# Allergomelissopalynology

A new process for the study of allergenic pollens of the honeys from around the world

Its scope in practice for beekeeper and honey consumer



### Allergomelissopalynology



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The global honey market exceeded 2 million tonnes last year.

The uses of honey are numerous:

- Honey was used by early humans as in modern times as a food and a medicine.
- Honey may be used as a natural means of giving colour, scent and emollient properties to cosmetics.
- Honey has still been used to impart both aroma and taste.





Honey contains many proteins:

Bee secretion proteins: Enzymes in honey are extremely important, playing a major role in converting the nectar.

Pollen proteins: The pollens present in honey vary from region to region. As honey is made from many plant sources, any of the « parent » plants pollen may be found in the honey, but not only: pollens from non-nectariferous plants are numerous.



### Table of allergenic pollens collected by bees in different states of USA

	California	Texas	Florida	Michigan
Spring	artemesia			fraxinus
				ligustrum
Summer		poaceae	poaceae	poaceae
			ambrosia	ligustrum
				plantago
Autumn		ambrosia	ambrosia	artemesia
			poaceae	poaceae
Winter	fraxinus			
	alnus			

Pierre Lau, Vaughn Bryant, James D. Ellis et all. PLoS ONE 2019





Honey is a potentially allergenic food:

Allergic reactions to honey are ranging from mild symptoms, such as an oral syndrome, to severe, such as an anaphylaxis accident (Vezir E1, Kaya A, Toyran M, et all. Allergy Asthma Proc. 2014).

Honey is a potentially allergenic cosmetic :

An urticaria stage 3 was induced by epicutaneous sensitization during skin care with honey (Katayama M, Inomata N, Inagawa N, et all. Contact Dermatitis. 2016)





In the literature, allergy to honey is most often attributed to the pollen content. Compositae pollens are a frequent source of allergens. (Lombardi C, Senna GE, Gatti B, et all. Allergol Immunopathol (Madr), 1998. Fuiano N, Incorvaia C, Riario-Sforza GG, et all. Eur Ann Allergy Clin Immunol, 2006.)

The allergens detected in pollens will depend on the source of honey.

Allergenic pollens retain their allergenic properties during the honey-making process. (Stanley RG and Linskens HF. Springer Verlag, Berlin, 1974.)

Cross-reactivity would depend on the component to which the honeyallergic individual is allergic, e.g. Compositae pollen





#### Honey appears to be a common allergy

(Eriksson NE, Moller C, Werner S, et all. J Investig Allergol Clin Immunol, 2004)

#### Prevalence is variable:

5% in Denmark and Sweden 31% in Estonia, Lithuania and Russia

Children > adults

Severity is variable : East > West

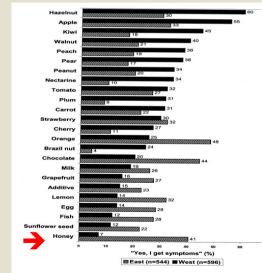


Figure 1. Foodstuffs most often eliciting symptoms in the East (Estonia, Lithuania, and Russia) and in the West (Sweden and Denmark)





However, it may be that honey not always induces symptoms of food allergy in individuals sensitised to pollen (Birnbaum J, Tafforeau M, Vervloet D, et all. Clin Exp Allergy, 1989)

To explain this we can imagine different reasons:

- honey does not contain pollen responsible for allergy in allergic
- the amount of allergenic pollen in honey is not enough to trigger an allergic symptom
- allergenicity of pollen is changed during the the honey-making process...

So, it takes a process to better assess the allergic risk due to honey pollens...





How the allergenicity of flower pollen is transformed when harvested by bees :

The allergenic properties of each allergenic floral pollen contained in bee products have been measured by the skin reactivity of allergics to this pollen.







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The method in broad outline: (Nonotte-Varly, C. Alergologia Polska-Polish Journal of Allergology, 2016)

First, it is experimentally characterized relationship between mass of one allergenic pollen (MassAP) content in bee products (BP) and its allergenic potential measured by skin reactivity testing.

MassAP is identified after having calculated the proportion of AP using the BP pollinic spectrum based on the European standard.

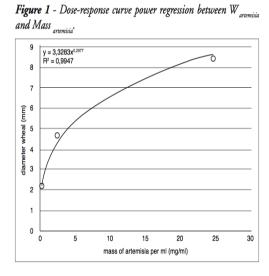
Relationship equations are calculated for several AP.

