

EFFECT OF CHOSEN AGRONOMIC FACTORS ON THE YIELD AND RAW MATERIAL QUALITY OF THREE POPULATIONS OF GREATER PLANTAIN (*PLANTAGO MAJOR* L.)

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Abstract

The effect of the time of seed sowing and herb harvesting on the yield and raw material quality of three populations of greater plantain (*Plantago major* L.) was assessed. Higher yield of air-dry herb was obtained from the plots where the seeds were sown in spring than from the plots where the seeds were sown in late spring. In all populations herb weight increased with delayed harvest time. The content of iridoid glycosides in dry herb ranged from 1395.0 to 2060.0 mg/100g. The highest content of iridoid glycosides was characteristic for the herb harvested at the stage of seed maturity. Content of polyphenolic acids ranged from 323.3 to 544.5 mg/100g, flavonoids from 350.0 to 540.0 mg/100g, tannins from 3.30 to 6.41%, and mucilages from 682.0 to 1151.0 mg/100g.

Key words: cultivation, sowing date, stage of plant development, phenolic compounds, mucilages

1. INTRODUCTION

Greater plantain (*Plantago major* L.) is an herbaceous perennial forming white rhizome with fibrous roots, a rosette of leaves, and flowering shoots up to 30 cm high, ended with the long dense spikes (Rumińska & Ożarowski 1990). Until the XIV century it only occurred in the Eastern hemisphere, but then it spread to other parts of the world. American Indians named this plant “a footprint of a white man” as it followed the colonisers (Samuelsen 2000). Nowadays, greater plantain commonly occurs all over the world. It prefers sunny places and the soils rich in nutrients. In Poland it grows in meadows, pastures, arable fields, roadsides, and wastelands. It is a component of the plant association *Festuco pratensis-Plantaginietum*. Botanical varieties of greater plantain occur in other associations, i.e. *Juncetum tenuis* (*P. major* var. *microstachya*), *Prunello-Plantaginietum* (*P. major* var. *brachystachya*) and *Lolio-Polygonetum arenastri* (*P. major* var. *typical*) (Matuszkiewicz 2001; Wysocki & Sikorski 2002). Numerous authors report that greater plantain protects the environment against the pollutions released in the reaction of fuel with oxygen during the work of car engines (Samuelsen 2000; Shepeleva, Baranov & Filimonova 2008). Medicinal properties of greater plantain have been known for ages. The raw materials used in phytotherapy are leaves or herb of this species (*Plantaginis majoris folium/herba*) collected from spring to autumn. The most important biologically active compounds in these raw materials are iridoid glycosides (incl. aucubin, asperuloside, catalpol, plantarenalloside, and geniposidic acid) (Ravn & Brimer 1988; Rønsted et al. 2000; Zubair 2010), flavonoids (baicalein, hispidulin, scutellarein, and plantagin) (Kandaswami & Middleton 1994; Samuelsen 2000), phenylpropanoid glycosides (verbascoside and plantamajoside) (Andrzejewska-Golec 2010) and mucilages. The latter are composed of arabinose (38%), galactose (49%), glucose, xylose, rhamnose (6%) and galacturonic acid (7%) (Gorin 1966; Samuelsen et al. 1998; Hetland et al. 2000). Sabirov & Khagi (1985) isolated the complex of polysaccharides and mineral compounds (Ca, Mg, K, Na) called plantaglucidum. Extracts from leaves or herb of great plantain has been used in the folk medicine in the inflammations of upper respiratory tract, bronchi, stomach and intestines, as well as in the case of wounds and abscesses (Samuelsen 2000; Chiang et al. 2003; Beara et al. 2009). There are also reports that different species of the genus *Plantago* may be useful in giving up smoking as they cause aversion to nicotine (Cody 2000). Nowadays the main species of *Plantago* used medicinally is ribwort plantain (*P. lanceolata*). It is collected both from natural sites and from the plantations. Greater plantain is collected in smaller amounts and only from the wild. It has been observed that the natural resources of this species decrease, mainly because of the human activity (house and industrial building, chemisation of agriculture, etc.). Therefore, like in the case of many other wild growing medicinal plants, there is a growing need to introduce it into cultivation. Plantations of the selected populations of this species may be the source of more uniform raw material preferred by the phytopharmaceutical industry.

