



Laboratory of  
Analytical Methods  
for Chemical Ecology

Biology of plant-insect interactions



# MONITORING NECTAR PRODUCTION

## DYNAMICS:

METHODS for SAMPLING, STORING and NECTAR EVALUATION

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*Flower Biology and Pollination Ecology: Module I - Concepts and practices*

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# DYNAMICS OF NECTAR PRODUCTION

## GENERAL CONSIDERATIONS

- The dynamics of nectar production fits the requirements of plant pollinators.
- The quantity and quality of nectar produced per flower depends on the energy demands of pollinators

NIGHT EXPOSURE

DAY EXPOSURE

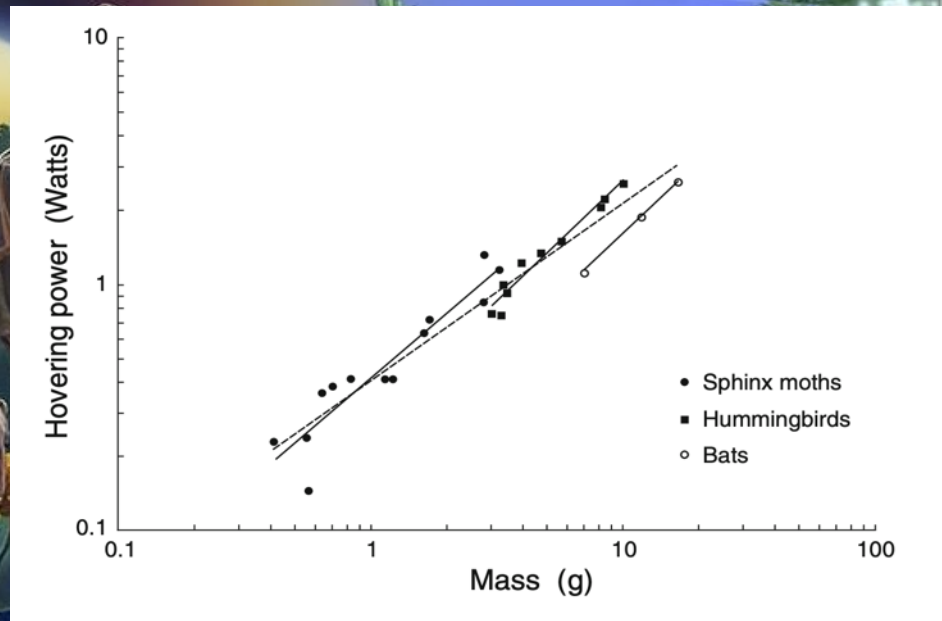
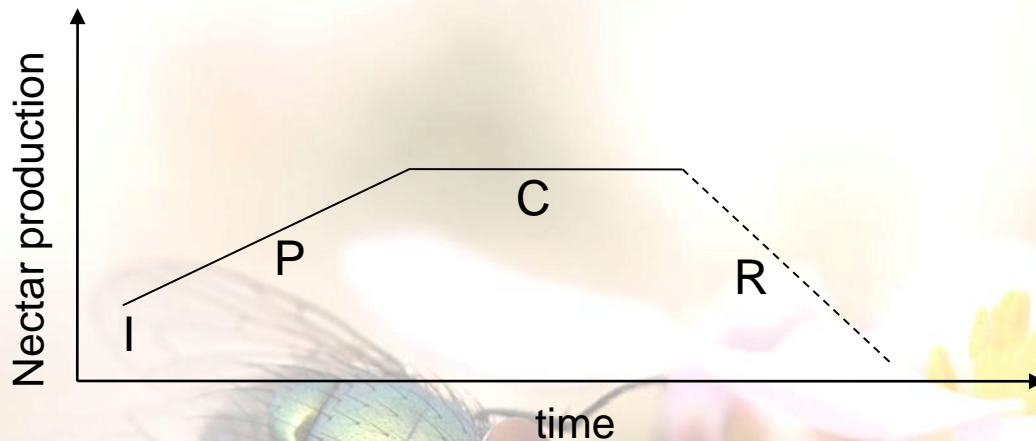


Figure from McCallum (2013), data from Voigt & Winter (1999)

## MONITORING NECTAR PRODUCTION DYNAMICS

when and how nectar is produced?



