ONE HEALTH 2022
PAESTUM

13th-15th SEPTEMBER 2022
5th MEDPALYNOS SYMPOSIUM

15th-18th SEPTEMBER 2022
16th AIA CONGRESS
ITALIAN SOCIETY OF AEROBIOLOGY ENVIRONMENT AND MEDICINE
"AEROBIOLOGIA 4.0"

15th-18th SEPTEMBER 2022
BASIC COURSE AND UPDATE ON AEROBIOLOGICAL MONITORING

MELISSOPALINOLOGY: THE LOUVEAUX METHOD ON HONEY SAMPLES AND THEIR ALLERGENIC POTENTIAL

Sistema Nazionale per la Protezione dell'Ambiente

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ARPAM AGENZIA REGIONALE PER LA PROTEZIONE AMBIENTALE

MELISSOPALINOLOGY: THE LOUVEAUX METHOD ON HONEY SAMPLES AND THEIR ALLERGENIC POTENTIAL

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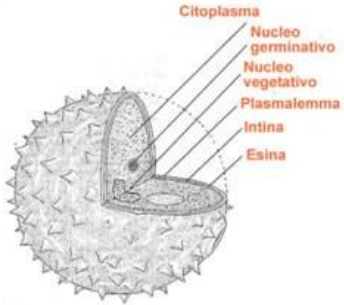
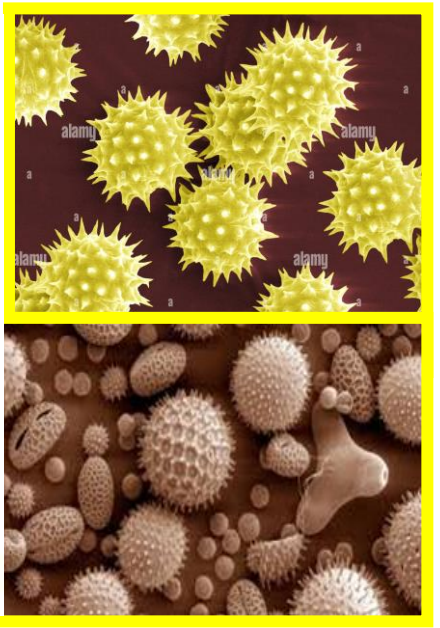
POLLEN IS THE ENTRANCE DOOR OF ALL APPLICATIONS OF PALYNOLOGY



- Aeropalinology
- Melissopalinology
- Paleopalinology
- Bioclimatology
- Copropalinology
- Pharmacopalinology
- Criminopalinology
- Bromatopalinology
- Sindology



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Melissopalinology is a branch of **palynology**, which studies **the pollen** and other **microscopic elements** that make up the honey sediment**.**

The morphological pollen characteristics, contained in honey, act as starting point for its origin taxa identification, useful to track it back to phylogenetic relationships between honey itself and plants.

While bees collect nectar from flowers, they unknowingly become vectors for pollen grains, which will end up in honey.

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HOW TO EXPLORE THE ADVENTUROUS WORLD OF PALYNOLOGY

MELISSOPALYNOLOGY

Botanical origin of honey

HONEY CONTAINS ITS OWN ORIGIN CERTIFICATE

Geographical origin of honey (L.753/82)

Intense research activity in honey samples (in our territory):

- **Assemble microscope slides, then create a palinoteca.**
- **Aerobiologist's specialization in the entomophilic pollen recognition.**
- **Floristic situation screening of places where bees produced honey samples.**

