



### Response study of olive (*Olea europae*) pollen to pyrogallol red dye

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In this work we report on changes that occur in olive's pollen, using "Pyrogallol-Red" (PR) dye, since its detection in atmosphere until it disappears completely. The period and the grade of pollination, with special emphasis in allergenic potential, periodicity and aggressiveness grade in correlation to clinical manifestation in atopic individuals were determined. Classical staining methods, based on fuchsin-glycerinated-gelatine, are used only to count and visualize the collected material, but they new methods, based on PR, can be used for both, to recount and to analyze details related to morphologic differentiation. Determination of pollen state after meteorological and atmospheric stress changes occurred during the recollection can also be studied.

PR dye a complexometric indicator, used for protein analysis, has shown pollen staining specificity, allowing analysis of morphological differentials of pollen granule, differentiating walls and cytoplasmatic proteins. The dye can be used for a better visualization, recognition of biologically active proteins and allergenic activity in pollen granule.

Optical and electronic microscopy results obtained with PR are shown in this communication.

### Poster session d2

### CLINICAL ASPECT OF ALLERGENIC POLLEN

#### Allergenic pollens in the working environment of Japanese orchard

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In Japanese orchards, artificial pollination and ground control with covered-grasses are strongly recommended in order to upgrade the fruit quality. However it has been reported that the artificial pollination and the grasses might cause serious pollinosis among some farmers.

To clarify the relation between the working environment and the pollinosis, we have performed airborne pollen surveys in Japanese pear orchards. At the same time, a questionnaire survey and IgE antibody examination were also conducted among the farmers.

From the airborne pollen survey using a volumetric personal air sampler (Burkard, England) during the pollination season, we found ten times higher pear pollen count in the ambient air of the farmers compared with the ordinary environment of the orchard. At the same time, we found many pollens of grasses such as annual bluegrass (*Poa annua* L., a major species in the undergrowth of orchards) and spores of *Lycopodium clavatum*.

A questionnaire was obtained from 198 pear farmers in Toyama City, 2001. 36.3% of pear farmers complained of pollinosis or similar symptoms during artificial pollination season from April to May. The rate of wearing mask remained to be 55.5% of the symptomatic farmers.

By IgE antibody examination, 7 farmers among 9 pollinosis farmers showed positive to the orchard pollens and negative to Japanese cedar pollen, which was the most popular allergic pollen in Japan. The annual bluegrass showed strong positive by the IgE antibody examination. Even the farmers who did not complain of any symptoms, some farmers showed positive IgE antibodies to the orchard pollens.

Because of the seasonal changes of the pollinosis symptoms and the results of IgE antibody examination, it was demonstrated that the pollinosis symptoms had a close relationship to the orchard work environment.

#### Environmental factors in relation to the increase of allergic pathologies: the pollinosis in Besançon (Doubs, France)

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The increase of cases of pollinosis in our western society is more and more important in urban environment and conversely, in rural environment, there is a protective effect on the development of allergy. The principal aim of this research is to oppose the city to the countryside by studying their own pollinic and epidemiologic specificities. Thus, the purpose of this investigation is to estimate and compare the exposure to allergenic pollen in a rural area and in an urban area. With this intention, daily pollen counts, and daily medicinal data were collected in the countryside (Pelousey, Doubs) and in the center of the city (Besançon, Doubs). The pollen counts were collected by three Hirst pollen traps which are located at one meter above the ground in Pelousey and at one meter above the ground in Besançon and on a roof too (at about 20 m height), because the collect of pollen by the RNSA is usually carried out on the roofs.

This study showed large similitude between the daily pollinic accounts observed on two pollen traps located at different heights in the town. Moreover, the results accentuated statically (Mantel test) that there are some pollinic contrasts between the countryside and the town. Therefore, one part of these contrasts is explained by an important representation of *Platanus* and Cupressaceae-taxaceae in the town and by the increase of the pollen production of some allergenic taxa in the town: it is the case of the hazel (*Corylus*), the birch (*Betula*) and the ash (*Fraxinus*). As regards the consumption of the anti-allergic drugs, this research highlighted a different behaviour between the urbane and the people who live in the country.