The aim of this study was to determine the effect of the chosen agrotechnical parameters on the yielding and accumulation of biologically active compounds in the aboveground parts of plants representing three populations of greater plantain.

2. MATERIAL AND METHODS

2.1. Plant material

The experiment was carried out in 2012 and 2013. The object of the study were 3 populations of greater plantain (*Plantago major* L.) originating from the natural sites in Poland (P1 – Elsnerów, N 52°16.766' E 21°04.980'; P2 – Krzywda, N 51°50.156' E 22°07.232'; P3 – Antonowo, N 52°44.736' E 21°57.280') and maintained in the *ex situ* collection at the experimental field of the Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences – SGGW. The experiment was established in the randomised block design in 3 replications. The individual plot area was 5 m². The seeds were sown in two dates: spring (April) and late spring (June). Sowing rate was 3 kg/ha, and row distance 30 cm. The raw material (aboveground parts of the plants) was harvested at the vegetative stage of plant development (second decade of July/first decade of August), at the stage of full blooming (first decade of August/second decade of August), and at the stage of seed maturity (second decade of September/third decade of September). The yield of fresh and air-dry aboveground organs was determined. The plant material was dried in the drying chamber at 45°C.

2.2. Chemical analyses

In the air-dry raw materials the content of iridoid glycosides, mucilages, phenolic acids, tannins, and flavonoids was determined. With the exception of mucilages, the above mentioned compounds were determined spectrophotometrically (using UV-1700 PharmaSpec. spectrophotometer, Shimadzu), according to the methods described in the Polish Pharmacopoeia (Farmakopea Polska VIII, 2008). All analyses were made in 3 replications.

2.2.1. Determination of iridoid glycoside content

The powdered plant material (0.5 g) was extracted under reflux with 100 ml of distilled water and 0.3 g of calcium carbonate for 30 min. After cooling, the crude extract was filtered through 1.2 g of aluminium oxide. The content of iridoid glycosides, calculated as aucubin, was determined by measuring the absorbance of the colour reaction product of these compounds with 1% solution of 4-dimethylaminobenzaldehyde in ethanol and concentrated hydrochloric acid. The absorbance was measured at 590 nm. The reference sample was the water solution of aucubin (0.015 g/ml) with the above mentioned reagents added.

2.2.2. Determination of phenolic acid content

1.0 g of air-dry raw material was extracted twice with 25 ml of distilled water for 30 min. The combined extracts were supplemented with distilled water to 50 ml. The absorbance of the solution containing 1 ml of this extract, 1 ml of NaOH (40 g/l), 1 ml of HCl (18 g/l), 1 ml of Arnov's reagent, and 6 ml of distilled water was measured at 490 nm. The content of phenolic acids was calculated for caffeic acid.

2.2.3. Determination of tannin content

1.0 g of the raw material was extracted with 150 ml of distilled water under reflux for 30 min. The extract was supplemented to 250 ml and filtered. The total content of polyphenolic compounds and the content of polyphenols that do not bind hide powder was determined on the basis of the reaction with Folin reagent. The absorbance was measured at 760 nm. The reference solution of pyrogallol was used.

2.2.4. Determination of flavonoid content

1.0 g of the raw material was extracted with 20 ml of acetone, 2 ml of 25% HCl (for hydrolysis of flavonoid glycosides) and 1 ml of 0.5% methenamine under reflux for 30 min. The extraction was repeated twice with 10 ml of acetone. The combined extracts were supplemented to 100 ml. 20 ml of this extract was exhaustively extracted with ethyl acetate by shaking. The content of flavonoids, calculated for quercetin, was determined spectrophotometrically after the reaction with 2% AlCl₃. The absorbance was measured at 425 nm.

