

## FLOWERING PHENOLOGY AND POLLEN PRODUCTION OF THREE EARLY SPRING *Pulsatilla* SPECIES

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**Abstract.** Blooming biology and pollen production of the following species: *Pulsatilla zimmermannii* Soó, *P. halleri* (All.) Willd., and *P. vulgaris* Mill. grown in UMCS Botanical Garden in Lublin, Poland (51°16'N, 22°30'E) was studied. The species attract insect visitors with large (size 6–8 cm in diameter), colourful, campanulate, actinomorphic flowers. The androecium is multistaminate (min–max: 142–282 anthers per flower), spirally arranged. Minute droplets of nectar were noted at the base of androecium and on staminodes. The anthers dehisced subsequently and pollen of a single flower was available to insects for 5–11 (7) days. On average, *P. zimmermannii* was found to produce the highest amount of pollen (9.16 g per 1 m<sup>2</sup>), which was 2 times more pollen than *P. vulgaris* (4.22 g per 1 m<sup>2</sup>), while *P. halleri* produced 7.63 g per 1 m<sup>2</sup>. Pollen grains of the *Pulsatilla* species are medium in size (*P* axis ranged 29.8–38.4 μm, and *E* axis ranged 32.3–39.6 μm) and are *oblate-spheroid* in shape (*P/E* index ranged between 0.90 and 0.95). If planted together, the species will provide pollen from first days of April until end of the month, i.e. during the period when seasonal activity of Apoidea insects begins. *Pulsatilla* species might contribute to lists of garden plants that attract insect visitors with abundant pollen reward and minute nectar.

**Key words:** flowering, staminodes, pollen output, pollen shape, *Pulsatilla* spp.

### INTRODUCTION

Ecosystem service that pollinators provide is a key issue in general biodiversity maintenance. In temperate climatic zone, approximately 80% of plants are insect-pollinated [Gallai 2008]. Both crop and wild entomophilous plants are regarded as im-

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portant for stabilizing pollination networks [Potts et al. 2010]. However, pollinators diversity and abundance primarily due to agricultural intensification and habitat fragmentation have decreased over past decades [Vanbergen 2013, Thomann et al. 2013, Goulson et al. 2015].

Much efforts have already been made to assess floral food resources in the landscape scale [e.g. Czarnecka and Denisow 2014, Denisow and Wrzesień 2015 a, Dicks et al. 2015, Turrini and Knop 2015, Wrzesień et al. 2016] and to improve the pollinators' food resources [Denisow et al. 2014 a, b, Hicks et al. 2016, Strzałkowska-Abramek et al. 2016]. Urban habitats can support low pollinator diversity [e.g. Colding et al. 2006, Banaszak-Cibicka and Zmihorski 2012], pollinator species richness and abundance [Carvalho et al. 2014, Sirohi et al. 2015]. It has already been documented that ornamental plants (both native and alien) cultivated in urban areas might be beneficial for pollinator's restoration [Denisow et al. 2014 a, b, Garbuzov and Ratnieks 2014, Bożek et al. 2015, Hicks et al. 2016, Strzałkowska-Abramek et al. 2016].

To reveal most suitable food resources for pollinators, the evaluation of the quality and quantity of floral reward is necessary [Fussell and Corbet 1992, Wrzesień and Denisow 2006, Wrzesień et al. 2016]. So far, the blooming phenology (flowering onset and duration), nectar production, sugar and pollen yield of many plants [e.g. Denisow and Bożek 2008, Denisow et al. 2014 a, b, Strzałkowska-Abramek et al. 2016] have been revealed. These features are crucial when selecting plant species for constituting bee pastures [e.g. Kumova and Korkmaz 2013, Hicks et al. 2016].

