# MONITORING NECTAR PRODUCTION DYNAMICS


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Aqueous solution dominated by three main simple sugars

(Sucrose, Glucose and Fructose); sugar content up to 80%.

Secondary components as amino acids, organic acids, proteins, lipids, vitamins,

minerals and other compounds are presents in minor concentrations.

Nicolson & Thornburg (2007) Nectar chemistry. In “Nectaries and nectar”. *Springer*. pp 215-263.
Herrera et al. (2013) Yeasts in nectar of an early-blooming herb: sough by bumble bees, detrimental to plant fecundity. *Ecology 94:273-279*
Nepi M. (2014) Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar. *Journal of Ecology* 102:108-115

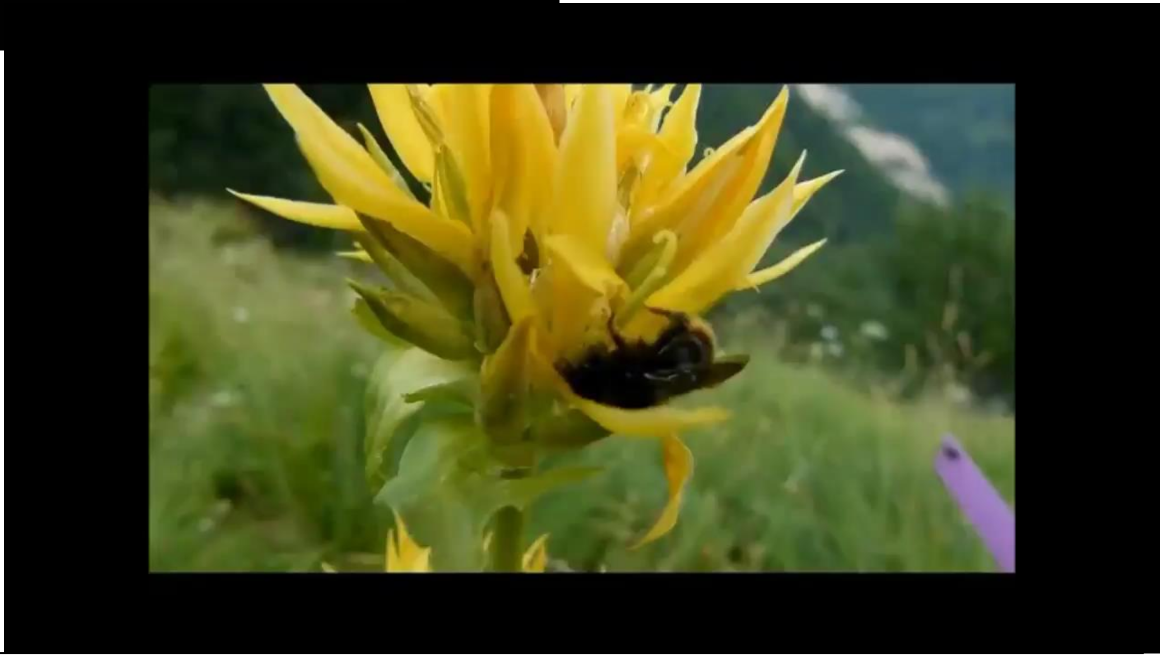

The **primary reward** directly consumed by floral visitors,

but can also be an **important determinant of plant fecundity**

Herrera et al. (2013) Yeasts in nectar of an early-blooming herb: sough by bumble bees, detrimental to plant fecundity. *Ecology 94:273-279*
Nepi M. (2014) Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar. *Journal of Ecology* 102:108-115
Schaeffer et al. (2017) Consequences of a nectar yeast for pollinator preference and performance. *Functional Ecology* 31: 613-621

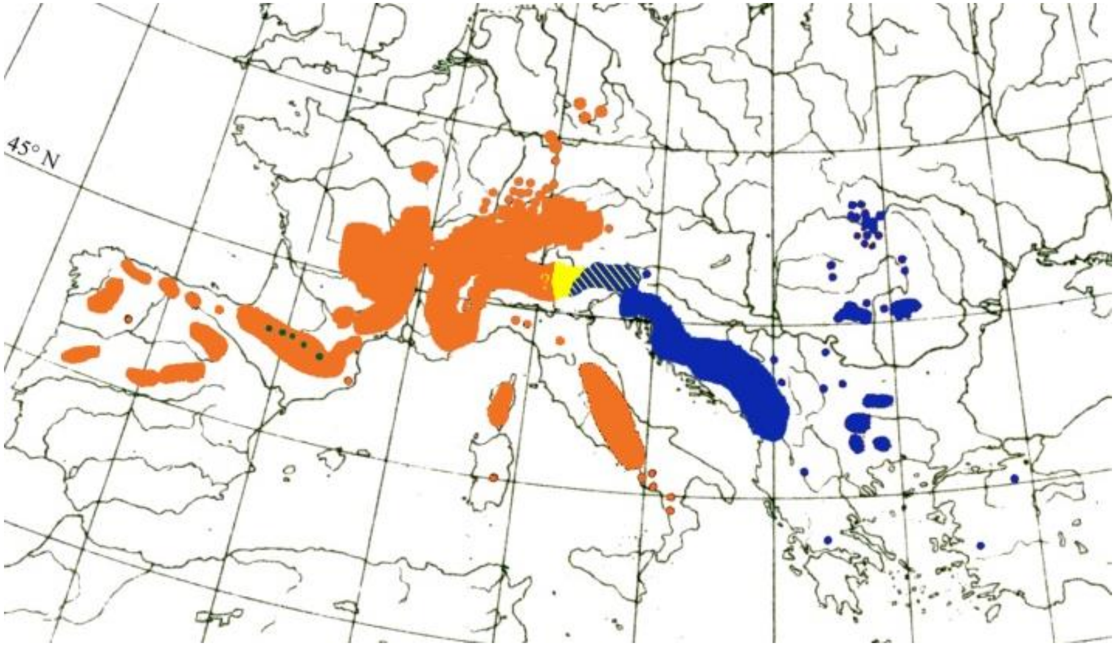
Generalist species

*Bombus* are the principal pollinators… but they are not equally efficient !



Rossi, Fisogni et al. (2014) Bouncy versus idles: On the different role of pollinators in the generalist *G. lutea* L. *Flora 209:164-171.*

# *Gentiana lutea* L.




Partially autogamous (geitonogamy)

Selfing → lower seed set, reduced fitness

Depends on insects for pollination


Rossi et al. (2016) Biosystematic studies on the mountain plant *Gentiana lutea* L. reveal variability in reproductive traits among subspecies. *Plant Ecology & Diversity, 9*, 97-104.

