

## The effect of nutrient and water availability on nectar secretion and nectary structure of the dominant Labiatae species of phrygana

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**Abstract.** – *The effect of irrigation and artificial nutrient supply on nectar secretion and nectary structure of three of the main Labiatae species occurring in a phryganic community (viz. Satureja thymbra, Stachys cretica, and Thymus capitatus) was studied. Experiments were carried out on potted plants taken from the wild, along with control plants (untreated or/and naturally growing). Only T. capitatus clearly secreted a higher nectar volume and sugars after treatment, whereas the yield of S. thymbra did not increase significantly when plants were irrigated or artificially supplied with nutrients. The case of S. cretica was more complicated showing different trends depending on the time of the year. Conclusions are: (i) nutrient supply does not always enhance nectar secretion in the poor-soil phrygana; (ii) irrigation is more important in increasing per flower nectar secretion than fertilization; (iii) nutrient supply results in the presence of nectarless flowers; (iv) nectar analyses made on three plants show that neither of the treatments affects amino acid concentration of the nectar, but they result in either glucose excess in the nectar of woody species (viz. S. thymbra, T. capitatus) or in sucrose excess in the nectar of herbaceous species (S. cretica). Most of these results can be explained through a differential resource allocation of the water-stressed plants grown in the wild on the one hand, and the treated plants on the other. This difference stems from the fact that woody species clearly distinguish between growth and reproductive phases, whereas in herbaceous perennials the growing phase overlaps with reproductive phase and blooming. Under conditions of artificial nutrient supply, plants may continue to allocate to vegetative growth rather than to pollinator allurements; on the contrary, wild growing plants may invest exclusively in pollinator allurements. It is assumed that the approach of the summer is very critical and conclusive for a shorter life cycle of the species. Some intraspecific differences in nectary structure were found as a result of the treatments. However, because these differences were not consistent either with the treatment effects on nectar secretion or with the nectar characteristics studied, it is concluded that the observed differences may be mere intraspecific variations normally occurring in nature.*

**Key words.** – *Labiatae, nectar secretion, sugars, amino acids, watering, nutrient supply, nectary structure, stomata, nectary size, Mediterranean communities, HPLC, SEM.*

## 1 Introduction

Much primarily descriptive work has been published on patterns of floral nectar secretion. Nectar secretion has been reported as a function of time in the season, time of day, flower age, flower location on the plant, flower size, plant size, plant location, weather conditions, nectar removal from flowers, defoliation and soil moisture. These phenomena have been reviewed by Zimmerman (1988). In addition, several authors reported that nectar secretion can be influenced by artificial fertilizing with several nutrients. Among the nutrients that positively enhance nectar secretion are potassium, phosphates, and boric acid (reviewed by Beutler 1953). On the other hand, water availability may also enhance nectar secretion as was recently reported by Wyatt et al. (1992). The authors found that *Asclepias syriaca* that has been first subjected to an early-summer drought, dramatically increased its nectar volume after watering with the equivalent of about 10 cm of rain, without a substantial change of its nectar concentration. On the other hand, Petanidou et al. (1995a) found that *Capparis spinosa* flowers secreted more nectar during the years of higher precipitation, but of a significantly lower concentration.

Areas of low rainfall have, indeed, an equally low nectar secretion, especially when low rainfall is combined with summer drought; this particularly holds for the Mediterranean climate area where the mild and wet winters are followed by dry and very hot summers (Aschmann 1973; Herrera 1985; Petanidou & Smets 1995; Petanidou & Ellis 1996). An additional limiting factor that may halt nectar secretion in Mediterranean ecosystems is suspected to be the nutrient-poor soils, especially as to nitrogen availability (Christensen 1973; Diamantopoulos 1983). The latter is limited by the frequent fires, and a slow, season-dependent decomposition rate.

Phrygana, the counterpart of the garrigue in the East Mediterranean Basin, occupies the driest parts of the precipitation gradient in the whole Mediterranean region; among the weather conditions prevailing there, the long-lasting water deficit has been considered as very harsh for nectar secretion (Herrera 1985; Petanidou & Vokou 1990). The latter two authors argued that under the Mediterranean climatic conditions it is very likely that pollen versus nectar has been selected to constitute the major reward to pollinators. Indeed, in a well-studied phryganic community consisting of 133 insect-pollinated species, 13.5% of the species did not produce nectar at all, while only 7.5% of them produced  $>0.5 \mu\text{l}$  nectar per flower (Petanidou 1991; Petanidou & Smets 1995). The main and consistent nectar producers of this community were the Labiatae, supporting with their nectar a fairly high number of bees, and making the maximal contribution to the apiculture potential of these landscapes (Petanidou & Vokou 1993; Petanidou & Smets 1995; Petanidou & Ellis 1996).

