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Ragweed pollen in France: origin, diffusion, exposure

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Summary

Purpose of the study: *To detect the origin of ragweed pollen and to measure the impact of this pollen exposure on allergic patients, so their sensitivity can be noted (using specific IgE production: sIgEw1) in order to inform the population about an "allergy" against those ragweed pollen grains.* ***Material and methods:*** *To measure population exposure to ragweed pollen, the R.N.S.A (National Aerobiological Monitoring Network, a French association) has a pollen trap network located in urban areas. These traps allow continuous recording of airborne pollen, the light microscope analysis (with a bi-hourly time step) allows one to know the daily concentrations of ragweed grains and the circadian rhythm of grains impaction. It is thus possible to follow the evolution of pollination during each day of each season and to compare seasons and years at each station. Biomnis is a biological Laboratory which performs more than 85% of ragweed specific IgE assay in France. It seems to be clear that when allergists ask ragweed IgE for a patient, it is because they think that this patient seems to be allergic to this specific pollen. The statistical analysis of results about specific IgE (for ragweed) from the Allergology laboratories Biomnis (located in Lyon and Paris) can determine the number of patients sensitized to ragweed in French departments.* ***Results:*** *The distribution of sensitized patients to ragweed is compared to ragweed pollen distribution studied by the R.N.S.A from the year 2005 to 2008 in France, whatever the ragweed plant' origin: local (closed to pollen trap) or imported (by wind).* ***Conclusion:*** *The biological database (Health impact) allows a correlation between the geographical distribution of ragweed pollen and the number of patients with specific IgE against ragweed (sIgEw1), i.e whose sensitization is due to local plants. That also permits one to estimate the expected number of allergy cases in the next years, because the sensitivity precedes the allergy.*

Introduction

Native North America *Ambrosia Artemisiifolia L.* (ragweed) was accidentally introduced in the late 19th century in Europe (1).

First, ragweed was strewed as a ruderal plant (derived from the Latin *rudus, ruderis*, rubble, meaning a plant growing spontaneously in the wasteland, the rubble along roads, often close to inhabited areas) (2). As a species able to develop on many soil types, its expansion is favoured by human activities through long-distance transport. As an invasive plant, it is now widespread in France (3) and in many European countries: Northern Italy, Switzerland, Sweden, Russia, Hungary, Ukraine, Spain etc. (4-6).

This is an annual plant that belongs to the family *Asteraceae*. Besides, ragweed may produce up to 2.5 billions pollen grains per plant (7). These phenomenal quantities of pollen grains are produced according to weather condi-

tions between late July and mid October, with maximum in August and September. These pollen grains are easily airborned due to their low density (0,63) and their low sedimentation velocity (1,56 cm/second) (8).
According to Girsh (9) and Cecchi (10), the ragweed pollen grains are able to cover a distance of 65 kilometers, and up to few hundreds kilometers (e.g. southern Hungary to Pistoia in Italy) depending on weather conditions, before setting down. Pollen emission by *Ambrosia artemisiifolia L.* occurs following a diurnal rhythm: the opening of the anthers takes place mainly in early morning, under the effect of temperature increasing and humidity decreasing (8). From his studies in the United States, Fischbach (11) advances that 45% of pollen grains are emitted between sunrise and noon (8).
The study of the circadian rhythm of ragweed pollen grain impaction allows one to estimate the either local or exogenous origin of seedlings accountable to the release of these pollen grains, and has been the topic of the first part of the study (12).
Moreover, the health impact of air content in ragweed pollen depends mainly on population exposure and the local origin of the seedlings. The diagnosis of allergy is based on skin tests or IgE antibodies assays in the serum, testifying the immune response (13). The statistical analysis of results from specialized laboratories for ragweed specific IgE assays gives a good representation of the patient number sensitized to ragweed in French departments: this study of health impact will be the second part of the study.
A correlation of observations and results of the two parts of the study will be conducted to highlight the link between the extent of ragweed pollination at spatial and temporal levels, with the direct health impact on the population, in order to detect sensitization and consequently to prevent allergy.