Rossi et al. (2016) The effect of pollination mode on seed performance of *Gentiana lutea*: a laboratory evaluation of seed germinability. *Nordic Journal of Botany* 34: 761-768.

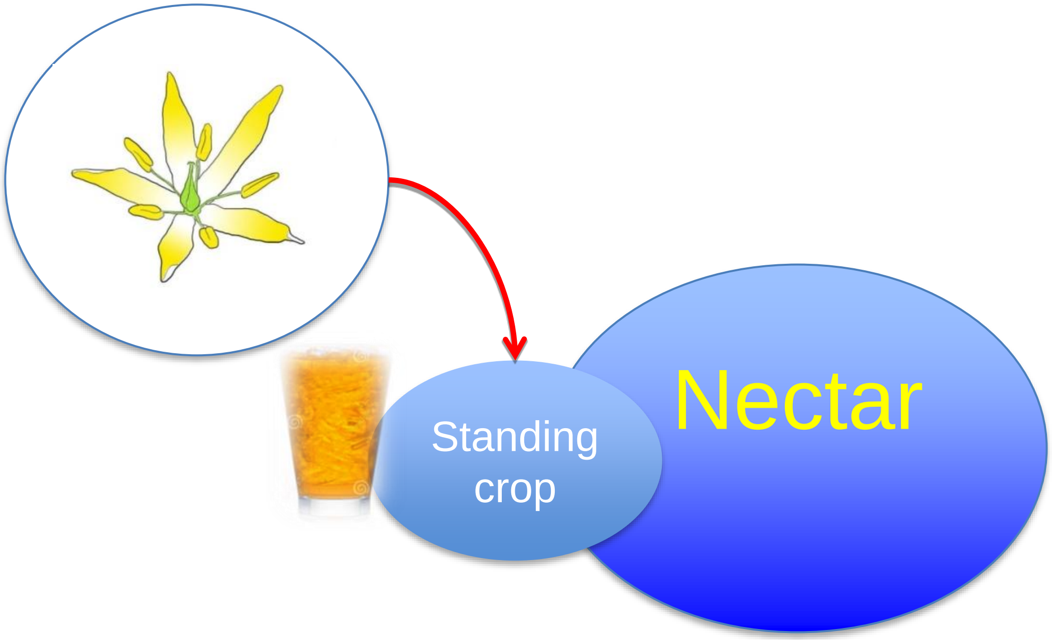
# Nectar is not the pure secreted solution

Nectar

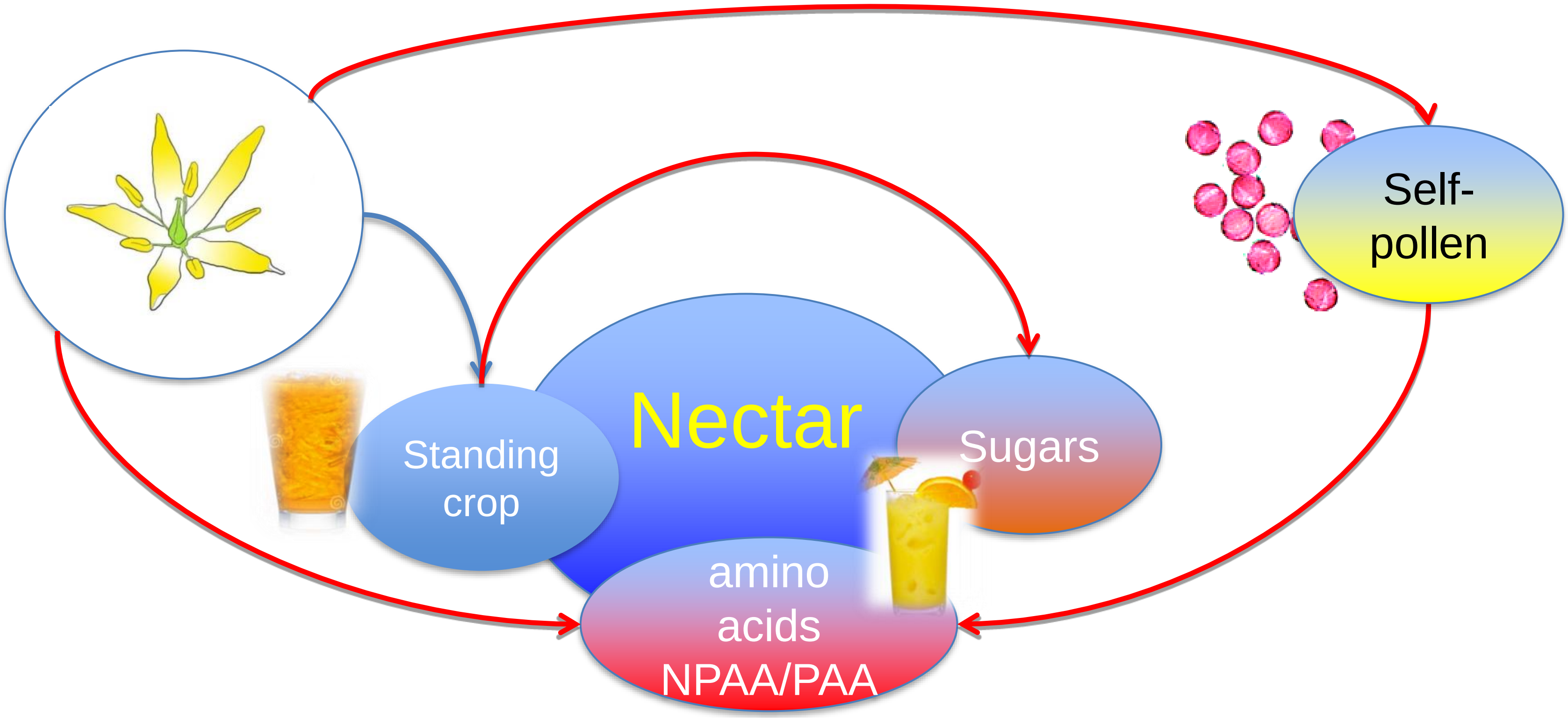
# Nectar is not the pure secreted solution