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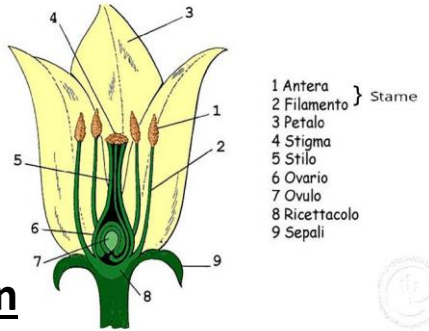
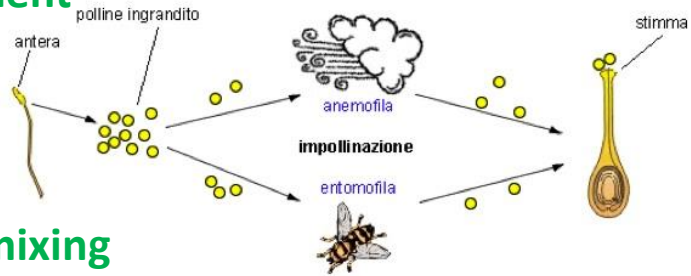
The pollen thus arrives in honey transported by bees, which guarantee the cross-pollination of distant plants, thus favoring genetic mixing, which is essential for the **survival** and **<u>strengthening of the species</u>**. Without forgetting another fundamental role that it plays: it is the **<u>only protein source for the nourishment of bees</u>**.



Nourishment of bees



Genetic mixing

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The pollen grain identifies the plant to which it belongs

NECTARIFEROUS

Fabaceae (Robinia)
Compositae
Fagaceae

NOT NECTAR

Graminaceae
Urticaceae
Oleaceae

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FIRST EXPERIENCES ANALYSIS OF POLLINI IN HONEY :

- **Pfister 1895**
- **Enoch Zander 1935 (1015 pages and 128 photographic plates))**
- **Recherches sur la rècolte du pollen per le abeilles (apis Mellifica)- Jean Louveaux (1959)**
- **Methods of Melissopalynology By Louveaux A.Maurizio and G.Worrol (published in Bee World -1970) International Commission Bee Botany of IUBS**

IN ITALY:

- **UNI 11299 – 2008** **The method is based on works of Louveaux and Von der Ohe-**

«.. Identification and quantification pollen, and of the microscopic elements present in the preparation of honey..»

- **Dlgs. 179/2004 - Distinction between wildflower and unifloral**
- **Norme UNI 201O - For five monoflorals italian..**
- **Norma UNI 10936 – Procedure for the preparation of the sample for chemical and melissopalonology analysis…**

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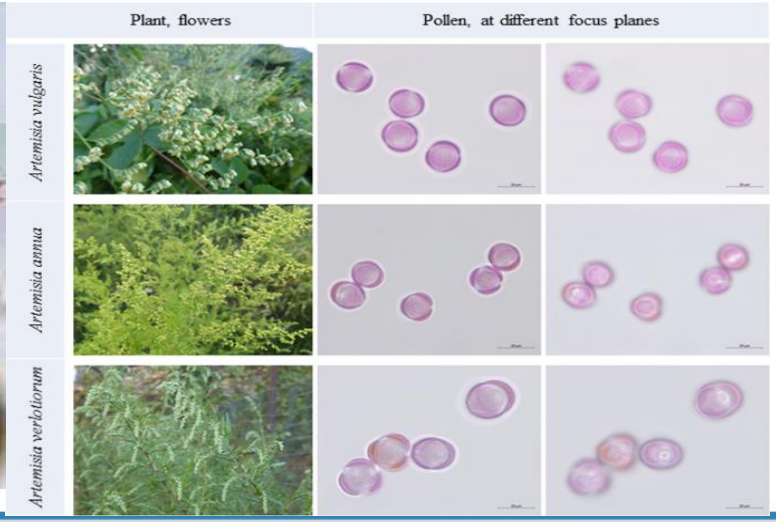
- In the LOUVEAUX Method and in the italian UNI 11299:2008 the microscopic elements are extracted and concentrated by centrifuging the honey dissolved in sterile water, and examining the sediment and evaluating it under the microscope
- Microscopical analisys of honey samples was carried out on fresh samples (no acetolysis performed)
- The result of the analysis consists of the list of pollens identified with the relative percentages found on the total