2.2.5. Determination of mucilage content

1.0 g of air-dry raw material was extracted with 20 ml of distilled water for 1 hour in a laboratory shaker (WU-4 type, Premed). The obtained extract was concentrated to 10 ml and filtered. Then, 40 ml of 1% acetic acid in ethanol was added and the sample was left for 6 hours. The precipitated mucilages were transferred into the weighed paper filter, washed with ethanol and ethyl ether, dried, and weighed on the analytical balance PS 2100/C/2 RADWAG.

2.3. Statistical evaluation of the results

The presented results are the mean values from the two-year experiment.

The results were subjected to statistical evaluation using ANOVA 3 programmes and Tukey's test at the significance level $\alpha=0.05$.

3. RESULTS

The highest yield of herb was obtained from the plants of the population P2 – Krzywda. This herb was also characterised by the lowest share of flowering shoots. The lowest yield of herb with the highest percentage of flowering shoots was characteristic for the population P3 – Antonowo. In the case of the populations P1 – Elsnerów and P2 – Krzywda higher yield of herb was obtained from the plantation established in spring, whereas for the population P3 – Antonowo late spring sowing appeared to be more favourable. The highest yield of herb was obtained from the plants harvested at the stage of seed maturity (Table 1 and 2).

Table 1. Yield of air-dry herb (g/m²)

Population	Time sowing	Time of harvest			Mean
		I	II	III	
P1	Spring	77.75	214.94	198.35	163.68
	Late spring	70.20	98.50	168.65	112.45
	Mean	73.98	156.71	183.50	138.07
P2	Spring	78.00	161.00	244.00	161.00
	Late spring	107.00	114.80	227.75	149.85
	Mean	92.50	137.90	235.88	155.43
P3	Spring	80.20	105.15	133.30	106.22
	Late spring	82.65	128.10	130.20	113.65
	Mean	81.43	116.63	131.75	109.94
Mean	Spring	78.65	160.36	191.88	143.63
	Late spring	86.62	113.80	175.53	125.32
Mean		82.64	137.08	183.71	

P1 – Elsnerów, P2 – Krzywda, P3 – Antonowo

A – population, B – time of sowing, C – time of harvest

LSD _{0.05} for A = 0.90	LSD _{0.05} for B = 0.61	LSD _{0.05} for C = 0.90
LSD _{0.05} for A/B = 1.27	LSD _{0.05} for B/A = 1.06	LSD _{0.05} for C/A = 1.56
LSD _{0.05} for A/C = 1.56	LSD _{0.05} for B/C = 1.06	LSD _{0.05} for C/B = 1.27
LSD _{0.05} for A/BC = 2.21	LSD _{0.05} for B/AC = 1.83	LSD _{0.05} for C/BA = 2.21

Table 2. Inflorescences share in the herb weight (%)

Population	Time sowing	Time of harvest			Mean
		I	II	III	
P1	Spring	0.0	11.8	20.4	10.7
	Late spring	8.9	14.8	15.9	13.2

	Mean	4.5	13.3	18.2	12.0
	Spring	0.0	11.6	17.9	9.8
P2	Late spring	4.7	13.6	16.8	11.7
	Mean	2.4	12.6	17.4	10.8
	Spring	10.0	12.0	21.7	14.6
P3	Late spring	8.9	17.2	24.8	17.0
	Mean	9.5	14.6	23.3	15.8
Mean	Spring	3.0	11.8	20.0	11.6
	Late spring	7.5	15.2	19.2	14.0
Mean		5.5	13.5	19.6	

P1 – Elsnerów, P2 – Krzywda, P3 – Antonowo

A – population, B – time of sowing, C – time of harvest

LSD_{0.05} for A = 0.4LSD_{0.05} for B = 0.3LSD_{0.05} for C = 0.4LSD_{0.05} for C/A = 0.7LSD_{0.05} for C/B = 0.6LSD_{0.05} for B/C = 0.5LSD_{0.05} for A/C = 0.7LSD_{0.05} for A/BC = 1.0LSD_{0.05} for B/AC = 0.8LSD_{0.05} for C/BA = 2.2

The highest content of iridoid glycosides in the herb was characteristic for the population P1 – Elsnerów, and the lowest – for the population P3 – Antonowo. With the exception of the population P3 – Antonowo, higher content of these compounds was found in the herb collected from the plantations established in late spring. The herb harvested at the stage of seed maturity was characterised by the highest content of iridoid glycosides. Only in the case of the plants of the population P2 – Krzywda obtained from late spring sowing and the population P1 – Elsnerów obtained from spring sowing higher content of these compounds was found at the stage of blooming (Table 3).