Nectar

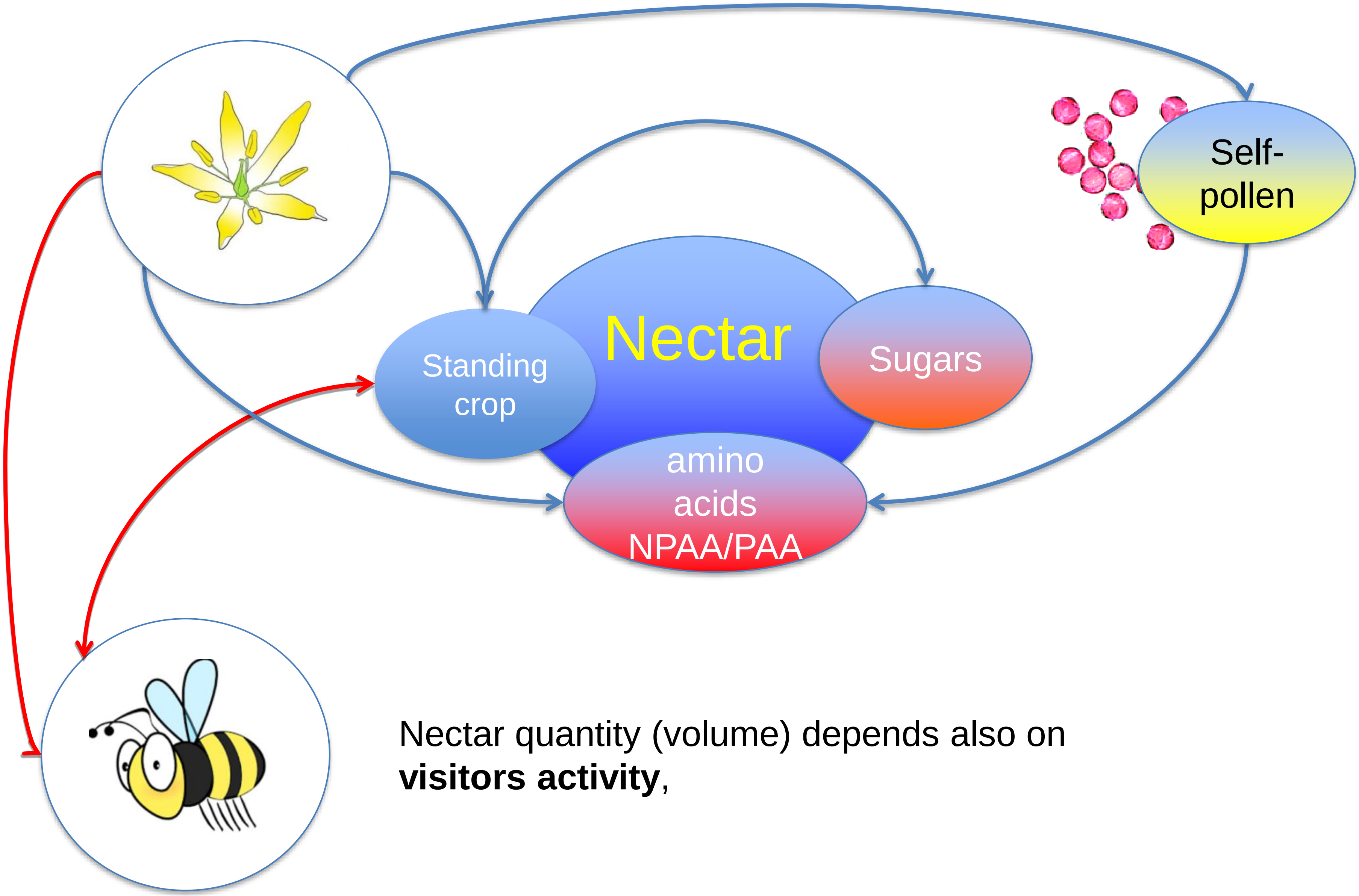
Floral nectar gets altered by numerous factors



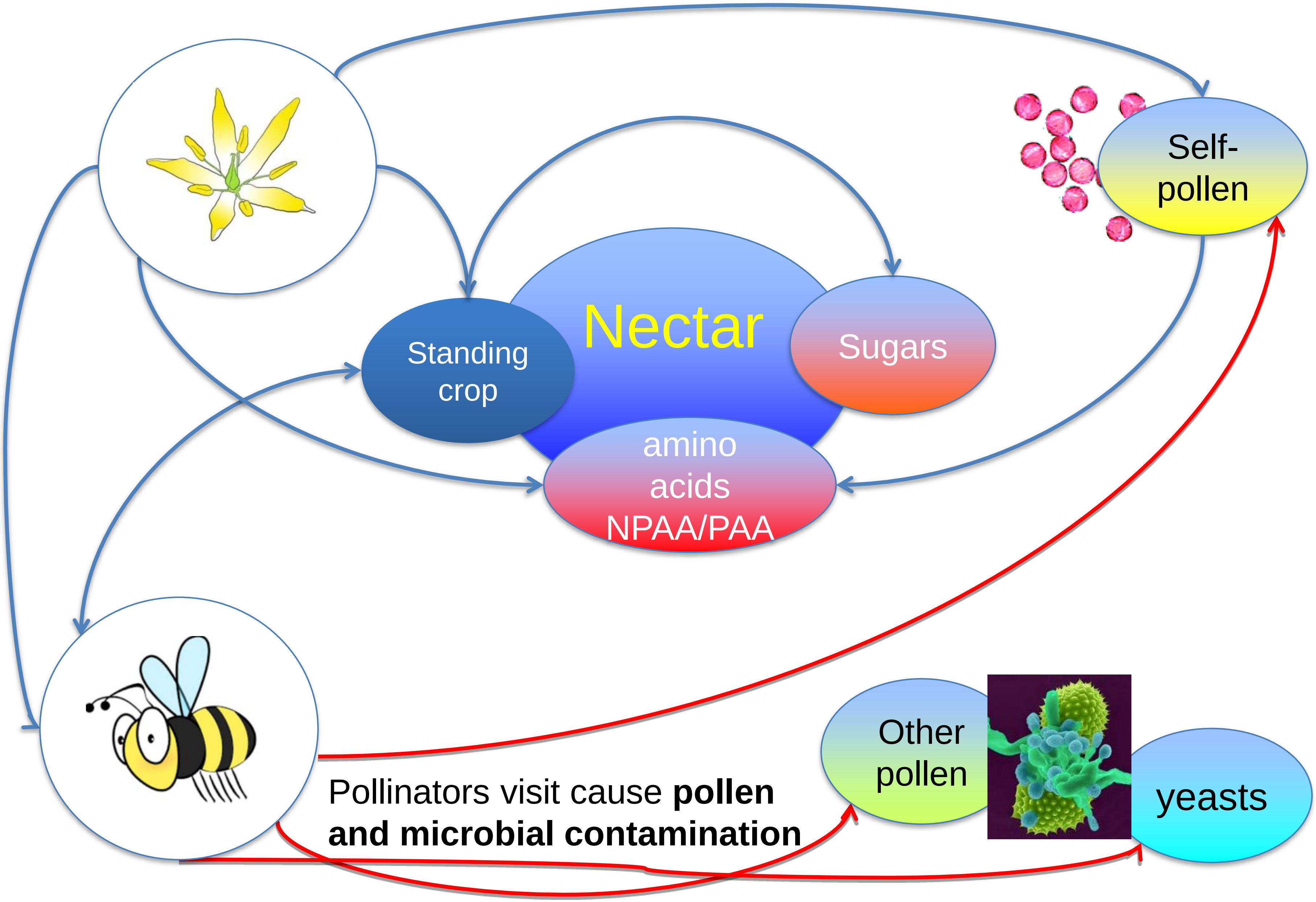

Flower phenology: Nectar evolves with **flower age both** in volume (quantity)



