NECTAR PRODUCTION IN THREE MELLIFEROUS SPECIES OF LAMIACEAE IN NATURAL AND EXPERIMENTAL CONDITIONS

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The nectar production of Lamium maculatum, Lamiastrum galeobdolon and Ajuga reptans was evaluated by determining the index of nectar production (INP), total nectar quantity per flower during 24h, nectar sugar concentration, flowering period, flower longevity, and flower number per plant and per square unit. The diurnal dynamics of nectar secretion in these three melliferous species, grown under different microclimatic habitat conditions (natural and experimental field) was also analyzed, by measuring the amount of nectar per flower at two hour intervals.

Nectar amount and sugar concentration varied among the studied species as a function of microclimatic habitat conditions (air temperature, air humidity and evaporation) and corolla morphology. Higher nectar production and lower sugar concentration were recorded in L. maculatum and L. galeobdolon grown in the forest, while for A. reptans in the same habitat, lower intensity of nectar secretion and higher sugar concentration were obtained. With regard to the average number of open flowers per plant during the nectar collecting day, total daily nectar volume per plant was the highest in L. maculatum (average volume 30.1 ml/plant). Considering the average size or density of the natural population of the species, the highest nectar yield per square unit was found in L. maculatum (1564.99 ml/m²) and the lowest in A. reptans (111.34 ml/m²). Diurnal variation in nectar production was also found and the secretion patterns were rather different in these three melliferous species. Regarding nectar secretion rate, A. reptans is a slow producer, secreting less than 0.02 ml/h.

With respect to secretion intensity (on average 0.22 ml/h), total daily nectar production per flower (on average 5.368 ml) and the density of species population, the most melliferous species was L. maculatum.

Key words: Ajuga, Lamiaceae, Lamium, Lamiastrum, nectar production
INTRODUCTION

Flowers produce nectar, an important source of food for many species of flower-visiting animals (insects, hummingbirds, small mammals), presenting a reward for well performed pollination. The nectar secretion period mainly corresponds with the pollinating phase and this is connected with an efficient reproductive strategy. Nectar is an aqueous solution of sugars (mainly fructose, glucose and saccharose, making up 95%), together with other less abundant constituents such as amino acids, lipids, fatty acids and other nutrients (Baker and Baker, 1983; Caldwell et al., 1986; Freitas et al., 2001; Galetto and Bernardello, 1992; Shuel, 1992; Vogel, 1983). This solution is released from secretory structures called nectaries, glandular tissues consisting of specialized cells. There are many differences in size, shape, ultrastructure and location of floral nectaries (Fahn, 1979; Smets, 1986; Vogel, 1983), although the majority of species keep the chemical composition of their secretion relatively constant (Percival, 1961).

The floral nectaries of Lamium, Lamiastrum and Ajuga have a circumgynoeceal position. According to Fahn (1979), the nectary of Lamiaceae is a disc surrounding the base of the ovary. The amount of secreted nectar and its sugar concentration vary widely among species and are influenced by internal and external factors. The internal ones are related to plant species-specific characteristics, such as flower size and location, nectary size and surface, phase of flower development etc. (Gottsberger et al., 1990; Petanidou et al., 1996). The external ones are related to habitat conditions or abiotic ecological factors, such as soil properties, air humidity and temperature, insolation, wind, rain etc. There is also intraspecies variability due to the influence of environmental conditions (Skenderov and Ivanov, 1986; Marden, 1984; Freeman and Head, 1990; Wayatt et al., 1992; Cruden et al., 1983).

The aim of this study was to establish the significance and contribution of three forest species Lamium maculatum, Lamiastrum galeobdolon and Ajuga reptans to bee pasture, as well as the influence of some abiotic ecological factors on their nectar production.

MATERIAL AND METHODS

The investigation was conducted in natural habitat conditions at a mixed-species forest in the Belgrade area, as well as in an experimental field, during spring 2003. For the determination of nectar potential, three melliferous spring-flowering plant species that bloom in March, April and May, were chosen: Lamium maculatum (spotted dead nettle), Lamiastrum galeobdolon (yellow archangel) and Ajuga reptans (common bugle). In order to analyze the influence of microclimatic parameters, natural populations of these species in the anthesis stage were removed to the experimental field. The Index of nectar production (INP), according to Jašmak (1980), Ricciardelli D’Albore and Persano Oddo (1981) and Umeljić (1999), shows the quality and melliferousness of plant
species. It is presented by scores ranging from 1 to 4 (1- minimal nectar production; 2- good melliferous plant, 3- very good, 4 – excellent).

