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Effect of harvest date on yield and secondary compounds of lemon balm (*Melissa officinalis* L.)

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Summary

The quality of the drug of lemon balm (*Melissa officinalis* L.) is influenced by several factors, among which the effect of ontogenesis has practically not been studied before. Five varieties ('*Lorelei*', '*Lemona*', '*Quedlinburger Niederliegende*,', '*Gold Leaf*', '*Soroksár*') were sampled at vegetative, budding, full flowering and after flowering phases at two locations (Budapest and Poznań) and their dried leaves analysed.

The accumulation of volatile compounds showed maximum values (0.08-0.46 ml/100 g dry weight) in budding phase (Budapest) or during flowering (Poznań). The content of total phenolics was highest (226-431 mg gallic acid equivalent/g dry weight) in vegetative stage and in some cases similar values were measured until budding. After a sharp decrease at flowering time in several cases, a second peak was detected at the end of the vegetation period. Similarly to the total phenolics, also the total flavonoid content reached the highest level (0.239-1.152% dry weight) at the first half of the vegetation period however, with characteristic differences between habitats.

In cultivation, the highest essential oil content may be reached later than highest polyphenol content, however harvesting at budding time may assure a good quality from both aspects with advantageous fresh and drug yields. The described dynamics of the accumulation of the investigated secondary metabolites was depending more on the habitat and less from the cultivar.

Keywords: phenological phase, essential oil, flavonoids, polyphenols, antioxidant capacity, habitat

Introduction

Lemon balm (*Melissa officinalis* L.) is a valuable perennial medicinal plant, which originates from the Mediterranean area and western Asia but it is already commonly cultivated under diverse ecological conditions in the temperate regions. The herb (*Melissae herba*) and the leaves (*Melissae folium*) are used as the components of digestive, antispasmodic and antiviral remedies (EMA, 2014).

Based on up-to-date results, phenolics, flavonoids and hydroxycinnamic acid derivatives are considered to be primarily responsible for most of the medicinal properties of the plant, most likely in connection with its considerable antioxidant activity (DASTMALCHI et al., 2008). The content of essential oil, which has a characteristic citrus like odor, varies in the herb of lemon balm from 0.01 to 0.3%. It consists mainly of monoterpene aldehydes (geranial, neral, citronellal) and sesquiterpenes (β-caryophyllene and β-caryophyllene oxide) (PATORA et al., 2003; SEIDLER-LOZYKOWSKA et al., 2017).

It is already a well-known fact, that growth, development and biologically active material content of medicinal and aromatic plants may be significantly influenced by genetic (internal) and environmental (external) factors, the effects of which are however, in many cases characteristic for the target species. Recent studies on the morphological and chemical intraspecific variability of lemon balm revealed considerable differences among accessions (TALLE et al., 2012; SZABÓ et al., 2016). The environment may also influence the performance of the species, concerning both morphological traits and active compounds (SARI and CEYLAN, 2002; MANUKYAN, 2011; MOQUBELI et al., 2011; NÉMETH-ZÁMBORI et al., 2017).

Surprisingly, the least studied factors seems to be the ontogenesis. AYANOGLU et al. (2005) provided data on the essential oil accumulation of lemon balm at two locations, depending on harvest time. However, a reproducible determination of sampling time and accurate information on the actual phenological phase of the plants is frequently missing in the references (PATORA et al., 2003; RUSACZONEK et al., 2007; DASTMALCHI et al., 2008; ONIGA et al., 2010; MOQUBELI et al. 2011; etc.). In other papers, a single sampling was carried out at diverse phases, e.g. before flowering (CARNAT et al., 1998), beginning of flowering (TALLE et al., 2012), when 50% of the plants had flowers (SARI and CEYLAN, 2002), at full bloom stage (KHALID et al., 2008) or at the end of flowering (FARAHANI et al., 2009).

Reliable complex studies about the changes of biomass and active ingredients of lemon balm during the development are still missing. Independent of the fact, that usually the leaves of lemon balm are used and processed, the optimal harvesting time may be of primarily importance concerning drug quality. The example of many species, – among others also numerous Lamiaceae species – show that there might be significant changes during ontogenesis in the accumulation of secondary compounds (NÉMETH-ZÁMBORI, 2015).

