The influence of habitat and cultivation conditions on generative shoot production of Sandy Everlasting [*Helichrysum arenarium* (L.) Moench] and the content of flavonoid compounds in their inflorescences

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S u m m a r y

Helichrysum arenarium (L.) Moench is a perennial belonging to the Asteraceae family. Its inflorescences are a valuable herbal material. Sandy Everlasting is also an ornamental plant: its blooming shoots are used for dry bouquets. The aim of this study was assessing the influence of habitat conditions on the number of generative shoots and dry mass of inflorescences per ramet (clump). Plants from experimental plantations and those growing in natural conditions were compared. The second aim was to measure the amount of flavonoids in inflorescences. It was determined that the number of generative shoots as well as dry mass of inflorescences depended significantly on the origin of Sandy Everlasting. Clumps of plants grown in plantations had on average 12-fold more generative shoots than those of plants from natural stands. Field-grown plants had also 17-fold higher dry inflorescence mass per ramet. Moreover, the amount of flavonoids in inflorescences from cultivated plants was higher (and attained its maximum at 1.19%). This means that Sandy Everlasting plantations could yield much more herbal material which is of a better quality than that from natural stands. An increased number of inflorescence shoots and a higher mass of flower heads in a clump obtained by *in vitro* methods can make Sandy Everlasting an attractive ornamental plant.

Key words: *Helichrysum arenarium (L.) Moench, field cultivation of plants obtained by in vitro method, generative shoots, dry mass of inflorescences, flavonoids*

INTRODUCTION

Helichrysum arenarium (L.) Moench is a perennial from the sunflower family (*Asteraceae*). This species is partially protected in Poland [1]. Its inflorescences are a source of valuable herbal material known since medieval times and used for medicinal and cosmetic preparations [2-4]. According to Polish Pharmacopoeia [5], the material referred to as *Helichrysi inflorescentia* should contain at least 0.5% of flavonoids (recalculated for quercetin).

Dried plants can be used for bouquets and Easter decorations.

Since the 1970s there have been some unsuccessful attempts at growing Sandy Everlasting in the fields [6]. Population studies conducted lately [7-10] have only explained the reason why those attempts failed. The next step in the study on cultivation of Sandy Everlasting was determining methods of acquiring plantlets from tissue cultures and then their adaptation to greenhouse and field conditions [11, 12].

The aim of this study was to compare the number of generative shoots and the dry mass of inflorescences for Sandy Everlasting ramets from field plantations and natural stands. The amount of flavonoids in inflorescences collected in the years 2007–2008 was also measured.

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MATERIAL AND METHODS

Analyzes and observations were carried out in growing seasons 2006–2008. The number of generative shoots of *Helichrysum arenarium* was determined according to commonly used methods [13]. The dry mass of flower heads was also estimated. In each of seven experimental fields (located at UTLS Experimental Station in Mochełek) 30 clumps (ramets) of Sandy Everlasting were analyzed. Samples consisted of plants from natural stands that were reproduced by tissue culture method on a modified MS medium [11]. Microseedlings were adapted to five different mineral and organic soil media [12]:

- highmoor peat supplemented with perlite,
- a medium for growing chrysanthemums,
- clayey soil,
- soil from a natural stand of *H. a.*,
- sterilized soil from a natural stand of *H. a.*

After adaptation period in a greenhouse, sandy everlasting specimens were transferred to experimental fields with clayey substratum. Generative shoots for biometric and chemical analyses were taken from these fields in three subsequent growing seasons.

In addition, for comparison purposes, the same attributes were analyzed for plants from two natural populations [7, 9]. The population I was located in the

outskirts of Bydgoszcz (Fordon district). The population II was situated in the village of Łosiny (Bory Tucholskie forest complex).

Weather conditions at the generative stage of Sandy Everlasting development were analyzed based on data gathered at meteorological stations in Bydgoszcz and Chojnice. Seljainov's *k* index [14] was applied for the evaluation of hydrothermal conditions:

$$k = \frac{\sum P}{0{,}1\sum t} = \frac{\textit{season's total precipitation}}{0.1\ (\textit{season's sum of temperature})}$$

, where $k < 1$ indicates a drought period.