Table 3. Content of iridoid glycosides in air-dry herb (mg/100g)

Population	Time sowing	Time of harvest			Mean
		I	II	III	
	Spring	1615.0	1730.0	1660.0	1668.3
P1	Late spring	1510.0	1785.0	2060.0	1785.0
	Mean	1562.5	1757.5	1860.0	1726.7
	Spring	1455.0	1710.0	1740.0	1635.0
P2	Late spring	1700.0	1860.0	1680.0	1746.6
	Mean	1577.5	1785.0	1710.0	1690.8
	Spring	1560.0	1735.0	1925.0	1740.0
P3	Late spring	1395.0	1355.0	1720.0	1490.0
	Mean	1477.5	1545.0	1822.5	1615.0
Mean	Spring	1543.3	1725.0	1775.0	1681.1
	Late spring	1535.0	1666.7	1820.0	1673.7

Mean	1539.2	1695.8	1797.5
P1 – Elsnerów, P2 – Krzywda, P3 – Antonowo			
A – population, B – time of sowing, C – time of harvest			
LSD _{0.05} for A = 9.6	LSD _{0.05} for B = 6.5	LSD _{0.05} for C = 9.6	
LSD _{0.05} for A/B = 13.5	LSD _{0.05} for B/A = 11.2	LSD _{0.05} for C/A = 16.6	
LSD _{0.05} for A/C = 16.6	LSD _{0.05} for B/C = 11.1	LSD _{0.05} for C/B = 13.5	
LSD _{0.05} for A/BC = 23.42	LSD _{0.05} for B/AC = 19.4	LSD _{0.05} for C/BA = 23.4	

The content of phenolic acids in the herb ranged from 323.3 mg/100g (population P1 – Elsnerów, spring sowing, herb harvested at the stage of vegetative growth) to 544.5 mg/100g (population P3 – Antonowo, late spring sowing, herb harvested at the stage of blooming). The mean content of polyphenolic acids was the highest in the plants of the population P2 – Krzywda (438.0 mg/100g). Late spring sowing resulted in higher content of these compounds in the herb. Herb collected at the stage of blooming was characterised by the highest content of phenolic acids (Table 4).

Table 4. Content of polyphenolic acids in air-dry herb (mg/100g)

Population	Time sowing	Time of harvest			Mean
		I	II	III	
P1	Spring	323.3	499.8	426.7	416.6
	Late spring	365.1	497.7	439.5	434.1
	Mean	344.2	498.8	433.1	425.4
P2	Spring	353.9	533.7	380.0	422.5
	Late spring	444.8	476.7	438.7	453.4
	Mean	399.4	505.2	409.4	438.0
P3	Spring	382.1	380.1	385.7	382.6
	Late spring	407.3	544.5	390.5	447.4
	Mean	394.7	462.3	388.1	415.0
Mean	Spring	353.1	471.2	397.5	407.3
	Late spring	405.7	506.3	422.9	445.0
Mean		379.4	488.8	410.1	

A – population, B – time of sowing, C – time of harvest

P1 – Elsnerów, P2 – Krzywda, P3 – Antonowo

LSD _{0.05} for A = 1.9	LSD _{0.05} for B = 1.3	LSD _{0.05} for C = 1.9
LSD _{0.05} for A/B = 2.7	LSD _{0.05} for B/A = 2.3	LSD _{0.05} for C/A = 3.4
LSD _{0.05} for A/C = 3.4	LSD _{0.05} for B/C = 2.3	LSD _{0.05} for C/B = 2.7
LSD _{0.05} for A/BC = 4.8	LSD _{0.05} for B/AC = 3.9	LSD _{0.05} for C/BA = 4.8

The tannin content ranged from 3.30% (population P1 – Elsnerów, late spring sowing, herb collected at the stage of plant blooming) to 6.41% (population P2 – Krzywda, spring sowing, herb harvested at the stage of blooming).

