

FLOWERING, POLLEN CHARACTERISTICS AND INSECT FORAGING ON *Campanula bononiensis* (CAMPANULACEAE), A PROTECTED SPECIES IN POLAND

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Received: 27.01.2014

Abstract

This study investigated the floral biology and pollen quantity and quality of *Campanula bononiensis* L. (Campanulaceae), a protected species in Poland. Observations and measurements were made during the years 2007–2009 in natural phytocoenoses from the Festuco-Brometea class situated within the Lublin area, SE Poland. A considerable decrease (approx. 87%) in population density was observed. Significant variations both in the amount of pollen (18.5%–34.8 % of pollen in the total anther dry weight, i.e. 0.5–1.5 mg per 10 anthers) and in pollen viability (38.8–97.0%) were noted. Both a low amount of pollen and low pollen viability may reduce the reproductive success of individuals. The most frequent visiting insects were bees (*Apoidea*), including solitary bees 45.7%, honeybees 20.4%, and bumblebees 11.4%. Dipterans, coleopterans (weevils), lepidopterans and ants were also recorded, implying a strong impact of *C. bononiensis* on insect biodiversity within grasslands.

Key words: *Campanula bononiensis*, grasslands, population size, pollen quantity, pollen viability, insect visitors

INTRODUCTION

The interaction of plants with pollinating agents is vital for the structural and functional integrity of natural ecosystems. The concept includes several aspects, i.e. the stability of pollination webs [1, 2]. Their maintenance is one of the most important challenges and can be obtainable by conservation of plant species biodiversity. It is believed that the understanding of the

floral biology of wild species threatened with extinction may contribute to their protection and through this our understanding of different interactions linking organisms within biocenosis may be improved [3]. In entomophilous plants, besides the nectar, pollen serves as an attractant [4]. Pollen of entomophilous species has a double function in a biocenosis. Firstly, it serves as a donor of male gametes and therefore has evolved to increase the efficiency of reproduction by qualitative and quantitative traits [5–8]. Secondly, it serves as a rich source of high-quality protein pollen, which is an important food for a variety of insect visitors that need a continuous food supply throughout the whole life cycle [9, 10].

The genus *Campanula* includes over 500 species of herbaceous plants, distributed across the temperate regions of the Northern Hemisphere, with the highest diversity in the Mediterranean region east to the Caucasus [11]. Among the genus *Campanula*, 9 species are recorded in natural phytocoenoses in Poland [12, 13]. Species from the genus *Campanula* are particularly important for oligolectic bees, i.e. specialized and with a narrow preference to collect pollen exclusively from *Campanula* flowers [14, 15]. To date, most studies on *Campanula* spp. focused on descriptions of style trichomes (PCHs-pollen collecting hairs), dichogamy and secondary pollen presentation mechanisms [9, 16–19]. The adaptive significance of the hairs in relation to pollination has been interpreted in relation to the plant reproductive success [20, 21].

The aim of the presented study was (1) to identify the phenology of blooming; (2) to determine the population density and blooming abundance; (3) to show the qualitative and quantitative pollen traits; and (4) to record the spectrum of insect pollinators on *Campanula bononiensis*, a protected species in Poland.

MATERIALS AND METHODS

Study site and species description. The observations and measurements were carried out during the years 2007–2009 in natural phytocoenoses from the Festuco-Brometea class situated within the Lublin area (FE2766, 51°16' N, 22°30' E, 200 m a.s.l.), the Lublin Upland, SE Poland.

Campanula bononiensis L. (Campanulaceae) is a species with high microhabitat demands and prefers edges of thermophilous woods and thickets as well as closed xerothermic tall-grass meadows. Detailed autoecological characteristics are shown in Figure 1. The species is considered to be characteristic of two alliances: *Cirsio-Brachypodium pinnati* grasslands of Festuco-Brometea and *Geranion sanguinei*-fringe communities of thermophilous herbs of Trifolio-Geranietea class [22]. The species is rare in Central Europe and is protected by law in Poland [23, 24].

