droughts, as 1988-89 (1 mm from December 3 to February 21), 1996-97 (5 mm from January 24 to May 4) and 1999-2000 (5 mm from December 17 to March 27).

Response of pollen dispersal to this temperature increase has been analysed in early works of the '70ies using the first aerosporological data on short periods (Lejoly-Gabriel, 1978), in the '80ies and '90ies applying mathematical models in particular to some early flowering arboreal taxa and both to rain and temperature data (for example Spieksma *et al.*, 1989, Caramiello *et al.*, 1994). Models and forecasting have been more and more applied to aerosporological data in the last years in which long series data are at disposal and best models have been pointed out to predict the start of the pollen season and also the productivity of some cultivated plant (Frei, 1998).

Our study is based on the start of the pollen season of *Betula, Alnus, Platanus* and *Juglans*, considered as the day in which 5% of the sum of the annual pollen concentration is reached, and on the temperature data of the period preceding the pollen start.

Aerosporological data have been collected in the period 1983-2003 with a Hirst spore-trap located in the centre of the city, in the same site all along the 20 years.

Meteorological data have been collected in urban observatories and mean daily temperature calculated as (Tmin + Tmax)/2 have been considered.

Temperature sums calculated from the beginning of January and in the 30 days before start of the pollen season, daily mean temperatures and rainfall have been considered for each of the four taxa.

The variables were processed in several combinations until the best model for the start of season prediction was constructed.

The elevated winter temperatures are always followed by an earlier start of the pollen season in all the taxa but rainfall values are equally important in determining the start of pollination.

Results were compared with the ones obtained for *Betula* in the United Kingdom (Adams-Groom *et al.*, 2002) in Neuchatel (Clot, 2001) and in Denmark (Rasmussen, 2002).

ADAMS-GROOM, B., EMBERLIN, J., CORDEN, J., MILLINGTON, W. & MULLINS, J. 2002. Predicting the star of the birch pollen season at London, Derby and Cardiff, United Kingdom, using a multiple regression model, based on data from 1987 to 1997. **Aerobiologia** 18: 117-123.

CARAMIELLO, R., SINISCALCO, C., MERCALLI, L. & POTENZA, A. 1994. The relationship between airborne pollen grains and unusual weather conditions in Turin (Italy) in 1989, 1990 and 1991. **Grana** 33: 327-332.

CLOT, B. 2001. Airborne birch pollen in Neuchâtel (Switzerland): onset, peak and daily patterns. **Aerobiologia** 17: 25-29.

FREI T. 1998. The effects of climate change in Switzerland 1969-1996 on airborne pollen quantities from hazel, birch and grass. **Grana** 37: 172-179.

LEJOLY-GABRIEL, M. 1978. Recherches écologiques sur la pluie pollinique en Belgique. **Acta Geogr. Lovanien**. 13.

RASMUSSEN, A. 2002. The effects of climate change on the birch pollen season in Denmark. **Aerobiologia** 18: 253-265.

SPIEKSMA, F. TH. M., FRENGUELLI, G., NIKKELS, A.H., MINCIGRUCCI, G., SMITHUIS, I.O.M.I.J., BRICCHI, E., DANKAART, W. & ROMANO, B. 1989. Comparative study of airborne pollen concentrations in central Italy and the Netherlands (1982-1985). Emphasis on *Alnus*, Poaceae and *Artemisia*. **Grana** 28: 25-36.

Poster session d1

MOLECULAR AND CELLULAR ANALYSIS OF POLLEN ALLERGENS

Ole e 10, a major olive pollen allergen, is the first member of a new family of plant proteins

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Type I allergy represents a major health problem affecting 25% of the population in industrialized countries. Isolation and characterization of the complete allergogram of an allergenic source is an important goal in the allergy research that contributes to understand the molecular bases of the disease and to improve diagnosis and therapy tools.