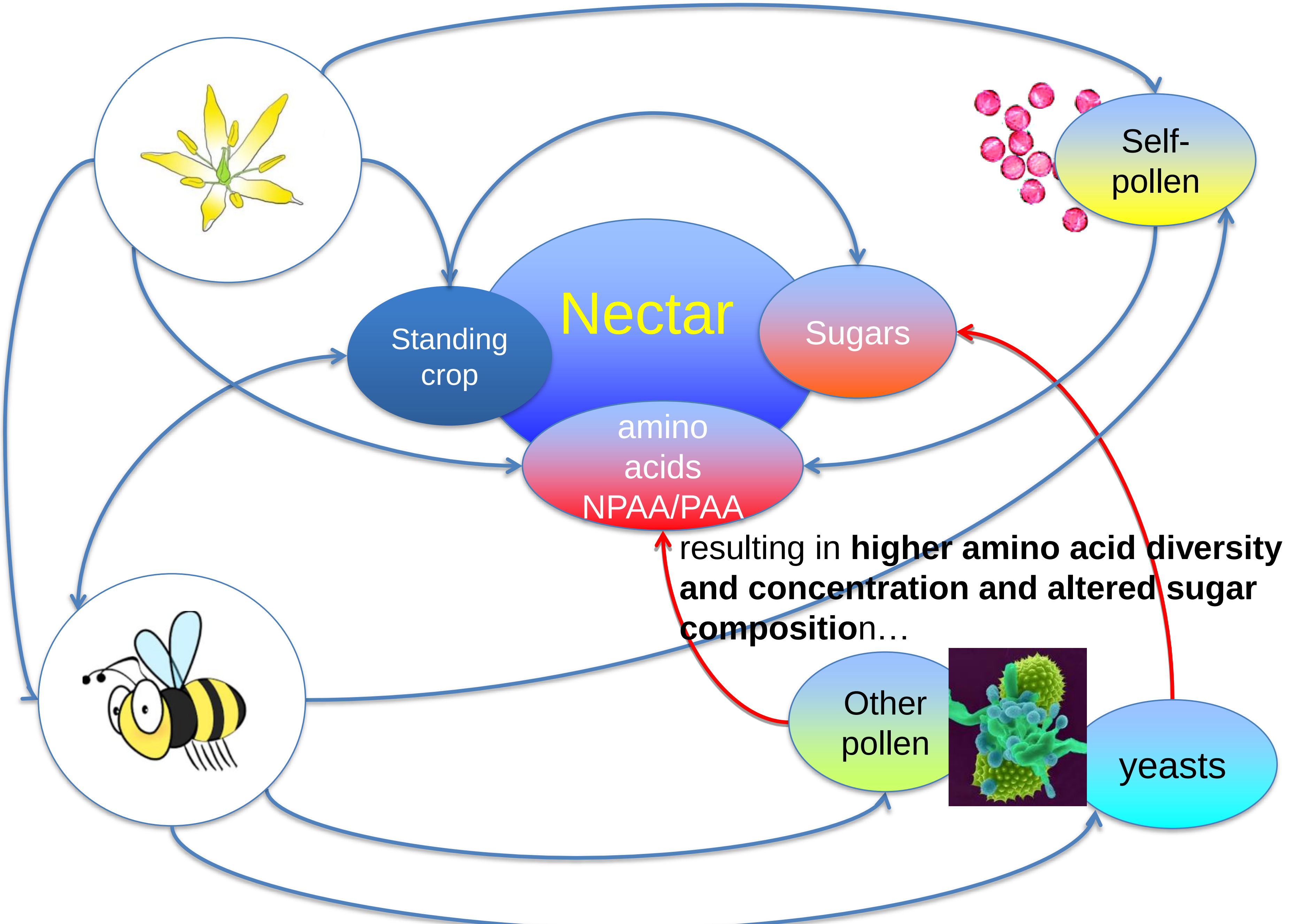

and in quality, (also because of self-pollen contamination)

