within 24 h and then declined at 48 h and 72 h later stages of desiccation stress. It seems likely that measuring MDA alone may give a rather poor indicator of oxidative stress in tissues and as suggested by De Dios Alché (2019), other molecules such as 4-hydroxy-nonenal (HNE) may be a more sensitive indicator of oxidative stress. Future studies on desiccation-induced changes in lipids in mosses should probably use indicator molecules other than MDA.

In the present study, the activities for all enzymes (APX, CAT, POD) tended to be lower in the rehydrated compared to the desiccated state. Previous studies in *S. ruralis* (Oliver 1991; Oliver and Bewley 1997; Oliver et al. 1998), and *A. viticulosus* and *R. lanuginosum* (Proctor and Smirnoff 2000) indicated the importance of constitutive protection with an induced repair mechanism upon rehydration. It appears that the H_2O_2 scavenging antioxidant enzymes form part of the inducible mechanism. During rehydration, processes such as photosynthesis, respiration and protein synthesis return to normal and suggesting recovery from stress (Oliver 1991; Cruz de Carvalho et al. 2011, 2014).

Although more frequent sampling occasions would have been desirable, our results also suggested that the antioxidant enzymatic activities might affected by increasing temperatures from spring to summer season (April to July 2018) and by declining temperatures from autumn to winter season (October to late March 2018). In summer and winter season, enzymatic activities differed greatly between collections and treatments, which indicated that anti-oxidative systems may be performed an important role in balancing the production of free radicals and adjust level of protective enzymes to provide protection in extreme environment. In the present study, collections were made on representative days of each of the four seasons, in an attempt to obtain an overview of how the activities of

the enzymes vary throughout the year.
In this present study, we investigated the activities of the antioxidant enzymes APX, CAT, POD with protein determination and MDA content in the desiccation-tolerant moss *S. ruralis*, comparing material collected from the NE and SW slopes in different seasons. Our results showed significant seasonal variations in antioxidant enzymatic activities in the rehydrated and desiccated states for the slopes. In general, higher activities of the antioxidant enzymatic activities were found in mosses collected from the NE slope. In both states, the highest activities occurred in mosses collected during summer and winter season and the lowest activities were found during the spring and autumn season. Besides seasonal differences in the activities of the antioxidant enzymes, the small spatial-scale exposures i.e., the NE and SW slope orientation also can modify the expression of these enzymes. The role of some antioxidant enzyme in desiccation tolerance may be different, basically depending on the actual metabolic balance of mosses. Their activity is influenced not only by water conditions but also by other environmental factors (e.g., exposures, light, and soil conditions) which need to be further investigated in the future.

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Investigation of the invasive plant infestation of the railway line between Gödöllő and Hatvan

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Abstract: Railway systems are considered as a special environment and they can play a key role in the spread of the invasive plant species. In Hungary, there was no survey that specifically examined the importance of these linear facilities before. Our survey aims to examine the condition of the Gödöllő-Hatvan railroad line in terms of the levels of invasive plant-infection and what can be the role of the railway itself in it. The data were collected manually by walking along the entire examined railway track section, where the invasive species were recorded within the 10 meter range of the outer axis of the open railway track. The latter was divided into 30 one-kilometer long sections and 120 two hundred and fifty meters long subsections. The exact position of each invasive species was recorded in these (sub)sections. The surveyed area was very diverse in habitat types. In the present study, the spatial distribution of the most common species is also presented in the studied trajectory section, highlighting the most infected areas. Contrary to our preliminary idea, the latter did not always occur in the immediate vicinity of the settlements. The survey did not demonstrate that this railway line promotes the spread of all occurring invasive species probably due to the very diverse habitats but for a general conclusion the study should be extended both in space and time.
Keywords: Invasive plants, railway main line, invasive plant-infection, abundance, spatial pattern
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Introduction

Invasive species are causing serious problems today in Hungary as well as nearly in the whole world. There are ecosystems without invasive species infections. Invasive species tend to make negative impacts on the environment even if it is artificial or natural (or semi-natural). These species can make an effect in the nature conservation and agricultural areas, and can be a threat for human health as well (for example *Ambrosia* & *Solidago* species) (Csiszár et al. 2012). Most endangered areas are the islands, in an ecological sense too, and the areas being some way geographically and ecologically separated ecosystems, because they have low if any resistance against newly introduced species. It is hard to find an exact definition for invasive species because almost all nations/governments have their own, but it is agreed by most definitions that invasive species are 1) non-native to the given ecosystem, and 2) their introduction on the new ecosystems cause negative effects. The EU Regulation 1143/2014 on Invasive Alien Species defines it as “Invasive Alien Species (IAS) are animals and plants that are introduced accidentally or deliberately into a natural environment where they cannot be normally found, with serious negative consequences for their new environment.” In the original document this definition is even more specified with several points by which these species are defined further.

