



Morphometric characteristics and nectar potential of *Ocimum basilicum* L. var. *genovese* (Lamiaceae) in relation to microclimatic and edaphic environmental factors

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Abstract

Background and Purpose: *Ocimum basilicum* L. var. *genovese* was grown from seed in selected soil types (eutric cambisol, fluvisol and humoglay) in order to analyse its morpho-physiological flower preference and morphometric characteristics of aerial parts in relation to microclimatic conditions and physico-chemical soil properties.

Materials and methods: The soil texture was analyzed using the »pipette method«, concentration of CaCO₃ was determined using the volumetric method and percentage of humus and carbon was detected using Tiurin's method. Electrometric method was used for chemical reaction of soil. The amount of nectar per flower was assessed using microcapillary method. Morphometric analysis comprised measurements of plant height, length and width of leaf, internode length, petiole length and leaf number.

Results and conclusions: Considering the whole flowering period, the most luxuriant growth and the highest intensity of secretion was recorded on eutric cambisol. Results of morphometric analysis showed that statistically significant difference existed between the plants on eutric cambisol and humoglay ($p < 0.05$). With respect to diurnal dynamics of nectar secretion, a pattern with a single daily peak was recorded, irrespective of the type of soil. Daily maximum was recorded at 8 am on eutric cambisol (0.104 µl/flower), and at 10 am on fluvisol (0.166 µl/flower) and humoglay (0.103 µl/flower). After reaching the highest values, secretion had decreasing tendency toward evening, and minimal nectar amount was sampled at 6 pm in all soil types (0.006–0.016 µl). Surprisingly, on nectar collecting day in June, the highest total daily nectar amount per flower was measured on humoglay (0.351 µl) and the lowest on eutric cambisol (0.288 µl). Air humidity and evaporation were positively and temperature negatively correlated with diurnal dynamics of nectar production in all soil types.

INTRODUCTION

Flower is the most important source of food for honeybees, because nectar, as an aqueous solution of sugars, secreted by floral nectaries, is the main raw material for honey production. Qualitative-quantita-

tive characteristics of the nectar are greatly influenced by endogenous and abiotic environmental factors (1, 2, 3, 4). Endogenous (factors) are species-specific and depend on flower and nectar characteristics (size, shape, position), timing and length of flower lifespan, flower development phase, beginning and duration of anthesis period etc. (5). Apart from endogenous rhythms of secretion, nectar production is strongly affected by microclimatic habitat conditions (air temperature, air humidity, evaporation, wind, rainfall), physical and chemical soil properties (structure and granulometric composition, chemical composition, fertility, moisture and acidity), light regime on the biotope, altitude, latitude etc. (6). Mutual action of the above mentioned factors creates the specific optimal (or suboptimal) living conditions for each plant species, that make it possible for them to develop capability of floral nectar exudation. Nevertheless, the most favourable conditions for this physiological process related to attracting pollinators, in the majority of melliferous species are windless and rainless weather, with air temperature between 10–30 °C (actually between 16 and 25 °C), sufficient soil moisture, and air humidity between 60 and 80% (7, 8). The floral nectar production in many melliferous plants has been widely studied from many aspects (9, 10, 11, 12, 13, 14, 15, 16, 17, 18), but basil in this respect was under-researched.

A number of basil varieties are used in commerce in Serbia. Because it is widely grown in plantations for traditional medicinal use, and has a long flowering phenophase (May–August), during which it is visited by bees extensively, basil could be very important for bee pasture. This paper is an extension of previous investigations of basil melliferousness that have dealt with morpho-anatomy of floral nectaries and nectar secretion, with special emphasis on the influence of microclimate and physico-chemical properties of soil on analysed morpho-physiological characteristics (19, 20). According to literature data, basil is best grown on eutric cambisol and fluvisol, and these two soil types together with humoglay are most widespread in Serbia (21). Since basil grows best in loose and deep soil and on the assumption that eutric cambisol has the best physical and chemical properties for its cultivation, which should be reflected in its growth and development, its positive effect on nectar production was also expected.

