ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 16(6) 2017, 67–79

www.acta.media.pl ISSN 1644-0692 DOI: 10.24326/asphc.2017.6.6

ORIGINAL PAPER

Accepted: 12.07.2017

ONTOGENETIC VARIABILITY IN THE QUANTITY AND QUALITY OF WINTER SAVORY (*Satureja montana* L.) HERB YIELD

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ABSTRACT

The chemical composition and activity of herbal raw material depend, among others, on ontogenetic variability. The present study investigated the effect of harvest date of winter savory (*Satureja montana* L.) herb and plant age on fresh and dry herb yield as well as on the content of L-ascorbic acid, carotenoids, chlorophylls, essential oil, flavonoids and tannins. *S. montana* herb from a one- and two-year-old plantation was harvested in June (vegetative stage), July (beginning of flowering), August (full flowering) and September (senescent plants). As the plants progressed to the successive growth stages, they were characterized by a higher fresh and dry herb yield. Two-year-old plants produced a significantly higher fresh and dry herb yield compared to one-year-old plants. The herb harvested before flowering contained most L-ascorbic acid, carotenoids and tannins, whereas the herb harvested at full flowering – most chlorophylls, essential oil and flavonoids. Two-year-old plants accumulated in the herb more L-ascorbic acid, chlorophylls and essential oil than one-year-old plants.

Key words: medicinal plants, development phase, harvest time, herb yield, essential oil content

INTRODUCTION

In their ontogeny, plants undergo several developmental stage changes, which are manifested in morphological and physiological changes. In the case of herbal plants, morphological changes are important due to differences in raw material yield, whereas physiological changes may contribute to chemical composition modifications and impaired biological activity of the herbal material [Kaya et al. 2012, Jakovljević et al. 2013]. Essential oil and monoterpene yield in inflorescences of *Salvia sclarea* L. (Lamiaceae) decreases during the full maturity period [Lattoo et al. 2006]. In the case of *Artemisia annua* L. (Asteraceae), in turn, the essential oil content in the above-ground part increases with flower development and senescence [Şenkal et al. 2015]. Moreover, essential oil production, associated with numerous biochemical processes occurring in the plant at different ontogeny stages, is subject to climatic variability [Sangwan et al. 2001, Lee and Ding 2016]. Likewise, the level of other active constituents changes at different plant growth stages [Çirak et al. 2007]. Harvest date has a significant effect on the content of L-ascorbic acid, chlorophyll, carotenoids and essential oil in hyssop herb [Zawiślak 2011] as well as on fresh and dry herb yield, dry leaf yield and essential oil content in sage [Zawiślak 2014].

A study on various species of the genus *Satureja* conducted by García-Rellán et al. [2015] shows the distinctiveness of *S. montana*, both in its morphological and chemical characteristics. *S. montana* is char-

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acterized by a strong aroma and wide biological activity, which is predominantly dependent on the genotype and habitat conditions [Dunkić et al. 2010, Ćavar et al. 2013]. Dudaš et al. [2013] obtained 1.00–2.33% of essential oil in collecting *S. montana* herb at different times and from different habitats. Similarly, Ibraliu et al. [2010] showed a relationship between the essential oil content in the herb (0.22–1.61%) and the location of the *S. montana* herb habitat. *S. montana* essential oil extraction yield (1.28–1.82%) is also dependent on the method of raw material preparation and distillation method [Damjanović-Vratnica et al. 2016]. The herb of *S. montana* contains essential oil [García-Rellán et al. 2015], phenolic compounds and flavonoids [Hassanein et al. 2014]. *S. montana* essential oil exhibits antibacterial [Miladi et al. 2013, Mihajilov-Krstev et al. 2014] and antioxidant activity [Miladi et al. 2013]. The antioxidant activity of *S. montana* herb is probably attributable to the presence of phenolic compounds and flavonoids [Hassanein et al. 2014]. Naghiloo et al. [2012] report that the content of phenolic compounds and antioxidant activity of *Astragalus compactus* L. (Fabaceae) leaf extracts increase starting from vegetative stage through flowering stage and reach the maximum level during fruiting stage. These authors explain the phenomenon of accumulation of antioxidant compounds by the plant under the influence of increased temperature and strong insolation by the need to protect tissues against radiation and/or against animals. The aim of the present study was to determine the effect of harvest date of *S. montana* herb and plant age on fresh and dry herb yield as well as on the content of L-ascorbic acid, carotenoids, chlorophylls, essential oil, flavonoids and tannins. In our opinion, these studies are interesting not only from the scientific but also practical point of view, and may be helpful in determining the best time to harvest the herb of winter savory.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm of the University of Life Sciences in Lublin (51°23'N, 22°56'E) over the period 2007–2009. The experiment was set up as a two-factor experiment with a randomized block design in four replicates; the plot area was 2.52 m^2. Weather conditions during the growing season are shown in Fig. 1. The study investigated the effect of ontogenetic variability (the following growth stages: vegetative stage, beginning of flowering, full flowering, senescent plants) and plant age-related variability (one- and two-year-old plants) on winter savory (*Satureja montana* L.) yield. *S. montana* seeds originated from the collection of the Botanical Garden of the Maria Curie-Skłodowska University in Lublin. The experiment was established using greenhouse-produced seedlings. Plants were planted in the field at the end of May at a spacing of 30 × 30 cm. After analysis of the chemical makeup of the soil (grey-brown podzolic soil derived from loess deposits with an organic matter content of 1.6%), nutrients were replenished at the following fertilizer rates on a per hectare basis: 60 kg N, 50 kg P_2O_5, and 100 kg K_2O. Soil loosening and manual weed control were carried out in the plantation, but due to the absence of pests or diseases no chemical plant protection treatment was performed.