There are approximately 12000 non native plant species throughout Europe, and 10–15 percent of it can be recognized as invasive. In Hungary, more than half of the natural and semi-natural habitats are endangered by invasive species. Most sensitive areas are open sand steppes, floodplains and aquatic habitats (Varga et al. 2016). Besides the destruction of the habitats their fragmentation contributes significantly to the declining species diversity. Ecological and green corridors are elements of the environment and landscape which connects the fragmented habitats, thereby counteracting the fragmentation effect. These corridors' connecting effect can not only be positive, it also can be negative since it can assist the spreading of the harmful species. Corridors can be the natural or semi-natural watercourses, wave fields, roadside grassy areas, forest strips and man-made habitats like roads and railroads. (Bartha et al. 2004).

The specific edaphic & microclimatic conditions of the railway embankment, the herbicide treatments and the antropochoric nature of the propagules together determine the species compositions of the railway tracks. The railway tracks are special habitats, their structure is special: the roof of the substructure functions as a watertight layer, above it the crushed stone bed and the soles in it form the superstructure (Dancza et al. 2002, Bartha et al. 2004). The water management of the track bodies is extreme, they dry out regularly. Weeding of railway tracks is not just an aesthetic problem. Accumulating dry plant parts or other oily or nitrogen-rich wastes clog the bed, thus altering the drainage capacity, but the roots of the weeds can even damage the structure of the substructure (Sargent et al. 1984, Bartha et al. 2004).

Open track bodies are generally weed-free as a result of regular herbicide treatments. Giant and Canadian goldenrod (*Solidago gigantea*, *Solidago canadensis*), Bohemian knotweed (*Fallopia* ± *bohemica*), Boxelder maple (*Acer negundo*), Tree of heaven (*Ailanthus altissima*) and the False indigo bush (*Amorpha fruticosa*) are common on the slopes accompanying the railway sections (Bartha et al. 2004). A different herbicide treatment is used on the railway stations than on open road sections. The dominance of annual species can be observed at the stations. Most typical species are Bindii (*Tribulus terrestris*) and Common sandbur (*Cenchrus incertus*) (Dancza et al. 2002).

Linear facilities, including railways and their possible connection to the spread of alien species have already attracted the attention of several researchers. Some theories suggest that the Turkish wartycabbage (*Bunias orientalis*) in Britain found its way from England to Scotland among the rubble used for railway embankments (Sargent 1984). Altay et al. (2015) carried out their research in Turkey along railroads, that aimed to investigate the occurence of plant species in these areas. 20 non-native species were recorded of which three tree species (Boxelder maple, Tree of heaven, Black locust) are also registered as invasive in Hungary. There are proofs that urbanization has a positive effect on the settlement of these species, they have more occurence data at populated areas and stations (Altay et al. 2015). Rutkovska et al. (2013) in Daugavpils, Latvia, examined the importance of railways in the distribution of alien species, but not found close correlation, except for three species (*Dracocephalum thymiflorum, Erysimum durum, Lappula squarrosa*), which settled exclusively along railways (Rutkovska et al. 2013). Hansen & Anthony (2005) observed additional effects in a different way. It has been proved by transective studies that in forest vegetation the frequency of non-native tree species decreases with increasing distance from the railway, while that of herbaceous species does not show any perceptable change in the grasslands. The same effect along roads was studied by Flory & Clay (2005) for shrubs in the decidious forests of Indiana, USA. Christen & Matlack (2009) also investigated the role of roads in the spread of invasive species in South-East

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Ohio, U.S., and found that more invasive plant species occur along roads than in surrounding areas (Flory & Clay 2005, Hansen & Anthony 2005, Christen & Matlack 2009).