Flowering and insect visitors' activity. Protocols described by Jabłoński and Szklanowska [25] as well as by Denisow [7] were applied. During the growing seasons, the onset and length of flowering were established. Due to time-consuming observations, the diurnal flowering pattern was established only in 2007 and 2008. The observations were conducted from 8.00 till 19.00 (GMT + 2.00 h) and in two-hour intervals newly opened flowers were counted ($n = 6$ individuals); night anthesis was excluded. Simultaneously to the blooming observations, insect visits were recorded ($n = 4$ random plots of 1 m²). These observations were made for three consecutive days, at the full bloom stage of the population. During each observation, the total number of visiting insects was recorded. The abundance of blooming was determined by the number of flowers produced during the life cycle, i.e. all buds, flowers and fruits per stem were counted. The population density was established on the basis of 10–30 random circular 0.1 m² areas (36.7 cm in diameter). The data was converted to the number of flowers per 10 m².

Quantity and quality of pollen. In order to measure the amount of pollen production, the ether method was used [8]. During each season, well-developed flower buds were randomly chosen at the full flowering stage, before the anthers dehisced. The anthers were dissected and placed in tarred vessels. Four samples were collected ($n = 50$ stamens). Stamen heads were assayed for fresh and dry weight and for

the weight of pollen contained in them. Samples were placed in a dryer (ELCON CL 65) at ca. 33°C. The pollen was rinsed once with pure ether (2 ml) and then 2–3 times with 70% ethanol (3–4 ml). Pollen viability ($n = 3 \times 100$) was examined in standard acetocarmine-stained slides, while the size of pollen grains in glycerogelatin slides ($n = 4 \times 50$). Pollen features were observed by light microscopy (Nikon Eclipse E-200).

The weather pattern. Weather data were obtained from a weather station located in Lublin (Felin). Monthly and 10-day means were compared to the long-term data (1951–2005). The general weather pattern differed between growing seasons. In 2007 the temperatures were above normal (May by +2°C, June by +1.9°C, July +1.4°C) and rainfall was higher than normal (May by 23.8 mm, June by 22.1 mm, July by 3.5 mm). The beginning of 2008 was colder compared to the long-term data. Spring was early with March and April temperatures higher than the long-term average (by +2.3°C and +1.9°C, respectively). May was very wet with rainfall approx. 50% higher than the long-term average. Summer was dry with rainfall in June 65% lower compared to the long-term average. The air temperatures in the spring of 2009 were at an average level, but the rainfall was twice higher than normal. In June the rainfall was approx. 130% higher than normal, whereas July was very dry (rainfall lower by 75%).

Data analysis. Analysis of variance (ANOVA) was applied [26]. The data are presented as means with SD. Parametric statistical analysis was applied for the pollen traits. *Post hoc* comparison of means was tested by Tukey's HSD test. The Kruskal–Wallis test for unequal size groups was used to determine the differences for non-normally distributed data (population density, number of flowers per individual). Paerson's correlation (r) of the mass of produced pollen against the dry weight of anthers was determined. The level of statistical significance for all analyses was at $P = 0.05$. All analyses were performed using Statistica ver. 6.0 (StatSoft Poland, Krakow).

RESULTS

Under the climate conditions of SE Poland, during 2007–2009 the flowering of *Campanula bononiensis* began in late June and lasted till the middle of September (Fig. 2). The phenology of flowering differed between the years of study. A two-week shift for the onset of flowering was noted between 2007 and 2009. The duration of seasonal flowering varied from 6 to 10 weeks. The flowers of *C. bononiensis* are perfect, actinomorphic and are day-opening. Most flowers opened between 9.00 h and 12.00 h, and the process continued till 17.00, GMT+2h (Fig. 3). The flowers are distinctly protandrous and therefore both functionally male-phase

and female-phase flowers were present on individuals (Fig. 4B, C). At the bud stage, the anthers were rigid and formed a narrow tube (Fig. 4D). The style with closed lobes was centrally positioned and the anthers touched closely to the style. Most anthers dehisced in well-developed buds and before corolla opening the male phase started. The release of pollen began in the apical part of the anthers and sticky pollen covered trichomes present on the style (Fig. 4E). The presentation of pollen to insects lasted for 1–2 days per flower. The centrally located style exhibited a 3-curved stigma during the female phase.