S. montana herb from the one- and two-year-old plantation was cut at about 8 cm above soil level in the following months: June (vegetative stage), July (beginning of flowering), August (full flowering), and September (senescent plants). The beginning of flowering of *S. montana* occurred in the first and second 10 days of July. Full flowering of one-year-old plants occurred later (August 22–28) than in the two-year-old plantation (August 4–11). There were also differences in harvest date of senescent plants, which was associated with the varying duration of flowering of *S. montana* plants in the individual years of the study. In the second year of cultivation, plants senesced earliest in 2009 (September 3), in 2007 seven days later, whereas in 2008 fifteen days later. Plants in the one-year-old plantation entered the flower senescence stage earliest in 2008 (September 12) and latest in 2007 (September 20). The harvested raw material was dried in a drying oven at a temperature of 30°C for about 10 days. Having dried the raw material, dry herb yield and yield of herb without stems were determined;

subsequently, after the dried herb was passed through a 3 mm mesh sieve, the percentage of herb without stems in dry herb was estimated.

Essential oil content in herb without stems was determined distilling directly 30 g of dried raw material from 400 ml of water in a 1000 ml flask for 2 hours [European Pharmacopoeia 6.0 2008]. Essential oil yield was determined according to the formula given by Sahzabi et al. [2010].

Chlorophyll and carotenoids extraction was performed by homogenization of 0.5 g fresh plant material with 50 ml 80% (v/v) aqueous acetone. After the extraction the samples were then filtered through the filter paper. The filtrate was quantitatively transferred to 50 ml flask and completed to 50 ml volume with acetone. The filtrate was quantitatively transferred to 50 ml flask and completed to 50 ml volume with acetone. Analyses of samples on the content of total carotenoids and chlorophylls a and b was carried quantified spectrophotometrically using a HITACHI spectrophotometer (Model U-2900). Absorbances of carotenoids and chlorophylls a or b extracts were determined at wavelengths of 470, 663 and 645 nm, respectively. The contents of chlorophyll A (C_A) chlorophyll B (C_B) and carotenoids (C_{X+C})were calculated according to the method of Charłampowicz [1966].

$$C_A = \frac{12.25A_{663.2} - 279A_{646.8}}{FW}$$

$$C_B = \frac{21.5A_{646.8} - 5.1A_{663.2}}{FW}$$

$$C_{x+c} = \frac{(1000A_{470} - 1.82_{CA} - 85.02_{CB})/198}{FW}$$

where: $A_{648.6}$ = Absorbance at 648.6 nm; $A_{664.2}$ = Absorbance at 664.2 nm; A_{470} = Absorbance at 470 nm; FW = fresh weight of plant tissue extracted (μg).

Determination of L-ascorbic acid: The fresh and comminuted plant material (10 g) were extracted twice for 30 min with 2.5 ml 4.0% (m/V) L-cysteine and 10.0 ml water by sonification. All aqueous extracts were combined and diluted with water to 25 ml. The samples were analyzed Using high performance liquid chromatography. Analyses were done with an LaChrom-Merck HPLC system with