Important to highlight, for example, that *Robinia pseudoacacia* is one of the first trees from North America that has been imported to Europe (e.g. Ernyey, 1927; Kolbek et al., 2004; Vadas, 1914), as nowadays it means a serious threat to nature conservation and has a very high negative environmental impact (Kumschick et al., 2015). This species also very important economically, as it is a fast growing tree and considered to give very good quality timber, firewood, leaf forage and nectar. It also has very good use for erosion control, amelioration and as a shade giving and nurse tree (e.g. Gröhe, 1952; Kasper-Szel et al., 2003; Keresztesi, 1988; Papanastasis et al., 1998; Rahmonov, 2009; Rédei et al., 2008; Yüsek, 2012). Forman and McDonald (2007) and Mortensen et al. (2009) found that railway provide good habitat for invasive plant species for dispersal, as vehicles, wildlife and wind can move propagules along roads which can increase the range of invasive species (Forman and McDonald 2007, Mortensen et al. 2009).

To examine the importance of surrounding landscape, we highlighted *Asclepias syriaca, Robinia pseudoacacia, Solidago canadensis*. For *Asclepias syriaca* linear infrastructure elements can be an important habitat (Knight et al., 2019), especially on sandy soils (Bagi, 1999). *Robinia pseudoacacia* is a widespread invasive species throughout Hungary that causes many conservation problems (Kumschick et al., 2015). *Solidago canadensis* can be found in very dry locations and also waterlogged ones (Milo, 1993), in the vicinity of settlements (Botta-Dukát et al., 2008).

The aim of our research was the comprehensive survey of the invasive plant infestation of the railway section between Gödöllő and Hatvan. This area is currently under renovation, so this pre-renovation data collection will provide an opportunity to establish a long term study as well as future monitoring.

Our objectives include to 1) survey of the invasive plant-infestation of the abovementioned railway track section, 2) evaluation of the spatial pattern and frequency of invasive plant species occurring on the Gödöllő-Hatvan section, 3) making proposals for the treatment of invasive plant species in the longer term.

Materials and Methods

The field survey was conducted from June to October, 2017. The whole area was examined twice, to be sure of the invasive plant species composition. The entire examined Gödöllő-Hatvan railway line (30 kilometers) was divided into 30 one kilometer long sections, and further into 120 two hundred and fifty meters long subsections. The 250 meters subsections were important because it was the maximum area, that we could surely view and still has the ability to determine most of the species along the railroad after we walked along the section and noted the invasive plant species being present. This way we could examine 5 to 10 km sections in a day efficiently.

The 250 meters long subsections were further examined as 2,5 meter pieces, in which the presence of the invasive species were recorded within the 10 meter range of the outer axis of the open railway track. We could maintain this distance with measuring with a tape measure in every subsection. In the case of stations the outer range of the survey was up to a 10 meter distance from the outer borders of the stations. This method could provide numerical data in terms of frequency, which could be interpreted as percentage. The 10 meters distance was important due to the fact that most of the herbicide treatments and manual weeding is usually carried out in this range. Spraying along railway tracks takes place up to a distance of 3.2 meters from the center of the railway pair (Szarka, ex verb., 2021). Chemicals usually being glyphfozate, 2,4-dichlorophenoxyacetic acid, clopyralid, florasulam, fluroxipir-meptil, styrene-acrylic emulsion polymers, drometrizole trisiloxane, propylene glycol, carboxymethyl cellulose, metsulfuron-methyl, pethoxamid, terbuthylazine (http1). Beyond this limit there are usually forests, agricultural fields and artificial objects (e.g. roads, gardens, houses).

The following categories have been selected during the survey in relation to the frequency of each invasive species: I. 0–5%, II. 5–10%, III. 10–25%, IV. 25–50%, V. 50–75%, VI. 75–100%.

