

NECTAR PRODUCTION AND POLLEN YIELD OF *Echium vulgare* L. IN THE CLIMATIC CONDITIONS OF LUBLIN

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Abstract. In Poland common viper's bugloss (*E. vulgare* L.) is the most common species. *E. vulgare* belongs to medicinal, ornamental and bee plants, since it is a source of nectar and pollen forage. These flowers are frequently visited by bumblebees and honey bees. Flowering specimens of common viper's bugloss were collected in the area of Lublin. Flowers and their parts were observed using stereoscopic microscopy. The micromorphology of the nectary at the initial stage of nectar secretion was observed in a scanning electron microscope. The rate of nectar production and pollen yield of the plants were determined as well as insects visiting flowers of this species were identified. The nectary, located at the base of the ovary, was found to be fused with its lower part. Nectar is secreted through numerous stomata located in the epidermis of the nectary. Nectar secretion, which starts at the bud stage, lasts throughout the flower life (3–4 days). Over this period, one flower of common viper's bugloss produces 2.14 mg of nectar with high sugar concentration (48%). Sugar yield of its flowers is on average 9.8 mg/10 flowers. The flowers start to shed pollen at bud break and release the largest amount of pollen during late morning hours. The weight of pollen produced by 10 flowers is 5.4 mg. The present study shows that the flowers of *E. vulgare* are a very good source of nectar and pollen forage.

Key words: *Echium vulgare*, nectary, morphology, nectar secretion, pollen yield

INTRODUCTION

The genus *Echium* comprises 30 species that are primarily native to the Mediterranean Sea area; among these species, *E. vulgare* L. and *E. lycopsis* Grufberg can be found most frequently in the natural environment. In Poland common viper's bugloss (*E. vulgare*) is the most common species. Red viper's bugloss (*E. russicum* J. F. Gmel.) is fully protected [Dz. U. 2004, nr 92, poz. 880], whereas purple viper's bugloss (*E. plantagineum* L.) is grown as an ornamental border plant [Szwejkowscy 2003, Rut-

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kowski 2008]. This is an alien species in a natural community that can appear as an ergasiofigophyte [Galera 2003]. Common viper's bugloss (*Echium vulgare*) is included in medicinal, ornamental and bee plants, since it is a source of nectar and pollen forage [Szweykowscy 2003, Kołtowski 2006, Lipiński 2010].

Currently, *E. vulgare* and *E. plantagineum* are of great pharmacological interest due to the chemical composition of their seeds, *inter alia*, the content of unsaturated fatty acids (γ -linolenic acid (GLA) and rarely found stearidonic acid (SDA)) [Cisowski et al. 2001; Król 2006]. But other studies show that the fruit of *E. vulgare* is poisonous due to the presence of several alkaloids, among others, echiine [Podhajska and Rivola 1992]. The nectar and pollen also contain poisonous pyrrolizidine alkaloids that may remain in honey [Beales et al. 2004, Boppre et al. 2005, Kędzia 2008; Kempf et al. 2010a, b]. According to Boppre et al. [2005], the content of pyrrolizidine alkaloids in the pollen of *Echium vulgare* ranged 0.8–1.4%. These compounds show hepatotoxic and carcinogenic activity. That is why their presence in flower pollen poses a threat to humans and animals [Kędzia 2008]. Pyrrolizidine alkaloids originating from the flower pollen of *Echium plantagineum* were found in Australian honey [Prakash et al. 1999]. Young leaves of *E. vulgare* were consumed as a vegetable. However, due to the content of carcinogenic pyrrolizidine alkaloids in the herb, *E. vulgare* cannot be treated as a medicinal and edible plant [Sadowska 2004].

The genus *Echium* includes species that are considered to be important bee plants. The flowers of *E. vulgare* are frequently visited by bumblebees and honey bees [Demianowicz 1953, Corbet 1978, Pappers et al. 1999, Kołtowski 2006]. Earlier studies have shown that plants of the genus *Echium* produce nectar in abundance in the climatic conditions of Poland [Kołtowski 2006, Chwil and Weryszko-Chmielewska 2007]. Monofloral honeys from viper's bugloss flowers are usually obtained in warmer climate zones [Maurizio and Grafl 1969]. Honey from *E. vulgare* has a light colour, a pleasant smell, and a delicate taste [Kołtowski 2006]. The aim of this research was to carry out a study to evaluate the apicultural value of *E. vulgare* in the conditions of Lublin. The rate of nectar production and pollen yield were estimated. The morphology of the nectary of this species was also determined.

