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In vivo biological potency of Fraxinus bee-collected pollen on patients allergic to oleaceae

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ABSTRACT

Oleaceae bee-collected pollen is identified as being potentially at the origin of allergic accidents but the biological potency of Oleaceae bee-collected pollen is not well known.

In this experiment, Fraxinus mass was identified in bee pollen mass and after having done so the proportion of Fraxinus mass using the bee pollen melissopalynology spectrum was calculated. Skin reactivity to Fraxinus was assessed by measuring wheal diameters (W) from skin prick tests using three serial dilutions of bee pollen on 10 patients allergic to Oleaceae pollen, in order to calculate the relationship between Fraxinus mass (Mass fraxinus) in bee pollen and skin reactivity.

The dose–response power regression curve ($W_{fraxinus} = 2.46\ (Mass_{fraxinus})^{0.21}$ $R^2 = 0.99$) and the linear function ($Log\ 10\ (W_{fraxinus}) = 0.21\ (Log\ 10\ [Mass_{fraxinus}]) + 0.39$ $R = 0.99$) were established using a bee pollen sample with 0.273 mg of Fraxinus pollen per mg.

Fraxinus allergens seem to be little or not altered by bee secretions. Fraxinus bee-collected pollen retains its allergenic capacity.

To the best of our knowledge this is the first time it has been shown that skin reactivity of patients allergic to Oleaceae pollen is proportional to the absolute Fraxinus mass contained in the bee-collected pollen.

Introduction

Pollen is flower sperm. It is the only source of certain macronutrients and is collected by worker honeybees. Collected from floral anthers at the tips of stamens, flower pollen grains stick to bee secretions. They are then assembled by the bee in loads, and placed in the baskets of the hind legs of the insect. Each load has a weight of 5–10 mg [1] and has several hundred thousand grains of a single floral species. Each load requires a visit to at least 80 flowers of the same plant type. The mixture of floral pollen is in the form of pellets and is what is commonly called "bee pollen". It consists of various loads from different plant species.

Bees visit numerous plant flowers. There are, for example, more than 268 species and varieties of plants in England [2]. G. Ricciardelli D'Albore and F. Intoppa have listed all the families of plants in Europe that are visited by bees [3].

Some floral pollen in bee products is responsible for allergies. Anaphylactic accidents related to the use of bee

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products are on the increase. There is substantial literature supporting this observation.

In spring, Oleaceae members represent as pollen suppliers for honeybees at locations where the olive and ash trees are an important pollen source [4].

Oleaceae bee-collected pollen is botanically closely related to common airborne allergenic pollen grains or could cross-react with unrelated allergenic plants. These allergic cross-reactions are caused by proteins sharing important structural homologies with several plant families [5], e.g., Profilin and Polcalcin panallergens from Ash pollen [6].

Oleaceae allergenic proteins appear to retain their allergenic properties in bee pollens from the time they enter the beehive to the harvesting by the beekeeper and their use by the end consumer [7].

To the best of our knowledge, however, there is currently no technical definition of the allergenic potential of Oleaceae in bee-collected pollen. The purpose of this study is to define the biological potency of pollen of Oleaceae in bee-collected pollen *in vivo* by skin prick tests on patients allergic to Oleaceae pollen.

Materials and methods

Analysis of bee pollen spectrum

A pollen analysis of bee products is usually performed in a specialist laboratory by analyzing the beehive products. In our case, we used Honey Expertise Laboratory – Naturalim France Miel, 39330 Port-Lesney, France.

Such an analysis defines the type and frequency of each botanical genus or family floral pollen and determines the total mass of floral pollen. Bee pollen is thoroughly cleaned when intended for human consumption. Cleaning the pollen separates pollen dust balls, bee body fragments, wax or propolis. Melissopalynology is based on the European Maurizo and Louveaux standard without acetolysis, as recommended by the International Commission for Bee Botany [8].

Counting and identifying floral pollen grains are made by examination under a microscope. Identification of pollen types is based on the laboratory pollen reference collection for local plants or guides specialized in the pollen morphology of floral species.

Ten grams of well-homogenized bee-collected pollen was dissolved and washed in distilled water, centrifuged, and then prepared using aqueous glycerine and paraffin for smear preparations. Each smear was studied under a microscope to identify 500 floral pollen grains in order to determine the percentage of each type of flower pollen.

With bee-collected pollen, the floral pollen mass is equated with the bee pollen mass because it is accepted that the bee-collected pollen pellets only contain kneaded floral pollen grains.

Calculation of the floral pollen allergen mass "$Mass_{p\text{-}allergen}$" in bee pollen

1. Calculate the volume "V_{pn}" of each of the 1 to *n* types of floral pollen from the bee pollen spectrum using the formula $V_{pn} = 4/3\pi r^3$ if the pollen grain is spherical or using the formula $V_{pn} = 4/3\pi e^2 l$ if the floral pollen has an ellipsoidal shape.

   The values of the radius *r* and of the mid-equatorial and longitudinal axes *e* and *l* are obtained from the literature from observations made on bee product pollen, including bee pollen (2). It is important to take into account changes in volumes of flower pollen due to orthodox or recalcitrant pollen qualities when pollen grains come into contact with aqueous bee fluids.
2. Calculate the proportion of volume $P_{p\text{-allergen}}$ of flower pollen allergen p-allergen

$$P_{p\text{-allergen}} = \frac{(V_{p\text{-allergen}} \times \%_{p\text{-allergen}})}{((V_{p\text{-allergen}} \times \%_{p\text{-allergen}}) + (V_{p2} \times \%_{p2}) + \cdots + (V_{pn} \times \%_{pn}))}$$

   (%pn is the percentage of flower pollen pn observed in the bee pollen analysis).
3. Calculate the mass of floral pollen allergen $Mass_{p\text{-allergen}}$

$$Mass_{p\text{-allergen}} = P_{p\text{-allergen}} \times Mass_{pollens}$$

Calculation using the equation defining the allergenic potential of flower pollen allergen in bee pollen

Before applying this equation, it is necessary to:
- Use bee pollen with only one floral pollen allergen,
- Calculate the mass of floral pollen allergen as indicated above,
- Use a bee pollen without any floral pollen allergen as a "bee pollen negative control" to eliminate a skin sensitization to bee specific allergens.

1. Preparation of bee pollen extracts:

Samples were prepared with the two types of bee pollen defined above. Five grams of fresh or frozen bee pollen was well homogenized on a glass plate and 450 mg of bee pollen was diluted in 4.5 ml of isotonic 0.4% phenol diluent (dilution weight/volume: 100 mg of bee pollen/ml of diluent) and was homogenized with a stirrer at a maximum speed for one minute. Samples were stored at room temperature for 24 h and homogenized one more time with the stirrer before being removed, then 0.5 ml of the 100 mg/ml diluted solution was diluted in 4.5 ml of isotonic 0.4% phenol diluent to produce a 10 mg/ml diluted solution. These steps were repeated one more time to produce at least three samples of bee pollen, i.e., 100 mg/ml, 10 mg/ml and 1 mg/ml, respectively.

The allergen pollen floral mass contained in per millilitre of each sample was deduced using the mass of floral pollen allergen in the bee pollen. Samples were kept at 5 °C and were used within five days.

2. Measurement of skin reactivity to floral pollen allergen contained in bee pollen:

Skin prick tests were duplicated on the inner side of the forearms of 10 subjects. Patients (three women/seven men) aged between 19 and 33 (mean: 25.3) had been referred for seasonal symptoms (rhino-conjunctivitis and/or asthma) produced in spring. They were recruited in the south of France. They were not hyposensitized and were positive