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Pyrrolizidine alkaloids and other plant toxins in honey and pollen

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5th Apiquality & 6th Apimedica International Symposium,
22-25 November 2016; Rome, Italy

www.agroscope.ch | gutes Essen, gesunde Umwelt

Plant toxins - plant defences against herbivory

- many plants produce secondary metabolites as chemical defences
- act as repellents or toxins to herbivores
- directed against insects or vertebrates, such as birds or mammals





bitter honey or mad honey

- 401 B.C.: field commander Xenophon
- 67 B.C.: roman army against the pontic king Mithridates VI



Honey from *Rhododendron ponticum* or *flavum* (rododendro)

- honey can contain grayanotoxins
 - hallucinations, vomiting, cardiac complication
 - traditional medicine: management of diabetes mellitus
- Black Sea Region, Turkey



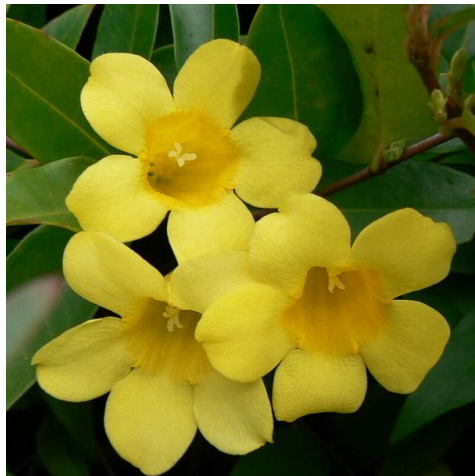
Rhododendron ponticum

- but not all *Rhododendron* species produce grayanotoxins



honey of *Gelsemium semper virens*

- State flower of South Carolina (Carolina jasmine)
- native to Guatemala, Mexico, southeastern and south-central United States (from Texas to Virginia)
- strychnine-related alkaloids
- Blooming: February to April



Gelsemium semper virens



Pyrrolizidine Alkaloids (PA) in honey?

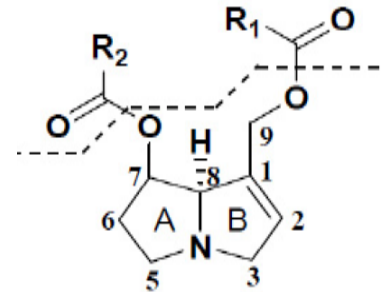
- 2007
 - BfR No. 028/2007 (Germany)
 - Salat containing *Senecio*
- 2011
 - BfR No. 038/2011
 - EFSA
 - health concern for toddlers and children who are high consumers of **honey**.
- 2013
 - BfR No. 018/2013
 - PAs in teas
- 2016
 - BfR No. 030/2016
 - EFSA
 - Tea, herbal infusions, **honey** as contributors to the total exposure to PAs

Ref: doi: 10.2903/j.efsa.2016.4572



Toxins from plants – Pyrrolizidine Alkaloids (PA)

- 3% of the flowering plants
- >350 structures
- toxicity:
 - acute: liver failure
 - chronic:
 - hepatotoxic
 - carcinogenic



Ref: Wiedenfeld *et al.*, 2011, *Food Additives and Contaminants* 28(3), 282.



Recommendation for food products

German Federal Institute for Risk Assessment (BfR) recommends:
 $\leq 0.007 \mu\text{g PA/day/kg}$ body weight



Ref.: Bundesinstitut für Risikobewertung: Stellungnahme Nr. 038/2011

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Important PA plants critical for the production of honey and pollen

- ***Boraginaceae***

- *Echium vulgare*

viper's bugloss, viperina azzurra



Echium vulgare

- ***Asteraceae***

- *Senecio spp.*

ragworts, erba di San Giacomo



Senecio jacobaea

- *Eupatorium spp.*

hemp-agrimony, canapa acquatica

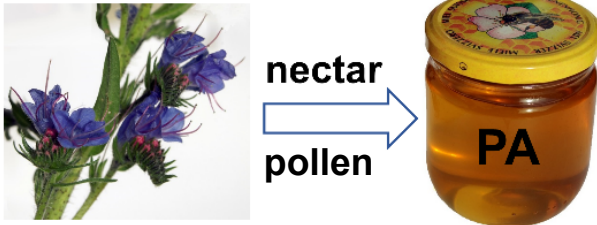


Eupatorium cannabinum

Photos: O. Zoller



Our study



- What is the concentration of PAs in Swiss honey and in pollen on the market?
- What is the source of PAs in honey?
- When do bees collect PAs?