Materials and methods

Pollen metrology
Circadian rhythm

A pollen trap network, located so as to cover a wide background (urban areas, buildings rooves), representative of the air that people breathe, has been established by the R.N.S.A (National Aerobiological Monitoring Network). The trap number is close to seventy, and these traps are distributed throughout the French territory. The traps, Hirst type (14), are aspirants weathervanes allowing the continuous impaction of pollen grains. The pollen grains are fixed on Melinex tape coated by coating medium (fluid silicone [polydimethylsiloxane, Trimethylsiloxi-terminated] and carbon tetrachloride [CCl4]). Then, this sample is prepared on a slide with a colored-medium (gelatin, glycerin, phenol, basic fuschin, 90° alcohol, phenol and distilled water). The readings and analyses are carried out by optical microscopy (x40 objective lens) on two fictitious axes located at 4.5 and 9.5 mm from the top of the sample (coated and colored Melinex tape), to record data with a bi-hourly time step (Figure 1). The orientation, reading and counting are controlled by computer programs: C. Scope®, Stamp® and Via-Voice®.

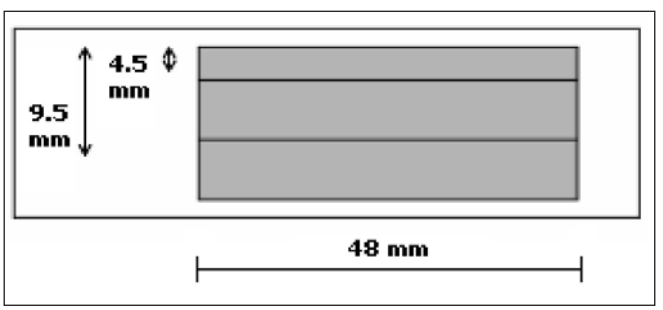
Total exposure of the population

The analysis of ragweed pollen grains impaction is performed for each trap, by one or several analysts belonging to R.N.S.A (about 70 analysts are recorded throughout France). The results of these analyses are in the form of bi-hourly daily/weekly data. They lead to highlight the circadian rhythm for seasonal and annual cumulative total for one or many specific pollens (in this study, only ragweed pollen is considered). A comparison of these results can then be performed, and is accessible to the general public via mapping.

Ragweed specific IgE assay

The ragweed specific IgE assay was perfomed by CAP System Phadia technique (Phadia 100) in both laboratories Biomnis Paris and Biomnis Lyon. This assay method is based on the antigen-antibody properties using secondary monoclonal antibodies (Anti-species Immunoglobulin) coupled to ß-galactosidase. Monoclonal antibodies are produced by hybridoma cells that originate from fusion between mouse myeloma cells and immune

Figure 1 - Slide caracteristics for 24 hours of impaction. The bi-hourly time step is about 48 mm, with a scale of 2 hours per 4 mm



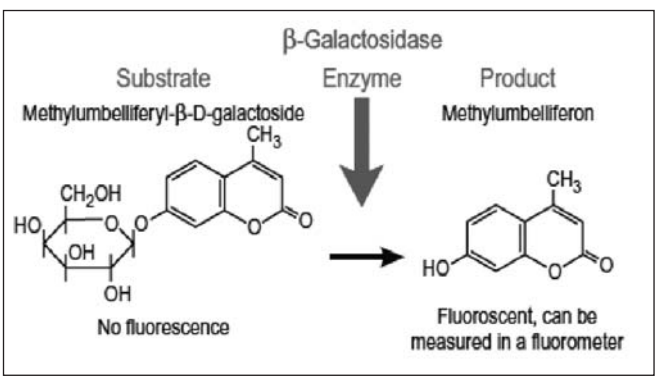
mouse spleen b-cells. Specific Immunoglobulin E w1 (sIgE w1) from human myeloma is used as immunogen.
The function of the substrate is to generate a measurable fluorescent product in the assay system. The 4-Methyl-umbelliferyl-β-D-galactoside is non-coloured and non-fluorescent. The enzyme β-galactosidase, which is the label molecule in the anti-IgE conjugate, splits off the galactose part from the substrate and the remaining Methylumbelliferon is a fluorescent molecule. The concentration of fluoroscent Methylumbelliferon generated is proportional to the concentration of IgE molecules in the sample.
Sera arriving to laboratories (Biomnis Paris and Biomnis Lyon) throughout the year derived from investigated patients with suspicions about ragweed allergen sensitizations, throughout France.

