

MedPalyn 2017



Pollens, Allergy and real time information

Michel THIBAUDON, Gilles OLIVER



Index

- **Pollen measurements**
Hirst pollen traps, CIP 10, PMF, Sigma2, SLT
- **Allergen measurements in the air:**
History, Devices, Monalisa project, Hialine project
- **New methods in aerobiology**
A.E.R.O.MEDI, BAA 500, KH3000, Rapid-E, FIDAS 200, modeling (SILAM, COSMO-ART, ZAMG)

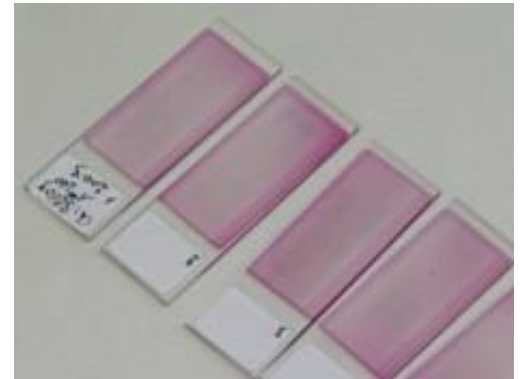
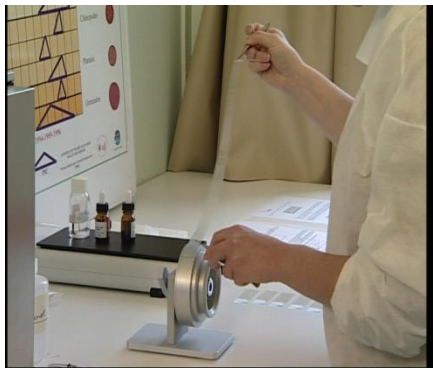
Pollen measurements

HIRST Type pollen trap

Currently standard method in Europe



HIRST Type pollen trap



Pollens concentrations (pollen grains/m³)

HIRST Type pollen trap: Technical specification

prEN 16868

Ambient air - Sampling and analysis of airborne pollen grains and fungal spores for networks related to allergy - Volumetric Hirst method

Portable pollen trap: CIP 10

CIP 10

Developed by the INERIS to answer the need of coalminers. It is autonomous and intended for "dusts" inhaled by human. It declines for the samples of silica, wood, bacteria, pollens, virus, endotoxines and molds.

Principle of functioning

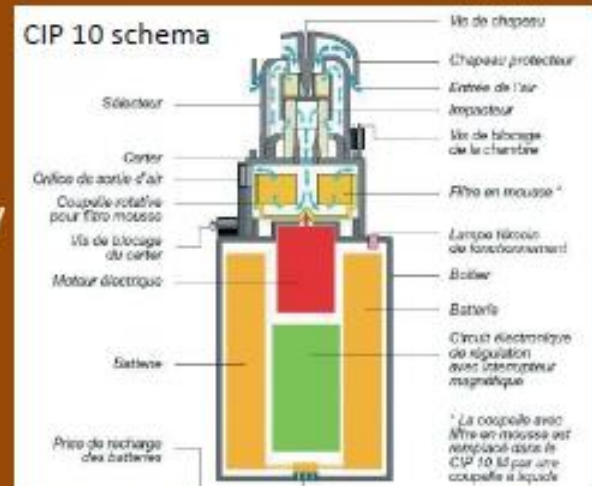
Sampling assured by the rotation of a small dish foams: engender a flow of inhalation of 10 l / mn (similar to the human respiratory flow).

Various analyses can then be practised: destructive, non-destructive, weight, PCR

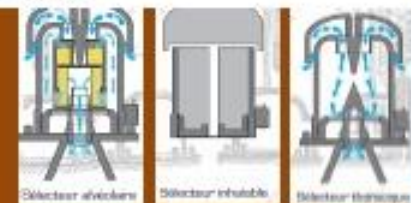
There are various selective heads: alveolar breathable fraction with a flow of 10 L / min, respirable fraction with a flow of 10 L / min, thoracic fraction with a flow of 7 L / min.

For our studies, the choice concerned to respirable heads.

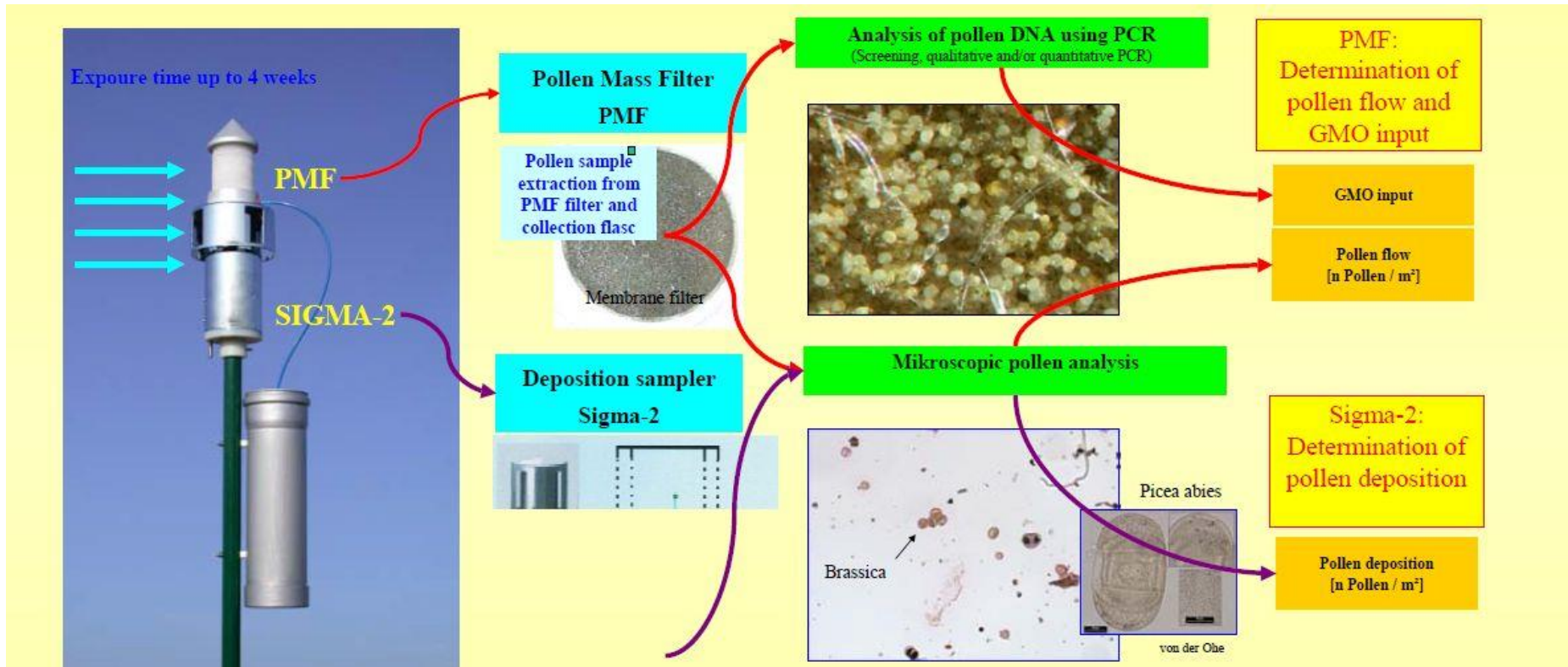
The system of inhalation allows a sampling in all directions.



Exchangeable heads for selection of the fraction of interest



PMF and Sigma 2 Slit



SLT: Sigma2 like trap



Allergen measurements

History

A lot of works about the necessity of airborne allergen measurements :

- Behrendt H et al in Int. Arch Allergy and Immunology - 1992**
 - . « Interaction between aeroallergens and airborne particulate matter »**
- Ghazaly G et al**
 - . « Orbicules in betula pendula and their possible role in allergy » Grana – 1995**
 - . « Localization of the major bet v 1 in betula using monoclonal antibody labelling » Grana -1996**

History

. A.Rantio – Lethimaki et al :

« Airborne birch pollen antigens in different particles sizes »

Clinical Experim. Allergy 1994

. E. Pehkonen and A.Rantio-Lethimaki

« Variation in airborne pollen antigenic particles caused by meteorologic factors » Allergy - 1994

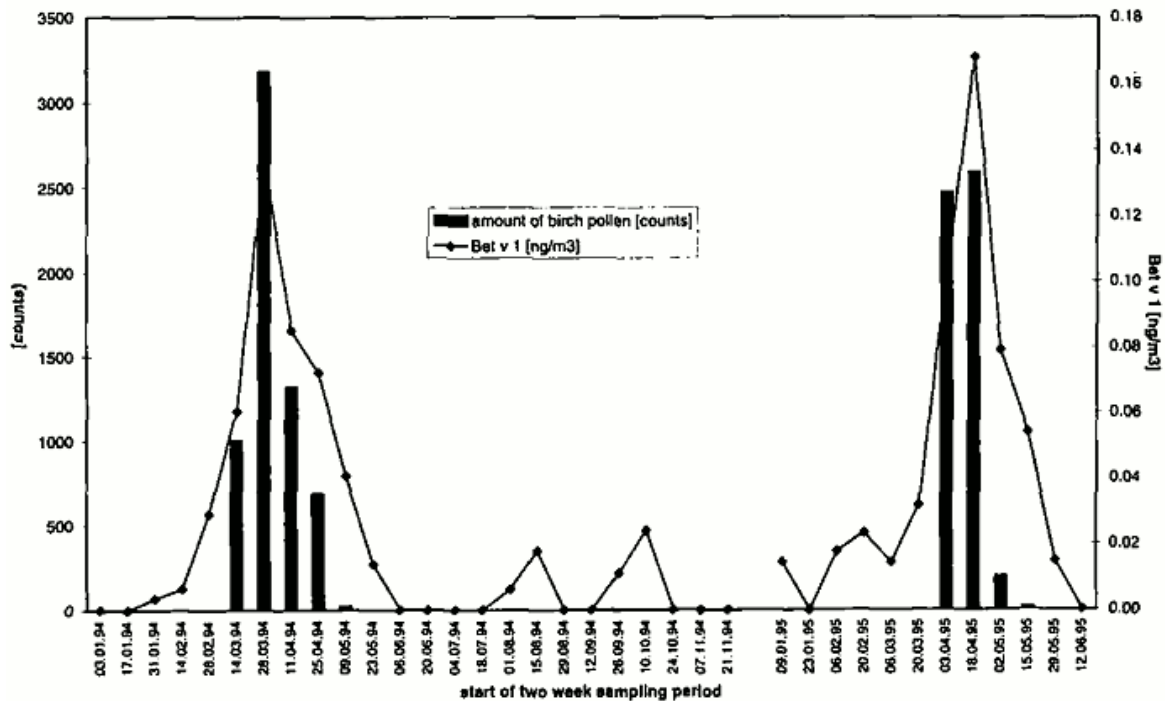
. F. TH. M. Spieksma et al.