Analysis

- Honey, bee collected pollen, QSI Bremen, Germany

LC-MS/MS: 18 PAs or PA-N-Oxides

group 1: *Echium-type*

group 2: *Eupatorium-type*

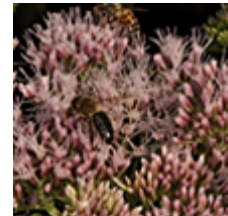
group 3: *Senecio-type*

- Nectar and plant pollen from *E. vulgare*

University of Neuchatel, Switzerland

UHPLC-HR-MS non-targeted analysis

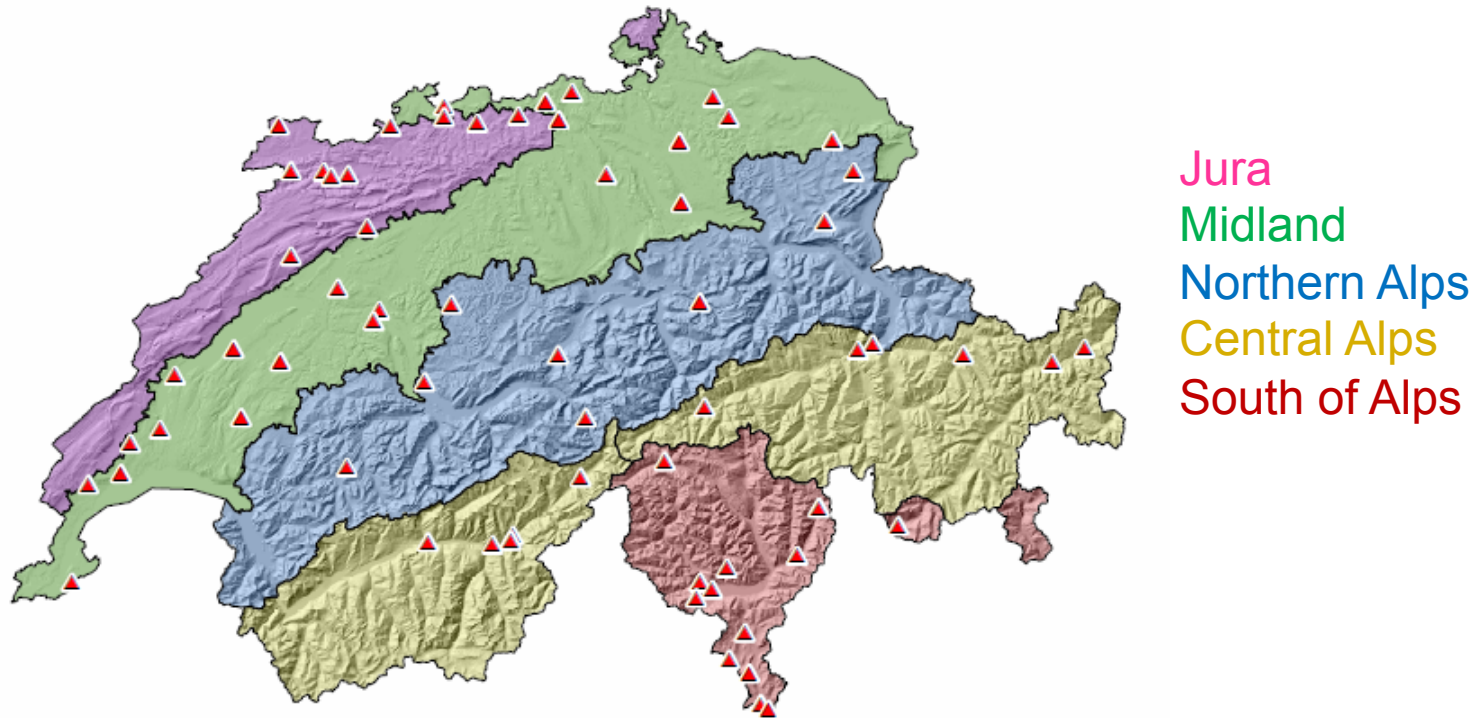
Identification of 10 *Echium-type* PAs or PA-N-oxides



Fotos: R. Ritter



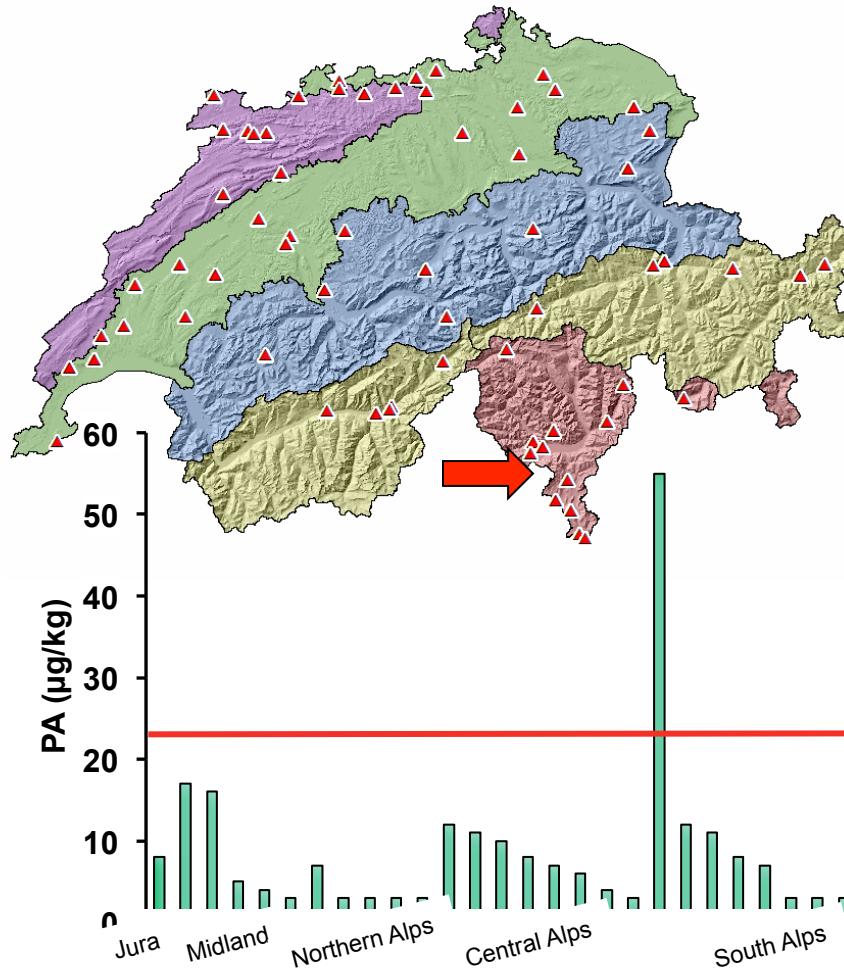
Honeys from five different geographical regions



- ▲ honeys (n = 71)
- 2009 – 2011
- polyfloral, alpine mountain flower, honeydew honeys, few monofloral



PA positive honeys



Half of the honeys contained PAs, usually at low concentrations, below the recommendations

PAs were mainly *Echium*-type PAs

Ref.: Kast *et al.*, 2014, *J. of Apicultural Research* 53 (1): 75.