A year effect was found for the number of flowers produced per plant ($P = 0.0328$) and for the population density ($P = 0.0287$) (Table 1). The number of flowers formed per individual was 66.9 ± 44.6 SD, ranging from 24 to 112. In 2009 the individuals produced 2 times fewer flowers, compared with the other years. The number of individuals decreased by 97% between 2007 and 2009. The total number of flowers per unit area also differed significantly between growing seasons ($P = 0.0314$).

A year effect was found for the size of anthers, measured as the (fresh and dry) mass of anthers ($F_{2,21} = 23.29$, $P = 0.021$, $F_{2,21} = 8.34$, $P = 0.035$, respectively). The smallest anthers were recorded in 2009, while almost twice larger anthers were observed in 2008 (Table 2). The mean value of the fresh weight for 10 anthers, including pollen, ranged between years from 4.3 to 7.9 mg (mean = 6.2). The mean dry weight of anthers was accordingly lower and ranged from

2.7 to 4.3 mg (mean = 3.3). The percentage participation of pollen in anthers varied and depended on the study year. In the anthers of *C. bononiensis*, pollen accounted for 18.5–34.8 % of the total anther dry weight (Table 2). The mass of pollen per 10 anthers correlated positively with anther dry mass (Pearson's correlation $r = 0.8622$). A significantly higher amount of pollen per 10 anthers was produced in 2008. The differences between means for years were significant ($F_{2,21} = 5.32$, $P = 0.014$). Pollen viability varied between the years of study. The lowest number of pollen grains with a viable protoplast was found in 2009 (mean = 38.8 %), while the highest one in 2008 (mean = 97.0 %). The pollen grain of *Campanula bononiensis* is trizonoporate circular in polar view and oblate-spheroidal in equatorial view (Fig. 4F, G). The mean value of the P/E ratio is 0.91. The polar axis (P) ranged 27.66–30.94 μm , mean 29.05 μm ; the equatorial axis (E) ranged 29.61–34.35, mean = 31.82 μm .

The percentage participation of insect visitors is presented in Figure 5. The primary visitors were *Apoidea* bees. The participation of *Apis mellifera* and solitary bees substantially varied between years. Solitary bees predominated on the flowers and contributed 45.7% of visits, on average (ranging 33.0–58.4%). *Apis mellifera* accounted for an average of 20.4% of visits (ranging 12.3–28.5 %). The inter-annual percentage of bumblebees (8.8–13.9%) and dipterans was almost steady (10–13%). Coleoptera and Formicidae accounted for ~ 5% of visits each. Butterflies were sporadically found on the flowers, only in 2007.

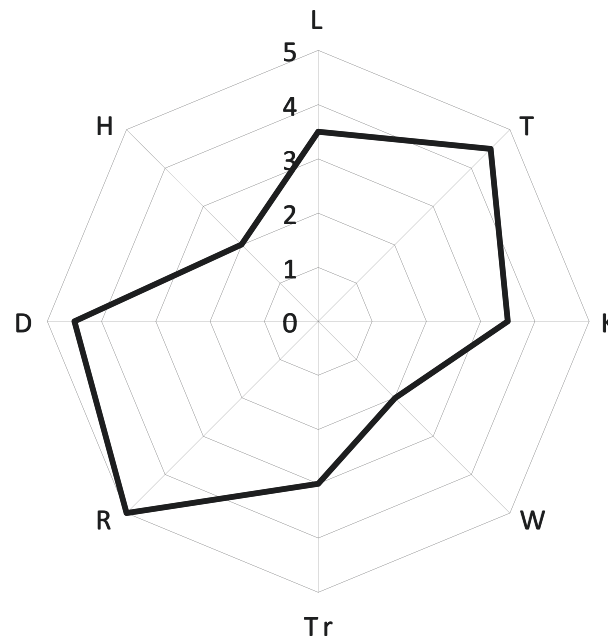


Fig. 1. The synthetic autecological characteristics of *Campanula bononiensis* based on Ellenberg ecological numbers; L – light, T – temperature, K – continentality, W – soil moisture, Tr – soil trophicity, R – soil reaction, D – soil granulometry, H – soil humic matter content