The percentage content of flavonoids recalculated for quercetin (see [5]) in dried herbal material (samples of inflorescences, 100 g each) was determined at Institute of Natural Fibres and Medicinal Plants in Poznań.

RESULTS AND DISCUSSION

Studies on generative productivity of *Helichrysum arenarium* were conducted during growing seasons 2006–2008. Weather conditions at that time were similar at both locations (tab. 1). Typical differences in pluviothermal conditions were reported: the precipitation in Bydgoszcz is usually lower but the air temperature higher than in Bory Tucholskie (data for Chojnice). A vigorous generative development of *H. arenarium* occurs in May-August. May 2006 was humid, June and July were very dry, followed by a humid August. These months in 2007 were exceptionally rainy and the weather conditions in August were close to the long-term average. Spring months and the beginning of 2008 were extremely dry, especially in Bydgoszcz. Only August brought significant precipitation.

Distribution of precipitation and temperature during the period of intensive plant development and the age of Sandy Everlasting individuals grown in experimental fields influenced the number of generative shoots developed (tab. 2). The number of generative shoots per ramet as well as per area unit was the highest in the year 2006. This was the 2nd year of cultivation and the 1st year of intensive blooming and harvest. The number of generative shoots produced in the subsequent years was constantly decreasing. The total number of these shoots per clump in the years 2007 and 2008 attained only 84.87% and 26.82% of the number recorded in 2006, respectively. The number of shoots per 1m^2 decreased in 2007 and 2008 by 15.84% and 90.04%, respectively.

Table 1.

Pluviothermal conditions at the generative phase of Sandy Everlasting development in 2006–2008. Data from meteorological stations at Bydgoszcz and Chojnice

year	month	location					
		Bydgoszcz			Chojnice		
		total precipitation [mm]	average temperature [°C]	*k* index	total precipitation [mm]	average temperature [°C]	*k* index
2006	May	59.5	12.5	1.54	79.1	12.3	2.07
	June	21.8	16.8	0.43	24.8	16.6	0.50
	July	24.2	22.4	0.35	12.6	21.9	0.19
	August	129.0	16.6	2.51	154.1	16.6	2.99
	May-August	234.5	17.1	1.11	270.6	16.9	1.30
2007	May	73.1	13.8	1.71	115.0	13.6	2.73
	June	105.5	18.2	1.93	121.9	17.5	2.32
	July	104.7	18.0	1.88	114.9	16.9	2.19
	August	42.1	17.8	0.76	68.3	17.3	1.27
	May-August	325.4	17.0	1.56	420.1	16.3	2.10
2008	May	11.5	13.2	0.28	2.1	13.0	0.05
	June	15.5	17.6	0.29	33.8	16.3	0.69
	July	58.7	19.2	0.99	70.3	17.8	1.27
	August	95.5	17.8	1.73	104.2	16.9	1.99
	May-August	181.2	17.0	0.87	210.4	16.0	1.07

Sandy Everlasting growing on a soil classified as clayey sand [15] fared better in humid spring and summer 2007 than in excessively dry May and June 2008. The plantation was grown for seasons and then deteriorated: 62.5% of individuals withered. The highest number of generative shoots per clump (within 2006-2008) was recorded in the plot with plants earlier adapted to an organic medium for growing chrysanthemums based on highmoor peat. In contrast, the lowest figure was obtained for sterilized sandy soil from a natural stand of *H. arenarium*.

The number of generative shoots per ramet collected in the years 2006-2008 from Sandy Everlasting plantations was 12-fold higher on average than that obtained for natural populations. The number of generative shoots per 1m^2 was 5-fold higher (tab. 2). The differences were statistically significant.

Weather conditions and the origin of Sandy Everlasting (plantation or a natural stand) influenced also the dry mass of inflorescences collected per ramet. The highest average amount of herbal material was collected in 2006: 12.56 g per clump from plantations compared to 0.72 g per clump from natural stands (tab. 3). In the subsequent years the amount was gradually decreasing: by 42.60% in 2007, and by 45.30% in 2008. In total, the clumps from plantations yielded 17-fold more herbal material that those from natural stands.

