Research Article

Variation in Nectar Volume and Sugar Concentration of Allium ursinum L. ssp. ucrainicum in Three Habitats

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Floral nectar volume and concentration of ramson (Allium ursinum L. ssp. ucrainicum) were investigated in three different habitats, including two types of sessile oak-hornbeam association on brown forest soil with clay illuviation and a silver lime-flowering ash rock forest association on rendzina. Daily nectar production ranged from 0.1 to 3.8 µL per flower with sugar concentrations of 25 to 50%. Mean nectar volumes and concentrations showed significant differences between freely exposed flowers and covered flowers, which had been isolated from flower visitors 24 h prior to nectar studies. Both the amount and quality of nectar were affected by microclimatic conditions and soil properties and varied between populations at different habitats. In the silver lime-flowering ash rock-forest association mean nectar volumes and concentrations were lower than in a typical sessile oak-hornbeam association on three occasions, the difference being significant in two cases. During full bloom, the date of sampling did not have a profound effect on either nectar volume or concentration.

1. Introduction

Allium ursinum L. (ramson or wild garlic) is a perennial plant, widely distributed in Europe, occurring in various deciduous woodlands, preferring damp shadow places, meso- and eutrophic, neutral to moderately acid soils of the hilly and the mountainous vegetation belt [1]. In Hungary, the largest populations can be found in Bakony and Mecsek hills, in the form of a continuous underwood layer in hornbeam-oak and beech forests [2, 3]. The flower stalk of ssp. ursinum is densely papillated and rough as opposed to the smooth pedicels of ssp. ucrainicum that lack papillae. The European distribution of ssp. ursinum is confined to the western and southern parts, being a subatlantic-submediterranean flora element, while ssp. ucrainicum is distributed in East Europe, with a western pontic-western sarmatic character [3]. The populations selected for the purposes of the present study belong to ssp. ucrainicum.

Besides being consumed fresh or cooked, ramson is a popular medicinal plant, lowering blood pressure, being effective against arteriosclerosis, diarrhea, and indigestion [4].

The plant is valued by bee keepers, as well, since ramson flowers can serve as pollen and nectar sources for honeybees, completing the spring bee pasture [5]. Ramson blooming starts in the second half of April and finishes in the first half of May. The umbel-like inflorescence comprises 8–12 trimeric flowers, with a septal nectary between the base of the ovary and the stamens of the inner circle, characteristic for the Alliaceae family [6–9].

In the genus Allium, nectar secretion starts at the time of anthesis and ceases parallel with the withering of the tepals, stamens, and style [10]. Allium species tend to secrete highly concentrated nectar: Akopyan [11] measured 70–75% sugar concentration in the nectar of A. cepa, while Hagler et al. [12] reported 52–65% for the same species. Kumar and Kumar Gupta [13] found similarly high concentrations in vegetable alliums, measuring 52.8–82.6% and 42.0–72.8% nectar sugar content in A. cepa and A. fistulosum, respectively. The 24 h sugar value in the latter two species varied between 0.219 and 0.767 mg/flower.

According to Silva et al. [14] nectar sugar concentration in A. cepa did not change significantly throughout the day,
while mid- to late-morning and late evening peaks were observed in nectar volume. Rate of nectar secretion was influenced by both floral age and environmental factors, from which relative humidity was the most important, being significantly and inversely related to nectar production. Similarly, environmental factors were found to affect the nectar production of ramson, ranging from 0.16 to 0.42 mg nectar/flower/day, with an average of 52.13% sugar content [5]. In this study, sugar value was 0.14–0.25 mg in sunny weather, but remained below 0.1 mg in changeable, cool weather.

Although the rewards offered by *A. ursinum* flowers can play an important role in the strengthening of bee colonies before the bloom of black locust (*Robinia pseudoacacia* L.), which is a major bee pasture in several countries, to date little is known about the nectar secretion process and nectar composition of ramson. Investigating the nectar traits of wild garlic can provide valuable information for beekeepers as well as for consumers of the honey derived from the floral nectar of *A. ursinum*. Although some data are available regarding the effect of environmental factors such as relative humidity and air temperature on nectar production in the *Allium* genus, the impact of different habitats on the nectar producing capacity of wild populations has largely been neglected. Therefore, the present study aims at demonstrating variation in nectar volume and sugar concentration in various populations of *A. ursinum* and at determining the possible role of habitat differences in this variation.