The aim of this paper was to investigate the effects of some abiotic environmental factors that make specific cultivation conditions, on the growth potential and nectar production of the most cultivated variety of basil used in Serbia. Furthermore, the results of this research were intended to be used in order to examine whether growing conditions can be modified in order to get (obtain) a higher nectar yield. Additionally, it was of an interest to estimate the melliferous potential of basil as a contribution to investigation of the melliferousness of other Lamiaceae species, that are valid as a good source of nectar, and except for (apart from) medicinal purposes can be used as good bee pasture (22).

MATERIAL AND METHODS

Study species

Basil (*Ocimum basilicum* L. var. *genovese*), belonging to the Lamiaceae family, has a characteristic flower morphology and inflorescence constitution. The flowers are concentrated in whorls of a terminal spike, surrounded by bracts. The morphology of the basil flower has been adapted to insect-pollination. Both the calyces and the petals are grown into tubes, but it is more pronounced in the case of the corolla. Four stamens and the pistil lie on the lower part (lip). The tube of the corolla is wide and not so deep, so several insect species may fit into it. Thus, basil is a bee-pollinated plant, but predominantly visited by short-tongued honeybees and short-tongued bumblebees (Photo 1).

Study soils

For the experiment, three types of soil were taken from the basin of the Sava river that belongs to the Pannonian plain (Srem) and covers the western part of Vojvodina (Northern Serbia).

Eutric cambisol is most widely distributed at heights of between 75 and 500 m above sea level in the Sava basin. Dominant factors for the formation of brown soil in this part of Serbia are the moderate continental climate (which is characteristic for cold winters and hot summers) and forest vegetation (23). The amount of precipitation is 550–700 mm, the mean annual temperature is



Photograph 1. Honey bee collecting nectar from basil flowers.

10–11 °C. Soil, taken for the experiment from the Bojčinska forest locality, belongs to the normal brown forest soil of A-(B)-C profile. The humus A horizon is 20–40 cm deep and it has a dark brown colour. Horizon (B), is well developed, contains less amount of humus and has rough and unstable structure. The water properties of this soil are favourable. Deficiency of air has not been observed over the whole profile. Brown forest soil belongs to moderately fertile soils.

Fluvisol appears along the whole course of the Sava on both sides of the river. Floods are frequent along this river, and fresh deposits are formed over the flooded terrain. The deposit of the Sava is distinguished by the fineness of material (24). In texture this deposit belongs primarily to sand, and to a lesser extent to loam and clay. The deposit along the Sava presents first class productive soil. Constant floods in the valley of the Sava preclude this first class deposit from being used universally for agriculture.

Humoglay belongs to a group of hydrogenic (semi-terrestrial) formations (25). This soil type has a profile of A-AC-C type, but in deeper layers horizon G occurs. It has formed in former bogs, under the effect of hydrolyte. The humus horizon A has black colour, unfavourable and unstable structure, no lime and variable humus amount. When it is dry, it is hard and heavy for tillage. With respect to granulometric composition, humoglay belongs to predominantly heavy soils. Its structure depends on clay amount. This soil is now drained and cultivated.

Cultivation conditions

Ocimum basilicum L. var. *genovese* was raised from seed in pots filled with dissimilar kinds of soil (eutric cambisol, fluvisol and humoglay). The surface layer of each soil (0–25 cm) was taken from selected sites. Seedlings in bifoliate stage were transplanted in experimental pots (46.5×19×14.5 cm) filled with three soil types. Ten seedlings per pot were transplanted and ten pots with each soil type were used.

The study was carried out during the vegetation period of 2003. Experiment was conducted in the experimental field of the Faculty of Veterinary Medicine in Belgrade. Plants were grown without fertilizing with balanced mineral solution, but were watered daily.

Soil analysis

The soil texture was analyzed by extracting soil particles of different size using the »pipette method« (26). Preparation was carried out with Na-pyrophosphate. The concentration of CaCO₃ was determined using the volumetric method (27). The percentage of humus and carbon was detected using Tiurin's method (28). Electro-metric method was used for chemical reaction of soil (in H₂O and 1M KCl solution 1:2.5).