To clarify the above questions and optimize lemon balm production, we investigated the role of the developmental phase on the growth, development and drug quality. Five cultivars were included into the experiment, which was conducted under two different environmental conditions.

Materials and methods

Plant material and experimental locations

The experiments were conducted in open field plots of the Szent István University of Budapest, Hungary (47º54'N 19º14'E) and of the Institute of Natural Fibers and Medicinal Plants of Poznań, Poland (52º25'N 16º58'E).

The main soil parameters of the experimental plots are presented in Tab. 1. The mean temperatures, marginal values of the temperatures and sum of precipitation before each harvest in both locations are summarized in Tab. 2. Irrigation was applied only in the very dry periods to avoid dying of the plants.

Five lemon balm (*Melissa officinalis* L.) varieties were included into the trial: '*Lorelei*' (MediSeeds, Switzerland); '*Lemona*', '*Quedlinburger Niederliegende*' (in the followings 'Quedlinburger'), *'Gold Leaf*'' (each from Jelitto, Germany) and '*Soroksár*' (Enkraft Bt., Hungary).

Seed sowing and seedling raising was made in greenhouse. Planting of 50 seedlings/genotype into open field plots was carried out at the beginning of June 2014, to a spacing of 45 × 45 cm. The harvesting treatments were carried out in the second vegetation year, in 2015. Five healthy representative individuals from each plot were cut in three replicates in the following phenological phases: 1. vegetative

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Tab. 1: Soil characteristics of the experimental locations

	pH	Humus %	NO_3-N mg/kg	P_2O_5 mg/kg	K_2O mg/kg	Ca %	Mg mg/kg
Budapest	6.91	0.87	7.73	583	189	0.51	32.1
Poznań	5.18	2.58	8.46	395	131	1.36	27.4

Tab. 2: Weather conditions during 30 days before lemon balm harvests in Poznań and Budapest

Phenological phase	Location	Harvest date	Average day temperature (°C)	Temperature range (°C)	Sum of precipitation (mm)
vegetative	Budapest	25 May	15.6	10.7 – 20.4	82.8
	Poznań	08 June	13.4	- 1.6 – 24.9	39.6
buds	Budapest	17 June	20.0	11.8 – 24.1	59.8
	Poznań	28 June	15.3	4.6 – 32.5	77.8
flowering	Budapest	25 July	21.5	15.0 – 21.8	32.8
	Poznań	03 August	17.0	6.3 – 34.1	82.2
after flowering	Budapest	31 August	22.3	7.6 – 39.1	84,2
	Poznań	06 September	20.7	7.2 – 37.1	68.8

stage (no flowering stems appear still), 2. plant budding (flowering shoots with buds without white petals), 3. full flowering (50% of flowers are open); 4. after flowering (no flowers are visible and green seeds are developing).

Sample preparation

The fresh mass of the plant samples was weighed individually, and then the shoots were dried in a shaded, well-ventilated place. The samples were separated into leaf and stem parts and only the leaves were used for chemical analysis. After drying, the individual leaf samples were mixed creating a representative bulk sample for each treatment and replicates. The phytochemical analyses were carried out on these homogeneous bulk samples in three further, technical replicates at the Department of Medicinal and Aromatic Plants, Budapest.

Phytochemical analyses

Essential oil content (EO): 50 g of each sample was hydrodistilled for three hours in a Clevenger-type apparatus recommended by the Pharmacopoeia Hungarica VII. The EO content was calculated as volume (mL) of oil per 100 g of dried weight (d.w.) determined in three hours, at 105 °C.

Total phenolic content (TPC): For the determination of the TPC 1 g powdered dried plant material was extracted by boiling in 100 mL distilled water and then was allowed to stand for 24 h. Then the extracts were filtered and stored frozen until the measurements took place. The total phenolic content was determined by the modified method of Singleton and Rossi (1965) and was expressed as mg of gallic acid equivalents (GAE) per g of dry weight of extract.

Total flavonoid content (TFC): The flavonoid content was determined according to the method given in the Pharmacopoeia Hungarica VIII for *Equiseti herba* using half of the amounts of materials described there. Shortly, 0.4 g dried and powdered plant material was extracted by 1 mL hexametilentetramine, 20 mL acetone and 2 mL HCl for 30 minutes, and then it was filtered. Afterwards the extraction was repeated by 20 mL acetone twice and diluted by water and ethyl-acetate. The absorbance was measured at 760 nm after incubation for 30 min and expressed in mg isoquercitroside (QE) per g of dry weight of plant material.

