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ORIGINAL ARTICLE

Allergenicity of Gramineae bee-collected pollen is proportional to its mass but is highly variable and depends on the members of the Gramineae family

C. Nonotte-Varly

Centre Hospitalier Marie-José Treffot, Avenue du Maréchal Juin – BP 50082, 83407 Hyeres Cedex, France

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Abstract
Background: Gramineae bee-collected pollen is identified as being at the origin of allergic accidents but the biological potency of Gramineae bee-collected pollen is not well known. Cereal grasses (e.g., Zea) and European wild forage grasses (FG) are contained in bee-collected pollen.
Method: In this experiment, Zea-mass and FG-mass were identified in bee pollen mass and the proportion of Zea and of FG was calculated using the bee pollen melissopalynology spectrum. Skin reactivity to Zea and to FG were assessed by measuring wheal diameters (*W*) from skin prick tests using three serial dilutions of bee-collected pollen on 10 allergic patients to Gramineae, in order to calculate the relationship between Zea mass ($Mass_{zea}$) or FG mass ($Mass_{FG}$) in bee pollen and skin reactivity.
Results: The linear function $Log_{10}(W_{FG}) = 0.24(Log_{10}(Mass_{FG})) + 0.33$ ($R = 0.99$) was established using a bee pollen sample with 0.168 mg of FG pollen per mg. The linear function $Log_{10}(W_{zea}) = 0.23(Log_{10}(Mass_{zea})) + 0.14$ ($R = 0.99$) was established using a bee pollen sample with 0.983 mg of Zea pollen per mg.

Gramineae allergens seem to be little altered by bee secretions. Gramineae bee pollen retains its allergenic capacity but it depends on the members of the Gramineae family.
Conclusions: To our knowledge this is the first time it has been shown that skin reactivity to Gramineae is proportional to the absolute Gramineae mass contained in the bee-collected pollen and that it depends on the members of the Gramineae family.

E-mail address: allergologue@limperialsante.net

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Introduction

Pollen is flower sperm. It is the only source of certain macronutrients 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 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 into pellets 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] Ricciardelli D'Albore and Intoppa[3] have listed all the families of plants in Europe visited by bees.

Some floral pollen in bee products is responsible for allergies. Anaphylactic accidents related to the use of bee products are on the increase. There is substantial literature supporting this observation (e.g., 4).

In summer, Gramineae represent pollen suppliers for honeybees at locations where cereal grasses (e.g., Zea) or wild grasses (e.g., animal forage grasses (FG)) are an important pollen source. 55% for Corn and 15% for FG are the percentages of studies in which Zea pollen and FG pollen are ranked among the five most common pollen sources.[5] Corn pollen proteins display specific IgE cross-reactions with FG pollen allergens.[6]

Gramineae pollen bee-collected 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,[7] e.g. Zea lipid transfer protein (LTP) which is highly homologous with the peach LTP, the major allergen of the Prunoideae subfamily.[8]

Gramineae 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.[9]

To our knowledge, however, there is currently no technical definition of the allergenic potential of Gramineae in bee-collected pollen. The purpose of this study is to define the biological potency of pollen of two Gramineae genera (Zea and FG) in bee-collected pollen in vivo by skin prick tests on patients allergic to Gramineae pollen.

Material and methods

Analysis of bee pollen spectrum

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

Such an analysis defines the 1 to n types (pn) and the 1 to n percentage (%pn) of the ''n'' botanical genus or family floral pollens, and determines the total mass of floral pollen ($\text{Mass}_{\text{pollens}}$).

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.[10]

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

Ten grams of well-homogenised bee-collected pollen were dissolved and washed in distilled water, centrifuged, 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 ''$\text{Mass}_{\text{p-allergen}}$'' in bee pollen

Calculate the volume ''V_{pn}'' of each of the 1 to n types of floral pollen ''pn'' from the bee pollen spectrum:

The formula $V_{\text{pn}} = (4/3)\pi r^3$ is used if the pollen grain is spherical, or the formula $V_{\text{pn}} = (4/3)\pi e^2 l$ 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.

Calculate the proportion of volume ''$P_{\text{p-allergen}}$'' of flower pollen allergen ''p-allergen'':

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

%pn is the percentage of flower pollen ''pn'' observed in the bee pollen analysis.