MATERIAL AND METHODS

The present study on common viper's bugloss (*Echium vulgare* L.) was conducted in the period 2007–2009. The flowers subjected to examination grew in the Botanical Garden of the Maria Curie-Skłodowska University in Lublin as well as in the Felin district area in this city. The flower life span was determined from perianth opening to the appearance of the first symptoms of corolla wilting. In order to capture this stage, observations were carried out at four-hour intervals from 8 am to 8 pm. Flowers sampled to collect nectar from their whole lifetime were isolated with tulle mesh in order to protect them against insect visitation. Among the insects visiting the flowers, attention was primarily drawn to bumblebees and bees. Observations were made at two-hour intervals (for three minutes) between 8 am and 6 pm.

The rate of nectar production was investigated during the period 2008–2009, using the pipette method [Jabłoński 2003]. Nectar was collected three times throughout the flower lifetime, examining 5 samples from each set of 10–15 earlier isolated flowers. The percentage sugar content was determined with an Abbe refractometer. The weight of nectar was determined using an analytical balance, while sugar yield of the flowers was calculated.

Pollen yield of the flowers was also calculated following the method described by Warakomska [1972] in which ether extraction and gravimetric measurement are used. Pollen weight was determined twice in five samples consisting of 100 stamens, and then it was calculated per 10 flowers.

A preliminary examination of the flowers was performed using stereoscopic microscopy, whereas the micromorphology of the epidermis of the nectary at the initial stage of secretion was observed in a TESCAN VEGA II LMU scanning electron microscope. Sections of the nectary were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer. The fixed plant samples were postfixed in 1% OsO₄ and stained with a 0.5% aqueous solution of uranyl acetate. After dehydration in alcohol series and acetone, they were critical-point dried in liquid CO₂, and then coated with gold using an EMITECH K550X sputter coater.

The results were statistically analysed by analysis of variance using Statistica 5.0 software. The significance of differences was verified by Duncan's test.

RESULTS

Our observations show that the flower life span in *Echium vulgare* is 3–4 days. The fact that flowers with different-coloured petals can be found simultaneously in the inflorescences of *E. vulgare*, which is dependent on the stage of their development, contributes to the ornamental value of the species under investigation. Budded flowers are pink-coloured, violet at full bloom, but when they are fading away, they become blue (Fig. 1 A, B). The stamen filaments, whose pink colour is most intense during pollen shed, are also colour attractants (Fig. 1 B, C).

Table 1. Characteristics of nectar production in *Echium vulgare*
Tabela 1. Charakterystyka nektarowania *Echium vulgare*

Year Rok	Nectar weight mg/10 flowers Masa nektaru mg/10 kwiatów		Nectar sugar concentration, % Koncentracja cukrów w nektarze, %		Sugar yield, mg/10 flowers Wydajność cukrowa, mg/10 kwiatów	
	min. – max.	mean średnio	min. – max.	mean średnio	min. – max.	mean średnio
2008	11.33–27.08	18.29 ^A	42.0–67	58.23 ^A	6.79–15.32	10.56
2009	18.67–32	24.83 ^B	34–38	36.5 ^B	7.03–12.16	9.03
Mean Średnio	-	21.43	-	47.8	-	9.82

^{A, B} means followed by different letters are significantly different for $P \leq 0.01$

^{A, B} średnie oznaczone różnymi literami w kolumnach różnią się istotnie przy $P \leq 0,01$

The nectary is located around the pistil's ovary and is fused with its lower part (Fig. 2A). From the outside, it is surrounded by the corolla tube in which nectar accumulates. Strongly haired sepals are an additional protection (Figs 1D, 2A). The nectary is characterized by a lighter colour (yellow-green) than the ovary (green). In the upper part of the nectary, there is a substantial projection in which numerous stomata are found (Fig. 2B, C).