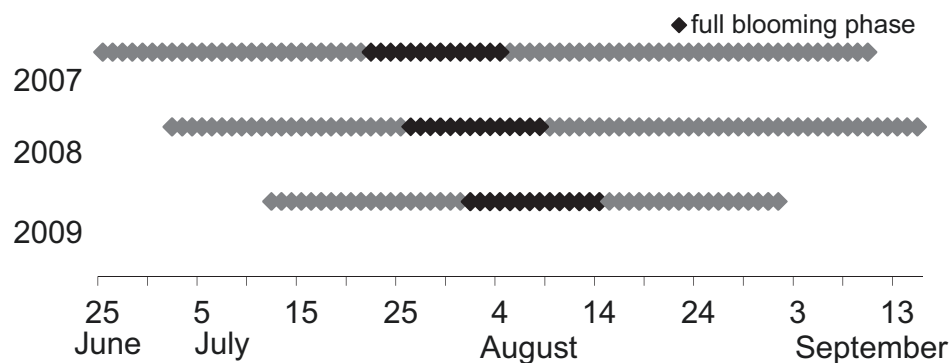


Fig. 2. Phenology of flowering of *Campanula bononiensis* in the years 2007–2009 observed in SE Poland.

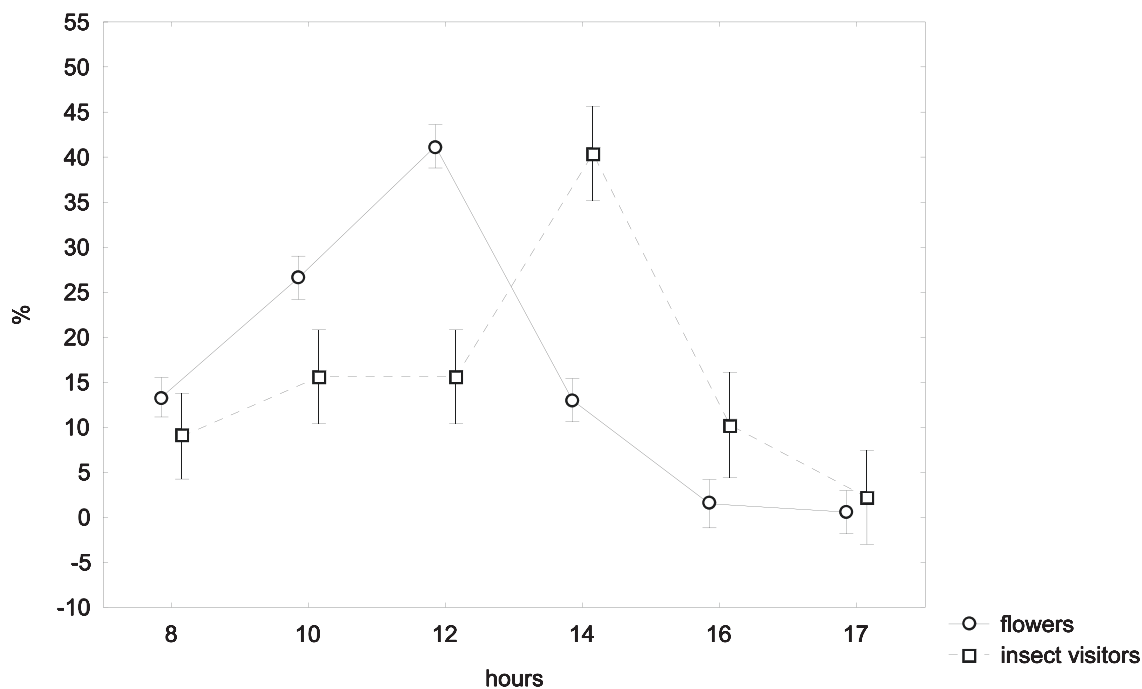


Fig. 3. Diurnal pattern of flowering of *Campanula bononiensis* expressed as the number of flowers opened in two-hour intervals in relation of the total opened during the day and the diurnal activity of insect visitors (mean for all species noted) observed in SE Poland (means calculated from 2007 and 2008). Vertical bars indicate 95% confidence intervals.