Results

Circadian rhythm

According to analysis and pollen counts from the traps in Valence and Dijon cities, two typical graphs are obtained (Figures 3 and 4) showing the circadian rhythm of ragweed.
The circadian rhythm of ragweed in Valence (Figure 3) shows a main peak of pollination between 7 a.m. and 1 p.m.
The circadian rhythm of ragweed in Dijon (Figure 4) shows pollen peaks spread over the day, with a maximum located about 4 p.m.
The maps below are derived from data stored in the R.N.S.A database. The bi-hourly data of the station and pollination characteristics (local pollen found on the morning while temperatures begin to rise) of ragweed allowded to delimit geographical areas where the plant is present.

Figure 2 - Action of the substrate with ß-Galactosidase: release of a fluorescent product



These maps show a migration of the distribution of ragweed pollen origin in France between 2006 and 2008, with an important area located in the Rhone Corridor. An unequal distribution is observed: the South of France is indeed is more affect.
In 2006, ragweed has been particularly abundant in the Lyon area, in the Dauphiné, in the Rhone Valley from Lyon to Montélimar and, in north from Lyon to Dijon and from Lyon to Nevers. A very low presence can be noted, without symptoms, in Pays de Loire, Charente, Midi Pyrénées and Mediterranean areas.

Figure 3 - Circadian rhythm of ragweed in Valence, for summer 2008

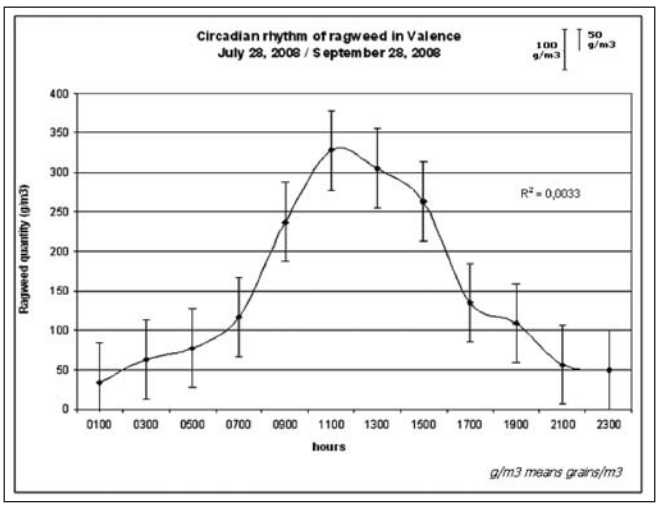
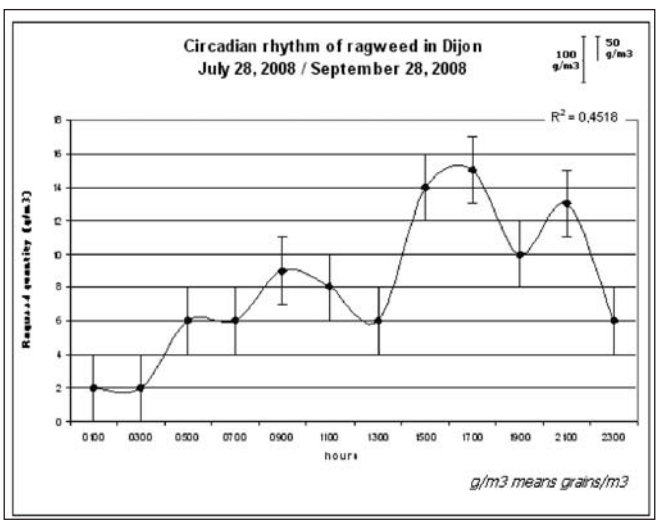


Figure 4 - Circadian rhythm of ragweed in Dijon, for summer 2008



Even if 2007 was not a « record-year » for ragweed pollens, allergic risk was high in Rhône Alpes, Auvergne and the south of Rhone Valley. High concentrations have been found in Charente and significant concentrations between Agen and Toulouse.
If the start of ragweed pollination in 2008 was ahead of schedule (late July), the number of raining days would have significantly reduced the allergic risk. However, it remained very high in Rhône Alpes and high in neighboring area.
The comparison of obtained maps with phenological observations maps, allows validating the interest of circadian rhythm study to assess the level of infestation of an area by the ragweed plant.