### Correlation between conjunctival provocation test and conjunctival symptoms in pollinosis. Preliminary report

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**Aim.** We sought to investigate the correlation between the level of reactivity in conjunctival provocation test and the severity of conjunctival symptoms that develop during pollinating season in grass pollen allergic patients.

**Material and methods.** Conjunctival Provocation Test (CPT) with allergen extracts (mixture of 6 grasses: *Holcus lanatus*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Festuca elatior*) was performed according to standardized procedure in 22 patients (aged 19 to 52 years), all diagnosed to suffer from intermittent (seasonal) allergic rhinoconjunctivitis due to grass pollen allergens. The procedure was performed prior to grass pollen season. During grass pollination season all patients monitored their symptoms with Symptoms Score Cards, assessing the presence of ocular manifestations. A parallel constant measurement of the level of grass pollen count was carried out on a daily basis with volumetric method (Burkard Seven-Day Recording Spore Trap).

**Results.** In all 22 patients CPT gave positive results; with none reacting at the low level of allergen concentration (5 or 50 SBU/ml). Every fourth patient developed positive CPT at medium concentration of 160 or 500 BU/ml. A half (n=11) showed positive reaction to CPT at allergen concentration of 1600 SBU/ml. Slightly more than one forth of the patients reacted at the level of 5000 BU/ml. In every fourth patient itching was the first reaction to the allergen provocation. - in every third patient at the allergen concentration of 160 or 500 BU/ml and in the following 13 patients at the level of 1600 BU/ml. In 3/4 of patients both symptoms appeared simultaneously - in every fourth patients at the allergen concentration of 160 or 500 BU/ml, in half of the patients at the level of 1600BU/ml, and in over one third of subjects at the allergen concentration of 5000BU/ml.

In the analysed early phase of grass pollination season (20 May to 5 June 2003) in Warsaw mean daily grass pollen count was 9 to 143 grains per 1 m<sup>3</sup> of the air (mean 51,5 ± 32,3). Itching developed as the first symptom in half of the patients and redness in 1/4 of subjects. 1/4 of patients developed both symptoms at the same time. Threshold grass pollen concentration was found to equal 22 grains per 1 m<sup>3</sup> of the air. Mean daily pollen count which triggered reaction to allergen in all patients who developed symptoms was 52 pollen grains per 1 m<sup>3</sup>. In cases of positive provocation test results appearing at the level of 1600 BU/ml, the grass pollen count values evoking conjunctival reaction during the season were significantly higher than in cases when positive CPT result was apparent at allergen extract concentration level of 500 BU/ml (F=2,423; p<0,05).

**Conclusions** The patients with pollinosis and high reactivity in the conjunctival provocation test develop conjunctival symptoms earlier during the grass pollination season (at lower levels of grass pollen count) than the patients who are characterised by the lower reactivity during the conjunctival provocation test (reacting to the allergen extract of the higher concentration). However, no statistically significant correlation was found between the sequence of the symptoms appearance during the conjunctival provocation test with the grass pollen allergen and the sequence symptoms appearance during grass pollen season in patients with pollinosis (chi<sup>2</sup> = 2,647; p>0,05).

### The relation of clinical picture of allergic disease to exposure to selected pollen and fungal allergens

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**Aim** The purpose of this study was to analyse the clinical picture of upper respiratory tract allergic disease and conjunctival symptoms that appear in response to different aeroallergens.

**Material and Method** The study was conducted in the years 2002 and 2003. We analysed the frequency and severity of allergic rhinitis, conjunctivitis and bronchial asthma in a group of 450 subjects living in Warsaw area with hypersensitivity to hazel, alder, birch, grasses, mugwort, plantain, nettle, *Alternaria* and *Cladosporium* allergens. During pollination season patients assessed their symptoms with Symptoms Score Cards. The results of physical examination: nasal cavity endoscopic pictures with colour and degree of nasal conchae swelling evaluation, the presence and intensity of nose blockage and conjunctiva symptoms were analysed. Constant measurement of the level of pollen count with volumetric method (Burkard Seven-Day Recording Spore Trap) was carried out on a daily basis.