Morphometric measurements

Analysis of morphometric characteristics included measurements of plant height, leaf length and width,

internode length, petiole length and leaf number. Data on all these parameters are related to the nectar collecting day at the end of June. Comparisons of these parameters between eutric cambisol and fluvisol (ab), eutric cambisol and humoglay (ac) and fluvisol and humoglay (bc) were made.

Nectar production

The intensity of nectar secretion was determined directly by the microcapillary method (29). The nectar was withdrawn from the flowers with glass microcapillaries (diameter 0.5–0.6 mm) carefully, without destroying nectaries. The length of the nectar column was measured with millimetre paper, immediately in the field. The results were converted into µl, presented as average value of repeated measurements (three sets with five flowers), by calculating from

$$\bar{V} = \Sigma(r^2\pi H) / \text{flower number} \pm \text{SE} (\mu\text{l per flower}),$$

where, r is a radius of the capillary glass tube (mm) and H is nectar height in the tube (mm)

The inflorescence was covered with fine mesh (20×20 cm) for 12 hours prior to nectar removal (evening of the previous day) and between daily measurements to prevent pollinator visits. Individual flowers were marked at random from different inflorescences. Only fully open flowers without mark of senescence were included.

Diurnal dynamics of nectar secretion was determined by measuring the nectar amount secreted at two hour intervals from 8 am to 6 pm. Total nectar amount per flower and per day, during the flowering season, was calculated as the sum of diurnal periodical measurements.

Microclimatic measurements

At the time of nectar collection (6 times a day), microclimatic measurements, carried out on 10 and 100 cm above ground level (average values are shown), included the next parameters: air temperature (°C), relative air humidity (%), evaporation (cm³).

Statistical analysis

Statistical analysis (ANOVA) comprised nectar production and morphometric characteristics of basil. Floral nectar production was comparatively analyzed between eutric cambisol and fluvisol (ab), eutric cambisol and humoglay (ac) and fluvisol and humoglay (bc). Results are presented as the mean values ±SD; n=30 (*p<0.05; **p<0.01; ***p<0.001; ns not significant).

RESULTS

Soil analysis

Mechanical composition and some chemical properties of the studied soil types are shown in Table 1 and Table 2. In texture, fluvisol belongs to sandy – loam (63.88 % sand). The reaction is slightly alkaline. This soil is middle carbonatic and slightly humous. In view of the granulometric composition, eutric cambisol belongs to

TABLE 1

Mechanical composition of three soil types.

Soil types	Texture (%)				
	Sand		Silt	Clay	total
	coarse	fine			
0.2–2 mm	0.02–0.2 mm	0.002–0.02 mm	<0.002 mm	0.02–2 mm	
Fluvisol	1.40	62.48	22.28	13.84	63.88
Eutric cambisol	15.70	31.78	33.56	18.96	47.48
Humoglay	4.30	39.30	25.20	31.20	43.60

TABLE 2.

Chemical properties of three soil types.

Soil types	Chemical properties				
	CaCO ₃ (%)	pH		C (%)	Humus (%)
		H ₂ O	KCl		
Fluvisol	5.82	7.85	7.21	1.78	3.07
Eutric cambisol	1.49	6.16	5.58	2.98	5.15
Humoglay	1.28	7.62	6.81	1.46	2.52

the type of loam. The reaction is slightly acid. This soil is slightly carbonatic and strongly humous. Texture of humoglay is variable from sandy loam to clayey-loam and clay. This soil is slightly alkaline, slightly carbonatic and slightly humous.

Morphometric measurements

Results of morphometric analysis showed that statistically significant difference exists between plants on eutric cambisol and humoglay ($p < 0.05$) (Table 3). The stems were the highest in basil grown on eutric cambisol in relation to other two soil types ($p < 0.001$). There is a statistically significant difference in leaf length and width of plants grown on eutric cambisol refer (compared) to the other two soil types ($p < 0.05$ and $p < 0.01$; $p < 0.05$ and $p < 0.001$), but not between fluvisol and

humoglay. There was no statistically significant difference with regard to petiole length and leaf number per plant in all three soil types.