Calculate the mass of floral pollen allergen ''$\text{Mass}_{\text{p-allergen}}$'':

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

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 sensitisation to bee specific allergens.

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 homogenised 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 homogenised with a stirrer at maximum speed for 1 min. Samples were stored at room temperature for 24 h and homogenised 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.

The allergen pollen floral mass contained 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.

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 (six women/four men) aged between 18 and 46 (mean: 28.9) had been referred for seasonal symptoms (rhino-conjunctivitis and/or asthma) produced in spring and early summer. They were recruited in Hyères, in the south of France. They were not hyposensitised and were positive skin prick tested with a commercially available pollen mixture of five grasses extract (Stallergènes). They were sensitised to rPhl p1/rPhl p5b by testing for specific IgE-antibodies (>0.38 kui/l). In addition to Gramineae, they were sensitive to mites (5), cats (2), cypress (6), olive (2) and parietaria (1) but none were sensitive to birch, to artemisia and to ambrosia and none had a history of bee sting reactions or food allergy.

Informed consent was obtained from each patient.

Skin reactivity was assessed by geometric measuring of the two largest wheal diameters observed 20 min after the pollen sample prick tests, positive (histamine 10 mg/ml) and negative (glycerinated saline) controls and commercial extract tests (Stallergenes pollen mixture of five grasses 100 IR/ml).

$W_{\text{p-allergen}}$ was defined by geometric measuring of skin reactivity to floral pollen allergen contained in bee pollen.

Analysis of the relationship between skin reactivity to floral pollen allergen in bee pollen $W_{\text{p-allergen}}$ and floral pollen allergen mass $\text{Mass}_{\text{p-allergen}}$
If the model curve was a power regression:

$$(W_{\text{p-allergen}}) = b(\text{Mass}_{\text{p-allergen}})^{a}$$

then the linear function was calculated as follows:

$$\text{Log}_{10}(W_{\text{p-allergen}}) = A(\text{Log}_{10}(\text{Mass}_{\text{p-allergen}})) + B$$

where A and B are specific pollen allergen constants.

Variance analysis was performed by calculating R^2, which records the results of the value dispersions associated with regression. The closer R^2 is to 1, the more the total variance is explained by the linear regression.

Results

Calculation of Mass_{FG} of bee pollen

Our bee pollen has a floral pollen allergen: FG. It was collected in July 2013 in Thezillieu (France) at GPS location: *X* 45.8833, *Y* 5.6.

Its spectrum includes 6.1% FG, 46.1% Robinia, 22.9% Brassicaceae, 19.6% Clematis, 3.4% Trifolium, 1.5% Cornus (<0.4% undetermined).

FG, Robinia, Brassicaceae, Clematis, Trifolium, Cornus are spherical pollens. Their respective diameters are 35, 23, 20, 22, 25 and 58 μm.

Indeterminate fractions are ignored.

Calculation of ''V_{pn}'' volumes:
FG: $V_{\text{FG}} = (4/3)\pi(35/2)^3 = 22{,}438\ \mu^3$
Robinia: $V_{\text{robinia}} = (4/3)\pi(23/2)^3 = 6367\ \mu^3$
Brassicaceae: $V_{\text{brassicaceae}} = (4/3)\pi(20/2)^3 = 4187\ \mu^3$
Clematis: $V_{\text{clematis}} = (4/3)\pi(22/2)^3 = 5572\ \mu^3$
Trifolium: $V_{\text{trifolium}} = (4/3)\pi(25/2)^3 = 8177\ \mu^3$
Cornus: $V_{\text{cornus}} = (4/3)\pi(58/2)^3 = 102{,}109\ \mu^3$

Calculation of ''P_{FG}'' proportion:

$$P_{\text{FG}} = \frac{V_{\text{FG}} \times \%\text{FG}}{(V_{\text{FG}} \times \%\text{FG}) + (V_{\text{robinia}} \times \%\text{robinia}) + (V_{\text{brassicaceae}} \times \%\text{brassicaceae}) + (V_{\text{clematis}} \times \%\text{clematis}) + (V_{\text{trifolium}} \times \%\text{trifolium}) + (V_{\text{cornus}} \times \%\text{cornus})}$$

$$= \frac{22{,}438 \times 6.1\%}{(22{,}438 \times 6.1\%) + (6367 \times 46.1\%) + (4187 \times 22.9\%) + (5572 \times 19.6\%) + (8177 \times 3.4\%) + (102{,}109 \times 1.5\%)} = \frac{136{,}872}{817{,}006} = 0.168$$

Calculation of ''Mass_{FG}'':

$$\text{Mass}_{\text{FG}} = P_{\text{FG}} \times \text{Mass}_{\text{pollens}} = 0.168 \times 1\ \text{mg} = 0.168\ \text{mg}$$

There was 0.168 mg of FG pollen per mg of bee pollen.

