# Potential Attractiveness of Olive Beneficial Insects to Flowering Weeds

Belo\*, A. F.<sup>1,2</sup>, Bennett R.N.<sup>4</sup>, Ganhão, E.<sup>2</sup>, Rei, F.<sup>1,3</sup>, Torres, L.<sup>4</sup>

<sup>1</sup>ICAAM, <sup>2</sup>Dept. Biology and <sup>3</sup>Dept. Crop Science, University Évora, Ap. 94; 7002-554 Évora Codex, Portugal <sup>4</sup>CITAB, Dept. Agronomy, University Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal \*e-mail: afb@uevora.pt



#### Introduction

Nowadays, there is a growing concern about keeping olive groves in a good sanitary condition with a decreasing input of pesticides. Increasing beneficial insect fauna is a novel, non-intrusive way to achieve this objective. To increase beneficial insect fauna, non-prey food availability must be enhanced, and that purpose can be attained by manipulating weed vegetation occurring on olive groves inter-rows. Amongst weeds naturally occurring in South Portugal olive groves, some were chosen regarding two natural olive beneficial insects - the parasitoid *Psyttalia concolor* Szep. and the pest predator Anthocoris nemoralis Fab. - accessibility and also as nectar and pollen producers. The aim of this research is to determine which weeds are more suitable as non-prey food source for these insects.

#### Results

Anchusa italica, Asparagus aphyllus, Calamintha baetica, Capsella bursapastoris, Diplotaxis catholica, Echium plantagineum, Foeniculum vulgare, Heliotropium europaeum, Lamium amplexicaule,Lathyrus clymenum, Lavandula stoechas, Malva hispanica, Medicago nigra, Melilotus segetalis, Origanum virens, Polygonum persicaria, Rosmarinus officinalis, Sinapis arvensis, Tolpis barbata, Urginea maritima were the species selected, according to the steps described on methods.

Until now just *Diplotaxis catholica* has been handled. For this species average width and depth of corolla tube were  $1,74 (\pm 0,426)$  and  $2,73 (\pm 0,474)$  mm, respectively. Daily average nectar production per flower was  $0,06 (\pm 0,044) \mu$ . A first screening from HPLC analysis to pollen and nectar can be seen in chromatograms A and B (Fig. 4).

## **Methods**

## a) Choosing 20 species.

- South Portugal olive groves natural weed flora was previously researched (Belo et al, 2009).
- The first selection criteria was to discard very competitive 2. perennial weeds from the existing list.
- To ensure that the studied insects could indeed reach the 3. nectaries of the chosen species, corolla morphometrics were assessed, namely the width and the depth of corolla tube; all measurements were made with a digital caliper and repeated on 30 corollas per species.
- Species ability as both nectar and pollen producers were, whenever possible, determined by consulting specialized literature.
- Flowering period described in portuguese botanical literature (Pereira Coutinho, 1939) was used to ensure that insects can feed on, at least, one flowering species at any time.

## b) Characterization of nectar and pollen

- Daily production of nectar will be quantified for each of the chosen species: flowers were covered with a bridal veil bag 24h before collection, at noon, to minimize nectar depletion by insects.