Olive pollen is one of the main causes of allergy in Mediterranean countries. Recently, a new allergen from this pollen, Ole e 10, has been isolated and characterized. It is a small (10.8 kDa) and acidic (pI 5.8) protein, which sensitizes 55% of patients allergic to olive pollen. Northern-blot experiments have demonstrated that Ole e 10 is not specific from pollen, so it is also presented in fruit, stem and leaves. Ole e 10 cloning has been performed in two PCR steps using total olive pollen cDNA as a template. The allergen consists in a single polypeptide chain with 102 amino acids, 6 cysteine residues and one consensus sequence of glycosylation. However, Ole e 10 staining with ConA lectin is negative, indicating the absence of mannose residues in its structure. Ole e 10 sequence shows homology with a family of genes from *Arabidopsis thaliana*, which would encode proteins comprised between 110 to 256 amino acids. The analysis of any protein product from these genes has not been reported so far, and its functional role is unknown. Hence, Ole e 10 would represent the first described and characterised member of a novel family of plant proteins. Ole e 10 also shows similarity with the C-terminal domain of 1,3-β-glucanases, such as the allergenic glucanase of olive pollen, Ole e 9. Finally, Ole e 10 aligns with the "Cys-box" domain of several families of glucanosyltransferases –Gas (glycophospholipid-anchored surface), Epd (essential for pseudohyphal development) and Phr (pH-regulated)- involved in yeast development.

Ole e 10 accounts for a new family of plant proteins that could perform, as an independent protein module, similar functions to those of homologous domains from glucosidases with catalytic role in carbohydrate metabolism.

Detection of *Parietaria judaica* allergenic proteins and comparison with *Urtica dioica*

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Loads of airborne pollen have been measured for many years in several countries to get information on daily quantities of allergenic pollen and their relations to symptoms of pollen allergy and to make forecasts of airborne pollen concentration in the air. Urticaceae family is a kind of plants which grows especially on soils rich in nitrogen, like overgrowing weeds in cultivations or abandoned lands near habited places. So, this is one of the allergenic family that appears in many pollen calendar (D´AMATO & col. 1991). Within this family there are two genera which are prominent significance in airborne pollen studies (*Parietaria* and *Urtica*) because these plants have great relevance as plants with allergenic pollen grains in diverse european regions, specially *Parietaria*.

Appllying a combination of transmission electron microscopy with immunocytochemical methods, the localization of the allergenic proteins in the *Parietaria judaica* and *Urtica dioica* mature and activated pollen

grains was investigated. Activation was induced in vitro for 10, 15 and 20 minutes. Serum from allergenic patient with specific IgE to *Parietaria* and *Urtica* was used.

In mature pollen grain of *P. judaica*, the localization of these allergenic proteins was remarkably different from that observed in activated pollen. The activated proteins, reacting with antibodies present in human serum from allergenic patient, are found in the cytoplasm, intine, exine and exudates from these pollen grains.

Our results show that the activation time plays an important role on the labeling intensity, the content of allergenic proteins is unstable, displaying variation relative to the progress of germination in *P. judaica* pollen grains. These proteins were activated at the moment of pollen hydration, prior to pollen tube formation, and was released and detected during the first 20 minutes of activation. The high allergenic activity of *P. judaica* pollen grains may be due to the rapid activation and release of these allergenic proteins.

In *U. dioica* pollen grain we have only observed a slight labeling in the apertural and non-apertural wall, especially in the oncus and in the material extruded from the pollen grain, in the 10 minutes hydrated activated pollen. In the cytoplasm there was no significant labeling. These proteins were less abundant than the allergenic proteins observed in *P. judaica* pollen. So, in the pollen-stigma recognition process of *U. dioica* takes part less allergenic proteins than in the pollen-stigma recognition process of *P. judaica*. Moreover, this study confirms the no existence of cross reaction between *P. judaica* and *U. dioica* through immunocytochemical methods.

D'AMATO, G., RUFFILLI A. & ORTOLANI C. 1991. Allergenic significance of *Parietaria* (Pellitory of the wall) pollen. In: G. D'AMATO, F.TH.M. SPIEKSMA & S. BONINI (eds.). **Allergenic pollen and pollinosis in Europe**. pp. 113- 118. Blackwell Sc. Publ. Oxford.

Intra and intercultivar variability of Ole e 1 in olive pollen. Preliminary analysis of patient's response to different cultivars extracts

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The presence of differences in both the allergenic content and the reactivity of patients to olive pollen from different cultivars is beginning to emerge (WAISEL et al., 1996; CARNÉS et al., 2002; CASTRO et al., 2003). These differences have been established up to date only in a limited number of olive cultivars from the extremely high number of cultivars (over 250 cultivars in Spain alone) available. It has been proposed that such differences may represent distinctive characteristics possessing both biological and clinical significance.

In the present study, we have analyzed the SDS-PAGE protein profiles of crude protein extracts corresponding to mature pollen from 30 well-defined olive cultivars. Our analysis indicate that significant inter-cultivar differences are clearly distinguishable, particularly concerning those polypeptides with Mws ranging 17-19 kDa, which correspond to different forms of the Ole e 1. Conspicuous differences have been also detected within individual cultivars, depending on either the specimen analyzed and/or the year of pollen collection.