Calculation of Mass_{zea} of bee pollen

Our bee pollen has a floral pollen allergen: Zea. It was collected in July 2013 in Villers le sec (France) at GPS location: *X* 47.5994, *Y* 6.21917.

Its spectrum includes 49.3% Zea, 24.3% Apiaceae, 22.7% Matricaria, 3.4% Trifolium, <0.4% undetermined.

Zea, Apiaceae, Matricaria, Trifolium are spherical pollens. Their respective diameters are 95, 25, 23, 28 μm.

Indeterminate fractions are ignored.

Calculation of ''V_{pn}'' volumes:

Zea: $V_{zea} = (4/3)\pi(95/2)^3 = 448{,}693\ \mu^3$

Apiaceae: $V_{Apiaceae} = (4/3)\pi(25/2)^3 = 8177\ \mu^3$

Matricaria: $V_{Matricaria} = (4/3)\pi(23/2)^3 = 6367\ \mu^3$

Trifolium: $V_{Trifolium} = (4/3)\pi(28/2)^3 = 11{,}488\ \mu^3$

Calculation of ''P_{zea}'' proportion:

$$P_{zea} = \frac{V_{zea} \times \%zea}{(V_{zea} \times \%zea) + (V_{apiaceae} \times \%apiaceae) + (V_{matricaria} \times \%matricaria) + (V_{trifolium} \times \%trifolium)}$$

$$= \frac{448{,}693 \times 49.3\%}{(448{,}693 \times 49.3\%) + (8177 \times 24.3\%) + (6367 \times 22.7\%) + (11{,}488 \times 3.4\%)} = \frac{221{,}206}{225{,}029} = 0.983$$

Calculation of ''$Mass_{zea}$'':

$$Mass_{zea} = P_{zea} \times Mass_{pollens} = 0.983 \times 1\ mg = 0.983\ mg$$

There was 0.983 mg of Zea pollen per mg of bee pollen.

Calculation of $Mass_{hedera\ helix}$ of bee pollen

Our bee pollen is a pure, unique, floral pollen, Hedera Helix (99%; indeterminate percentage <0.9%). It was collected in September 2013 in Thezillieu (France) at GPS location: *X* 45.8833, *Y* 5.6.

This is a spherical pollen with a diameter of 25 μm.

Calculation of volume ''V_{pn}'':

Hedera helix : $V_{hedera\ helix} = (4/3)\pi(25/2)^3 = 8177\ \mu^3$

Calculation of proportion ''$P_{hedera\ helix}$'':

$$P_{hedera\ helix} = \frac{V_{hedera\ helix} \times \%hedera\ helix}{V_{hedera\ helix} \times \%hedera\ helix} = \frac{8177 \times 99\%}{8177 \times 99\%} = 1$$

Calculation of ''$Mass_{hedera\ helix}$'':

$$Mass_{hedera\ helix} = P_{hedera\ helix} \times Mass_{pollens} = 1 \times 1\ mg = 1\ mg$$

There was 1 mg of Hedera helix pollen per mg of bee pollen.

Measurements of skin reactivity to FG, Zea and Hedera helix pollen and analysis of the relationship between ''$W_{p\text{-}allergen}$'' and ''$Mass_{p\text{-}allergen}$''

Skin prick test results with three 10-fold dilutions of bee pollen with 0.168 mg of FG pollen per mg, with 0.983 mg of Zea pollen per mg and with 1 mg of Hedera helix pollen per mg are shown in Table 1.

Skin reactivity and forage grasses:

The model dose–response curve of FG bee pollen is a power regression:

$$W_{FG} = 2.16(Mass_{FG})0.24 \quad R^2 = 0.99$$

The dose–response curve power regression is shown in Fig. 1 and the linear function is:

$$Log_{10}(W_{FG}) = 0.24(Log_{10}\ (Mass_{FG})) + 0.33 \quad R = 0.99$$

The dose–response curve linear function is shown in Fig. 2.