Total exposure of the population

Data used (analysis and pollen counts) allows monitoring of the evolution of exposure to ragweed pollens on the territory. Cumulative daily totals of pollens define areas of distribution of ragweed pollen grains collected by the trap.
A decreased amount of ragweed pollen is found in France between 2006 and 2008. Moreover, the maps (Figure 6) show the progress of the areas concerned by ragweed pollens in France between these two dates. The North of France is not concerned by this progress.

Ragweed specific IgE assay

Results are expressed by the absence or presence of specific circulating IgE (sIgE) to ragweed (w1). The number of French patients who have been screened between 2005 and 2008 is presented in Table 1.
The number of patients sensitized or not appears rather constant during these four years (1800 persons per year). In addition, specific circulating IgE to ragweed are found in less of 50% screened cases (≈ 47%).
In Table 2, the distribution of patients is indicated in some selected French department in percentages (number

Figure 5 - Distribution of the origin of ragweed pollen in France from 2006 to 2008

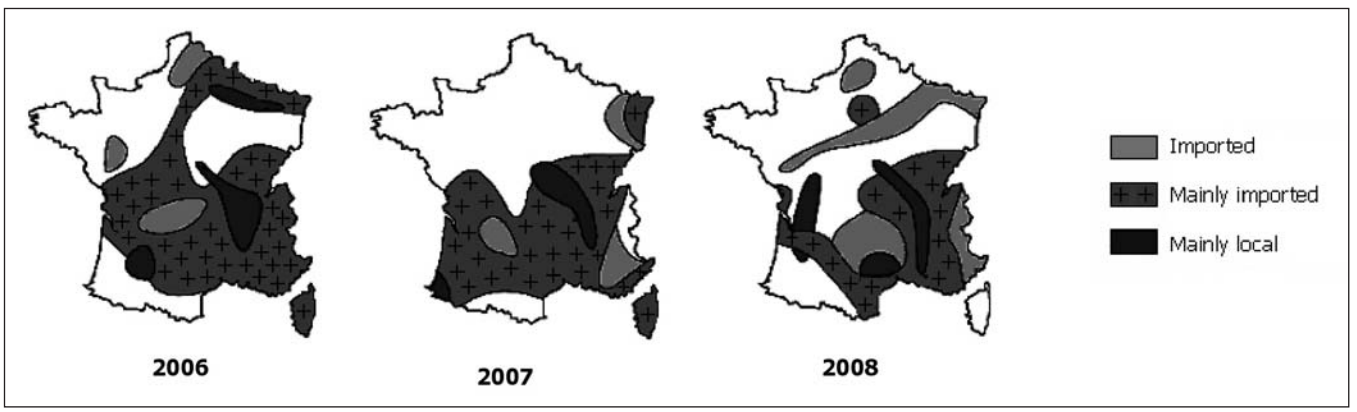


Figure 6 - Distribution of ragweed pollen (in total quantities) in France in 2006, 2007 and 2008