In this paper we examine whether water shortage and nutrient-poor soil are, indeed, the causal factors for the observed low nectar secretion in phrygana. We asked whether water- or nutrient-limitation does affect nectar secretion in phrygana. Our hypothesis is that if, indeed, nectar secretion is water-limited in plants growing in the wild, then artificial water supply will enhance nectar yield. In addition, if nectar secretion is further nutrient-limited in phrygana, then artificial fertilization will increase its nectar secretion. In parallel, these changes in nectar yield are expected to be accordingly reflected in the structure of the floral nectaries. Indeed, there is evidence that nectar secretion increases with nectary size within a family (Fahn 1949; Dafni et al. 1988; Petanidou, Goethals & Smets unpublished). We tested our hypotheses on three Labiatae species, grown in the wild and in transplants. We selected Labiatae to experiment on, because, as mentioned above, they are the highest nectar producers in the phrygana, therefore the easiest to handle the nectar. Besides, Labiatae are the most common representatives of phrygana, and the most important species for apiculture (Petanidou & Vokou 1993; Petanidou & Smets 1995; Petanidou & Ellis 1996).

## 2 Material and methods

### 2.1 Plant material

We studied the effect of irrigation and artificial nutrient supply on the herbaceous perennial *Stachys cretica* L. ssp. *cretica* as well as on *Satureja thymbra* L. and *Thymus capitatus* (L.) Hoffmanns. & Link (both woody perennials). We performed our experiments in the nature reserve of the "I. and A. Diomedes Botanical Garden of the University of Athens" which has been described in detail in previous studies (Petanidou & Vokou 1993; Petanidou & Ellis 1993; Petanidou et al. 1995b).

**2.1.1 Experiment 1: potted plants.** For each species 30-40 wild plants were selected at random in January 1992 and transplanted in field soil. We used black plastic bags of 16 cm diameter filled with soil from the spot to a depth of 20 cm. All potted plants were kept in their natural environment, buried in soil so that they experience the same limitations as the ones left in the wild. During the first year all of them were regularly irrigated (up-to-twice per week, depending on the season). In the beginning of the second year a small number of plants was left aside without any treatment to be used as controls. However, in our nectar collection we had to replace this group with plants naturally grown in the wild (also referred to as controls) because untreated plants often did not flower synchronously to the treated ones. The remaining plants continued to be irrigated regularly until two months before flowering, i.e. beginning of March. Then they were divided into two groups: one continued to be regularly irrigated with water only (hereafter called irrigated) while the other was supplied with water containing nutrients (hereafter called fertilized group). As a nutrient mixture we applied the standard mixture consisting of  $\text{KNO}_3$  0.9 g/l,  $\text{K}_2\text{HPO}_4$  0.14 g/l,  $(\text{NH}_4)_2\text{SO}_4$  0.06 g/l,  $\text{HBO}_3$  0.0015 g/l (Schwarz 1968, p. 113) because it contained the elements that promote nectar secretion (see above). For both the irrigated and fertilized plants we followed the same weekly schedule. We carried out the main part of the experiment during the flowering season of 1993 (May-June), and in 1994, due to availability of plant material, we repeated the experiment only on *S. cretica*. All nectar measurements and flower collection for laboratory analyses were carried out during the peak of the flowering period.

**2.1.2 Experiment 2: Plants naturally grown in the wild.** In order to find what is the immediate response of nectar secretion to irrigation, an irrigation experiment was carried out on two individual plants of *T. capitatus* in flower, selected in the wild. We measured their nectar for two consecutive days, then irrigated them with an abundant quantity of water and re-measured their nectar the following day.

### 2.2 Nectar measurements

Flowers to be handled/collected were marked and covered in bud stage with bridal veil to prevent insects from feeding on the nectar. Nectar was collected the following day, always in the early afternoon. Weather conditions during all consecutive sampling days for a given species were more or less constant. Nectar volume was measured by inserting calibrated microcapillaries (Drummond) ranging between 0.1 and 5  $\mu\text{l}$  into the flower. Nectar concentration (almost entirely due to sugars and expressed in % w/w sucrose) was measured in the field with a pocket refractometer (Bellingham and Stanley, Tunbridge Wells). Nectar measurements were followed by collection of nectar on Whatman  $\text{N}^\circ$  1 paper wicks to be analysed for sugars and amino acids in the laboratory. Flowers were preserved in FAA (5 ml formaldehyde 40%, 85 ml ethanol 70%, 10 ml acetic acid) in order to study the nectary structure. Sugar and amino acid analyses of nectar were performed by means of HPLC. A detailed description of the methods of collection and analyses are available in Petanidou & Smets (1995) and Petanidou et al. (1995a). Flower samples for all sets of measurements were selected at random.