Skin reactivity and Zea:

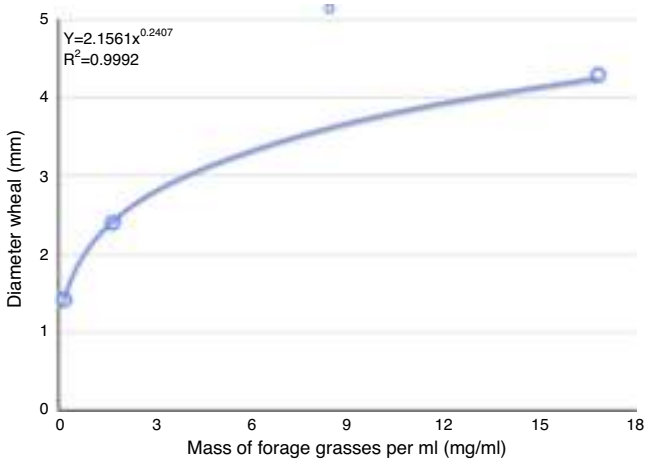

Figure 1 Dose–response curve power regression between W_{FG} and $Mass_{FG}$.

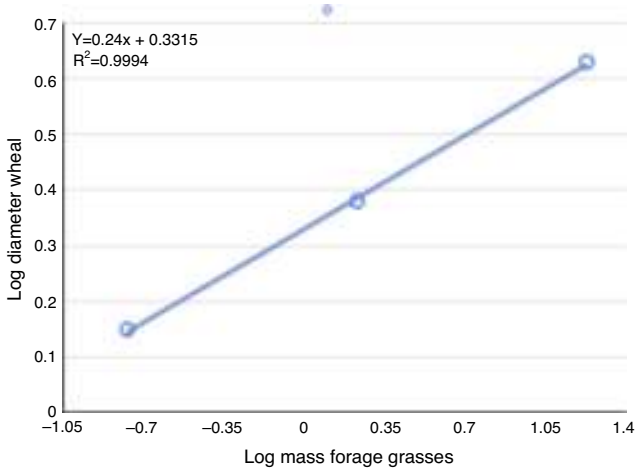

Figure 2 Dose–response curve linear regression between Log W_{FG} and Log $Mass_{FG}$.

Table 1 Skin prick test results with three 10-fold dilution of bee pollen with 0.168 mg forage grasses pollen per mg, 0.983 mg Zea pollen per mg or with 1 mg of hedera helix pollen per mg.

Patient	Forage grasses*				Zea*		Control*		Hedera helix**		
	16.8 mg/ml	1.68 mg/ml	0.17 mg/ml	98.3 mg/ml	9.83 mg/ml	0.98 mg/ml	Positive	Negative	100 mg/ml	10 mg/ml	1 mg/ml
P1	3.87	2	1	2.45	1.41	1	6.48	0	1	0	0
P2	4	1.73	1	4	3.46	1.73	4.24	0	0.5	0	1
P3	4.47	3	1.41	3.87	1.73	1	4.24	0	0.5	0.5	0
P4	7.48	4.47	2.83	8.49	6	3	6.92	0	0	0	0.5
P5	6.48	3	1.73	3.46	2	1	8.48	0	0	0.5	1
P6	2.45	1	1	2.45	1.73	1	7.93	0	0.5	0	1
P7	4.90	3	1.41	5	2.83	1.73	3.87	0	1	0	0.5
P8	4.47	3.87	2.45	5	4	2	6.92	0	0	0	0
P9	5.92	2.83	1.41	3.87	2	1.41	8.48	0	0	0.5	1
P10	2	1.41	1	4	1.73	1	6.92	0	0.5	0	0.5
Mean wheal	4.29	2.40	1.42	4.00	2.42	1.38	6.45	0	0.4	0.15	0.55

* Geometric mean wheal (mm).
** Mean wheal (mm).