### 2. Materials and Methods

#### 2.1. Location and Time of Studies

Field studies were done at three different locations in the Meceke hills (South Transdanubia, Hungary) in the springs of 2007, 2008, and 2010. The selected sampling sites included two of the most dominant wood types and an edaphic one (for details see Tables 1 and 2).

#### 2.2. 24-Hour Nectar Production Studies

Nectar was extracted with glass capillaries from 30 to 50 pollen-shedding flowers each day, at the time of peak nectar secretion, which was found to occur either at 9 hr or 17 hr in our pilot study. Each sampled flower represented a separate individual. In certain experimental designs the flowers have previously been isolated with a tulle net in order to exclude visiting insects (covered flowers). The volume of nectar produced in the preceding 24 hours was determined directly upon sampling the flowers with calibrated 5 µL micro pipettes (DURAN), by reading the length of the nectar column within the capillary. The refractive index—corresponding to the concentration of nectar—was measured immediately with hand refractometers (ATAGO N-50E and OG 101/A). Since sucrose refractometers are calibrated directly in g sucrose per 100 g solution (% Brix) and the presence of hexose sugars scarcely affects the relationship between solute concentration and refractometer reading [15], the refractive index was directly used for characterizing the concentration of nectar.

In addition, at site 3, repeated nectar sampling was performed from previously covered, pollen-shedding flowers on 5 consecutive days (15–19 April, 2007). All 5 study days fell within the main bloom of ramson. Each day, 25 to 30 flowers were sampled. Each flower was sampled only once during this period, that is, nectar was measured in different flowers on different days.

#### 2.3. Statistical Analysis

Means of data measured in covered/uncovered flowers, at different sites and on different days were compared with either two-sample t-test or ANOVA with Tukey’s multiple comparisons test. Homogeneity of variances was tested with F-test or Bartlett’s test. If the variances differed significantly, Welch test was applied. The normality of data series was checked by using Kolmogorov-Smirnov test. If the normality assumption was violated, either Mann-Whitney test or Kruskal-Wallis test with Dunn’s multiple comparisons post test was applied. For statistical evaluation of the results, the software GraphPad InStat (release 3.0.5) was used.

### 3. Results

#### 3.1. The Effect of Flower Isolation on Nectar Volume and Concentration

Ramson flowers produced low to medium volumes (extreme values: 0.1–3.8 µL/flower) of highly concentrated (extreme values: 25–55%) nectar at all three sampling sites on all occasions, with sugar values varying between 0.17 and 0.69 mg/flower in the three years of our study. The 24 h sugar values were within the range (0.219 to 0.757 mg/flower) calculated for the flowers of *A. cepa* and *A. fistulosum* ([13], but were higher than the values determined in a previous study on *A. ursinum* (0.14–0.25 mg) [5].

The effect of 24-hour isolation of flowers preceding nectar measurements was investigated at site 3 on two different occasions (covered versus uncovered flowers in Table 2). In both cases, mean nectar volumes in covered flowers were significantly higher than in uncovered flowers (Table 3). Similarly, mean nectar concentration values of covered flowers exceeded those of freely exposed flowers in both years, but in 2010 the difference was not statistically significant (Table 4). The above results were taken into account in further evaluation of data, that is, data from covered and uncovered flowers were not pooled, and comparisons between various sites or dates were done either for covered flowers or freely exposed flowers.

#### 3.2. Effect of the Habitat on Nectar Volume and Concentration

In order to analyze the effect of the habitat on nectar volume and concentration, ramson flowers that had not been previously isolated were sampled on three occasions. On April 27 mean nectar volumes differed significantly in 2007, but in 2008 we did not find any statistically relevant differences between the three study sites (Table 5). On 9 May, 2008 there was a significant difference in the mean nectar volumes of site 1 and site 2, and mean values at site 3 differed from those at the other two sites. Mean nectar volumes at site 2 were lower than at site 1 on all three days of investigation, the difference being significant in two cases.