Commercial pollen samples

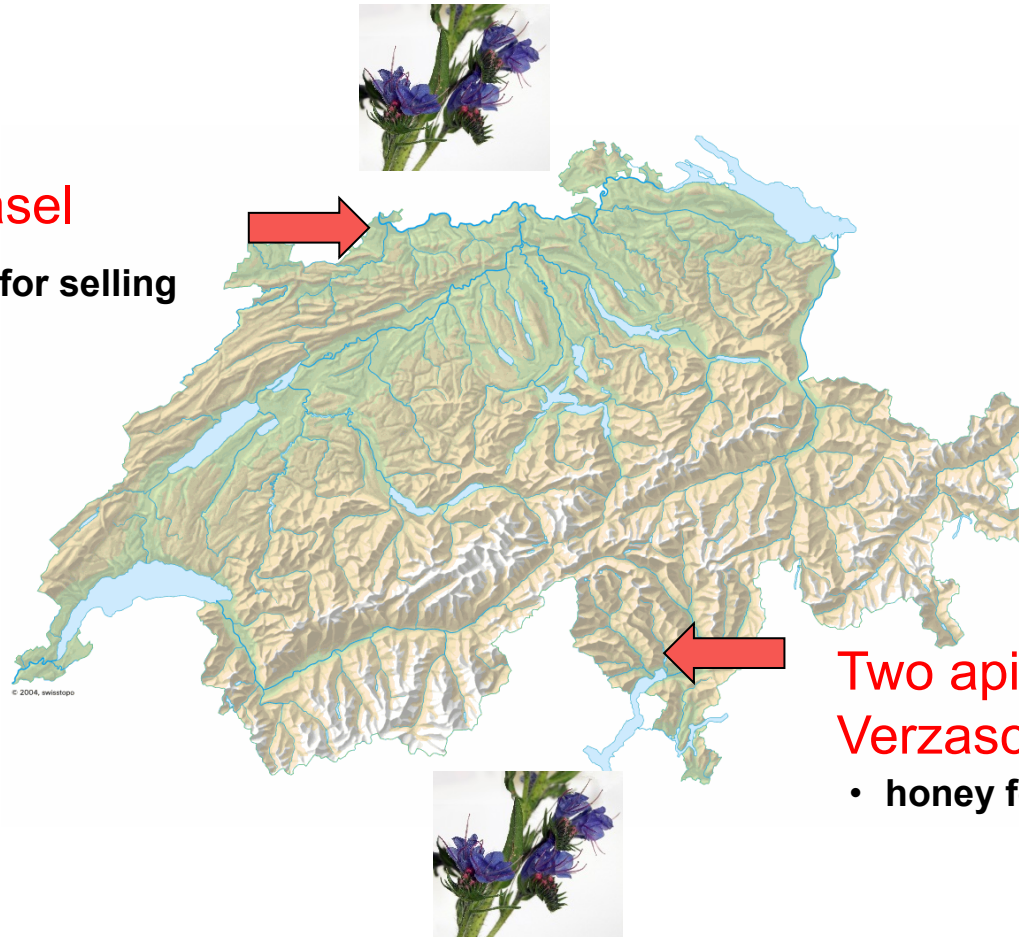
25 pollen samples, 2011 or 2014

20% of the samples contained PAs above the recommendations

Honey, pollen from observation sites

Apiary in Basel

- honey, pollen for selling



Two apiaries in the Verzasca-Valley

- honey for selling

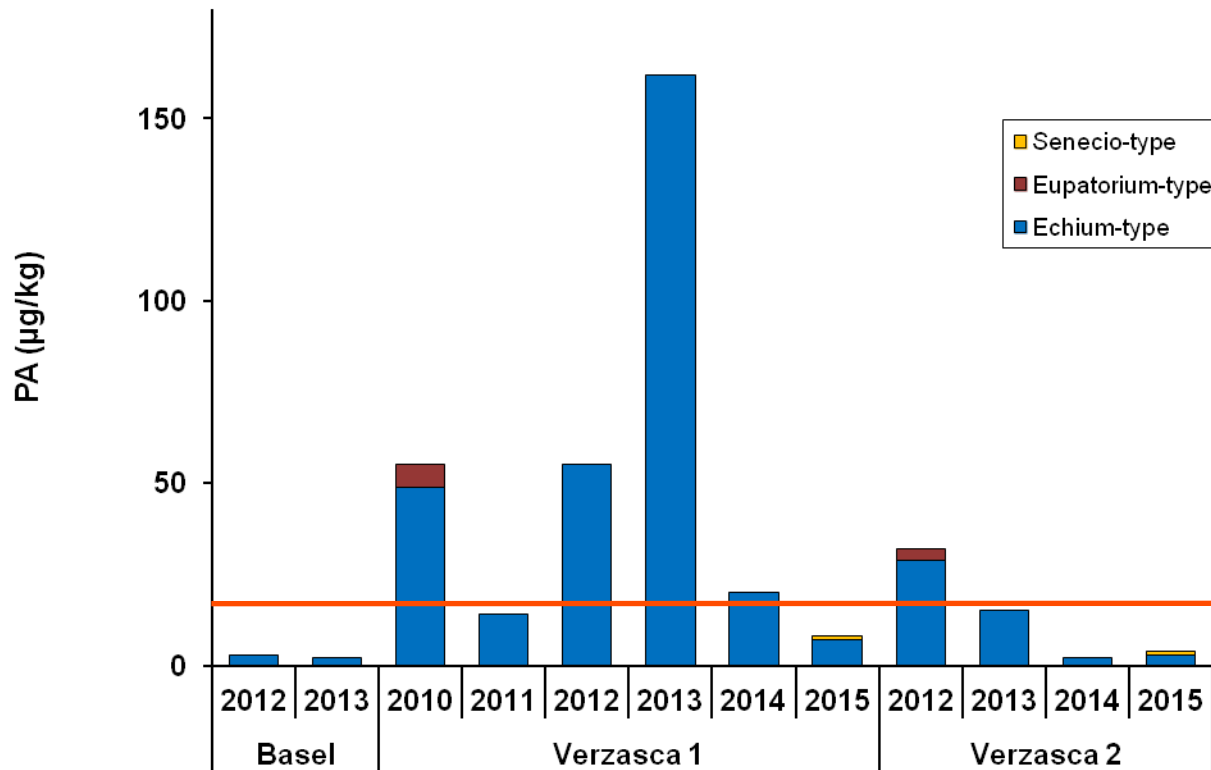


Echium vulgare at observation site





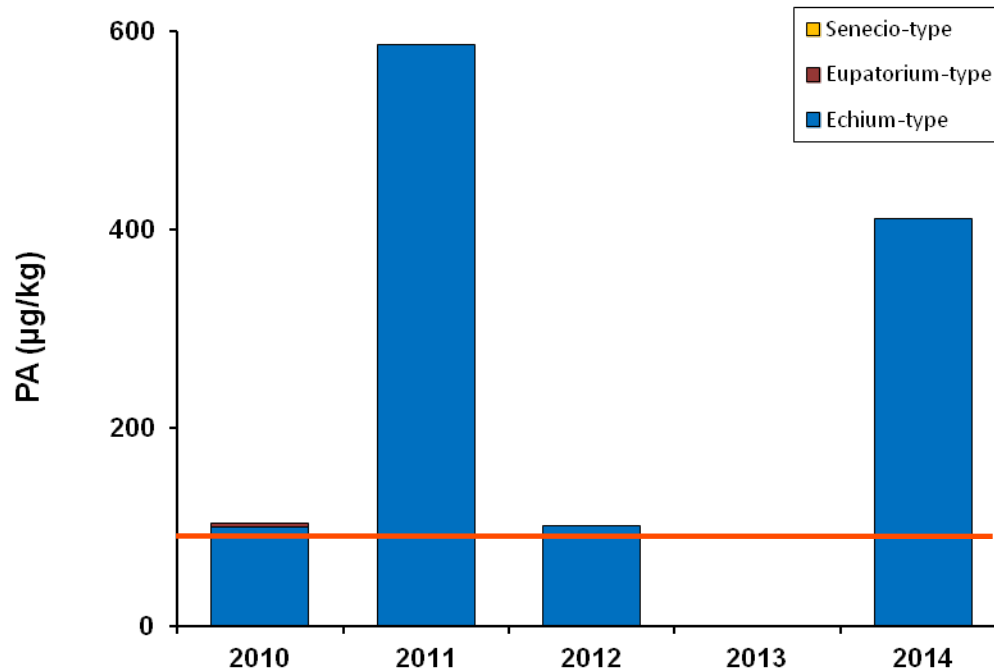
Honeys from observation sites



The PA content of honeys varied substantially between the production years, probably depending on other attractive plants.



Pollen from observation site Basel



The PA content in pollen varied substantially between the production years.

Summary: PAs in Swiss honey/pollen

- Swiss honeys and pollen usually pose no risk for consumers