« Seasonal appearance of grass pollen allergen in natural, pauci-micronic aerosol of various size fractions. Relationship with airborne grass pollen concentration » Clinical and Experimental Allergy, 1995

History

. GF Schäppi et al. *Aerobiologia* 1996

« Analysis of allergens in ambient aerosols : comparison of areas subjected to different levels of air pollution »



Total Bet v 1 concentrations and the corresponding birch pollen counts in Zürich, 1994 and 1995

History

. G.F. Schäppi,
Source of Bet v1 loaded
inhalable particles from
birch revealed,
Sex plant reprod, 1997

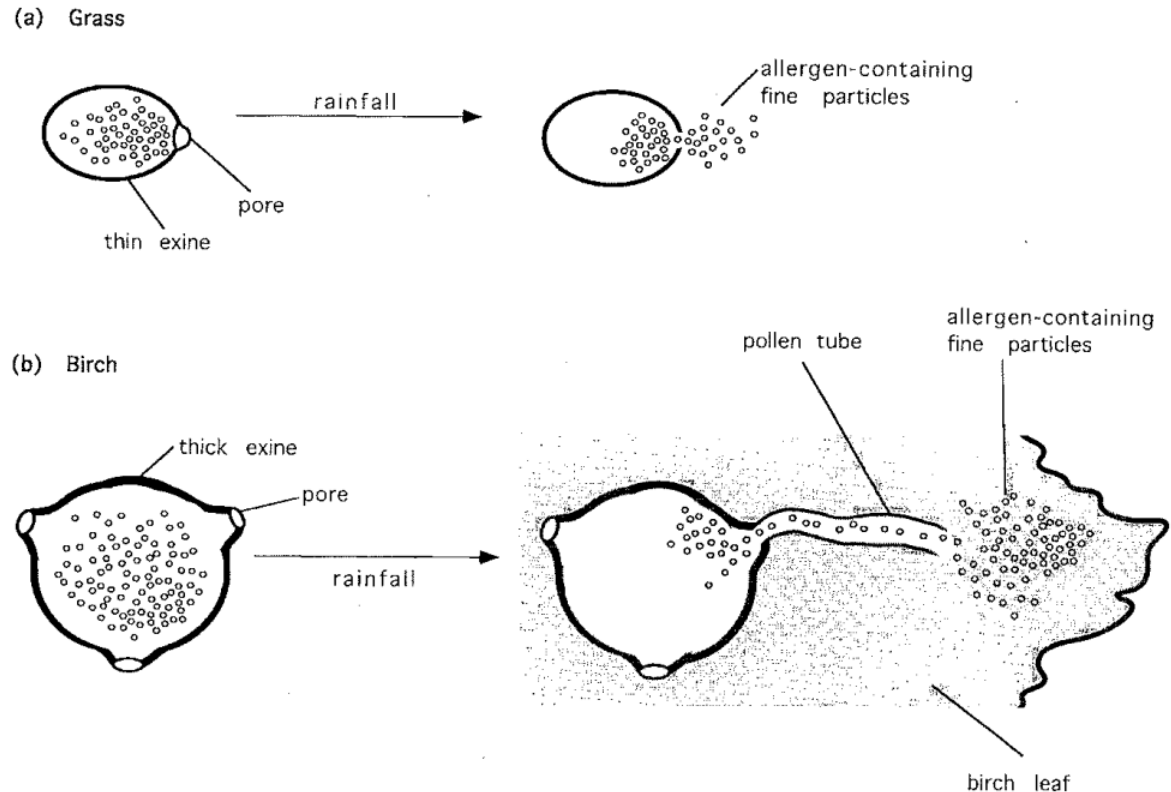


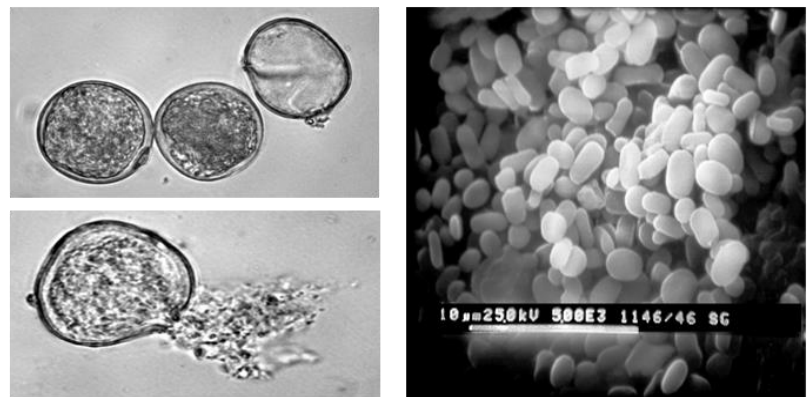
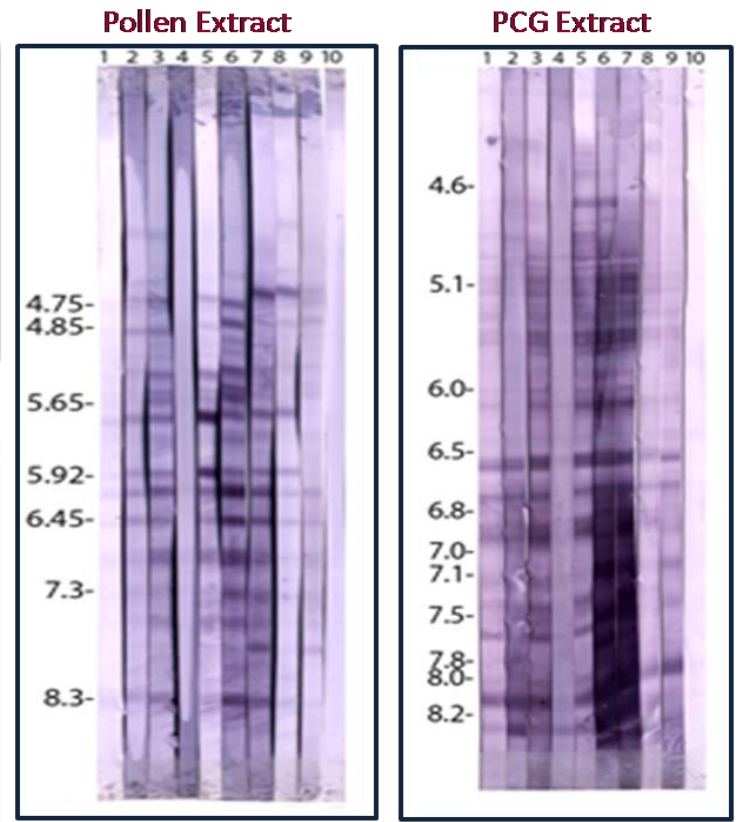
Fig. 1a, b Diagram showing the two pathways for release of natural allergen-containing inhalable particles from pollen grains. **a** A grass pollen grain ruptures in rainwater by osmotic shock, releasing about 700 allergen-loaded starch granules. **b** A birch pollen grain germinates on a leaf; the pollen tube ruptures, releasing about 400 allergen-loaded starch granules

Pollen Cytoplasmic Granules

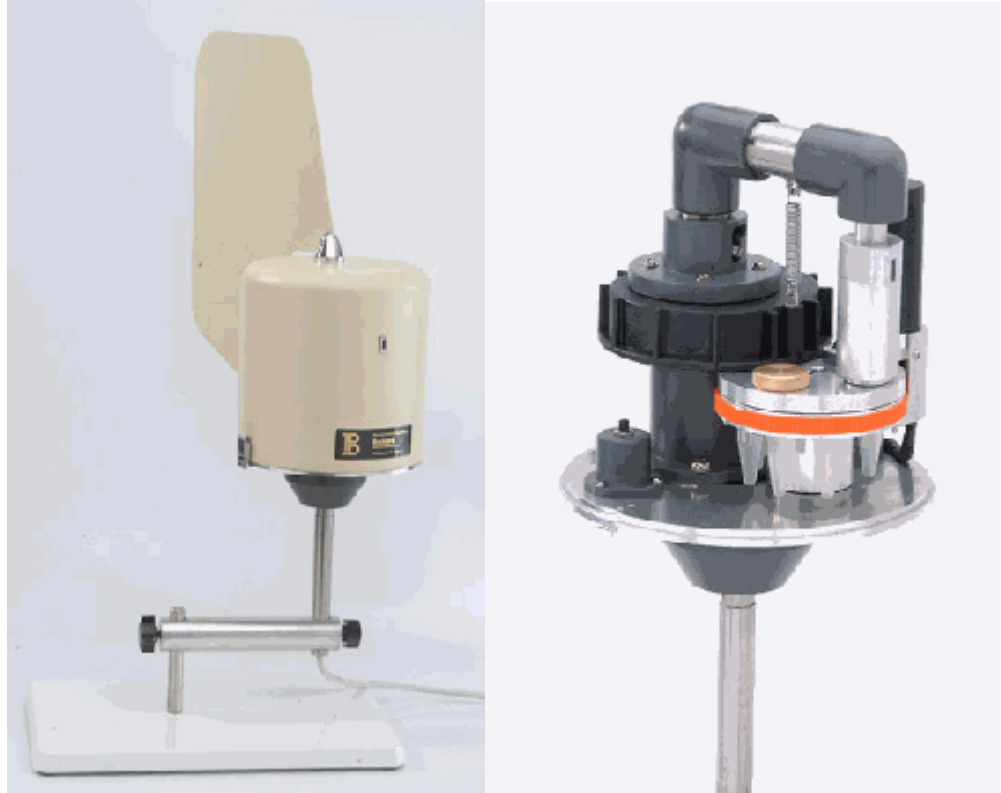
(Results from ABOU CHAKRA Thesis 2009)