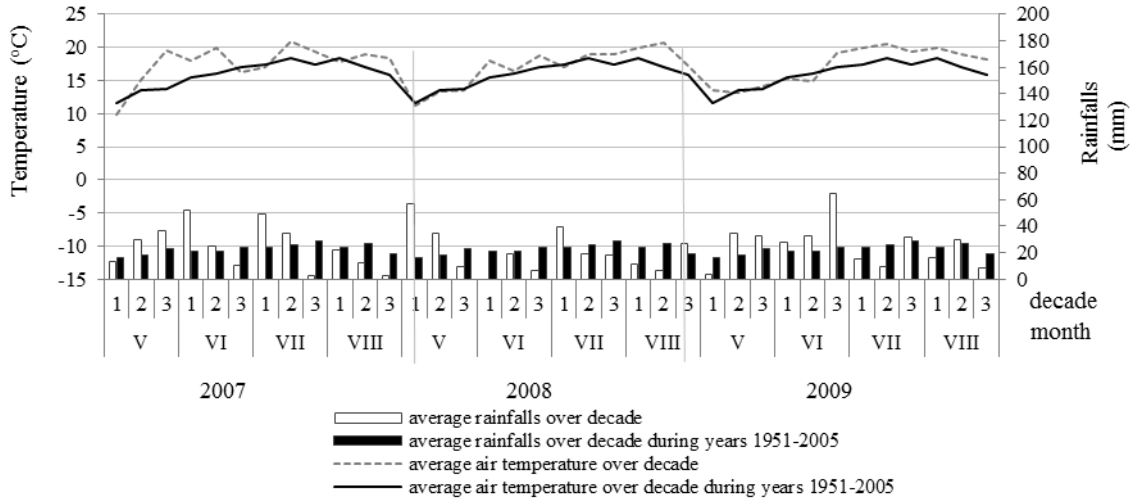

Fig. 1. Weather conditions during the *S. montana* growing season background multiannual data

a photodiode array detector (DAD L–7450), and all separations were on a Lichrospher 100 RP18 column (250.0 × 4.0 mm, 5.0 μm; Merck). The mobile phase consisted of 0.0272 g L^{-1} KH_2PO_4 adjusted to pH 2.40 with H_3PO_4, applied in isocratic elution for 30 min. The flow rate was adjusted to 1.0 ml/min. The detection wavelength was set to DAD at λ = 254.0 nm. 20.0 μL samples were injected. All separations were performed at 24.0°C. Peaks were assigned by spiking the samples with standard compounds and comparing the UV spectra and retention times (ascorbic acid 5.66 min). Calibration curves were obtained from 5 concentrations of each external standard (0.01–1.20 mg mL^{-1}). The regression coefficient (R_2) of the calibration curve for ascorbic acid (Y = 83780x – 19.693). The RSD values for the repeatability (n = 4) of standard solution were 0.40% (0.01 mg ml^{-1} ascorbic acid). The limits of quantitation (LOQ) and detection (LOD) of ascorbic acid were 0.18 and 0.06 mg L^{-1}, respectively. All solvents used were HPLC grade (Merck). Reference standards were obtained from Sigma-Aldrich.

Estimation of flavonoids was carried out according to the Christ and Müller method described by Polish Pharmacopoeia 8.0 [2008] as recommended by the European Pharmacopoeia 6.0 [2008]. For this purpose, 0.5 g dried and comminuted plant material were added to a round-bottomed flask, 20 ml of acetone, 2 ml of HCl (281 g L^{-1}), and 1 ml of methenamine (5 g L^{-1}) were then added and the mixture was maintained for 30 min under reflux in a water bath. The hydrolysate was filtered through into a 100-ml volumetric flask, then placed in a flask together with the filter and re-added 20 ml of acetone, and refluxed for 10 min. Next, 20 ml of solution were dispensed into a separatory funnel with 20 ml of water and extracted with ethyl acetate in 15-ml portions 3 times with 10 ml. The combined organic layers were washed twice with 40 ml of water, filtered into a 50 ml volumetric flask, and supplemented with ethyl acetate. To determine the flavonoid content, 2 samples were prepared: 2 ml of a solution of $AlCl_3$ (20 g L^{-1}) supplemented with a mixture (1 : 19) of acetic acid (1.02 kg L^{-1}) and methanol (25 ml) were added to 10 ml of the stock solution. To prepare the comparative solution, the stock was supplemented with 10 ml of a mixture (1 : 19) of acetic acid (1.02 kg L^{-1}) and methanol (25 ml). After 45 min, the absorbance of the solutions was read at λ = 425 nm on a HITACHI U-2900 spectrophotometer using the reference solution for comparison. The samples were analysed in 3 replicates. The total content of flavonoids (mg 100 g^{-1}) was expressed as quercetin equivalent QE according.