- caution should be used at locations with a lot of PA-containing plants, such as *Echium vulgare* around the bee hives
- *Echium vulgare* was the most relevant plant for PA contamination of honey and pollen
- This correlates well with other European studies
 - *Echium vulgare* is also the most relevant plant for PAs in honey

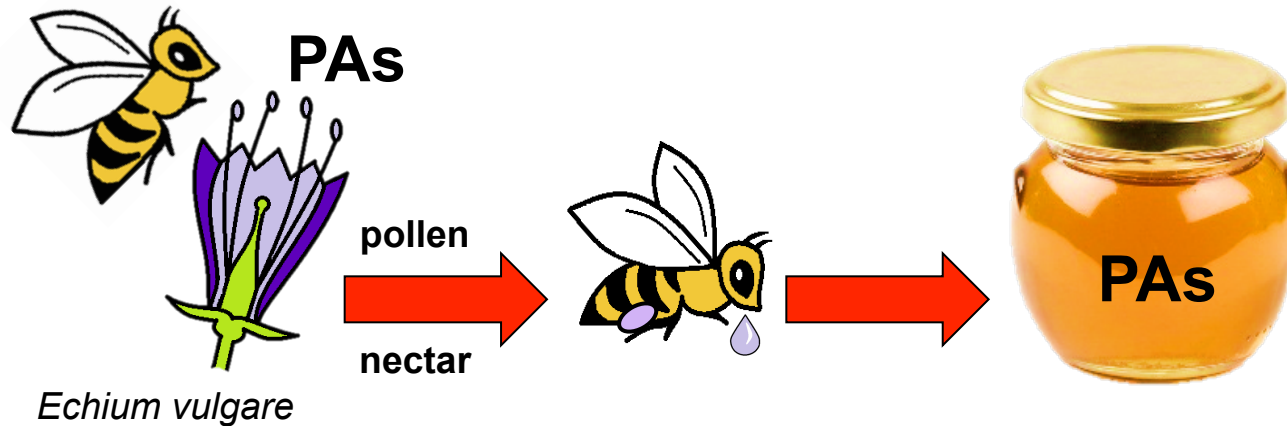
Ref: Dübecke *et al.*, 2011, *Food Additives & Contaminants: Part A* 28(3): 348.

Martinello *et al.*, 2014, *Food Control* 37: 146.

Kast *et al.*, 2014, *J. of Apicultural Research* 53 (1): 75.



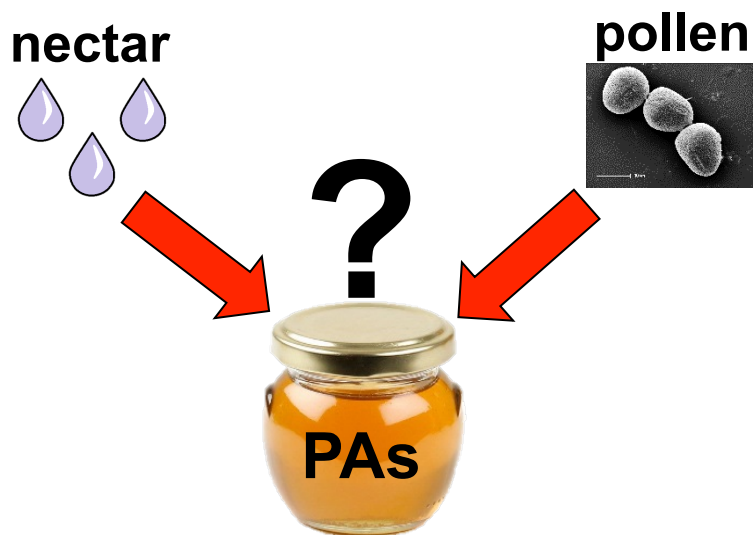
Source of PAs in honey?



- Pollen proposed as the major source of PA contamination of honey (Ref: Edgar *et al.* 2011; Boppré *et al.* 2005)

.....and nectar?