The sampling was carried out manually by walking along the entire examined railway track section (30 kilometers). The right and left sides of the sections were observed at the same time, they were not examined separately. The examined invasive species were based on the list of the 31 most aggressive terrestrial invasive plant species of Hungary, valid at the time of the survey. Data were collected only about the listed species:

Acer negundo, Amorpha fruticosa, Asclepia syriaca, Ailanthus altissima, Aster novi-belgii agg., Celtis occidentalis, Chencrus incertus, Echynocystis lobata, Elaeagnus angustifolia, Fallopia japonica, Fallopia sachalinensis, Fallopia × bohemica, Fraxinus pennsylvanica, Helianthus tuberosus, Heracleum mantegazzianum, Heracleum sosnowskyi, Hordeum jubatum, Humulus scandens, Impatiens glanduifera, Impatiens parviflora, Juncus tenuis, Prunus (Padus) serotina, Parthenocissus inserta, Parthenocissus quinquefolia, Phytolacca americana, Phytolacca esculenta, Robinia pseudoacacia, Rudbeckia laciniata, Solidago gigantea, Solidago canadensis, Vitis vulpina. Ambrosia artemisiifolia was left out of this survey due to its wide range distribution in the country.

The results were evaluated and the species' percentage group distribution groups were made according to Raunkiaer's life-forms, Borhidi's social behavior types, and the spatial distribution and density of each species.

Results

Altogether the following 19 invasive plant species were recorded on the entire length of the surveyed railway track section:

- *Acer negundo* L. – Boxelder maple
- *Amorpha fruticosa* L. – False indigo bush
- *Asclepias syriaca* L. – Common milkweed
- *Ailanthus altissima* (Mill.) Swingle – Tree of heaven
- *Aster novi belgii agg.* L. – New York aster species
- *Celtis occidentalis* L. – Hackberry
- *Cenchrus incertus* M.A. Curtis – Common sandbur
- *Elaeagnus angustifolia* L. Russian Olive
- *Fallopia* × *bohemica* (Chrtek et Chrtková) J.P. Bailey – Bohemian knotweed
- *Fraxinus pennsylvanica* Marshall Green ash
- *Helianthus tuberosus* L.s.l. – Jerusalem arthichoke
- *Juncus tenuis* Willd. – Slender rush
- *Padus serotina* (ehrh.) Borkh. – Black cherry
- *Parthenocissus inserta* (A. Kern.) Fritsch – Thicket creeper
- *Parthenocissus quinquefolia* (L.) Planch. – Virginia creeper
- *Robinia pseudoacacia* L. – Black locust
- *Solidago gigantea* Aiton – Giant goldenrod

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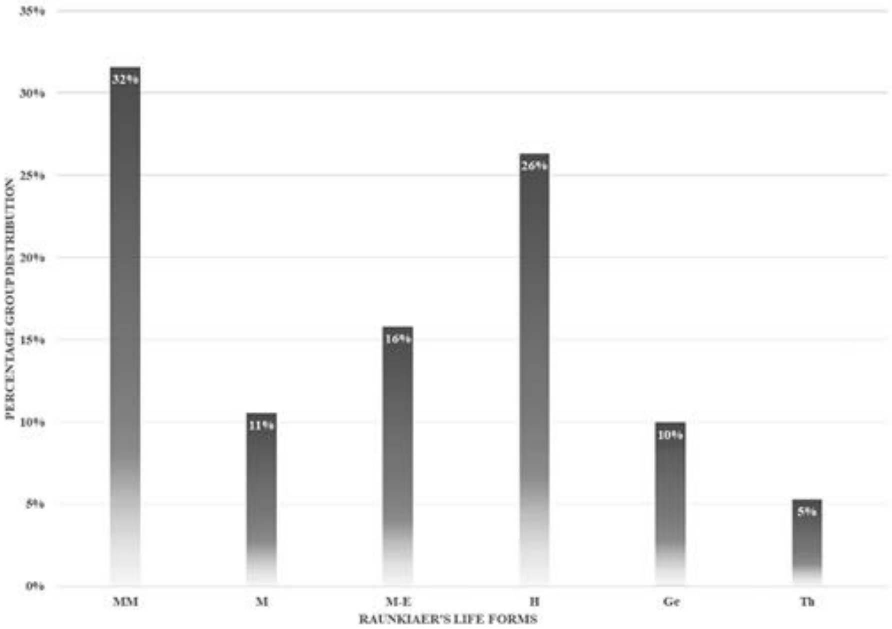

Figure 1. The Raunkiaer's life form types of the identified invasive species and their percentage group distribution

- *Solidago canadensis* L. – Canada gondenrod
- *Vitis vulpina* L. – Riverbank grape

Based on the percentage group distribution of the recorded species by Raunkiaer's life-form types, the species can be classified into 7 different life form categories (Fig. 1). Out of the categories mega-mesophanerophyta (woody plants) were present in the largest abundance, represented by 6 species (*Amorpha fruticosa, Ailanthus altissima, Celtis occidentalis, Fraxinus pennsylvanica, Padus serotina, Robinia pseudoacacia*). This group includes, but is not limited to, *Robinia pseudoacacia* and *Celtis occidentalis*, which are the most common species along the railway line. They followed by the hemicryptophyta species, which were represented by 5 taxa (*Asclepias syriaca, Aster novi belgii agg., Juncus tenuis, Solidago gigantea, Solidago canadensis*).