The clinical relevance of the reported biochemical differences was assessed by performing skin tests using individual extracts from pollen of each cultivar, on patients considered to be allergic to olive pollen on the basis of medical history and previous SPT and RAST tests to commercial olive extracts. Sharp differences in the skin reactivity of patients to the individual extracts were detected using this method.

Western blots corresponding to SDS-PAGE gels of cultivar pollen extracts were also probed with patient's sera, in order to define the IgE reactivity of such sera to the major allergen Ole e 1 and other pollen allergens.

This study confirms the need to take into account the intraspecific differences in the allergenic content of pollen in order to standardize the extracts used for clinical diagnosis of allergy and for the preparation of vaccines.

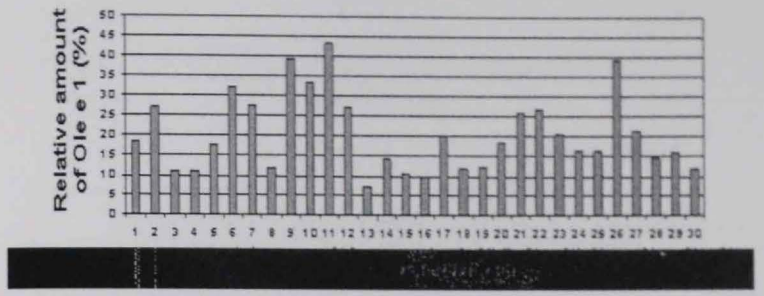

Fig. 1: Relative percentages of Ole e 1 polypeptides with respect to the total protein content of the cultivars studied.

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

CARNÉS SÁNCHEZ, J.; IRAOLA, V. M.; SASTRE, J.; FLORIDO, F.; BOLUDA, L. & FERNÁNDEZ-CALDAS, E. 2002. Allergenicity and immunochemical characterization of six varieties of *Olea europaea*. **Allergy** 57:313-318.

CASTRO, A. J.; ALCHÉ, J. D.; CUEVAS, J.; ROMERO, P. J; ALCHÉ, V. & RODRÍGUEZ-GARCÍA, M. I. 2003. Pollen from different olive tree cultivars contains varying amounts of the major allergen Ole e 1. **Int. Arch. Allergy Immunol.** 131:164-173.

WAISEL, Y.; GELLER-BERNSTEIN, C.; KEYNAN, N. & ARAD, G. 1996. Antigenicity of the pollen proteins of various cultivars of *Olea europaea*. **Allergy** 51: 819-825.

Study of *Olea europae* pollen proteins: An proteomic approach

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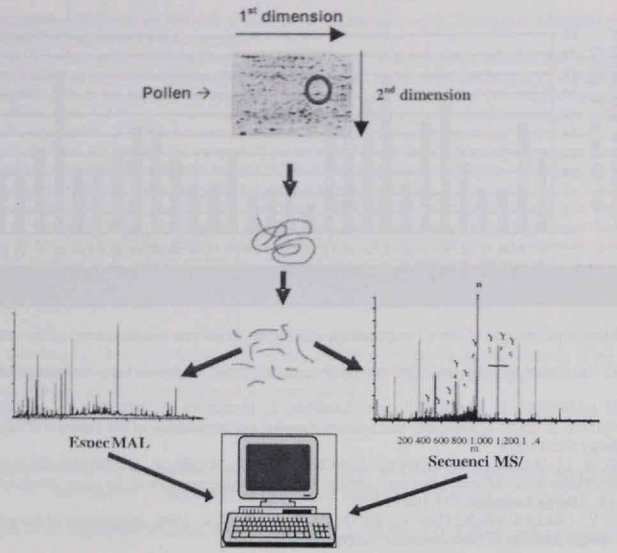
[3] Münter, (Alemania).

The study of pollen proteins and specially those responsible of its allergenic power, so as the modifications suffered along the time and by different stress conditions, are of special interest, both from a basic and practical point of view.

In this communication we report on preliminary results obtained in the study of *Olea europae* pollen proteins, using 2D electrophoresis and mass spectrometry (MS). Theses results can be of great importance for the preparation of a Data Base of pollen proteins.

The procedure used in our study are shown schematically in Figure-1



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 disappear 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 fuchsina-glicerinated-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 atmospherical 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 the 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.