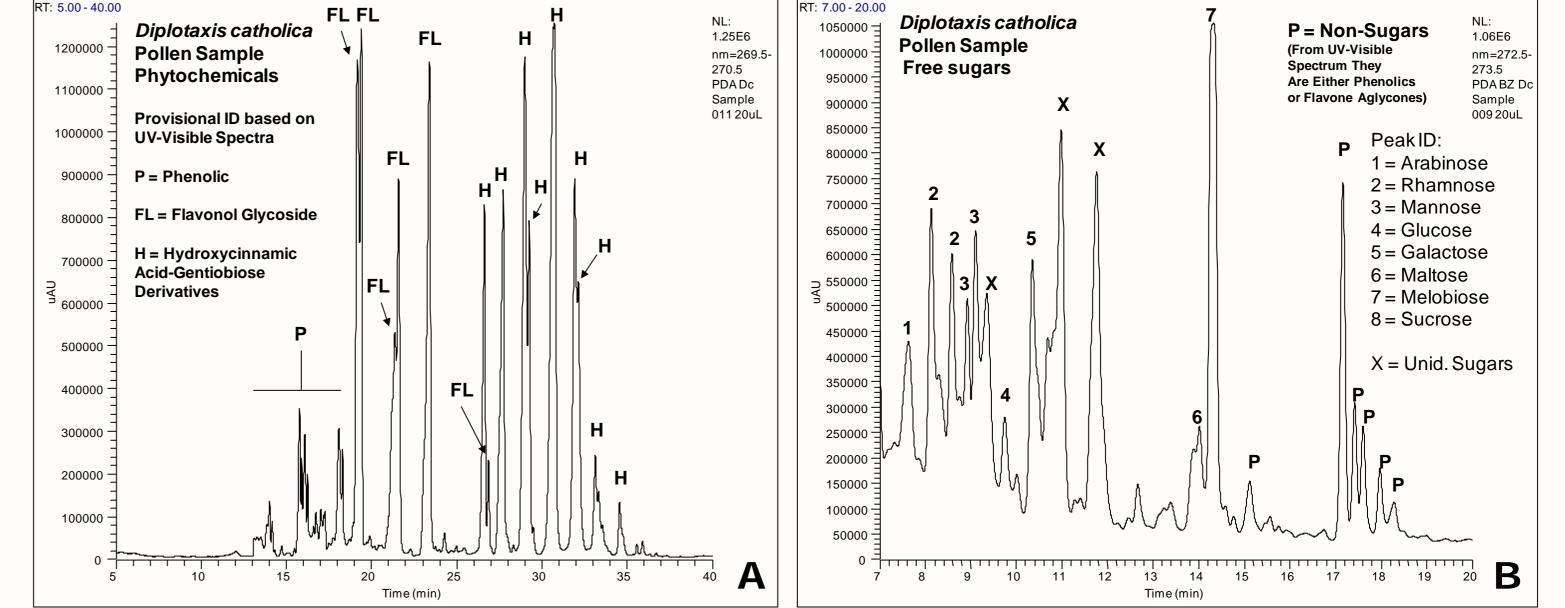


Fig 4. HPLC first screening of Diplotaxis catholica pollen phytochemicals (A) and free sugars (B).

Collecting pollen from small entomophilous flowers is a very laborious and time consuming work and, therefore, we are now adjusting the available methodology to make it more feasible: we have analyzed Rosmarinus officinalis phytochemicals (Fig.5) and free sugars (Fig.6) using samples with just pollen and with pollen+anthers to see with which error could we take one for the other.

Г: 5.00 - 40.00	)	P2	RT: 5.00 - 40.00	P2	
300000 -	Rosmarinus officinalis "Clean" Pollen	3.00E5	1200000	Rosmarinus officinalis "Anther-	NL: 1.25E6
280000	Sample Phytochemicals	nm=269.5- 270.5		Contaminated" Pollen Sample Phytochemicals	nm=269.5 270.5
260000	Provisional ID based on	PDA Ro Sample	1100000	Provisional ID based on	PDA Ro Sample
240000 -	UV-Visible Spectra and Retention Time	001 20uL	1000000	UV-Visible Spectra and Retention Time	002 20uL
220000	FV = Flavone Gkycoside			H = Hydroxycinnamic Acid/Derivative	
20000	P = Phenolic FL = Flavonol (Aglycone)		900000	FV = Flavone Glycoside P = Phenolic	
200000			800000	FL = Flavonol (Aglycone)	
100000	P2 may be Rosmarinic Acid or a				

Nectar from 30 flowers will be extracted and quantified with capillary micropipettes (Drummond Microcaps®), and sugar concentration was determined with a hand held refractometer (Atago®). Nectar will be lyophilized for posterior determination of its composition through gas chromatography.

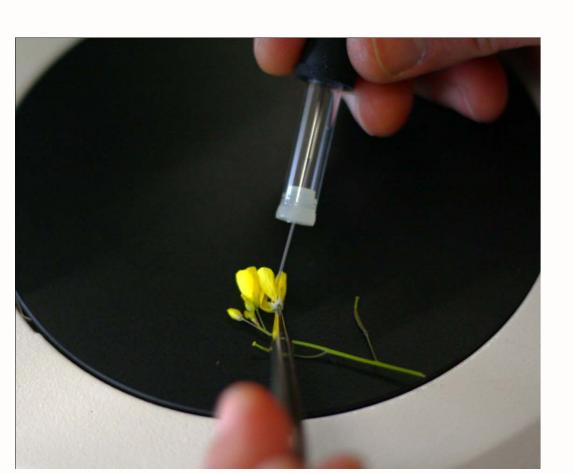


Fig 2. Nectar collection with micropipettes.



Fig 1. Bridal veil to minimize nectar depletion.