Total tannin content was determined by the weight method with hide leather powder according to the European Pharmacopoeia 6.0 [2008]. according to the Polish Pharmacopoeia 6.0 (2006). The results were statistically analyzed by two-way analysis of variance (ANOVA), determining the significance of differences at 0.05. Correlation coefficients were determined at significance levels of 0.01 and 0.05 [Oktaba 1986].

RESULTS AND DISCUSSION

Significant variation was revealed in fresh and dry *S. montana* herb yield depending on growth stage at harvest (tab. 1). As the plants progressed to the successive growth stages, they were characterized by a higher fresh and dry herb yield. Moreover, two-year-old plants produced a significantly higher fresh and dry herb yield compared to one-year-old plants. The highest fresh and dry herb yield was obtained when the raw material was harvested at senescence stage (respectively: 112.15 and 38.88 kg·100 m^{-2}) and in the second year of cultivation (respectively: 88.38 and 30.10 kg·100 m^{-2}). The interaction of the ontogenetic factors studied was also shown to have a significant effect on fresh and dry *S. montana* herb yield. Similar relationships were demonstrated by Baranauskiene et al. [2011] in the case of sage, who found fresh and dry herb yield to continuously increase until the time of seed maturation when these parameters significantly decreased, and by Zawiślak [2014] who proved that sage harvested in August gave a higher yield than that collected in May. In the case of hyssop, the fresh and dry herb yield was highest after plant senescence compared to the other growth stages [Zawiślak 2011].

The study also found a significant increase in the yield of herb without stems with plant growth (tab. 2). The highest yield of herb without stems was obtained in the case of harvest of full flowering plants, compared to the other ones. The percentage of herb without stems in dry herb is an important element that determines raw material quality and production profitability. Dry *S. montana* herb that contains a lot of stems, which are usually hard and thick, does not have a high marketable value and is rejected in the production of spice raw materials due to its low quality. The present study demonstrated that despite the dry herb yield obtained from plants at vegetative stage was lowest, the percentage of herb without stems in dry herb was highest at this stage – 68.29% (tab. 2). The dry herb obtained from plants at the next growth stages was characterized by a gradual increase in the proportion of stems. A study on hyssop [Zawiślak 2011] reveals that the yield of herb without stems increases with plant growth, whereas the per-

Table 1. Herb yield of *S. montana* (kg·100 m^{-2}) (mean from 2007–2009)

Growth stage (A)	Fresh herb			Dry herb		
	Plant age (B)					
	1 year old	2 years old	Mean	1 year old	2 years old	Mean
Vegetative	17.24	51.43	34.33	4.74	18.02	11.38
Beginning of flowering	55.37	80.76	68.06	16.26	25.15	20.70
Full flowering	102.83	107.85	105.34	29.95	35.37	32.66
After flowering	110.82	113.49	112.15	35.87	41.88	38.88
Mean	71.56	88.38	79.97	21.70	30.10	25.90
$LSD_{0.05}$						
Growth stage (A)			5.340			2.453
Plant age (B)			2.827			1.298
A×B			9.063			4.163

Table 2. Yield of herb without stems and share of herb without stems in dry herb of *S. montana* (mean from 2007–2009)

Growth stage (A)	Herb without stems (kg·100 m^{-2})			Herb without stems in dry herb (%)		
	Plant age (B)					
	1 year old	2 years old	Mean	1 year old	2 years old	Mean
Vegetative	3.41	11.65	7.53	71.94	64.65	68.29
Beginning of flowering	10.49	17.72	14.10	64.51	70.45	67.48
Full flowering	17.12	19.01	19.73	57.49	57.50	57.49
After flowering	12.89	17.18	15.03	56.98	45.39	51.18
Mean	12.89	17.18	15.03	59.40	57.07	58.23
$LSD_{0.05}$						
Growth stage (A)			1.502			
Plant age (B)			0.795			
A×B			2.549			

Table 3. Correlation between some morphological characteristics of *S. montana* plants and herb yield (mean from 2007–2009)

Specification	Correlation coefficient	
	Yield of fresh herb	Yield of herb without stems
	1 year old plants	
Yield herb without stems	0.973**	–
Essential oil content	–0.012	–0,077
	2 years old plants	
Yield herb without stems	0.929**	–
Essential oil content	0.256	0.246

**correlation significant at $\alpha = 0.01$; *correlation significant at $\alpha = 0.05$

Table 4. L-ascorbic acid and carotenoids content (mg·100 g^{-1} fresh weight) in *S. montana* herb (mean from 2007–2009)