The population P2 – Krzywda was distinguished by distinctly higher content of tannins (5.64%, on average) in comparison with two other investigated populations (Table 5).

Table 5. Content of tannins in air-dry herb (%)

Population	Time sowing	Time of harvest			Mean
		I	II	III	
P1	Spring	3.65	4.52	3.62	3.93
	Late spring	3.72	3.30	3.77	3.60
	Mean	3.69	3.91	3.70	3.77
P2	Spring	5.69	6.41	5.00	5.70
	Late spring	5.67	5.57	5.50	5.58
	Mean	5.68	5.99	5.25	5.64
P3	Spring	4.50	4.44	3.95	4.30
	Late spring	3.77	5.06	4.43	4.42
	Mean	4.14	4.75	4.19	4.36
Mean	Spring	4.60	5.12	4.19	4.64
	Late spring	4.39	4.64	4.57	4.53
Mean		4.50	4.88	4.38	

A – population, B – time of sowing, C – time of harvest

P1 – Elsnerów, P2 – Krzywda, P3 – Antonowo

LSD_{0.05} for A = 0.35

LSD_{0.05} for C = 0.35

LSD_{0.05} for A/B = 0.41

LSD_{0.05} for B/A = 0.41

LSD_{0.05} for C/A = 0.61

LSD_{0.05} for A/C = 0.61

LSD_{0.05} for A/BC = 0.86

LSD_{0.05} for B/AC = 0.71

LSD_{0.05} for C/BA = 0.86

Late spring sowing resulted in higher content of flavonoids in the herb of the studied populations, especially the population P3 – Antonowo. With the exception of this population, herb collected at the stage of blooming was characterised by slightly lower content of flavonoids than the raw material harvested at the stage of vegetative growth of the plants or at seed maturity (Table 6).

Table 6. Content of flavonoids in air-dry herb (mg/100g)

Population	Time sowing	Time of harvest			Mean
		I	II	III	
P1	Spring	460.0	400.0	400.0	420.0
	Late spring	490.0	440.0	480.0	470.0
	Mean	475.0	420.0	440.0	445.0
P2	Spring	430.0	380.0	490.0	433.3
	Late spring	480.0	430.0	450.0	453.3
	Mean	455.0	405.0	470.0	443.3
P3	Spring	350.0	370.0	360.0	360.0

	Late spring	500.0	540.0	530.0	523.3
	Mean	425.0	455.0	445.0	441.7
Mean	Spring	413.3	383.3	416.7	404.4
	Late spring	490.0	470.0	486.7	482.2
Mean		451.7	426.7	451.7	

A – population, B – time of sowing, C – time of harvest

P1 – Elsnerów, P2 – Krzywda, P3 – Antonowo

LSD _{0.05} for B = 12.3	LSD _{0.05} for C = 18.2	LSD _{0.05} for A/B = 25.7
LSD _{0.05} for B/A = 21.3	LSD _{0.05} for C/A = 31.5	LSD _{0.05} for A/C = 31.5
LSD _{0.05} for B/C = 21.3	LSD _{0.05} for C/B = 25.7	LSD _{0.05} for C/BA = 44.5
LSD _{0.05} for A/BC = 44.5	LSD _{0.05} for B/AC = 36.9	

There was no clear relation between the factors of the experiment and the content of mucilages in the herb. The highest content of these compounds was found in the herb harvested at the stage of vegetative growth from the plantation of the population P1 – Elsnerów established by late spring sowing, followed by the raw material harvested at the stage of blooming and seed maturity from the plantation of the population P2 – Krzywda established by spring sowing (Table 7).