Similarly to the amount of nectar, its mean concentration also showed significant differences at the three different
Table 1: Characteristics of the sampled forest stands.

<table>
<thead>
<tr>
<th>Stand ID</th>
<th>Location; latitude (°); longitude (°); elevation (m); aspect; slope (°)</th>
<th>Plant association; status; dominant species in canopy layer (c); shrub layer (s); herb layer (h)</th>
<th>Site description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>West-Mecsek hills; N 46°07.041′; E 18°10.825′; 370 m; NE; 26°</td>
<td>Sessile oak-hornbeam association: Asperulo taurinae-Carpinetum Soó et Borhidi in Soó, 1962; zonal; c: Carpinus betulus, Fagus sylvatica; Quercus dalechampii; s: scarce; h: Allium ursinum ssp. ucrainicum</td>
<td>The middle of a typical occurrence of sessile oak-hornbeam forest.</td>
</tr>
<tr>
<td>Site 2</td>
<td>Mid-Mecsek hills; N 46°06.652′; E 18°11.899′; 535 m; S-SW; 26°</td>
<td>Silver lime-flowering ash rock forest association: Aconito anthorae-Fraxinetum orni (Borhidi-Kevey 1996); edaphic; c: Tilia argentea, Quercus cerris, Q. pubescens and Q. virgiana, Fraxinus ornus s: Cornus mas h: Allium ursinum ssp. ucrainicum</td>
<td>Close to the border of the calciphilous oak association (Tamo-Quercetum virgilianae).</td>
</tr>
<tr>
<td>Site 3</td>
<td>East-Mecsek hills; N 46°08.511′; E 18°15.386′; 410 m; NE; 8°</td>
<td>Sessile oak-hornbeam association: Asperulo taurinae-Carpinetum Soó et Borhidi in Soó 1962; zonal; c: Quercus dalechampii, Carpinus betulus; s: sparse, Crataegus oxyacantha, h: Melica uniflora, Allium ursinum ssp. ucrainicum</td>
<td>Next to the border of Turkey oak wood. This habitat is receiving relatively more irradiation from the direction of the Turkey oak wood.</td>
</tr>
</tbody>
</table>

Table 2: Sampling dates and sites, with bloom stage. C: covered flowers; UC: uncovered flowers.

<table>
<thead>
<tr>
<th>Year</th>
<th>Date</th>
<th>Bloom stage</th>
<th>Site 1 Orfu valley</th>
<th>Site 2 Tubes hill</th>
<th>Site 3 Arpad peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>April 14</td>
<td>Full</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
</tr>
<tr>
<td></td>
<td>April 15</td>
<td>Full</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>April 16</td>
<td>Full</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>April 17</td>
<td>Full</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>April 18</td>
<td>Full</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>April 19</td>
<td>Full</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>April 26</td>
<td>End</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
</tr>
<tr>
<td></td>
<td>April 27</td>
<td>End</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>April 28</td>
<td>End</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>2008</td>
<td>April 25</td>
<td>Full</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
</tr>
<tr>
<td></td>
<td>April 27</td>
<td>Full</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
</tr>
<tr>
<td></td>
<td>April 29</td>
<td>Full</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>May 9</td>
<td>End</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
</tr>
<tr>
<td>2010</td>
<td>May 4</td>
<td>End</td>
<td>C and UC</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

Habitats on both April 27, 2007 and May 9, 2008, but no such differences were found on April 27, 2008. Mean nectar concentrations were lower at site 2 on all three sampling dates compared to those measured at site 1—the difference being significant in two out of three cases (Table 6).

3.3. Effect of the Sampling Dates on Nectar Production. In 2007, previously isolated flowers were sampled on five consecutive days during full bloom at site 3. Neither nectar volume (Figure 1) nor concentration (Figure 2) changed significantly during this period.

