FLOWERING, POLLEN PRODUCTION AND INSECT VISITATION IN TWO Aconitum SPECIES (Ranunculaceae)

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Abstract

Flowering phenology, diurnal dynamics of blooming, insect visitation and pollen production in Aconitum lycoctonum L. and Aconitum carmichaelii Debaux were investigated in 2012–2013 in the Lublin area, SE Poland. Flowering of A. lycoctonum occurred in June/July, whereas A. carmichaelii bloomed in September/October. Both Aconitum species differed in terms of the diurnal pattern of flowering. The flowers of A. lycoctonum started opening at 5.00, whereas those of A. carmichaelii started blooming at 8.00 (GMT+2h). The species differed in the number of anthers per flower, the size of anthers, and the mass of pollen produced in anthers. As a result, the flowers of A. lycoctonum produced less pollen (mean = 1.0 mg per 10 flowers) than the flowers of A. carmichaelii (mean = 8.2 mg per 10 flowers). The estimated pollen yield was 0.2 g per m² for A. lycoctonum and 1.6 g per m² for A. carmichaelii. The flowers of both Aconitum species were foraged exclusively by bumblebees with the predominance of the long-tongued Bombus hortorum. Nectar was a more attractive floral reward than pollen. The propagation of Aconitum lycoctonum and A. carmichaelii in ornamental gardens may support the conservation of bumblebees whose populations are steadily declining.

Key words: Aconitum lycoctonum, Aconitum carmichaelii; blooming phenology, mass of pollen, insect visitors, Bombus spp.

INTRODUCTION

The genus Aconitum L. includes about 300 species of perennial plants in the family Ranunculaceae. The species are native to Eurasia, occur from lowlands to subalpine zones and are chiefly found in forest habitats [1, 2]. Even though Aconitum spp. are known for their toxicity, due to the presence of aconitine-type alkaloids [3], they are used in several regions of Asia for their various therapeutic and pharmacologic effects [4]. In Europe these plants are widely cultivated, since they are well-suited for park or garden planting [2, 5, 6].

The flowers of Aconitum are zygomorphic with a highly specialized perianth. The corolla consists of 5 petaloid sepals, while the posterior sepal is helmet-shaped and conceals modified petals [7, 8]. The genus Aconitum is described as bee-pollinated, and the distribution of Aconitum corresponds to the range of Bombus spp. [7, 1, 9].

The importance of the conservation of general insect biodiversity via supporting pollinators with the great diversity of plant species, including ornamentals, has been underlined by many authors [10–12]. Since garden plants are often non-native, their usefulness for pollinator-friendly gardens requires the observations of phenology, flowering biology and the insect visitor guilds as well as the evaluation of floral rewards [13–15]. These studies are of great importance as the variations in the total number of insects attracted by ornamental species are very large (80–300-fold) [16]. Although the pollen or nectar of several Ranunculaceae have been reported to be toxic [e.g. 17, 18], but these species are readily visited by insects and considered food plants for various groups of floral visitors [19–23].

The purpose of this study was to 1) examine the phenology and diurnal dynamics of flowering for two ornamental perennials, Aconitum lycoctonum L. and A. carmichaelii Debaux, 2) estimate pollen production as a source of floral reward for insects, 3) monitor the spectrum and activity of the floral insect visitors.
MATERIALS AND METHODS

Study site

The study of flowering and pollen production was carried out in 2012 and 2013. The perennial species Aconitum lycoctonum subsp. lycoctonum L. em. Koelle (A. lycoctonum hereafter) and A. carmichaelii Debeaux were grown on loess soil, at a pH of 6–7, at a site fully exposed to the sun in the Botanical Garden of Maria Curie-Skłodowska University, Lublin, SE Poland (51°15′44″ N, 22°30′48″ E). Both Aconitum species differed in terms of their origin: A. lycoctonum is native to Europe and Northern Asia and is widespread throughout Central and Southern Europe [2], whereas A. carmichaelii is native to Eastern Asia [3].

Flowering and insect observations

Detailed observations of flowering biology of these two Aconitum species were performed. The duration of flowering was noted; the beginning of flowering was defined when 2–5% of flowers opened, while the end of flowering when almost 90% of individuals finished blooming. The diurnal dynamics of flowering was estimated in accordance to the protocol described by Denisow [24]. The diurnal pattern of flowering was expressed as the percentage of newly opened flowers in relation to the total number of flowers opened during the day. The observations of flower development were carried out for three consecutive days in one-hour intervals, between 5.00–18.00 (GMT+2h). Additionally, the average number of flowers produced per inflorescence was determined (n = 30 per each species, per year). At full flowering stage, morphological measurements (n = 20 flowers per species, per year) were made: (i) the length of the corolla was measured from the base to the tip of the petaloid sepals; (ii) the diameter of the corolla was measured at the entrance to the flower, in the region of the androecium. These measurements were performed using a digital caliper.