Table 2

Number of generative shoots of Sandy Everlasting per ramet (clump) and per 1 m^2 of experimental plantation or phytocoenosis

origin of Sandy Everlasting	year (of cultivation)			
	2006 (II)	2007 (III)	2008 (IV)	Total
number of generative shoots per ramet (clump)				
origin of plants: A. plantation: plants from tissue cultures before being transferred to experimental fields were adapted to five media:				
- peat + perlite	60.28	54.33	15.56	130.17
- a medium for chrysanthemums	63.74	62.8	16.92	143.64
- clayey soil	52.96	49.61	13.45	116.02
- soil from a natural stand of *H. a.*	57.75	38.37	17.40	113.52
- sterilized soil from a natural stand of *H. a.*	44.19	31.39	11.46	87.04
B. natural stands:				
- population I	5.73	2.72	1.68	10.13
- population II	5.39	3.29	1.37	10.05
number of generative shoots per 1 m^2				
origin of plants: A. plantation: plants from tissue cultures before being transferred to experimental fields were adapted to five media:				
- peat + perlite	491.82	443.28	29.30	964.40
- a medium for chrysanthemums	519.87	513.67	78.00	1111.54
- clayey soil	432.00	404.67	44.70	881.37
- soil from a natural stand of *H. a.*	471.43	297.56	35.51	804.50
- sterilized soil from a natural stand of *H. a.*	360.50	256.08	39.21	655.79
B. natural stands:				
- population I	87.50	41.50	12.50	141.50
- population II	158.50	31.50	8.50	198.50

The highest dry mass of inflorescences was recorded for plants that were adapted to sandy soil taken from natural stands of the species. The total amount of herbal material collected for these plants in the years 2006–2008 was 32.19 g as compared to average amount of 26.59 g per ramet recorded for all experimental plantations. The differences were statistically significant only for dry masses from plantations on the one hand, and the masses from natural stands, on the other.

An increased number of inflorescence shoots and a higher mass of flower heads for clumps obtained by *in vitro* methods can make Sandy Everlasting an attractive ornamental plant suitable for poorer soils.

The percentage of flavonoids (recalculated for quercetin) in dried herbal material was determined for inflorescences collected in the years 2007–2008. The highest amount of flavonoids in 2007 (1.19% of dry mass) was recorded for plants originating from *in vitro* plantlets and growing on clayey soil since 2005 (tab. 3).

The amount obtained is almost twice as high as the content determined for plants from natural stands (0.6%).

Table 3.

Dry mass of inflorescences obtained from Sandy Everlasting ramets (clumps) and the amount of flavonoids in inflorescences (measured by S. Mielcarek, Ph.D., at Research Institute of Medicinal Plants in Poznań)

origin of inflorescences	year (of cultivation)			
	2006 (II)	2007 (III)	2008 (IV)	Total
dry mass of inflorescences [g]				
origin of plants: A. plantation: plants from tissue cultures before being transferred to experimental fields were adapted to five media:				
- peat + perlite	10.65	7.59	4.95	23.19
- a medium for chrysanthemums	12.66	9.19	7.75	29.60
- clayey soil	10.50	6.95	6.73	24.18
- soil from a natural stand of *H. a.*	14.59	7.62	9.98	32.19
- sterilized soil from a natural stand of *H. a.*	14.40	4.69	4.69	23.78
B. natural stands:				
- population I	0.94	0.51	0.44	1.89
- population II	0.50	0.39	0.31	1.20
the amount of flavonoids in inflorescences (recalculated for quercetin) [%]				
origin of plants: A. plantation: plants from tissue cultures before being transferred to experimental fields were adapted to five media:				
- peat + perlite	-	1.06	0.83	-
- a medium for chrysanthemums	-	1.07	0.64	-
- clayey soil	-	1.19	0.84	-
- soil from a natural stand of *H. a.*	-	1.03	0.60	-
- sterilized soil from a natural stand of *H. a.*	-	0.95	0.81	-
B. natural stands:				
- population I	-	0.70	0.84	-
- population II	-	0.49	0.83	-

In 2008 the amount of flavonoids in inflorescences from plantations was lower than in 2007 by 1.58% on average, but the content determined for flower heads from natural stands was higher by 0.24% on average.