From the Borhidi social behavior types, 4 weed categories were represented by the species recorded (Fig. 2.), out of them the share of 'Alien competitors' (AC) was exceptionally high, with more than 50%. Alien competitors include, for example, *Acer negundo, Amorpha fruticosa* and *Asclepias syriaca*. The second most populous group was 'Introduced alien species' (I), represented 26% of the species (e.g. *Celtis occidentalis, Elaeagnus angustifolia* and *Fraxinus pennsylvanica*). Category „I" could mean the species once was cultivated, so therefore it means it was a conscious planting. Among the examples, *Celtis occidentalis* and *Fraxinus pennsylvanica* came to Hungary as park trees, while *Elaeagnus angustifolia* as raw materials for the wood industry. This could be an important factor when it comes to understanding the behavior of an invasive species.

The number of species present in the subsections (Fig. 3.) are unequal along the railway line. The map presents the difference between the settlements and the open track sections – those without constant trampling and other human activities, other than maintenance work. The species number is higher near the settlements, than the open track sec-

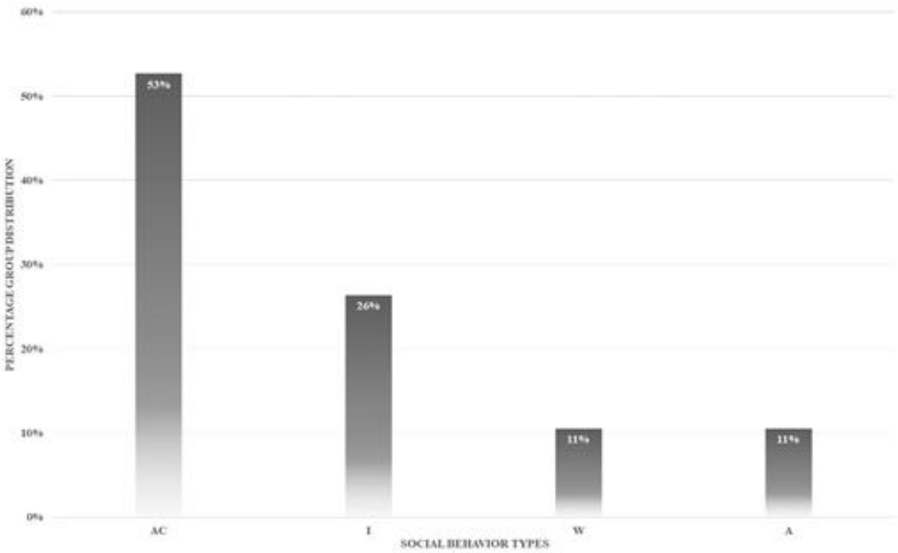

Figure 2. Group distribution of Borhidi Social Behavior Types of recorded taxa (A – Adventives, AC – Alien Competitors, I – Introduced alien species, W – Weeds)