I : initiation

P : production

C : cessation

R : **reabsorption**

### **Initiation of nectar production**

In most cases flowers begin to secrete nectar before pollinators start their foraging activity and in some cases before the flowers open and in some rare case most is produced during the flower bud stage (e.g. *Mendevillea pentlandiana* (Apocynaceae) – Torres & Galetto, 1998)

### **Production rate (NPR)**

Cruden *et al.* (1983) recognised three classes of nectar producers based on the quantity of nectar secreted in a unit of time (generally an hour)

1. slow producers: 5 to 10% of their maximum accumulation;
2. fast producers: 22 to 68% of their maximum accumulation;
3. super fast producers: double to triple that of fast producers.

## FIELD MEASUREMENT TO ASSES NECTAR DYNAMICS

### NECTAR VOLUME

The range of variability is from about 50 nL, as a single florets of Asteraceae (Wist & Davis, 2006) to 9.4 mL in *Ochroma lagopus* (Bombacaceae, bat pollinated).

### NECTAR CONCENTRATION

It varies between less than 10% (*Aloe castanea*) to 60-70% as reported for *Carum carvi*. In the field it is generally determined with refractometers and it is expressed by % (w/w) of sucrose equivalent.

### How to convert from °BRIX (% w/w) to mg/μl ?

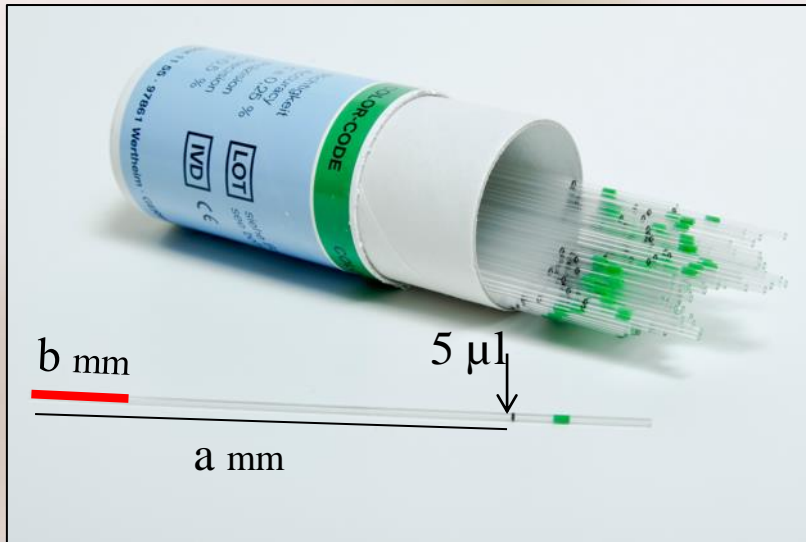
$$0,00226 + (0,00937 * X) + (0,0000585 + X^2) \text{ [mg/}\mu\text{l]}. \{X = \text{°Brix \% w/w}\}$$

there is a conversion table from Handbook of Chemistry and Physics (1978-1979)

### TOTAL SUGAR QUANTITY PER FLOWER

nectar volume [μl] \* concentration [mg/μl] = mg of sugar per flower (or better for sample)

## MEASURE NECTAR VOLUME



### What we need?

Calibrate microcapillaries

various volumes, most used from 0,5 µl to 20 µl

a = total length [mm]

b = measured length [mm]

c = total volume [µl] (example for 5µl)