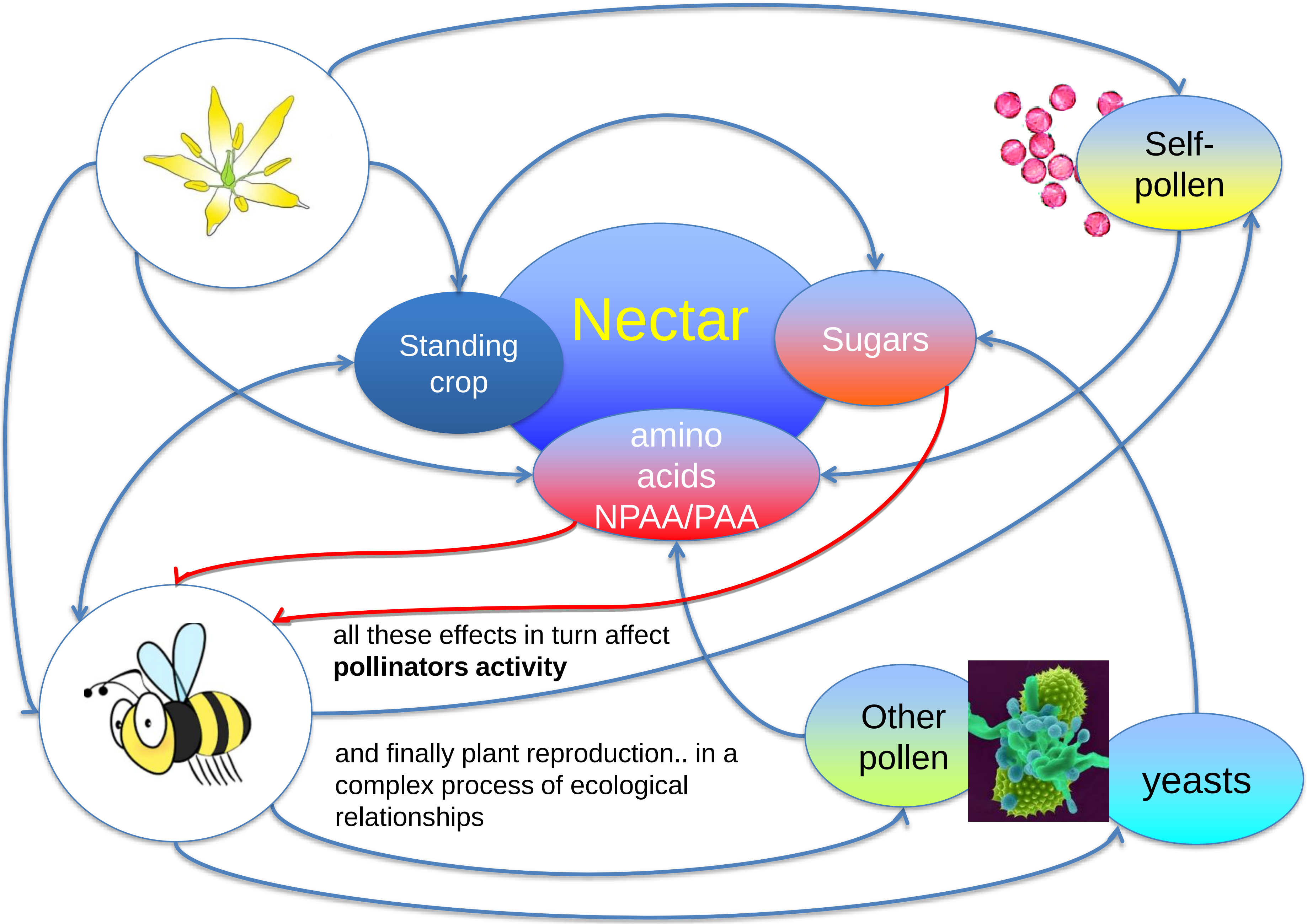



Nectar quantity (volume) depends also on **visitors activity**,

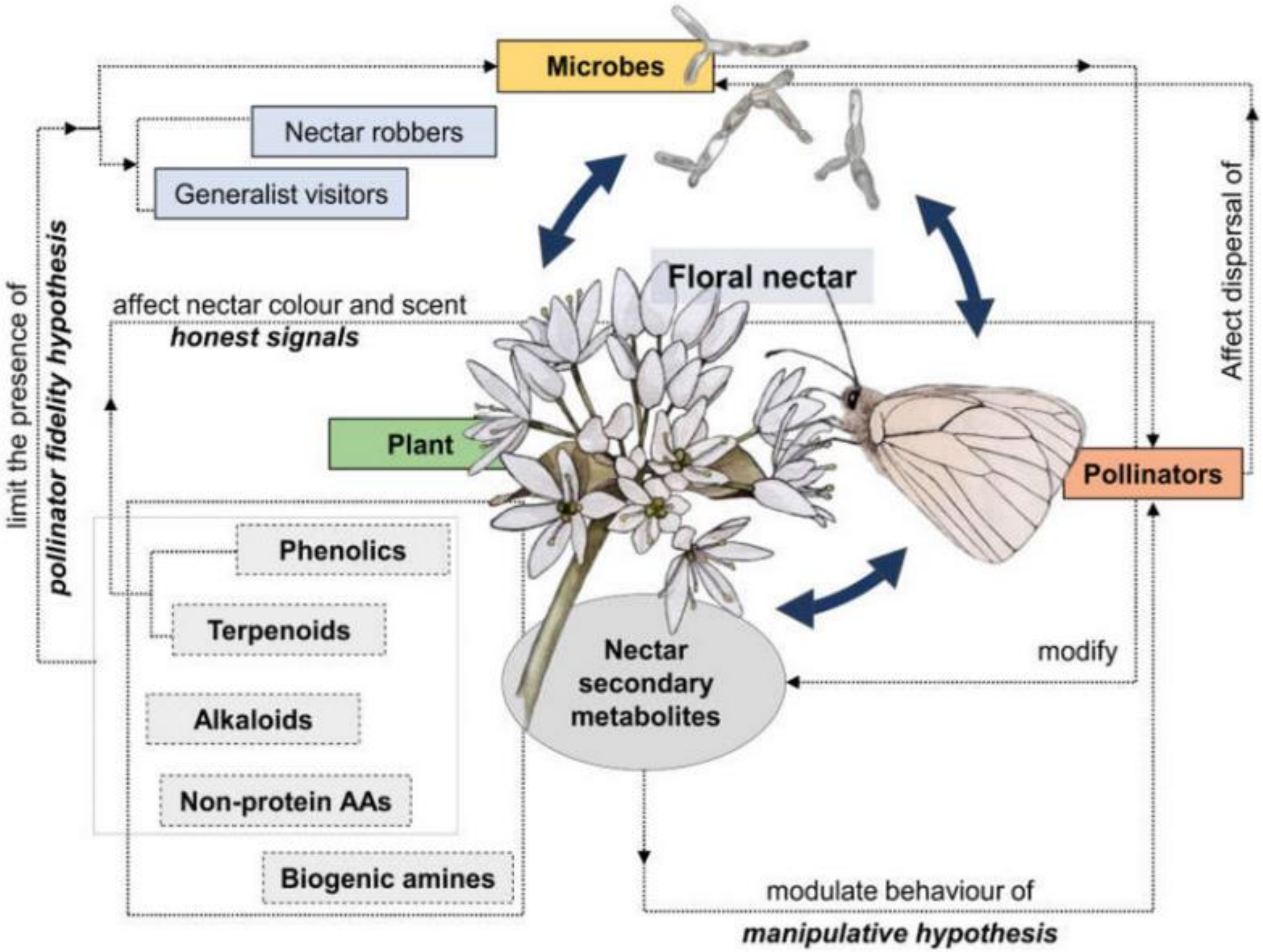


Nectar
Standing crop
Sugars
amino acids NPAA/PAA
Self-pollen
Other pollen
yeasts
Pollinators visit cause **pollen and microbial contamination**


Nectar
Standing crop
Sugars
amino acids NPAA/PAA
Self-pollen
Other pollen
yeasts
resulting in **higher amino acid diversity and concentration and altered sugar composition**…


Self-
pollen
Nectar
Standing
crop
Sugars
amino
acids
NPAA/PAA
all these effects in turn affect
**pollinators activity**
and finally plant reproduction.. in a
complex process of ecological
relationships
Other
pollen
yeasts

# One Thousand and One functions played by floral nectar in shaping plant-animal interactions




Barberis et al. 2023. **Secondary Metabolites in Nectar-Mediated Plant-Pollinator Relationships** *Plants* **2023**, *12*(3), 550
https://www.mdpi.com/2223-7747/12/3/550

