Sequence polymorphism of the major olive pollen allergen (Ole e 1) in defined cultivars

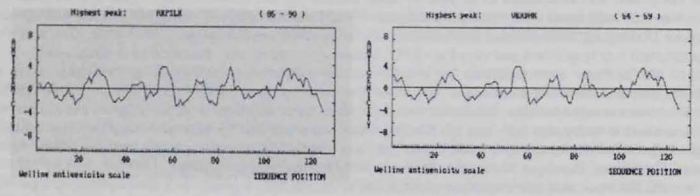
Hamman-Khalifa A. M.; Alché, J. D. ; Jiménez, J. C.; Castro, A. J. & Rodríguez-García, M. I.

Departamento de Bioquímica, Biología Celular y Molecular de Plantas. Estación Experimental del Zaidín. CSIC. Profesor Albareda, 1, 18008 Granada (Spain).

Ole e 1 is considered to be the major allergen from olive pollen. The protein presents several glycosylation forms, and its amino acid sequence displays relevant homology to pollen proteins from maize, tomato, ryegrass, birch, rice, *Arabidopsis* etc. This identity rises to >85% when compared to Ole e 1-like proteins from members of the *Oleaceae* family (lilac, privet, ash, forsythia) (see review of Rodríguez et al., 2002). Ole e 1 itself exhibits microheterogeneities at several positions of its amino acid sequence (Villalba et al., 1994; Lombardero et al., 1994).

In this work, Ole e 1 sequences were amplified by RT-PCR procedures using total RNA from mature pollen of eight different cultivars of olive (*Olea europaea* L.). Ole e 1 amplified sequences were cloned and sequenced. The sequences obtained were submitted to the GenBank™/EMBL Database. The analysis of the obtained sequences showed the existence of a high number of microheterogeneities in the analysed sequences, which were particularly profuse in the 5' and the 3' coding regions. Tree-view analysis of microheterogeneities showed that the inter-cultivar viariability detected was higher than the intra-cultivar variability present in at least three clones of Ole e 1 for each cultivar. An additional N glycosylation motif was detected in one of the cultivars examined. The changes detected within the Ole e 1 molecule affect in many cases immunodominant T-cell epitopes, and produce differences in the hydrophilicity and antigenecity profiles, also affecting the predicted secondary structures of the allergen in the majority of the cultivars studied.

The procedure described here offers a very useful molecular tool to establish discrimination between olive tree cultivars, and to study the basis of the interaction between the allergens and the human immune system. The expression of the obtained clones could be used to define homogeneous Ole e 1 molecules valuable for the improvement of clinical diagnosis and therapy of olive pollen allergy.



Figs. 1 and 2: Antigenicity profiles of the Ole e 1 allergen deduced sequences in two olive cultivars.

This work was funded by projects CAO99-003 (INIA) and BCM2000-1484 (both from the Spanish MCT)

LOMBARDERO, M.; BARBAS, J.A.; MOSCOSO DEL PRADO, J. & CARREIRA, J. 1994. cDNA sequence analysis of the main olive allergen, Ole e I. **Clin. Exp. Allergy** 24: 765-770.

RODRÍGUEZ, R.; VILLALBA, M.; BATANERO, E.; GONZÁLEZ, E.M.; MONSALVE, R.I.; HUECAS, S.; TEJERA, M.L. & LEDESMA, A. 2002. Allergenic diversity of the olive pollen. **Allergy** 57(Suppl. 71): 6-16.

VILLALBA, M.; BATANERO, E.; MONSALVE, R.I.; GONZÁLEZ DE LA PEÑA, M.A.; LAHOZ, C. & RODRÍGUEZ, R. 1994 Cloning and Expression of Ole e I, the major allergen from olive tree pollen. **J. Biol. Chem.** 269: 15217-15222.

Session d2

CLINICAL ASPECT OF ALLERGENIC POLLEN

Comparison of *Betula* pollen allergens and *Betula* pollen grains concentrations in the air during pollination season 2003 in Cracow (Poland)

Madeja J.[1]; Wypasek E.[2] & Harmata K.[1]

[1] Department of Palaeobotany, Institute of Botany, Jagiellonian University, Cracow, Poland.
[2] Department of Evolutionary Immunology, Institute of Zoology, Jagiellonian University, Cracow, Poland.