Growth stage (A)	L-ascorbic acid			Carotenoids		
	Plant age (B)					
	1 year old	2 years old	Mean	1 year old	2 years old	Mean
Vegetative	19.80	25.66	22.73	16.91	16.38	16.64
Beginning of flowering	27.98	31.31	29.64	21.52	17.12	19.32
Full flowering	26.28^{B}	30.66	28.47	16.33	19.16	17.74
After flowering	23.43	22.04	22.73	17.81	18.08	17.94
Mean	24.37	27.41	25.89	18.14	17.68	17.91
$LSD_{0.05}$						
Growth stage (A)			3.073	1 year old		2.396
Plant age (B)			1.609			n. s.
A×B			5.258			4.101

centage of herb without stems in dry herb is highest at vegetative stage and decreases until senescence stage. In the present study, the percentage of herb without stems in dry herb was very similar in the raw material harvested from one- and two-year-old plants, respectively 59.40% and 57.07%. A significant positive correlation was demonstrated between yield of herb without stems and fresh herb yield, both in one- and two-year-old plants (tab. 3). It can be concluded based on these data that technological quality of *S. montana* herb, as determined by the percentage of stems in the herb, is primarily related to plant growth, not plant age.

The L-ascorbic acid content in herbs is from 18.51 to 27.05 mg·100g^{-1} of fresh weight (FW) [Dumbravă et al. 2012]. The average L-ascorbic acid content in the *S. montana* herb studied was 25.89 mg·100g^{-1} FW and significantly dependent on growth stage at harvest, plant age and the interaction of these factors (tab. 4). More of this compound was determined in the herb cut at the beginning of flowering (29.64 mg·100g^{-1} FW) and at full flowering (28.47 mg·100g^{-1} FW) than in the raw material obtained during the other stages. The herb of two-year-old plants had a significantly higher content of L-ascorbic acid than the herb of one-year-old plants.

Likewise, hyssop plants are characterized by the highest L-ascorbic acid content (31.19 mg·100g^{-1} FW) in the herb harvested at the beginning of flowering [Zawiślak 2011]. Baranauskiene et al. [2011] showed that the L-ascorbic acid content in sage herb increases with plant growth, reaching the maximum level (17.6 mg·100g^{-1} FW) right before flowering. Harvest date also affects the L-ascorbic acid content in summer savory grown for bunching [Dzida et al. 2015]. A high vitamin C level in the plant is primarily associated with light intensity and temperature during the growing season [Lee and Kader 2000]. Analyzing the obtained results, it can be concluded that the L-ascorbic acid content in leaves of herbal plants depends not only on weather conditions during the growing season, but also on growth stage and plant age at harvest.

The study showed growth stage of *S. montana* plants to have a significant effect on the carotenoid content in the herb, which was on average 17.91 mg·100g^{-1} FW and was not dependent on plant age (tab. 4). Nevertheless, the interaction of growth stage and plant age was found to significantly affect the accumulation of carotenoids in the herb (fig. 2). The carotenoid content in *S. montana* herb increased from vegetative stage until the beginning of flowering (maximum content – 19.32 mg·100g^{-1} FW), and subsequently decreased during and after flowering. Similar results have been found for hyssop [Zawiślak 2011] and basil [Politycka and Golcz 2004]. Carotenoids protect photosynthesizing organisms against potentially harmful photo-oxidation processes leading to membrane and protein damage. In plants, some of these compounds are the precursors of abscisic acid, a phytohormone that modulates developmental and stress processes [Bartley and Scolnik 1995, Cazzonelli 2011]. Research on the content of carotenoids in fruits reveals that these compounds are intensively synthesized during the first stages of fruit formation, whereas after ripening their concentration distinctly decreases, which is probably related to enzymatic degradation or bioconversion of these compounds [Mendes-Pinto 2009]. Fraser et al. [1994] proved an increased level of carotenoids in ripening fruits and developing leaves of tomato. The varying content of these compounds is also attributable to genetic variability. Analyzing various mint varieties, Straumite et al. [2015] showed the highest level of carotenoids in stems and leaves in *Mentha spicata* 'Marokko' (respectively: 16.9 and 10.3 mg 100 g^{-1}). Some data indicate that root carotenoid content depends on plant age [Ortiz et al. 2011]. It should be presumed that the varying level of carotenoids in various plant raw materials primarily depends on the plant's organ, performing different physiological functions, as well as on light and thermal conditions during harvest.