Ph.D. of M. Lucchetti



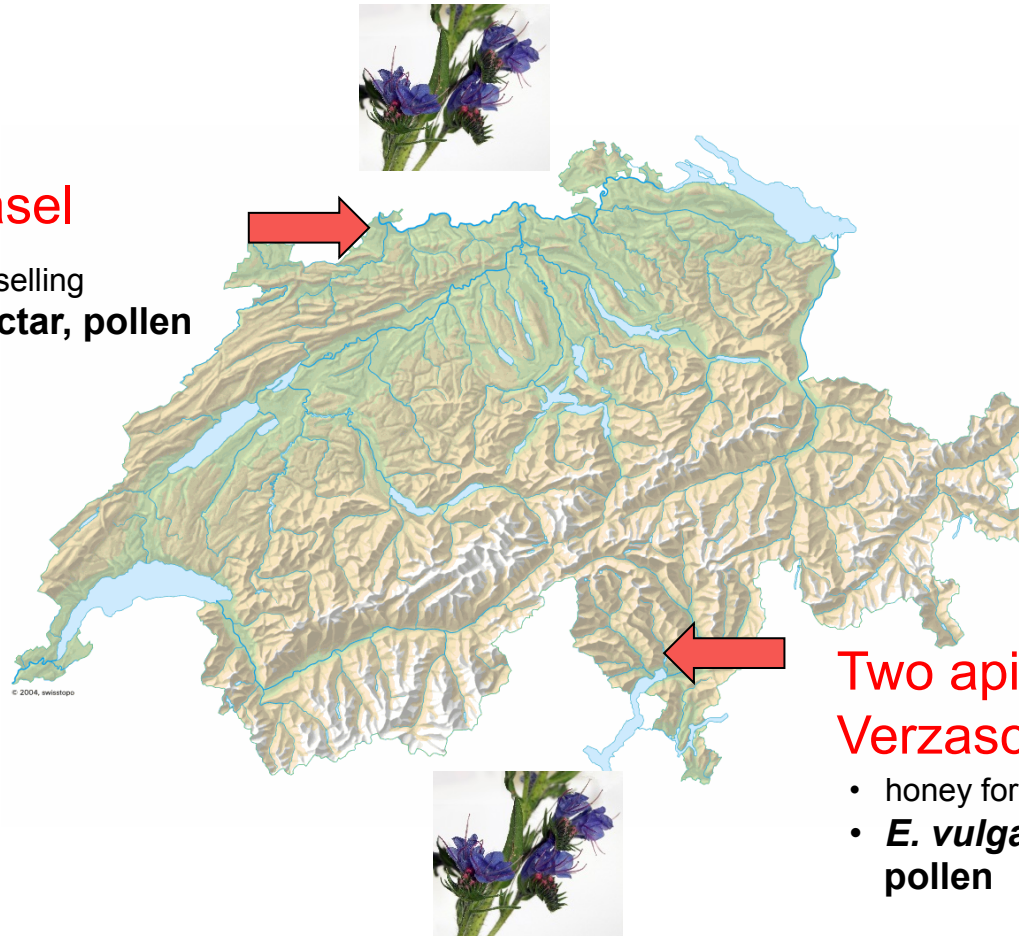
- Concentration of PAs in floral rewards
- Differences in the PA profile



Nectar and pollen from *E. vulgare* from the observation sites

Apiary in Basel

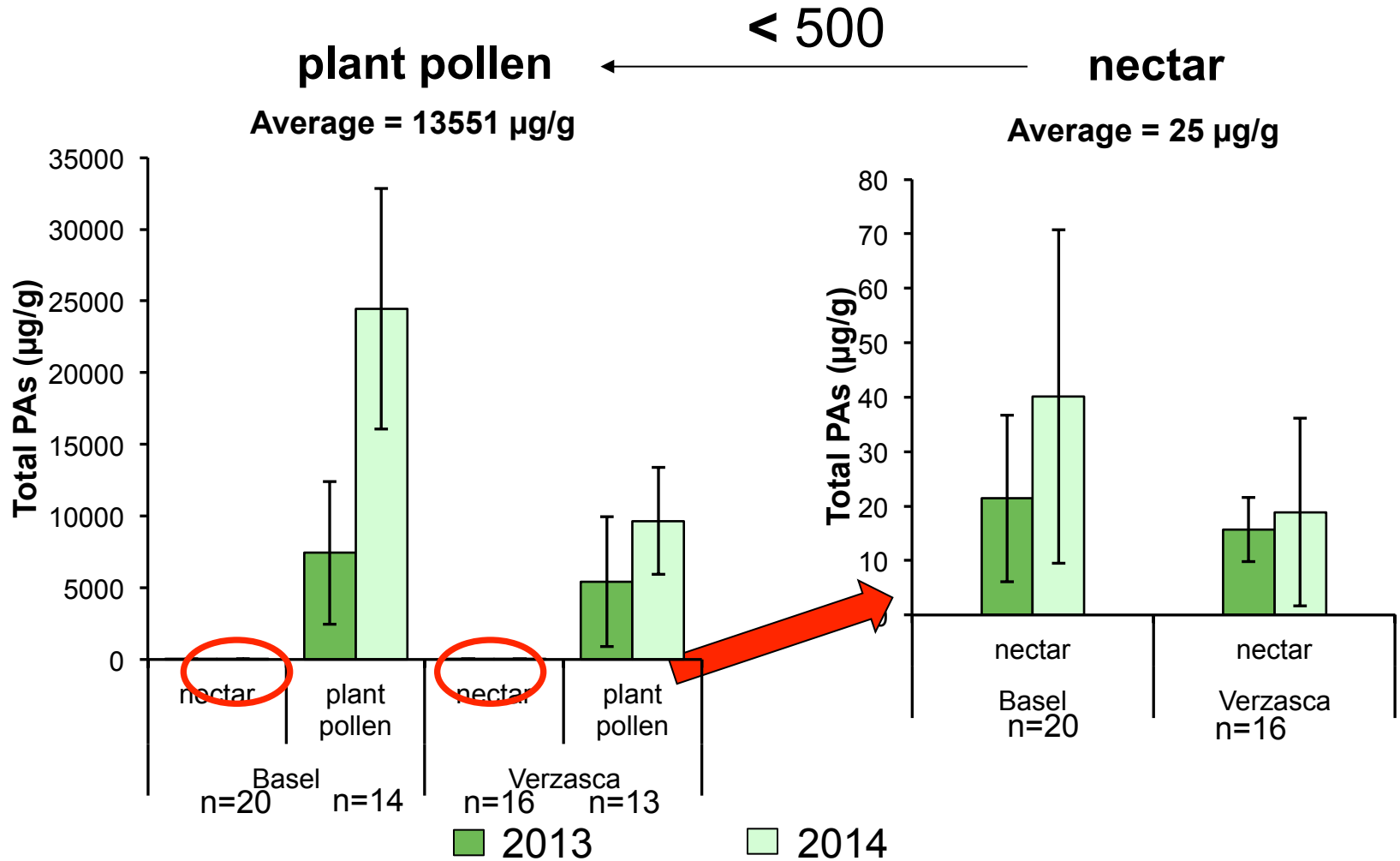
- honey, pollen for selling
- *E. vulgare*: nectar, pollen