Betula pollen belongs to the most important allergens source and are the main cause of seasonal allergies in Central and Northern Europe. A number of authors have described possibility of releasing the micronic and submicronic allergen-bearing particles from pollen grains especially under moist conditions. These small particles might easy penetrate to the lower respiratory tract and cause symptoms of bronchial asthma. In Cracow volumetric pollen and spores trap monitor its concentration in the air all year round since 1982. In our study we showed that ordinary pollen counts do not reflect concentration of the small particles having antigenic activity in the air.

The concentration of *Betula* air borne allergens was measured on PFDV membrane after capillary blotting from Lanzoni pollen and spores trap tape. For detection and quantification allergens polyclonal antibodies against *Betula* pollen antigens and secondary antibodies conjugated with HRP were used. In the chemiluminescence reaction the light orginating from the antibody-enzyme complex bound to the *Betula* antigens are visualized on x-ray film as black spots. The light was emited at positions in the membrane where antigen was present. The quatification of the allergens was done by the x-ray film densitometry. The resultes of measeurements are given as optical density units (ODu). Obtained results were confronted with palynological data showing pollen grains concentration.

Antigens concentration varied during the whole study period (Marz 10 – May 27, 2003). For this paper the example showing the *Betula* pollen allergens presence whereas no pollen grains was noticed in the air at that time was chosen (Fig. 1.). It might be evoked by relatively high humidity (70 – 90 %) or some unknown conditions caused to attendance of earlier released allergens in the air.

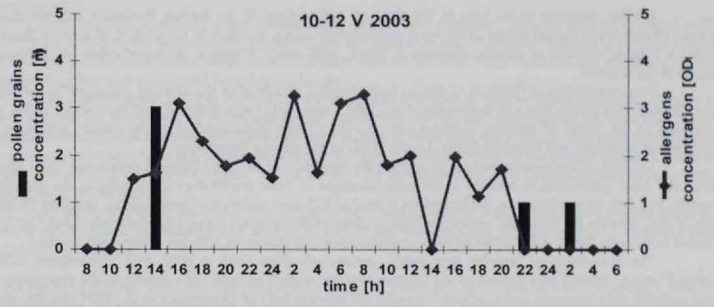


Fig. 1. Comparison of *Betula* pollen allergens and *Betula* pollen grains concentrations in the air on 10-12 of May 2003 in Cracow.

Aerobiological clinical and immunochemical studies on *Carica papaya* L.pollen

Gupta-Bhattacharya, S.; Chowdhury, I.; Chakraborty, P. & Ghosh, D.

Department of Botany,Division of Palynology and Environmental Biology , Bose Institute, 93/1 Acharya P.C. Road Kolkata – 700 009, India

Carica papaya L. is an important fruit yielding tree in the tropics and sub-tropics of Indian subcontinent. There are reports of that pollen to be airborne and allergenic.

In the present study, the seasonal and diurnal periodicities of the relevant pollen were recorded in a five-year survey using Burkard Volumetric Sampler and it was found that *carica* pollen occurred in the air round the year with peaks during January and September-October. Among a patient population of one thousand , skin test results showed 27.8% +1 level and 5.6 % +2 level reactions. In aero allergen immunoblotting of exposed Burkard tape, the detected allergen spots showed a good correlation with relevant aero pollen count. In IgE imunoblot, the pollen extract showed six allergenic components. Later, the total pollen extract was fractionated by Sephacryl S-200 column, and out of the eluted five fractions, fraction 1 was found to be highly allergenic in IgE ELISA inhibition. This fraction was resolved into five major components in RP-HPLC. The fraction contained a component with esterase like activity in activity gel. This component was eluted by RP-HPLC and its homogeneity was checked in native PAGE and crossed immunoelectrophoresis with rabbit antisera. In non-reducing SDS-PAGE,it was found to be a 100 kDa component. Its allergenicity was confirmed by ELISA inhibition and immunoblotting.