Simultaneously to flowering observations, the intensity and spectrum of floral insect visitors were noted. The observations were conducted for three consecutive days in one-hour intervals, between 5.00 to 18.00 (GMT+2h). Each census of observation was 5–10 min long. During the observations, the weather conditions were as follows: daily temperature above 10°C, wind speed <10 km·h⁻¹, and no precipitation. In case of very strong wind or rain, the observations were halted and completed on the subsequent day. In each observation period, all insect visitors were recorded. Insect identification was based on Pawliowska [25].

Pollen production

Pollen production of both Aconitum species was estimated at the full bloom stage of each species. Mature but unopened anthers (n = 100) were extracted and placed in glass containers of known weight in four replications. The glass containers with collected anthers were placed in a dryer (Elcon CL 65) for several days, at a temperature of ca. 30°C. In order to determine the dry mass of anthers with pollen, the dried samples were reweighed on a WPS 36 electronic balance (RADWAG, Poland). Subsequently, pollen was extracted from the anthers 4–6 times with 70% ethanol (2–8 ml). The accuracy of pollen extraction was checked using a dissecting microscope under x 5 power. The mass of pollen produced was calculated for 100 anthers, 10 flowers and 1m² [19].

Data analysis

Standard ANOVA procedures were applied to assess differences in the mean values of the analyzed criteria (number of flowers per inflorescence, number of anthers, dry mass of anthers = anther size, pollen mass in anthers) between species and within species between years of study. Post hoc comparison was made with the Tukey HSD test. Data are presented as mean values ± SD (standard deviation). The level of statistical significance for all the analyses was P = 0.05. All data analyses were performed using STATISTICA 6.0 (Statsoft Inc.) software.

RESULTS

The flowers of A. lycoctonum are yellow and have a narrow corolla tube, 5.1 mm in diameter and 35.5 mm in length, whereas the flowers of A. carmichaelii are violet-blue and have a broader corolla tube, 8.8 mm in diameter, and longer flowers, 52.7 mm in length on average (Fig. 1A–D).

The details concerning the flowering period of both Aconitum species are shown in Table 1. In general, the flowering of A. lycoctonum lasted from June to mid-July, whereas the flowering of A. carmichaelii began in late September and lasted until late October. The duration of flowering varied slightly between years for both studied species from 47 to 49 days (A. lycoctonum) and from 32 to 37 days (A. carmichaelii). The species also differed in respect to the diurnal pattern of flowering. The flowers of A. lycoctonum started opening at 5.00 and peaked between 6.00–9.00 when approx. 59% of newly open flowers were observed. The flowers of A. carmichaelii began opening at 8.00 and peaked between 11.00–13.00 when approx. of 60% of daily installment of flowers opened (Fig. 2).
Fig. 1. Macro photographs of *Aconitum lycoctonum* (A–B) and *Aconitum carmichaelii* (C–D). A – yellow flowers with a narrow corolla tube; B – *Bombus hortorum* foraging flowers; C – overall habit of inflorescences; D – violet-blue flowers with a broad corolla tube.
### Table 1
Flowering period, duration of flowering and abundance of flowering in two *Aconitum* species during two years of study in SE Poland.

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Flowering period</th>
<th>Duration of flowering (days)</th>
<th>Number of flowers per inflorescence</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>min - max</td>
<td></td>
</tr>
<tr>
<td><em>Aconitum lycocotonum</em></td>
<td>2012</td>
<td>02.06 - 20.07</td>
<td>49</td>
<td>5 - 26</td>
<td>14.4 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>29.05 - 14.07</td>
<td>47</td>
<td>12 - 26</td>
<td>18.3 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td>48.0</td>
<td>12 - 26</td>
<td>16.4 ± 5.2</td>
</tr>
<tr>
<td><em>Aconitum carmichaelii</em></td>
<td>2012</td>
<td>18.09 - 24.10</td>
<td>37</td>
<td>12 - 26</td>
<td>19.1 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>28.09 - 29.10</td>
<td>32</td>
<td>18 - 29</td>
<td>21.6 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td>34.5</td>
<td>18 - 29</td>
<td>20.4 ± 3.7</td>
</tr>
</tbody>
</table>

ANOVA procedures were performed separately for each analyzed feature. Mean values followed by the same small letters are not statistically significant between seasons within species, whereas means with the same capital letters are not statistically different between species at *P* < 0.05, based on HSD Tukey test.