Two apiaries in the Verzasca-Valley

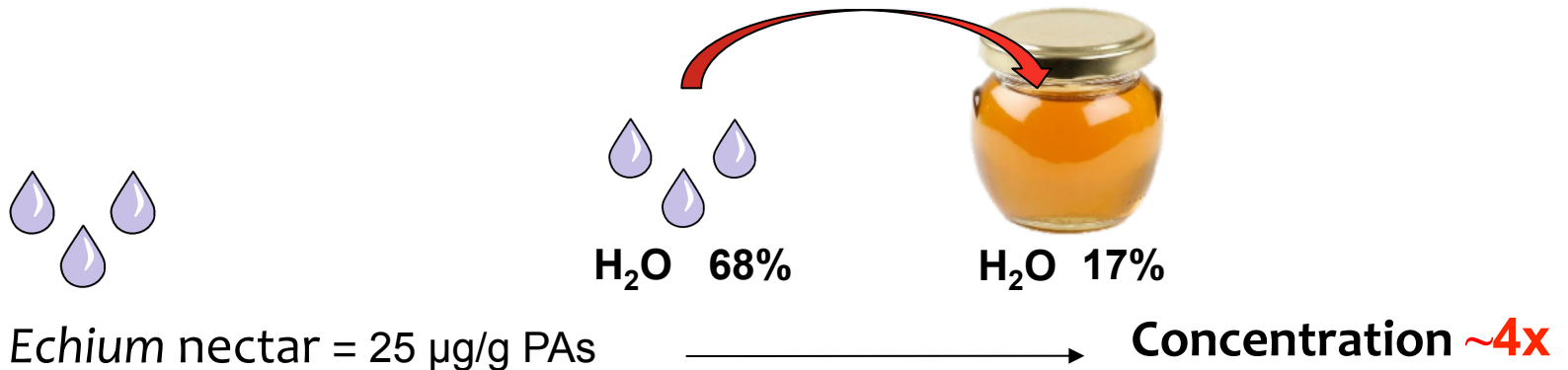
- honey for selling
- *E. vulgare*: nectar, pollen