Antioxidant capacity (AC): The FRAP assay was performed according to the method of Benzie and Strain (1996), and the FRAP values of samples were calculated from a standard curve equation and expressed as mg ascorbic acid equivalent (AAE) per g of dry weight of extract.

Statistical analysis

The results were analysed with an IBM SPSS 22.0 statistical program. MANOVA was used for evaluating the effects of variety and phenological phase for data obtained in each location. Homogeneity of variances was tested by Levene's method. Depending the homogeneity of variances measurement, a *Tukey HSD* or *Games-Howel* test was used for the pairwise comparisons of the variances. Confidence level was 5%.

Results and discussion

Yields

Highest fresh mass of the plants was produced in the flowering period (Tab. 3). In Budapest, highest yields were achieved in full flowering (in case of 'Lorelei' and 'Soroksár' not significantly different from the previous phase). Unfortunately, in this growth area, the development of 'Gold leaf' was severely disturbed, several individuals exhibited a decay and after budding phase the measurements could not be continued. In Poznań, all of the five cultivars gave the highest yields at the beginning of flowering (budding phase), although in case of two cultivars the values did not differ significantly from the previous ('Gold leaf') and the following ('Quedlinburger') phases. In case of the fresh shoot mass the interaction between cultivar and phenophase was not significant in either location.

The dry leaf mass – drug yield of *Melissae folium* – followed similar tendencies (Tab. 3). In Budapest, highest drug yield were achieved at full flowering stage. In this aspect, the cultivar × phenophase interaction was significant only in Poznań. In Poland, the full flowering stage proved to be optimal only for cultivar 'Quedlinburger', while in case of the other varieties budding ('Lorelei' and 'Soroksár') or vegetative phase ('Gold leaf' and 'Lemona') assured the largest drug mass. The latter results might have been influenced also by the decreasing leaf proportion in the shoot mass, which was detected already from budding stage. Leaf ratio in the total shoot mass was 66.6% in vegetative phase, 41.0% in budding, 38.4% in full flowering and 40.0% after flowering as a mean of the data of the examined cultivars.

Tab. 3: Fresh mass and drug yield of the tested cultivars in four phenological phases in two location (*Between subject effect Variety × Phenophase according to MANOVA*: fresh mass: $p_{Budapest}$=0.167; p_{Poznan}=0.455; dry mass: $p_{Budapest}$=0.213; p_{Poznan}=0.011)

Cultivar	Fresh mass of shoots (g/plant)				Dry mass of leaves (g/plant)			
	phenological phase				phenological phase			
	vegetative	budding	flowering	after flowering	vegetative	budding	flowering	after flowering
				Budapest				
'Gold Leaf'	36 Bc	55 Ad	-	-	6 Bb	10 Ac	-	-
'Lemona'	140 Aab	122 ABc	150 Ab	106 Bc	24 Ba	22 BCb	29 Ab	20 Cb
'Lorelei'	158 Ba	193 Aa	197 Aa	161 Ba	21 Ca	32 Ba	44 Aa	41 Aa
'Quedlinburger'	116 Bb	107 Bc	198 Aa	105 Bc	20 Ca	24 Bb	45 Aa	27 Bb
'Soroksár'	143 Ba	166 Ab	153 Ab	124 Cb	26 ABa	31 Aa	30 Ab	22 Bb
Mean	119	129	175	124	19	24	37	28
				Poznań				
'Gold Leaf'	78 Ab	72 Ac	42 Bc	34 Bc	50 Ab	33 Bc	12 Cc	8 Cd
'Lemona'	94 Ca	152 Ab	124 Bb	70 Cb	63 Aa	61 Ab	47 Bb	29 Cc
'Lorelei'	56 Cc	180 Aa	124 Bb	110 Ba	38 Cc	70 Aa	51 Bb	51 Ba
'Quedlinburger'	86 Bab	152 Ab	154 Aa	96 Bab	57 ABa	61 Ab	66 Aa	42 Bb
'Soroksár'	50 Dc	172 Aa	146 Ba	106 Ca	35 Cc	69 Aa	61 Aa	46 Bab
Mean	73	146	118	83	49	59	47	35

Capital letters indicate sign. diff. ($p<0.05$) among phenophases in the same variety each location separately; Lower case letters indicate sign. diff. ($p<0.05$) among varieties at the same phenophase in each location separately

In the practice, this crop is harvested before flower development and repeated cuttings can be carried out in the same year depending on the region and climate (Hoppe, 2013). Seidler-Lozykowska et al. (2013) and Szabó et al. (2016) described significant variation in yields of different lemon balm accessions. Nevertheless, no detailed former study is known about the dynamics of biomass production of the species during the vegetation cycle, comparable with the recent findings.