### 2.3 Nectary structure

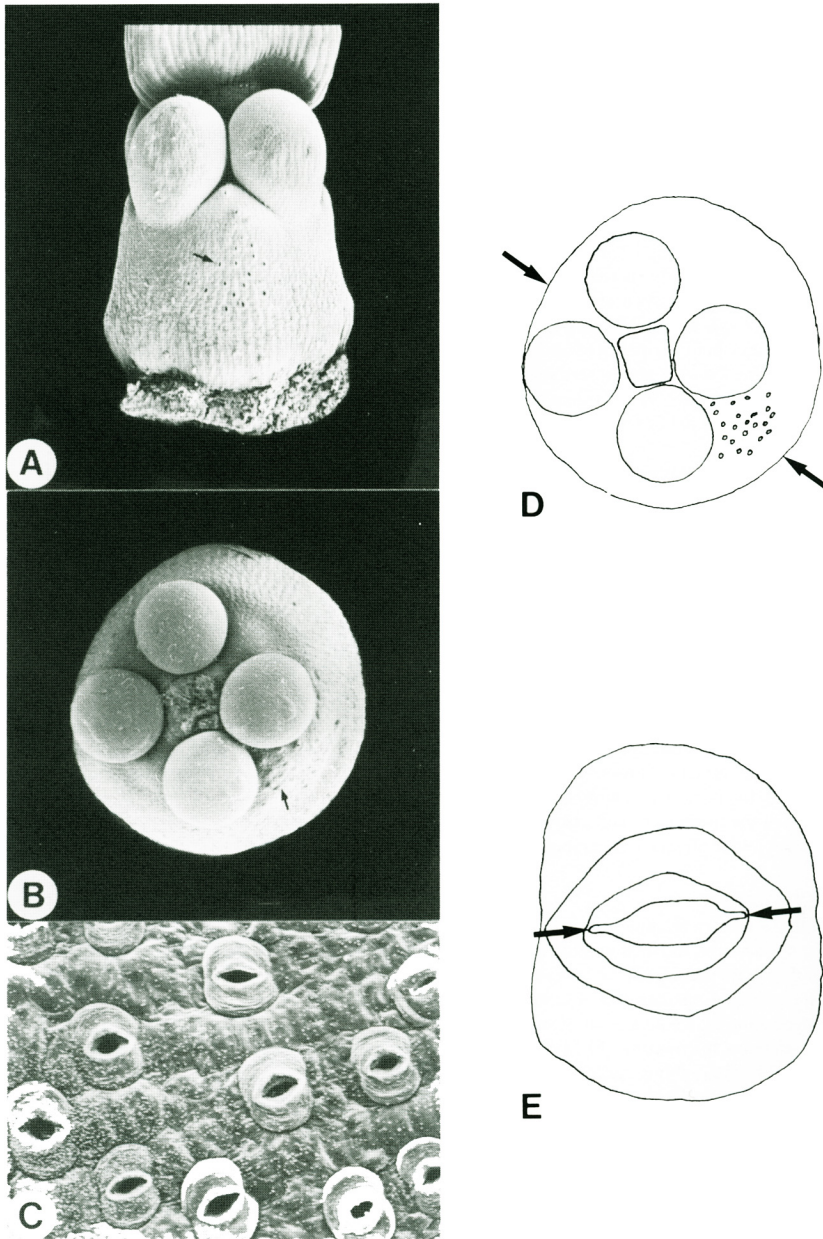
We recorded all those nectary features that according to Petanidou, Goethals, and Smets (unpublished) are related to nectar secretion, viz. the nectary size, the total number of modified stomata on the nectary, and finally, the size and the position of the stomata. As nectary size we considered the longest cross-section axis passing through the active nectary and as stomatal size the longest axis of the stomatal opening (fig. 1). Stomata were characterized as sunken, elevated, or isobathic to the adjacent epidermal cells, depending on the alignment of stomata on the surface of the nectariferous tissue. Nectary structure was studied through scanning electron microscopy (SEM). The material was dissected in alcohol 70% under a Wild M3 microscope equipped with a cold-light lighting system (KL 1500, Scott) followed by a dehydration with dimethoxymethane. The dehydrated material was critical point dried (CPD 030, Balzers Union) using liquid  $\text{CO}_2$ . The dried material was mounted on aluminium stubs using Leit C (after Gscheke) and coated with a thin layer of gold (approximately 180 nm). Finally, it was examined in a Jeol SM 6400 scanning electron microscope using an acceleration voltage of 10 kV.