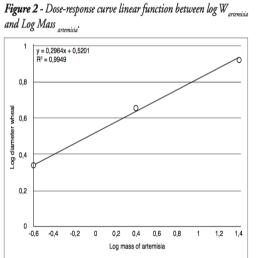




# Dose-response curves linking allergic skin reactivity and allergen pollen mass (Nonotte-Varly, C. European annals of allergy and clinical immunology, 2015)

Mugwort pollen









# Dose-response curves linking allergic skin reactivity and allergen pollen mass (Nonotte-Varly, C. Alergologia Polska-Polish Journal of Allergology, 2015)

# Ash tree pollen

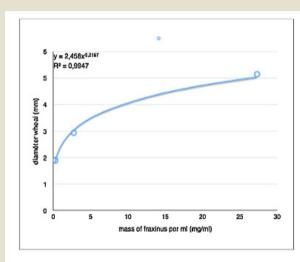


Fig. 1 – Dose–response curve power regression between  $W_{\rm fraxinus}$  and  $Mass_{\rm fraxinus}$ 

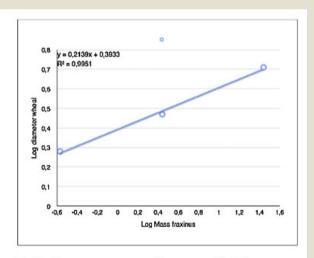


Fig. 2 – Dose–response curve linear regression between Log  $W_{fraxinus}$  and Log Mass $_{fraxinus}$ 



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The method in broad outline (Nonotte-Varly, C. Alergologia Polska-Polish Journal of Allergology, 2016):

Then, for a bee product having several AP, allergenic potentials of AP are assessed by :

- the bee product spectrum analysis and the AP identifications,
- the calculations of MassAP
- the allergic skin reactivity calculations using equations.

The Allergenic Potency Index System (APIS) assess the allergenic potential of an AP from the bee products.





The method in broad outline (Nonotte-Varly, C. Alergologia Polska-Polish Journal of Allergology, 2016):

Allergenic Potency Index System (APIS): scale of risk of occurrence of allergic accident

APIS: scale of risk of occurrence of allergic accident

APIS	APIS ≤ 9	10 ≤ APIS ≤ 19	20 ≤ APIS ≤ 29	30 ≤ APIS ≤ 39	40 ≤ APIS
occurrence of allergic accident	negligeable	very low	low	moderate	important





The method in broad outline (Nonotte-Varly, C. J. Reval., 2019):

Allergenic Potency Index System (APIS) and Honey:

To have the best informations for honeys, APIS is calculated :

- for 10 grams of honey (tea spoon of honey)
- for 30 grams of honey (soup spoon of honey)

APIS: scale of risk of occurrence of allergic accident								
APIS	APIS ≤ 9	10 ≤ APIS ≤ 19	20 ≤ APIS ≤ 29	30 ≤ APIS ≤ 39	40 ≤ APIS			
occurrence of allergic accident	negligeable	very low	low	moderate	important			



### Allergomelissopalynology: Materiel



226 honey samples from 4 continents received in 2017 were studied in collaboration with the research and bee ecology center of moselle – lorraine (CETAM - Lorraine, 1 B rue Jeanne d'Arc , 57310 Guénange - France).







(Nonotte-Varly, C. J. Reval., 2019)

#### Origin of the honey samples:

- 190 honey samples have a precise geographical origin
- 34 have a origin referred to as "European Union (EU)" or "European Union and outside European Union (EU & no EU) "according to the European regulations. These are assemblies of honeys of multiple geographical origins.
- 2 honey samples have no pollen (ultrafiltered)





(Nonotte-Varly, C. J. Reval., 2019)

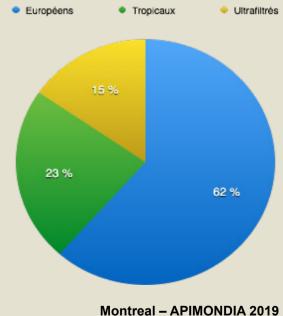
- 5% of honeys have no allergenic pollen (ultrafiltered excluded)
- 95% of honeys contain allergenic pollens :
  - 7/10 in trace amounts,
  - 3/10 with one or more allergenic pollens > 3% with a maximum of 88%.