4. Discussion

According to our previous studies, the nectar producing period lasts for 4 days in individual ramson flowers, with peak production on the 2nd day of anthesis [16]. This was in contrast with the study of Zimmermann and Pyke [17],...
who found that individual flowers of another mass-flowering
species, Polemonium foliosissimum, produce equivalent nec-
tar volumes every day of their lives within a single blooming
season. Although the intensity of nectar production in A. ursinum
flowers was expected to vary also at the popula-
tion level on different days of full bloom, no significant
differences were found either in volumes or concentrations
of nectar on five consecutive days during full bloom. This
might be explained by the different approach applied in
the two studies: our previous investigation [16] monitored
nectar secretion from the bud stage until flower senescence,
sampling the same flowers on each consecutive day; whereas
in the present study all flowers were at the stage of anthesis,
and they were sampled on a single occasion.

Standing crop, that is, the quantity of nectar found in
freely exposed flowers at a given time [15] tends to be lower
than nectar volumes measured in isolated flowers, as demon-
strated by several studies (e.g., [18]). The significantly higher
nectar volumes of covered versus uncovered ramson flowers
might be explained by the foraging activity of pollinators
from freely exposed flowers. Various bees, including Apis
mellifera L., A. cerana F., A. dorsata F., A. florea F. and
Trigona iridipennis Smith, and flies like Musca domestica L.,
Calliphora vicina Robineau-Desvoidy, Epiyrphus balteatus
De Geer, Eristalis aeneus Scopoli, and Eupeodes sp. have
been reported as frequent visitors of Allium species [19–23].

In our field studies, the most important visitors of wild garlic
flowers were honeybees and ants. The highly concentrated
nectar reported for various Allium species [10–13] make
it difficult for honeybees to collect the secretion product. In
our experience, ramson flowers might also produce nectar
with concentration values exceeding 50%; however, the
average values are in the range of 25 to 40%, which is suitable
for honeybees, allowing even the production of unifloral wild
garlic honey. Besides foragers, the slightly changed microcli-
mate due to the coverage of inflorescences, which results in
higher temperature and humidity, can contribute to differen-
tes in nectar production between covered and freely exposed
flowers.

Differences in microclimate can also lead to variation ob-
served between populations at different habitats. The rather
diluted nectar in covered flowers at site 1 can be explained by

![Changes of nectar volume between 15 and 19 April, 2007](image1)

**Figure 1:** Nectar volume (mean and standard deviation) in covered ramson flowers at site 3, on five consecutive days of full bloom in April 2007.

![Changes of nectar refraction between 15 and 19 April, 2007](image2)

**Figure 2:** Nectar concentration (mean and standard deviation) in covered ramson flowers at site 3, on five consecutive days of full bloom in April 2007.
the more humid microclimate in the closed oak-hornbeam association mixed with beech. The drier microclimate of the border of the sessile oak-hornbeam and sessile oak-Turkey oak woods in site 3 may stand in the background of large amounts of concentrated nectar even in isolated flowers. Interpopulational differences in nectar production were found in other plant species, as well: for example, in Impatiens capensis the variation in nectar volume was not significant among plants, but was nearly significant among populations [24]. Microclimatic conditions were found to influence nectar peaks were measured at various times depending on the three investigated soil types, the highest intensity in the monocarpic perennial Ipomopsis aggregata in the year of treatment; whereas in the perennial Linum lewisii reproductive output was not influenced by fertilization in the first year, but delayed effects were seen in the next year. The nectar secretion rate of Vaccinium macrocarpon was unaffected by fertilizer application [32]. Species-specific responses of nectar traits to variation in soil nitrogen availability were observed also by Baude et al. [33], who found that litter amendment to the soil led to an increase in total nectar sugar content in Lamium amplexicaule, but not in two other temperate grassland species, Mimulus guttatus and Medicago sativa. Besides sugar content, amino acid levels of the nectar can also be affected by soil conditions. Total amino acid concentrations varied significantly at both the plant and population level in Impatiens capensis [24]. In Agrostemma githago, total amino acid concentrations increased significantly with increasing fertilizer treatment [34].