The genus *Pulsatilla* (Ranunculaceae) consists of 38 herbaceous perennials that inhabit mainly Europe and Asia. These plants occur mostly in dry, fully sun-exposed habitats, like subalpine and alpine regions or dry heathlands. Some of them are cultivated as ornamentals. European *Pulsatilla* species are listed among rare and endangered ones [Szczecińska and Sawicki 2015]. They are usually endemics with strongly restricted population size. Given that information, some investigations concerning population size, biology and ecology or *in vitro* regeneration of *Pulsatilla* spp. have been conducted [e.g. Pilt and Kukk 2002, Šaulienė and Brinkyte 2009, Grzyl et al. 2014]. In Poland, 5 species: *Pulsatilla alba*, *P. patens*, *P. pratensis*, *P. slavica* and *P. vernalis* are considered native. *Pulsatilla vulgaris* is now thought to be extinct [Mirek et al. 2002]. However, many *Pulsatilla* spp. are popular and cultivated in gardens [Šaulienė and Brinkyte 2009].

The aim of our study was to (i) investigate flowering phenology of three *Pulsatilla* species and (ii) to establish to what degree the pollen yield can differ among years for the same perennials. In addition, we determined the size of pollen grains. This feature can be useful for identifying pollen grains while analyzing bee products.

## MATERIAL AND METHODS

**Study site and study species.** The observations were carried out in 2012, 2013 and 2016. Three species were grown in the alpine section of the Botanical Garden of Maria Curie-Skłodowska in Lublin, Poland (51°16'N, 22°30'E) and were fully exposed to the

sun. The following species were chosen for the study: *Pulsatilla zimmermannii* Soó, *P. halleri* (All.) Willd., and *P. vulgaris* Mill.

*P. zimmermannii* occurs almost exclusively in central and northern Hungary and eastern Slovakia. The species is calciphilous and grows mostly on sunny mountain slopes or hillsides. It can be also found on dry pastures developed on non-calcareous soils [Mártonfióvá 2004].

*P. halleri* is distributed mainly in the southern, central and eastern parts of Europe [Turner 2005]. The species can be found in subalpine and alpine zones on calciferous soil exposed to full sunlight. Stem is covered by floss silk and basal leaves that develop after the initiation of blooming are hairy [Hashani and Shuka 2013]. The corolla is campanulate. Petals range in colour from white, brown-reddish to different shades of violet (light violet, lavender blue). This perennial grows up to 30 cm and is quite freeze-resistant [Grabowska and Kubala 2012].

*P. vulgaris* occurrence is limited to dry calcareous grasslands of Western and Central Europe. The plant forms a rosette of basal pinnate leaves arising after flower buds opening [Walker and Pinches 2011]. Bell-shaped flowers are usually blue-violet or red-violet, but cultivars are quite popular and represent various colours of corolla (e.g. white 'Alba', red 'Rubra') [Grabowska and Kubala 2012].

**Flowering phenology and abundance.** The flowering duration and the onset of each stage of blooming were investigated according to the protocol described by Denisov and Božek [2006]. We established the pheno-phases as follows: the beginning of flowering was when 10% of flowers started to bloom, the full blooming phase when 70% of flowers bloomed and the end of blooming when 80% fell off.

To assess the flowering abundance, we counted the number of all buds and flowers developed on individual plants ( $n = 5-8$ ), and then, we established the number of individuals on the experimental plots ( $0.5-1 \text{ m}^2$ ;  $n = 3-4$ ). The data were converted and the number of flowers per  $1 \text{ m}^2$  have been obtained. The abundance of blooming per unit area was necessary for total pollen yield evaluation.

**Pollen measurements and nectar secretion.** Pollen production was estimated in the full blooming stage. Mature, unopened anthers ( $n = 100$ ) were put in tarred glass containers. Four replications for each species were performed. The containers were then placed into ELCON CL 65 dryer (ca.  $33^\circ\text{C}$ ) for a couple days. The pollen was swilled out from anthers using 70% ethanol thereafter. The pollen production was then calculated for 100 anthers (in mg), 10 flowers (in g) and  $1 \text{ m}^2$  (in g). We checked the secretion of nectar in 2012 and 2013 by bagging flowers with tulle isolators and applying the pipette method [Jabłoński 2002]. To determine pollen grains size, we measured the length of 50 equatorial and 50 polar axes in glycerogelly slides (in 2013 and 2016).

