Study Group
Environment, Migration, Epidemiology, Occupational Diseases of the Italian Society of Allergy and Clinical Immunology (SIAIC)
Italian Association of Aerobiology (AIA)

Review of Allergy and Clinical Immunology
2013;23 - 14-20

Key words
Allergens • Outdoor monitoring • Pollen

# The European project HIALINE (Health Impacts of Airborne Allergen Information Network): results of pollen and allergen of Betula monitoring in Parma (2009)

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***Summary***

**Introduction.** Exposure to allergens is pivotal in determining sensitization and allergic symptoms in individuals. Pollen grain counts in ambient air have traditionally been assessed to estimate airborne allergen exposure. However, the exact allergen content in ambient air is unknown. HIALINE therefore monitored atmospheric concentrations of *Betula*, Poaceae and *Olea* pollen grains and matched their major allergens Bet v1, Phl p5 and Ole e1 across Europe. Monitoring the allergens themselves together with pollen in ambient air might be an improvement in allergen exposure assessment. New knowledge through the use of new experimental approaches in the field of aerobiological monitoring will enable better in the prevention and clinical management of pollinosis. In order to disseminate the knowledge of the project we present the results of first year of birch pollen grains and the matched major pollen allergen Bet v1 monitored in Parma (UNIPR), Italy with a short reference to the results obtained by the other participants and about developing models of dissemination and forecasts of pollen and allergens.

**Materials and methods.** The pollen was sampled by a Hirst pollen trap. Allergens was collected with a CHEMVOL® high-volume cascade impactor, extracted from pollen and quantified by ELISA. Antibodies for analysis of Bet v1 are delivered by the industrial partner in this project. Quality control has been carried out for the monitoring pollen activities and allergen concentrations.

**Results.** The project has highlighted that it is possible also to measure pollen allergen in ambient air in different European areas. The results obtained from the center of Parma and other European partners have highlighted the different allergenic powers of pollen of *Betula*, in different geographical areas. Moreover, daily in each area the allergenic power of pollen grains was very variable.

**Discussion.** HIALINE has been a very important project to understand the mechanisms of sensitization, clinical management of pollinosis and to improve immunotherapy towards a tailored immunotherapy. The results of the project will help medical doctors, authorities and patients, to better manage the different aspects related to pollinosis.

## Introduction

This article does not address the issues concerning the causes of the increased prevalence of allergic respiratory diseases observed in the last three decades and the clinical aspects arising there from, to enter instead some preliminary remarks immediately concerning the topic. First, it is appropriate to underline some aspects of the biology of pollen that may be useful to frame the context in which it has been consolidating the design idea of HIALINE (Health Impacts of Airborne Allergen Information Network). HIALINE was funded by the Executive Agency for Health and Consumers. In the early 1990s were reported evidences for the presence of allergens in respirable particles [1] even at times when the pollen concentration was low or absent. Other studies have shown that allergens of Poaceae are contained predominantly in the cytoplasm of mature pollen [2]. The release of allergens by different types of pollen, such as Poaceae pollen, and the pollen of Fagales, occurs in very different ways [3]. In the first case occurs the loss of integrity of the outward structure while in the second case occurs an abortive germination. This determines different response times to the