**Results** The strongest clinical symptoms followed exposure to high concentrations of alder, birch, grass and mugwort pollen. First symptoms were visible during exposure to the concentration of 20 pollen grains in 1m<sup>3</sup> of air (25% subjects sensitized to grass pollen). Symptoms were noted in all the subjects sensitized to grass pollen at the concentration of approximately 50 grains/m<sup>3</sup> of air. During exposure to the concentration of 65 grass pollen grains per m<sup>3</sup> the symptoms were strengthened and included both nasal passage blockage, watery nasal secretion, sneezing, palate pruritus, patency disturbances of Eustachian tubes and conjunctival symptoms. A several hours' exposure to grass pollen concentration exceeding 120 grains/m<sup>3</sup> caused dyspnoea in some patients. Similar intensified symptoms occurred after exposure to alder, birch and mugwort pollen. Patients with positive skin prick tests to plantain and nettle pollen allergens experienced symptoms of low intensity or even none symptoms during pollination period.

Subjects with hypersensitivity to *Alternaria* allergens experienced their symptoms during exposure to the concentration of approximately 80 spores in 1 m<sup>3</sup> of air, while patients sensitized to *Cladosporium* allergens at the exposure to the concentration of over 2800 spores in 1 m<sup>3</sup> of air. The dominant symptoms occurring in patients sensitized to fungal spores' allergens were loss of nasal patency, and dyspnoea as well as recurring cough at higher concentrations.

**Conclusion** Clinical manifestation of allergic disease was related to the type and the concentration of aeroallergen that patients were exposed to.

### Clinical study of pollinosis in children to Poaceae pollen in the province of Granada (S. Spain)

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The family Poaceae, comprised of some 9000 different species, includes annual and perennial grasses with a widespread distribution. These grasses represent nearly 20% of the world's plant cover, not only natural, but also ornamental and agricultural. In the province of Granada, more than 230 species have been catalogued, many of these with allergenic pollen, particularly those belonging to the genera *Dactylis*, *Festuca*, *Lolium*, *Poa*, *Phleum* and *Cynodon*. (Díaz de la Guardia et al., 1995). Their pollen is the principal cause of allergy in Europe, affecting probably more than 5% of the population. In Spain, the incidence of Poaceae allergy is very high among

the atopic population, and in Andalusia more than half of the patients with pollinosis are believed to present symptoms of this pollen.

To establish a relationship between the aerobiological behaviour of grass pollen and pollinosis in children in Granada (S Spain), we analysed the seasonal and annual behaviour of this type of pollen and the symptoms of sensitive patients. A total of 825 cases of first-time complaints of paediatric allergy were studied from the hospitals Materno Infantil and Clínico San Cecilio of Granada during the year 2000. These clinical data were related to the pollen concentrations measured by aerobiological sampling performed from 1998 to 2003. The collector used (Hirst volumetric) was installed on a terrace of the Science Faculty of the University of Granada at a height of 23 m a.s.l., this building being situated in the centre of the city of Granada. The method used to prepare and analyse the samples was that of Domínguez et al. (1991) for the Spanish aerobiology network "REA".

The results show that Poaceae pollen is the second cause of allergy among the infant population of Granada. Of the 825 case studies, some 65% presented sensitivity to grass pollen. Of these, 61% of the cases were male and 39% female. The highest percentage (47%) of the allergic patients corresponded to 6 to 10 years of age. The most frequent symptoms were asthma followed by rhinoconjunctivitis and atopic eczema. Also, this analysis indicated that 12% of the patients were monosensitive.

The aerobiological study revealed that Poaceae pollen appears in the atmosphere of Granada practically the entire year, although incidence peaks in March and July. March (at the end of the month concentrations approach 100 grains/m<sup>3</sup>) begins a constant register of atmospheric pollen, reaching maximum levels in May and June. This pollen is the fourth most abundant type of pollen in the overall pollen spectrum in Granada.

DOMÍNGUEZ, E., GALÁN, C., VILLAMANDOS, & INFANTE, F. (1991). Manejo y evaluación de los datos obtenidos en los muestreos aerobiológicos. *Monogr. REA/EAN* 1.

DIÁZ DE LA GUARDIA, C., ALONSO, R., ALBA, F. & VALLE, F. (1995). Airborne grass pollen in Granada (Spain). *Aerobiologia* 11: 47-50.