**Data analysis.** The data presented in this paper are expressed as means with standard deviation (SD). The significance of differences was tested by one-way ANOVA and means were compared post hoc using Tukeys test at  $P = 0.05$ . Statistica ver. 6.0 (StatSoft Poland, Kraków) was applied to all calculations [Stanisz 2007].

## RESULTS

During the study seasons, flowering of all the investigated species began in the first decade of April and ended in the third decade of the month or at the very beginning of May (fig. 1) *Pulsatilla zimmermannii* was the first to bloom, followed by *P. halleri* and *P. vulgaris*. There were no significant differences in the duration of blooming neither between species nor between years of study (min–max = 22–27 days).

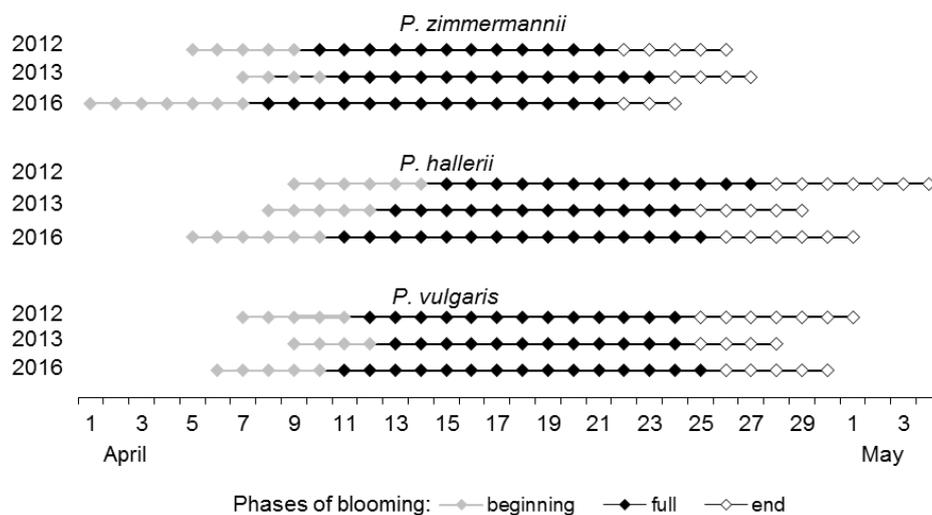


Fig. 1. Time and duration of blooming of *Pulsatilla* species in the years 2012–2013 and 2016 in Lublin, SE Poland

The number of flowers produced per 1 m<sup>2</sup> differed between species (df = 2, F = 14.35, P = 0.032) (tab. 1). The lowest number of flowers was recorded for *P. halleri*, approx. 20-percent more flowers was established for *P. vulgaris*. For each species, the year effect was also found (*P. zimmermannii* – df = 2, F = 24.25, P = 0.036; *P. halleri* – df = 2, F = 38.54, P = 0.028; *P. vulgaris* – df = 2, F = 14.31, P = 0.048). For example, for *P. halleri* up to 2-fold disparities were noted between years.

Flowers of all *Pulsatilla* spp. are hermaphrodite and protogynous. The life-span of a single *P. zimmermannii* flower was 5–11 days (mean = 7.3 ± 5.2 SD), 4–9 days (mean = 6.7 ± 3.7 SD) of a single *P. halleri* flower, and was 5–10 days (mean = 7.3 ± 5.2 SD) of a single *P. vulgaris* flower. The androecium is multistaminate, spirally arranged (fig. 2 A, B). A diverse high staminodes were present (fig. 2 C-F). At the base of androecium, minute droplets of nectar were noted (fig. 2 D), however we were not able to measure the nectar quantity using the pipettes applied in this study. The secretion was also visible on staminodes (fig. 2 F). The anthers dehisced subsequently and

pollen was available to insects for 5–11 (7) days. Most anthers dehiscid in mid-day hours, between 11.00 and 14.00 (GMT + 2 h). Usually pollen was released simultaneously in 10–40 anthers per single flower. The intensive pollen release was observed at a mean air temperature 10–15°C. In such conditions the entire quantity of pollen was released in 3–4 hours per anther. Temperature drops (mean = 2–5°C) slowed the process and pollen release per anther lasted even 1–2 days.