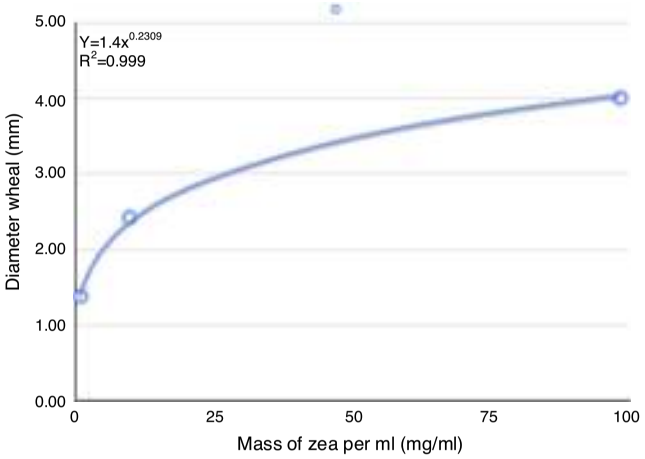

Figure 3 Dose–response curve power regression between W_{zea} and $Mass_{zea}$.

The model dose–response curve of Zea bee pollen is a power regression:

$$W_{zea} = 1.4(Mass_{zea})0.23 \quad R^2 = 0.99$$

The dose–response curve power regression is shown in Fig. 3 and the linear function is:

$$Log_{10}(W_{zea}) = 0.23(Log_{10}(Mass_{zea})) + 0.14 \quad R = 0.99$$

The dose–response curve linear function is shown in Fig. 4.

Skin reactivity and Hedera helix:

The model dose–response curve of Hedera helix bee pollen is not a power regression:

$$W_{hedera\ helix} = 0.38Mass_{hedera\ helix} - 0.07 \quad R^2 = 0.06$$

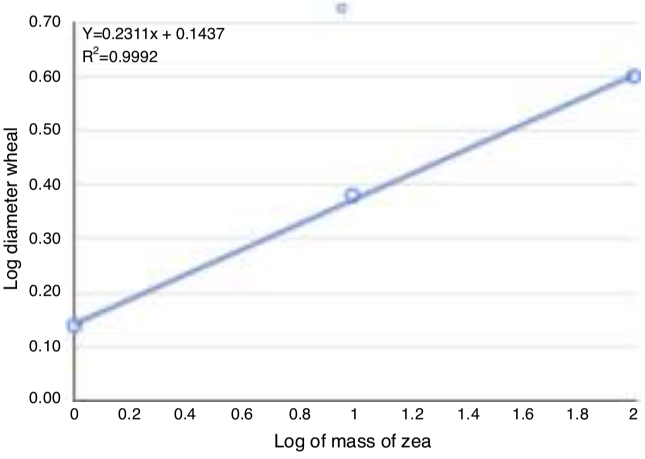

Figure 4 Dose–response curve linear regression between Log W_{zea} and Log $Mass_{zea}$.

Discussion

Forage grasses and Zea Mays are plant species that provide bees with pollen but not nectar. Patients sensitised to Gramineae pollen, e.g., to timothy grass[11] who ingested bee products may experience an immediate allergic reaction.[11] It might be caused by cross-reaction between Gramineae bee product pollen and airborne Gramineae pollen.

However, patients who are allergic to bee products (honey, royal jelly, bee pollen) may also be sensitised to honeybee secretion proteins, pollen proteins contained in bee products[12] or bee venom components.[13] This is why we tested our patients with bee pollen not containing airborne pollen allergens, which was used as a bee pollen negative control. This was to eliminate skin sensitisation to allergens other than Gramineae allergens (i.e., bee specific component allergens). Our bee pollen negative control was 100% Hereda helix bee pollen. Hedera helix pollen is not a common allergic pollen. In some rare cases, it might be responsible for cross-reaction to pollen panallergens among Mexican allergic patients with dermatitis.[14]

None of our patients had positive skin prick reactions to Hedera helix bee pollen. No relationship was established between Hedera helix bee pollen and skin reactivity.