PAs in *Echium* plant pollen and nectar



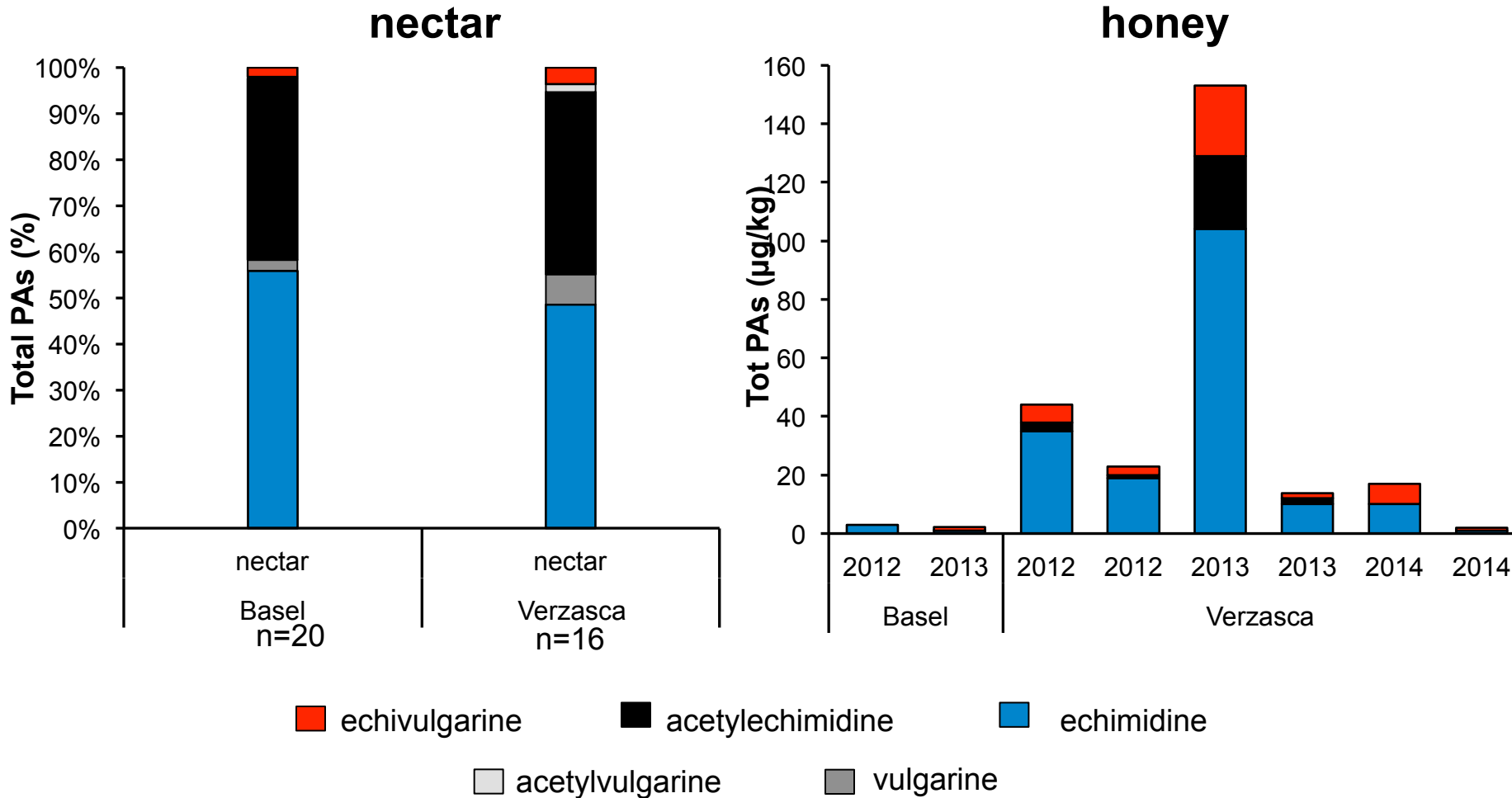


Contribution of natural sources to honey



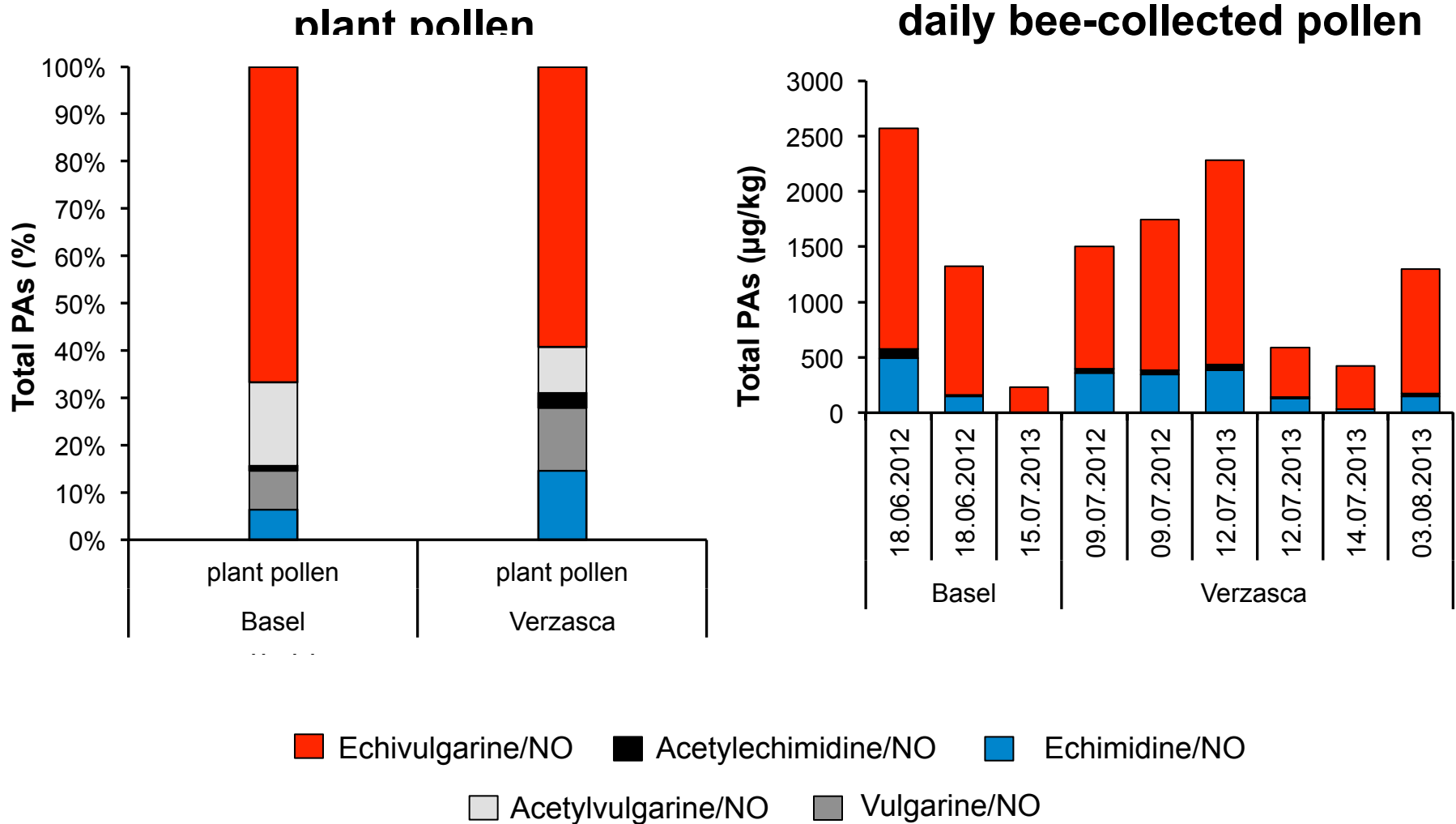


Differences in the PA profile



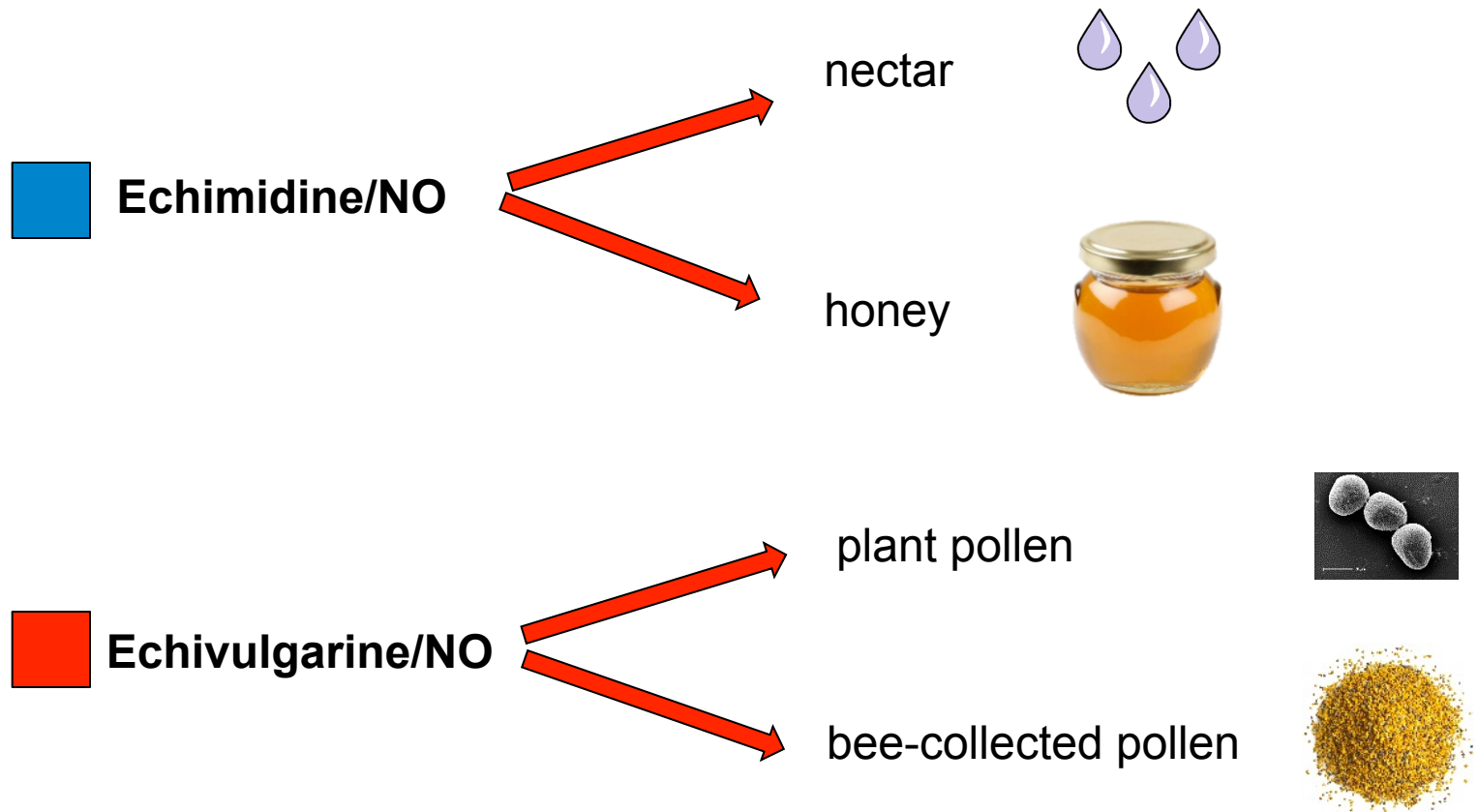


Differences in the PA profile





Main PAs found in floral rewards and bee products





Summary: Source for PAs in honey

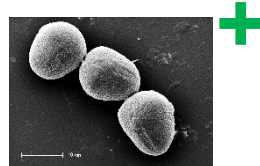
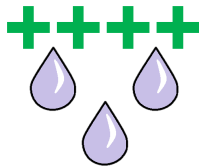
Pollen and nectar contribute to the PA contamination of honey, but...

- Nectar concentrated
- Pollen diluted

- PA profile of nectar similar to the PA profile of honey
- PA profile of plant pollen similar to the PA profile in bee-collected pollen

Conclusion

Results suggest nectar of *Echium vulgare* contributes to a higher extent to PA contamination of honey than pollen.



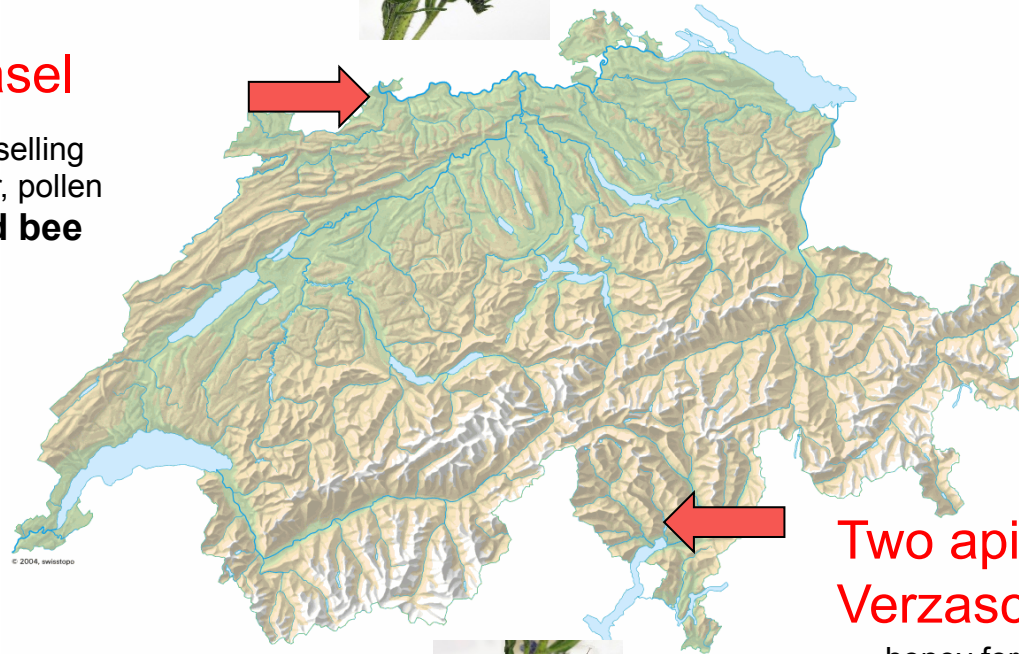
Ref: Lucchetti *et al.*, 2016, *J. Agric. Food Chem.*, 64 (25), 5267.



When do bees collect PAs?

Apiary in Basel

- honey, pollen for selling
- *E. vulgare*: nectar, pollen
- **daily collected bee pollen**



Two apiaries in the Verzasca-Valley

- honey for selling
- *E. vulgare*: nectar, pollen
- **daily collected bee pollen**