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- **Centrifuge the solution and decant the supernatant liquid**
- Add 20 ml of distilled water and dissolve the honey
- **Weight 10 g of honey in glass centrifuge tube**
- Add another 20 ml of distilled water to the centrifuge tube
- **Centrifuge the solution and decant the supernatant liquid**
- Dry the sediment on a hot plate at 40 °C
- **Add a drop of liquefed glycerine gelatine with fuchsin 40 °C to the sediment**

Observation and counting under the microscope

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Vegetative coverage of Marche region

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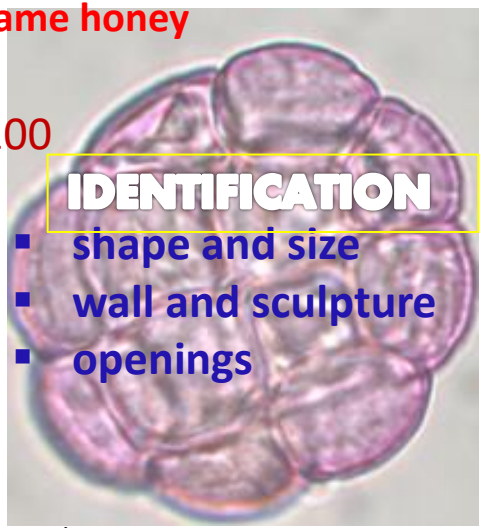
FOR THE IDENTIFICATION OF POLLEN TYPES AND THE INTERPRETATION OF POLLEN SPECTRA

1. Specific training and extensive experience are required.
2. A collection of reference *pollen slides* and photographic atlas are very helpful
3. **Make counts on two slides, prepared independently, from the same honey**

$$\textbf{FREQUENCES } \% = \frac{\textbf{nectariferous pollen counted granules}}{\textbf{total nectariferous pollen grains}} \textbf{ x } 100$$



Terms used in estimate of pollen grain frequencies:

"**Very frequent"** for grains constituting more than 45 % of the total

"Frequent" for grains constituting 16-45 % of the total

"**Rare"** for grains constituting 3-15 % of the total

"Sporadic" for grains constituting less than 3 %

Pollen dominant > 45% - by the Method 3.62 Presentation of frequencies

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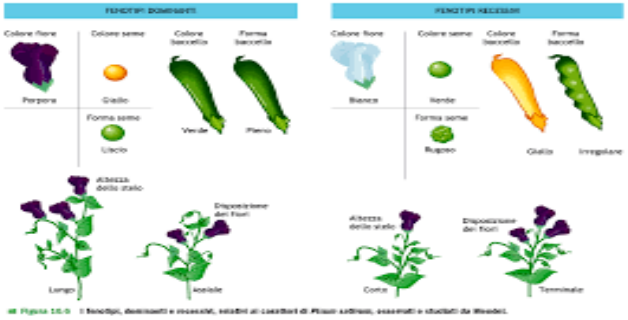
Expression of results:

It is necessary to count at least 300 pollen grains for an estimation of the relative frequencies of pollen types and 500 to 1000 pollen grains for the determination of relative frequencies (Behm et al., 1996).

