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Yield and quality of plantain (*Plantago major* L.) herb in the second year of cultivation

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Abstract: *Yield and quality of plantain (*Plantago major *L.) herb in the second year of cultivation.* The aim of this study was to evaluate the effect of time of plantation establishment and time of harvesting on the quality of plantain herb in the second year of cultivation. Seeds for the establishment of a plantation were collected in 2011 from natural habitats located in Mazowsze, Lublin and Podlasie provinces. The seeds were sown in 2012 and 2013 in spring (April) and in late spring (June). The herb was harvested in 2013 and 2014, both when the plants were in the vegetative phase and during their flowering. Dry mass of herb per 1 m^2, as well as content of mucilages, iridoid glycosides, phenolic acids and flavonoids, were determined. A significantly higher mass of herb was obtained from plots where the seeds were sown in late spring than from those sown in mid spring. Plants collected in the flowering stage yielded a higher mass of herb than those collected in the vegetative phase. The highest content of iridoid glycosides (the main chemical compounds) was recorded on plots established in late spring. The herb from the population from Podlasie province contained significantly more of these compounds than those from Mazowsze and Lublin provinces.

Key words: plantain, cultivation, mucilages, iridoid glycosides, phenolic acids, flavonoids

INTRODUCTION

Plantain is a popular medicinal plant growing wild all over the world. It is a characteristic element of cosmopolitan, synanthropic and semi-synanthropic habitats. In Poland plantain occurs in gardens, on boundaries, meadows and pastures, overgrown roads, roadsides, dumps etc. It is also found among crops, where it is treated as an onerous weed. The main raw material of plantain consists of leaves harvested from natural sites during the flowering stage of the plant's development. The active substances are mucilages, iridoid glycosides (aucubin, catalpol, asperuloside etc.) [Ravn and Brimer 1988, Rřnsteda et al. 2000, Zubair et al. 2011], bitterness, tannins, organic and phenolic acids [Makhudov et al. 2011], saponins, minerals and fiber [Grandi et al. 1982, Samuelsen 2000, Andrzejewska-Golec 2010]. An important component of the herb is flavonoids: plantagin, quercetin, isorhamnetin, hyperoside, rutin, scutellarin [Lebedev-Kosov et al. 1976, Pourmorad et al. 2006, Kobeasy et al. 2011, Makhudov et al. 2011], luteolin, baicalein and baicalin, which affect the stimulation of mononuclear cells of peripheral blood, as well as apigenin and hispidulin, which have a protective action. Plantain has expectorant, anti-inflammatory, protective, diuretic and astringent effects. It is

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used internally for treatment of the gastrointestinal tract, diarrhea, and the upper respiratory tract, and externally for stomatitis, burns, wounds, pimples and boils (it has shielding properties and accelerates the healing process) [Ringbom et al. 1998, Noor et al. 2000, Samuelsen 2000]. Nowadays plantain is a species of less importance than ribwort plantain (in Poland both species provide equivalent raw material). Nevertheless, it is still an important medicinal plant and subject of research, especially in relation to its pharmacological activity. Research carried out around the world has revealed a number of new properties of this species. In studies on mice, for example, it was found to have a prophylactic effect against breast cancer [Samuelsen 2000]. Noor et al. [2000] found a positive effect of plantain extracts on fertility in men. It has also been shown that the terpenoids present in plantain inhibit the growth of tumors, as well as having hepatoprotective and hypolipernic properties [Ringbom et al. 1998].

Observations at natural sites show numbers of populations to be decreasing, largely due to human activity. For this reason an increasing number of wild species are being introduced into cultivation. Plantain is one of those species. Research relating to its cultivation has been carried out for several years. Plantain is a perennial plant, and so its cultivation can be carried out on a multiannual basis. Control of the process of growth and development of plants under cultivation means that the raw material obtained is controlled in terms of both morphological and chemical traits, and therefore meets the requirements of the pharmaceutical industry.

In the present study the effect of certain agronomic factors on the yield and quality of plantain herb harvested from a two-year-old plantation was evaluated.