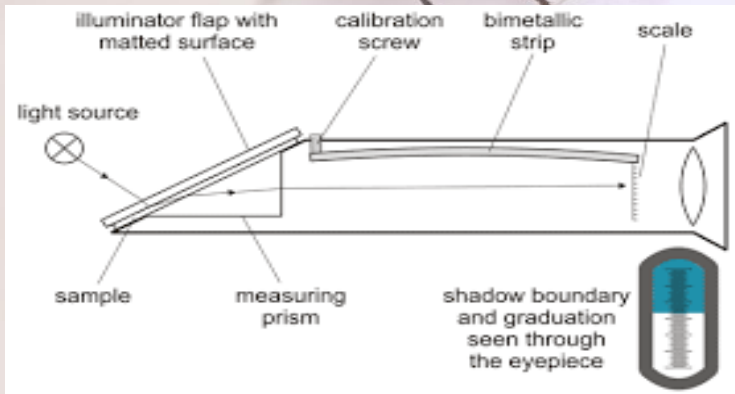
x = measured volume [µl]

$$x = \frac{b \cdot c}{a}$$

### Problems:

- too viscous nectar;
- very low amount of nectar;
- difficulties in the access to nectar.

## MEASURE NECTAR CONCENTRATION



### What we need?

Hand held refractometers

it measure the concentration of a solution according to its refractive index in Brix %  
**not a real concentration!**

20% Brix means that the solution have the same refractive index of a 20% sucrose solution (w/w)  
**sucrose equivalent!**

### Problems:

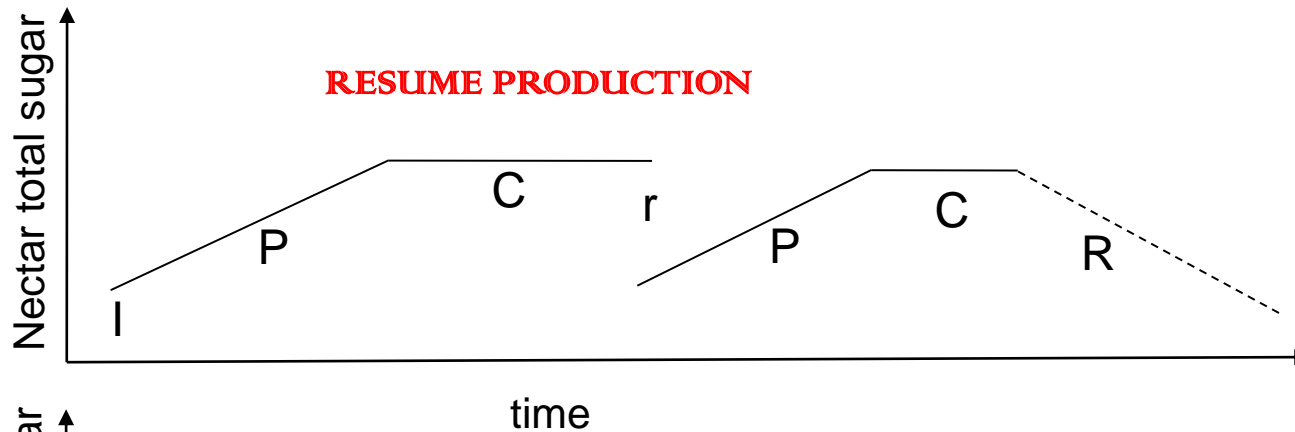
- very low volume of nectar;
- possibility to dry out very quickly;
- concentration out of the scale.