## DYNAMICS OF NECTAR PRODUCTION

### GENERAL CONSIDERATIONS

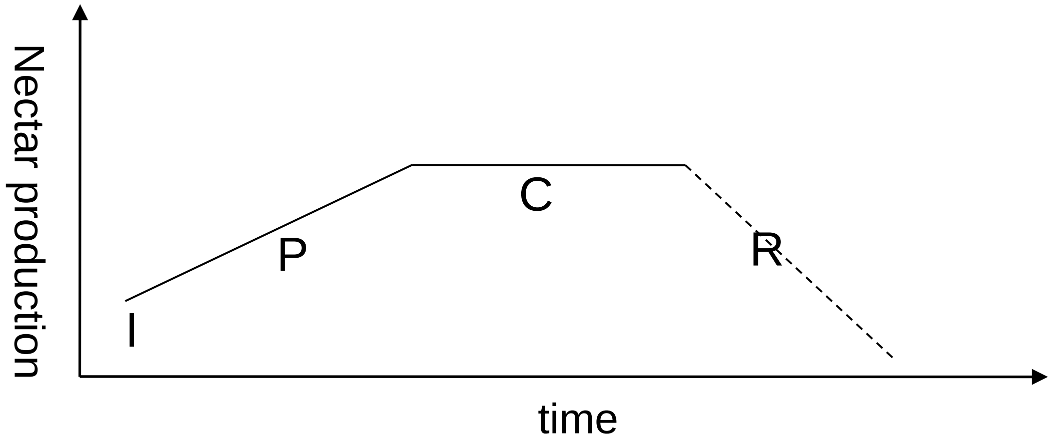
It is generally accepted that the dynamics of nectar production fits the requirements of plant pollinators. For example flowers pollinated by diurnally active animals produce nectar and expose it during the day. Flowers pollinated by nocturnally active animals expose nectar at night

### The quantity and quality of nectar

As far as the quantity of nectar produced per flower is concerned, numerous works demonstrate that flowers pollinated by high-energy requiring animals, such as bats, hawkmoths and birds, produce significantly more nectar than flowers pollinated by low-energy requiring animals, such as butterflies, bees, and flies

MONITORING NECTAR PRODUCTION DYNAMICS

When and how nectar is produced?




I initiation

P production

C cessation

R **resorption**

**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 (e.g. Pleasants, 1983; Witt et al., 1999; Nepi et al., 2001). In *Mandevillea pentlandiana* (Apocynaceae) most nectar is produced during the flower bud stage (Torres & Galetto, 1998).

**Production rate** (NPR)

Considering the nectar secretion rate, i.e. the quantity of nectar secreted in a unit of time (generally an hour), Cruden et al. (1983) recognised three classes of nectar producers:

slow producers secrete 5 to 10% of their maximum accumulation per hour;

fast producers secrete 22 to 68% of their maximum per hour;

super producers secrete double or triple that of fast producers.

# What is possible to measure in the field in order to asses nectar dynamics?

## NECTAR VOLUME

the range of variability is from about 50 nL, as in single florets of Asteraceae (Wist and 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*.
Is generally determined with refractometers and it is expressed by % (w/w) of sucrose equivalent

After converting the concentration from % w/w to mg/µl (conversion tables)
$0{,}00226+(0{,}00937*X^2)+(0{,}0000585*X^2)$ X=% w/w
it is possible to calculate the **TOTAL SUGAR QUANTITY PER FLOWER**:

nectar volume (µl) * concentration (mg/µl) = mg of sugar per flower

and also energetic calculations taking into account that:

1mg sugar (sucrose) = 4 cal = 16.8 Joule

# MEASURING NECTAR VOLUME

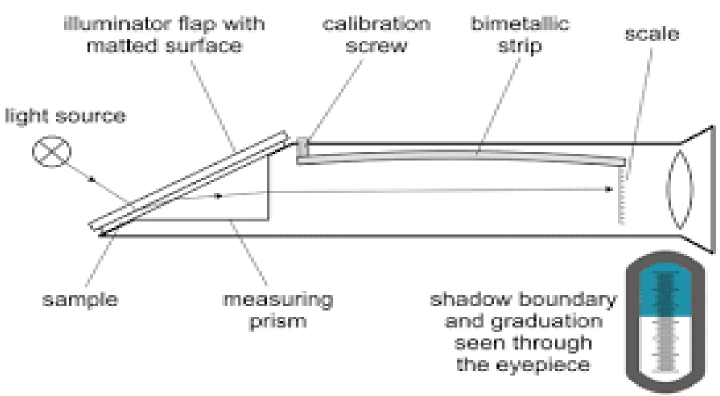

Calibrated microcapillaries
20, 5, 2, 0.5 µl

$a : 5 = b : x \qquad x = 5/a \times b$

$x$ = nectar volume in µl

**Problems**: too viscous nectar, very low amount of nectar, difficulties in the access to nectar

# MEASURING NECTAR CONCENTRATION


Hand held refractometers

They measure the concentration of a solution according to its refractive index in Brix % (not a real concentration!).

6% Brix means that the solution have the same refractive index of a 6% (w/w) sucrose solution (sucrose equivalents)