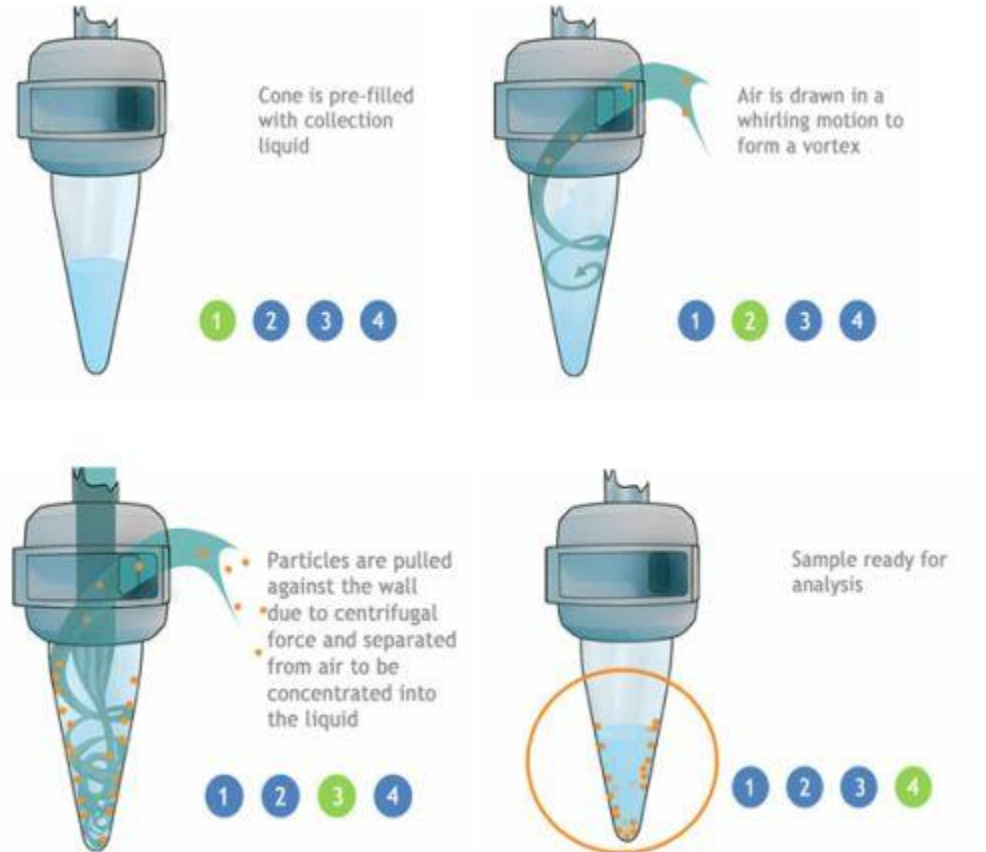
- Water extracts of pollen and PCG
- Protein characterization
 - * IEF (pH 2-11)
- IgE immunodetection
 - * Blotting with sera from grass allergic patients
 - * Antibody anti-human IgE conjugated with AP
 - * Revelation of AP



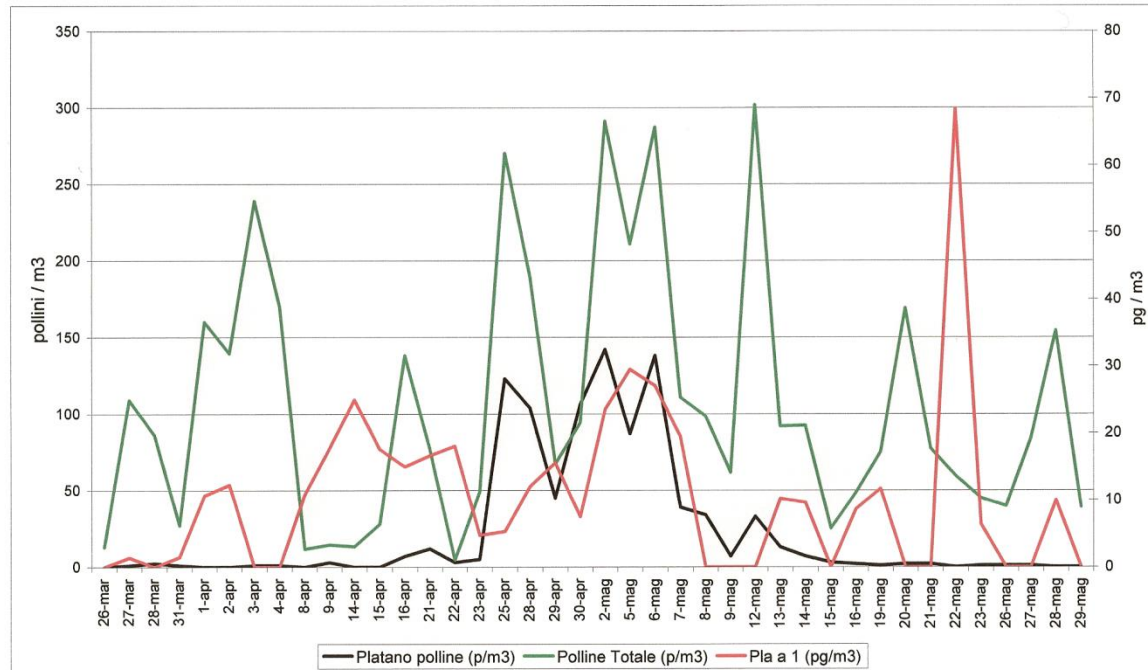
Devices: Burkard Microcyclone



Devices: Coriolis[®] Air Sampler

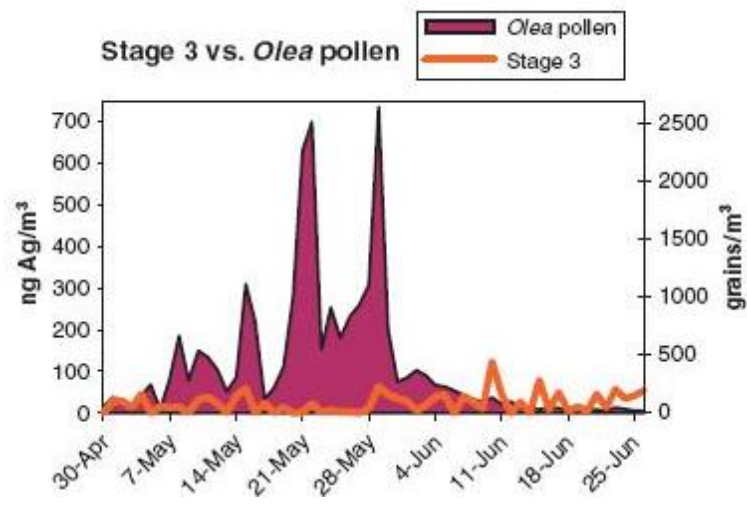
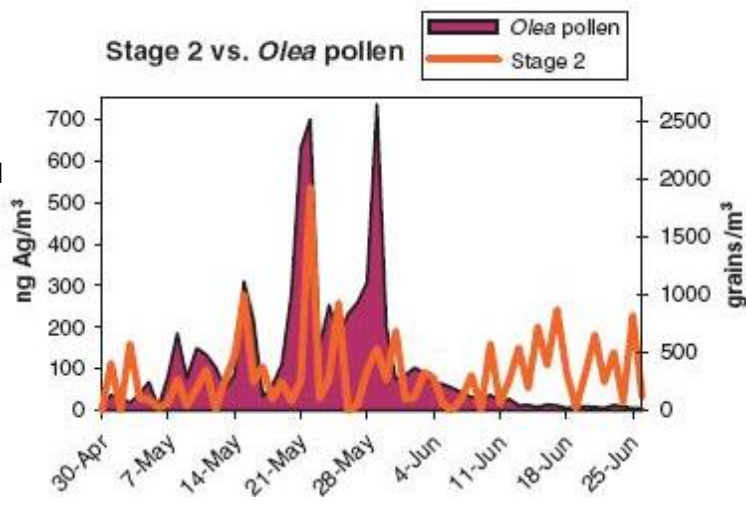
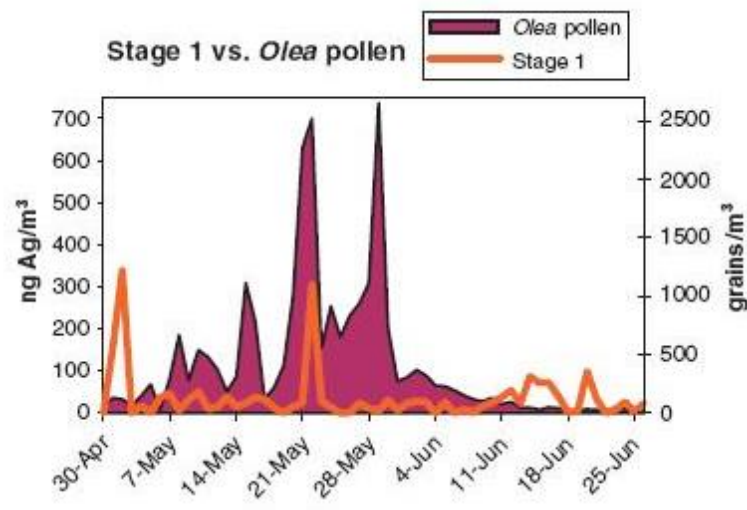
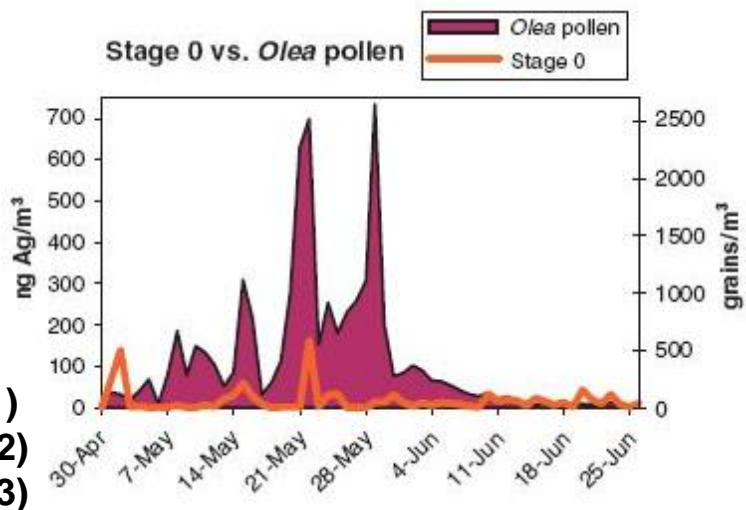