Table 1. The abundance of flowering of three *Pulsatilla* spp. observed in the years 2012–2013 and 2016. Means followed by the same small letters are not significantly different between years and values followed by the same capital letters are not significantly different between species at  $P = 0.05$  according to Tukeys HSD test

Species	Year	Number of flowers per 1 m <sup>2</sup>			
		min–max	mean	±SD	CV%
<i>P. zimmermannii</i>	2012	182–305	280.6 <sub>b</sub>	76.2	27.2
	2013	215–412	398.5 <sub>c</sub>	124.3	31.2
	2016	169–398	248.5 <sub>a</sub>	96.4	38.8
	mean	–	309.2 <sub>AB</sub>	–	39.9
<i>P. hallerii</i>	2012	168–298	189.8 <sub>a</sub>	106.0	55.8
	2013	256–427	379.5 <sub>b</sub>	84.0	22.1
	2016	187–425	305.7 <sub>b</sub>	54.0	17.7
	mean	–	291.7 <sub>A</sub>	–	31.9
<i>P. vulgaris</i>	2012	325–480	420.2 <sub>c</sub>	103.0	24.5
	2013	268–325	280.9 <sub>a</sub>	36.0	23.4
	2016	236–389	321.9 <sub>b</sub>	83.0	25.7
	mean	–	341.0 <sub>B</sub>	–	24.5

SD – standard deviation, CV – coefficient of variation

The species ( $df = 2$ ,  $F = 32.2$ ,  $P = 0.0024$ ) and year ( $df = 2$ ,  $F = 26.13$ ,  $p = 0.0021$ ) effects were established for the number of anthers developed per flower (tab. 2). The lowest number of anthers was assessed for *P. vulgaris*. On average, 12–14% more anthers was recorded in *P. zimmermannii* and *P. hallerii*.

Significant differences were recognized for the size of anthers (expressed as the dry mass of 100 anthers) between species ( $df = 2$ ,  $F = 22.5$ ,  $P = 0.027$ ) and between years ( $df = 2$ ,  $F = 42.5$ ,  $P = 0.034$ ).

The mass of pollen calculated per 100 anthers was species-specific ( $df = 2$ ,  $F = 42.5$ ,  $P = 0.0002$ ) and varied between years of study ( $df = 2$ ,  $F = 36.1$ ,  $P = 0.022$ ) with disparities reaching up to 1.6-fold (see *P. zimmermannii*) (tab. 3). Pollen production calculated for 10 flowers did not differ significantly between *P. zimmermannii* and *P. hallerii*, but twice outnumbered those calculated for *P. vulgaris*. The mass of produced pollen per 1 m<sup>2</sup> differed between species (fig. 3). On average, *P. zimmermannii* was found to produce the highest amount of pollen (9.16 g per 1 m<sup>2</sup>), which was 2 times more pollen

than *P. vulgaris* (4.22 g per 1 m<sup>2</sup>). The pollen yield strongly depended on the year of study in case of *P. halleri*. In 2013 the amount of pollen produced per 1 m<sup>2</sup> was approx. 2.7-fold higher than in previous year of study.