**Problems**: very low volume of nectar, concentration out of the scale

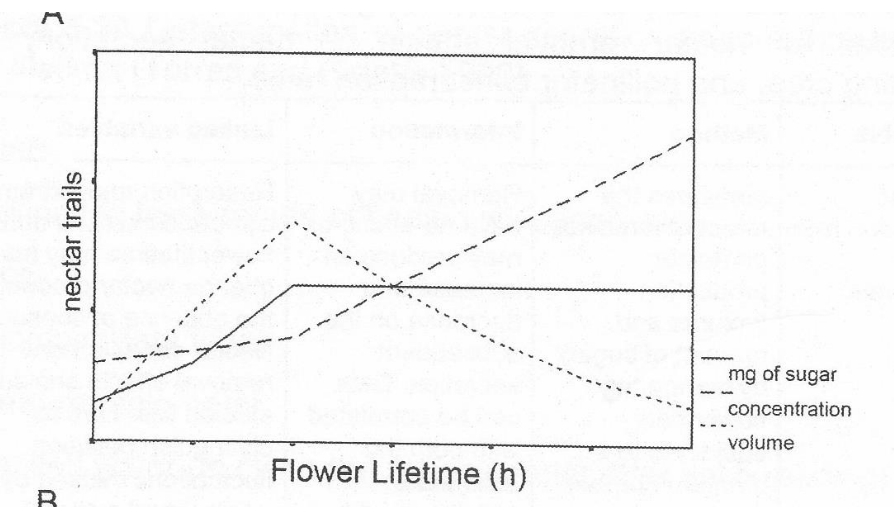
# POST SECRETORY CHANGE OF NECTAR

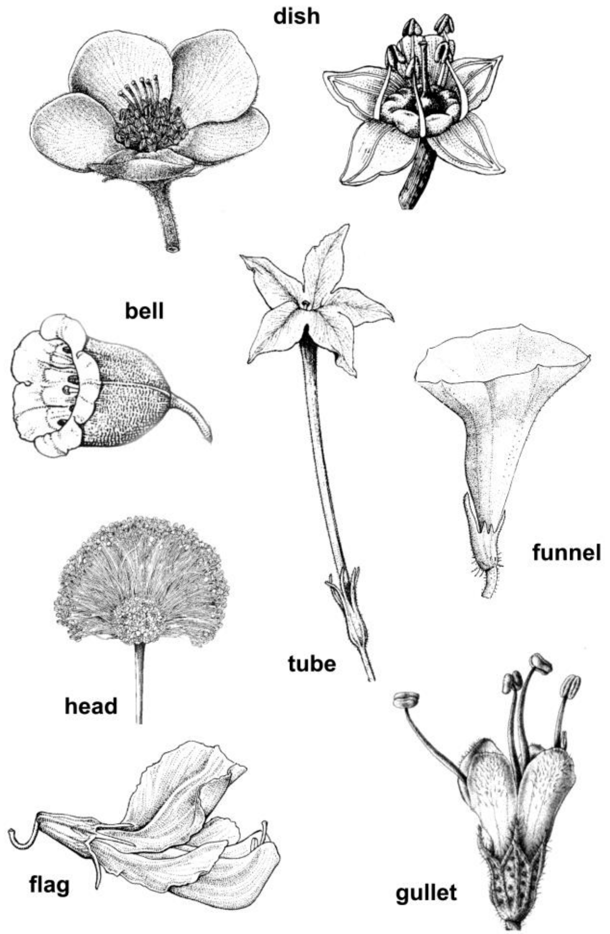
Due to environmental parameters:

Nectar volume and concentration are highly affected by RH of the air around the flower

High T and low RH values decrease nectar volume and increase concentration because **water loss by evaporation**

Since this inverse effect  on volume and concetration, water loss by evaporation does not affect total sugar per flower that is relatively indipendent from environ pars



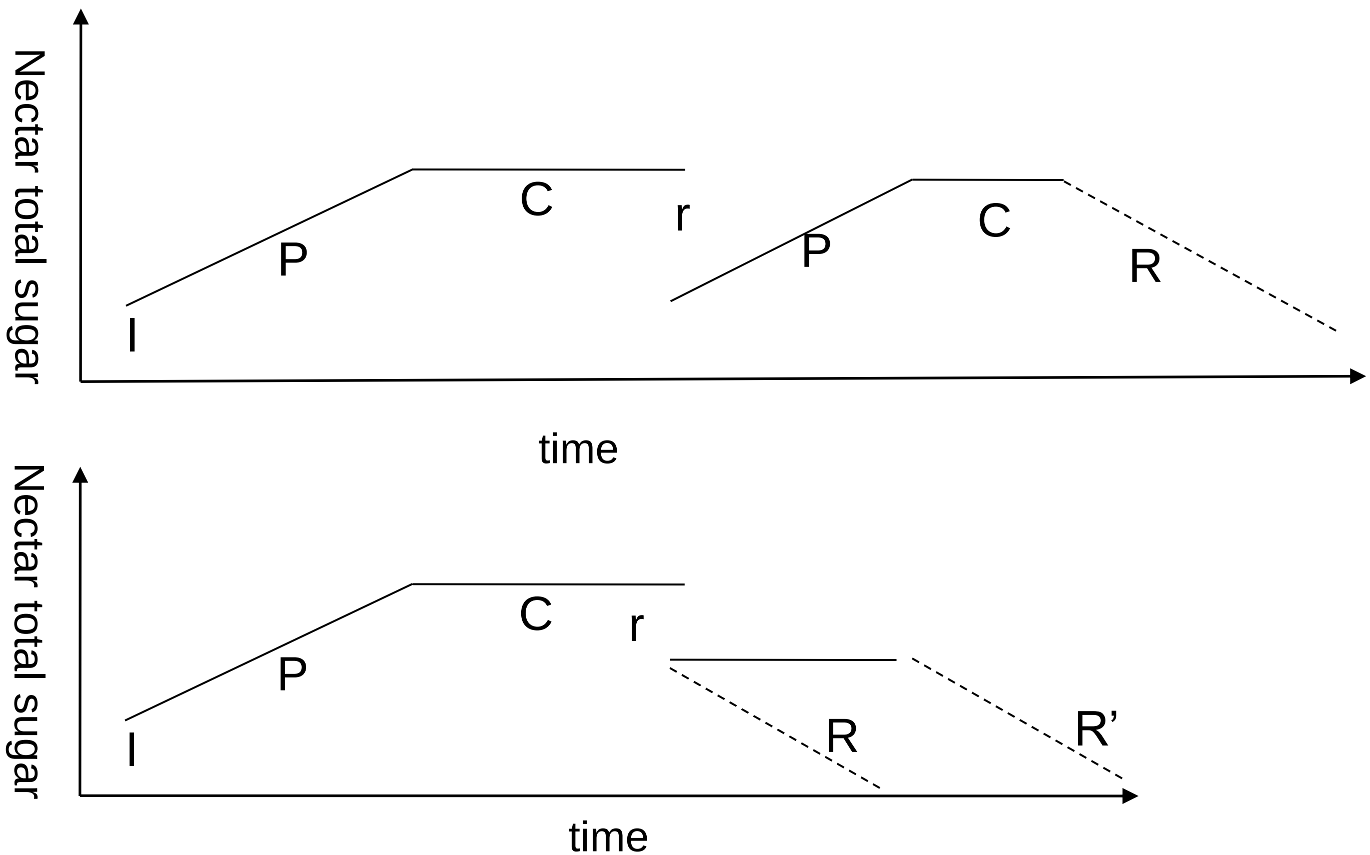




Nectar protection and flower morphology

# POST SECRETORY CHANGE OF NECTAR

Due to interaction with pollinators:

Decreased nectar volume due to nectar foraging, no effect on concentration (….?). Consequently decreased total sugar per flower




I initiation

P production

C cessation

R resorption

r removal

nectar removal by pollinators after cessation may or may not resume production

**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:

1) Study nectar secretion pattern through the flower lifetime:

Several sets of tagged flowers on **different plants**.
**Each set is sampled only once** at a scheduled time (time depends on flower lifespan)

2) Asses the effect of nectar removal on nectar production pattern:

Several sets of tagged flowers **on different plants**
Each set received a different number of repeated sampling at scheduled time during the flower lifespan (set 1 removed once, set 2 removed twice, set 3 removed 3 times... controls)

## NECTAR STANDING CROP

is defined by Kearns and Inouye (1993) as the "*quantity and distribution of nectar determined by randomly sampling flowers, that have not been protected from pollinators by bagging, at a given moment*".