MATERIAL AND METHODS

Experiment location

The experiment was carried out at the experimental field of the Department of Vegetable and Medicinal Plants of the Warsaw University of Life Sciences – SGGW. The study involved three populations of plantain (*Plantago major* L.) originating from natural sites in Poland (P1 – Mazowsze province, Elsnerów, N 52°16.766′ E 21°04.980′; P2 – Lublin province, Krzywda, N 51°50.156′ E 22°07.232′; P3 – Podlasie province, Antonowo, N 52°44.736′ E 21°57.280′).

Experiment procedure

Seeds for the establishment of a plantation were collected from the above sites in autumn 2011. Until sowing the seeds were stored in airtight seals in a cool, dark room. In 2012 and 2013 the seeds were sown at two times: spring (last 10 days of April) and late spring (first 10 days of June). The sowing rate was 3 kg·ha^{-1}, and the row distance was 30 cm. The individual plot area was 5 m^2. The experiment was established in a randomized block design in three replications.

Cultivation was continued for two years. In both years a dose of mineral fertilizers was applied per 1 ha: 30 kg N, 20 kg P and 40 kg K. Nitrogen fertilizers

were applied in early spring, and phosphorus and potassium fertilizers in the autumn, of the year preceding the experiment, and in the second year in early spring. In the first year only, nurturing work was carried out.

The raw material (above-ground parts of the plants) was harvested in 2013 (for the plantation established in 2012) and in 2014 (for the plantation established in 2013). Each plot was divided into two parts. From one part of the plot the raw material was collected at the vegetative stage of plant development (last 10 days of April), while on the other it was collected when the plants were at the stage of full blooming (last 10 days of May). Both parts of plot regrowth were also harvested (plants at harvesting were in the vegetative stage – first 10 days of July). The yield of air-dry above-ground organs was determined. The plant material was dried in a drying chamber at 45°C.

Chemical analyses

In the air-dry raw material, the content of mucilages was determined according to Klimek [1991], and the contents of iridoid glycosides, phenolic acids and flavonoids were determined according to the methods described in the Polish Pharmacopoeia (Farmakopea Polska VIII 2008). All analyses were made in three replications.

Determination of mucilage content

The air-dry raw material (1 g) was extracted with 20 ml of distilled water for 1 h in a laboratory shaker (WU-4 type, Premed). The 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 h. The precipitated mucilages were transferred to a weighed paper filter, washed with ethanol and ethyl ether, dried, and weighed on an analytical balance (PS 2100/C/2 RADWAG).

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 aluminum oxide. The content of iridoid glycosides, calculated as aucubin, was determined by measuring the absorbance of the color 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 a water solution of aucubin (0.015 $g \cdot ml^{-1}$) with the above-mentioned reagents added.

Determination of phenolic acid content

The air-dry raw material (1g) 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 a solution containing 1 ml of this extract, 1 ml of NaOH (40 $g \cdot l^{-1}$), 1 ml of HCl (18 $g \cdot l^{-1}$), 1 ml of Arnov's reagent and 6 ml of dis-

tilled water was measured at 490 nm. The phenolic acid content was calculated for caffeic acid.

Determination of flavonoid content

The raw material (1g) 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. Amount of 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% $AlCl_3$. The absorbance was measured at 425 nm.

Statistical evaluation of the results

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

The statistical analysis showed no significant differences between 2013 and 2014, hence the results presented are the mean values from the two-year experiment.

RESULTS AND DISCUSSION

Plantain (*Plantago major* L.) is a popular medicinal plant, naturally occurring in the temperate climatic zone. According to Wysocki and Sikorski [2002], in Poland this species can be found on farmland and in places that are heavily trampled and rich in nutrients. Due to the wide possibilities of using plantain herb, interest in the species is increasing. Research relating to its introduction into cultivation is also being conducted. In this study the effect of the time of establishment of the plantation and time of harvesting on the yield and quality of plantain herb was investigated. The study involved three populations of plantain (*Plantago major* L.) in the second year of cultivation.