Fig. 2. Parts of *Pulsatilla halleri* and *P. vulgaris* flowers and pollen grains. *P. halleri*: A – the part of flower with many stamens (visible anthers) and stigmas, B – staminodes located in lower part of androecium (arrow), C – staminodes differ in height, *P. vulgaris*: D – nectar at the base of androecium (arrow), E – stamens and staminodes, F – staminodes producing nectar; pollen grains of *P. halleri*: G – in polar view, H – in equatorial view; pollen grains of *P. vulgaris*: I – in polar view, J – in equatorial view; pollen grains of *P. zimmermani*: K – in polar view, L – in equatorial view; a (anther), s (stigma), st (staminodes)

The pollen grain characteristics are presented in Table 4 and Figure 2 G-L. The species effect was found for the length of both polar (P) and equatorial (E) axes (df = 2, F = 8.02, P = 0.000 for P axis, df = 2, F = 19.03, P = 0.000 for E axis). The pollen grains size differed between years of study (df = 1, F = 100.80, P = 0.000 for P axis; df = 1, F = 16.69, P = 0.000 for E axis). Significantly larger pollen grains were observed in 2016 compared to 2013. The shape index was in the range 0.90–0.95.

Table 2. The number of anthers per flower and the mass of dry anthers (= anther size) of *Pulsatilla* species in the years 2012–2013 and 2016. Means followed by the same small letters are not significantly different between years and values followed by the same capital letters are not significantly different between species at  $P = 0.05$  according to Tukeys HSD test

Species	Year	Number of anthers per flower				Mass of 100 dry anthers with pollen (mg)		
		min–max	mean	±SD	CV %	mean	±SD	CV %
<i>P. zimmermannii</i>	2012	159–282	237.8 <sub>b</sub>	31.0	13.0	21.5 <sub>b</sub>	1.4	6.5
	2013	179–259	199.6 <sub>a</sub>	16.7	8.4	16.8 <sub>a</sub>	0.9	5.4
	2016	183–248	215.3 <sub>ab</sub>	27.5	12.8	19.3 <sub>b</sub>	1.2	6.2
	mean	–	217.6 <sub>B</sub>	19.2	–	19.2 <sub>B</sub>	2.4	–
<i>P. halleri</i>	2012	159–269	215.8 <sub>ab</sub>	28.5	13.2	16.7 <sub>a</sub>	0.6	3.5
	2013	138–235	199.8 <sub>a</sub>	18.4	9.2	20.4 <sub>c</sub>	1.4	4.9
	2016	202–252	231.0 <sub>b</sub>	21.2	9.2	18.5 <sub>b</sub>	1.9	10.2
	mean	–	215.5 <sub>B</sub>	15.6	–	18.5 <sub>B</sub>	1.9	–
<i>P. vulgaris</i>	2012	179–230	191.5 <sub>b</sub>	8.8	4.6	10.4 <sub>a</sub>	1.2	11.5
	2013	142–228	169.5 <sub>a</sub>	12.2	7.2	13.5 <sub>c</sub>	1.6	11.8
	2016	191–202	195.5 <sub>b</sub>	4.7	2.4	11.8 <sub>b</sub>	2.2	18.6
	mean	–	185.5 <sub>A</sub>	14.0	–	11.9 <sub>A</sub>	1.6	–

SD – standard deviation, CV – coefficient of variation

Table 3. The mass of pollen produced per 100 anthers and per 10 flowers of *Pulsatilla* species in the years 2012–2013 and 2016. Means followed by the same small letters are not significantly different between years and values followed by the same capital letters are not significantly different between species at  $P = 0.05$  according to Tukeys HSD test

Species	year	Mass of pollen per 100 anthers (mg)			Mass of pollen per 10 flowers (g)		
		mean	±SD	CV %	mean	±SD	CV %
<i>P. zimmermannii</i>	2012	16.7 <sub>c</sub>	4.3	25.7	0.40 <sub>c</sub>	0.21	52.0
	2013	10.5 <sub>a</sub>	1.5	14.2	0.21 <sub>a</sub>	0.18	85.7
	2016	14.9 <sub>b</sub>	3.8	25.5	0.32 <sub>b</sub>	0.22	68.7
	mean	14.0 <sub>C</sub>	4.3	–	0.31 <sub>B</sub>	–	–
<i>P. hallerii</i>	2012	9.8 <sub>a</sub>	2.9	29.5	0.21 <sub>a</sub>	0.14	66.7
	2013	14.2 <sub>c</sub>	7.2	50.7	0.28 <sub>b</sub>	0.16	57.1
	2016	11.5 <sub>b</sub>	4.9	42.6	0.27 <sub>b</sub>	0.20	74.1
	mean	11.8 <sub>B</sub>	6.1	–	0.25 <sub>B</sub>	–	–
<i>P. vulgaris</i>	2012	5.9 <sub>a</sub>	1.1	18.6	0.11 <sub>a</sub>	0.06	54.5
	2013	8.0 <sub>b</sub>	3.2	40.0	0.14 <sub>b</sub>	0.11	78.6
	2016	6.5 <sub>a</sub>	2.7	41.5	0.13 <sub>ab</sub>	0.09	69.2
	mean	6.8 <sub>A</sub>	1.4	–	0.13 <sub>A</sub>	–	–

