

The role of nectar aminoacidic composition in pollinator preference

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Introduction

Amino acids are the main nectar components after sugars. In addition to protein amino acids (among which proline is the main one) non-protein amino acids (eg β -alanine) are also present and sometimes very abundant, but their contribution to floral attraction to pollinators is not clear (Nepi, 2014). Model organism for the study is *Gentiana lutea* subsp. *symphyandra*, a perennial and generalist plant that in the studied area is mainly pollinated by bumble bees. Previous analyses have revealed high concentration of β -alanine in *G. lutea* nectar (Rossi *et al.*, 2014).



G. lutea subsp. *symphyandra*

The aim of the study is to investigate if the presence of proline and β -alanine in the nectar has an influence on the choice of *Bombus terrestris* and *Apis mellifera* for *G. lutea* nectar.

Materials and methods

Nectar preference was tested by dual choice feeding test. Four different solutions simulating *G. lutea* nectar were administered to bees:

1. enriched with β -alanine (B);
2. enriched with proline (P);
3. enriched with both amino acids (P+B);
4. no amino acids enriched (C).

Consumption was checked at 24, 48, 72, and 96 hours. Five trials were performed on *B. terrestris* workers (1-5 W), two trials on *B. terrestris* males (2 and 4 M), and one trial on *A. mellifera* foragers (6 A).

Trials Theses	Theses in the different trials.						
	1W	2W	2M	3W	4M	5W	6A
C vs P	X				X	X	X
C vs B	X				X	X	X
C vs P+B	X	X	X	X	X	X	X
P vs P+B	X			X	X	X	X
P vs P+B		X	X	X	X	X	X
B vs P+B		X	X	X	X	X	X

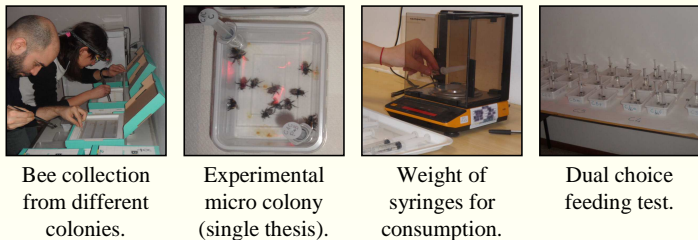
Sucrose	Glucose	Fructose	β -alanine	Proline
1.90 mg/mL	177.8 mg/mL	164.9 mg/mL	204.9 mg/L	138.16 mg/L

G. lutea nectar composition.

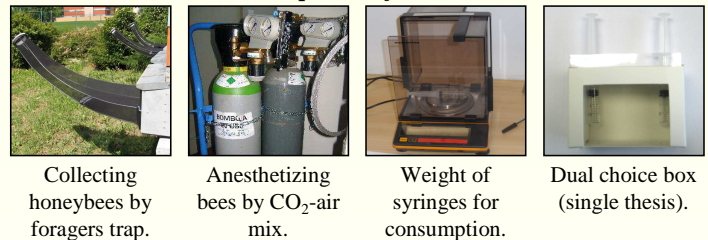
	Trials 1, 2, 3		Trials 4, 5		Trial 6	
	Proline	β -alanine	Proline	β -alanine	Proline	β -alanine
P	138.16	-	686	-	733.33	-
B	-	204.9	-	686	-	733.33
P+B	138.16	204.9	343	343	366.67	366.67

Amino acids concentration (mg/L) in the tests solutions.

Bombus terrestris

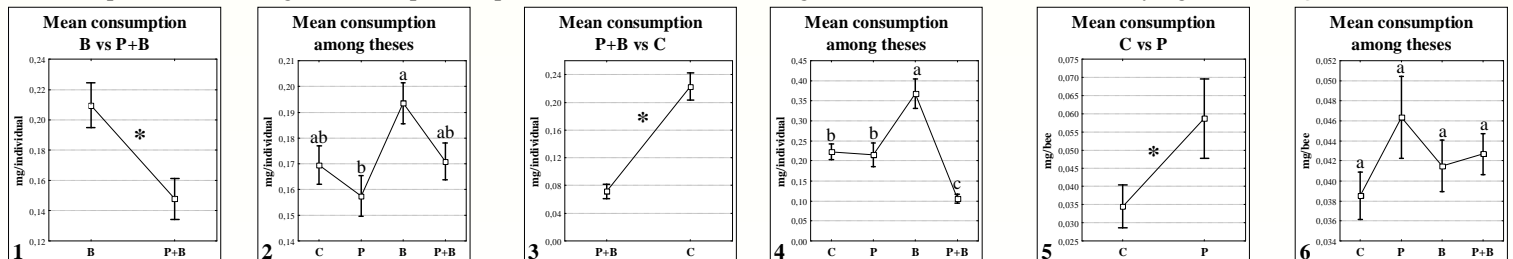


Apis mellifera



Results

- The results of the single paired comparisons on *B. terrestris* workers, analysed over the five trials by t-test, show a significant preference for β -alanine enriched nectar (B solution) vs P solution ($p=0,008$) and P+B solution ($p=0,004$) (Fig. 1).
- Moreover ANOVA test on mean individual consumption shows that nectar enriched with β -alanine is the most consumed ($p=0,018$) (Fig. 2).
- Nevertheless, the same analyses performed separately on the single trials give some inconsistent results.
- MANOVA analysis shows that the nectar preference is influenced not only by the presence of amino acids, but also by the colony of origin and the kind of paired choice (colony*thesis*amino acid: trials 1-2-3 $p<0,000$; trials 4-5 $p=0,042$).
- In the two tests performed on *B. terrestris* males, a preference for the C vs P+B solution (ANOVA, $p<0,000$) (Fig. 3) and for B vs P+B solution ($p<0,000$) is observed. Among the four solutions, also males consume more β -alanine enriched solution (ANOVA, $p<0,00$) (Fig. 4).
- The results of honey bees test show a significant preference for proline in the choice C vs P (Fig. 5). Consistently, ANOVA test on the mean total consumption shows a higher consumption of proline enriched nectar, although this difference is not statistically significant (Fig. 6).



Figs 1-2: Analysis on bumble bees workers. Figs 3-4: Analysis on bumble bees males. Figs 5-6: Analysis on honeybees foragers (□ Mean; I Mean \pm 0,95*SE).

Conclusions

Our study indicates a preference of *B. terrestris* workers and males for nectar enriched with the amino acid β -alanine. This can explain the strict relationship between *G. lutea* and bumble bees. The inconsistency of the results obtained in the five *B. terrestris* worker tests is probably due to the effect of the colony of origin on the nectar preference.

The results on both bumble bee workers and males seem to exclude a preference for proline enriched nectars.

On the contrary, the test on honeybee foragers confirms the results of Bertazzini *et al.* (2010), in finding a preference of this pollinator for proline enriched solutions.

Bertazzini, M., Medrzycki, P., Bortolotti, L., Maistrello, L., Forlani, G. (2010): Amino acid content and nectar choice by forager honeybees (*Apis mellifera* L.). *Amino Acids* 39: 315-318.
 Nepi, M. (2014): Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar. *Journal of Ecology* 102: 108-115.
 Rossi, M., Fisogni, A., Nepi, M., Quaranta, M., Galloni, M. (2014): Bouncy versus idles: On the different role of pollinators in the generalist *Gentiana lutea* L. *Flora* 209: 164-171.