$$50\% = \frac{50}{100} = 0{,}5$$

Il 50% di 750

$750 \times 0{,}5 = 375$



DETERMINING THE FREQUENCY CLASSES GIVES THE HONEY PRECISE IDENTITY OF BOTANICAL ORIGIN

THE GRAPH DATA OF THE NEXT SLIDE IS PROVIDED AS A PERCENTAGE % OF THE TOTAL COUNTED

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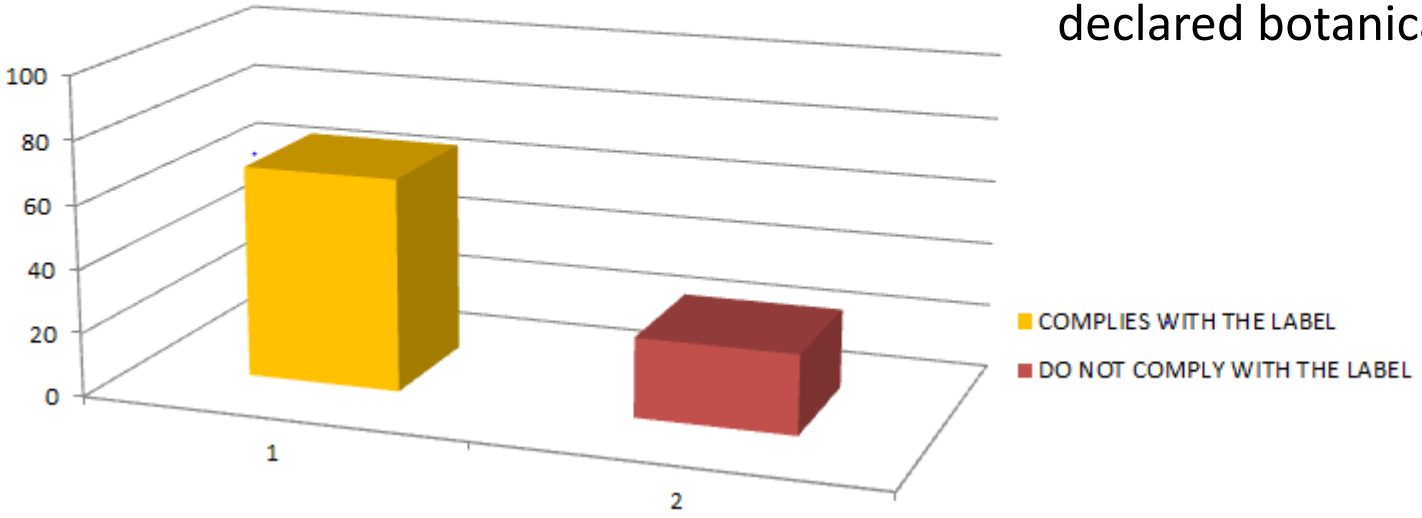
Compliance of artisan honey



Sample compliance compared to the declared botanical origin = 67%

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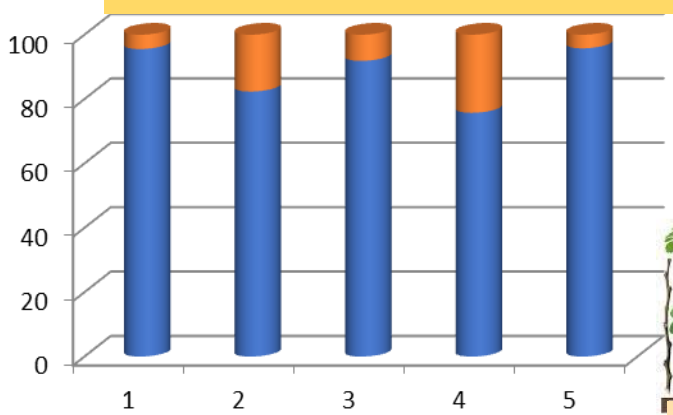
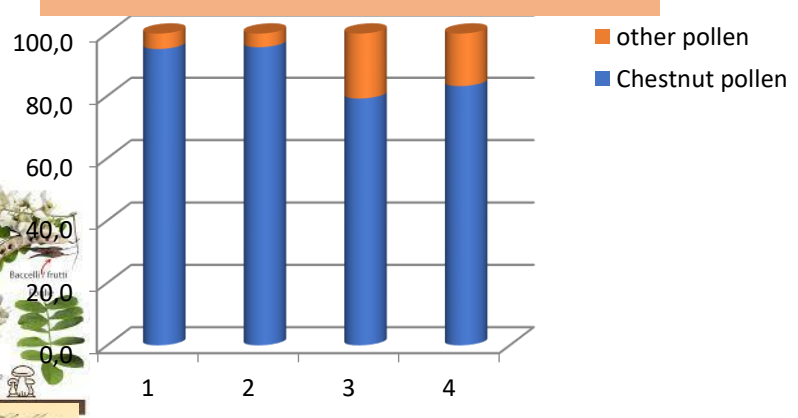
Honey Acacia