Fig. 1. Portion of an inflorescence (A) and flowers of *Echium vulgare* (B, C), visible pink colour of stamen filaments (arrow), D. Portion of a flower of *E. vulgare* with the visible nectary (n) around the ovary (o)

Ryc. 1. Fragment kwiatostanu (A) i kwiaty *Echium vulgare* (B, C), widoczne różowe nitki pręcików (strzałka). D. Fragment kwiatu *E. vulgare* z widocznym nektarium (n) wokół zalążni słupka (o)

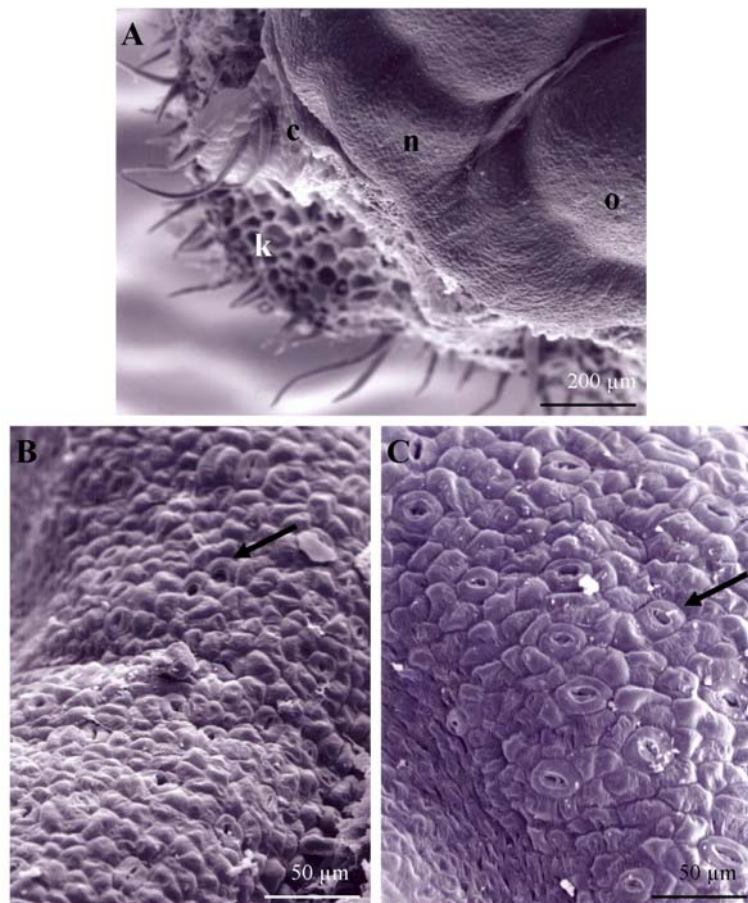


Fig. 2. Nectary surface in scanning electron microscope (SEM). A. Portion of a flower with the nectary (n), ovary (o), cutting sepals (k), and the corolla tube (c). B. The middle part of the nectary visible from the top with stomata (arrows). C. The lateral part of the nectary with numerous stomata at the apex (arrows)

Ryc. 2. Powierzchnia nektarnika w skaningowym mikroskopie elektronowym (SEM). A. Fragment kwiatu z nektarnikiem (n), zalążnią (o), przeciętymi działkami kielicha (k) i rurką korony (c). B. Środkowa część nektarnika ze szparkami widoczna z góry (strzałki). C. Boczna część nektarnika z licznymi aparatami szparkowymi na szczycie (strzałki)

The flowers of *E. vulgare* provide mainly a nectar reward to insects. Throughout its lifetime, a single flower of common viper's bugloss produces nectar with a weight ranging between 1.1 and 3.2 mg, with an average value equal to 2.1 mg. Nectar sugar concentration in analysed plants is within a range of 34–67 %, with its average value of ca. 47.8%, while sugar yield is 0.98 mg (tab. 1).

Table 2. Pollen yield of *Echium vulgare*
Tabela 2. Wydajność pyłkowa *Echium vulgare*

Year Rok	Pollen weight (mg) – Masa pyłku (mg)			
	per 100 stamens – ze 100 pręcików		per 10 flowers – z 10 kwiatów	
	min. – max.	mean – średnio	min. – max.	mean – średnio
2008	10.01–10.68	10.47	5.01–5.34	5.24 ^a
2009	10.46–11.73	11.09	5.23–5.87	5.55 ^b
Mean Średnio	-	10.78	-	5.39

^{a, b} means followed by different letters are significantly different for $P \leq 0.01$

^{a, b} średnie oznaczone różnymi literami w kolumnach różnią się istotnie przy $P \leq 0,01$

The stamens in the flowers of common viper's bugloss start to shed pollen at the bud break stage. An intense release of pollen from the anthers was found during late morning hours. The weight of pollen per 100 anthers is from 10.01 mg up to 11.73 mg (on average 10.78 mg). Calculated per 10 flowers, the weight of pollen of common viper's bugloss is within a range of 5.01–5.87 mg, with an average value equal to 5.39 mg (tab. 2). Observations of insects foraging on the flowers of *E. vulgare* showed that its nectar and pollen were collected primarily by honey bees, but also bumblebees and beetles.