Because research on the introduction of plantain into cultivation has been carried out only for a few years, there is still an absence in the available literature of information about the cultivation of that species. More information can be found about another plant of the genus *Plantago* – ribwort plantain (P. *lanceolata*) [Volák and Stodola 1983, Kołodziej 2006]. Assessment of yields of ribwort plantain has shown that between 0.8 and 2 t of leaves can be collected from 1 ha. Results obtained in this work for plantain are similar, especially in the case of material collected in the third and fourth harvesting stages (regrowth). For example, for the third harvesting stage the air-dry mass of herb was 170.7 $g \cdot m^{-2}$ (i.e. 1.7 $t \cdot ha^{-1}$). The air-dry mass of raw material depended on experimental factors. Higher air-dry mass was obtained from a plantation established in late spring (average 109.9$\cdot m^{-2}$) compared with that established in mid spring (average 90.7 $g \cdot m^{-2}$), and from the second harvest (plants in the flowering stage – average 69 $g \cdot m^{-2}$) compared with the first harvest (plants in the vegetative phase – average 48.6 $g \cdot m^{-2}$) – Table 1. The results confirm the observations of other

TABLE 1. Mass of air-dry herb ($g \cdot m^{-2}$)

Specification	Time of sowing	First harvest	Second harvest	Third harvest	Fourth harvest	Mean for population
P1	spring	45.0	66.0	187.0	88.0	96.4
	late spring	17.7	60.7	126.0	113.0	79.4
	mean					
	×	31.3	63.3	156.0	101.0	87.9
P2	spring	93.3	49.3	225.0	105.0	118.0
	late spring	37.0	76.0	160.0	93.3	91.6
	mean					
	×	65.2	62.7	193.0	99.0	105.0
P3	spring	35.3	22.7	115.0	59.0	58.2
	late spring	63.0	139.0	211.0	211.0	156.0
	mean					
	×	49.2	81.0	163.0	156.0	107.0
Mean for time of harvest		48.6	69.0	170.7	111.7	–
Mean for spring sowing		–	–	90.7	–	–
Mean for late spring sowing		–	–	109.0	–	–

P1 – population at Elsnerów, P2 – population at Krzywda, P3 – population at Antonowo; A – population, B – time of sowing, C – time of harvest; $NIR_{0.05}A = 3.19$; $NIR_{0.05}B = 2.17$; $NIR_{0.05}C = 4.06$; $NIR_{0.05}A/B = 4.42$; $NIR_{0.05}B/A = 3.76$; $NIR_{0.05}B/C = 4.34$; $NIR_{0.05}C/B = 5.75$; $NIR_{0.05}A/C = 6.39$; $NIR_{0.05}C/A = 7.04$; $NIR_{0.05}A/BC = 9.04$; $NIR_{0.05}B/A\,C = 7.51$; $NIR_{0.05}C/AB = 9.95$.

authors regarding the yields of other medicinal plants. Jadczak [2007] and Jadczak and Grzeszczuk [2008] report that a higher yield of herb is usually obtained at a later sowing, as well as at later collection of the raw material. Different results in studies of ribwort plantain were reported by Kołodziej [2006], who obtained a higher yield of herb when plants were collected in the vegetative phase. Cultivation of plantain may be continued for two or more years. In the second and subsequent years the herb may be collected twice or three times during the growing season. In the reported experiment, in both years material was collected only twice, because from the end of July the plants were strongly attacked by powdery mildew. The mass of the regrowth (third and fourth stage of harvesting) was clearly higher than the mass of herb collected in the first and second stages. For plots where the first harvest was made when the plants were in the vegetative phase (first stage), air-dry mass was three times lower than the mass of herb collected at the third stage, and when the plants were collected for the first time in the flowering phase (second stage) the mass of regrowth (fourth stage) was about 1.5 times higher. The total mass of herb (sum for harvest I + + III and harvest II + IV) ranged from + 161.5 to 216.0 $g \cdot m^{-2}$. Population P3 gave the highest air-dry mass of herb (average 214.3 $g \cdot m^{-2}$); a lower (but statistically insignificantly) mass was obtained for population P2; and a significantly lower mass of herb was obtained for population P1 (176.0 $g \cdot m^{-2}$) – Table 2.

TABLE 2. Influence of first cut on the total mass of air-dry herb ($g \cdot m^{-2}$)

Specification	First harvest + + regrowth	Second harvest + + regrowth	Mean for population
Population P1	188.0 c*	164.0 d	176.0 B
Population P2	258.0 a	161.5 d	209.8 A
Population P3	212.5 b	216.0 b	214.3 A
Mean for harvest	219.3 A	180.5 B	–

* Means marked with the same letter do not differ significantly at $\alpha = 0.05$.
Other symbols – see Table 1.