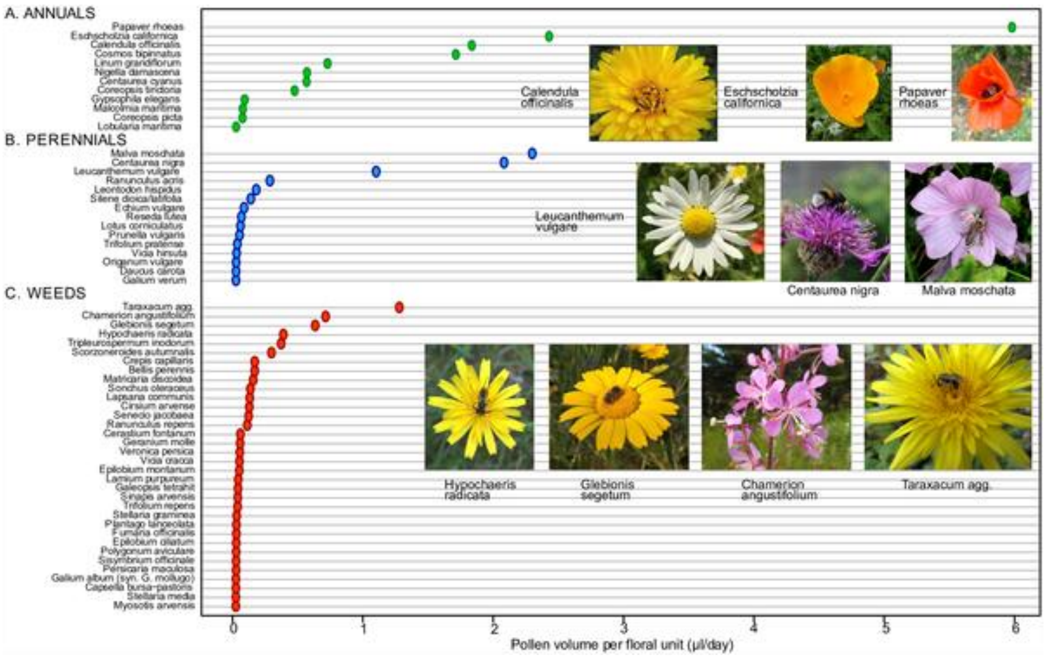
Corresponds to the nectar availability that the polinators encounter in their foraging activity

its correlation with nectar production in protected crop will be strong if pollinator visits are rare but weak when pollinator foraging is high

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

if nectar is patchily distributed within plants, foragers encountering nectar-rich flowers will move to neighbouring flowers, while foragers encountering nectar-poor flowers will move longer distances in order to avoid visits to neighbouring flowers.

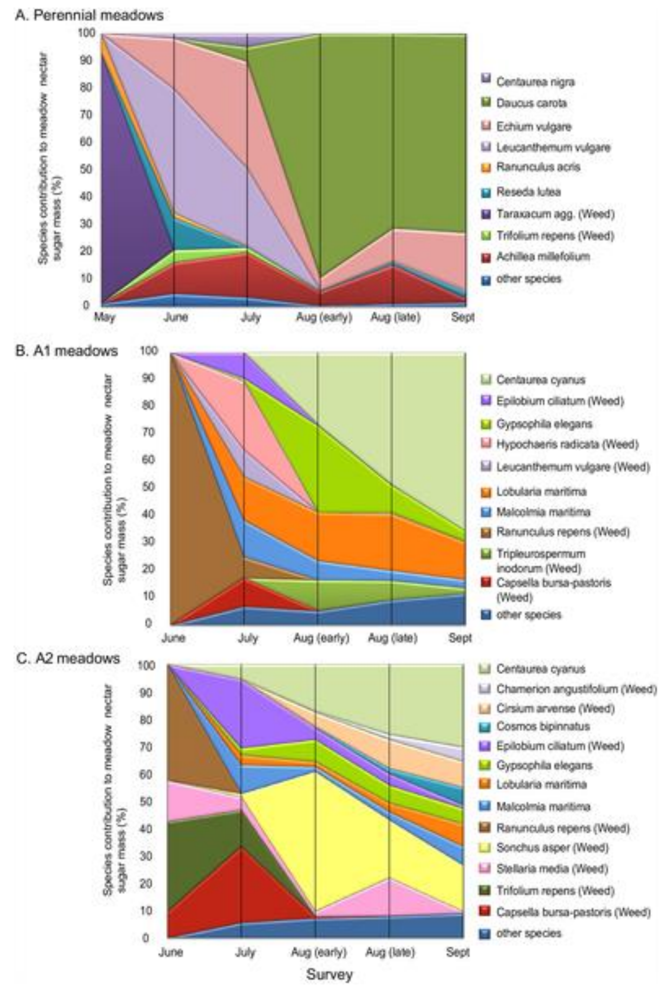
## Fig 2. Mean pollen volume per 24h per floral unit for species in A. the annual seed mix, B. the perennial seed mix, and C. native weeds in either mix.




| Meadow treatment | Mean nectar sugar mass (mg) | Median nectar sugar mass (mg) | $25^{th}$ percentile | $75^{th}$ percentile | Significance group |
|---|---|---|---|---|---|
| Perennial | 67.547 | 35.981 | 6.076 | 83.603 | a |
| Annual A1 | 10.821 | 3.072 | 0.013 | 17.015 | b |
| Annual A2 | 10.594 | 5.129 | 0.148 | 13.277 | b |
| Amenity grassland Control | 0.486 | 0.090 | 0.000 | 0.367 | c |

doi:10.1371/journal.pone.0158117.t001

## Fig 5. Seasonal patterns in the proportion of available daily nectar sugar contributed by individual plant species for Edinburgh meadows sown with A. perennial, B. annual A1, and C. annual A2 treatments.




Hicks DM, Ouvrard P, Baldock KCR, Baude M, Goddard MA, et al. (2016) Food for Pollinators: Quantifying the Nectar and Pollen Resources of Urban Flower Meadows. PLOS ONE 11(6): e0158117. https://doi.org/10.1371/journal.pone.0158117
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0158117

## PRACTICAL ACTIVITY: what are we going to do?

Monitoring nectar dynamics (volume and concentration)

- through flower lifespan in protected crop (bagged flowers)
- within the population in not protected flowers (standing crop)

**We can work** on different nectariferous plants...

*Chasmanthe* sp.

*Oxalis pes caprae*