. Fernandez et al. 2009 – GEA – v(1) : 95-97



Platanus pollen count – Total pollen count – Platanus allergen Pla a 1

Comparison between relative *Olea* pollen allergenic activities in different article-size fractions vs. daily fluctuations in the airborne *Olea* pollen counts per cubic metre of air from April to June.



**49 mm (stage 0),
49–5.8 mm (stage 1)
5.8–4.7 mm (stage 2)
4.7–3.3 mm (stage 3)**

**De Linares et al
Clinical and Experimental
Allergy, 37**

Monalisa project



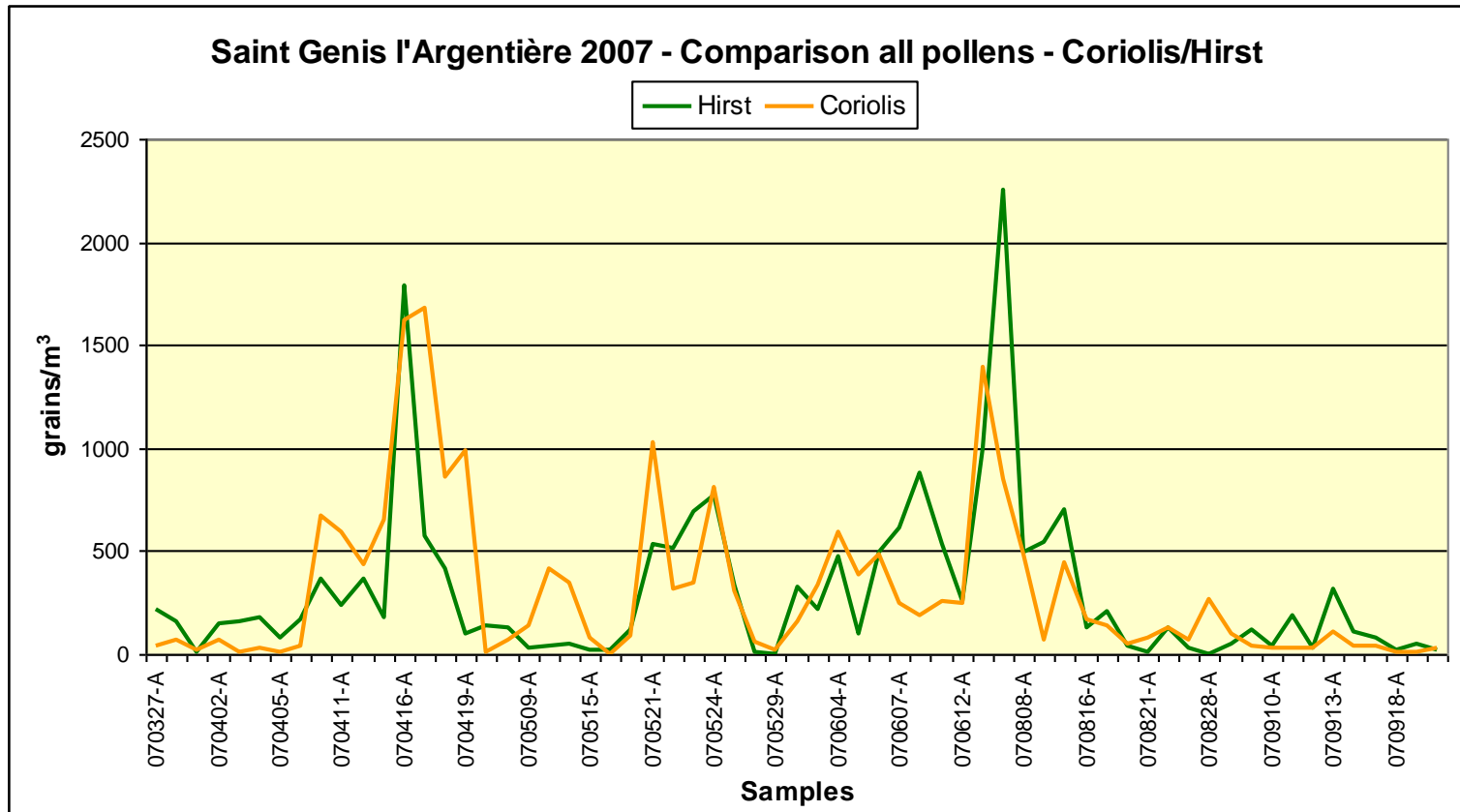
. First step :

- Congress of palynology in Granada 2004.

M.Thibaudon presents a study about new techniques to try to measure allergen and not only to count pollen using Chemsan[®] system