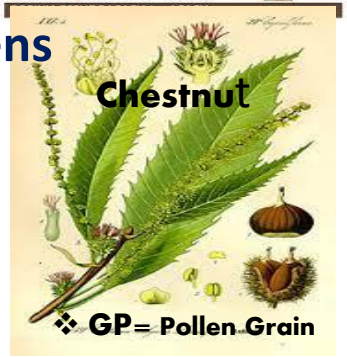


Honey Chestnut



"Under-represented" pollens

Samples	❖ PG	❖ PG Acacia
1	90	86
2	102	84
3	160	147
4	165	125
5	474	454



"Over-represented" pollens

Samples	❖ PG	❖ PG Chestnut
1	2547	2421
2	1669	1596
3	192	152
4	677	563

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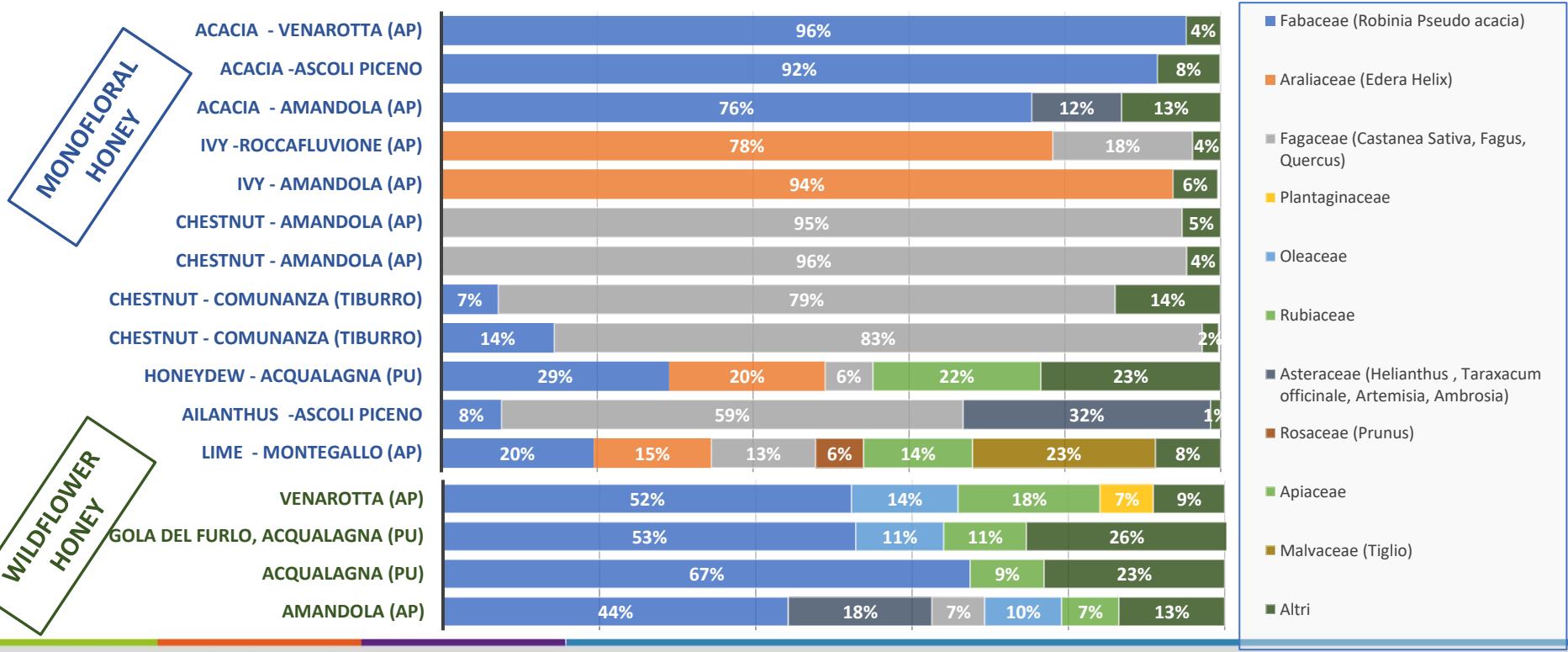
Pollen spectrum of each home-made honey sample