Essential oil content

The EO content of the harvested drug showed significant differences thorough the phenophases (Tab. 4). Budding phase was detected as the optimal time for all cultivars in Budapest. In the following period, the essential oil content decreased sharply again except 'Lemona'. In Poland, the maximum values of the volatile accumulation were reached at full flowering except 'Gold leaf'. This phenomenon and the later maximum accumulation compared to Budapest may stay in connection with the very cold weather during spring and the fact that the plants could grow slowly. The cooler environment (Tab. 1) might be an explanation also for the generally much lower essential oil values in Poznań, where the highest mean value is only 42% of the maximum mean value in Budapest. No variety × phenophase interaction could be determined in Budapest, while it was a significant one ($p<10\%$) in Poznań.

The range of the EO content is fully comparable with other references (Bomme et al., 2002; Patora et al., 2008; Seidler-Lozykowska et al., 2017). In own earlier investigations in a pot experiment on the same cultivars 0.07-0.29 ml/100 g EO content was determined in vegetative phase (Szabó et al., 2016). Based on investigation of 15 accessions, Chizzola et al., (2018) mentioned that first cut leaves (in June) gave more oil, than those of the second cut (in August). Ayanoglu et al. (2005) declared the before flowering phase as assuring highest oil contents but differences were significant only in one of the experimental locations. In related species frequently the early flowering or full flowering period has been detected as the optimal phase for volatile accumulation, both in our own works and in those of other authors: in *Hyssopus officinalis* L. (Németh et al., 2001), in *Mentha piperita* L. (Zámboriné and Tétényi, 1988), in *Majorana hortensis* Mönch. (Sellami et al., 2008), *Salvia officinalis* L. (Mirjalili et al., 2006). Latter authors suggest that the ecological role of the higher volatile accumulation during flowering might be attracting of pollinators and defeating some diseases.

Total phenolic content

In general, the TPC was higher at the beginning of plant development (Tab. 5). In Budapest, it showed maximum values already during the first sampling time at least for 'Lorelei' (431.9 mg GAE/g d.w.) and 'Soroksár' (340.2 mg GAE/g d.w.) cultivars. At flowering time phenolic content decreased in all of the samples while interestingly, at the end of the vegetation time it increased again to variable extent (except 'Lemona').

In Poznań, all of the cultivars provided the highest phenolic content at the vegetative phase. Compared to the later phases the advantage was significant in each case. Similarly to the findings in Budapest, in three cultivars also a second peak could be observed. However, this second peak was detected during flowering, not in the last phase, as in Budapest. No variety × phenophase interaction has been established at either of the locations.

Rusaczonek et al. (2007) and Chizzola et al. (2018) stated that TPC was higher in leaves compared to stem parts. Considering the larger leaf proportion in the earlier phases, it could contribute to the peak values of phenolics in the beginning of vegetation. However, it cannot be an explanation for the second increase because leaf ratio has already dropped at this time (see above). It lets to assume, that changing metabolomic processes during ontogenesis could be responsible for changing values. In case of two cuts carried out at different times (in June and August) but in the same, vegetative phase after regrowth, as in the trial of Chizzola et al. (2018), no significant change in the TPC was measured and this ascertains the role of the phenological phase in phenolic accumulation. At the same time, the effect of weather conditions cannot be excluded either (Németh-Zámbori et al., 2017).