Pollen analysis was done according to the following scheme 3. (Bennet et al, 2006; Daniel et al, 1981):



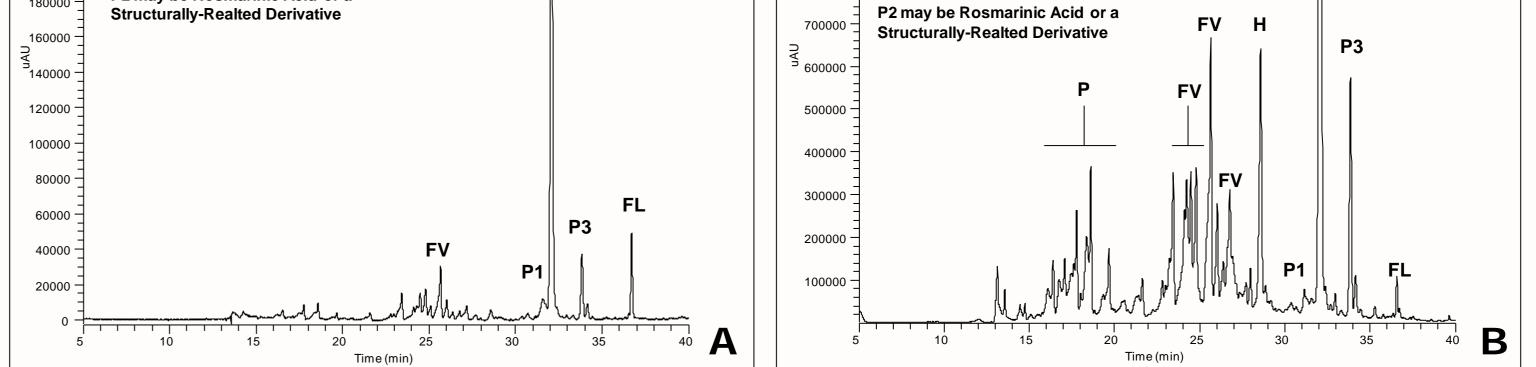


Fig 5. HPLC first screenings of Rosmarinus officinalis pollen and pollen+anthers phytochemicals (A and B, respectively).

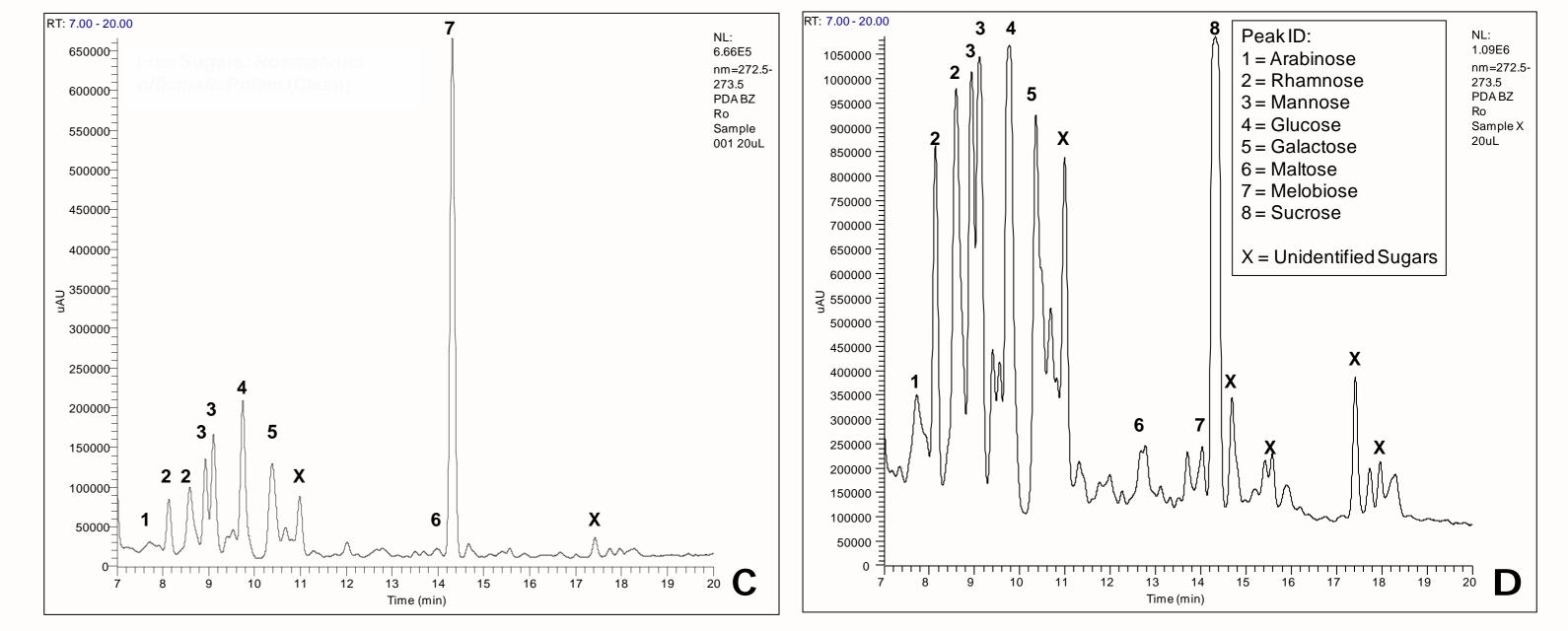
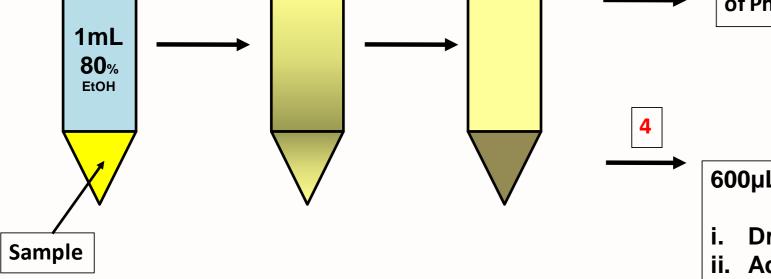


