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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.

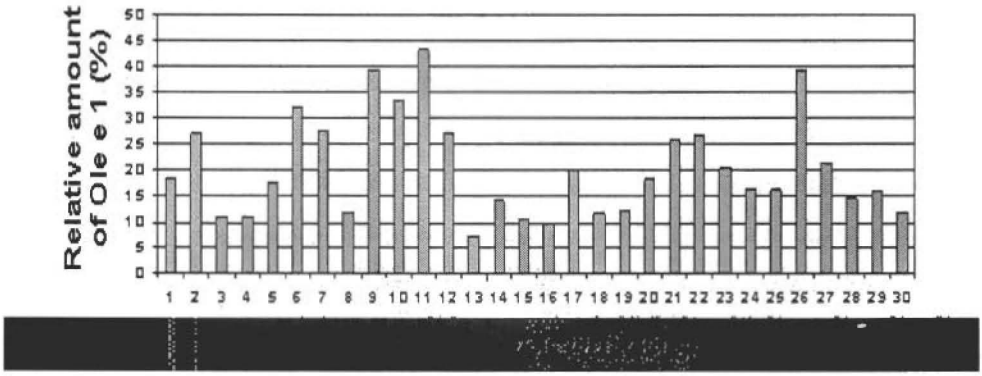


**Fig. 1:** Relative percentages of Ole e 1 polypeptides with respect to the total protein content of the cultivars studied.

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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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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