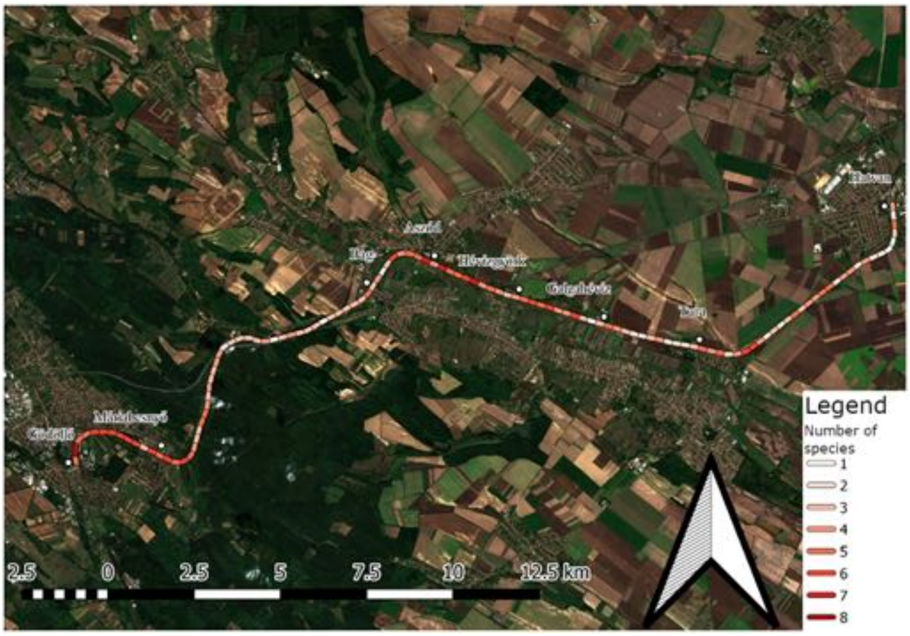

Figure 3. The number of species present in the subsections is indicated by a color scale. Between Gödöllő and Hatvan (October 2017). Source: ESA, Sentinel 2/A; QGIS 3.8.2, self-editing

tions. Certain species, such as *Juncus tenuis* and *Cenchrus incertus* are species specifically associated with stations, where there are poor quality, trampled and mowed grassy patches.

These facts indicate that the species composition is highly affected by the surrounding landscape. We highlight 3 species to investigate this phenomenon.

Figure 4 shows the presence of *Robinia*

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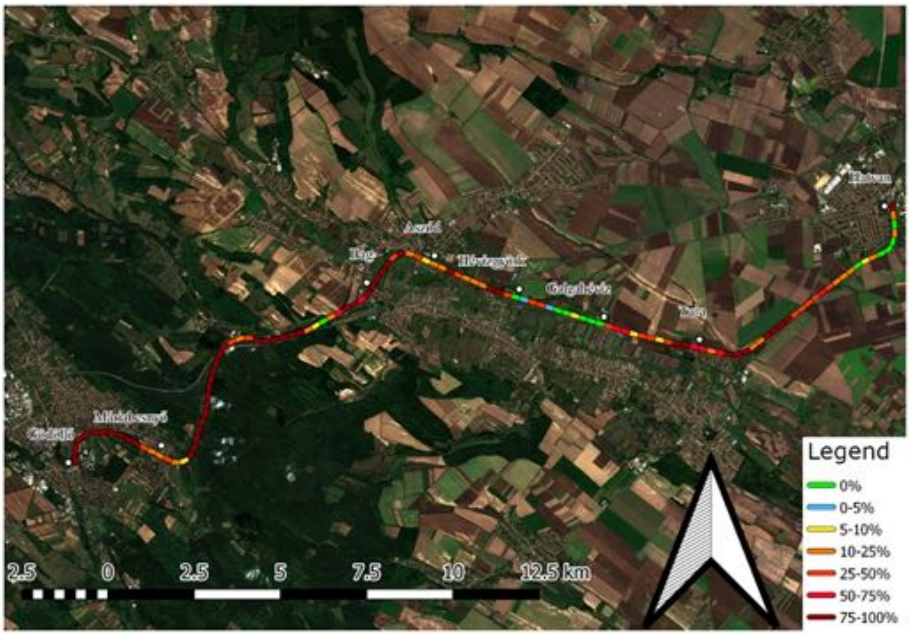

Figure 4. Black locust (*Robinia pseudoacacia*) infection on the surveyed road section between Gödöllő and Hatvan (October 2017). Source: ESA, Sentinel 2 / A; QGIS 3.8.2, self-editing

pseudoacacia on each small section. The most abundant invasive species of the survey, *Robinia pseudoacacia* was present at each recorded kilometer, and occurred in 108 out of the 120 subsections. *Robinia pseudoacacia* is a widespread invasive species throughout Hungary that causes many conservation problems (Kumschick et al., 2015).