Studies of the chemical composition of the plantain herb show that these parts of the plant contain many groups of active compounds. The most important are mucilages and phenolic compounds (iridoid glycosides, polyphenolic acids, flavonoids etc.). Both polysaccharides and polyphenols have been suggested to act as bioactive compounds in this species. Mucilages are the most commonly available plant ingredients with a wide range of applications in the pharmaceutical and cosmetic industries. Literature data suggest that in the case of plantain, mucilages occur primarily in the seeds (up to 13%) [Andrzejewska-Golec 2010], but are also present in the herb. In the literature there is no information about the content of these compounds in the aforementioned raw materials, although the qualitative composition of this fraction of polysaccharides is well known [Gorin 1965]. The results obtained in this study show that the content of mucilages ranges from 3,630 (3.63%) to 9,690 $mg \cdot 100\ g^{-1}$ (9.69%), and depends on many factors, including the cultivated population and agronomic factors such as time of sowing and time of harvest. The highest content of mucilages was recorded in the herb from population P3 (average 7185 $mg \cdot 100\ g^{-1}$), and the lowest in population P1 (average 6812.5 $mg \cdot 100\ g^{-1}$). Significantly greater quantities of mucilages were detected in the raw material from spring-sown plots compared with those sown in late spring, and in the herb collected at the first harvesting stage compared with the third (regrowth from the first harvest) and fourth (regrowth from the second harvest) – Table 3. Observations that the mucilage content in plants is modified by many factors have been made by various authors. Kaewmanee et al. [2014], for example, reported great variation in the mucilage content of flaxseed between seven different populations. Similar observations were made by Klimek [1991] in studies of six species of the genus *Verbascum*. The influence of agronomic factors on the content of these compounds in the roots of marshmallow was studied by Andruszczyk and Wiśniewski [2006], who showed that this parameter is favorably affected by the establishment of the plantation from seedlings, compared with sowing and cultivation of the plant under cover of yarn.

Among the phenolic compounds in plantain, an important group is the iridoid glycosides. The presence of these compounds in the aerial parts of this species is confirmed by numerous authors [Rønsteda et al. 2000, Taskova et al. 2002, Jurišić et al. 2004, Jurišić-Grubesic et al. 2005; Stanisavljevic et al. 2008]. Studies on iridoid glycoside content in the spe-

TABLE 3. Content of mucilages in air-dry herb (mg·100 g^{-1})

Specification	Time of sowing	First harvest	Second harvest	Third harvest	Fourth harvest	Mean for population
P1	spring	9 050.0	9 460.0	3 630.0	4 090.0	6 557.5
	late spring	8 660.0	8 650.0	4 950.0	6 010.0	7 067.5
	mean					
	×	8 855.0	9 055.0	4 290.0	5 050.0	6 812.5
P2	spring	9 820.0	9 110.0	6 210.0	5 400.0	7 635.0
	late spring	8 070.0	8 060.0	4 340.0	5 620.0	6 522.5
	mean					
	×	8 945.0	8 585.0	5 275.0	5 510.0	7 078.8
P3	spring	9 690.0	7 760.0	8 930.0	7 210.0	8 397.5
	late spring	7 170.0	8 770.0	5 830.0	5 950.0	6 930.0
	mean					
	×	8 430.0	8 265.0	7 380.0	6 580.0	7 663.8
Mean for time of harvest		8 743.3	8 635.0	5 648.3	5 713.3	7185.0
Mean for spring sowing		–	–	7 529.8	–	–
Mean for late spring sowing		–	–	6 840.0	–	–

P1 – population at Elsnerów, P2 – population at Krzywda, P3 – population at Antonowo; A – population, B – time of sowing, C – time of harvest; $NIR_{0.05}A = 422.81$; $NIR_{0.05}B = 287.02$; $NIR_{0.05}C = 537.90$; $NIR_{0.05}A/B = 597.95$; $NIR_{0.05}B/A = 497.13$; $NIR_{0.05}B/C = 574.04$; $NIR_{0.05}C/B = 760.70$; $NIR_{0.05}A/C = 845.63$; $NIR_{0.05}C/A = 931.67$; $NIR_{0.05}A/BC = 1195.80$; $NIR_{0.05}B/AC = 994.27$; $NIR_{0.05}C/AB = 1317.58$.