## POST SECRETORY CHANGE OF NECTAR

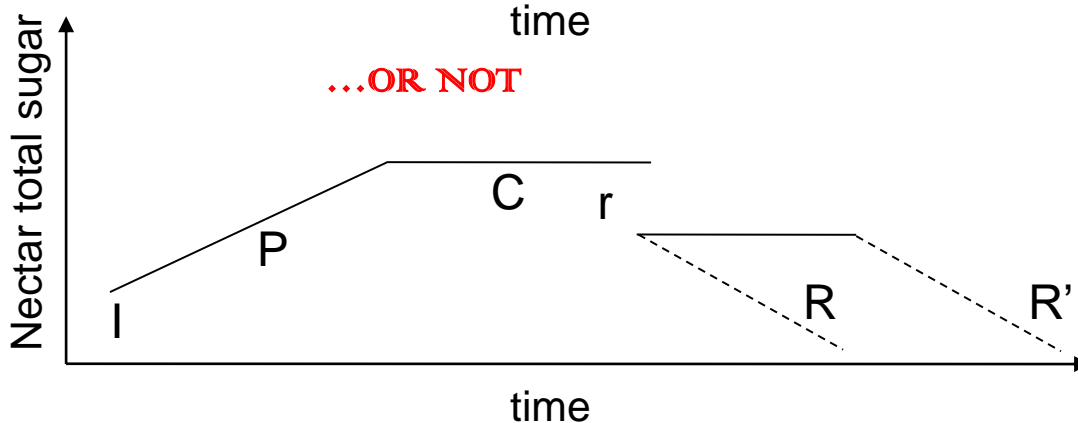
### DUE TO INTERACTION WITH POLLINATORS

Decrease nectar volume due to nectar foraging, no effect on concentration (?!)

Consequently decreased total sugar per flower



I : initiation  
P : production  
C : cessation  
R : reabsorption  
r : removal



## NECTAR PRODUCTION in protected flowers (protected crop)

This methodology measure the **potential total production** of nectar by the plant **excluding the visits by pollinators** (generally bagging the flowers, inflorescences, whole plants)





Two types of experiments can be performed with protected crop:

- **Study nectar secretion pattern through the flower lifetime:**  
Several sets of bagged flowers on different plants.

Each set is **sampled only once** at a scheduled time (depending on flower lifespan)

- **Assess the effect of nectar removal on nectar production pattern:**  
Several sets of bagged flowers on different plants

Each set received **different number of repeated sampling** at scheduled time during the flower lifespan (control, set 1 removed once, set 2 removed twice, set 3 removed three times and so on...)