Table 1
The number of flowers per individual, the population density
and the total abundance of blooming of *Campanula bononiensis* in the years 2007–2009.
Year effect was tested according to Kruskal-Wallis test

Variable	Year						P- value
	2007		2008		2009		
	Mean	± SD	Mean	± SD	Mean	± SD	
flowers per individual	71.2	21.4	86.1	32.2	43.6	21.8	0.0328
individuals per 10 m ²	47.8	32.2	22.6	6.6	4.3	1.6	0.0287
flowers per 10 m ² (thousands)	3.4	1.2	1.9	1.4	0.2	0.1	0.0314

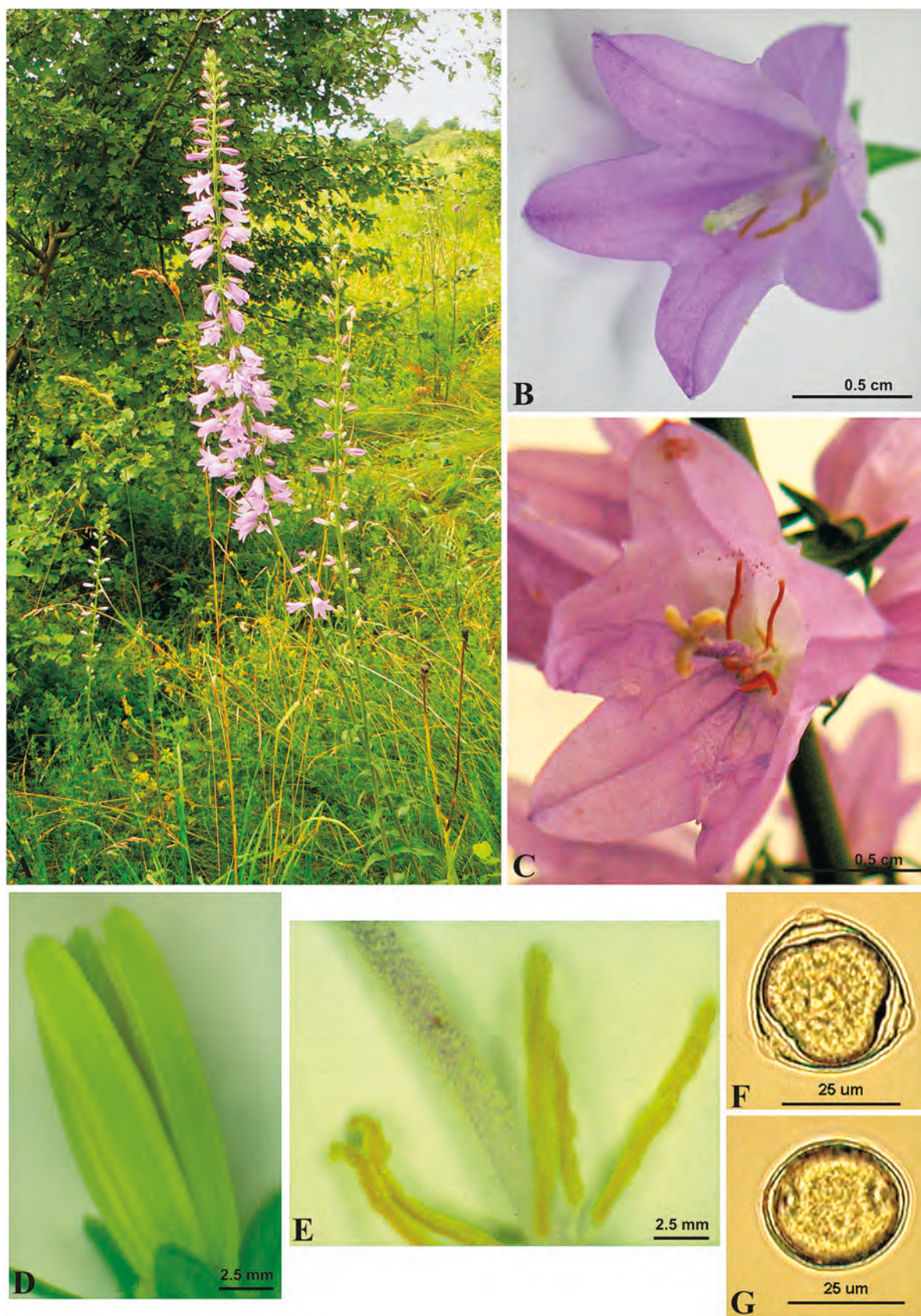


Fig. 4. *Campanula bononiensis* A – at full bloom in the phytocoenoses from Festuca-Brometea class; B – male-phase flower; C – female-phase flower; D – anthers in the bud stage; E – pollen presentation on the style; F – pollen grain – polar view; G – pollen grain – equatorial view

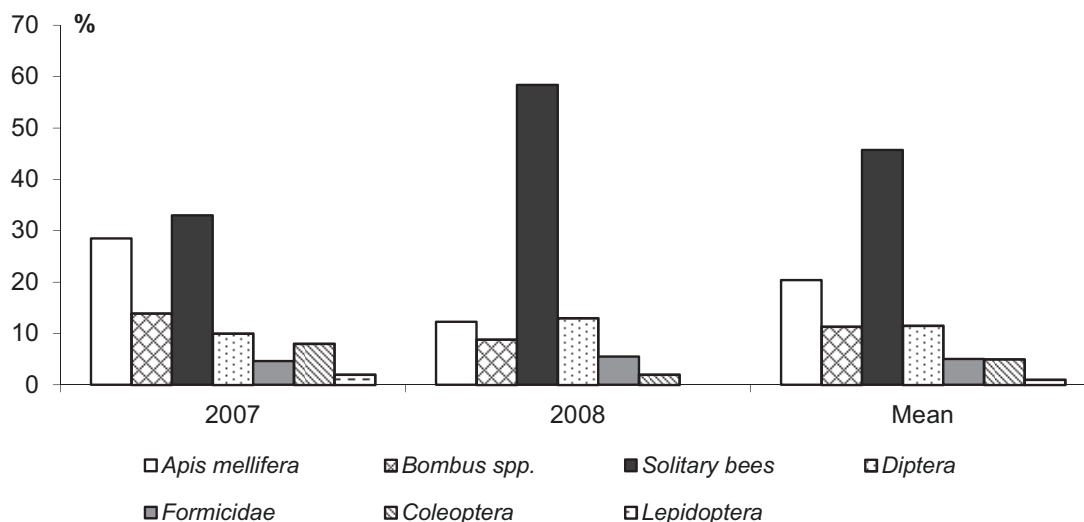


Fig. 5. The relative abundance of insect visitors to the flowers of *Campanula bononiensis* observed in 2007 and 2008 in SE Poland.

Table 2

The fresh and dry mass of 10 anthers and the mass of pollen produced per 10 anthers, the percentage participation of pollen in anthers and pollen viability of *Campanula bononiensis* in the years 2007–2009. Values within columns followed by the same letters are not significantly differ between years at $P=0.05$ according to Tukey's HSD test.