cies have shown that the most important compound of this group is aucubin; therefore in this work the content of iridoid glycosides is expressed in terms of that compound. Andrzejewska-Golec [2010] reports that the content of iridoid glycosides in the leaves of plantain ranges from 0.3 to 2.5% (300 to 2,500 mg·100 g^{-1}). In the raw material analyzed in the present study the content of iridoid glycosides ranged from 1,37 to 2,01 mg·100 g^{-1}. The results are comparable to the content of iridoid glycosides in other species of the genus of *Plantago*. For *P. lanceolata*, for example, it amounts to 1,224 mg·100 g^{-1}, for *P. holosetum* 1,452 mg·100 g^{-1}, and for *P. atrata* 1,252 mg·100 g^{-1} [Jankovic et al. 2012]. According to Samulsen [2000] and Andrzejewska-Golec [2010], iridoid glycoside content depends on the plant's stage of development. The greatest amount of these compounds (mainly aucubin) in the aerial parts of plantain is found in June and July (over 1.3%). A similar pattern was observed by Rosłon et al. [2014] in research on plantain cultivation in the first year of vegetation. Results obtained in this work for biennial plants confirm the observations of those authors. A distinctly greater quantity of iridoid glycosides was found in the raw material collected in June (third and fourth harvesting stage) compared with that collected in April and May (first and second stage). The content of iridoid glycosides also significantly depends on when the plantation was established. A higher content of these compounds was determined in raw material harvested from plots established by late spring sowing

(1,725 mg·100 g^{-1}) compared with those sown in mid spring (1,645 mg·100 g^{-1}). The highest content of iridoid glycosides was found in population P1 (2,010 mg·100 g^{-1}), in the case of raw material from plots established in the late spring and at the fourth stage of harvesting, while the lowest was also obtained from population P1, from spring-sown plots and for material harvested at the first stage (1,440 mg·100 g^{-1}) – Table 4. Another group of phenolic compounds present in the above-ground organs of plantain is phenolic acids. They are also very important compounds in most of the plants of the genus *Plantago*. Jankovic et al. [2012], in studies of seven species of *Plantago*, demonstrated that the content of phenolic acids in this genus is from 1,670 (*P. atrata*) to 8,160 mg·100 g^{-1} (*P. lanceolata*). Pourmorad et al. [2006] in turn report that in the case of plantain the content of phenolic acids is about 600 mg·100 g^{-1}. In the present study the results were two to three times higher. The content of these compounds was from 1,290 to 2,260 mg·100 g^{-1} and was comparable to the content of phenolic acids in *P. atrata*, *P. coronopus* and *P. reniformis* [Jankovic et al. 2012]. The content of phenolic acids also depended on experimental factors. Population P2 had the highest average content of phenolic acids, while the lowest was found in population P1, and in the herb harvested from plots established in spring as opposed to late spring. As regards the time of harvesting, a distinctly greater quantity of phenolic acids was found in the raw material collected in the flow-

TABLE 4. Content of iridoid glycosides in air-dry herb (mg·100g^{-1})

Specification	Time of sowing	First harvest	Second harvest	Third harvest	Fourth harvest	Mean for population
P1	spring	1 540.0	1 590.0	1 800.0	1 860.0	1 697.5
	late spring	1 370.0	1 660.0	1 570.0	2 010.0	1 652.5
	mean					
	×	1 455.0	1 625.0	1 685.0	1 935.0	1 675.0
P2	spring	1 640.0	1 520.0	1 790.0	1 690.0	1 660.0
	late spring	1 650.0	1 690.0	1 980.0	1 790.0	1 777.5
	mean					
	×	1 645.0	1 605.0	1 885.0	1 740.0	1 718.8
P3	spring	1 440.0	1 490.0	1 690.0	1 690.0	1 577.5
	late spring	1 450.0	1 710.0	1 930.0	1 890.0	1 745.0
	mean					
	×	1 445.0	1 600.0	1 810.0	1 790.0	1 661.3
Mean for harvest time		1 515.0	1 610.0	1 793.3	1 821.7	1 685.0
Mean for spring sowing		1 645.0				
Mean for late spring sowing		1 725.0				