DISCUSSION

The colour of *Echium vulgare* flower changes, depending on the stage of flower development, from pink to violet to blue. In Boraginaceae, the above mentioned colours come from anthocyanins contained in the vacuoles [Harborne 1997, Chwil and Weryszko-Chmielewska 2009]. According to Szafer and Wojtusiakowa [1969], these pigments frequently crystallize in cells. Coloured spots on the stems may also perform the role of an attractant. Glandular trichomes on the sepals also ensure scent attractiveness of the flowers [Weryszko-Chmielewska and Chwil 2008].

Food attractants (nectar and pollen) in the tubular flowers of *E. vulgare* are available to many groups of insects. We observed in these flowers not only bees and bumblebees, but also beetles. Other authors also report that flowers of this species are visited by various insects [Corbet 1978, Pappers 1999]. Proctor et al. [1996] stated that the flowers of *E. vulgare* are visited by *Conopidae* as well as by *Bombylius*, *Rhingia*, and bees.

We presented the specifics of the structure of the nectary glands in *E. vulgare* in our earlier paper [Weryszko-Chmielewska and Chwil 2008]. Nectar in the flowers of *E. vulgare* is secreted by the nectary that forms a ring around the basal part of the ovary. The tissues of the nectary form four indentations in the respective portions of the 4-loculed ovary. Nectar is secreted onto the surface of the epidermis through anomocytic stomata that are found in great numbers on the entire surface of the nectary.

At the initial stage of nectar secretion, the nectary of *E. vulgare* has a green colour, but at a later stage of development it changes its colour to yellow and green. As described in our earlier papers, the nectary of *E. russicum* [Chwil and Weryszko-Chmielewska 2007] and of *Anchusa officinalis* [Weryszko-Chmielewska and Chwil 2008] has a similar colour. Colour change in the nectary during its development was also observed in *Erica carnea* [Weryszko-Chmielewska et al. 2009]. A mature nectary is orange-yellow coloured, which is attributable to the presence of chromoplasts in the parenchymal cells [Weryszko-Chmielewska et al. 2009].

Different colours of the nectariferous tissue during the successive stages of its development in tobacco are described by Horner et al. [2007]. The green colour is determined by the presence of chloroplasts occurring at the early stages of their development. In a mature nectary, the yellow colour results from the presence of carotene in the plastids. The parenchymal cells of the nectary become filled with starch grains before nectar secretion. Then, the accumulated starch is quickly degraded into sugars and substrates for β -carotene and ascorbate biosynthesis. The plastids perform the double function of amylo- and chromoplasts.

Nectar secretion in the flowers of *E. vulgare* under investigation started already at bud break. According to Maurizio and Grafl [1969], the optimal time for nectar release in the flowers of plants of the genus *Echium* is during afternoon hours. Sucrose is predominant in the nectar of *E. vulgare* [Maurizio and Grafl 1969]. Table 3 shows the rate of nectar production in the flowers of the species under study and of other species of the genus *Echium* described in the literature.

Table 3. The rate of nectar production in some species of the genus *Echium*. Comparison of literature data with the results of the present study.

Tabela 3. Obfitość nektarowania wybranych gatunków z rodzaju *Echium*. Porównanie danych z literatury z wynikami prezentowanej pracy

Species Gatunek	Nectar sugar concentration, % Koncentracja cukrów w nektarze, %	Sugar weight mg/10 flowers Masa cukrów mg/10 kwiatów	Honey yield kg per ha Wydajność miodowa kg z ha	Author Autor
	-	-	250–300	Demianowicz 1953
	17–43	2.3–25.6	182–429	Maurizio and Grafl 1969
<i>Echium vulgare</i>	30–50	-	300–400	Bornus 1989
	-	0.8–9.2	200–400	Prabucki 1998
	-	9	400	Kołtowski 2006
	34–67	6.8–15.3	-	The present study
	25–50	7–15	400–600	Bornus 1989
<i>Echium creticum</i>		3.7–15.6	700	Prabucki 1998
	-	7–15	600	Kołtowski 2006
<i>Echium russicum</i>	53.3–56 (58)	16.48	-	Chwil and Weryszko- -Chmielewska 2007

Nectar weight per 10 flowers of *E. vulgare* growing in the climate conditions of Lublin is 21.4 mg. This value is much higher than the data reported by Demianowicz [1953] (3–5 mg/10 flowers), but it is in the range given by Maurizio and Grafl [1969] (5–88 mg/10 flowers). On the other hand, it is lower than the weight of nectar in *E. russicum* (29 mg /10 flowers) [Chwil and Weryszko-Chmielewska 2007].