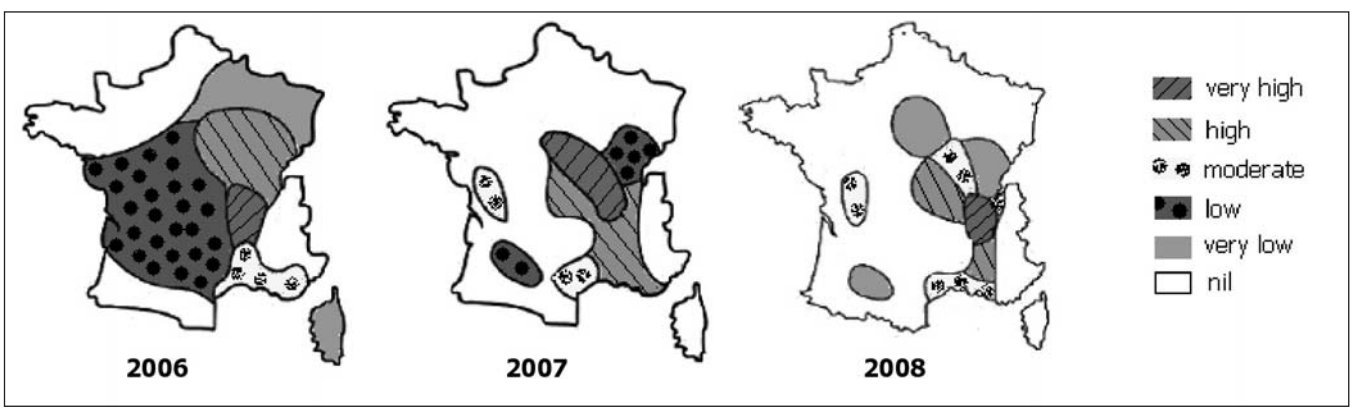


Table 1 - Number of patients sensitized or not in France, from 2005 to 2008

Number of patients	2005	2006	2007	2008
total screened	1709	1757	1876	1766
without sIgE w1	920	918	1000	950
with sIgE w1	789	839	876	816
% with sIgE w1/ total screened	46,2%	47,8%	46,7%	46,2%

Table 2 - Distribution of sensitized patients to ragweed in key departments in France from 2005 to 2008

Department		2005	2006	2007	2008
Ain	***01***	***3.04***	***5.84***	***4***	***3.68***
Allier	03	1.14	1.19	1.6	1.72
Ardèche	***07***	***2.79***	***1.67***	***2.74***	***2.7***
Bouches du Rhône	***13***	***2.15***	***1.79***	***1.6***	***1.84***
Drôme	***26***	***11.53***	***10.25***	***11.53***	***11.4***
Gard	30	2.03	2.86	2.28	7.38
Isère	***38***	***10.39***	***10.61***	***11.3***	***9.8***
Loire	***42***	***0.38***	***2.15***	***3.42***	***4.9***
Loiret	45	1.14	0.6	0.11	0.12
Manche	50	1.01	0.36	0.34	0.25
Nord	59	1.01	0.12	0.11	0.37
Rhône	***69***	***35.23***	***43.5***	***41.89***	***41.3***
Saône et Loire	71	0.89	0.48	1.14	1.59
Savoie	73	1.14	0.6	1.14	1.1
Paris	75	2.28	1.19	0.46	0.98
Yvelines	78	1.01	0.36	0.34	0
Var	83	0.63	0.95	1.71	1.1
Vaucluse	***84***	***3.8***	***2.15***	***3.2***	***3.8***
Vienne	86	1.01	0.48	0	0.12
Hauts de Seine	92	1.52	0.36	0.34	0.12
Val de Marne	94	1.27	0.36	0.46	0.25
Val d'Oise	95	1.01	0.24	0.23	0.37
Rhone Corridor	***01-07-13-26-38-42-69-84***	***66.52***	***76.29***	***76.94***	***79.42***

of patients with sIgE W1 in the department/total number of patients in France with sIgE W1)

In bold and italics, are written the 8 departments belonging to the Rhone Corridor (Table 2).

Few departments have a very low number of sensitized patients to ragweed (Loiret, Manche, Nord, Yvelines, Vienne, Hauts de Seine, Val de Marne, Val d'Oise), contrarily to others, all located in the Rhone Corridor (Rhône, Drôme, Isère).

From results presented in Table 2, three maps were constructed (Figure 7). To simplify, the map only includes data for the years from 2006.

This distribution of sensitized patients to ragweed in France between 2006 and 2008 shows clearly the importance of Rhone Valley, with Rhône department (69) lodging more than 30% of French patients secreting sIgE W1.