SD – standard deviation, CV – coefficient of variation

On average, pollen grains of the *Pulsatilla* species are medium. The size of P axis ranged 29.8–38.4  $\mu\text{m}$ , and E axis ranged 32.3–39.6  $\mu\text{m}$ , i.e. within a range of 25–50  $\mu\text{m}$  characteristic for medium-sized pollen grains (Erdtman, 1954). The mean shape index of the studied ornamental *Pulsatilla* species ranged between 0.90 and 0.95, which classifies the pollen grains as *oblate-spheroid*.

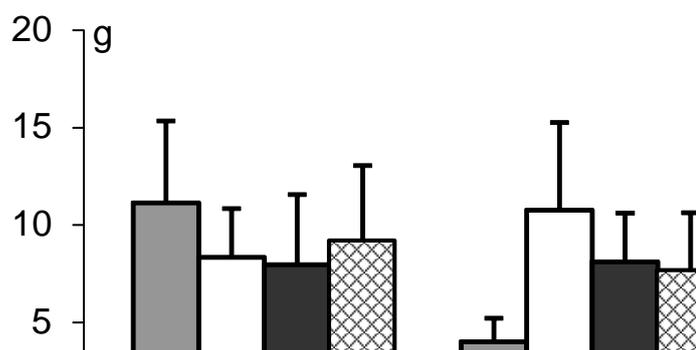


Fig. 3. The mass of pollen produced per 1 m<sup>2</sup> of three *Pulsatilla* spp. in the years 2012, 2013 and 2016 (SE Poland). Mean values are given.

Table 4. Morphological characteristics of pollen grains from three *Pulsatilla* species in 2013 and 2016. Means followed by the same small letters are not significantly different between years and values followed by the same capital letters are not significantly different between species at  $P = 0.05$  according to Tukey's HSD test

Species	Year	Length of axis ( $\mu\text{m}$ )						Shape index P/E
		polar (P)			equatorial (E)			
		min-max	mean	$\pm\text{SD}$	min-max	mean	$\pm\text{SD}$	
<i>P. zimmermannii</i>	2013	27.5–37.5	32.5 <sub>a</sub>	2.9	35.0–42.5	38.5 <sub>a</sub>	2.4	0.8
	2016	35.4–40.4	38.4 <sub>b</sub>	1.4	36.6–43.2	39.6 <sub>b</sub>	1.7	1.0
	mean	–	35.5 <sub>B</sub>	–	–	39.1 <sub>B</sub>	–	0.90
<i>P. hallerii</i>	2013	27.5–35.0	30.8 <sub>a</sub>	2.4	32.5–40.0	35.3 <sub>a</sub>	2.5	0.9
	2016	30.7–37.5	34.5 <sub>b</sub>	1.6	32.4–39.7	36.2 <sub>b</sub>	1.8	1.0
	mean	–	32.7 <sub>A</sub>	–	–	35.8 <sub>A</sub>	–	0.95
<i>P. vulgaris</i>	2013	27.5–32.5	29.8 <sub>a</sub>	1.8	30.0–35.0	32.3 <sub>a</sub>	2.2	0.9
	2016	31.3–39.2	36.4 <sub>b</sub>	2.1	33.9–40.7	37.6 <sub>b</sub>	2.0	1.0
	mean	–	33.1 <sub>A</sub>	–	–	35.0 <sub>A</sub>	–	0.95

SD – standard deviation

## DISCUSSION

Our study can be considered as a contribution to lists of garden plants that attract insect visitors mainly with pollen reward. The species from the genus *Pulsatilla* can attract insect visitors with large (size 6–8 cm in diameter), colourful, campanulate, actinomorphic flowers, that produce a large amount of pollen and trace amounts of nectar.