The chlorophyll content in the *S. montana* herb studied was significantly dependent on the investi-

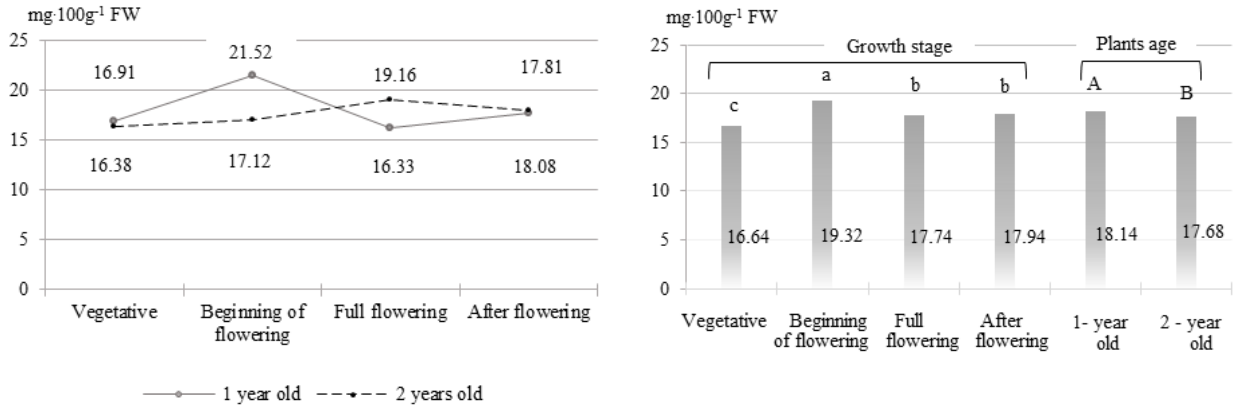

Fig. 2. *S. montana*: Seasonal changes in total amount of carotenoids (mean from 2007–2009)

Table 5. Chlorophyll content (mg·100 g^{-1} fresh weight) in *S. montana* herb (mean from 2007–2009)

Growth stage (A)	Chlorophyll A			Chlorophyll B			Chlorophyll A+B		
	Plant age (B)								
	1 year old	2 years old	Mean	1 year old	2 years old	Mean	1 year old	2 years old	Mean
Vegetative	33.37	45.50	39.43	17.10	24.07	20.58	50.48	69.52	60.02
Beginning of flowering	34.40	51.22	42.81	16.01	18.09	17.05	50.41	69.31	59.86
Full flowering	32.80	58.95	45.87	17.15	23.96	20.55	49.95	82.91	66.43
After flowering	28.29	42.62	35.45	11.21	18.14	14.67	39.50	60.76	50.13
Mean	32.21	49.57	40.89	15.36	21.06	18.21	47.58	70.64	59.11
$LSD_{0.05}$									
Growth stage (A)			5.471			4.915			8.923
Plant age (B)			2.866			2.574			4.674
A×B			9.362			n. s.			n. s.

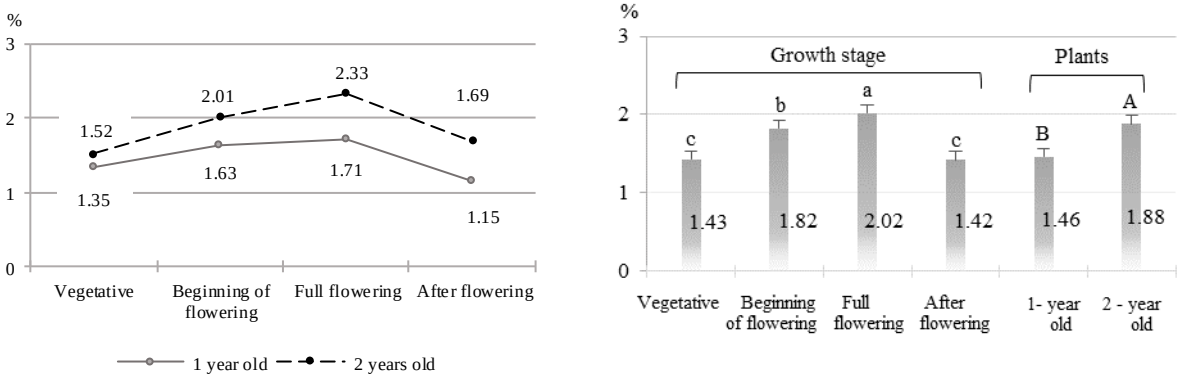

Fig. 3. *S. montana*: Seasonal changes in the essential oil content (mean from 2007–2009)