### 2.4 Data analysis

In general, the flowering of treated plants (both irrigated and fertilized) lasted longer than that of untreated or those growing in the wild. In some experiments a given set of plants either did not produce flowers at all, or its flowering was not congruous with that of the rest of the same species; these cases are not included in the following data analyses.

Total sugar weight was calculated on the basis of the nectar values measured in the field [volume x (concentration/100) x density of sucrose at the observed concentration], the latter values taken from existing tables (Dafni 1992). Any missing value (volume or concentration) was replaced by the mean of the whole group of these particular values.

Throughout the paper values are followed by standard error (SE). Prior to statistical analysis, the data were tested for normality (Kolmogorov-Smirnov/Lilliefors tests). Because normality in most cases could not be achieved by transformation, non-parametrical tests were used throughout the study. In all post-hoc comparisons following a (non-parametric) ANOVA, we applied the Bonferroni correction (Pagano & Gouvreau 1993).



*Figure 1. Thymus capitatus. A, Frontal view of the abaxial side of the gynoecium with its gynobasic nectary (arrow). 88X. B, Top view of the same; the style has been removed. 88X. C, Detail of the nectariferous surface with nectarostomata. 680X. D, Drawing of the nectary, illustrating the longest cross-section axis passing through the nectary. 88X. E, Drawing of a nectarostoma, illustrating the longest axis of the stomatal opening. 4080X.*

**Table 1. Per flower nectar production (volume, concentration, sugar content) of three labiates under different conditions and treatments (irrigation or fertilization).**

Not specified plants are the controls (mostly plants measured in the wild). N (volume) is higher than n (concentration), since some flowers were nectarless.

Treatment	Date of collection	Volume ( $\mu\text{l}\pm\text{SE}$ )	Sugar content per flower ( $\mu\text{g}\pm\text{SE}$ )	$\underline{N}$	Nectarless flowers (%)	Concentration (% w/w)	$\underline{n}$
<b>A. Treatment in pots</b>							
<i>Satureja thymbra</i>							
control	16.5.93	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01	22	0	71.9 $\pm$ 1.18	13
fertilized	17-18.5.93	0.14 $\pm$ 0.04	0.09 $\pm$ 0.03	18	17	56.8 $\pm$ 3.34	9
irrigated	18.5.93	0.17 $\pm$ 0.03	0.14 $\pm$ 0.01	2	0	62.8 $\pm$ 3.90	2
fertilized	14.4.93	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	20	5	72.1 $\pm$ 0.93	13
fertilized	12-17.6.93	0.03 $\pm$ 0.004	0.03 $\pm$ 0.004	83	11	71.7 $\pm$ 0.81	47
<i>Stachys cretica</i>							
control	12.5.93	0.37 $\pm$ 0.09	0.31 $\pm$ 0.07	21	5	66.6 $\pm$ 2.41	16
fertilized	17-18.5.93	0.83 $\pm$ 0.14	0.28 $\pm$ 0.06	10	10	30.7 $\pm$ 2.79	9
irrigated	17-18.5.93	1.02 $\pm$ 0.18	0.36 $\pm$ 0.05	7	0	33.4 $\pm$ 3.85	7
fertilized	13-16.6.93	0.09 $\pm$ 0.03	0.06 $\pm$ 0.02	44	36	59.8 $\pm$ 2.37	19
irrigated	13-15.6.93	0.37 $\pm$ 0.04	0.19 $\pm$ 0.02	47	4	47.7 $\pm$ 2.24	42
control	9-10.6.94	0.47 $\pm$ 0.08	0.34 $\pm$ 0.05	24	17	59.4 $\pm$ 2.70	17
fertilized	9-10.6.94	0.22 $\pm$ 0.07	0.12 $\pm$ 0.03	22	14	55.4 $\pm$ 5.12	11
irrigated	9-10.6.94	0.36 $\pm$ 0.09	0.28 $\pm$ 0.06	11	0	61.8 $\pm$ 1.91	10
<i>Thymus capitatus</i>							
control	15.6.93	0.03 $\pm$ 0.004	0.02 $\pm$ 0.003	23	0	64.3 $\pm$ 1.90	22
fertilized	12-17.6.93	0.07 $\pm$ 0.01	0.04 $\pm$ 0.01	62	16	50.2 $\pm$ 1.40	45
<b>B. Treatment in the wild</b>							
<i>Thymus capitatus</i>							
control	18-19.6.94	0.15 $\pm$ 0.01	0.10 $\pm$ 0.005	93	0	52.5 $\pm$ 0.85	93
irrigated	20.6.94	0.22 $\pm$ 0.01	0.15 $\pm$ 0.009	47	0	55.1 $\pm$ 0.95	47