stimulus, and in the case of Poaceae already after 5' of contact with rainwater 70% of the pollen releases outwards its content [4]. Moreover the Poaceae dehydrated pollen lose the ability to eject the cytoplasm [5]. Certainly, linked to some of these aspects are the epidemics of asthma that occur during or immediately after storms [6]. Another aspect which needs attention is the role that climate change is playing relatively to the increased prevalence of respiratory allergies [7] and duration of pollination season of some pollen types, (eg. Parietaria with +85 days, Cupressus +18 days and Olea +18 days) [8], with an overall advance of the beginning of pollination. In some cases, the amount of pollen produced is increasing, as is often the case for tree pollen, while in some cases the amount of pollen produced tend to decrease as often happens for the pollen of herbaceous plants. On the other hand, the air pollution and increased concentrations of $CO_2$ can determine qualitative and quantitative changes in pollen allergen content [9-11] and in the capacity of release of pollen. The various species of grasses release their pollen grains at different times during the pollen season, and this information is not visible through the analysis of pollen calendars [12]. This can have a significant effect for the choice of an appropriate approach with specific immunotherapy. An important role in the spread of pollen and consequently of their allergen content is also determined by the long-distance transport as has been demonstrated on several occasions especially in the case of the ragweed pollen [13-15]. In fact, some studies have revealed the existence of interannual and geographic variations and during the same season of the allergen content of pollen of *Betula* taken directly from the catkin and the relationship between concentration of birch pollen and of allergen Bet V1 in ambient air [16 17]. Some studies a little older [18] and other recent studies [19] showed the trends in the concentrations of allergens related to the pollen concentrations. In some cases or at certain times of the observations was found a correlation while in other cases or periods the correlation was less or not evident. Has been shown that Bet v1 concentration, measured in fractions of different sizes, are correlated with the amount of rainfall. In particular, the particles of aerodynamic diameter greater than 7.2 µm are predominant in dry days, and probably represent allergen associated with the pollen. On days with mild rainfall (< 1 mm per day), particles smaller than 7.2 µm increase, representing particles from cytoplasm containing allergens. Among these particles can be included starch granules derived from pollen tubes or broken orbicolae. After heavy rainfall (> 1 mm per day), particles larger than 7.2 µm tend to be "washed" from the atmosphere along with their allergen content. These studies have been very important for understanding some phenomena, but very often the observations were point source, and methodological and instrumental approaches non-standardized were used, with some difficulties in interpretation. Already in 2005 the European project MONALISA (Monitoring Network of Allergens by Immuno-Sampling) [10 21] had tried to overcame it. Only with the european project HIALINE [22] (February 2009-May 2012) was activated a task force on European scale. A project on a European scale facilitates more general results than individual groups can do locally. This, for example, is linked to the climate and vegetation contexts that can be studied simultaneously. In fact, through HIALINE we could use the large climatic differences existing between the participating European countries to identify some of the factors that can affect qualitative and quantitative aspects of the content of allergenic pollen. Only by the identification of the mechanism of action the real impact of climate change on the spread of respiratory allergic diseases could be better understood.

## Materials and methods

For the pollen sampling spore traps type Hirst were used (Fig. 1A) [23]. The method is described in Italy by UNI 11108/2004 [24] and used by the Italian Monitoring Network in Aerobiology (Rete Italiana di Monitoraggio in Aerobiologia, R.I.M.A.®) of the Italian Association of Aerobiology (AIA) which operates since 1985. The pollen concentrations were measured according to methods that are historically different in some countries. For example, three centers have performed three horizontal sweeps (Lyon) or four horizontal sweeps (Munich and Parma). In U.K. 12 vertical sweeps was done, while Finland used the randomized method. The differences between the methods were normalized taking into account the extent of the surfaces read. The season of pollination was defined as the period in which is included the presence from 1% to 95% of total seasonal pollen of *Betula*, in agreement with the recommenda-


**Fig. 1A.** Hirst-type pollen trap.

tions of the European Aeroallergen Network (EAN). The monitoring of the allergens was carried out using solid phase samplers [25] with daily head (CHEMVOL®) and high volume (pump control with microprocessor Digitel DHM-60®) (Fig. 1B). Polyurethane filters pre-treated with buffers of ammonium carbonate have been used. The CHEMVOL® samplers were installed at the same height of the Hirst samplers and not more than 5 m away. In the case of Parma they were 18.2 m above the ground level on the meteorological tower at the University of Parma in the city center (Fig. 1C). The city of Parma is located south of the Po River, about 100 km from the Tyrrhenian coast and about 200 km from the Adriatic coast, 52 m above sea level, latitude 44°48'15 North, longitude 10°19' East. We used two stage version of CHEMVOL® to sample particles between PM10 and PM2.5 and larger than PM10. After extraction of the particulates collected and centrifugation of the washing liquid, the samples were freeze-dryed for 48 hours and stored at -80°C until the time of the immunoassay. Particular attention has been paid to the quality control both in terms of the monitoring and quantification of pollen and as regards monitoring and quantification of allergens. In the first case inter-laboratory checks were performed for the definition of operating range as reported in the literature [26 27]. In the second case intra laboratory controls were performed to assays low and high dosage positive controls. These should not differ to a greater extent of 25% between the various immuno assays performed. The calibration curves were used only in case of linearity of the data (Fig. 2) [28]. Each collected filter was divided into three equal parts. The determinations were carried out on two filter parts and in case there was no consistency between the results was also used the third part for further determinations. For each assay were used three dilutions in duplicate to demonstrate the linearity of the results obtained. In case of failing the assay was repeated amending, as needed, the dilutions, for example in the case of samples too concentrated. The meteorological data (average daily temperature, relative humidity, wind speed and