Fig. 2. Diurnal pattern of flowering of two *Aconitum* species expressed as the percentage of newly opened flowers in relation to the total number of flowers opened during the day and the activity of bumblebees (means calculated across 2012 and 2013).

The flowers of both *Aconitum* species are arranged in raceme inflorescences (Fig. 1A–D) and start to bloom from the bottom to the top. The number of flowers per inflorescence was a species-specific feature (*F*<sub>1,118</sub> = 23.469, *P* < 0.001), and a single inflorescence of *A. carmichaelii* produced more flowers (mean = 20.4) than an inflorescence of *A. lycocotonum* (mean = 16.4). Considerable year-to-year variations in the number of flowers per inflorescence were also found in both studied species (*F*<sub>1,58</sub> = 9.523, *P* < 0.004 for *A. lycocotonum* and *F*<sub>1,58</sub> = 12.408, *P* < 0.008 for *A. carmichaelii*).

A species effect was found for the number of anthers developed per single flower (*F*<sub>1,120</sub> = 79.845, *P* < 0.001), the size of anthers, expressed as the dry mass of anthers (*F*<sub>1,14</sub> = 267.164, *P* < 0.001) and for the mass of pollen produced in anthers (*F*<sub>1,14</sub> = 10.896, *P* < 0.006). The number of anthers developed per flower varied from 17 to 35 (*A. lycocotonum*) and from 38 to 55 (*A. carmichaelii*), and was significantly different between years for both studied species (*F*<sub>1,60</sub> = 152.058, *P* < 0.001 and *F*<sub>1,58</sub> = 15.544, *P* < 0.001, respectively; Table 2). In contrast, no significant year effect was noted for the dry mass of anthers of *A. lycocotonum* (*F*<sub>1,6</sub> = 2.387, *P* = 0.173) and of...
A. carmichaelii ($F_{1,6} = 0.169, P = 0.695$). However, both studied species showed differences between years as regards the amount of pollen produced. Namely, the pollen output of A. lycoctonum was relatively stable between years ($F_{1,6} = 4.909, P = 0.069$), whereas the flowers of A. carmichaelii produced 3.2-fold more pollen in 2012 than they did in 2013 ($F_{1,6} = 17.253, P < 0.006$). The estimated pollen yield differed between studied species (Fig. 3) and amounted to 0.2 g per m$^2$ (A. lycoctonum) and 1.6 g per m$^2$ (A. carmichaelii).

We observed five insect species visiting flowers of the studied taxaons. All insects belonged to the Hymenoptera order and represented the following species: Bombus hortorum (L.), B. lapidarius (L.), B. pascuorum (Scopoli), B. soroensis (Fabricius), and B. terrestris (L.). Nocturnal observations excluded insect visits after dusk. The activity of bumblebees on A. lycoctonum started at early morning hours at 5.00 and was relatively constant throughout the entire day. Bumblebees started to visit the flowers of A. carmichaelii at 8.00, with the highest number of visits recorded between 10.00–14.00 (Fig. 2). We also observed that the participation of bumblebee visitors on the flowers of two Aconitum species slightly changed between years (Fig. 4). On average, the most frequent insect visitor recorded both for A. lycoctonum and A. carmichaelii was B. hortorum, which was responsible for 75 % and 72 % of total insect visits, respectively. Floral visitors collected both nectar and pollen; however, nectar was a more attractive goal.

### Table 2

Characteristics of the androecium and pollen mass produced in anthers and flowers of two Aconitum species during two years of study in SE Poland. Data represent mean values ± SD (standard deviation).