Due to the abundance of *Robinia pseudoacacia*, we characterized those subsections where it did not occur or occurred with a small number of individuals.

i The first section was located between Domony-valley and Bag. This small section was almost completely free of invasive species. We found only a few *Solidago canadensis* individuals. It is a forested habitat patch dominated by *Acer* trees located between an agricultural area and the Bag train station, but its even more important feature is that it is much higher than the level of the rails, so the steep hillside and tree shade makes it difficult for invasive species to invade aggressively.

ii The second area is the small section in front of the Hévízgyörk railway station, where treatment is often done. Since the survey only examined the railway track up to 10 meters from the outer axis, the result does not mean that some seedlings or shoots could not appear here in a few years.

iii The third area is between the Hévízgyörk and Galgahévíz railway stations, which runs from halfway to the latter station. This section is covered rarely with trees, consists of 6 sub-sections, and only one of them has 2 specimens of Black locust. The surrounding areas are agricultural fields, so these populations are not stable, so this area is considered as one whole. There is agricultural activity on one side close to the rails, so regular eradication and the pressure of other species (including *Fraxinus pennsylvanica, Fraxinus excelsior* and some *Populus* trees) gives no opportunity for the species to settle.

iv The fourth area is the section next to the Hatvan marshalling yard. There are some stems from the 111th to the 118th small section, but due to regular eradication and agricultural activity, they are mainly seedlings.

Asclepias syriaca is one species that can be linked to linear infrastructure elements, such as roads or railways. Since this species prefers sandy soils and open, disturbed habitats, its presence near railway is expected.

Figure 5 shows that the species is present in the examined area, but not in great quantities. The most frequent subsections were placed between Máriabesnyő and Bag. The most dense subsections were present before and after Máriabesnyő station, where the vicinity of the inhibited area demands proper mowing, what creates good habitat for the species. Other sections were less frequently used by the species. The proximity of the railway is treated with herbicides, and the vegetation a little bit further is more and more closed, giving the species less opportunity to settle.

Figure 6 shows the presence of *Solidago canadensis* on the examined area. This species prefers more humid conditions. Between Gödöllő and Domonyvölgy it is more frequent. The area is well supplied with water by old drainage channels and the Besnyői-stream. After that there is two major occurence, one before Bag, near the Egres-stream; and the other near Hévízgyörk station, where there is an abandoned, wide, drainage channel under the railway embankment, which has been filled with dirt and occupied by weeds.

Discussion

Most of the found invasive species are not explicitly connected to the special habitats created by the railway structure, but they find their habitats in these areas as well. Stable populations run along railroads. These can be identified as borderline habitats, for these are in most cases line-like habitat patches neglected during maintenance work between railway areas and surrounding areas. Our results were not able to determine whether or not the found invasive plant species are explicitly connected to the habitats connected to the railway. In this regard, the species composition is more determined by the surrounding landscape than the railway structure for most species. Forman and McDonald (2007) and Mortensen et al. (2009) found that railway provide good habitat for invasive plant species for dispersal, as vehicles, wildlife and wind can move propagules alond roads which can increase the range of invasive species (Forman and McDonald 2007, Mortensen et al. 2009).

The 19 invasive species that were found along the railway lines were close to the 20 non-native species found in the survey carried out by Altay et al. (2015) in Turkey, where the three out of the found species (*Acer negundo, Ailanthus altissima, Robinia pseudoacacia*) are also registered as invasive in Hungary (Altay et al. (2015)).

Since lateral transect study wasn't performed, it cannot be said that more stable populations would be present along the railway line. The sudden appearance of large numbers of species may also indicate a larger population what has been cut in half by the railway. The examined railway line is well-bordered by trees in some areas, where most of the weedings take place only in the immediate vicinity of the railway tracks. In addition to plough fields, it is mainly *Robinia pseudoacacia* and *Asclepias syriaca* that line the railway embankments, as is often the case along other roads. Economic activities (timber planting, beekeeping) and poor quality treatments might encourage this phenomenon. During the study, the railway track section was not divided into right and left sides.