### Clinical usefulness of regional and individual aerobiological monitoring for diagnostics and therapy of pollen and fungal spore allergy

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A project of the State Committee for Scientific Research nr 3 POSD 034 24 is aimed at the practical use of Partrap FA 52 to evaluate the treatment efficiency of patients suffering from pollen allergy. Because of a rapid increase in pollen allergy frequency which is observed in the whole world attention is paid to the individual monitoring.

The aim of the study was to compare pollen concentrations in the air, measured using the Burkard trap and Partrap FA 52, provoking pollen allergy symptoms. For this study the volumetric method has been employed. Presented results, regarding the first season, are preliminary ones. On the basis of aeropalynological studies: - 40 taxa of plants and 2 taxa of fungi (*Cladosporium*, *Alternaria*) were identified using the Burkard trap from February to October 2003 - pollen concentrations obtained using Partrap FA 52 located on the Burkard trap and the Burkard trap during a 10 h period within 7 warm and dry days were compared in July and August 2003. Seven persons, of the group of 30 patients suffering from pollen allergy, carried Partrap FA 52 on themselves 4-6 h daily in different surroundings from April to August 2003. Concentrations obtained using Partrap FA 52 and the Burkard trap were compared during the same time periods and their influence on allergy symptoms were estimated. Our study resulted in pollen calendar consisting of selected 13 allergenic taxa (including *Betula*, *Poaceae*, *Artemisia*) occurring most abundantly in the atmosphere.

Pollen concentrations obtained using Partrap FA 52 located on the Burkard trap and the Burkard trap showed strong significant correlation especially for *Artemisia* pollen (Pearson's correlation,  $p < 0,05$ ). The concentrations counted from Partrap are about 0,96-1,65 the value from the Burkard trap. Comparing pollen concentrations obtained from Partrap FA 52 carried by patients and from the Burkard trap it appeared the Partrap concentration was clearly higher which could result from different places visited by patients.

### Comparative study of the aerobiological record of San Carlos de Bariloche city and the upper valley of Rio Negro and Neuquen, Patagonia, Argentine

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Airborne pollen and spore records from two patagonian regions, Bariloche city (41°S, 78°W) and the Río Negro and Neuquén Upper Valley (39°S, 68°W) were compared. In both regions the climate is continental (cold in Bariloche, temperate in Upper Valley), with a huge thermic width: Bariloche 8.4°C mean temperature (absolute values max. 35.5°C, min. -18°C), Upper Valley 15°C mean temperature (absolute values max. 42°C, min. -13.2°C), and very windy: Bariloche 22.6 km/h per year average, Upper Valley 9.9 km/h per year average.

The Upper Valley is the most populated region in Patagonia: a lineal irrigated oasis crossing the semiarid steppe (199 mm average rainfall per year, 56% relative humidity), with a high agricultural development (orchards of apples, pears and grapes). Bariloche is a touristic city developed into the forest-steppe boundary under a striking west to east precipitation gradient (1600-800 mm, 68% relative humidity). In the area of the Upper Valley hourly aerobiological records were carried out from March 1996 to September 1997 while in Bariloche city daily records were carried out from September 2001 to September 2002. At both sites impact samplers were used: Burkard and Lanzoni automatic volumetric spore traps respectively.

In Upper Valley synchronous to the aerobiological studies, 180 outpatients with respiratory allergic disease were prick-tested. 40% were sensitized to Poaceae, 28.7% to *Chenopodium*, 22.1% to *Plantago* and 26% to *Alternaria*. Allergy symptoms suffered by patients showed to be consistent with aerobiological findings.

At the Upper Valley, main pollen season extended from September to May, at Bariloche city from October to January. The main pollen taxa were Chenopodiaceae-Amaranthaceae, Poaceae and *Plantago* in Upper Valley and native taxa *Austrocedrus* and *Nothofagus* in Bariloche city. Considering spore concentration, *Alternaria* achieved the maximum value during the Autumn in Upper Valley and in October (Spring) in Bariloche city. *Cladosporium* achieved the maximum value during the Autumn in Bariloche city (March, April), while it was absent in Upper Valley. Both records shared about 57% of the identified types.

Each region has its own aerobiological fingerprints and data cannot be extrapolated between localities. Data from different cities within regions should allow the development of aerobiological networks and should set the basis for biometeorological forecasts. Further research, adequately founded is needed to refine this preliminary approach.

**Key words:** aerobiology, pollen, spores, Upper Valley, Bariloche, allergy