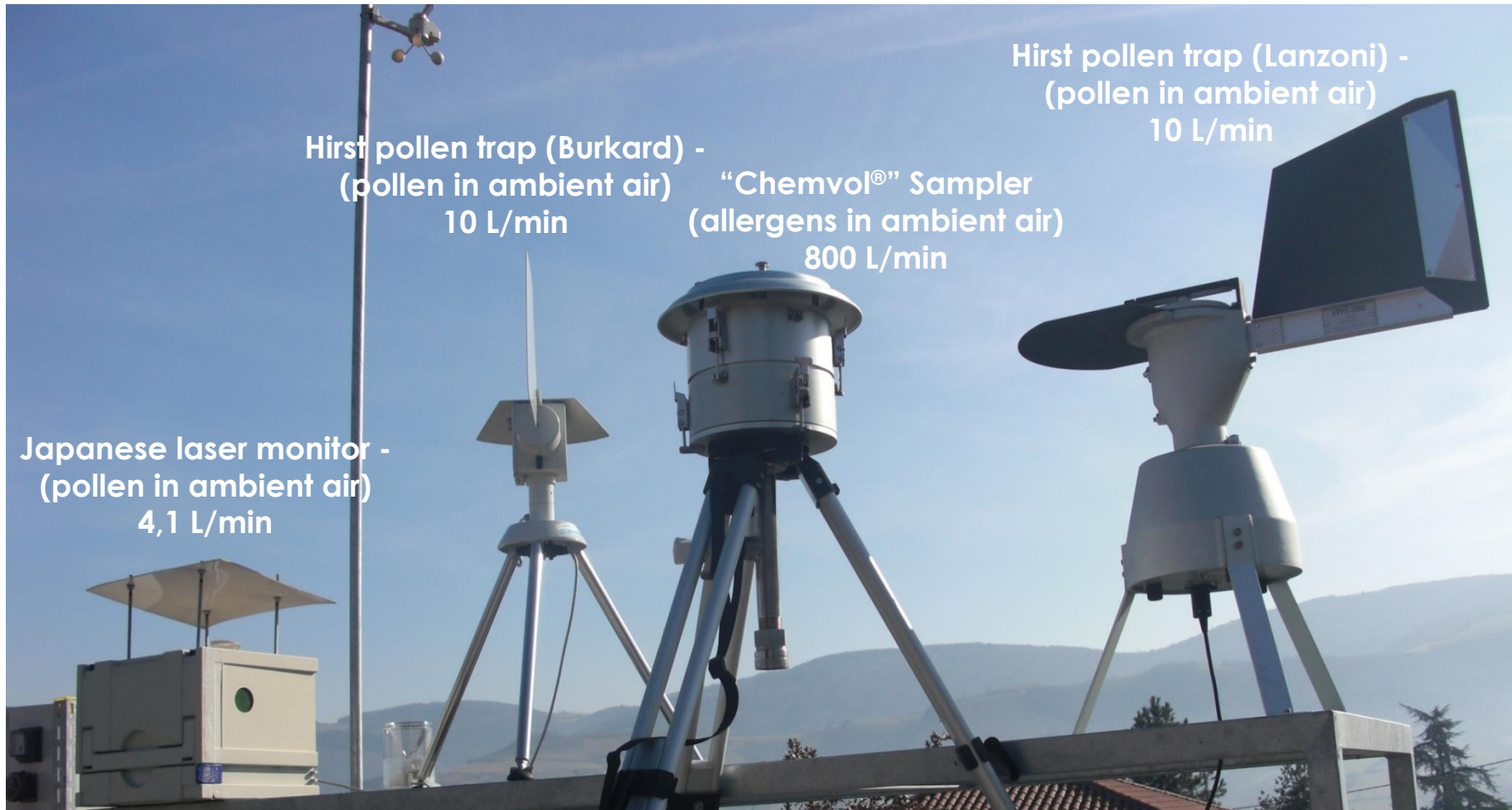


Comparison Coriolis[®] /Hirst

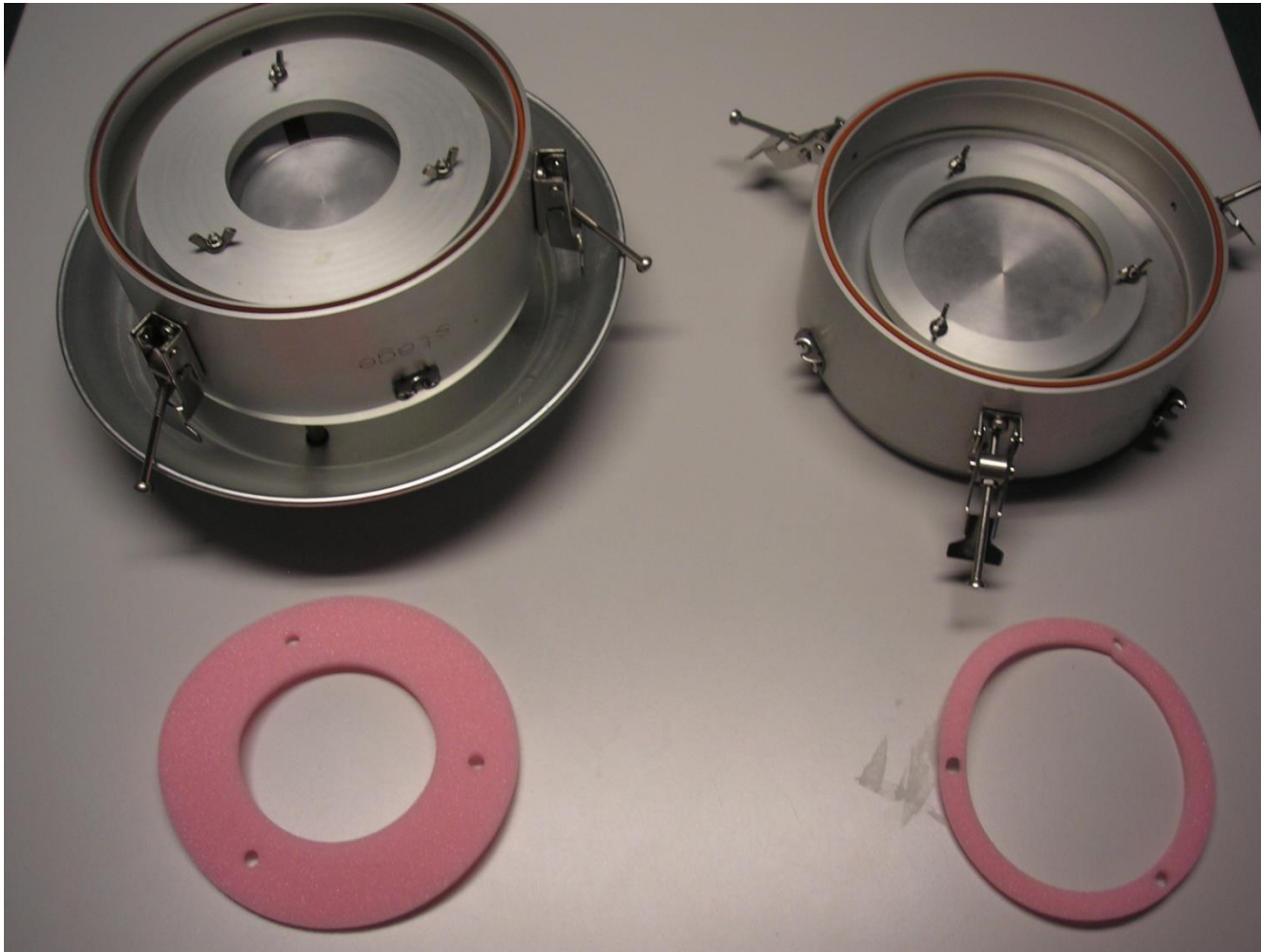


HIALINE Experimental Set-Up

Brussieu, 2.00 m over ground, 450 m above sea level



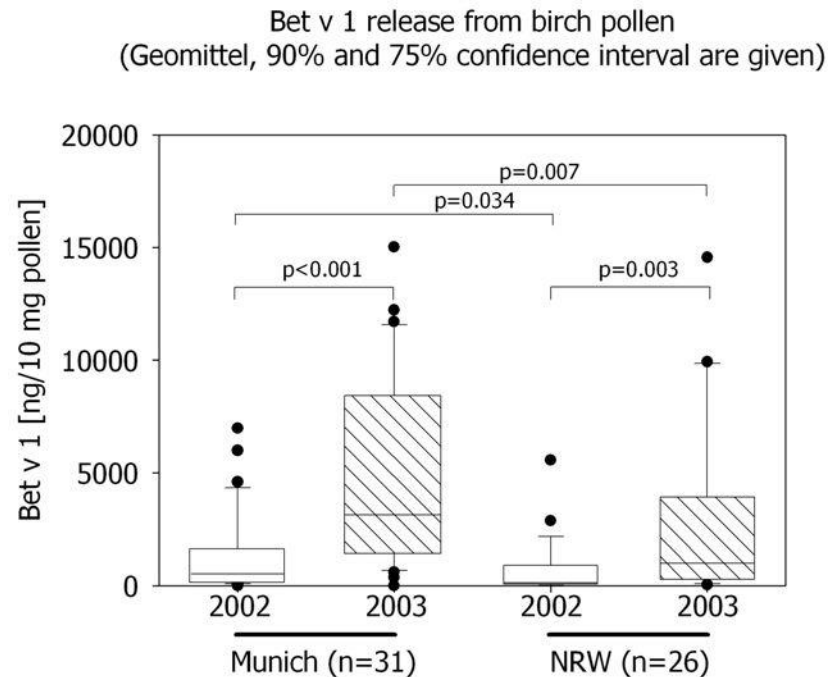
HIALINE: Chemvol



HIALINE

. J. Buters et al

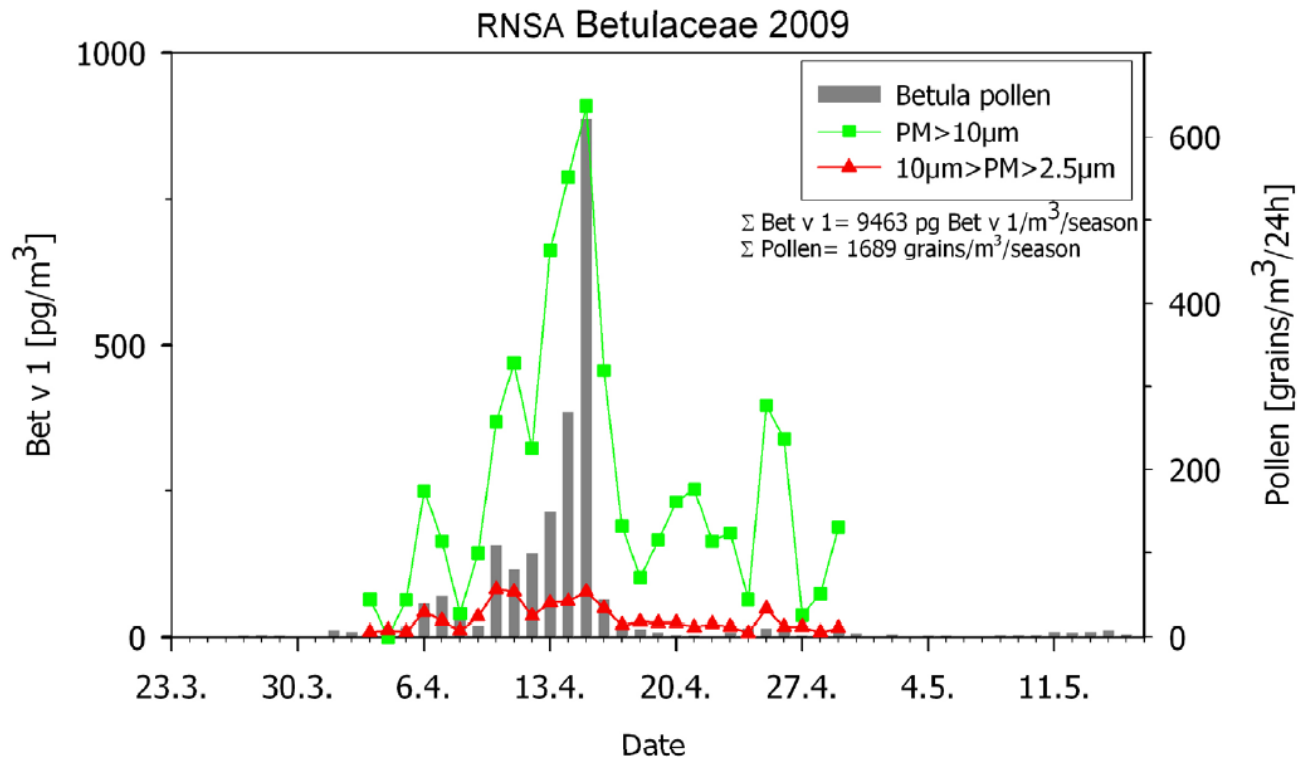
« Year-to-year variation in release of Bet v 1 allergen from birch pollen :
evidence for geographical differences between west and south Germany »
Int. Arch. Allergy Immunology - 2008