Table 7. Content of mucilages in air-dry herb (mg/100g)

Population	Time sowing	Time of harvest			Mean
		I	II	III	
P1	Spring	974.0	881.5	840.0	898.5
	Late spring	1151.0	844.0	937.0	977.3
	Mean	1062.5	862.8	888.5	937.9
P2	Spring	936.5	1017.0	1000.5	984.7
	Late spring	967.0	730.0	975.5	890.8
	Mean	951.8	873.5	988.0	937.8
P3	Spring	682.0	860.5	916.5	819.7
	Late spring	940.5	897.0	989.0	942.2
	Mean	811.3	878.8	952.8	881.0
Mean	Spring	864.2	919.7	919.0	901.0
	Late spring	1019.5	823.7	967.2	936.8
Mean		941.9	871.7	943.1	

A – population, B – time of sowing, C – time of harvest

P1 – Elsnerów, P2 – Krzywda, P3 – Antonowo

LSD _{0.05} for A = 4.6	LSD _{0.05} for B = 3.1	LSD _{0.05} for C = 4.6
LSD _{0.05} for A/B = 6.5	LSD _{0.05} for B/A = 5.4	LSD _{0.05} for C/A = 8.0
LSD _{0.05} for A/C = 8.0	LSD _{0.05} for B/C = 5.4	LSD _{0.05} for C/B = 6.5

LSD_{0.05} for A/BC = 11.3LSD_{0.05} for B/AC = 9.2LSD_{0.05} for C/BA = 11.3

4. DISCUSSION

The effect of the time of establishing the plantation of greater plantain and the time of herb harvesting on the yield and quality of this raw material was investigated. Three populations of greater plantain were compared in respect of herb yield and quality as affected by the above-mentioned factors. The yield of herb and the content of biologically active compounds in this raw material were significantly affected by both the source of the raw material (population), time of plantation establishing and time of plant harvesting. However, the interactions between these factors were found.

The highest yield of herb was obtained from the population P2 – Krzywda (155 g/m², on average). Higher yield was obtained from the plantation established in spring (143.6 g/m², on average) than in late spring (125.3 g/m², on average). Such a relation was observed in the case of two of the investigated populations. There is no data in the literature on the effect of agrotechnical treatments on the yielding of greater plantain. In the case of other species the influence of the time of plantation establishing on the yield is not clear. Jadczak (2007) and Jadczak & Grzeszczuk (2008) report that higher yields of raw materials are usually obtained when later sowing term is applied. As regards the effect of the time of plant harvesting on the yield of herb, it was found that later harvesting is favourable. On the contrary, Dragland & Aslaksen (1995) and Kołodziej (2006) in the studies on ribwort plantain obtained the highest yield when the plants were collected at the vegetative stage of development. In the herb harvested later higher share of inflorescences was noted.