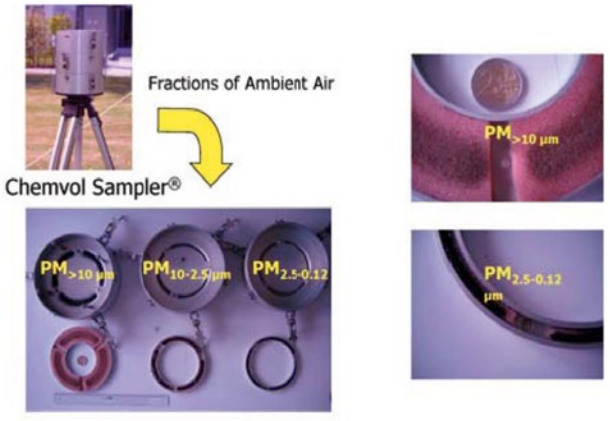


**Fig. 1B.** High volume solid phase sampler.

**Fig. 1C.** Devices of the Parma Monitoring Center.

total daily rainfall) were measured using devices located in the nearness of aerobiological samplers. To process the aerobiological observations to calculate the periods of flowering the System for Modelling of Atmospheric Composition (SILAM) was used. The model evaluated with a time step of 15' the transport of particulate matter for 60 hours back in time every day, in every sampling site. The configuration has worked on 8 vertical layers up to 6 km from the ground. The size of the observational horizontal cells was 25 km and simulations have covered almost the European continent. Weather information were acquired from the operational archives of the European Centre for Medium-range Weather Forecasts (ECMWF). The project involved 15 research team from 11 European countries (Fig. 3): 1) Lyon, Réseau National de Surveillance Aérobiologique de France - RNSA (F), 2) Munich, Center for Allergy and Environment of the Technische Universität München - Zaum (D), 3) Parma - Laboratory of Allergology, Department of Clinical and Experimental Medicine , University of Parma, Unit of Clinical and Medical Immunology, University Hospital of Parma (I), 4) Poznan, Adam Mickiewicz University - AMU (PL), 5) Turku, Aerobiology Unit,

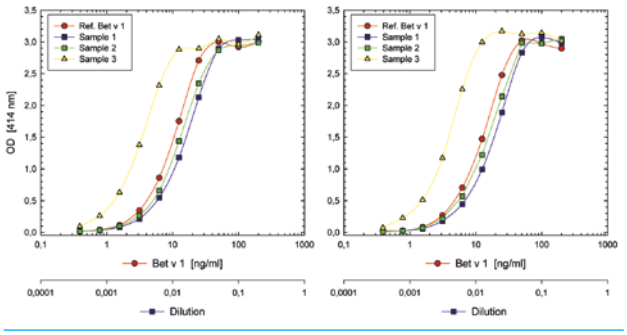


**Fig. 2.** Calibration curve: two examples of calibration curve of the Bet v1 ELISA assay.


**Fig. 3.** Team participating to HIALINE project.

University of Turku (FIN), 6) Vienna, ENTDepartment, Medizinische Universität, (A), 7) Worcester, National Pollen and Aerobiology Research Unit (NPARU), University of Worcester (UK), have worked with the monitoring of pollen from Poaceae and *Betula* and their major allergens Phl p5 and Bet v1. The team of Bursa, Laboratory for Aerobiology and Palynology, Uludag University (TR) [volunteer team], Cordoba, Aerobiology Group of the Department of Botany, University of Córdoba (E), Evora, Centro de Estudios da Mitra, Universidade de Évora (P) have worked with the monitoring of pollen from Poaceae and *Olea* and their major allergens Phl p5 and Ole e1; team of Helsinki, Finnish Meteorological Institute - FIM (FIN) and Vilnius, Siauliai University - SU (LT) directed their work to study forecasting models and dissemination of pollen and allergens. The team of Reinbek, Allergopharma Joachim Ganzer KG (D), industrial partner, has produced monoclonal antibodies, positive controls and reference standards of Bet v1 and Phl p5; the team of Florence, Interdepartmental Centre of Bioclimatology (CIBIC), University of Florence - UNIFI (I) took care the dissemination activities and the team of Munich GABO: milliarium KG (D) managed the administrative project. In March 2009, in Reinbek, laboratories Allergopharma hosted the kick-off meeting to meet members of the various team to work together for a week on various aspects of the project, including the quality control. At the end of February 2010 and 2011 were held respectively in Lyon and in Florence two meetings to discuss the activities carried out, the problems identified and the future activities. In February 2012 were held in Munich the final meeting of the project, that has officially ended its activity on May 2, 2012. Currently, all evaluations of the activity are ongoing, some of which, concerning results already established for 2009 are anticipated as follows.