- Bees collected pollen with
 - *Echium*-type PAs: June until July
 - *Eupatorium*-type PAs: July to August
 - *Senecio*-type PA: June to September



Echium vulgare



Eupatorium cannabinum



Senecio jacobaea



- PAs present in nectar and pollen are transferred into bee products
- PAs in honey originate mainly from nectar
- *Echium vulgare*, *Eupatorium cannabinum* and *Senecio spp.* are important plants with respect of PAs in European bee products
- Beekeepers should try to avoid large numbers of PA-containing plants around the apiaries



Avoiding PAs in honey and pollen

Echium vulgare (Viperina azzurra)

- no seed mixtures with *Echium*
- remove or cut plants before blooming



Eupatorium cannabinum (Canapa acquatica)

- stop collecting pollen beginning of July



Senecio spp. (Erba di San Giacomo)

- landscape maintenance



Photos: O. Zoller



Good quality of bee products

Beekeeping

- Varroa treatment
- Wax foundation



Photo: M. Salathe

Environment

- Pesticides
- Plant toxins



Matteo Lucchetti



Verena Kilchenmann



Jean-Daniel Charrière



Beekeepers



Christophe Praz



Gaetan Glauser

Arne Dübecke
Quality Services International



Grazie per la vostra attenzione



Photo: R. Ritter