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>No. of anthers per flower</th>
<th>Dry mass of 100 anthers with pollen (mg)</th>
<th>Pollen mass per 100 anthers (mg)</th>
<th>Pollen mass per 10 flowers (mg)</th>
<th>% of dry mass mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aconitum lycoctonum</td>
<td>2012</td>
<td>25 - 35</td>
<td>31.0 ± 2.0</td>
<td>10.2 ± 0.7</td>
<td>0.3 ± 0.1</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>17 - 26</td>
<td>22.2 ± 2.4</td>
<td>8.9 ± 1.5</td>
<td>0.5 ± 0.1</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td>26.7 ±</td>
<td>9.5 ±</td>
<td>0.4 ±</td>
<td>4.4 ±</td>
</tr>
<tr>
<td>Aconitum carmichaelii</td>
<td>2012</td>
<td>38 - 54</td>
<td>47.3 ± 4.1</td>
<td>17.8 ± 0.9</td>
<td>2.6 ± 0.7</td>
<td>14.2 ±</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>46 - 55</td>
<td>50.6 ± 2.1</td>
<td>18.1 ± 0.4</td>
<td>0.8 ± 0.5</td>
<td>4.4 ±</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td>48.9 ±</td>
<td>17.9 ±</td>
<td>1.7 ±</td>
<td>9.3 ±</td>
</tr>
</tbody>
</table>

ANOVA procedures were performed separately for each analyzed feature. Mean values followed by the same small letters are not statistically significant between seasons within species, whereas means with the same capital letters are not statistically different between species at $P < 0.05$, based on HSD Tukey test.

Fig. 3. Pollen yield of two Aconitum species during two years of study in SE Poland.
DISCUSSION

The flowering period of Aconitum lycoctonum differs from that of A. carmichaelii. In SE Poland A. lycoctonum blooms in June/July and the blooming period lasts approx. 6 weeks, which is 4 weeks shorter than the flowering period for Poland [26] or even 6 weeks shorter than the one observed in Western Europe [1]. The differences in the flowering period and duration of the same species are usually caused by various abiotic (e.g. habitat, weather conditions, soil type) or biotic (e.g. phenotypic plasticity) factors [27–29].

In SE Poland the blooming of A. carmichaelii occurs in September/October. In the range of its origin, it is also considered to be an autumn-blooming species [3]. The autumn season of blooming is very attractive, as only a few native species (e.g. Linaria vulgaris, Lamium album, Ballota nigra) may contribute to the feeding of insects at the end of the growing season in Poland. Moreover, bearing in mind that the frequency of climate anomalies increases [30] and the phenological shifts of native plants will be extended, a reduction in floral resources available to 17–50% of all pollinators is expected [31]. Therefore, any species that can provide forage, particularly during the end of the growing season, is indeed worthy of propagation.

In our study, both Aconitum species differed in terms of the diurnal pattern of flowering. The flowers of A. lycoctonum start opening at 5.00, whereas those of A. carmichaelii start blooming at 8.00. The difference in the diurnal blooming pattern of both Aconitum spp. confirms that this feature is highly species-specific [e.g. 20, 14]. Likewise, the different periods of flowering of both species (summer vs. autumn) and hence the different length of daytime undoubtedly impact the differences observed. Various environmental conditions (duration of daylight, average daily temperatures, air humidity, precipitation) are well-known to modify the diurnal pattern of blooming even of the same species [24, 32]. Furthermore, the different patterns of diurnal flower opening observed in this study may be adaptively beneficial with respect to the activity of insect visitors, including true pollinators. The convergence in the diurnal flowering pattern and insect activity pattern has been previously documented [13, 21, 24, 33, 34]. The intensity of flower opening during the day is presumably one of the variables in plant strategies to attract potential pollinators at the most suitable moment for effective pollination [35–37].

In our study, the number of flowers produced per inflorescence differed between growing seasons only for A. lycoctonum, which shows this species to be sensitive to external factors in respect of flower formation. The abundance of flowering usually varies significantly between seasons and the influence of weather conditions, e.g., on the different aspects of flowering has been described for many other species [e.g. 38, 39].

The flowers of entomophilous taxa from the genus Aconitum attract visitors by primary attractants – nectar and pollen [1, 8]. These floral rewards were also present in both studied Aconitum species, but the amount of nectar was not measured in the present study. The studied species formed a multi-staminate androecium, but both Aconitum species differed in the number of anthers per flower; A. lycoctonum produced...
fewer anthers (mean = 26.7) than that of A. carmichaelii (mean = 48.9). Likewise, a significant year effect on the number of anthers per flower was found in the studied species. These variations between years can be explained by the influence of external conditions during androecium formation. A significant effect of climatic factors on the number of anthers produced has already been recorded in several Ranunculaceae [19, 33] and other multi-staminate species [e.g. 34].