The herb of greater plantain was evaluated in respect of the content of phenolic compounds (iridoid glycosides, polyphenolic acids, flavonoids, and tannins) and mucilages. The presence of these compounds determines physiological activity of this raw material (Chiang et al. 2003; Budryn & Nebesny 2006; Türel et al. 2009). Iridoid glycosides are taken into consideration as a marker useful for chemotaxonomy of the genus *Plantago* (Rønsted et al. 2000). Some herbal raw materials (e.g. leaf of ribwort plantain) are standardised on the content of these compounds. Andrzejewska-Golec (2010) reports that the content of iridoid glycosides (calculated for aucubin) in the leaves of greater plantain ranges from 0.3 to 2.5% (300-2500 mg/100g). In the raw material analysed in the present study the content of iridoid glycosides ranged from 1355.0 to 2060.0 mg/100g. The investigated populations differed significantly in the content of these compounds in herb. The highest content of iridoid glycosides was characteristic for the plants of the population P1 – Elsnerów (1726.7 mg/100g, on average) and for the herb harvested at the stage of seed maturity (1797.5 mg/100g). Higher content of these compounds was found in the herb collected from the plantation established by spring sowing (1681.1 mg/100g, on average) in comparison to late spring sowing (1673.9 mg/100g). The content of polyphenolic acids, tannins, and flavonoids was also affected by all three factors of the experiment. The highest content of phenolic acids and tannins was characteristic for the population P2 – Krzywda (438.0 mg/100g and 5.64%, respectively). The content of phenolic acids in the investigated raw materials ranged from 323.3 to 544.5 mg/100g, and it was much lower (about 10 times) than that reported by Pourmorad, Hosseinimehr & Shahabimajd (2006). The highest content of polyphenolic acids was characteristic for the herb harvested at the stage of blooming and the lowest at the stage of vegetative growth. In the studies on stinging nettle (*Urtica dioica* L.) Rosłon & Węglarz (2003) found similar relations, whereas in the studies on sweet woodruff (*Galium odoratum* L.) highest content of polyphenolic acids was characteristic for the herb harvested at the vegetative stage of development (Węglarz & Rosłon 1998). The content of tannins in the investigated herb of greater plantain was slightly lower in comparison with that reported in the literature (Ghedira, Goetz & Le Jeune 2008). This parameter was affected by the source of the raw material (population) and time of plantation establishing. The lowest content of tannins (3.30%) was found in the herb of the plants of the population P1 – Elsnerów collected from the plantation established in late spring, and the highest – in the herb of the plants of the population P2 – Krzywda from the plantation established in spring (6.41%). Herb harvested at the stage of blooming was distinguished by slightly higher content of tannins than that collected at the stage of vegetative growth or at the stage of seed maturity. On the contrary, in the studies on sweet woodruff, herb collected during plant blooming was characterised by the lowest content of tannins (Osińska & Prusik 2004).

According to Pourmorad, Hosseinimehr & Shahabimajd (2006) flavonoid content in the leaves of greater plantain reaches 25 mg/g. Zubair et al. (2011) reported much lower content of these compounds (6.41 mg/g), more comparable to the results obtained in the present study (350.0-540.0 mg/100g). The content of these compounds in the herb of greater plantain was mainly affected by the time of plantation establishing and the time of harvest. Lower flavonoid content was characteristic for the raw material obtained from the plantations established in spring and for that harvested at the stage of plant blooming. Similar relations were observed for

sweet woodruff (Osińska & Prusik 2004).

An important group of biologically active compounds in the aboveground organs (leaves and seeds) of greater plantain are mucilages. They are partly responsible for expectorant, demulcent, and wound-healing properties of this herb (Samuelsen et al. 1995). According to Ghedira, Goetz & Le Jeune (2008) the content of these compounds ranges from 2.0 to 6.5%. In the herb investigated during the present experiment it was distinctly lower (682.0-1151 mg/100g) and was affected by all the experiment factors. The populations P1 – Elsnerów and P2 – Krzywda were characterised by similar mucilage content (937.9 and 937.8 mg/100g, respectively) and higher in comparison to the third investigated population (881.0 mg/100g, on average). The tendency to higher content of these compounds in the herb obtained from the plantations established by late spring sowing was observed.

5. CONCLUSIONS

1. The obtained results show intraspecific variability of *Plantago major* in respect of herb yield and the content of biologically active compounds (iridoid glycosides, phenolic acids, tannins, and mucilages) in this raw material.
2. The time of plantation establishing (seed sowing) and the time of plant harvesting determine the yield of greater plantain herb.
 - a. Spring sowing allow to obtain higher yield of herb in comparison to late spring sowing.
 - b. Late harvesting (at the stage of seed maturity) is the way to increase the yield of the raw material.
3. The content of biologically active compounds in the herb of greater plantain is also affected by the above-mentioned agrotechnical factors.
 - a. The herb harvested from the plantation established in spring is characterised by higher content of iridoid glycosides and tannins, but lower content of phenolic acids, flavonoids, and mucilages, in comparison with the raw material originating from the plantation established by late spring sowing.
 - b. The highest content of iridoid glycosides is characteristic for the herb harvested at the stage of seed maturity.
 - c. Herb collected at the stage of plant blooming is characterised by the highest content of phenolic acids and tannins, but the lowest content of flavonoids and mucilages.

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