P1 – population at Elsnerów, P2 – population at Krzywda, P3 – population at Antonowo; A – population, B – time of sowing, C – time of harvest; $NIR_{0.05}A = 12.91$; $NIR_{0.05}B = 8.76$; $NIR_{0.05}C = 16.42$; $NIR_{0.05}A/B = 18.26$; $NIR_{0.05}B/A = = 15.18$; $NIR_{0.05}B/C = 17.53$; $NIR_{0.05}C/B = 23.23$, $NIR_{0.05}A/C = 25.82$; $NIR_{0.05}C/A = 28.45$; $NIR_{0.05}A/BC = 36.51$; $NIR_{0.05}B/AC = 30.36$; $NIR_{0.05}C/AB = 40.29$.

ering phase (second harvesting stage) compared with plants in the vegetative phase (first stage). The lowest quantity of these compounds was found in the herb from the fourth harvesting stage (Table 5). Comparing the results with those obtained by Rosłon et al. [2014] for one-year-old plants, it was observed that the content of phenolic acids in two--year-old plants was several times higher. Another group of polyphenols present in species of the genus *Plantago*, including plantain, is flavonoids [Pourmorad et al. 2006, Andrzejewska-Golec 2010, Makhudov et al. 2011]. Jurišić-Grubesic et al. [2007] report that in the *Plantago* genus flavonoid content depends on the species, ranging from 65 (*P. holosteum* subsp. *holosteum*) to 131 mg·100 g^{-1} (*P. maritima*). Pourmorad et al. [2006] report that, in the case of plantain, the content of flavonoids in the leaves is higher, at 2,500 mg·100 g^{-1}, whereas according to Zubair et al. [2011] and Makhudov et al. [2011] it is about 641 mg·100 g^{-1}. The results obtained in this study showed the content of flavonoids to range from 350 to 530 mg· ·100 g^{-1}. These results are lower than those reported by Paramount et al. [2006], but similar to those of Makhudov et al. [2011] and Zubair et al. [2011]. Experimental factors modified the flavonoid content in the tested raw material. A distinctly higher content of these compounds was found in the herb from a plantation established in late spring than in plants sown in mid spring. In the case of late spring sowing, the content of flavonoids was the highest for popu-

TABLE 5. Content of phenolic acids in air-dry herb (mg·100 g^{-1})

Specification	Time of sowing	First harvest	Second harvest	Third harvest	Fourth harvest	Mean for population
P1	spring	1 780.0	1 960.0	1 590.0	1 500.0	1 707.5
	late spring	1 530.0	2 260.0	1 580.0	1 290.0	1 665.0
	mean					
	×	1 655.0	2 110.0	1 585.0	1 395.0	1 686.3
P2	spring	1 750.0	2 180.0	1 960.0	1 710.0	1 900.0
	late spring	1 620.0	2 280.0	1 610.0	1 980.0	1 872.5
	mean					
	×	1 685.0	2 230.0	1 785.0	1 845.0	1 886.3
P3	spring	1 450.0	2 380.0	1 910.0	1 400.0	1 785.0
	late spring	1 300.0	2 080.0	1 700.0	1 460.0	1 635.0
	mean					
	×	1 375.0	2 230.0	1 805.0	1 430.0	1 710.0
Mean for time of harvest		1 571.7	2 190.0	1 725.0	1 556.7	1 760.8
Mean for spring sowing		–	–	1 797.5	–	–
Mean for late spring sowing		–	–	1 724.2	–	–

P1 – population at Elsnerów, P2 – population at Krzywda, P3 – population at Antonowo; A – population, B – time of sowing, C – time of harvest; $NIR_{0.05}A = 8.84$; $NIR_{0.05}B = 6.00$; $NIR_{0.05}C = 11.25$; $NIR_{0.05}A/B = 12.51$; $NIR_{0.05}B/A = = 10.40$; $NIR_{0.05}B/C = 12.01$; $NIR_{0.05}C/B = 15.91$; $NIR_{0.05}A/C = 17.68$; $NIR_{0.05}C/A = 19.49$; $NIR_{0.05}A/BC = 25.01$; $NIR_{0.05}B/AC = 20.80$; $NIR_{0.05}C/AB = 27.56$.

lation P3, and the lowest for population P1 (respectively 522.5 and 450.0 mg·100 g^{-1}). The time of harvesting does not significantly affect this parameter, but the highest content of flavonoids was recorded at the first harvesting stage, and the lowest at the fourth stage (Table 6).