The intensity of nectar secretion was determined directly by the capillary method of Kuliev (1951). The nectar was withdrawn from the flowers with glass microcapillaries (diameter 0.5 or 0.6 mm) without destroying the nectaries. The length of the nectar column was measured with millimeter paper, immediately in the field. The results were converted into ml (mm³), presented as the mean value of repeated measurements, by calculating from:

\[
V_{\text{mean value}} = \frac{r^2 \rho \ H}{\text{flower number}} \ SE \ (\text{ml per flower})
\]

\( r \) – radius of the capillary glass tube (mm); \( H \) – nectar height in the tube (mm)

The nectar production of the three melliferous species was measured at the peak blooming period. The inflorescences were covered with fine mesh or perforated plastic bags (20 x 20 cm) for 24 hours prior to nectar removal and between daily measurements (6 times) to prevent visitors or wind and rain influence. Individual flowers were marked at random from different inflorescence whorls. Only fully open flowers without signs of senescence were included. Four to twelve flowers from each plant species were used for nectar collection. Estimate of the nectar production, based on flowers bagged for twenty-four hours, is significantly less than the amounts produced by flowers that were visited several times during the day. Therefore, total daily nectar amount per flower was obtained from removing accumulated nectar from flowers periodically (at two hour intervals) during the day, and presented as the sum (mean value) of the single measurements. Sugar concentration in nectar was measured by a portable field refractometer immediately after nectar removal once a day at 10:00.

Diurnal dynamics of nectar production was determined on plants grown in two ecologically different localities, by measuring the amount of nectar secreted at two hour intervals from 8:00 to 18:00. At the time of nectar removal, microclimatic parameters were measured: air humidity, air temperature and evaporation.

Floral longevity was determined by monitoring flower development from the bud to signs of senescence.

The flowering period was registered by observing the duration of anthesis (flowering phenophase) and by using data from the literature (Josifović et al., 1977). The number of plants per square metre was estimated by counting the plants inside the placed wooden frame.

At the time of nectar collecting, microclimatic measurements were carried out on both localities at 10 and 100 cm above ground (average values are shown) at two hour intervals from 8:00 to 18:00: air temperature (°C) (thermometer), relative air humidity (%) (hygrometer) and evaporation (cm³) (evaporimeter). Evaporation intensity describes the vapour volume from the rounded leaf surface (radius – 1.5 cm) at two hour intervals. At the same time evaporation intensity shows air humidity level and specific conditions for transpiration.
RESULTS

The nectar potential of *L. maculatum*, *L. galeobdolon* and *A. reptans* was determined by analyzing the index of nectar production (INP), total daily nectar amount per flower, number of open flowers per plant and plants per square metre and nectar sugar concentration (Table 1). These parameters describe the total nectar yield of each species per square unit during 24 hours. The highest amount of nectar per flower during 24 hours was measured in *L. maculatum* in the natural habitat (5.368 ml ± 0.240), somewhat less in *L. galeobdolon* (3.938 ml ± 0.154), and the least in *A. reptans* (0.541 ml ± 0.297). All results are presented as the mean value of five repeated measurements with the standard error (± SE). The highest number of open flowers per plant as well as the highest nectar sugar concentration were obtained in *A. reptans*. In the order of the beginning of flowering (median date) these species were: *A. reptans* (first half of April), *L. maculatum* and *L. galeobdolon* approximately at the same time (second half of April). The average flower life span was the greatest in *A. reptans*, 3.9 days. The number of open flowers per plant was the greatest in *A. reptans* (42 flowers/plant), but the mean number of plants per square unit was the highest for *L. maculatum* (52 plant/m²).