gated factors and their interactions (chlorophyll a) as well as on growth stage and plant age (chlorophyll b and total chlorophyll a and b) (tab. 5). Taking into account the ontogeny of the *S. montana* plants studied, senescent plants contained significantly the least chlorophyll a + b (50.13 mg·100g^{-1} FW). No significant differences were found in the content of this pigment in plants at the earlier growth stages. With increasing plant age, the level of these compounds significantly increased. The chlorophyll a + b content in two-year-old *S. montana* was higher by 32.64% than in one-year-old *S. montana*, chlorophyll a – higher by 35.02%, while chlorophyll b – by 27.06%. The raw material studied had a higher proportion of chlorophyll a (40.89 mg·100g^{-1} FW) than chlorophyll b (18.21 mg·100g^{-1} FW). This is in agreement with other information concerning the level of chlorophylls in leaves of plants of the family Lamiaceae [Dumbravă et al. 2012, Śledź et al. 2012, Straumite et al. 2015] and concerning the relationship between plant growth stage and their concentration [Zawiślak 2011]. Differences in the leaf content of pigments (chlorophyll and carotene) and their rela-

tionships may result from internal factors (physiological transformations) and external factors (habitat conditions). Chlorophyll content may change in response to biotic and abiotic stress such as pathogen infection or light stress [Ndukwe et al. 2016, Živanović et al. 2017]. During ripening of tomato fruits, total carotenoid content increases with decreasing chlorophyll concentration [Fraser et al. 1994]. The results of Ndukwe et al. [2016] reveal the existence of a positive correlation between chlorophyll a content, chlorophyll b content, total chlorophyll content (a + b), and carotenoids in maize leaves.

Essential oil is the major biologically active substance in winter savory raw material. The essential oil content determines the aroma of a raw material and significantly affects its quality and use. The *S. montana* herb studied contained on average 1.67% of essential oil. Essential oil content was significantly affected by plant age and growth stage as well as by their interaction (fig. 3). A similar oil level (1.7%) was determined by Bezić et al. [2009] in plants growing in the mountains of Biokovo (Croatia). García-Rellán et al. [2015], on the other hand, found a lower essential oil content (0.79–0.85%) in the herb of *S. montana* growing in the area of Valencia (Spain). These differences were probably related to other variability factors, but the essential oil content obtained should be considered to be high, in particular under temperate climate conditions. The *S. montana* plants studied were characterized by an increase in the oil content in the herb from vegetative stage to full flowering stage (the maximum oil content was 2.02%). At senescence stage, the oil content decreased to the level determined at vegetative stage (respectively: 1.42 and 1.43%). Mastelić and Jerković [2003] showed a higher essential oil content in *S. montana* herb before flowering (1.46%) than during flowering (0.80%). Collecting the raw material from June to August, Dudaš et al. [2013] found a decrease in the oil concentration in *S. montana* herb with plant growth. Essential oil content changes throughout the plant growth period [Sangwin et al. 2001, Uyanik and Gurbuz 2015, Lee and Ding 2016]. Tahmasebi et al. [2016] obtained most essential oil in the herb of *Origanum vulgare* L. and *O. majorana* L. at flowering stage. Angioni et al. [2009] demonstrated that the essential oil yield obtained from flowers of *Lavandula stoechas* L. ssp. *stoechas* decreases from the beginning until the end of the flowering stage, whereas the oil yield extracted from leaves remains the same throughout the entire year. Latto et al. [2006] report that in *Salvia sclarea* L. (Lamiaceae) the essential oil content is low at flower bud stage, it is highest at full flowering, and then decreases quickly during maturation. Plants in the second year of cultivation accumulated significantly more oil (1.88%) than one-year-old plants (1.46%) (fig. 3). Similar results were obtained for the oil content in the individual years of the study. Essential oil content was not correlated with fresh herb yield and yield of herb without stems both in the first and second year of cultivation (tab. 3). Research on lemon balm [Nurzyńska-Wierdak et al. 2014] and lemon grass [Rocha et al. 2014] shows that plant age may cause differences in the content of essential oil to a lesser extent than in its chemical composition. Santos et al. [2012], on the other hand, demonstrated that in the case of harvest of *Alpinia zerumbet* (Zingiberaceae) leaves, with increasing age there is a significant increase in plant biomass as well as in essential oil content and yield. These differences can be explained by the specificity of this plant species and the type of raw material. Transport of essential oils from one region to another one in a plant cell or outside the plant body occurs with the participation of various mechanisms. In the case of many plant species of the families Lamiaceae, Poaceae and Asteraceae, leaf development has been shown to be closely correlated with essential oil biosynthesis and accumulation [Rehman et al. 2016]. El-Zaeddi et al. [2016] showed that harvest date and related plant age as well as plant developmental stage modify essential oil content and composition as well as sensory qualities of raw material.