### 3 Results

#### 3.1 Nectar secretion

Table 1 shows the nectar secretion measured in the field (volume, concentration, and deriving sugar content) under different conditions. Table 2 summarizes the statistical differences between the sets of data contained in Table 1. They suggest that (i) among the species studied, only *T. capitatus* showed significant increase both in volume and sugar content of nectar after all treatments. *S. thymbra* showed also a similar trend in both treatments, but the increases shown

**Table 2. Summary statistics for the data presented in Table 1.**

Only non-parametric tests were applied, viz. Kruskal-Wallis H and Mann-Whitney U tests. In all a posteriori comparisons we applied the Bonferroni correction. ns is when  $P > 0.05$ .

Time	Groups tested	Test	Volume		Sugar content	
			Test value	<i>P</i>	Test value	<i>P</i>
<b>A. Treatment in pots</b>						
<i>Satureja thymbra</i>						
May 93	Control-fertilized-irrigated	K-W H	3.048	ns	3.162	ns
April, May, June (93)	Fertilized	K-W H	2.508	ns	1.880	ns
<i>Stachys cretica</i>						
May 93	Control-fertilized-irrigated	K-W H	10.961	0.004	1.116	ns
"-	Control-irrigated	M-W U	20.000	0.014		
"-	Control-fertilized	M-W U	49.500	0.057		
"-	Fertilized-irrigated	M-W U	24.500	ns		
June 93	Fertilized-irrigated	M-W U	331.500	0.000	379.000	0.000
June 94	Control-fertilized-irrigated	K-W H	5.702	0.006	8.980	0.011
"-	Control-irrigated	M-W U	116.500	ns	119.000	ns
"-	Control-fertilized	M-W U	171.500	ns	141.000	0.021
"-	Fertilized-irrigated	M-W U	68.500	ns	62.000	ns
<i>Thymus capitatus</i>						
June 93	Control-fertilized	M-W U	387.500	0.001	495.000	0.031
<b>B. Treatment in the wild</b>						
<i>Thymus capitatus</i>						
June 94	Control-irrigated	M-W U	1173.500	0.000	1135.000	0.000

were not statistically significant. Finally, *S. cretica* showed a very strange response to treatments, i.e. an increase in nectar volume -but not in sugars- after both treatments in May, and a decrease in volume and sugars after fertilization in June (1993 and 1994); (ii) when different, nectar yield of irrigated plants was, in general, higher than that of fertilized ones; (iii) an unexpected result in all species and treatments was the presence of nectar-blank flowers. This was much more common after fertilization (cf. Table 1).

Table 3 gives the sugar ratios and the amino acid content and concentration (= content/volume, the latter taken from Table 1) of the floral nectar from treated and control plants of the three species studied. The amino acid concentration remained constant in each of the species sets (M-W U and K-W H tests;  $P > 0.05$ ). After treatment, the sucrose/hexose ratio dropped in *S. thymbra*, but increased in *S. cretica*, and remained constant in *T. capitatus*. Glucose/fructose ratio increased after treatment in all cases except *S. cretica*.

### 3.2 Nectary structure

The nectary characteristics of the species raised in the wild as well as under all treatments are given in Table 4. Table 5 shows the statistical results in order to assess the impact of each treatment on the four nectary features studied.

**Table 3. Total amino acid content, concentration, and sugar ratios (in weight) of the floral nectar of three labiates raised under special conditions.**

Chemical analyses were carried out through HPLC. Statistical tests were K-W H and M-W U tests for 3- and 2-sample analyses, respectively. \*\* 0.001≤P≤0.01, \* 0.01≤P≤0.05, ns P>0.05.

Plant group	Date	Amino acids					Sugar ratios				
		nmoles/flower	SE	<u>N</u>	nmoles/ml	<u>P</u>	S/G+F	<u>P</u>	G/F	<u>N</u>	<u>P</u>
<i>Satureja thymbra</i>											
Control	16.5.93	4.29	0.96	3	0.086		3.03		0.57	6	
Fertilized	17.5.93	5.08	2.73	2	0.036	ns	1.36	**	0.75	5	*
<i>Stachys cretica</i>											
control	12.5.93	5.57	0.64	8	0.015		2.17		0.47	10	
fertilized	17.5.93	6.31	5.03	2	0.008		3.71		0.50	5	
irrigated	17.5.93	12.92	12.23	2	0.013	ns	6.19	*	0.41	5	ns
<i>Thymus capitatus</i>											
control	15.6.93	0.75	0.18	10	0.025		1.20		0.21	11	
fertilized	16.6.93	1.76	0.21	3	0.025	ns	1.31	Ns	0.41	5	*