A honeybee collects pollen grains at maturity from the male organs of flowers in order to obtain certain proteins or lipids. It gathers using an elaborate strategy based on pollen research of the highest quality for optimal protein and nutrient collecting. It takes advantage of the plant fertilisation mechanisms in order to attain its objective, which is why the bee is not interested in wet pollen. As with floral nectar, wet pollen swells on contact with the secretions of sugar-water pollen grains that then release the soluble nutrient content. Based on comparisons between hand and bee-collected pollens, it appears that half or more of the mass of bee-collected pollens can be attributed to the addition of nectar-derived sugars to the pollen.[15] The protein content of the grain decreases and this causes a leakage of the proteins in the external environment.[16]

It seems that the forage grasses bee pollen sample and the Zea bee pollen contain protein allergens that are exclusively Gramineae allergens:

Robinia, Brassicaceae, Clematis, Trifolium, Cornus pollens were contained in forage grasses bee pollen sample. Apiaceae, Matricaria, Trifolium pollens were contained in the Zea bee pollen sample. To our knowledge they are common in bee pollens. Literature searches in Medline were performed and no paper has described these pollens, except Matricaria, as being allergic pollens when they are included in bee pollens.

This fact should be compared with what we know of the allergen qualities of these pollens:

- Clematis, Trifolium and Cornus are strictly entomophilous pollens and are not known as allergenic pollens.
- Robinia belongs to the Fabaceae family. The prevalence of Robinia sensitisation is 49% among the patients with pollinosis in Spain[17] but it is likely to be due to cross-sensitisation to panallergens from other common pollens.[18]
- Brassicaceae pollen allergens are well known in cabbage, oilseed rape or mustard and might cross-react with foods.[19,20] Sensitisation to rapeseed pollen is correlated to exposure level[21] but oilseed rape pollen allergy is below 2% even in areas of high production,[22,23] and the symptoms may be due to both allergens and irritant potentials of oilseed rape.[24] In addition, our forage grasses bee pollen was harvested in a mountain pasture area where there is no rapeseed or mustard cultivation.
- Apiaceae are well known in spice allergy and pollen-food syndrome. Sensitisation to Apiaceae is frequent but allergy to spices is below 2% of the totality of food allergies and risk factors are sensitisations to mugwort or birch.[25] Apiaceae might cause oral allergy syndrome related to Bet v1 family PR10 and of severe allergic symptoms[26] related to LTP.[27] Our Zea bee pollen had a very small amount of Apiaceae (0.85 mg per 100 mg) and our patients were not sensitive to birch, to artemisia and to ambrosia and none had a history of food allergy.
- Asteraceae that attract bees, e.g., Matricaria, have been found to cross-react with the Allergenic anemophilous Asteraceae, e.g., mugwort and ambrosia. Mugwort and Asteraceae are implicated in the origin of bee-collected pollen-induced accidents.[13] Patients sensitised to Ambrosia have developed in 50% of cases systemic allergic reactions after ingestion of bee-collected pollen that contained Asteraceae pollen.[28] Cross-reactivity within Asteraceae family seems to be a major mechanism of bee-collected pollen-induced anaphylaxis.[29] Our Zea bee pollen had a very small quantity of Matricaria (0.64 mg per 100 mg) and our patients were not sensitive to artemisia and to ambrosia.

Furthermore, there is no Robinia, Brassicaceae, Clematis, Trifolium, Cornus, Apiaceae or Matricaria pollen in the analysis of the contents of the pollen traps of the French aerobiology network in the area neighbouring Hyères.

It seems that our bee pollen samples with Gramineae contain Gramineae protein allergens:

In the literature, a strong correlation has been noted between cutaneous reactivity to bee pollen containing Gramineae pollen and the cutaneous reactivity in patients with a positive skin prick test to a Gramineae commercial extract.[9] Pitsios et al. found that approximately 55% of patients were sensitised to both bee pollen and floral Gramineae pollen. They considered that it might be due to Gramineae pollen in their samples, which contained 20 mg of bee pollen per ml of solution.

This correlation was observed in their five bee pollen samples, but only two melissopalynology analyses of bee pollen samples have shown Gramineae pollen. This might be due to the qualitative and quantitative methods used to analyse bee pollen. Only five spherules of different tinges were chosen from each bee pollen sample. Tinge loads are subjective. Colours change with time, if the loads are dry or are exposed to sunlight.[30] Many plant species have pollen loads with very similar colours and sometimes up to three

colours are observed for a single genus.[2] Gramineae pollen is often a minor bee pollen and choosing five pellets can raise the risk of non-homogenised samples.

On the contrary, our bee pollen was analysed using the standard European melissopalynologycal method recommended by the International Commission for Bee Botany.[10] This method is based on the study of 10 g of well-homogenised bee pollen and 10 g composed of more than 1000 pellets.