Discussion

About the circadian rhythm, in Valence (department of Drôme) the graph (Figure 3) shows a main peak of pollination between 7 a.m. and 1 p.m. As pointed out by Fischbach (11) as well as by C. Déchamp and H. Méon (8), the opening of the anthers of ragweed plant takes place mainly in the early morning under the temperature increasing effect and humidity change. This peak is consistent with the time of dispersion of ragweed pollen grains since diurnal temperatures increasing: this fact confirms the local origin of ragweed plants in this city. In contrast, the circadian rhythm in Dijon (department of Côte d'Or) shows (Figure 4) more spread pollen peaks in the day, with a maximum located about 4 p.m. These late arrivals to traps show a mixture of ragweed pollen from local plant and imported pollens from remote areas.

Distribution of ragweed pollen origin in France from 2006 to 2008 (Figure 5) shows a migration of the origin with a critical situation in the Rhone Corridor. Distribution indeed of ragweed pollen origin is uneven on the French territory, with a larger amount of pollen in the South of France than in the North. Other regions don't seem to remain constant during these three last years.

Regarding ragweed specific IgE assay (sIgE w1), numbers of patients sensitized or not were rather constant during the years 2005, 2006, 2007 and 2008, with an average about 1 800 (Table 1). In addition, sIgE w1 are found in less than 50% of screened patients (47%).The distribution of patients studied in Table 2 and Figure 6 for some key departments shows a high concentration of individuals secreting sIgE w1 within the Rhone Corridor, with 66,52% in 2005 and 79,42% in 2008 of French patients producing sIgE w1.

A strong analogy is seen between Figures 5 and 6. After comparison indeed, the Rhone Corridor appears as a common area and very important on both. This region contains probably a significant amount of ragweed plants (pollen origin mainly local) and is the French region containing the largest number of patients sensitized to rag-

Figure 7 - Distribution in France of patients sensitized to ragweed from 2006 to 2008