Geographical origin



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CROSS-REACTIVITY AEROALLERGENS AND FOOD ALLERGENS

Food allergy

Allergene alimentare di Classe 1

- allergeni resistenti al calore
- degradazione enzimatica
- causano sensibilizzazioni a livelli intestinali

Principalmente bambini

Cross allergies

Allergene alimentare di Classe 2

- allergeni sensibili al calore e alla degradazione enzimatica
- non causano sensibilizzazioni a livelli intestinali

Principalmente adulti

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CROSS-REACTIVITY AEROALLERGENS AND FOOD ALLERGENS

Food allergy

Cross allergies

Class 1 food allergen

- heat resistant allergens
- enzymatic degradation
- they cause sensitization at intestinal level

Mostly children

Class 2 food allergen

- allergens sensitive to heat and enzymatic degradation
- they do not cause intestinal sensitization

Mostly adults

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Table 2 Significant syndromes and associations due to cross-reactivity between aeroallergens and food allergens of plant origin[9,11]

Syndrome or association (sensitivity to heat and proteases)	Relevant allergen components involved (allergen sources)
Birch-apple syndrome	Bet v 1 homologue Mal d 1
Cypress-peach syndrome	Pru p 3 non-specific lipid transfer protein (nsLTP)
Celery-mugwort-spice syndrome	Art v 4 profilin, Art v 60 kDa homologue to Api g 5
Mugwort-peach association	Art v 4 profilin, Art v 3 LTP
Mugwort-chamomile association	Art v 1 defensin (possible candidate)
Mugwort-mustard syndrome	Art v 3 LTP, Art v 4 profilin, Art v 60 kDa (possible candidates)
Ragweed-melon-banana association	Amb a 6 LTP, Amb a 8 profilin (possible candidates)
Goosefoot-melon association	Che a 2 profilin (possible candidate)

IGE CROSS-REACTIVITY:

- **between allergenic molecules closely related species**
- **Between well preserved molecules in widely different species**

Allergic syndromes

HONEY PROTEIN

GENERAL POPULATION **0,001 %**

POLLEN-FOOD SYNDROME

GENERAL POPULATION **5 %**

In cross allergies (pollen-food syndrome) pre-sensitization to the pollen allergen is necessary for the allergic disease to occur.

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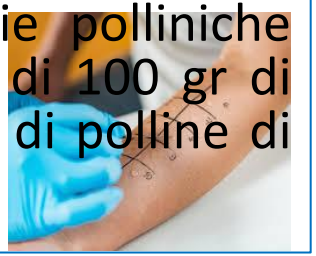
SOME of all CASE STUDIES REPORTED IN LITERATURE :

1.Sia le proteine derivate dalle secrezioni delle ghiandole faringee e salivari delle teste delle api sia le proteine del polline contenute nel miele hanno provocato reazioni allergiche dopo il consumo di miele (**Food allergy to honey**:..)

2.Ruolo eziologico dei pollini di Compositae nelle manifestazioni cliniche varie in pazienti sensibilizzati alla specie stessa dopo ingestione di miele(**Allergy to honey**..)