Nectar secretion as a function of time

Diurnal dynamics of the nectar production in the studied species is shown in Figures 1-3. Nectar secretion rate was different in the flowers of the three species. In *L. galeobdolon*, growing in the forest, the minimal nectar amount was sampled at 8:00 (0.283 ml ± 0.108; 4 flowers), and the maximal at noon (1.187 ml ± 0.115; 4 flowers). No nectar was found at the first and last collections in the second locality, where the maximum was reached at 12h (0.791 ml ± 0.089; 4 flowers). In *L. maculatum* secretion began early in the morning and it was minimal at 8:00 (natural loc.- 0.339 ml ± 0.103; 4 flowers; experim. local.- 0.196ml ± 0.119; 4 flowers). It subsequently increased, reaching two maxima during the day in both localities. Each of the two secretion peaks was lower and reached about two hours earlier in the experimental field. With the exception of small fluctuations, the amount of nectar in *A. reptans*, remained at a low level during the day in both habitats (from 0.056ml ± 0.019; 4 flowers, to 0.459ml ± 0.06; 12 flowers). It slightly increased towards evening in the experimental habitat (max. at 18:00 – 0.459 ml ± 0.06). Nectar values for *A. reptans* were much lower than in the other two species and higher in experimental conditions than in the forest.

Microclimatic habitat conditions

The spring of 2003 was warm, without intensive rain and with high maximum daily temperatures (about 39°C). Microclimatic parameters, for the two study localities are presented in Figures 4-6. As expected, there were obvious microclimatic differences between these two habitats. Forest air temperature (Fig. 4) had a smaller daily variation range and was about 10 degrees lower than in the experimental field. The highest
Table 1. Nectar potential and flowering phenology of the studied species

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>nectar quantity per flower in 24h MV±SE (ml)</th>
<th>nectar sugar conc. (%)</th>
<th>flower/plant MV RANGE</th>
<th>plant/m² MV RANGE</th>
<th>flower longevity (days) RANGE</th>
<th>flowering period</th>
<th>INP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamiastrum galeobdolon</td>
<td>F 3.938±0.154</td>
<td>20</td>
<td>31 18-40</td>
<td>40 37-44</td>
<td>3,3 2,5-4</td>
<td>IV-VI</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>EF 1.535±0.066</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamium maculatum</td>
<td>F 5.368±0.240</td>
<td>24</td>
<td>22 14-31</td>
<td>52 48-55</td>
<td>2,8 2-4</td>
<td>IV-VI</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>EF 3.143±0.185</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ajuga reptans</td>
<td>F 0.541±0.297</td>
<td>30</td>
<td>42 36-48</td>
<td>11 5-15</td>
<td>3,9 3,5-4,5</td>
<td>IV-V</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>EF 1.754±0.497</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: F – forest; EF – experimental field; MV – mean value
Figures 1-3. Diurnal dynamics of nectar secretion in the studied species grown in forest and experimental habitat conditions.
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Figures 4-6. Microclimatic parameters during the nectar collecting day in natural and experimental conditions.
temperature in the forest was at noon (25°C), and in the second locality in the afternoon (34.6°C).

During nectar collecting days, relative air humidity showed an opposite pattern to air temperature, and was constantly higher in the forest than in the experimental field, due to the added secondary radiation from the surrounding rocky surface. The maximum air humidity in the forest was at 18:00 (81%), when it was slightly raining, and in the second locality in the morning (62%).

In the Figure 6, evaporation measurements for the forest phytocoenoses Querceto-Carpinetum, and the experimental field with meagre vegetation are presented. Evaporation intensity was less in the forest (lower temperature, higher humidity) than in the controlled conditions. Also there was more intensive evaporation between 10:00 and 14:00 than between 14:00 and 18:00 in the forest. The opposite situation was registered in the second locality.