## Results

The results of the first year of project activity are summarized [29-33]. Due to the problems related to the delivery and installation of equipment for the detection of allergens, the activities of HIALINE 2009 in Parma began with *Betula* pollination season in progress and this has resulted in the loss of some data. Table I shows the parameters of *Betula* pollen and allergens detected in Parma. 93,9% of the allergen of the *Betula* pollen was detected in the fraction PM > 10 µm, similarly to that observed at the other centers (data not shown). In Table II are reported some of the observations made by other partners. The highest number of seasonal pollen was observed in Poland (Poznan) with 10,160 pollen (grains/m$^3$). The lowest number of seasonal pollen was observed in Italy (Parma) with 235 pollen (grains/m$^3$). The highest peak value was recorded in France with 622 pollen (grains/m$^3$). The lowest was observed in Italy with 31 pollen (grains/m$^3$). The peak value of Bet v1 has been detected in Germany with with 2229 pg/m$^3$, while the lowest was observed in Italy with 82 pg/m$^3$. The highest seasonal value of Bet v1 was in Germany with 13,030 pg/m$^3$, the lowest was observed in Italy with 681 pg/m$^3$. In Figure 4 has been showed relationship between concentration of pollen and of allergen detected in Parma. Overall, a good correlation was obtained in the whole season. Only during some period, which can be observed by disaggregating the seasonal data, the correlation was lower. However, perhaps the most interesting data is in Figure 4, which shows significant differences in the daily allergen single pollen grain indicating that the allergen was basically higher when pollen concentrations were lower. Similar results have also

**Table I.** Betula pollen and Bet v1 allergen parameters, measured in Parma during 2009.

| *Year* | *Pollen Day of peak date* | *Pollen Peak value pollen/m$^3$* | *Seasonal sum of pollen grains/m$^3$* | *Pollination Duration days* | *Bet v1 Day of peak date* | *Bet v1 Day of peak pg/m$^3$/24h* | *Bet v1 Annual pollen pg/pollen* | *Bet v1 annual sum pg/m$^3$* | *% Bet v1 > PM 10* |
|---|---|---|---|---|---|---|---|---|---|
| 2009 | 8/4 Season<br>15/4 HIALINE | 31<br>21 | 235 | 48 | 7/5 | 81.99 | 2.569 | 681 | 93.88 |

been observed by other monitoring centers (data not shown). Figure 5 shows the trend of some climatic parameters during the observations of pollen and allergens in Parma (daily relative humidity, average temperature, total rainfall and wind speed). Figure 6 illustrates the differences in allergenic potency observed on an annual basis in different European locations calculated based on the regression curves. The highest allergenic potency was measured in Germany with 3.91 pg/pollen grain of Bet v1, the lowest was observed in the UK with 2.26 pg/pollen grain of Bet v1. The European Bet v1 average stands at a value of 3.2 pg / pollen grain [32].

The development of the SILAM [33] is particularly complex, starting from addressing aspects of the pollen grains. Of course it gets harder when these aspects alongside and overlap those related to the spread of allergens. In fact, the project HIALINE showed that the allergen load of *Betula* pollen was highly variable and tends to be higher when pollen concentrations are low. In this situation becomes more important, even if it is a low percentage, the allergen extracted from the particulate fraction of smaller size (10 µm > PM > 2.5 µm). Consequently it would be very important to understand if this value is derived from free allergen. If this hypotesis is true, diffusion models may be closer to what is observed in reality. When we will have the results of three years, probably many behaviors may be easier to understand.

## Discussion

With HIALINE we were able to build an European network devoted to the measurement of airborne allergens using standardized methods. The monitoring of allergens is then possible on a large scale and, improving technical aspects of sampling and analysis, in a not too far future, will be able to flank the routine aerobiological monitoring. The results obtained in all European monitoring centers showed a close correlation between the presence of pollen and allergen, indicating that the pollen of *Betula* appears to be the main if not the only source of allergen Bet v1. We could show that exposure to aeroallergens does not only depend on the concentrations of pollen in the atmosphere, but also by the amount of allergen content in pollen and that concentrations of allergen for single pollen grain may vary on a daily basis more than 10 times. It will be important to understand if the allergen content is similar in the different countries and in the case it is different for what reason (climatic conditions or other). Equally important are the feedback that can be made after the analysis of the results obtained as compared with Poaceae and *Olea* with Phl p5 and Ole e1. This is related to numerousness of Poaceae species involved, much greater than for example of *Betula,* or to high number of varieties cultivated or spontaneous distributed in the three areas involved in the project concerning *Olea*. It will be very important also to verify if sometimes the observed behavior may be due to long-range transport of pollen with a different allergenic potency from that locally produced.