3.Caso particolare di tossicità al miele dovuto a specie polliniche presenti,(Cina Sud-Occidentale) in seguito ad ingestione di 100 gr di miele. L'analisi melisso palinologica riportava la presenza di polline di Tripterygium hypoglaucum (**Fatal honey poisoning**…)



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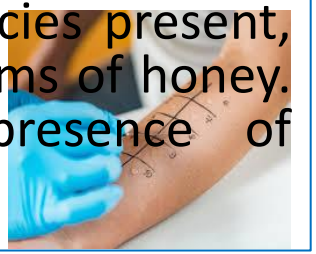
SOME CASES REPORTED IN THE LITERATURE:

1.Both the proteins derived from the secretions of the pharyngeal and salivary glands of the bee heads and the pollen proteins contained in honey have caused allergic reactions after the consumption of honey(**Food allergy to honey**:..)

2.Etiological role of Compositae pollen in some clinical manifestations in patients sensitized to the species itself after ingestion of honey(**Allergy to honey**..)

3. Particular case of toxicity to honey due to pollen species present, (South-Western China) following the ingestion of 100 grams of honey. The melisso palynological analysis reported the presence of Tripterygium hypoglaucum pollen(**Fatal honey poisoning**…)



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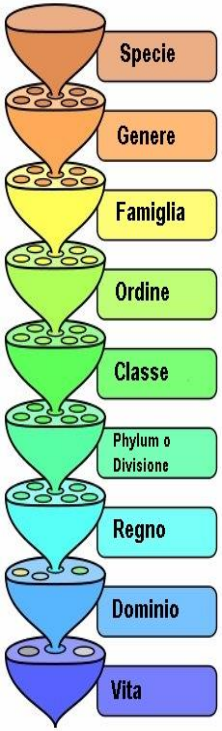
CASE REPORTS OF ADVERSE REACTIONS TO HONEY FOUND IN LITERATURE

1. The proteins present in the pharyngeal secretions of bees and the pollen proteins are responsible for allergic manifestations (**Food allergy to honey**:..).
2. Etiological role of Compositae pollen in allergic manifestations, caused by honey, in sensitized patients. miele(**Allergy to honey**..)
3. Toxicity of honey caused by the presence of Tripterygium hypoglaucum pollen, detected by melissopalinological analysis **(Fatal hone poisoning…).**

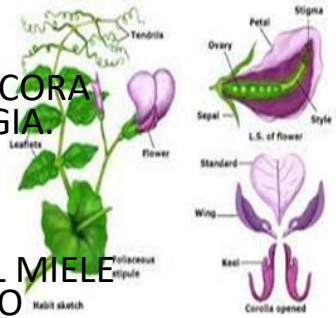
CONCLUSIONS

LO SPETTRO POLLINICO DEI CAMPIONI ESAMINATI RISPECCHIA FORTEMENTE LA SITUAZIONE FLORISTICA DEL LUOGO DI PROVENIENZA (ORIGINE GEOGRAFICA)



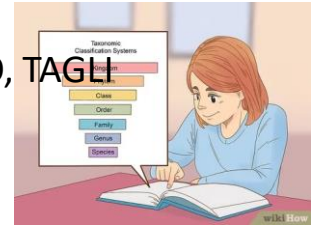
LA MAGGIOR PARTE DEI CAMPIONI ANALIZZATI CORRISPONDE ALL'ORIGINE BOTANICA DICHIARATA

I DIVERSI CASI STUDIO NELLE FORME ALLERGICHE TRATTATE NON HANNO ANCORA ISOLATO LA COMPONENTE PROTEICA DEL MIELE CHE FA SCATENARE L'ALLERGIA.



PROPOSALS

- ESTENDERE SUL TERRITORIO LA VALUTAZIONE DELL'ORIGINE BOTANICA DEL MIELE COME MONITORAGGIO DEGLI AGROECOSISITEMI, IN QUANTO E'UN VALIDO TRACCIANTE PER EVENTUALI INGRESSI DI SPECIE NUOVE O PERDITA DI SPECIE IN ESTINZIONE.
- CONTROLLO SPETTRO POLLINICO NEGLI ANNI DEGLI STESSI MIELI, PER INDIVIDUARE VARIAZIONI DEGLI ECOSISTEMI DOVUTI AL CONSUMO SUOLO, TAGLI ALBERI, EDIFICAZIONE INCONTROLLATA ECC..ECC..)