If planted together in spring ornamental gardens, the species will provide pollen from first days of April until end of the month, i.e. during the period when seasonal activity of Apoidea insects begins. We have to point out that the onset and duration of blooming for single species differed slightly (only 3–5 days) between growing seasons indicating that the repeatable pollen resources can be expected in the early spring period, in April. It seems to be a very important issue because many early spring flowering species indicate remarkable (even 25–40 days) differences in the beginning and duration of flowering e.g. *Corydalis* spp. [Denisow et al. 2014b] or *Anemone sylvestris* [Denisow and Wrzesień 2015 b].

Irrespective of the species, we established the differences in the seasonal flowering abundance. Generally, plants differ greatly with respect to the number of flowers displayed in the season, depending on flowering time, environmental conditions or fertilization type [Denisow et al. 2015 a]. For the same ornamental species, the year-to-year disparity in the number of developed flowers can differ greatly, e.g. 2–10 fold [Garbuzov and Ratnieks 2014, Wrzesień et al. 2016].

In the genus *Pulsatilla*, the multistaminate androecium is spirally arranged. A high number of stamens, characteristically inserted in a spiral on a conical apex is an attribute of several genera within the Ranunculaceae family, i.e. *Caltha*, *Ranunculus*, *Helleborus*, *Anemone* [e.g. Denisow and Božek 2006, Jensen and Kadereit 2012, Denisow and Wrzesień 2015 b].

In the *Pulsatilla* flowers most anthers dehisced in mid-day hours. This is a common feature in many early spring plant species [Denisow et al. 2014 b, Denisow et al. 2015]. The mechanism of mid-day anther dehiscence is related to the environmental conditions, i.e. the decline in the relative humidity and the rise of air temperature. Such conditions enhance water loss from the anther tissues and pollen release [Pacini 2000]. Mid-day pollen release in the early spring flowers is an advantage and correspond to the daily insect visitor activity pattern.

The significant year effect on the number of anthers developed per flower was noted. This indicate the influence of weather factors on the androecium formation and is in accordance with observations concerning other multi-staminate flowers from the Ranunculaceae family [e.g. Szklanowska 1995, Denisow and Wrzesień 2015 b].

In our study species, pollen was the main flower reward available for insect visitors. The nectarines were reported in the flowers of *P. vulgaris* by Weryszko-Chmielewska and Sulborska [2011]. We identified the nectary glands in the flowers of *Pulsatilla* species, however the nectar amount was very minute (c.a. < 0.5 ml per flower). Nectar was found to attract bumble bees to flowers of *P. pratensis* [Torvik et al. 1998] and both bumble bees and solitary bees to flowers of *P. cernua* [Huang et al. 2002]. We observed some insect visitors (bumblebees and ants), which were not interested in pollen reward and had been found at rest in the base of the ovary. However, we are not sure if these

insects searched for or used nectar as an attractant. Many spring flowers are reported to give shelter for mating or sleeping pollinators e.g. *Adonis vernalis* [Denisow et al. 2014] or *Anemone sylvestris* [Denisow and Wrzesień 2015b, Denisow et al. 2016]. Besides the food these functions are very important for insect pollinators biodiversity maintenance [Chittka et al. 1999]. According to Kugler [1970], *Pulsatilla* flowers are visited for nectar by butterflies, however we did not find Hymenoptera representatives during our observations.