In conclusion, it can be said that *Carica papaya* contribute significantly to the aero pollen and aeroallergen load of the suburban outskirts of Calcutta metropolis, our study area. The pollen extract contains a 100 kDa allergenic protein component with esterase like enzyme activity

Relationship between the concentration of airborne allergens (pollen and spores) and the cases of allergy in the province of Granada (S Spain)

De Linares, C.[1]; Lara, M. A.[2]; Martínez, R.[2]; Rojas, M. J[2]; Díaz de la Guardia, C.[1]; Alba, F.[1] & Nieto, D.[1]

[1] Department of Botany. University of Granada. (REA) 18071 Granada (Spain).
[2] Allergy Unit. Hospital Clínico Universitario "San Cecilio". Granada.

Studies currently under way in the field of Aerobiology in the Iberian Peninsula indicate that the presence of pollen and fungal spores in the air is continuously rising, and this, in the opinion of many authors, has spurred a notable increase in allergic illnesses in Spain, with nearly 8 million estimated cases of allergopathic patients in the country.

Previous studies in Granada (S Spain) have revealed that most of the allergies registered in the city are due to atmospheric pollen (Díaz de la Guardia et al., 1991), especially *Olea*, Poaceae, and *Parietaria*. In this light, knowledge of the trends of airborne particles can provide a better understanding of the causes, processes, and treatments of pollinosis and therefore better quality of life of the allergic population.

The aim of the present study is to analyse the incidence of the most frequent pollen and spore allergies among the adult population in relation to the concentrations of these aerobiological particles in the province of Granada. For this, an analysis was performed on a total of 825 case histories of new patients between 12 and 80 years of age, who for the first time visited the allergy clinic of the Hospital Clínico Universitario "San Cecilio" of Granada in the year 2000. All of these patients registered positive skin prick tests for pollens and spores.

The aerobiological samples were made continuously from 1998 to 2003 with a volumetric collector (Burkard model) located on a terrace of the Science Faculty of the University of Granada in the city centre. The aerial samples were prepared and analysed following the methodology of Domínguez et al. (1991) for the spanish aerobiology network "REA".

Of the 60 biological particles currently detected in the atmosphere of Granada, 14 have the highest incidence in the province of Granada (12 pollen types and 2 spore types). Of all of these, *Olea*, Poaceae, Urticaceae, *Platanus*, *Plantago*, Chenopodiaceae/Amaranthaceae, *Populus*, *Ulmus* and *Alternaria* are recognized

as major aeroallergens and consequently are tested in allergy clinics. Other pollen types, such as *Quercus* and *Pinus*, also appearing at high levels in the atmosphere, have a substantial incidence in the population.

With respect to the clinical study, *Olea* pollen is the main cause of allergy among the population of Granada, with more than 73% of the cases studied, followed by Poaceae, Chenopodiaceae, Cupressaceae, and *Artemisia*. Among fungal spores, *Alternaria* is the ninth cause of allergy, followed by *Cladosporium* and *Aspergillus*. Some 21% of the cases analysed refer to monosensitive patients, while the rest (79%) are polysensitive. With respect to the symptomology, asthma registered the highest incidence, with 60% of the total, followed by rhinoconjunctivitis and atopic eczema.

DÍAZ DE LA GUARDIA, C., ALONSO, R. & BOCIO, I. (1991). Análisis de las recetas de vacunas antialérgicas en la provincia de Granada. **Monog. Fl. Veg. Béticas** 6: 83-97.

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

The clinical relevance of pollen monitoring in the Western Cape, South Africa

Berman, D. M.[1]; de Villiers, S.[2] & Motala, C.[1]

[1] Allergy and Respiratory Unit, Red Cross Children's Hospital, Cape Town.
[2] Department of Environmental and Geographical Science, University of Cape Town

Pollen and fungal spore levels have been continuously monitored for 20 years at Red Cross Children's Hospital in Cape Town, a suburban area close to Table Mountain. In addition, aerspora have been monitored at Table View, a town on the western coastline, since 1994. Two additional Burkard spore traps have been run for shorter periods in a semi-rural inland area, as well as an urban area. The weather patterns in Cape Town and surrounding areas of the Western Cape vary widely, due to the long coastlines and mountainous terrain that exist within a relatively small area. It is therefore important to assess the different pollen and fungal loads in several areas, despite the fact that they might be in fairly close proximity to each other.