The nectar sugar concentration in the flowers under study ranged 34–67%. This value is close to the nectar sugar concentration of this species reported by Bornus [1989], but higher than the results obtained by Maurizio and Grafl [1969] (tab. 3). However, the average sugar concentration in the nectar (48%) of *E. vulgare* is lower than the sugar concentration in the flowers of *E. russicum* (58%) [Chwil and Weryszko-Chmielewska 2007].

Sugar yield per 10 flowers of *E. vulgare* is comparable to the results of other authors (tab. 3). But it is lower than the weight of sugars in the nectar of *Echium russicum* [Chwil and Weryszko-Chmielewska 2007].

Various studies show that honey yield of *E. vulgare* flowers ranges from 182 kg to 429 kg per ha. Much higher honey yield (400–700 kg per ha) was found in the flowers of *E. creticum* (tab. 3).

Pollen weight per 10 flowers of *E. vulgare* is 5.4 mg. This value is 40% higher than pollen yield of *Echium russicum* (3.1 mg) [Chwil and Weryszko-Chmielewska 2007]. On the other hand, Koltowski [2006] estimated pollen productivity of *E. vulgare* at 70–80 kg per ha. High pollen viability (88%) is evidence of the good value of pollen grains of a related species – *E. russicum* [Chwil and Weryszko-Chmielewska 2007]. *E. vulgare* pollen loads are light or dark blue coloured [Maurizio and Grafl 1969, Prabucki 1998].

The obtained results on nectar and pollen rewards offered by the flowers of *Echium vulgare* to pollinating insects suggest that this species can be used as a good supplement of food resources for insects.

CONCLUSIONS

1. Nectar secretion, which starts at the bud stage, lasts throughout the flower life (3–4 days).
2. The nectar, flowing out onto the surface of the nectary through the stomata, is characterized by high sugar concentration (34–67%).
3. Sugar yield of the flowers of *E. vulgare* is on average 9.5 mg/10 flowers.
4. Due to a small number of stamens, pollen weight per 10 flowers of *E. vulgare* reached 5.4 mg.

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NEKTAROWANIE I WYDAJNOŚĆ PYŁKOWA *Echium vulgare* L. W WARUNKACH KLIMATYCZNYCH LUBLINA

Streszczenie. W Polsce pospolitym gatunkiem jest żmijowiec zwyczajny (*E. vulgare* L.). *E. vulgare* należy do roślin leczniczych, ozdobnych i pszczelarskich, gdyż jest źródłem pożytku nektarowego i pyłkowego. Kwiaty te licznie odwiedzają trzmiele i pszczoły. Kwitnące okazy żmijowca zwyczajnego zbierano w rejonie Lublina. Obserwacje kwiatów i ich fragmentów przeprowadzono przy użyciu mikroskopii stereoskopowej. Mikromorfologię nektarnika w początkowej fazie sekrecji nektaru obserwowano w skaningowym mikroskopie elektronowym. Określono obfitość nektarowania i wydajność pyłkową roślin oraz grupy owadów odwiedzające kwiaty tego gatunku. Stwierdzono, że nektarnik położony u nasady załączni słupka jest zrośnięty z jej dolną częścią. Nektar wydzielany jest za pośrednictwem licznych aparatów szparkowych położonych w epidermie nektarnika. Rozpoczynająca się w pąku sekrecja nektaru trwa przez całe życie kwiatu (3–4 dni). W ciągu tego okresu jeden kwiat żmijowca zwyczajnego wydziela 2,14 mg nektaru o wysokiej koncentracji cukrów (48%). Wydajność cukrowa kwiatów wynosi średnio 9,8 mg/10 kwiatów. Kwiaty rozpoczynają pylenie w stadium pękającego pąka i uwalniają najwięcej pyłku w godzinach przedpołudniowych. Masa pyłku wytwarzana przez 10 kwiatów wynosi 5,4 mg. Z badań wynika, że Kwiaty *E. vulgare* są bardzo dobrym źródłem pożytku nektarowego i pyłkowego.

Słowa kluczowe: *Echium vulgare*, nektarnik, morfologia, sekrecja nektaru, wydajność pyłkowa

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