Tab. 4: Essential oil content (ml/100g d.w.) in herb of lemon balm cultivars in four phenological phases in two location (*Between subject effect Variety × Phenophase according to MANOVA*: $p_{Budapest}$=0.333; p_{Poznan}=0.068)

Cultivar	Phenological phase							
	vegetative		budding		flowering		after flowering	
	mean	s.d.	**mean**	s.d.	**mean**	s.d.	**mean**	s.d.
				Budapest				
'Gold Leaf'	**0.30 Ba**	0.001	**0.46 Aa**	0.037	**-**	-	**-**	-
'Lemona'	**0.13 Cb**	0.021	**0.41 Aa**	0.042	**0.28 Ba**	0.005	**0.37 ABa**	0.021
'Lorelei'	**0.10 Bc**	0.020	**0.13 Ac**	0.020	**0.05 Cd**	0.020	**0.08 Bb**	0.001
'Quedlinburger'	**0.06 Cd**	0.021	**0.32 Ab**	0.035	**0.10 Bc**	0.020	**0.07 Bb**	0.001
'Soroksár'	**0.07 Bc**	0.003	**0.12 Ac**	0.021	**0.12 Ab**	0.021	**0.05 Bc**	0.021
Mean	**0.13**		**0.29**		**0.11**		**0.12**	
				Poznań				
'Gold Leaf'	**0.09 C**	0.004	**0.11 C**	0.001	**0.17 B**	0.001	**0.38 A**	0.039
'Lemona'	**0.09 B**	0.004	**0.10 B**	0.015	**0.14 A**	0.001	**0.13 A**	0.021
'Lorelei'	**0.01 C**	0.001	**0.06 B**	0.015	**0.09 A**	0.001	**0.07 A**	0.001
'Quedlinburger'	**0.02 C**	0.004	**0.05 B**	0.001	**0.08 A**	0.004	**0.02 C**	0.016
'Soroksár'	**0.05 B**	0.004	**0.07 A**	0.016	**0.00 C**	-	**0.00 C**	-
Mean	**0.05**		**0.08**		**0.11**		**0.12**	

Capital letters indicate sign. diff. ($p<0.05$) among phenophases in the same variety each location separately; Lower case letters indicate sign. diff. ($p<0.05$) among varieties at the same phenophase in each location separately

Tab. 5: Total phenolic content (mg GAE/g d.w.) of herb of lemon balm cultivars in four phenological phases in two location (*Between subject effect Variety × Phenophase according to MANOVA*: $p_{Budapest}$=0.123; p_{Poznan}=0.566)

Cultivar	Phenological phase							
	vegetative		budding		flowering		after flowering	
	mean	s.d.	**mean**	s.d.	**mean**	s.d.	**mean**	s.d.
				Budapest				
'Gold Leaf'	**226.0 Bd**	30.5	**249.1 Ad**	15.0	**-**	-	**-**	-
'Lemona'	**303.3 Bc**	29.8	**394.8 Aa**	33.9	**222.1 Ca**	11.5	**160.0 Dd**	12.8
'Lorelei'	**431.9 Aa**	35.8	**352.6 Bb**	18.5	**163.7 Cb**	9.4	**336.2 Ba**	17.0
'Quedlinburger'	**334.0 Ab**	31.3	**360.8 Ab**	38.2	**165.4 Bb**	14.4	**193.6 Bc**	19.7
'Soroksár'	**340.2 Ab**	27.5	**308.1A Bc**	27.3	**167.9 Cb**	26.5	**294.4 Bb**	40.6
Mean	**327.1**		**333.1**		**179.8**		**246.1**	
				Poznań				
'Gold Leaf'	**354.7 Ab**	21.2	**244.1 Cc**	18.9	**270.4 Bc**	44.4	**222.4 Ca**	29.4
'Lemona'	**410.8 Aa**	56.6	**289.6 Cb**	36.4	**331.9 Bb**	22.3	**205.5 Da**	21.4
'Lorelei'	**361.6 Ab**	22.4	**316.3 Ba**	38.12	**221.3 Cd**	23.7	**163.5 Db**	19.9
'Quedlinburger'	**369.0A Bb**	32.5	**321.5 Ba**	40.14	**410.2 Aa**	27.1	**220.2 Ca**	12.7
'Soroksár'	**364.4 Ab**	27.1	**300.8 Bab**	37.91	**249.5 Ccd**	51.2	**146.9 Db**	19.2
Mean	**372.1**		**294.5**		**296.7**		**151.7**	

Capital letters indicate sign. diff. ($p<0.05$) among phenophases in the same variety each location separatel; Lower case letters indicate sign. diff. ($p<0.05$) among varieties at the same phenophase in each location separately