Further studies on the effects of climate change and on the distribution and seasonality of pollen and their allergenic potency are needed. The results analyzed at the end of the project period of three years could certainly confirm some observations, even if the project was too short to properly evaluate this factor. In addition, it would be very important to being able to make correlations with clinical data which, unfortunately, were not foreseen in the HIALINE project. However, the results obtained during the HIALINE project will open new ways to understand the mechanisms that control the sensitization and the symptoms in patients with pollinosis and could be very useful to improve diagnostic procedures, clinical trials designed to evaluate the effectiveness of new drugs and aspects related to specific immunotherapy, through a molecular view of aerobiology and allergology. In addition, the authorities and individuals will be able to manage with

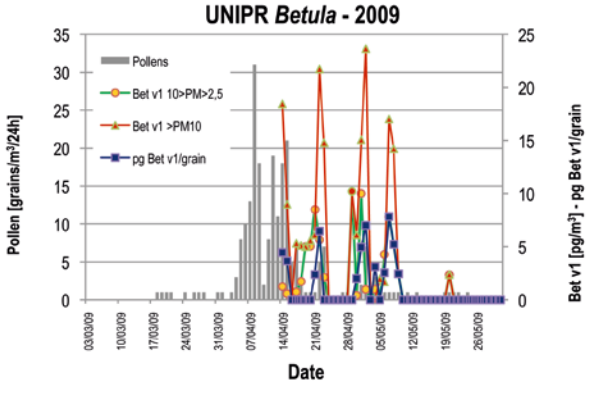


**Fig. 4.** Monitoring season 2009: Betula pollen and Bet v1 allergen. During 2009 the HIALINE detection of allergens, started on April 15.

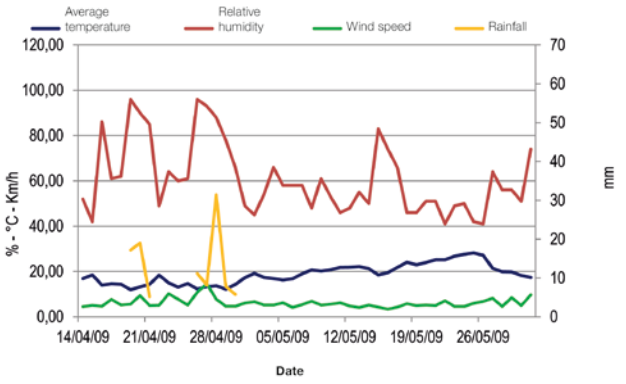


**Fig. 5.** Meteo-climatic parameters during the aerobiological monitoring.

**Table II.** *Betula* pollen and Bet v1 allergen parameters during 2009 in other countries (Buters et al., 2012, mod.) [32].

| | | *UK* | *France* | *Germany* | *Finland* |
|---|---|---|---|---|---|
| *Betula* pollen | | | | | |
| Peak value | grains/m$^3$ | 278 | 622 | 495 | 253 |
| Day of peak value | | 15 april | 14 april | 11 april | 27 april |
| Seasonal sum of pollen grains | Σ grains/m$^3$ | 2586 | 1705 | 3144 | 2923 |
| Duration | days | 24 | 28 | 19 | 30 |
| | | | | | |
| Bet v 1 | | | | | |
| Peak value | pg Bet v 1/m$^3$ | 755 | 986 | 2299 | 978 |
| Seasonal sum | pg Bet v 1/m$^3$ | 5969 | 7374 | 13030 | 7634 |

**Table III.** Allergenic potency of Betula pollen during 2009 in the European countries (Buters et al., 2012, mod.) [32].

| *Nation* | *Bet v1 pg/pollen grain* |
|---|---|
| Germany | 3.91 |
| France | 3.06 |
| Finland | 2.62 |
| Italy | 2.57 |
| UK | 2.26 |

greater awareness maintenance and design of public and private green, helping to improve the biological quality of the air in our cities, that too often is not adequately assessed as it is done with the pollution in general.

## ACKNOWLEDGEMENTS

The authors thank the Italian Association of Aerobiology (A.I.A.) which funded a postdoctoral fellowship in aerobiology. The authors thanks Paolo Fantini, Department of Physics and Earth Sciences (University of Parma) for providing meteorological data.

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