Year	Mass of 10 anthers with pollen (mg)				Mass of pollen per 10 anthers (mg)			Viability (%)	
	Fresh		Dry		Mean	± SD	%	Mean	± SD
	Mean	± SD	Mean	± SD					
2007	6.4 _a	6.0	3.0 _a	1.1	0.8 _a	0.4	26.7	75.5 _a	6.5
2008	7.9 _b	0.9	4.3 _b	0.7	1.5 _b	0.5	34.8	97.0 _b	4.5
2009	4.3 _c	1.8	2.7 _a	1.8	0.5 _a	0.3	18.5	38.8 _c	2.0
Mean	6.2		3.3		1.6		31.5	70.4	

DISCUSSION

Campanula bononiensis was a component of typical xerothermic phytocoenoses from the Festuco-Brometea class, analogous to that observed in other central European countries [22]. A decrease in the number of individuals and in the abundance of flowering between growing seasons was observed and this was presumably related to the overgrowing processes present in the patch. The encroachment of shrubs (e.g. *Prunus spinosa*, also noted in the phytocoenoses studied) is considered as the main factor for the decline in many herbaceous plant populations in communities from the Festuco-Brometea class [27, 28]. The decrease in the abundance of flowering, combined with the effect of patch overgrowing, may also be influenced by fluctuations in environmental conditions, e.g. weather factors during the growing period. A highly significant correlation between the abundance of flowering (density of individuals, the number of developed flowers) and meteorological factors was noted for species from the genus *Campanula* [29].