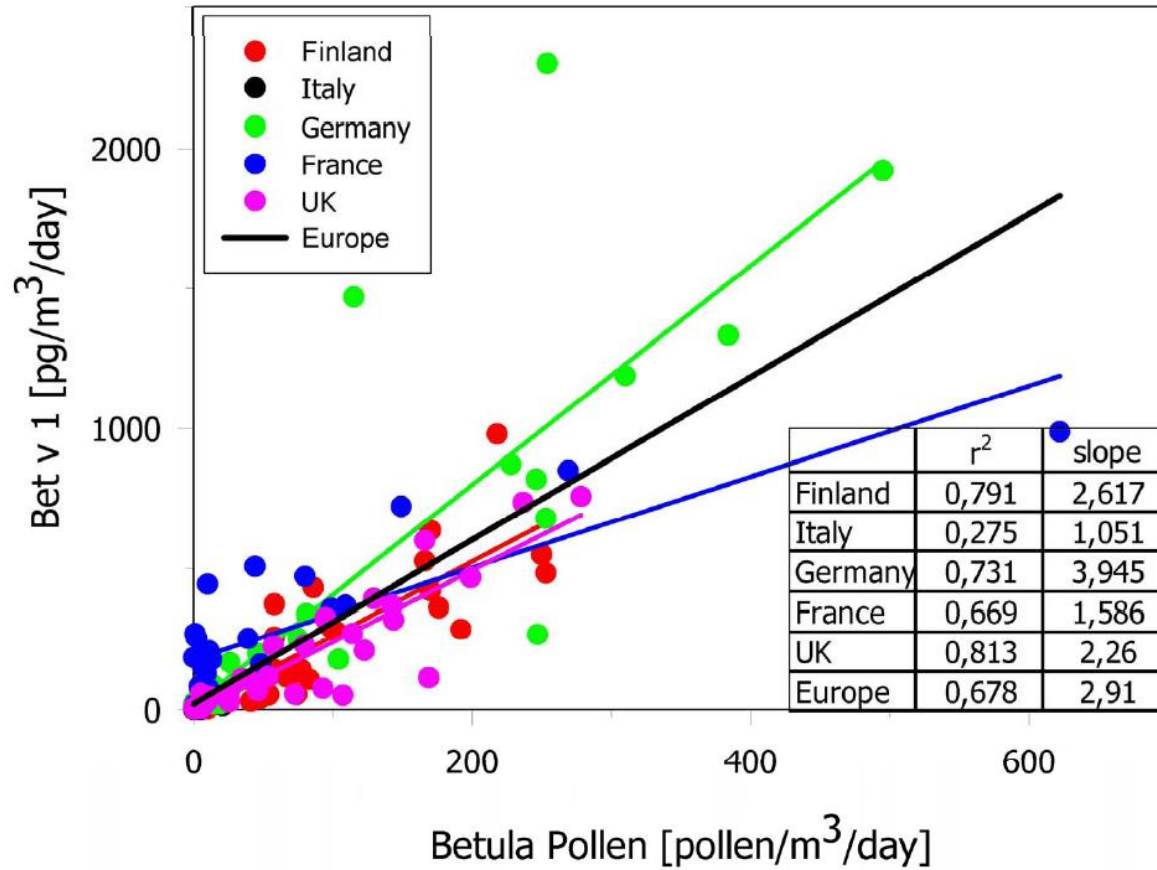
HIALINE – 2009 Results

Birch/Bet v1

RNSA (France): Birch pollen and allergen Bet v 1 in ambient air in 2009



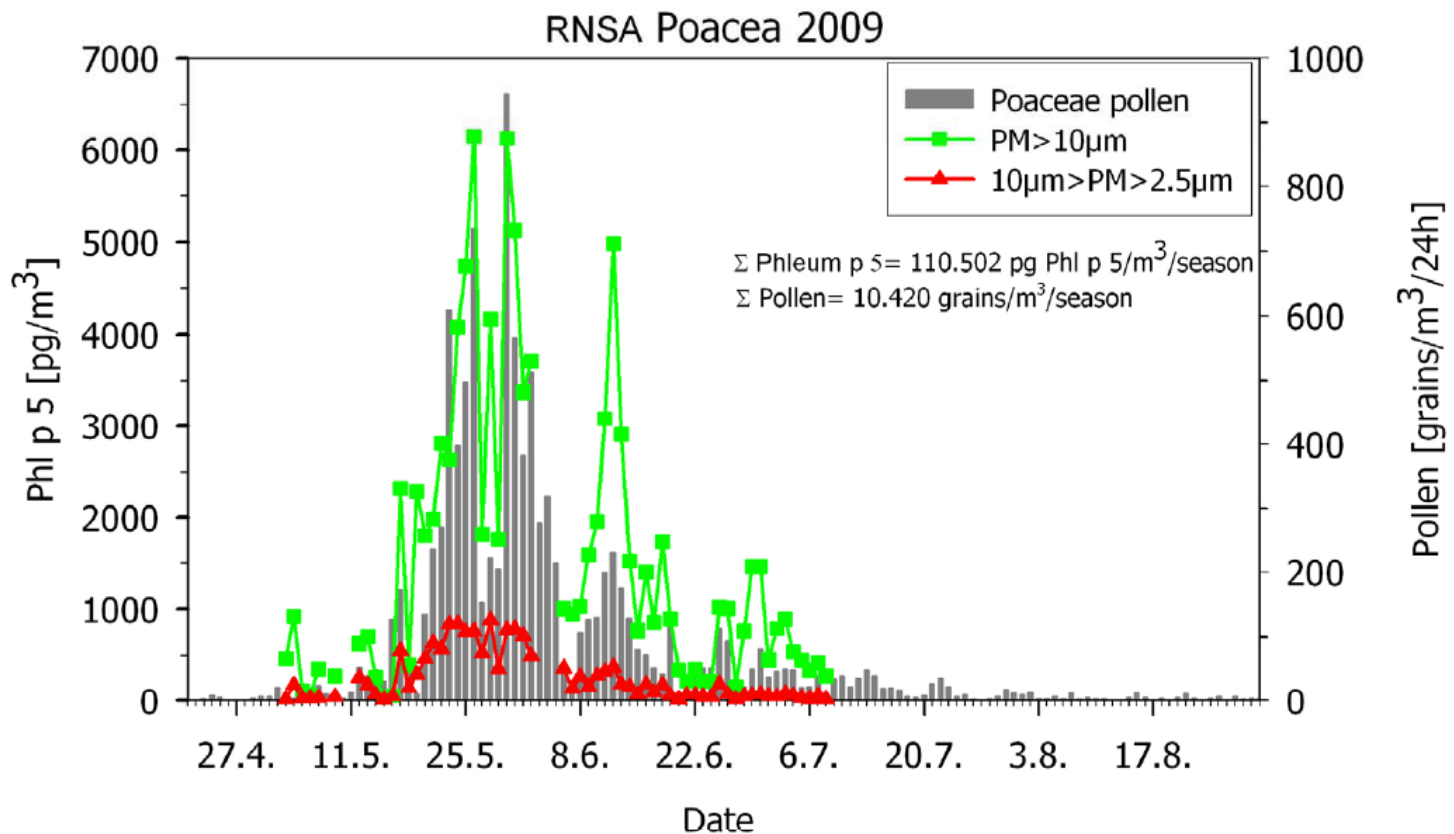
Europe: correlation between birch pollen and Bet v 1 in 2009



HIALINE – 2009 Results

Grasses/Phl p5

RNSA (France): Grass pollen and allergen Phl p 5 in ambient air in 2009



Average yearly grasses pollen count (pollen index) and allergen release per pollen for the stations in the different countries.

		1999-2008	2009		2010		2011	
		Pollen index	Pollen index	Potency	Pollen index	Potency	Pollen index	Potency
		Σ grains/m ³ /year	Σ grains/m ³ /year	Phl p 5/grain	Σ grains/m ³ /year	Phl p 5/grain	Σ grains/m ³ /year	Phl p 5/grain
Austria	Vienna				2556	2.52		
Finland	Turku		654	2.17	738	1.76	782	2.05
France	Lyon		10420	2.74	7027	2.90	6152	3.03
Germany	Munich	1813 ± 627 ¹	1999	3.06	1821	2.58	2184	2.24
Italy	Parma		4165	0.98	4362	2.98	5409	0.88
Poland	Poznan		6083	2.62	7898	3.31	4482	2.16
Portugal	Evora		5643	1.95	17,07	1.68	22536	1.72
Spain	Cordoba		3876	2.49	8166	4.26	6850	2.47
Turkey	Bursa				2892	3.12		
UK	Worcester		4882	6.20	4996	3.52	3383	4.08
Average ± s.d.			4715 ± 2932	2.77 ± 1.52	5757 ± 4743	2.86 ± 0.78	6472 ± 6800	2.33 ± 0.94
s.d. (%)			(62%)	(54.8%)	(82.4%)	(27.3%)	(105%)	(40.3%)

¹ not for all stations a complete preceding decade was available

New methods in aerobiology

Image analyses

A.E.R.O.MEDI

MIAS

Microscope

Image

Analysis

System

To analyze directly
slides

MIAS
Microscope
Image
Analysis
System

Microscope Image Analysis System

aeroScope® **NEW**

New developed apparatus takes automatically 200 images from each of 10 slides.

PCS-Software
Easy to use PC-Software identifies, counts and classifies the different pollen from these 200 images per slide.