**Table 4. Nectary characteristics of three labiates raised in the wild, under irrigated conditions and under additional nutrient supply.**

Plant Group	Date	Level	Stomata						Nectary size		
			Number			Length of opening					
			#	SE	n	(µm)	SE	n	(mm)	SE	n
<i>Satureja thymbra</i>											
control	16.5.93	Isobathic	18.4	1.25	10	7.0	0.27	23	0.80	0.02	4
fertilized	15.6.93	Tendency to	23.7	5.52	10	5.5	0.53	19	0.66	0.03	7
irrigated	15.6.93	"-	52.1	4.39	10	4.2	0.38	20	0.71	0.02	4
<i>Stachys cretica</i>											
control	8.6.94	Elevated	35.8	2.96	10	8.67	0.51	29	0.98	0.03	7
fertilized	6.6.94	"-	28.8	1.62	10	8.20	0.49	21	1.01	0.02	6
irrigated	6.6.94	"-	37.0	2.24	10	12.09	0.87	16	1.07	0.02	9
control	12.5.93	Elevated	24.1	2.09	10	8.84	0.64	10	1.18	0.03	5
fertilized	12.5.93	"-	18	2.34	10	5.58	0.48	13			
<i>Thymus capitatus</i>											
control	15.6.93	Isobathic	41.4	1.77	11	6.7	0.29	40	0.55	0.03	3
fertilized	15.6.93	"-	46.3	9.81	8	7.2	0.34	20	0.43	0.01	4
irrigated	15.6.93	"-	49.8	3.76	5	7.2	0.34	20	0.42	0.00	4

The level of nectarostomata remained constant in fertilized and irrigated plants of *S. cretica* and *T. capitatus*; *S. thymbra*, however, showed a type of structural variation, as the nectarostomata of treated plants became elevated versus isobathic which was the norm. The size of the nectary did not appear to be related to any type of nectar change, as no species showing a change in nectar features showed a consistent change in nectary size. The same holds true for the length of the stomatal opening, as well as the number of stomata per nectary. Table 6 summarizes all comparisons on nectar and nectary characteristics of untreated and treated plants of the above three species.

## 4 Discussion

### 4.1 The effect of water and nutrient supply on nectar secretion

Out of the three species studied only *T. capitatus* secreted clearly more nectar (i.e. both in volume and sugars) after a treatment. *S. thymbra* appears also to follow the same pattern, but the increase shown is not significant, probably due to the big number of nectar-blank flowers present after

**Table 5. Summary statistics for nectary features between flower sets from plants of the three labiates grown under different conditions (cf. Table 4).**

*P* in post hoc comparisons has been corrected after Bonferroni (ns is when  $P > 0.05$ ; c= control, f= fertilized, i= irrigated).

Groups tested	Test	# stomata			Length of stomata opening			Nectary size		
		df	Test value	<i>P</i>	df	Test value	<i>P</i>	df	Test value	<i>P</i>
<i>Satureja thymbra</i>										
c-f-l	K-W H	2, 30	16.582	0.000	2, 62	20.476	0.000	2, 15	7.996	0.018
c-f	M-W U	10, 10		Ns	23, 19	133.000	0.090	4, 7	1.000	0.040
c-l	M-W U	10, 10	0.000	0.000	23, 20	45.000	0.000	4, 4	0.000	0.063
f-l	M-W U	10, 10	9.000	0.006	19, 20	112.000	0.083	7, 4	9.000	Ns
<i>Stachys cretica</i>										
c-f-i June 94	K-W H	2, 30	7.406	ns	2, 89	22.736	0.000	2, 22	7.175	0.028
c-l	M-W U				16, 39	143.500	0.005	7, 6	8.500	0.044
c-f	M-W U				34, 39	430.000	0.030	7, 9	18.000	Ns
f-l	M-W U				34, 16	57.500	0.000	6, 9	10.500	Ns
c-f May 93	M-W U	10, 10	29.000	ns	10, 13	11.000	0.002			
c-c 93-94	M-W U	10, 10	14.000	0.006	29, 10	145.000	ns	5, 7	0.000	0.004
f-f 93-94	M-W U	10, 10	12.000	0.004	21, 13	51.000	0.002		-	-
<i>Thymus capitatus</i>										
c-f-l	K-W H	2, 24	1.492	ns	2, 49	4.625	ns	2, 11	6.430	0.040
c-f	M-W U							3, 4	0.000	Ns
c-l	M-W U							3, 4	0.000	Ns
f-l	M-W U							4, 4	0.000	Ns



**Table 6. Comparative results for nectar and nectary features of the three labiates with and without treatments, summarized from Tables 1-5. Abbreviations are as in Table 5.**