The flowering period of *Campanula bononiensis* in SE Poland may last from 6 up to 10 weeks. On average, the flowering begins in late June and may extend till the middle of September. Seasonal shifts are due to weather conditions varying between years. Generally, high temperatures speeded up the blooming process, while prolonged rainfall inhibited flowering. The dependence of flowering on heterogeneous climate conditions is highlighted for many plant species [i.e. 7, 25, 30], including species from the genus *Campanula* [29]. The majority of flowers open between 10.00 and 16.00 h, similarly to *C. rapunculoides* flowers described by Schlindwein et al. [15].

The proportion of pollen in anthers differed (18.5–34.8 %) and therefore the mass of pollen produced in anthers varied (0.5–1.5 mg per 10 anthers) between growing seasons. The lowest pollen production was probably attributed to a lack of rainfall, as the decrease in the mass of produced pollen was noted during the year with a rainfall deficit (precipitation approx. 75% lower compared to the long-term average). The effect of drought on the decline in pollen produc-

tion has been found by different authors [8, 31]. The lowest pollen viability (only 38.8 %) was also recorded in the year with low precipitation. Pollen viability can vary considerably depending on different weather factors (relative humidity, temperature) and water deficit is considered as particularly important for protoplast formation, although the response of plants to stress conditions can differ [5, 7, 32, 33].

The corolla of *C. bononiensis* shows subtle changes in the color during flower development. This could be used by pollinators to help in distinguishing between rewarding and unrewarding flowers and is common among entomophilous taxa [4, 9]. The flowers of *C. bononiensis* are strictly protandrous. This kind of dichogamy is characteristic for the family Campanulaceae [9] and is considered as a mechanism to avoid self-pollination [20]. In the flowers of *C. bononiensis*, the dehiscence of anthers begins at the bud stage; the process of pollen release is very rapid and when the corolla opens the pollen from all the anthers is presented on the style. A rapid mechanism of pollen presentation is known in a few families, e.g. Orchidaceae and Asteraceae, and is associated with the androecium consisting of a few stamens [5, 30]. This mechanism contrasts with flowers that develop numerous stamens and show a prolongation of pollen presentation by a regular pattern of pollen release [8, 32]. Due to the secondary pollen presentation, the process of pollen exposition extended for approx. 2 days per single flower of *C. bononiensis*. In the case of protandrous species, the prolongation of the male phase is an advantage based on phenology and the availability of ovules increases rapidly during the flowering season [34, 35]. The secondary pollen presentation in *C. bononiensis* definitely increases the mating opportunities by the extension of the availability of male gametes for reproduction and allows the transfer of pollen for cross-pollination.

The style presenter of *C. bononiensis* is covered with trichomes, a feature known widely in Campanulaceae [e.g. 4, 9]. Similarly to other *Campanula* species described [20], the elongation of the style and the unfolding of the three lobes took place after anther dehiscence; thus the stigma could receive substantial quantities of cross-pollen. We also observed that the disappearance of trichomes of the style correlates with the end of pollen presentation and with the unfolding of the stigma lobes, similar to that described by Weryszko-Chmielewska and Bartyś [19]. When the function of the style's trichomes ended, the female phase followed the male phase. It seems that the disappearance of the trichomes additionally restricts the risk of self-pollination. Otherwise, the plant saves energy when a component of the flower that has finished functioning is destroyed [18].

The pollen grain of *C. bononiensis* is medium sized, within a range (26–50 µm) defined by Erdtman [36] and Moore et al. [37] as characteristic for *Campanula* type.