First flowers appeared on the plants grown in fluvisol on June the 16th (Figure 1). However, the most luxuriant plants were in eutric cambisol, while plants grown in humoglay were poorly developed, low growth and less numerous. The number and size or even the existence of side-branches within the inflorescence was also very variable. In each dichasium the terminal flower bloomed



Figure 1. *Ocimum basilicum* L. var. *genovese* growing in three soil types (left to right: eutric cambisol, fluvisol and humoglay). On June 16th, the first flower appeared on a basil plant raised in fluvisol.

TABLE 3

Morphometric characteristics of basils raised on different soil types (June 30th).

Morphometric characteristic	(a) Eutric cambisol	(b) Fluvisol	(c) Humoglay
plant height (cm)	23.58(±1.2)ab,ac***	20.38(±0.887)bc*	18.16(±3.136)
leaf length (cm)	5.2(±0.652)ab*,ac**	4.72(±0.580)bc ^{ns}	4.32(±0.567)
internode length (cm)	4.476(±1.981)ab ^{ns} ,ac*	3.328(±1.726)bc ^{ns}	2.8(±1.343)
petiole length (cm)	1.18(±0.370)ab,ac,bc ^{ns}	1.36(±0.364)	1.18(±0.311)
leaf width (cm)	3.34(±0.498)ab*,ac***	2.9(±0.332)bc*	2.5(±0.406)
leaf number	29.2(±4.382)ab,ac,bc ^{ns}	29.6(±2.191)	26.0(±8.155)

Results are presented as mean values (±SD); n=30; (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ^{ns} not significant). Comparisons between a-b, a-c and b-c were made.

as the first one, after it the neighbouring flowers, followed by the marginal ones. The time shift among them showed that the last ones open only when the terminal flower faded. Flower lifespan was recorded and a single flower was open for an average of 4 days. However, if no fertilization occurred, it may last longer. Completion of flowering phenophase in experimental conditions was in the first half of November.

Nectar production of *Ocimum basilicum* L. var. genovese

Diurnal dynamics of basil nectar secretion has similar patterns (with one daily maxima value) for all three types of soil (Figure 2). The nectar secretion reached maximum at 10 am for the fluvisol (0.166 µL/flower), and humoglay (0.103 µL/flower) and at 8 am for eutric cambisol (0.104 µL/flower) at the peak blooming period. Subsequently, basil nectar production in all three soil types had decreasing tendency toward evening.

The individual data on nectar production for each soil were variable during the season. Maximal total daily nectar amount per flower was measured in basil grown on eutric cambisol (0.445 µL/flower/day) in September. These values vary during the season between 0.288 and 0.445 µL/flower/day for eutric cambisol; between 0.109 and 0.352 µL/flower/day for humoglay and between 0.130 and 0.367 µL/flower/day for fluvisol. Seasonal dynamics of basil nectar production during the flowering period of 2003 is shown in Figure 3.

Microclimatic condition influence on nectar production

Diurnal changes in air temperature, air humidity and evaporation during the nectar collecting day in the peak blooming period of basil (June the 30th) are shown in Table 4. The highest air temperature was recorded at 4 pm (28.8 °C) and air humidity at 8 am (67%). Evaporation was the most intensive around 2 pm (1.95 cm³).

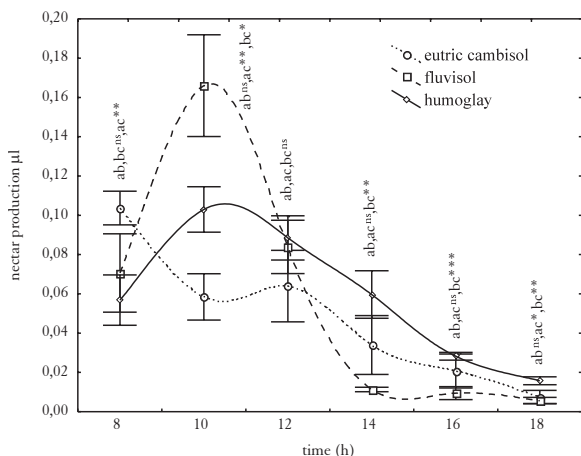


Figure 2. Diurnal dynamics of nectar secretion in *Ocimum basilicum* L. var. genovese (on June 30th 2003.), on three different soil types.