Two peaks for the accumulation of TPC were published also by KINDLOVITS et al. (2016) in yarrow (*Achillea collina*), the first in green bud - early flowering phase (178.0-233.4 mg GAE/g) and the next one in overblown phenological phase (170.9-258.5 mg GAE/g). In marjoram, SELLAMI et al. (2009) established that TPC of the marjoram herb was highest in vegetative phase before flowering. In parallel, they found that EO content showed a maximum at full flowering. Although they did not investigate the after flowering phenological phase, the results show large similarity with our ones. The authors suggest, that phenolic accumulation in this early phenological phase might have a role in plant defence mechanism, which is taken during flowering by volatile secondary compounds.

Total flavonoid content

The TFC of the tested cultivars exhibited a decreasing tendency during the phenological phases (Tab. 6). In Budapest, highest flavonoid contents were measured in the samples collected during vege-

Tab. 6: Total flavonoid content (mg QE/g d.w.) in herb of lemon balm cultivars in four phenological phases in two location (*Between subject effect Variety × Phenophase according to MANOVA*: $p_{Budapest}$=0.865; p_{Poznan}=0.0622)

Cultivar	Phenological phase							
	vegetative		budding		flowering		after flowering	
	mean	s.d.	**mean**	s.d.	**mean**	s.d.	**mean**	s.d.
				Budapest				
'Gold Leaf'	**0.239 Aa**	0.011	**0.222 Ab**	0.027	**-**	-	**-**	-
'Lemona'	**0.648 Aa**	0.002	**0.547 Ba**	0.007	**0.463 Ca**	0.010	**0.523 Ba**	0.002
'Lorelei'	**0.693 Aa**	0.087	**0.560 Ba**	0.007	**0.414 Cb**	0.016	**0.299 Db**	0.014
'Quedlinburger'	**0.624 Aa**	0.004	**0.498 Bb**	0.010	**0.389 Cb**	0.038	**0.223 Dc**	0.022
'Soroksár'	**0.641 Aa**	0.029	**0.482 Bb**	0.003	**0.407 Cb**	0.020	**0.303 Db**	0.009
Mean	**0.569**		**0.462**		**0.418**		**0.337**	
				Poznań				
'Gold Leaf'	**0.520 Ac**	0.015	**0.393 Bc**	0.022	**0.354 Cd**	0.025	**0.409 Bc**	0.026
'Lemona'	**0.736 Ba**	0.009	**0.852 Ab**	0.036	**0.617 Cc**	0.033	**0.432 Dbc**	0.003
'Lorelei'	**0.642 Cb**	0.039	**0.871 Ab**	0.031	**0.723 Ba**	0.004	**0.543 Da**	0.013
'Quedlinburger'	**0.752 Ba**	0.001	**0.854 Ab**	0.043	**0.663 Cb**	0.011	**0.573 Da**	0.002
'Soroksár'	**0.667 Bb**	0.049	**1.152 Aa**	0.026	**0.644 Bb**	0.026	**0.473 Cb**	0.015
Mean	**0.663**		**0.824**		**0.600**		**0.486**	

Capital letters indicate sign. diff. ($p<0.05$) among phenophases in the same variety each location separately; Lower case letters indicate sign. diff. ($p<0.05$) among varieties at the same phenophase in each location separately

tative phase in case of all cultivars. The values did not show large deviations among varieties (0.624-0.693 mg QE/g d.w.) except 'Gold leaf' (0.239% d.w.). After the first sampling, a continuous decrease of the TFC could be measured in all cultivars. In 'Lemona' however, the change was only a moderate one and this variety showed also a smart second peak at the end of the vegetation cycle.

The mean values of the flavonoid content were by 18-44% higher in Poznań, than in Budapest. The maximum contents could be detected at budding phase and the decrease started after that. The only exception is 'Gold leaf', which showed the highest contents in the earliest phase, similarly to the findings in Budapest. After the first peak, a continuous decreasing tendency and significantly lower values of TFC were measured in each of the cultivars. The interaction between variety and phenophase was a significant one only in Poznań ($p<10\%$).

There are practically no former data on the role of plant development in this aspect. The TFC detected by Carnat et al. (1998) in lemon balm leaves is comparable with our data. However, only leaves harvested just before flowering of a single accession were investigated by those authors. The values of the TFC were characteristically different between the two growing areas, therefore investigating the spectrum of components depending on location might be of interest in the future.