Treatment	Nectar				Nectary size	Nectarostomata		
	Volume	Sugar content	Amino acid concentration	S/F+G		Level	Length	Number
<i>Satureja thymbra</i>								
c-f	ns	Ns	ns	c>f	c>f	-> elevate	ns	ns
c-i	ns	Ns	-	-	c>i	-> elevate	c>i	i>c
<i>Stachys cretica (May 1993)</i>								
c-f	f>c	Ns	ns	f>c	-	constant	c>f	n0s
c-i	i>c	Ns	ns	i>c	-	constant	-	ns
<i>Stachys cretica (June 1994)</i>								
c-f	ns	c>f	-	-	ns	constant	c>f	ns
c-i	ns	Ns	-	-	i>c	constant	i>f	ns
<i>Thymus capitatus</i>								
c-f	f>c	f>c	ns	ns	ns	constant	ns	ns
c-i	-	-	-	-	ns	constant	ns	ns

fertilization. That nectar yield may even drop after artificial treatment (*viz.* *S. cretica* in June) is more intriguing and this is the only example that could imply that nectar secretion in phrygana is not by definition water- or nutrient-limited as has been suggested elsewhere (Petanidou & Vokou 1990). However, because this concerns nectar production in June, *i.e.* beyond the regular flowering time of the species (Petanidou 1991; Petanidou et al. 1995b), we believe that the phenomenon is rather linked to the differential resource allocation of the plant, as it will be explained below. In total, our findings imply that (i) nutrient supply enhances not always nectar secretion; (ii) as to the nectar yield produced per flower, irrigation is more favourable than additional nutrient supply; (iii) fertilization very often results in the presence of nectarless flowers; and (iv) amino acid concentration is not influenced by any treatment, while sugar ratios are.

The fact that irrigated plants yielded at least the same, and in the case of *S. cretica* (June 1993, but also June 1994 if we disregard the ultraconservative statistics) more nectar volume/sugars per flower than fertilized ones, shows that it is the water that plays the major role in nectar secretion in phrygana - not the nutrients, at least not the tested ones. That nutrient supply has no impact on nectar secretion sounds strange. Moreover, it is even stranger that fertilization results in the presence of nectarless flowers. In seeking an explanation we cannot exclude that excessive nutrient supply may result in different resource allocation between woody (*e.g.* *S. thymbra* and *T. capitatus*) and herbaceous perennials (*e.g.* *S. cretica*). The latter species may be more flexible in distinguishing between growth and reproductive phase compared to the typical, seasonally dimorphic shrubs of phrygana such as *T. capitatus* and *S. thymbra*. This is supported by the fact that the flowering stems of *S. cretica* develop *ca.* one month before they come into bloom. *i.e.* in early summer. Therefore, under favourable conditions herbaceous plants may produce more and longer stems, larger photosynthetic area (*e.g.* more leaves), and more

flowers than the plants grown under typical, wild conditions. Although we have no data to support this, we can testify that fertilized as well as plants grown in the National Botanic Garden of Belgium of *S. cretica*, as well as of *Ballota acetabulosa*, another herbaceous perennial labiate, followed this rule (Petanidou personal observation). Moreover, the growing phase of *S. cretica* overlapped with that of flowering which is not the case under typical Mediterranean conditions. This phenomenon did not apply to the seasonally dimorphic woody shrubs, at least not to the same extent (Petanidou personal observation).

We hypothesise that under favourable conditions of nutrient supply the plants may produce less nectar per flower than expected due to a differential allocation of the photosynthetic products, i.e. to vegetative growth or reproductive potential. Under Mediterranean conditions, on the other hand, a great deal of the photosynthetic product is exclusively or to a major extent allocated in reproduction (rewards to pollinators, seed development, etc.) in order to face summer and its critical drought successfully. Because of the latter, the growing phase is utterly distinguished from flowering, which is also favoured by the long duration of the growing season (Petanidou et al. 1995b). As a result, photosynthesis in the wild may result in carbohydrate synthesis rather than in protein synthesis - which may be the case in fertilized plants, especially in herbaceous plants. The latter, however, may produce unusually high number of flowers per plant, which on average may result in lower - and sometimes no - phloem sap flow to some of the flowers; thus, these flowers appear as nectar-poor or nectar-blank. This phenomenon is not surprisingly new: Shuel (1955) also found that nectar yield in plants of "luxuriant growth" i.e. after artificial nitrogen supply, was comparatively poor, whereas it was comparatively good in plants of meagre growth. The same author reports that small-sized plants of a given species produce more nectar per flower than large-sized ones (cf. also Bonnier 1879; Shuel 1964). Quite similar is the phenomenon described by Corbet & Delfosse (1984) who found lower nectar secretion rates as flower density per m<sup>2</sup> increased.