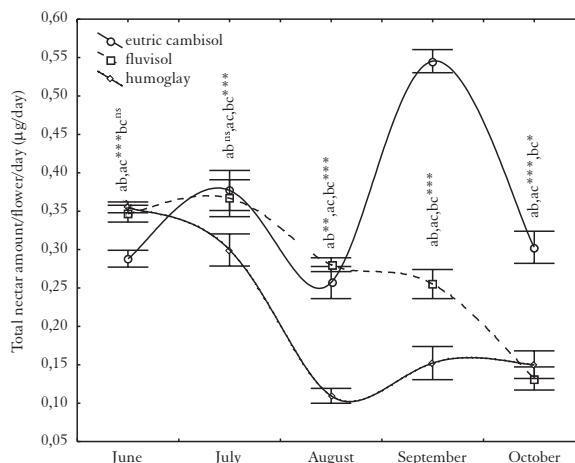


Figure 3. Seasonal dynamics of basil nectar production grown on three soil types during the anthesis period June-October 2003.

Negative correlation between air temperature and nectar secretion during the day in basil grown in all three soil types is shown on Figure 4. Thus, nectar production decreased with air temperature augmentation. In contrast to the temperature, air humidity during the day was positively correlated with diurnal dynamics of basil nectar secretion, thus the amount of collected nectar per flower decreased with air humidity diminution (Figure 5).

Evaporation and diurnal nectar production were positively correlated in basil grown in all three soil types (Figure 6).

DISCUSSION

Due to the favourable weather conditions of the moderately continental climate of Belgrade, and controlled microclimatic conditions, the flowering phenophase of basil in 2003 lasted around five months (from June till the beginning of November) independently of soil type. Our observation concerning basipetal blooming sequence within a dichasium, that was described earlier in basil (30) confirmed that within an inflorescence, flowers

TABLE 4

Microclimatic parameters (air temperature, relative humidity and evaporation rate) during the nectar collecting day in June 2003.

Time (h)	Air temperature (°C)	Relative humidity (%)	Evaporation rate (mL)
8	21.0	67	0.6
10	23.2	62	0.9
12	24.9	58	1.0
14	26.0	52	1.98
16	28.8	50	0.67
18	23.6	59	0.9