DISCUSSION

Numerous studies, concerning nectar production in relation to pollination and pollinator effectiveness, have been made (Lange and Scott 1999; Lange et al., 2000; Perret et al., 2001; Scobell and Scott, 2001). Also, some evaluations about herbivorous effects on nectar production were carried out (Wäckers et al., 2001). Several experiments about the influence of elevated CO₂ on nectar production (Lake and Hughes, 1999; Rusterholz and Erhardt, 1998) as well as microclimatic influences have been done (Jakobsen and Kritjansson, 1994). A few studies have investigated breeding (within species and interspecific) influences on nectar volume or nectar – sugar production (Davis and Gunning, 1991; Galetto and Bernardello, 1995; Marshall et al., 1995; Davis, 2001). Several studies have evaluated intergeneric and infrageneric differences of nectar production influenced by environment (Cruden et al., 1983; Marden, 1984; Freeman and Head, 1990; Wyatt et al., 1992). Rare studies have been devoted to analysis of the contribution of some melliferous plant species to bee pasture (Danon et al., 1990) and the significance of floral nectar in providing the most important source of honey production (Eisikowitch and Masad, 1980; Shuel, 1989). According to Blaženčić (1987), investigations of plant species melliferousness include: - phenological investigations (study of the impact of the climate on the seasonal occurrence of plant species, dates of flowering, duration of blooming period ), - analysis of nectar potential including diurnal and seasonal dynamics of nectar production, total nectar amount per flower connected with population abundance, nectar sugar composition, concentration and content and - mellissopalynology (studies of contemporary pollen are useful for the examination and quality control of honey).

The investigation of forest plant species that provide pollen and nectar to bees as raw material for the production of honey is also significant for mainly forest-based beekeeping regions. Forest flowering plants are useful as major or minor sources of nectar and pollen and represent an immense potential for the development of beekeeping. Lamiaceae species are predominantly bee-pollinated plants (Van der Pijl, 1972; Stebbins, 1974; Faegri and Van der Pijl,
1979). It was recorded that in the Mediterranean region, for instance, Lamiaceae are pollinated by solitary bees (Herrera, 1987; Dafni, 1991). According to the literature data, Lamiaceae species were by far the most nectariferous species in the phrygana, both in volume and sugar content (Herrera, 1985).

Among the ground-floor flora in Košutnjak forest, precocious flowering melliferous plants are of special significance, appearing directly before tree foliation. These are herbaceous plants with a short vegetation period. Many of them are ephemeroids such as *Scilla bifolia*, *Corydalis cava*, *C. solida*, *Anemone ranunculoides*, *Muscari botryoides* etc. They precede and supplement shrub and tree flowering, so that bee pasture is continuous for a long period of time. The three studied species, *L. maculatum*, *L. galeobdolon* and *A. reptans* otherwise show a long lasting flowering period (till October) in habitats different from forest. However, forest conditions shorten their blooming period by inhibiting it at the end of May or beginning of June.

The highest total nectar volume per flower in 24 hours was measured in *L. maculatum* grown in the forest (5.368 ml ± SE) in relation to the other studied species in both localities. *L. galeobdolon* flowers augmented the natural level of nectar in the forest nearly up to twice that in the experimental field, but that was not observed for *A. reptans*. *Ajuga* flowers secreted about three times more nectar per day in the experimental field than in the forest. Consequently, nectar production of *L. maculatum*, in response to habitat adaptability, was the least affected by environment. The differences became even greater if, instead of the per flower nectar production of a species, the nectar production per population was taken into consideration. Regarding the average size and density of the natural population of a species, the highest nectar yield per square metre was found in *L. maculatum*. Although the most abundant population and the longest flowering period were recorded for *L. maculatum*, *A. reptans* had the greatest flower longevity.

As mentioned earlier, nectar amount and sugar concentration are influenced by internal and external factors. According to Škenderov and Ivanov (1986), the most intensive secretion occurs before and during the flowering stage and corresponds with the pollinating phase (Malahova-Akovleva, 1966). After pollination, nectar secretion decreases or completely ceases, although in some species it continues during the fruit ripening phase (Daumann, 1931).

Comparative analysis of nectar secretion per flower in Lamiaceae species, indicated three models of diurnal dynamics for this process. *L. galeobdolon* had a secretion maximum between 10:00 and 12:00 with a decreasing tendency afterwards in both localities. *L. maculatum* had an increasing tendency of nectar production during the nectar collection day with two secretion maxima. Nectar amount per flower and per hour was the lowest in *A. reptans* with minor fluctuations during the day. The high values for standard error could be explained by minor amounts or total absence of nectar in some marked flowers during the nectar collection. *L. maculatum* secreted somewhat more nectar per flower during 24 hours than *L. galeobdolon*, and both species secreted significantly more nectar than *A. reptans*, for which the floral morphology could be the reason. The species differed in the length of the corolla tube, position, appearance and
presence of the upper corolla lip. The lack of the upper corolla in Ajuga allowed a greater influence of weather conditions on secretion and sugar concentration. Considering the classes stated by Cruden et al. (1983) regarding nectar secretion rate, A. reptans is a slow producer, secreting less than 10% per hour at its maximum accumulation.