Table 6. Bioactivity component content (% dry weight) in *S. montana* herb (mean from 2007–2009)

Growth stage (A)	Flavonoids			Tannins		
	Plant age (B)					
	1 year old	2 years old	Mean	1 year old	2 years old	Mean
Vegetative	0.34	0.33	0.33	0.82	1.12	0.97
Beginning of flowering	0.44	0.53	0.48	1.16	1.39	1.27
Full flowering	0.50	0.52	0.51	1.22	1.25	1.23
After flowering	0.43	0.33	0.38	1.05	1.16	1.10
Mean	0.42	0.43	0.42	1.06	1.23	1.41
$LSD_{0.05}$						
Growth stage (A)			0.034			0.144
Plant age (B)			n. s.			n. s.
A×B			0.059			n. s.

On average, the *S. montana* herb contained 0.42% of flavonoids. The content of the above-mentioned compounds was significantly dependent on growth stage and the interaction of growth stage and plant age (tab. 6). It was found that the flavonoid content in *S. montana* herb increases from vegetative stage until full flowering stage (maximum content 0.51%), and subsequently it decreases at senescence stage. This confirms the results obtained for hyssop [Zawiślak 2011]. In the case of *Hypericum origanifolium* Willd, Çirak et al. [2007] found a strict relationship between the content of chemical compounds and growth stages during the phenological cycle. These authors obtained the highest content of quercetin and hypericin in St. John's wort herb at flower bud and full flowering stages, concluding that for medicinal purposes the raw material should be harvested during flowering stage when the content of bioactive substances reaches the highest level. Ozkan et al. [2010] showed the highest percentage of rutin, hesperidin and luteolin in the herb of *Origanum onites* L. at full flowering stage, whereas the highest percentage of apigenin and genistein was found at fruiting stage. Changes in the content of flavonoids in aerial parts of plants during growth should also be associated with the photoprotective role of these compounds. Živanović et al. [2017] demonstrated the highest accumulation of epidermal flavonoids in the leaves of field-grown tomato plants compared to plants grown in polytunnels covered with polyethylene plastic that reduces photosynthetically active radiation as well as UV-A and UV-B radiation. These authors conclude that significant accumulation of flavonoids in leaves improves overall plant resistance to environmental conditions during maturation.

Tannins, secondary metabolites that occur most commonly in plants, are used in medicine as antidiarrhoeal, hemostatic and antihemorrhoidal drugs [Ashok and Upadhyaya 2012]. The physiological role of tannins has not been fully identified, but they are known to perform the role of antiseptics protecting plants against pathogens and herbivores as well as protective compounds against adverse environmental conditions [Close and McArthur 2002]. The average tannin content in the *S. montana* herb studied was 1.41% and significantly dependent on plant growth stage (tab. 6). Most tannins were determined in the herb harvested at the beginning of flowering (1.27%), whereas the herb obtained from plants at vegetative stage contained the least tannins (0.97%). Plant age and the interaction of the investigated ontogenetic factors did not cause significant differences in the tannin content in *S. montana* herb. The study by Zawiślak [2011] reveals that the level of tannins in

hyssop herb is highest at the beginning of flowering and at full flowering stage and subsequently decreases to the vegetative stage level. Mudau et al. [2007] demonstrated seasonal variability to significantly affect the content of condensed and hydrolyzable tannins in the leaves of *Athrixia phylicoides* DC. Jürgenson et al. [2012] proved that the tannin content in *Epilobium angustifolium* L. (Onagraceae) plants is highest in leaves collected in May, followed by flowers and fruits picked in July.

CONCLUSIONS

Ontogenetic factors largely affect the quantity and quality of *S. montana* yield. Harvest at senescence stage produces the highest fresh and dry herb yield as well as yield of herb without stems. A higher herb yield is obtained in the second year of cultivation compared to the first year. Technological quality of *S. montana* herb, which is related to the proportion of stems in the herb, is more associated with plant growth than with plant age.

The chemical composition of *S. montana* herb changes during its ontogeny. The herb harvested before flowering contains most L-ascorbic acid, carotenoids and tannins, whereas the herb collected during full flowering – most chlorophylls, essential oil and flavonoids. Two-year-old plants accumulate in the herb more L-ascorbic acid, chlorophylls and essential oil than one-year-old plants. In growing *S. montana*, it can be recommended that this herb should be harvested during the flowering period because at this time the raw material, which is primarily dry herb, contains most active compounds that determine its seasoning qualities.

ACKNOWLEDGMENT

The present research was supported by the Ministry of Science and Higher Education of Poland as a part of statutory activities of the Department of Vegetable Crops and Medicinal Plants, University of Life Science in Lublin. The authors would like to thank Dr Barbara Mysiak for help with field trials and to Mrs Klaudia Świca for analytical help.

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