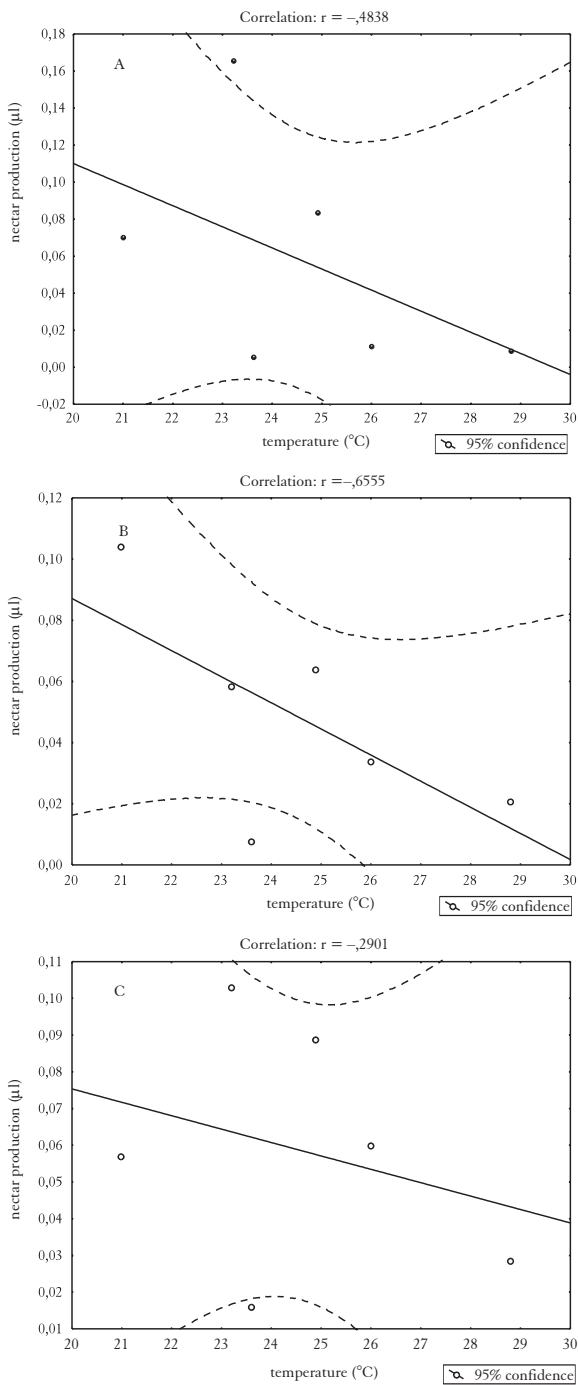


Figure 4. Correlation between air temperature and diurnal dynamics of nectar secretion in basil grown in three soil types (A-fluvisol; B-eutric cambisol; C-humoglay).

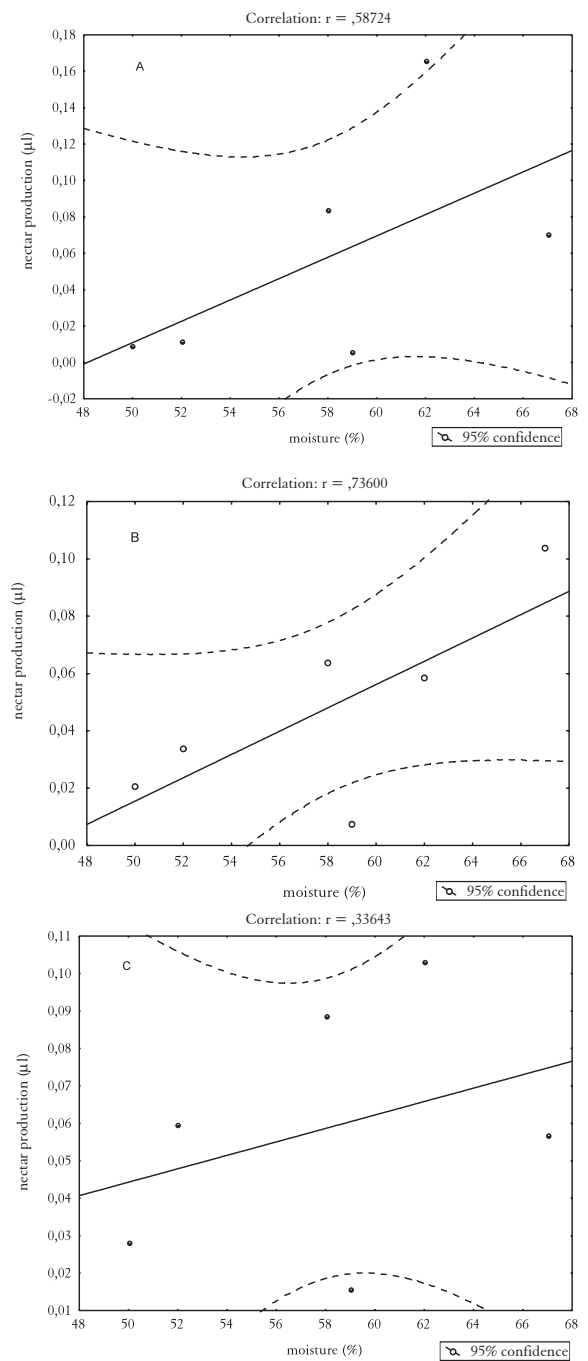


Figure 5. Correlation between air humidity and nectar secretion in basil grown in three soil types (A-fluvisol; B-eutric cambisol; C-humoglay).