* A. ursinum* applies Clan-of-Clone strategy which can be characterized among other things with relatively small allocation to vegetative reproduction, which prolongs local persistence [35]. Despite being a clonal plant, sexual reproduction is prevalent over clonal reproduction in the majority of natural populations [27, 28, 36]. Accordingly, *A. ursinum* can be characterized with extraordinarily high values of reproductive allocation, compared both to other woodland perennials and related species of the Liliales [37]. In a habitat that cannot provide enough nutrients during the time of flowering, the plant is not able to invest sufficiently into nectar production. This was demonstrated by our measurements as well. From the three study sites, Tubes (site 2) was the driest and warmest habitat, whose rendzina soil was characterized by the highest humus content and pH values. The high humus content can be advantageous if there is enough precipitation in spring—typically in April, at full bloom of ramson—since in this case nutrients are available in high

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### Table 5: The effect of habitat on nectar volume.

<table>
<thead>
<tr>
<th></th>
<th>27 April 2007, end of bloom</th>
<th>27 April 2008, full bloom</th>
<th>9 May 2008, end of bloom</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>n</em></td>
<td>mean (μL)</td>
<td>std</td>
</tr>
<tr>
<td>Site 1</td>
<td>33</td>
<td>1.339*</td>
<td>0.549</td>
</tr>
<tr>
<td>Site 2</td>
<td>31</td>
<td>0.936*</td>
<td>0.526</td>
</tr>
<tr>
<td>Site 3</td>
<td>49</td>
<td>1.318</td>
<td>0.677</td>
</tr>
</tbody>
</table>

Method: *t*-test, *P* = 0.0039 ANOVA, *P* = 0.4298 Kruskal-Wallis test, *P* < 0.0001

Abbreviations: *n* = sample size; std = standard deviation; * indicates significant differences between sites.

### Table 6: The effect of habitat on nectar concentration.

<table>
<thead>
<tr>
<th></th>
<th>27 April 2007, end of bloom</th>
<th>27 April 2008, full bloom</th>
<th>May 9, 2008, end of bloom</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>n</em></td>
<td>mean (%)</td>
<td>std</td>
</tr>
<tr>
<td>Site 1</td>
<td>33</td>
<td>36.182*</td>
<td>3.860</td>
</tr>
<tr>
<td>Site 2</td>
<td>31</td>
<td>32.516*</td>
<td>3.548</td>
</tr>
<tr>
<td>Site 3</td>
<td>49</td>
<td>35.898</td>
<td>0.685</td>
</tr>
</tbody>
</table>

Method: *t*-test, *P* = 0.0002 ANOVA, *P* = 0.1655 ANOVA, *P* < 0.001

Abbreviations: *n* = sample size; std = standard deviation; * indicates significant differences between sites.
amounts. Furthermore, rendzina soil is well-drained, which is important for ramson. Later on—typically in May, at the end of bloom—when there is less or no rain, the thin rendzina soil becomes warmer and drier, therefore humus decomposition is hindered and nutrients cannot be properly absorbed by ramson. This may account for the fact that nectar production was twice as high in April 2008 compared to May 2008 at site 2, as opposed to the less pronounced decrease in nectar production during the same period at site 1 (Table 5), characterized by a more humid microclimate and medium humus content. At site 3 the humus layer is rather shallow, and as ramson plants develop, the deeper penetrating roots reach a nutrient-poor soil layer, where lower levels of potassium, phosphorous, and nitrate-nitrogen can be measured [36]. The poorly drained soil with higher proportion of clay and the lack of sufficient nutrients may explain lower vigour of plants and consequently lower nectar sugar production.

5. Conclusion

Our study demonstrated that floral nectar volume and concentration varies in different populations of A. ursinum, which can be largely attributed to the varying conditions provided by different habitats. Populations in the sessile oak-hornbeam association, which is the typical habitat of ramson and provides sufficient nutrient levels for nectar secretion, produced higher volumes of nectar with higher nectar sugar concentrations, compared with the population in the silver lime-flowering ash rock forest, where A. ursinum cannot find its optimal living conditions.

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References