Our bee pollen samples are rich in Gramineae pollen, with 6.1% and 0.168 mg of FG pollen per mg for the first sample, and 49.3% and 0.983 mg of Zea pollen per mg for the second sample. Quantifying the absolute mass of Gramineae pollen with bee pollen per gram requires knowing the pollen spectrum of bee pollen and measuring pollen grain sizes. More particularly, this requires knowing pollen sizes when in contact with aqueous fluids. In contact with water, the pollen grain is in osmotic shock. Hydrated grain results in a change of its volume and opens pores and fissures[15] depending on the recalcitrance and orthodoxy of the pollen.[31]

Furthermore, two pollens of the same genus can have different reactions, e.g., *Helianthus annuus* pollen is orthodox and swells in contact with water, whereas *Helianthus tuberosus* pollen maintains the same volume.[31]

Strong relationships were established between the absolute mass of the two genera of Gramineae pollen in bee pollen and skin reactivity despite our patient group including a small number of individuals sensitised to pollen mixture of five grasses extract and to rPhl p1/rPhl p5b.

The first dose–response curve (forage grasses) was a power regression curve:

$$W_{FG} = 2.16(\text{Mass}_{FG})0.24 \quad R^2 = 0.99$$

from which we were able to deduce the linear curve:

$$\text{Log}_{10}(W_{FG}) = 0.24(\text{Log}_{10}(\text{Mass}_{FG})) + 0.33 \quad R = 0.99$$

The second dose–response curve (Zea) was a power regression curve:

$$W_{zea} = 1.4(\text{Mass}_{zea})^{0.23} \quad R^2 = 0.99$$

from which we were able to deduce the linear curve:

$$\text{Log}_{10}(W_{zea}) = 0.23(\text{Log}_{10}(\text{Mass}_{zea})) + 0.14 \quad R = 0.99$$

Gramineae allergens in bee pollen appear to be little or not altered by bee secretions and the allergens retain their allergenic capacity. In fact, the bee secretions contain digestive enzyme sugars[32] but are devoid of proteases. There is no protein digestion, as salivary and hypopharyngeal glands do not produce proteolytic enzymes.[33]

It seems that the allergenicity of Gramineae bee-collected pollen depends of the members of the Gramineae family:

It is remarkable to note the two linear curves have similar slopes (0.24 versus 0.23). In contrast, the constant terms are different (0.33 versus 0.14). This fact should be compared with what we know of the allergen qualities of Gramineae pollens because our patients were sensitised to rPhl p1/rPhl p5b by testing for specific IgE-antibodies (>0.38 kui/l).

The major and most widespread allergenic component of grass pollen are the group I grass allergens, e.g., Zea m1 from Zea Mays and Phl p1 allergens of Timothy, an example of forage grass pollen.[34] Phl p1 cross-reacts with most grasses and corn-derived group 1 allergens.[35] Zea m1 has been reported to have 72% sequence identities to Phl p1.[36] Zea m1 has been reported to be responsible for cross-reactivity to grasses growing in temperate climate zones.[36] Maize pollen also contains Zea m2 and Zea m3 from the minor group 2 and group 3 grass allergens and Zea m5, a group 5 grass allergen, another major allergenic component of grass pollen. A monoclonal human IgE antibody has been shown to cross-react with group 5A allergens from several grasses and Corn.[37]

But cross-reactivity between Zea m1 and other derived Group 1 allergens grasses, and between Zea m5 and other derived group 5 allergens grasses, has been shown to be highly variable, and might be high with specific grasses rather than grasses in general.[38] Cross-reactivity for group 1 allergens did not imply the same for group 5 allergens[38] and Zea mays might in fact not contain group 2 and group 5 allergens.[39] The differences found in maize and other grasses reflect the fact that maize belongs to the Andropogneae tribe of the Panicoideae subfamily, while the animal forage grasses belong most likely to the Poeae and Agrostideae tribes of the Pooideae subfamily.[40]

Conclusion

To our knowledge this is the first time it has been shown that the skin reactivity of patients who are allergic to Gramineae is proportional to the absolute Gramineae mass contained in bee pollen and that it depends on the members of the Gramineae family.

Further studies are needed to determine how Gramineae allergens retain their allergenic qualities.

Conflict of interest

The author declares no conflict of interest

Ethical disclosures

Protection of human subjects and animals in research. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical

Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

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