Antioxidant capacity

In Budapest, the FRAP AC of the samples showed peak values in budding stage (Tab. 7). After budding, a sharp decrease was measured in all cultivars and only in 'Lorelei' a smart second peak detected at the end of the vegetation.

Similarly to this, in Poznań maximum values for AC were measured in budding phase (with the exception of 'Gold leaf'). After that, a continuous decrease could be observed, however, the differences among phenological phases were less characteristic, than in Budapest. 'Gold leaf' exhibited a very different behavior than the other varieties, as it showed increasing AC during the developmental phases. In the first two phases, the AC data are comparable with the values in Budapest, but in the last two phases the values in Poznań are more than twofold compared with the Hungarian data. The variety × phenophase interaction is not significant at either habitat.

A lower variability of FRAP AC of lemon balm compared to the active ingredients (volatile, polyphenols) was described also in other studies (Szabó et al., 2016; Chizzola et al., 2018). Drought stress may elevate it to some extent (Németh-Zámbori et al., 2017) however, no data were found on the changes of AC during ontogenesis of this species.

In summary, we established that based on the investigation of five lemon balm cultivars at two different locations, the accumulation of volatile compounds showed a single peak with maximum values after the appearance of the generative organs. Phenoloid type compounds showed highest concentrations in earlier phases, and in several samples, another peak appeared towards the end of the vegetation period.

In practice, determination of optimal harvesting time may be oriented according to the goal of the production. Highest essential oil yield may be reached later than highest polyphenol content. However, harvesting at budding time seems to assure the best quality from both aspects. This timing may also assure an advantageous fresh and drug yield. The study revealed, that the described dynamics of accumulation of the investigated secondary metabolites is depending more on the habitat and much less on the intraspecific cultivar.

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Tab. 7: FRAP antioxidant activity (mg AAE/g d.w.) of herb of lemon balm cultivars in four phenological phases in two location (*Between subject effect Variety × Phenophase according to MANOVA*: $p_{Budapest}$=0.556; p_{Poznan}=0.848)

Cultivar	Phenological phase							
	vegetative		budding		flowering		after flowering	
	mean	s.d.	**mean**	s.d.	**mean**	s.d.	**mean**	s.d.
				Budapest				
'Gold Leaf'	**351.1 Ab**	29.72	**357.4 Ac**	20.35	-	-	-	-
'Lemona'	**431.6 Ba**	47.10	**476.8 Aa**	69.16	**186.8 Ca**	9.81	**119.7 Dc**	3.52
'Lorelei'	**432.0 Aa**	35.82	**425.3 Ab**	29.88	**131.5 Cb**	18.72	**184.1 Ba**	8.29
'Quedlinburger'	**430.0 Aa**	30.73	**445.9 Aab**	53.51	**126.0 Bb**	14.53	**98.2 Cc**	14.13
'Soroksár'	**378.9 Bb**	33.41	**420.9 Ab**	29.55	**142.5 Cb**	13.63	**146.3 Cb**	15.82
Mean	**404.7**		**425.3**		**146.7**		**137.1**	
				Poznań				
'Gold Leaf'	**349.3A Bb**	28.00	**301.3 Bb**	16.53	**373.1 Ad**	10.52	**399.5 Ab**	18.53
'Lemona'	**404.2 Ba**	20.50	**462.7 Aa**	57.82	**450.1 Aa**	24.53	**396.6 Bb**	19.48
'Lorelei'	**361.7 Cb**	22.41	**450.0 Aa**	29.57	**401.2 Bc**	15.55	**429.0A Ba**	26.83
'Quedlinburger'	**346.4 Db**	16.22	**460.4 Aa**	34.32	**424.8 Bb**	26.56	**383.7 Cc**	73.13
'Soroksár'	**368.4 Cb**	41.26	**447.4 Aa**	33.75	**423.6 Ab**	17.18	**410.5 Bab**	8.99
Mean	**366.0**		**424.4**		**329.8**		**321.8**	

Capital letters indicate sign. diff. ($p<0.05$) among phenophases in the same variety each location separately; Lower case letters indicate sign. diff. ($p<0.05$) among varieties at the same phenophase in each location separately

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