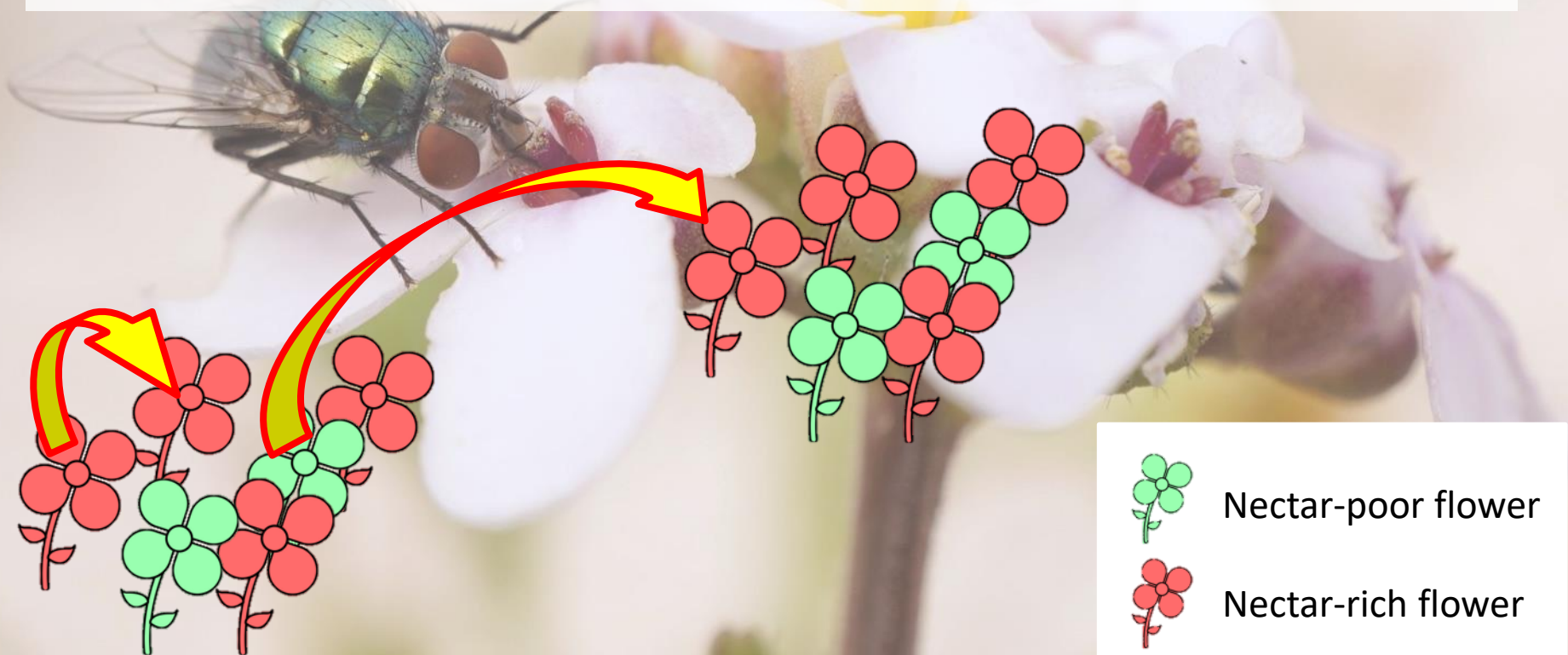


## NECTAR STANDING CROP

Defined by Kearns & Inouye (1993)

*«quantity and distribution of nectar determined by randomly sampling flowers, that have not been protected from pollinators by bagging, at a given moment»*

**The spatial distribution of standing crop** within a plant or within a population may show some spatial patterning that affects pollinators' movements between flowers of an individual plant or between individuals of a population



## STUDYING NECTAR CHEMISTRY

Where and how do we start?

### SAMPLING AND STORING NECTAR:

two type of information:

**in the field** – data on production per flower or per sample (pool)

**in the Lab** – quality and quantification of chemical compounds

What do we need?