The prevalence rate for 12-14 year olds, resident in Cape Town was found to be 15% in 1995. Patients attending the Allergy Clinic at our hospital are skin-prick tested to a panel of inhalant allergens, which includes locally occurring grasses, trees and fungal spores in accordance with the findings of the aerobiological data. Grass is an important inhalant allergen and in severe cases, immunotherapy is advised. Charts of the seasonality of the wild grasses are consulted in order to choose the months when the grass count is consistently <10 grains/m^3. Most of these grasses have been introduced and naturalised, but the allergenicity of a limited number of African grasses have been studied.

Start times for flowering times for the major grasses and allergenic trees, as well as the total pollen count vary according to the area and will be compared. In addition, a petro-chemical refinery is situated close to the monitoring site at Table View, where some pollutants are also continuously monitored and published in local newspapers together with pollen and fungal spore levels.

The allergologists in Cape Town at all the academic hospitals as well as those in private practice are assisted, not only by the reports of pollen and fungal spore levels but by the interpretation of these data., which should not be examined in isolation but against the background of other inhalant allergens that are known to occur in the area, such as housedust mite. Although pollen sensitivity is seasonal >80% of the allergic children attending our clinic are sensitive to *D.pteronyssinus*. As few of these are monosensitive, they frequently have severe rhinitis during the peak pollen months.

An interpretation of the pollen and fungal spore report as well as a bar chart showing these levels has assisted in the diagnosis and treatment of allergic rhinitis in the Western Cape and this service is currently being extended to other regions.

THE INTERNATIONAL STUDY OF ASTHMA AND ALLERGIES IN CHILDHOOD (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, rhinoconjunctivitis and atopic eczema: ISAAC. **Lancet** .35: 1225-32.

PRESCOTT, R. A. & POTTER, P. C., 2000. Allergenicity of the Indigenous Grasses of Southern Africa. **ACI** 12/6: 282-7.

Risk evaluation of exposure to *Olea europaea* L. pollen in Andalusia (S Spain)

Díaz de la Guardia, C.[1]; Alba, F.[1]; Girón, F.[1]; Nieto, D.[1]; De Linares, C.[1]; Candau, P.[2]; Galán, C.[3]; Trigo, M. M.[4]& Ruiz, L.[5]

[1] Department of Botany. University of Granada. E-18071 Granada. Spain.
[2] Department of Plant Biology and Ecology. University of Sevilla. E-41012 Sevilla. Spain.
[3] Department of Botany. University of Córdoba. E-14071 Córdoba. Spain.
[4] Department of Plant Biology. University of Málaga. E-29080 Málaga. Spain.
[5] Department of Ecology, Animal and Plant Biology. University of Jaén. E- 23071 Jaén. Spain.

In recent years olive cultivation has surged in Andalusia (S Spain), cultivation expanding from 1.3 million ha in 1997 to 1.5 million ha in 2002 (IEA, 2003). Favourable economic conditions since admission to the EU largely explain the increase. Spain is the European country with the largest surface area of olive orchards, with some 2.5 million ha, followed by Italy (1.5 million) and Greece (1 million). Within Spain, the region of Andalusia claims more than 65% of the overall area devoted to this crop, with the province of Jaén occupying first place (590,000 ha). Spain is the greatest producer of olive oil worldwide, at roughly 5 million tonnes, with Andalusia contributing up to 75% of this production.

Pollen from the olive tree (*Olea europaea* L.), the most abundant type in the pollen spectrum of the region of Andalusia, has in the last 5 years increased in total annual production by 33% in Córdoba, 100% in Granada and Jaén , and up to 200% in Málaga. It has been shown that during the pollination of these trees, the human population of Andalusia is exposed to extremely high levels of pollen, making this airborne allergen the prime cause of allergy in this region, with high percentages of the population being allergic according to cutaneous tests: 46% in Málaga (Burgos, 1991), 72% in Córdoba (Domínguez et al., 1993), 77% in Granada (Martinez Cañavate et al., 1995) and 89% in Jaén (Florido et al., 1999). As a means of providing preventive information, spatio-temporal modelling was conducted for the seasonal evolution of this pollen in the different provinces of the region by creating risk categories for pollen exposure based exclusively on aerobiological criteria.