The content of active compounds in Sandy Everlasting inflorescences depends on weather conditions (harsh in spring and summer 2008 because of prolonging drought period) and, probably to a lesser degree, on the age of plants.

In 2007, the amount of flavonoids (recalculated for quercetin) in herbal material of *in vitro* origin varied from 0.95% (plantlets adapted to sterilized soil from a natural stand of Sandy Everlasting) to 1.19% (adaptation to clayey soil). The amount for

inflorescences from natural stands was 0.70% and 0.49%, for population I and II, respectively (tab. 3). In the subsequent year the content of flavonoids varied from 0.60% to 0.84%; there were no significant differences between inflorescences from plantations and those from natural stands.

Bryksa-Godzisz et al. [16] analyzed the amount of flavonoids in inflorescences collected in July 2004 from 22 Sandy Everlasting populations growing in Bug Valley. The content ranged over the interval 0.15 - 0.70%. Pluviothermal conditions in 2004 were close to the many-year average.

A higher content of flavonoids in *Helichrysi inflorescentia*, varying from 0.37% to 1.10% is reported by Czinner et al. [17].

The most important result of this work is confirmation that *Helichrysi inflorescentia* obtained from cultivated plants of *in vitro* origin is of considerably higher quality than herbal material collected from natural stands.

CONCLUSIONS

1. The number of generative shoots and the dry mass of inflorescences per clump (ramet) depend significantly on the origin of Sandy Everlasting individuals. Ramets from plantations have on average 12-fold more generative shoots than those from natural stands. Analogously, the dry mass of inflorescences per ramet for plantations exceeds 17-fold the amount obtained for plants from natural stands.
2. The content of flavonoids in inflorescences from plantations, varying from 0.60% to 1.19%, exceeded that for plants growing in natural stands. This means that a plantation can yield much more herbal material (of a better quality) in comparison with natural stands.
3. An increased number of inflorescence shoots and a higher mass of flower heads for clumps obtained by *in vitro* methods can make Sandy Everlasting an attractive ornamental plant.

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WPŁYW WARUNKÓW SIEDLISKOWYCH UPRAWY I STANOWISK NATURALNYCH NA TWORZENIE PĘDÓW GENERATYWNYCH PRZEZ KOCANKI PIASKOWE [*HELICHRYSUM ARENARIUM* (L.) MOENCH] ORAZ ZAWARTOŚĆ W ICH KWIATOSTANACH ZWIĄZKÓW FLAWONOIDOWYCH

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Streszczenie

Helichrysum arenarium (L.) Moench jest byliną z rodziny Asteraceae. Jej kwiatostany są cennym surowcem zielarskim, a pędy kwiatostanowe wykorzystuje się do układania suchych bukietów. Celem badań było porównanie liczby pędów generatywnych i suchej masy kwiatostanów ramet (kęp) otrzymanych z uprawy na poletkach doświadczalnych oraz z naturalnych zbiorowisk, na tle warunków siedliskowych. W kwiatostanach oceniono również zawartość flawonoidów. Stwierdzono, że liczba pędów kwiatostanowych na roślinie oraz sucha masa kwiatostanów są w istotny sposób uzależnione od pochodzenia kocanek piaskowych. Kępy uzyskane z uprawy mają przeciętnie 12-krotnie więcej pędów generatywnych od pochodzących ze stanowisk naturalnych. Uprawa pozwala również na osiąganie 17-krotnie wyższych suchych mas kwiatostanów z ramety. Ponadto zawartość flawonoidów w kwiatostanach pochodzących z uprawy była wyższa – maksymalnie 1,19%. Oznacza to, że z plantacji można otrzymać wielokrotnie więcej lepszego surowca zielarskiego w porównaniu ze stanowiskami naturalnymi. Zwiększona liczba pędów kwiatostanowych oraz duża masa koszyczków w kępie kocanek piaskowych pozyskanych metodą *in vitro* sprawia, że mogą one stać się również atrakcyjną rośliną ozdobną.

Słowa kluczowe: *Helichrysum arenarium (L.) Moench, uprawa polowa roślin otrzymanych metodą in vitro, pędy generatywne, sucha masa kwiatostanów, flawonoidy*