We observed that both Aconitum species varied in respect to pollen mass produced in the flowers. Namely, the flowers of A. lycocotonum produced less pollen (mean = 1.0 mg per 10 flowers) than the flowers of A. carmichaelii (mean = 8.2 mg per 10 flowers). These differences result from the different number of anthers observed in flowers as well as the different anther size and pollen productivity of a single anther. These features are highly species-specific and a correlation between the mass of pollen produced in flowers and androecium characteristics have been observed for plants from various families [14, 40, 41], including species from the Ranunculaceae family [22]. The average productivity of archesporial, expressed as the percentage contribution of pollen to the dry mass of anthers, was stable between years in A. lycocotonum, but varied significantly in A. carmichaelii. The decrease in pollen production noted in the latter species was threefold. Generally, microsporogenesis and pollen production are very sensitive to weather conditions and during adverse weather even empty anthers are developed [36]. Species sensitivity may differ greatly, however, a decrease in pollen production has been associated mainly with water stress, e.g. a shortage of rainfall [40, 42]. A considerable precipitation deficit, before and during the flowering period, was a probable reason that contributed to the lower pollen production observed in the case of A. carmichaelii in 2013.

Floral morphology, nectar characteristics and/or pollen traits are considered to influence the plant-pollinator interaction [42, 43, 44]. Both Aconitum lycocotonum and A. carmichaelii have evolved deep corolla tubes (on average, 30 mm and 60 mm, respectively) and present nectar in deep spurs. These characteristics are associated with all species from the genus Aconitum, regardless of their geographical origin or habitat [1, 2]. Such floral morphology is attributable to pollination by long-tongued bees [7]. We observed that the flowers of both Aconitum species were foraged exclusively by bumblebees, with the predominance of the long-tongued Bombus hortorum. Similarly, exclusive foraging by bumblebees was recorded for Aquilegia vulgaris (Ranunculaceae), also with a highly morphologically specialized corolla [34]. However, Utelli [14] and Roy [9] observed beetles and small insects, such as flies, in the flowers of Aconitum lycocotonum in the natural populations in Switzerland. Interestingly, in the case of both Aconitum species studied, the contribution of particular insect species hardly changed between years. Insect visitors usually change significantly between growing seasons even for the same sites [23, 37]. A stable contribution of insect visitors in our observations indicates that the insect guild was similar at the study area in both years.

In conclusion, due to the phenology of both Aconitum species and the floral reward that attracts different Bombus species, the propagation of Aconitum lycocotonum and A. carmichaelii may support the conservation of bumblebees whose populations are steadily declining.

Acknowledgements

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Authors’ contributions

The following declarations about authors’ contributions to the research have been made: designed the experiments: BD; performed the experiments: SA, KM; analyzed the experimental data: BD, SA; wrote the paper: BD, SA; photographs: SA.

REFERENCES


Flowering, pollen production and insect visitation in two *Aconitum* species (Ranunculaceae)

Kwitnienie, obfittość pylenia oraz oblot przez owady dwóch gatunków z rodzaju *Aconitum* (Ranunculaceae)

**Streszczenie**

W latach 2012–2013, na terenie Lublina prowadzono obserwacje pory kwitnienia, dzienne dynamiki rozkwitania, obfittości pylenia oraz oblotu kwiatów przez owady dwóch ozdobnych gatunków *Aconitum lycoctonum* L. i *A. carmichaelii* Debeaux. W warunkach Polski południowo-wschodniej kwitnie *A. lycoctonum* przypada w okresie czerwiec/lipiec, zaś *A. carmichaelii* kwitnie na przełomie wrześni/a wrześniowa. W ciągu doby kwiaty *A. lycoctonum* rozkwitają od 5.00 (GMT+2h), otwieranie kwiatów *A. carmichaelii* rozpoczyna się o 8.00. Gatunki różnią się liczbą pręcików, wielkością oraz produktywnością pylników. Kwiaty *A. lycoctonum* dostarczają mniej pyłku (średnio = 1.0 mg z 10 kwiatów) niż kwiaty *A. carmichaelii* (średnio = 8.2 mg z 10 kwiatów). Przeciętna wydajność pyłkowa wyniosła 0.2 g/m² (*A. lycoctonum*) oraz 1.6 g/m² (*A. carmichaelii*). Kwiaty odwiedzane były wyłącznie przez trzmiele (*Bombus* spp.), a długojęczkowy trzmiel ogrodowy (*Bombus hortorum*) pojawiał się z najwyższą częstotliwością. Trzmiele chętniej korzystały z nектaru niż pyłku. Propagacja badanych gatunków z rodzaju *Aconitum* do różnicego typu zużycia ogrodowych może urozmaicić bazę pożytkową trzmieli, których liczność systematycznie spada m.in. na skutek braku pokarmu.

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