The aerobiological analysis was made over several years with Burkard or Lanzoni volumetric collectors placed in the cities of Almería, Cadiz, Córdoba, Granada, Huelva, Jaén, Málaga and Sevilla, using the methodology proposed by the Spanish Aerobiology Network. The spatio-temporal models were constructed following the procedure of Alba et al. (2002) using three-dimensional coordinate systems at the same time as the pollen data were interpolated by traditional geostatistical methods. The use of averaged pollen data from the temporal series pertaining to each city will enable the preparation of maps of pollen dispersion, for which four risk categories of exposure were established (low, medium, high, and extreme). Finally, the use of these models together with digital thematic cartography (vegetation, topography, orthoimage, etc.) has provided a high degree of reliability in establishing the risk areas on a regional scale.

The results indicate that the bioclimatic diversity of this region promotes a stepped flowering of the olive tree in such a way that in March the first pollen grains are detected in the warmest and coastal zones of Andalusia, reaching levels of little significance. In the thermomediterranean level of the Guadalquivir Basin (Córdoba, Sevilla, Jaén), the olive trees flower at the beginning of April, reaching medium levels both in the coastal zones (Málaga and Almería) as well as in the interior during the two following weeks. Pollen production does not begin in Granada until the end of April. May represents the greatest risk for the population exposed to this pollen, given that, except for the coastal belt, levels range from high to extreme. Despite that during June the pollen values decline, interior zones such as Jaén, Córdoba and Granada continue to maintain moderate to high risk values. In early July, this pollen practically disappears from the air of Andalusia, remaining at low levels in the province of Granada.

Alba, F., Díaz de la Guardia, C.; Trigo, MM.; Galán, C.; Ruíz, L. & Sabariego, S. (2002). Modelización espacio-temporal del polen de *Olea europaea* L. en el Sur de España. **XIV Simposio de Palinología de la APLE**. Salamanca.

BURGOS, F. (1991). Pólenes y Medio ambiente: sensibilizaciones polínicas en Andalucía. AAIS. Sevilla.

DOMÍNGUEZ et al. (1993). Variations in the concentration of airborne Olea pollen and associated pollinosis in Córdoba (Spain): A study of the ten years period 1982-1991. J. Invest. Allergol. Clin. Immunol. 3: 121-129.

FLORIDO, J. et al. (1999). High levels of *Olea europaea* pollen and relation with clinical finding. Int. Arch. Allergy Immunol. 119: 133-137.

IEA (2003). Anuario Estadístico de Andalucía 2002. Ed. IEA

MARTÍNEZ CAÑAVATE, A. et al. (1995). Estudio epidemiológico en la unidad de alergia infantil en el área hospitalaria norte. APAOE. Sevilla.

Tolerance of specific immunotherapy (SIT) with*Olea europaea* and grass pollen extract depending on patient's habitat and region

Salz Sanchez, V.; Justicia Ruano, J. L.; Moreno Aguilar, C. & Guerra Pasadas, F.

Allegy Unit. Hospital Universitario Reina Sofía. Córdoba-Spain.

SIT consists in administration of allergenic extract to a sensitized patient, in order to induce an immunologic and clinical tolerogenic response. Safety is an important concern about this procedure wich can be influenced by genetic factors and environment.

To verify habitat and region as influence factors (genetics or environment) on appearance of sistemic reactions (SR) in patients treated with SIT to *Olea europaea* and grass pollen extracts.

A prospective trial of patients receiving build-up phase of SIT to *Olea europaea* and grass pollen from 1998 until 2002 in allergy unit of Hospital Reina Sofía, Córdoba (Spain). Patients were classified according region of Cordoba province where they live, and also the habitat (rural, semi-rural and urban). Logistic regression was used to determine association among regions, habitats and SR.

Data from 611 patients were collected: 287 (47 %) female and 324 (53 %) male, aged 18 years on average, that received SIT with *Olea europaea* pollen extract or *Olea europaea* – grass pollen 50 % mixed extract.

Thirty four per cent of patients came from Cordoba city; 21 % from Subbética region; 20 % from Campiña-Sur region and 25 % from others. No significant difference was found in rate of SR among regions ($p=0.74$) either habitats ($p=0.31$).

Results of trial can't establish region and habitat as influential factors on rate of SR among patients receiving SIT with pollen vaccines.