spring and when harvesting takes place during the flowering stage, but given the opportunity to harvest the raw material twice during the season, a higher total mass of herb is obtained when the first harvest takes place in the vegetative phase. The time of plantation

TABLE 6. Content of flavonoids in air-dry herb (mg·100 g^{-1})

Specification	Time of sowing	First harvest	Second harvest	Third harvest	Fourth harvest	Mean for population
P1	spring	480.0	400.0	410.0	350.0	410.0
	late spring	510.0	450.0	480.0	400.0	460.0
	mean					
	×	495.0	425.0	445.0	375.0	435.0
P2	spring	440.0	390.0	500.0	370.0	425.0
	late spring	470.0	440.0	460.0	430.0	450.0
	mean					
	×	455.0	415.0	480.0	400.0	437.5
P3	spring	450.0	480.0	440.0	500.0	467.5
	late spring	500.0	550.0	530.0	510.0	522.5
	mean					
	×	475.0	515.0	485.0	505.0	495.0
Mean for time of harvest		475.0	451.7	470.0	426.7	455.8
Mean for spring sowing		–	–	434.2	–	–
Mean for late spring sowing		–	–	477.5	–	–

P1 – population at Elsnerów, P2 – population at Krzywda, P3 – population at Antonowo; A – population, B – time of sowing, C – time of harvest; $NIR_{0.05}A = 35.87$; $NIR_{0.05}B = 24.35$; $NIR_{0.05}A/C = 71.73$; $NIR_{0.05}C/A = 79.03$.

CONCLUSIONS

In this study the effect of the time of establishment of the plantation and the time of harvesting on the yield and quality of plantain herb was investigated. The results indicate that the cultivation of plantain can be carried out on a multiannual basis. In the second and subsequent years of cultivation, harvesting may be performed twice. A third harvest was not possible due to attack by powdery mildew. A higher mass of herb is obtained when the plantation is established in late

establishment and time of harvesting affect the content of active compounds in the herb. The herb harvested from a plantation established in mid-spring has a higher content of mucilages and phenolic acids, but a lower content of iridoid glycosides and flavonoids, than the material from the plantation sown in late spring.

The highest content of iridoid glycosides and phenolic acids was recorded for the herb harvested for the first time at the flowering stage. The herb collect-

ed at the vegetative stage has the highest content of flavonoids and mucilages. When introducing wild plants to cultivation, it is possible to select a population with optimal traits (yield, content of biologically active compounds). In this experiment the highest mass of herb was obtained from population P3, and the lowest from population P1. Population P3 had the highest content of mucilages and flavonoids, but the lowest content of iridoid glycosides, although still within the limits laid down in Polish standards (the content of iridoids should be not less than 1.5%).

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Streszczenie: *Plonowanie i jakość surowca babki większej (*Plantago major *L.) w drugim roku uprawy*. W pracy oceniono wpływ terminu zakładania plantacji oraz zbioru surowca na jakość ziela babki większej w drugim roku uprawy. Nasiona do założenia plantacji zebrano w 2011 roku na naturalnych stanowiskach tego gatunku położonych w województwach mazowieckim, lubelskim i podlaskim. Nasiona te wysiano w latach 2012 i 2013 wiosną (w kwietniu) i późną wiosną (w czerwcu). Surowiec zebrano w latach 2013 i 2014, gdy rośliny znajdowały się w fazie wegetatywnej i w fazie kwitnienia. Określono powietrznie suchą masę ziela z 1 m^2 oraz zawartość śluzów, glikozydów irydoidowych, kwasów polifenolowych i flawonoidów w powietrznie suchym surowcu. Wyraźnie większą masę ziela uzyskano z poletek, na których wysiano nasiona późną wiosną w porównaniu z siewem wiosennym oraz z roślin zebranych w fazie kwitnienia w porównaniu z roślinami zebranymi w fazie wegetatywnej. Większą zawartością glikozydów irydoidowych (głównej grupy związków czynnych) charakteryzował się surowiec zebrany z poletek założonych późną wiosną. Ziele populacji z Podlasia zawierało wyraźnie więcej tych związków w porównaniu z populacjami z województw mazowieckiego i lubelskiego.