Staining Solution

aeroScope®

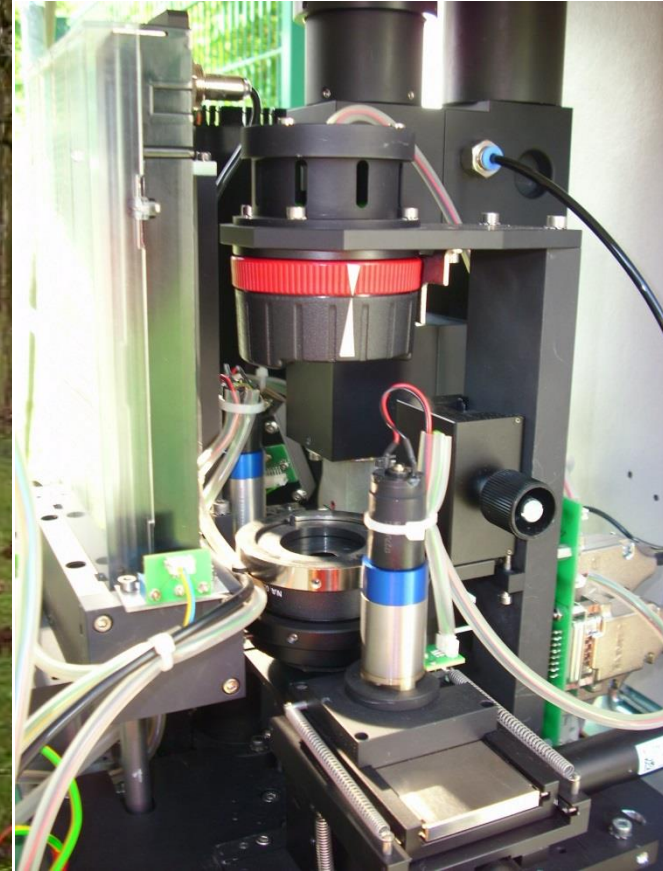
PCS-Program

The advertisement features a central image of the aeroScope machine, a microscope with a slide tray. To the left, there are bottles of staining solutions. Below the machine, a computer monitor displays the PCS-Program software interface. A red curved line connects the staining solutions, the machine, and the software, indicating the workflow.

Image analyses

BAA 500

Pollen monitor for
Fully-Automated
Analysers Airborne
Particles



Particle counters

KH3000

S.Kawashima,
University of Kyoto

Information about pollen
concentration without
discrimination.

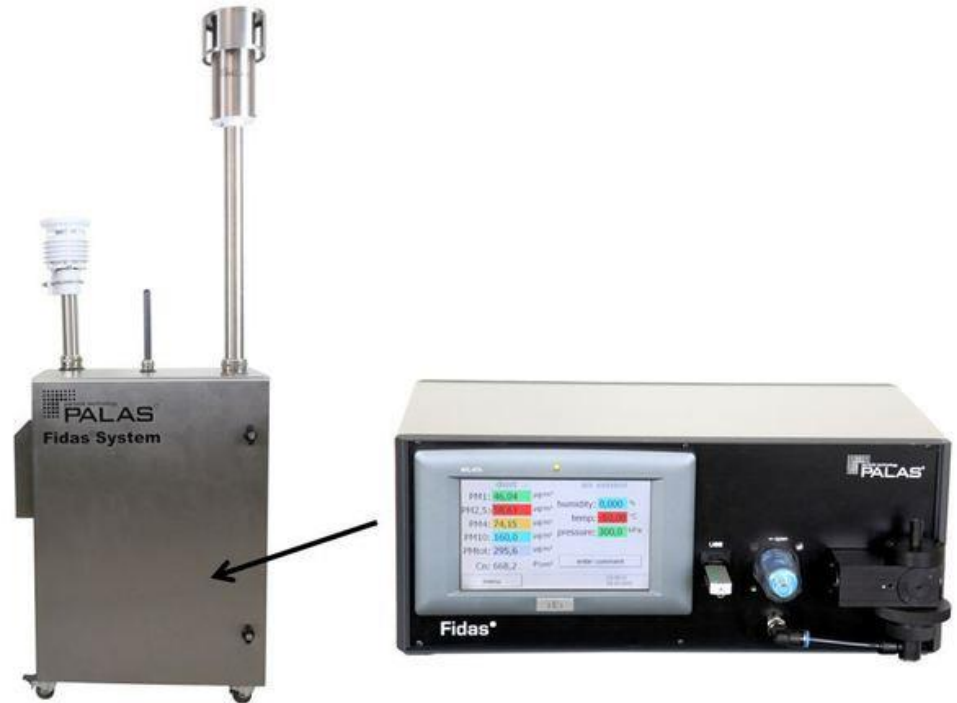


Particle counters

FIDAS 200

Size segregated particle counter adapted for the measurement of Particulate Matter from 2 to 100 μm .

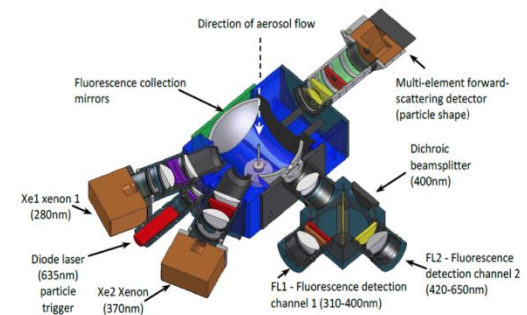
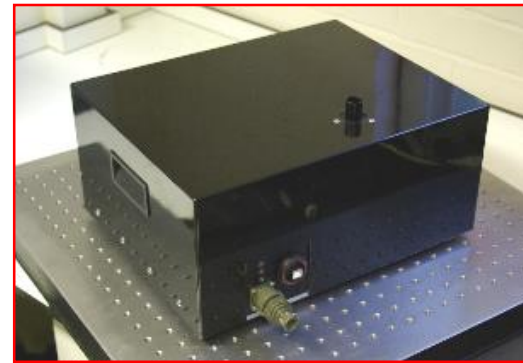
Real time granulometric distribution of the particles in two modes: number and mass



Particle counters

WIBS

Optimization of the real-time measurements of Bioaerosols with the Wideband Integrated Bioaerosols Sensor



Particle counters

RAPID-E

New pollen and molds counter using fluorescence.

Good correlation with some pollens (>90 % for Dactylus for instance)



Molecular biology

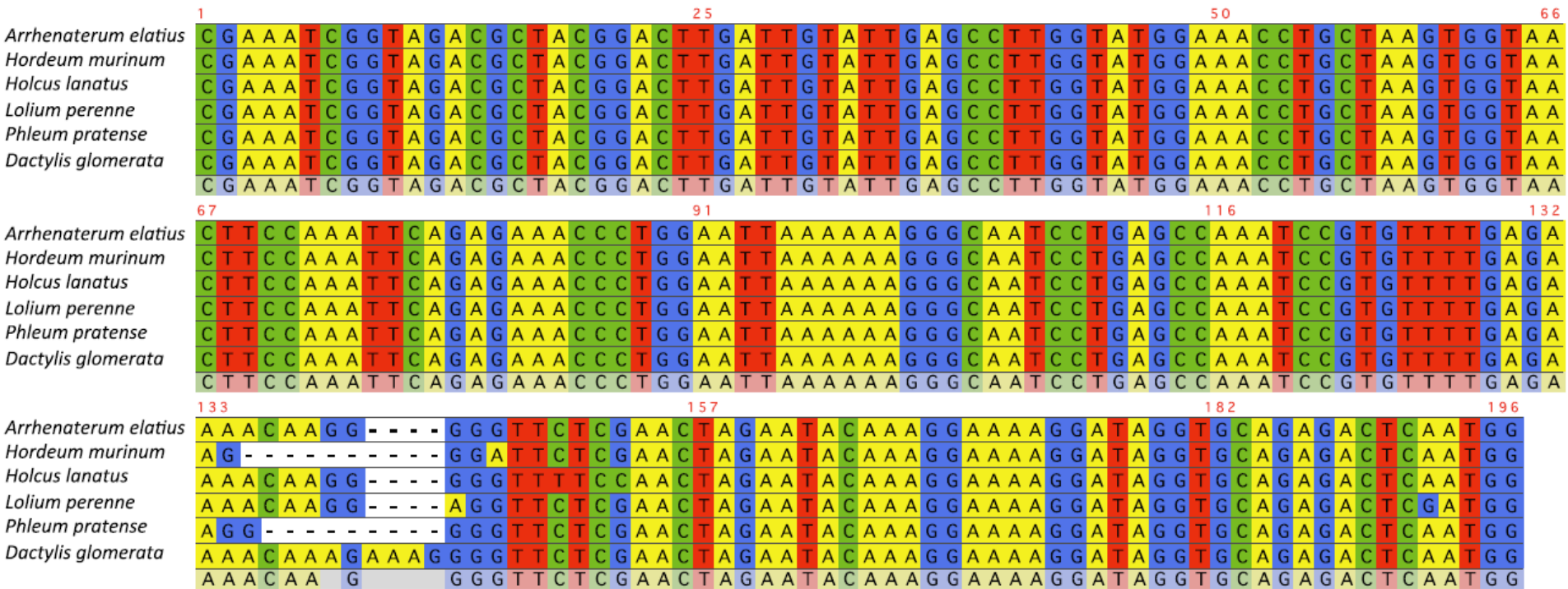


Fig. 3 Alignment of the reference sequences for the six grass species used in the grass mix. The sequences were generated using Sanger sequencing from the individual pollen samples before they were mixed.