start to bloom from the top of the shoot, further on at the side-branches into the basal direction. As the different flowers open continuously within a spike, it is rather hard to define the blooming stages of a plant as whole. Of all the analysed morphometric characteristics of basil raised on different soil types, it could be suggested that

the majority of them have the greatest values if grown on the most fertile soil. This suggests that the plants grown on eutric cambisol were the best developed and had the highest nectar production, while plants grown on humoglay had the lowest growth and nectar potential. Of course, with the uniform microclimatic conditions, dif-

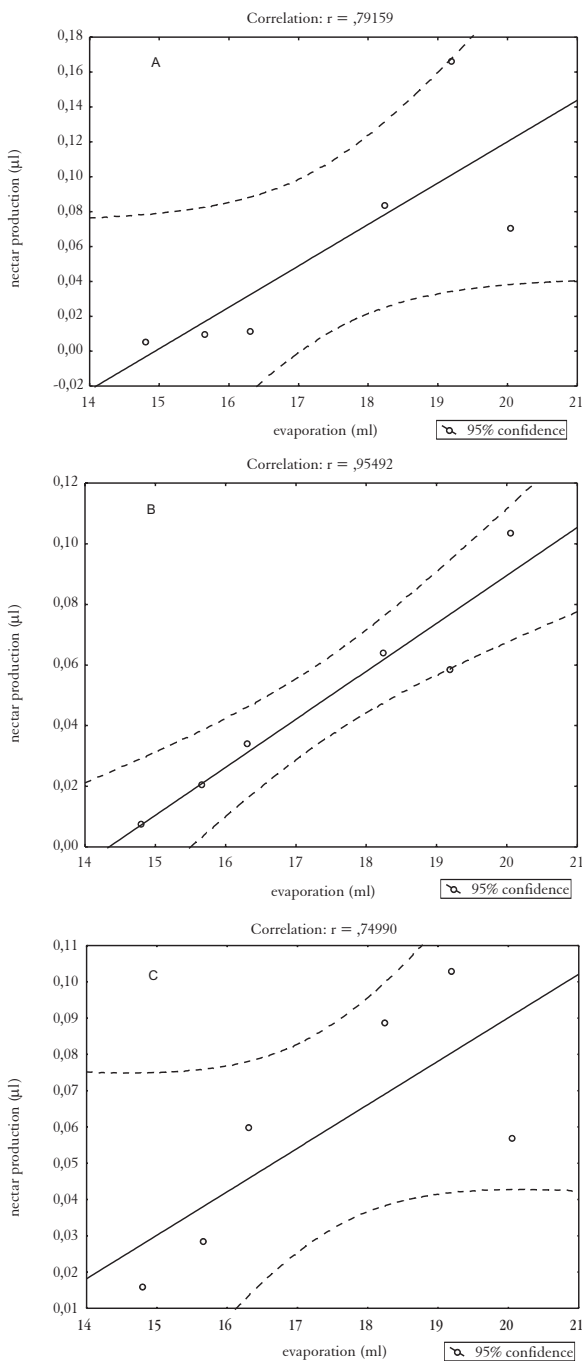


Figure 6. Correlation between evaporation and diurnal dynamics of nectar secretion in basil grown in three soil types (A-fluvisol; B-eutric cambisol; C-humoglay).

ferences in these parameters could be attributed to the influence of different soil characteristics, especially soil fertility. Although this general conclusion can be applied to the entire flowering season, providing the diurnal dynamics of nectar secretion, this was not always the case, because on some days, plants that were better developed produced less nectar per flower. Our observations indicate that soil condition such as fertility, moisture and

acidity may affect not only the growth of the plant but also the secretion of nectar, although only indirectly and when viewed in the long term. However, luxuriant plant growth (in eutric cambisol) does not necessarily imply that maximal nectar secretion will take place. With reference to correlation between nectar production and soil type, we have shown that basil flowers do not necessarily produce small amounts of nectar when grown on non-fertile soil. It was evident that basil grown on humoglay (humus poor soil) produced a significant amount of nectar in favourable weather on the nectar collecting day in June, in spite of the fact that the plants were the least developed. At times, limited growth results in increased nectar production.