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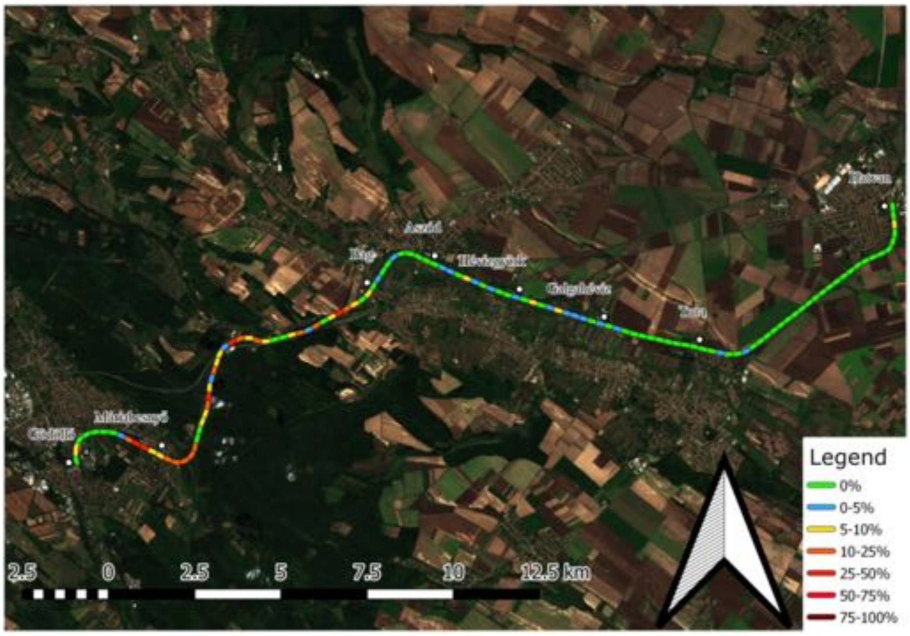

Figure 5. Common milkweed (*Asclepias syriaca*) infection on the surveyed road section between Gödöllő and Hatvan (October 2017). Source: ESA, Sentinel 2 / A; QGIS 3.8.2, self-editing

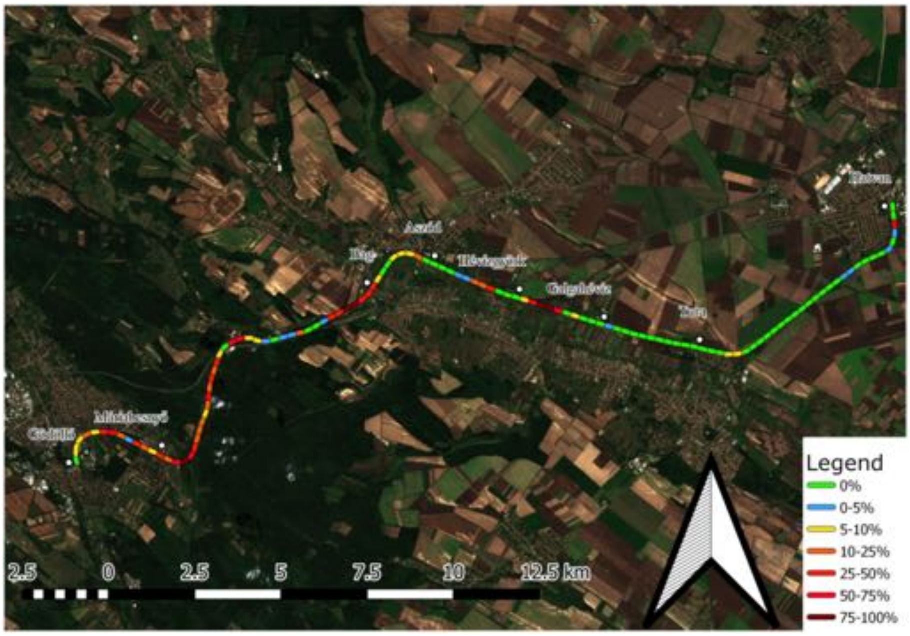

Figure 6. Canada goldenrod (*Solidago canadensis*) infection on the surveyed road section between Gödöllő and Hatvan (October 2017). Source: ESA, Sentinel 2 / A; QGIS 3.8.2, self-editing

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Source of the graphics

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Gallo-Roman harvesting machine, called Vallus. Source: U. Troitzsch - W. Weber (1987): Die Technik : Von den Anfangen bis zur Gegenwart

Rear cover:
Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones. Lugduni: Apud Ioan. Tornaesium 1559. Centre d'Études Supérieures de la Renaissance - Tours

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Lucius Junius Moderatus Columella

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His De Re Rustica in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19th Century.