- 5% of honeys have no allergenic pollen (ultrafiltered excluded):

- These are all monofloral honeys
- But monofloral honey does not induce do not have allergenic pollen

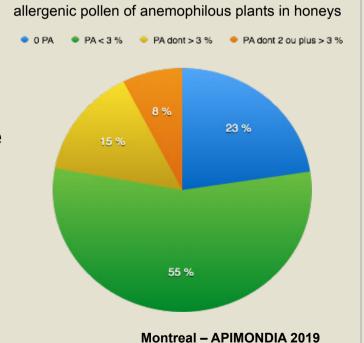




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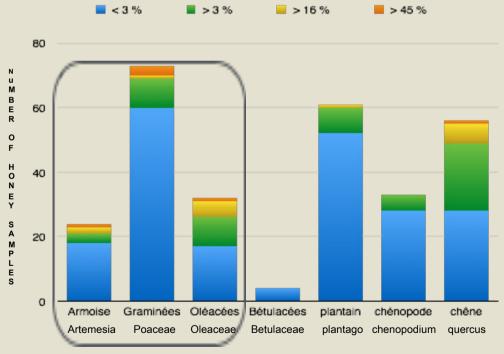
- 95% of honeys have allergenic pollen
- -These are pollens of :
- melliferous asteraceae (sunflower, thistle and dandelion)
- non-melliferous anemophilous plants (eg mugwort, olive, grasses).







#### Table of allergenic pollen of anemophilous plants in honeys





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Focus on honeys with olive pollen grains

Sample S 167 Orange honey from Spain with 10 500 pollen grains /10 g

Olive pollen grains: 74 %

Mass of olive pollen grains: 0,0703 mg for 10 grams of honey (tea spoon of honey)

APIS olive = 14

The risk of occurrence of allergic accident is very low

Mass of olive pollen grains: 0,2109 mg for 30 grams of honey (soup spoon of honey)

APIS olive = 18

The risk of occurrence of allergic accident is also very low





Focus on honeys with grass pollen grains

Sample S 86 Jujube honey from Pakistan with 13 200 pollen grains /10 g

Grass pollen grains: 55 %

Mass of grass pollen grains: 0,2303 mg for 10 grams of honey

APIS grass = 15

The risk of occurrence of allergic accident is very low Mass of grass pollen grains: 0,6909 mg for 30 grams of honey

APIS grass = 20

The risk of occurrence of allergic accident becomes low





Focus on honeys with mugwort pollen grains

Sample S 102 Jujube honey from Yemen with 15 700 pollen grains /10 g

Mugwort pollen grains: 81 %

Mass of mugwort pollen grains: 0,0757 mg for 10 grams of honey

**APIS mugwort = 15** 

The risk of occurrence of allergic accident is very low Mass of mugwort pollen grains: 0,2271 mg for 30 grams of honey

**APIS** mugwort = 21

The risk of occurrence of allergic accident becomes low





Focus on honeys with mugwort pollen grains

Sample S 12 multi-flower honey from France with 148 600 pollen grains / 10 g

Mugwort pollen grains: 26 %

Mass of mugwort pollen grains: 0,2262 mg for 10 grams of honey

**APIS mugwort = 21** 

The risk of occurrence of allergic accident is low

Mass of mugwort pollen grains: 0,6786 mg for 30 grams of honey

**APIS mugwort = 30** 

The risk of occurrence of allergic accident becomes moderate





Focus on honeys with mixed-asteraceae pollen grains

Sample S 98 Sunflower honey from EU & no EU with 18 900 pollen grains /10 g

Mixed-asteraceae pollen grains: 83 %

Mass of asteraceae pollen grains: 0,4034 mg for 10 grams of honey

APIS mixed-asteraceae = 21

The risk of occurrence of allergic accident is low

Mass of asteraceae pollen grains: 1,2102 mg for 30 grams of honey

**APIS** mixed-asteraceae = 28

The risk of occurrence of allergic accident is also low



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# Allergomelissopalynology: its scope in practice for beekeeper



#### The beekeeper or the honey conditioner/packager/distributor:

- often conducts analyzes of quality including honey pollen analysis
- has relevant information to communicate

So, with **APIS**, he can value his products because he can:

- master the allergenic characteristics of his products,
- do assemblies of honeys to reduce the power of the allergenicity of the flower pollens of his products
- communicate on the allergenic safe qualities of his products and, thus, give their an added value for the benefit of the greatest number of honey consumers.





# Allergomelissopalynology: its scope in practice for honey consumer



With **APIS**, consumer knowing or not his allergy to flower pollens can :

- fully appreciate the natural qualities of honey by benefiting from reliable information, with the certainty of the control of the safety of honey
- benefit from the development of innovative products and benefit without fear of the natural properties of honey such as, for example, supporting immunity.



# Allergomelissopalynology: its scope in practice for honey consumer



In case of allergic accident with honey, Allergomelissopalynology permits the etiological diagnosis more easy in case of allergy to floral pollens.

This method **APIS** avoids the expensive extraction of allergic proteins.