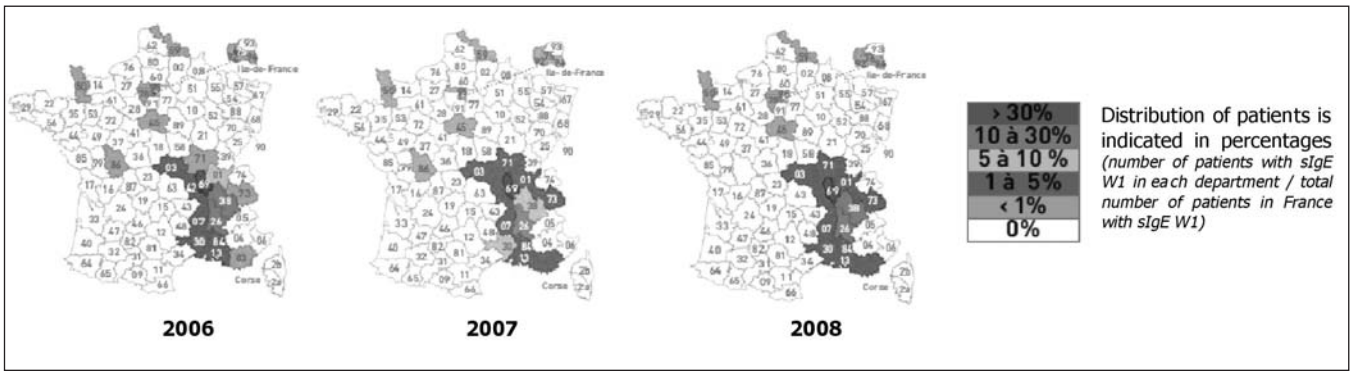
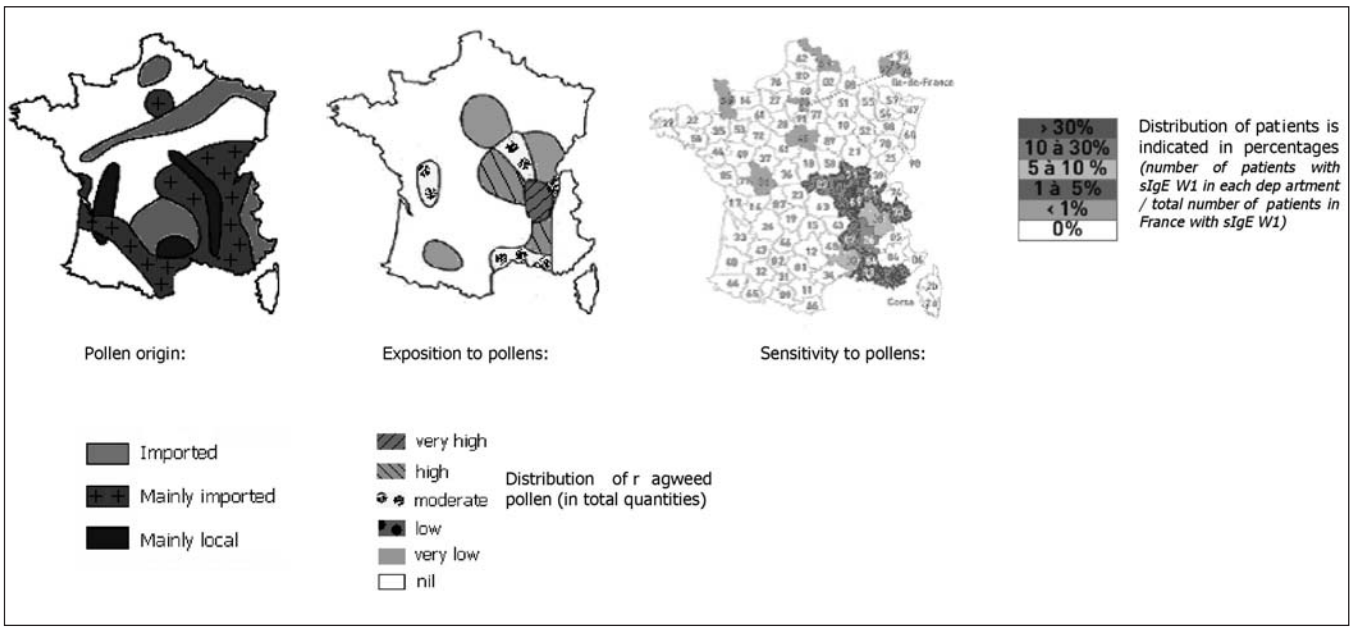


Figure 8 - Distribution of ragweed pollen origin (left), distribution of ragweed pollen in total quantities (middle) and distribution of patients sensitized to ragweed (right), in 2008 in France.



weed, producing sIgE w1. *It is important to note that numbers obtained of sensitized patients are undervalued in relation to reality, because only a subset of patients were examined. Only patients potentially allergic according to allergists have been screened on the one hand, and on the other hand, not all patients potentially allergic visited an allergist.*

A cause-and-effect relationship between the presence of pollen grains of local ragweed and the population sensitivity (with sIgE w1 secretion) is in the Rhone region is indisputable.

These maps confirm the relationship between « local pollen » and « population sensitivity », that is to say between the local presence of ragweed plants and the health impact caused.

Raweed pollen counts in non invasive areas but coming from invasive areas don't produce allergy symptoms.

Conclusion

The traps network established by the R.N.S.A (National Aerobiological Monitoring Network) allowed the measurement of the exposure of the French population to ragweed. The statistical analysis of results about IgE assay

(specific to ragweed) from laboratories (specialized in allergology) Biomnis (located in Lyon and in Paris) allowed one to assess the number of patients sensitized to ragweed in the French departments.

The distribution of sensitization to ragweed with the distribution of ragweed pollen studied by the R.N.S.A for years 2006 to 2008, reveals an undeniable cause-effect relationship between the presence of pollen grains of local ragweed and the sensitivity of population (sIgE w1 secretion) within the Rhone Corridor. Considering the migration of the distribution of ragweed pollen origin in France, and with work conducted by Dr. Peter Gumowski (INRAAIC_Switzerland), a growing number of sensitized and allergic individuals to ragweed pollen is feared in France over the years to come…

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