When compared to other species of Lamiaceae, Balota nigra, Prunella vulgaris and Lamium album (Maćukanović and Blaženčić, 1998), there are some similarities in the diurnal dynamics of nectar production between L. maculatum and B. nigra. The last mentioned ruderal species with a long lasting flowering period, growing in a natural habitat, had the L. maculatum pattern of nectar production, with a higher total daily nectar production per flower.

There were obvious and expected differences in nectar production and sugar concentration among the three species grown in natural and experimental conditions, due to microclimatic factors: air temperature and humidity. According to Vogel (1983), periodicity of nectar secretion (in both quantitative and qualitative sense) is primarily under the influence of temperature. The beginning, maximum and end of nectar secretion in plants are determined by different temperatures. Škenderov and Ivanov (1986) concluded that the optimal nectar secretion interval is between 10 and 30 °C (more closely between 16 and 25°C) and above 30°C it decreases and ceases. Our investigation confirmed that this process started to decrease above 29°C in L. galeobdolon and above 27°C in L. maculatum. Nectar production is also greatly influenced by air humidity. According to data in the literature (Belčić et al., 1982) the optimal relative air humidity for the majority of plants is between 60 and 80%. However, this rule cannot be applied to all species. For instance, in Borago officinalis and Fagopyrum esculentum elevated air humidity level led to increased secretion, and in Phacelia tanacetifolia, Trifolium pratense and Melilotus albus, the same situation caused an opposite reaction. In our investigation, both Lamium and Lamiastrum species had a greater production in the mentioned range in forest microclimatic conditions, and A. reptans had minimal, or negligible production.

Lower air humidity and greater evaporation during warm and dry days resulted in an elevated nectar sugar concentration in all three species in the experimental field. The highest concentration measured in Ajuga was related to flower openness and absence of the upper corolla lip, so the flowers were more exposed to sunlight and evaporation effects.

It could be concluded that Lamium maculatum is the most melliferous among the three investigated species, with respect to secretion intensity (on average 0.22 ml/h), total daily plant nectar production (on average 5.368 ml) and the density of species population.

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NEKTARSKA PRODUKCIJA TRI MEDONOSNE VRSTE FAMILIJE LAMIACEAE U PRIRODNIM I EKSPERIMENTALNIM USLOVIMA

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SADRŽAJ

Nektarska produkcija kod Lamium maculatum, Lamiastreum galeobdolon i Ajuga reptans, određivana je proučavanjem dnevne dinamike nektarske sekrecije, indeksa nektarske produkcije (INP), ukupne dnevne količine nektara po cvetu, koncentracije šećera u nektaru, broja cvetova po biljci i po jedinici površine, fenofaze cvetanja i dužine trajanja cveta. Analizirane medonosne biljne vrste su rasle u različitim mikroklimatskim uslovima stanja (prirodni i ogledna parcela).

Količina nektara i koncentracija šećera variraju u zavisnosti od temperature i vlažnosti vazduha i evaporacije. Veća količina nektara po cvetu i niža koncentracija šećera zabeležena je kod L. maculatum i L. galeobdolon u prirodnim, šumskim uslovima uvećane relativne vlažnosti vazduha, smanjene temperature i evaporacije, a kod A. reptans u eksperimentalnim. Najveća dnevna količina nektara po biljci izmerena je kod L. maculatum (prosečno 30.1 ml). S obzirom na prosečnu brojnost, tj. gustinu prirodne populacije, najveći prinos nektara po jedinici površine je nađen kod L. maculatum (1564.99 ml/m²) a najniži kod A. reptans (111,34 ml/m²). U odnosu na dinamiku nektarske sekrecije, A. reptans proizvodi najmanje nektara po jedinici vremena (manje od 0.02 ml/h).

U odnosu na intenzitet sekrecije (prosečno 0.22 ml/h), ukupnu dnevnu produkciju nektara po cvetu (prosečno 5.368 ml) i gustinu populacije, najveći medonosni potencijal ima vrsta Lamium maculatum.