Fig 6. HPLC first screenings of Rosmarinus officinalis pollen and pollen+anthers free sugars (C and D, respectively).

Differences were quite evident, which means that collecting pollen with the anthers is not an accurate methodology to analyze either sugars or phytochemicals. In both *Rosmarinus* and *Diplotaxis* pollen, melobiose is by far the most abundant sugar.



600µL Supernatant (in 2mL Screw-cap Tube):

- Dry Completely with N<sub>2</sub> Gas
- ii. Add 500µL Benzoyl Chloride & 200µL Pyridine
- iii. Incubate at 37°C for 16 Hours
- iv. Add 1mL Diethyl Ether and Vortex Mix
- v. Centrifuge (13,000 rpm, 4°C, 20 min)
- vi. Remove 1mL of Supernatant
- vii. Dry Completely with  $N_2$  Gas
- viii.Resuspend Residue in 500µL 100% MeOH
- ix. Centrifuge (13,000 rpm, 4°C, 20 min)
- x. Transfer Supernatant to HPLC Vial
- xi. Analyse by RP-HPLC Mehod (20µL Injection)

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### **Final Considerations**

Because methodological questions are very important regarding results accuracy and time is a limited resource we are now optimizing all methods. Next step will be phytochemicals and sugars quantification from pollen extracted from anthers with ultra-sounds vs. pollen extracted by hand, under the binocular microscope.

#### **References**

Belo, A.F, Simões, M.P, Pinto-Cruz, C. e Castro, M.C. (2009). Efeitos da gestão do coberto do solo na diversidade vegetal de olival. Actas do XII Congresso da SEMh/XIX Congresso da ALAM/II Congresso da IBCM, vol.1:61-64. Bennett, RN, Rosa, EAS, Mellon, FA, Kroon, PA (2006). Ontogenic profiling of glucosinolates, phenolics, flavonoids and other secondary metabolites in Eruca sativa (salad rocket), Diplotaxis erucoides (wall rocket), Diplotaxis tenuifolia (wild rocket), and Bunias orientalis (Turkish rocket). J. Agric Food Chem. 54: 4005-4015. **Corbet** SA. 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. Apidologie 34: 1–10. Coutinho, A. X. P. (1939) - Flora de Portugal : plantas vasculares.- 2ª ed.- Lisboa: Bertrand, 1939, 938 p. Daniel, PF, De Feudis, DF, Lott, IT, McCluer, RH (1981). Quantitative microanalysis of oligosaccharides by high-performance liquid chromatography. Carbohydrate Research 97: 161-180

Morrant DS, Schumann R, Petit S (2009) Field methods for sampling and storing nectar from flowers with low nectar volumes. Ann Bot Lond 103:533-542.

**Processing Steps** 1: Ultra-sound Extraction from anthers (3 min) 2: Removal of anthers 3:Centrifugation (10,000rpm, 4°C, 10min) 4: Sub-sample for Phytochemicals

5: Sub-sample for Free Sugars

Fig 3. Pollen analysis procedures.