Campanula bononiensis offered both nectar and pollen as the floral reward and the flowers were attractive to a variety of insect visitors, not only Apoidea. Mating and sleeping dipterans as well as ants were observed in the flowers. According to Szafer and Wojtusiakowa [16], *Campanula* flowers emit heat radiation. The pollen presenter is presumably involved in the process. Dipterans and ants, known to be temperature sensitive, were observed close to the pollen presenter. Their behavior in the flowers provide evidence for the combined function of the flower (food, shelter), thereby implying a strong impact of *C. bononiensis* on insect biodiversity.

The participation of insect visitors varied between years. The temporal changes in the guild of insect visitors to *C. bononiensis* flowers could be associated with different factors, e.g. the variable density of individuals, the varying abundance of blooming, the variations in the quantity of flower reward offered or the changes in accompanying vegetation. A similar conclusion was made by Goulson [10] who found that insect foragers preferred to visit abundantly flowering areas that ensure high quantity of resources. Also co-flowering species likely impact the differences in the participation of insect visitors [10, 38]. We observed that the accompanying species varied between years, i.e. *Centaurea scabiosa*, a taxon regarded as very attractive for the honeybee [30], was present in 2008 but not in 2007. This potentially could affect the lower participation of *Apis mellifera* on the *C. bononiensis* flowers in 2008, compared with 2007. Additionally, the population size of *Apis mellifera* undoubtedly varied annually and is related to the activity of local beekeepers. The other possible important factor in the distribution of foragers, e.g. solitary bees (33–58%), was likely due to the location of nests within the area and to the insect population size which fluctuates between growing seasons. Incredibly intriguing was a very low frequency of butterflies and their absolute absence in one year of the study. Butterflies have been regularly observed on various *Campanula* species across European countries [4, 15, 16, 39, 40]. However, according to a report from the European Environment Agency (EEA), grassland butterflies have declined dramatically between 1990 and 2011 [41]. Likewise, this negative trend may explain the lack of butterflies in our biocenosis.

Authors' contributions

Concept of the study – BD, MW; field work – BD, MW, MS-A; photographs – BD, MW, MB;

laboratory analysis – BD, MB, AJ; statistical analysis – BD; writing the manuscript – BD.

Acknowledgements

The material from protected species was collected in compliance with Polish law under the permit from the Regional Nature Conservator in Lublin. We gratefully thank Michał Wrzesień for his logistic support during the field study. This research was supported financially by the Ministry of Science and Higher Education of Poland as part of the statutory activities of the Department of Botany, University of Life Sciences in Lublin.

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Kwitnienie, cechy pyłku oraz owady wizytujące kwiaty chronionego gatunku *Campanula bononiensis* (Campanulaceae)

Streszczenie

W pracy przedstawiono wyniki badań dotyczących aspektów kwitnienia, cech ilościowych i jakościowych pyłku *Campanula bononiensis* L. (Campanulaceae), gatunku objętego w Polsce ustawową ochroną. Obserwacje prowadzono latach 2007–2009, w naturalnej fitocenozie z klasy Festuco-Brometea zlokalizowanej w Lublinie. Zanotowano znaczny, ok. 87% spadek zagęszczenia populacji. Poza procesami sukcesyjnymi, prowadzącymi do zarastania murawy, zanikanie populacji *C. bononiensis* może być związane z wewnętrznymi czynnikami biologicznymi, np. ilością i jakością wytwarzanego pyłku. W latach badań zanotowano znaczne wahania w ilości produkowanego pyłku (18.5%–34.8 % suchej masy pylników, tj. 0.5–1.5 mg z 10 pylników) oraz istotne zróżnicowanie jego żywotności (38.8–97.0%).

Najczęściej obserwowanymi owadami wizytującymi kwiaty były owady pszczołowate (*Apoidea*). Pszczoły samotnice stanowiły 45.7% ogólnej liczebności owadów, 20.4% wyniósł udział pszczoły miodnej, a 11.4% stanowiły różne gatunki z rodzaju *Bombus*. Obserwowano również muchówki, chrząszcze – ryjkowce, motyle oraz mrówki, co świadczy o dużym wpływie *C. bononiensis* na bioróżnorodność owadów w obrębie muraw ciepłolubnych.

Handling Editor: Elżbieta Pogorzewska

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