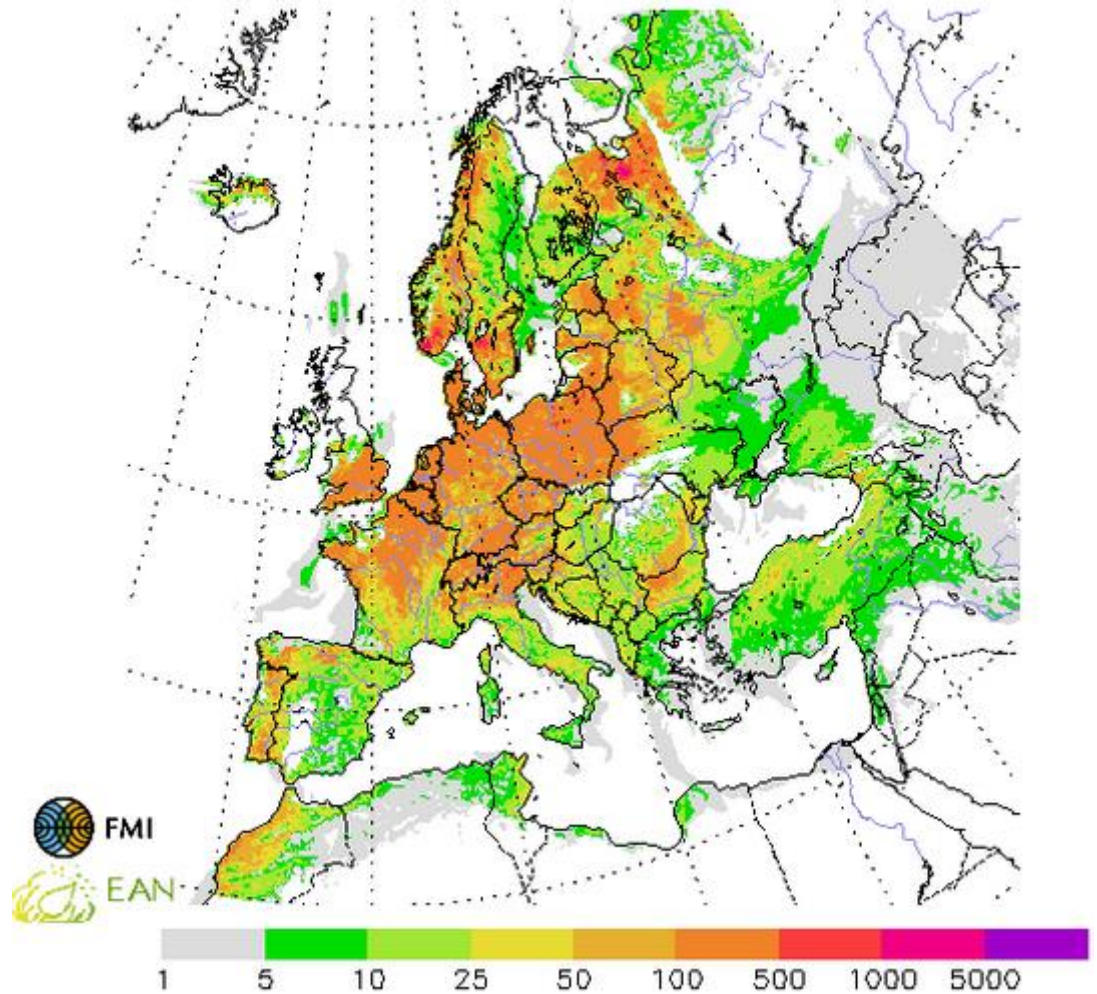
K.KRAAIJEVELD and al.,

Efficient and sensitive identification and quantification of airborne pollen using next-generation DNA sequencing,

Molecular Ecology Resources (2015) 15, 8–16 doi: 10.1111/1755-0998.12288

Modeling

Pollen concentration grass, (#/m³)
09Z06JUL2017



SILAM

5-days forecasts of pollen distribution over **Europe and Northern Europe**,
The forecasted species include birch, grass, olive, and ragweed pollen.

Modeling

COSMO-7 FORECAST
Mean Grass Pollen Concentration of the previous 24h

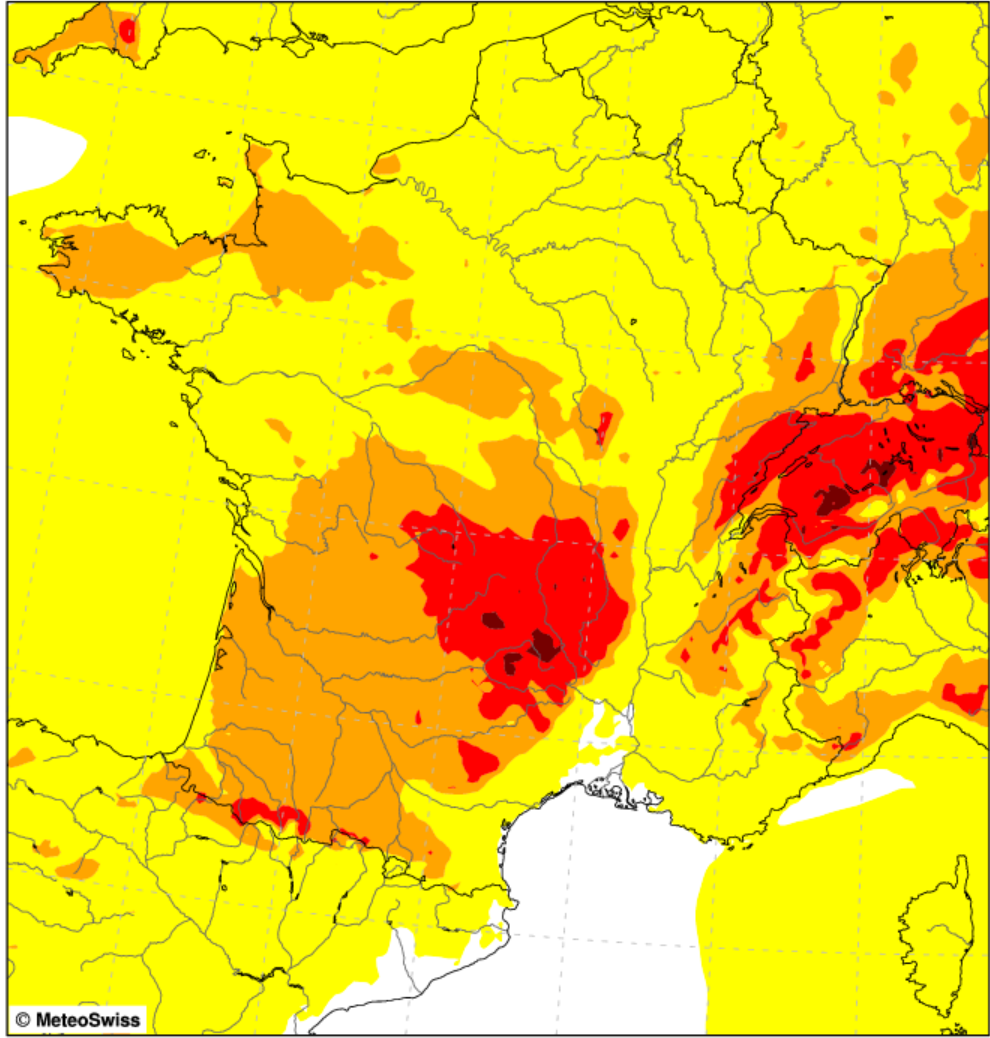
Version: 952

Thu 06 Jul 2017 00UTC

05.07.2017 00UTC +24h

COSMO-ART

3-days forecasts of pollen distribution,
The forecasted species include alder, birch, grass and ragweed pollen.



Concentration of graminaceae pollen [m-3]

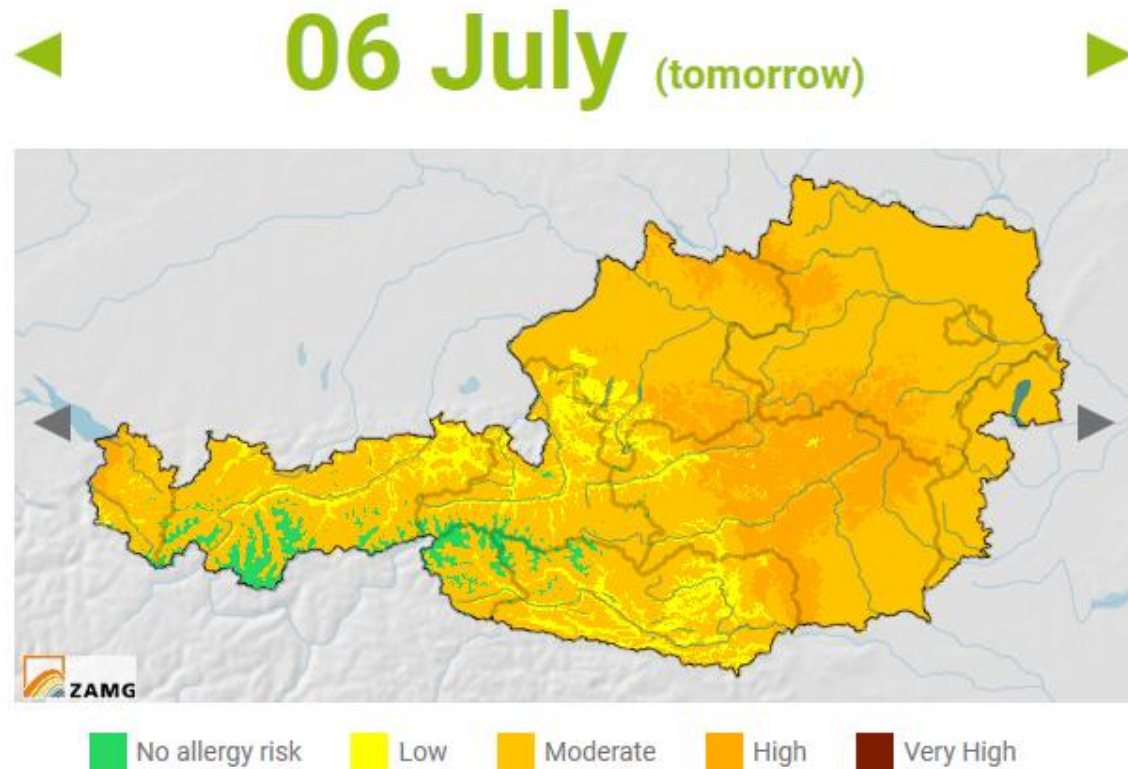
Max: 219.1 m-3

Modeling

ZAMG

Cooperation with
Pollenwarndienst.at

Provides the short
and medium-term
pollen forecasts
for hazel, alder,
birch, grasses,
mugwort and ragweed.



Conclusion

Allergen measurements in the air could be the exposure assessment of the future, but pollen counts will stay the reference of analysis for a long time.

**Thank you
for your attention !**