Different authors [e.g. Szklanowska 1995, Denisow et al. 2014, Denisow and Wrzesień 2015b] established the mass of pollen produced in Ranunculaceae flowers and underlined substantial disparities from 2.0 mg to 16.0 mg per 10 flowers. Also in *Pulsatilla* species the differences in the mass of pollen produced in flowers were observed, which is due to multi-staminate androecium. In agreement with Denisow and Bożek [2006] the mass of pollen per flower depend also on the anther size, in our study measured as anther dry mass.

We calculated the pollen production per 1 m<sup>2</sup> (4.22–9.16 g, on average), therefore *Pulsatilla* species can be indicated as good pollen source, delivering more pollen than other early spring perennials, i.e. *Caltha palustris* (1–3 g per 1 m<sup>2</sup>) [Szklanowska 1995], *Adonis vernalis* (0.6–1g per 1 m<sup>2</sup>) [Denisow and Wrzesień 2006] or *Anemone sylvestris* (0.4–2.1 g per 1 m<sup>2</sup>) [Denisow and Wrzesień 2015 b].

Pollen grains of the *Pulsatilla* species are medium in size, within a range of 25–50 µm [Erdtman 1954]. The mean shape index of the studied ornamental *Pulsatilla* species ranged between 0.90 and 0.95, which classifies the pollen grains as *oblate-spheroid* [Ziemińska-Tworzydło and Kohlman-Adamska 2003].

## CONCLUSIONS

1. In the conditions of SE Poland, blooming of *Pulsatilla halleri*, *P. vulgaris* and *P. zimmermannii* began at the beginning of April and lasted until the end of the month.
2. Pollen production of the studied species ranged, on average, from 4.22 g/m<sup>2</sup> (*P. vulgaris*) to 9.16 g/m<sup>2</sup> (*P. zimmermannii*).
3. Minute droplets of nectar were found both at the base of androecium and on the staminodes.
4. Pollen grains of three studied *Pulsatilla* spp. are medium in size (P axis ranging 29.8–38.4 µm and E axis 32.3–39.6 µm) and *oblate-spheroid* (P/E ratio ranging 0.90–0.95).

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## FENOLOGIA KWITNIENIA I PRODUKCJA PYŁKU TRZECH WCZESNOWIOSENNYCH GATUNKÓW Z RODZAJU *Pulsatilla*

**Streszczenie.** Przeprowadzone badania dotyczyły biologii kwitnienia i produkcji pyłku trzech gatunków sasanki: *Pulsatilla zimmermannii* Soó, *P. halleri* (All.) Willd. oraz *P. vulgaris* Mill., uprawianych w Ogrodzie Botanicznym UMCS w Lublinie. Rośliny te wytwarzają duże (średnica kwiatu 6–8 cm), dzwonkowate kwiaty o symetrii promienistej. Pręcikowie składa się z licznych pręcików (142–282 pręcików/kwiat) ułożonych spiralnie. U podstawy pręcikowia oraz na pręciczkach zauważalne były drobne krople nektaru. Pożytek pyłkowy z pojedynczego kwiatu był dostępny dla owadów przez 5–11 (7) dni. Największą wydajność pyłkową obliczono dla *P. zimmermannii* (średnio 9,16 g/m<sup>2</sup>).

Średnie masy pyłku produkowanego przez kwiaty *P. halleri* i *P. vulgaris* wynosiły odpowiednio 7,63 g/m<sup>2</sup> i 4,22 g/m<sup>2</sup>. Ziarna pyłku *Pulsatilla* spp. są średniej wielkości: długość osi biegunowej (P) zawarta była w przedziale 29,8–38,4 μm, a równikowej (E) – w granicach od 32,3 μm do 39,6 μm. Stosunek P/E wynosił od 0,90 do 0,95, co wskazuje, że ziarna są kształtu płasko-kulistego. Badane gatunki sasanki mogą uzupełniać zasoby pokarmowe owadów zapylających wczesną wiosną.

**Słowa kluczowe:** kwitnienie, prątniczki, pożytek pyłkowy, ziarna pyłku, *Pulsatilla* spp.

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