In accordance with some earlier studies, growing conditions, especially water supply and air temperature, may strongly influence growth potential, blooming dynamics and periodicity in nectar production (1, 31, 32, 33, 34, 35, 36). According to Fahn (37), stress by water shortage reduces secretion, but with some delay. Our observations agree with the view/conception of Cruden *et al.* (38), that differences in nectar production may reflect differences in soil moisture. Extremely dry or moist soil may cause nectar production decrease or cessation. Also, extremely high or low temperatures may delay the start of nectar secretion or decrease the secretion rate. At high temperatures, evaporation is greater than secretion, and the weather is dry enough to concentrate the nectar and it becomes too viscous (bees prefers sugar concentration from 14 to about 30%). Boëtius and Kleber (39, 40) reported that the volume of nectar was greater at high humidity, but that the concentration of sugar was less. They attribute this to absorption of water from the atmosphere. Earlier observations indicated that nectar tends to equilibrate with ambient air humidity, most water exchange occurring with more concentrated nectar (4). Nectar amount in flowers increases with enhancement of air humidity, although there is a limit for this positive correlation. Further increase of air humidity causes nectar dilution. During the season, wet flowers in the shade on cloudy days contained less nectar than those that had been in direct sunlight for a period of time. Our observations confirmed that nectar volume decreases as a consequence of high evaporation. Conditions favouring evaporation frequently occur at mid-day or early afternoon when relative humidity is low and temperatures high.

Based on our results obtained during the season, ambient temperature was considered to be the major governing factor since nectar production of the investigated species was lower in all three types of soil on the warmest days of our study, but was much higher on the days with much cooler weather. On the other hand, nectar production was completely prevented in days when the weather was very hot (above 30 °C daily maximum in August) during the blooming period of basil. Our observations suggest that the type of soil to a lesser extent, and air temperature to a greater extent, are limiting factors for timing and quantity of nectar exudation. Basil plants raised in eutric cambisol had the highest and those in humoglay

the lowest nectar production, during the whole flowering season. The gradual increase in nectar secretion undoubtedly reflects the gradually increasing temperature during the period of observation, although there is a limit to this correlation. Our research supports those of Everett (41) who reported that clear, warm, windless days are likely to favour nectar secretion.

Diurnal nectar production and secretion were extremely dependent on weather conditions during the day, as indicated for other honey plants in some earlier publications (42, 43). Our observations support those of Lazarov et al. (44) who indicated that temperature is a dominant factor influencing nectar secretion during the day. The sudden temperature rise and resulting rise in vapour pressure were partly responsible for the recorded daily maximum secretion in the early morning hours (at 8 and 10 am), but temperatures above 25–30 °C, definitely reduced or ceased nectar exudation in all soil types during the day (and season). This conclusion agrees with several other studies of nectar production of other medicinal Lamiaceae species in which similar patterns of diurnal dynamics of nectar secretion with one secretion peak were recorded (22). The rate of nectar secretion between 8 and 10 am in humoglay and fluvisol was higher than that between 10 and 2 pm, which can be attributed to the influence of diurnal changes of temperature. Subsequently, secretion rate also showed decreasing tendency. It remains unclear why the total daily nectar amount per flower measured on June the 30th was the greatest for humoglay despite poorly developed plants.

Given that the nectar production is strongly affected by microclimatic habitat conditions, it has been established that air humidity and evaporation were positively, and temperature negatively, correlated with diurnal dynamics of nectar secretion in all soil types.

Analysis of some vegetative characteristics of plants individuals cultivated in different soil types proved that growth potential and development depend on soil properties and microclimatic conditions, but did not reveal close positive correlation with nectar secretion. Taking into account the ecological circumstances, age and health state of the plant, the total daily nectar volume per flower and the rate of nectar secretion in basil were under the indirect influence of the soil properties which, viewed for a longer time during the vegetation period, manifested through the impact on growth and photosynthesis. This comparative study of the nectar secretion dynamics of *Ocimum basilicum* (diurnal and seasonal), emphasises out its good nectar potential, describing its importance, apart from medicinal purposes, as a significant nectar source predominantly for honeybees, *Apis mellifera*.

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