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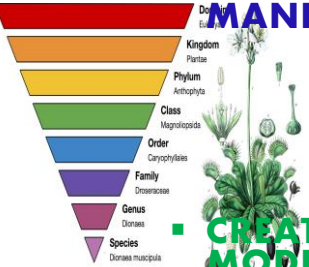
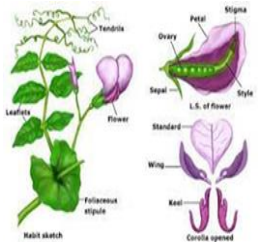
CONCLUSIONS

- THE POLLENS FOUND IN THE SAMPLES REFLECT THE FLORISTIC SITUATION OF THE PLACE WHERE THE HONEY IS PRODUCED (GEOGRAPHICAL ORIGIN)
- THE FREQUENCY OF POLLEN SPECIES REFLECTS A PRECISE BOTANICAL ORIGIN; IN OUR CASE THE DECLARED ORIGIN OF OUR SAMPLES IS CONSISTENT WITH THE RESULTS OF THE ANALYSIS.
- THE NATURE OF THE ANTIGEN RESPONSIBLE FOR THE ALLERGIC MANIFESTATIONS CAUSED BY HONEY IS NOT YET CLEAR.

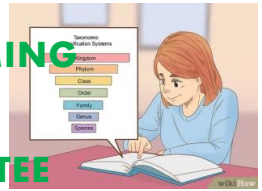




PROPOSALS

EXTEND THE RESEARCH TO A WIDER TERRITORY :

- CREATE A POLLEN SPECTRUM TYPICAL OF THE REGIONAL TERRITORY ON THE MODEL OF THE TRENTINO ALTO ADIGE
- PROMOTE THE ASSESSMENT OF THE BOTANICAL ORIGIN OF HONEY AS A METHOD FOR MONITORING AGROECOSYSTEM OVER TIME: MONITOR INCOMING OR LOSING SPECIES (INFLUENCE OF HUMAN ACTIONS EX:UNCONTROLLED BUILDING, LAND CONSUMPTION)
- THE ASSESSMENT OF THE GEOGRAPHICAL ORIGIN OF HONEY AS A GUARANTEE FOR THE CONSUMER



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Methods of Melissopalinogy – J.Louveaux, A. Maurizio, and G.Worrol (1970)
Recherches sur la recolte du pollen par les abeilles - Louveaux – (1958)
Linee guida per il monitoraggio aerobiologico – Pollnet (2017)
Atlante del polline delle principali specie allergeniche in Italia – Travaglini A, Brighetti M.A. and Vinciguerra (2014)
Spettro pollinico dei mieli dell'Alto Adige – Edith Buker, Veronica Kofler, Gunther Vorwohi, Emanuela Zieger
Le analisi del Miele (Ape nostra amica vol .5) – L.Piana
La diagnosi delle allergie alle profiline – Pucci N., Asero R, Calvani M, Indirli M.C., La Grutta S. (2011)
Cross-reactivity between aeroallergens and food allergens – Popescu FD (Giugno 2015)
Food allergy to honey pollen or bee product? Caracterizazion of allergenic proteins in honey by means of immunoblotting – Bauer L., Kohlich A., Hirschwehr R., et al. (1996/97)
Fatal honey poisoing in southwest china:a case series of 31 cases – Zhang Q., Xinguang Chen, Shunan Chen, Yinlong Ye, Jiancheng Luo, Juanjuan Li, Siyang Yu, Hui Liu and Zhitao Liu (2017)
Allergy to honey: relation to pollen and honey bee allergy- A.Hebling,Ch Peter, E.Berchtold, S.Bogdanov, U.Muller (1992)

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THANKS FOR YOUR ATTENTION