Whether the differential allocation to nectar has an evolutionary explanation is doubted. Nevertheless, several researchers have already mentioned the existence of empty flowers which they characterized as cheaters (Feinsinger 1978; Heinrich 1979; Brink & de Wet 1980; Thomson & Plowright 1980; Bell 1986; Gilbert et al. 1991; Sakai 1993). Because of the particular water-limited conditions in the Mediterranean, we believe this point should be handled and interpreted with caution; when doing this, the differential allocation of photosynthetic products during flowering should also be taken into consideration. In this case, a more profound study on the differential resource allocation in Mediterranean plants through their vegetative and reproductive phase is essentially needed.

The pattern in which sugar ratios of the three species studied are influenced by treatment indicates that it is not a simple phenomenon of sucrose breakdown when nectar volume increases and concentration drops, as is normally the case (Petanidou et al. 1995a). A careful look at Table 3 reveals that, when treated, flowers may produce comparatively either more glucose (*S. thymbra*, *T. capitatus*) which may be combined with sucrose drop (*S. thymbra*), or more sucrose (*S. cretica*). This, again, provides evidence to the above mentioned hypothesis that under treatment herbaceous plants may respond by enhancing their anabolic rate (*S. cretica*), whereas in woody perennial shrubs any photosynthetic excess is simply and passively invested as reproductive success. As the main transported sugar from phloem to nectary cells is sucrose, a high sucrose content appears then in the secreted nectar (Nichol & Hall 1988; Roshchina & Roshchina 1993).

#### **4.2 The effect of water and nutrient supply on nectary structure**

The level of nectarostomata may change with treatment, as is the case of *S. thymbra*; in this species nectarostomata tended to be rather elevated in treated plants versus isobathic which is the

norm. This finding is also supported by Dafni et al. (1988) who found that in plants of *S. thymbra* cultivated in a botanical garden the stomatal level was higher than the adjacent epidermal cells.

The number of nectarostomata may increase with treatment (in only one of the three species cases examined), but this does not result in a greater volume of nectar secretion. That this feature alone does not influence nectar secretion has also been noted in another study in the Mediterranean (Petanidou, Goethals & Smets unpublished) as well as elsewhere in the literature (Teuber et al. 1980; Davis & Gunning 1991).

The length of stomatal opening of the nectary does not seem to be related to nectar secretion in treated plants, as was the case within a set of labiate species in the wild (tested specieswise; Petanidou, Goethals, and Smets, unpublished). In fact, here we found that nectar secretion may increase with decreased opening (e.g. *S. cretica*-fertilized, but marginally), remain constant (*S. thymbra*-all treatments), and in only one case increased with the opening (*S. cretica*-irrigated). Contrary to these, nectar secretion in *T. capitatus* increased despite the fact that no nectary feature did change.

Nectary size was the parameter that has been found to be related to nectar secretion within Labiatae tested specieswise (Fahn 1949; Dafni et al. 1988; Petanidou, Goethals & Smets unpublished). However, as showed above, our data do not support this point since any increase in nectar secretion is not equally followed by an increase in the nectary size as we expected (Table 6). For instance, although fertilized plants of *T. capitatus* had higher nectar secretion, it was the wild plants that had bigger nectaries. Again, wild plants of *S. thymbra* had bigger nectaries than all treated plants, but this was not followed by greater nectar secretion. Similar, even more complicated is the case of *S. cretica* (Table 6). Apparently, within a species the relation between nectar secretion and nectary size is not as easy to describe as it is in the interspecific context, e.g. within the Labiatae (Dafni et al. 1988; Petanidou, Goethals & Smets unpublished).

The above findings show that conditioning nectar secretion does not affect nectary structure and vice versa, as was hypothesised in the beginning of this study. One explanation might be that the classes of difference between the "wild" and the "mutant" type may stem from the long-lasting evolution of a species and cannot be achieved just by mere irrigation or nutrient supply. Such treatments may have a measurable effect on the nectar secretion, as has been shown in this paper, but not on the nectary structure. If this is really true, the structural differences we found here may merely reflect the naturally existing intraspecific variations or even variations within a plant. To detect whether intraspecific variation in nectary size also reflects an equal variation in nectar secretion, we need to experiment on the same clones with a different, more drastic method than just watering or fertilizing. On the other hand, the observed differences in nectary size may also be an artifact caused by the additional nutrient supply/irrigation. It might be that under conditions of ongoing vegetative growth some species invest much less in nectary development than they do under wild conditions (e.g. *T. capitatus*). We need certainly more research to clarify this point.

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