### MATERIALS FOR TYPE OF SAMPLING:

**microcapillary tubes:** microcapillary tubes; caliber; vial; distilled water or ethanol/methanol.

**filter paper wicks:** filter paper; scissors; forceps; vial; distilled water.

**washing or rinsing with water:** micropipette; distilled water; vial.

**micro-rinse:** microcapillary tubes, distilled water, vial.

## MICROCAPILLARY TUBES METHOD



**pros:** most used, volume, narrow (low contamination), simulate the foraging method of the insect

**cons:** sugar concentration lower than other methods (Amato & Petit, 2017), sometimes difficult to use

## FILTER PAPER WICKS METHOD



**pros:** easy to use, good results for concentration and composition, don't need to know exactly where the nectary is

**cons:** easy to contaminate your sample, no volume indication

## WASH AND/OR RINSE METHODS

### CUT FLOWER

**pros:** very high sugar and AA concentration, easy to use

**cons:** it is always contaminated with floem!!!

### INTACT FLOWER

**pros:** very high sugar and AA concentration, easy to use

**cons:** it is always contaminated with concretions of sugar or other solutes!!!

### MICRO-RINSE METHOD

**ADDING A KNOWN VOLUME OF  
DISTILLED/DEIONIZED WATER  
BEFORE USE MICROCAPILLARY TUBES**

**pros:** very accurate!!

**cons:** time and energy

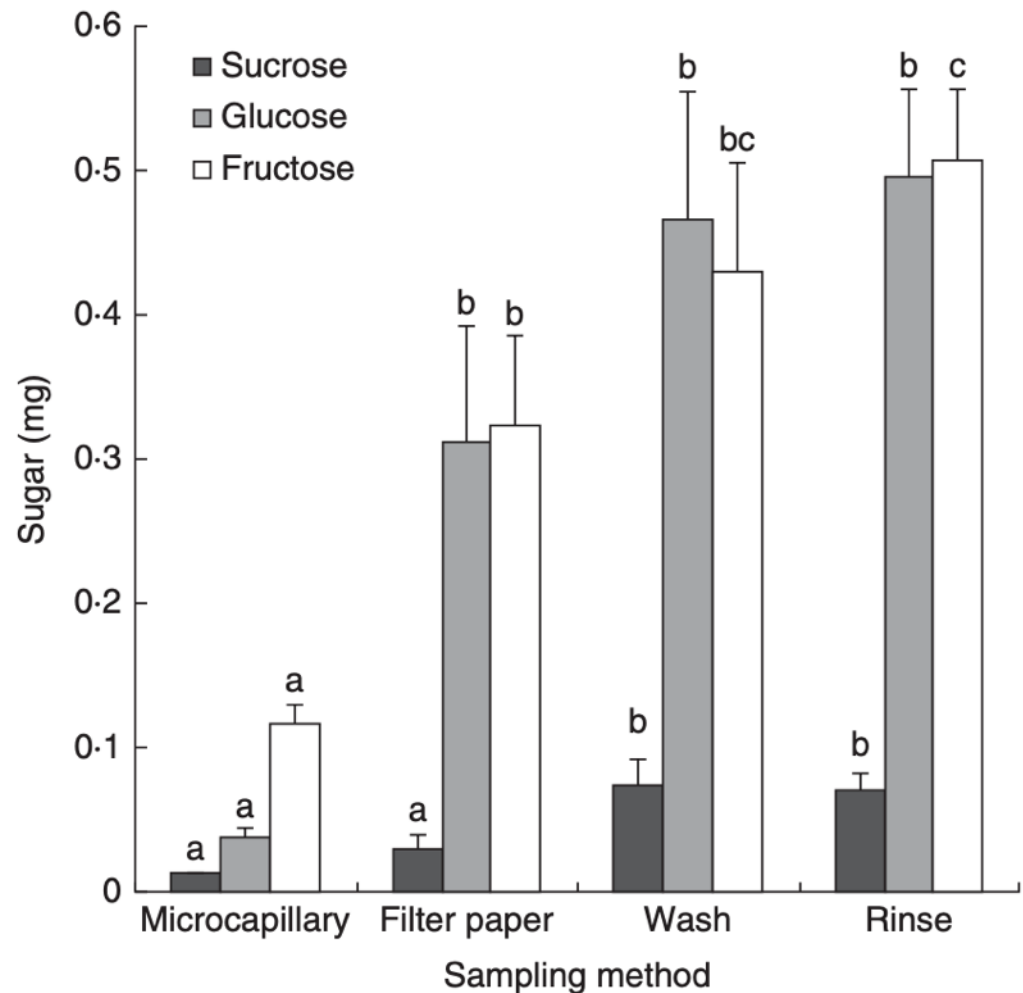


# STUDYING NECTAR CHEMISTRY

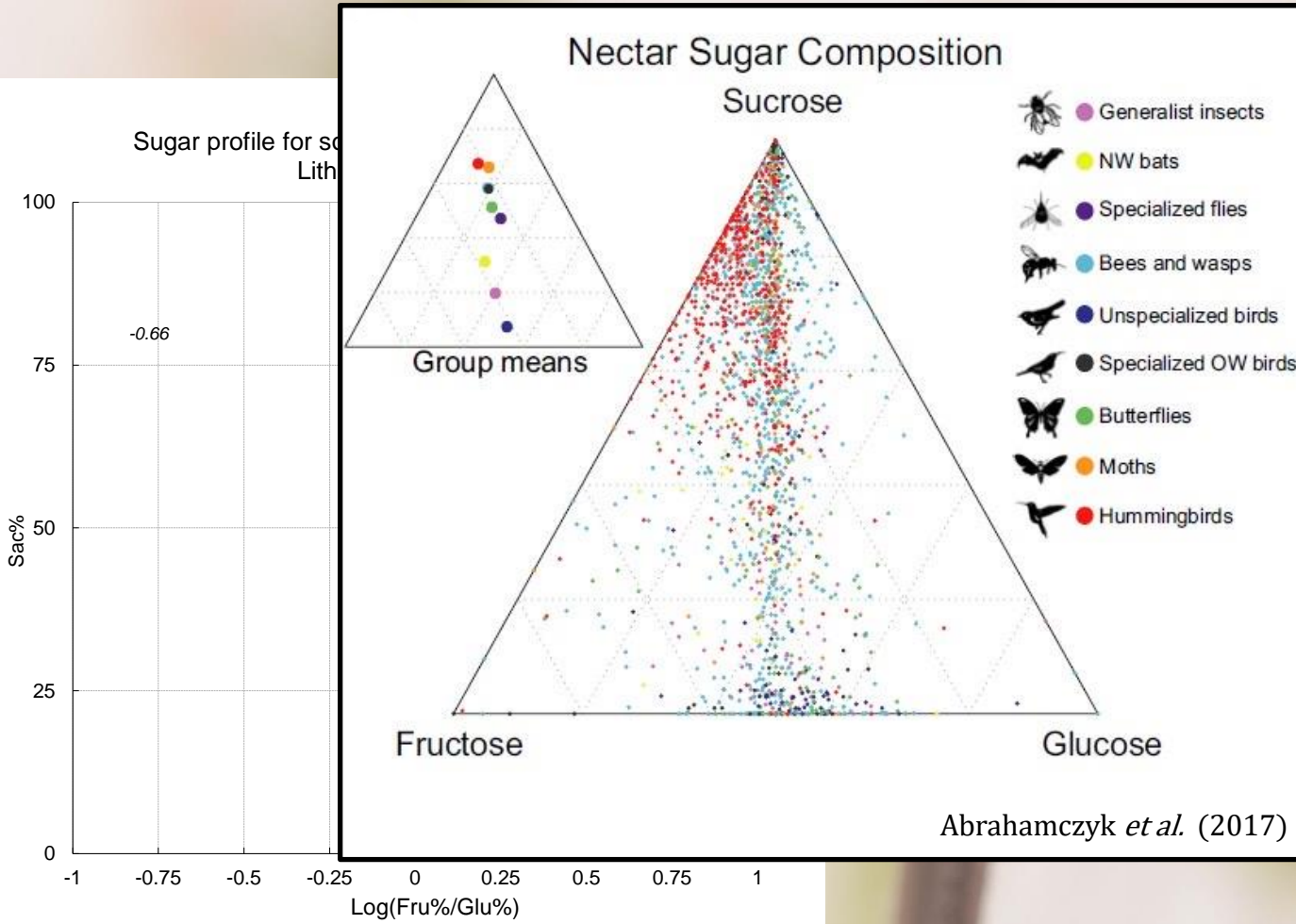
## Which sampling method to choose?

Morrant *et al.* (2009) test the quality of sampling and storing methods for sugar quantification. but there are **no comparative studies** on Low Mol. Weight Comps.

**The most used is microcapillary tubes!**



# STUDYING NECTAR CHEMISTRY – what is important of sugar profile?



Key species:

*...* sp. 75-80%

*...* sp. and

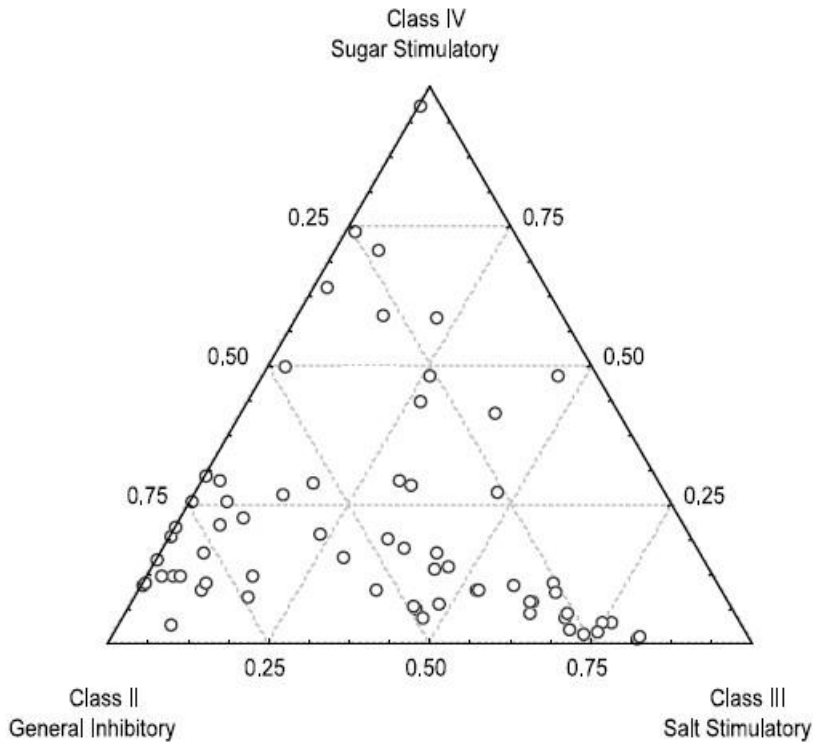
*...* spp. 3-50%

46,7%

3%

Nocentini, D. (2014)

# STUDYING NECTAR CHEMISTRY



Gardener & Gillman (2002)

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## Amino acid taste classes

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*Class I – no effect*

Asn, Gln, Ala, Cys, Gly, Ser, Thr, Tyr

*Class II – general inhibitory*

Arg, Asp, Glu, His, Lys

*Class III – salt cell stimulatory*

Hyp, Pro

*Class IV – sugar cell stimulatory*

Ile, Leu, Met, Phe, Trp, Val

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+

Non-Proteogenic Amino acid class

*Class V - no effect*

GABA,  $\beta$ -Alanine, Ornithine, Taurine



NECTAR CHEMISTRY



GUILDS OF POLLINATORS

Chemical Profile:

- Sugar
- Amino acid
  - Proteinogenic
  - NPAAAs
- LMWC

Data on Pollinators:

- Field observations
- bibliographic data

## How much nectar do I need to carry on my analysis?

### VOLUMES REQUIRED FOR ANALYSIS WITH DIFFERENT METHODS

$$V_{nect} = \frac{\sum V_{in}}{100}$$

denominator = dilution 1:10 → 1:100

	LC-MS	UV-HPLC	HPLC-FD	HPLC-DAD	HPLC-RI
SUGAR	∥	∥	∥	∥	20μl
PROTEIN	5μl	∥	∥	∥	∥
AMINOACID	5μl	∥	10μl (after derivatization)	∥	∥
LIPID	5μl	∥	∥	50μl	∥
LOW-MOL. WEIGHT COMPOUND	5μl	20μl	20μl (just BioAmines or Ring Compounds)	50μl	∥

## NECTAR ENERGETICS

### **MATERIALS:**

- microcapillary tubes;
- hand-held refractometer;
- distilled water.

**in the field** – obtain the concentration and volume data per flower

**in the Lab** – obtain the mg of sugar present per  $\mu\text{l}$  of nectar of nectar corresponding to a particular concentration using the previous formula or the conversion table and multiplying this value by the volume obtained

- every mg of sugar (sucrose equivalent) represents 4 cal:

$$\text{sugar mass [mg]} * 4 \text{ cal/mg} = \text{nectar energetics in cal}$$

**to be considered:** digestion rate and effective quantity of nectar digested; ability to digest sucrose; other nectar compounds that could bring energy to the consumer.