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CROSS-RECOGNITION OF FAGACEAE POLLEN ALLERGEN BY IgE RAISED AGAINST ALLERGEN OF *CUPRESSUS ARIZONICA* POLLEN

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This study investigated cross-reactivity between allergens of Cupressaceae and Fagaceae pollen. Human IgE raised against *Cupressus arizonica* pollen allergen was used to demonstrate the presence of related allergens on ultrathin sections of Fagaceae pollen (*Quercus ilex*, *Castanea sativa*, *Fagus sylvatica*). Tissue localization of the cross-reactive allergen was investigated by immunogold electron microscopy. TEM observations showed that IgE raised against *C. arizonica* allergen recognizes epitopes on Fagaceae pollen. The cross-reactive allergens can be found on the wall and in the cytoplasm.

Key words: Pollen allergen, immunogold labelling, cross-reactivity.

INTRODUCTION

The release of pollen in the air is a normal part of the sexual cycle in anemophilous plants. Before its dispersion, pollen is dehydrated and its metabolic activity is reduced. Metabolic activity reengages when the pollen is in contact with a source of humidity, which can be in the atmosphere, on the stigma, or in the respiratory system of a subject. When pollen rehydrates and activates, it releases molecules which are probably involved in the process of sexual reproduction.

Some of these molecules can interact with the human immune system, inducing it to produce antibodies (IgE), and eliciting allergic symptoms as hay fever and asthma (Knox and Suphioglu, 1996). Allergenic molecules are usually proteins or glycoproteins with molecular weights ranging from 10 to 70 Kda, localized variously in the pollen grain: in the wall, the cytoplasm, or in various structures such as the endoplasmic reticulum, starch grains or the nucleus (Grote, 1999). Pollinosis, pollen allergy, has been increasing continuously for a century (EUROPEAN ALLERGY WHITE PAPER, 1997). Allergenic proteins often have epitopes in common with other proteins present in the pollen of many other species or in plant-derived food, so there can be high cross-reactivity among them. A way to establish whether or not a pollen is allergenic is to check the reaction of human IgE to the suspected pollen.

Several species widely diffused in Italy belong to the Fagaceae family, including *Quercus ilex* L., *Castanea sativa* Mill., and *Fagus sylvatica* L. These species grow spontaneously in Latium, are used for reforestation, and are also used along city avenues and in urban parks for ornamental purposes.

Fagaceae are anemophilous plants, so during their flowering season they produce a great amount of pollen which is released into the air. At "Tor Vergata" University there is an active airborne monitoring center managing three stations set up in the city of Rome. From May to July, high levels of pollen of the three genera studied here are reported at the three Rome stations, with average daily concentrations of 40 grains/m^3 air, and maximum peaks of 450 g/m^3 for *Quercus* and 350 g/m^3 for *Castanea* (Travaglini et al., 2002). In surveys of the extent of pollinosis based on question-

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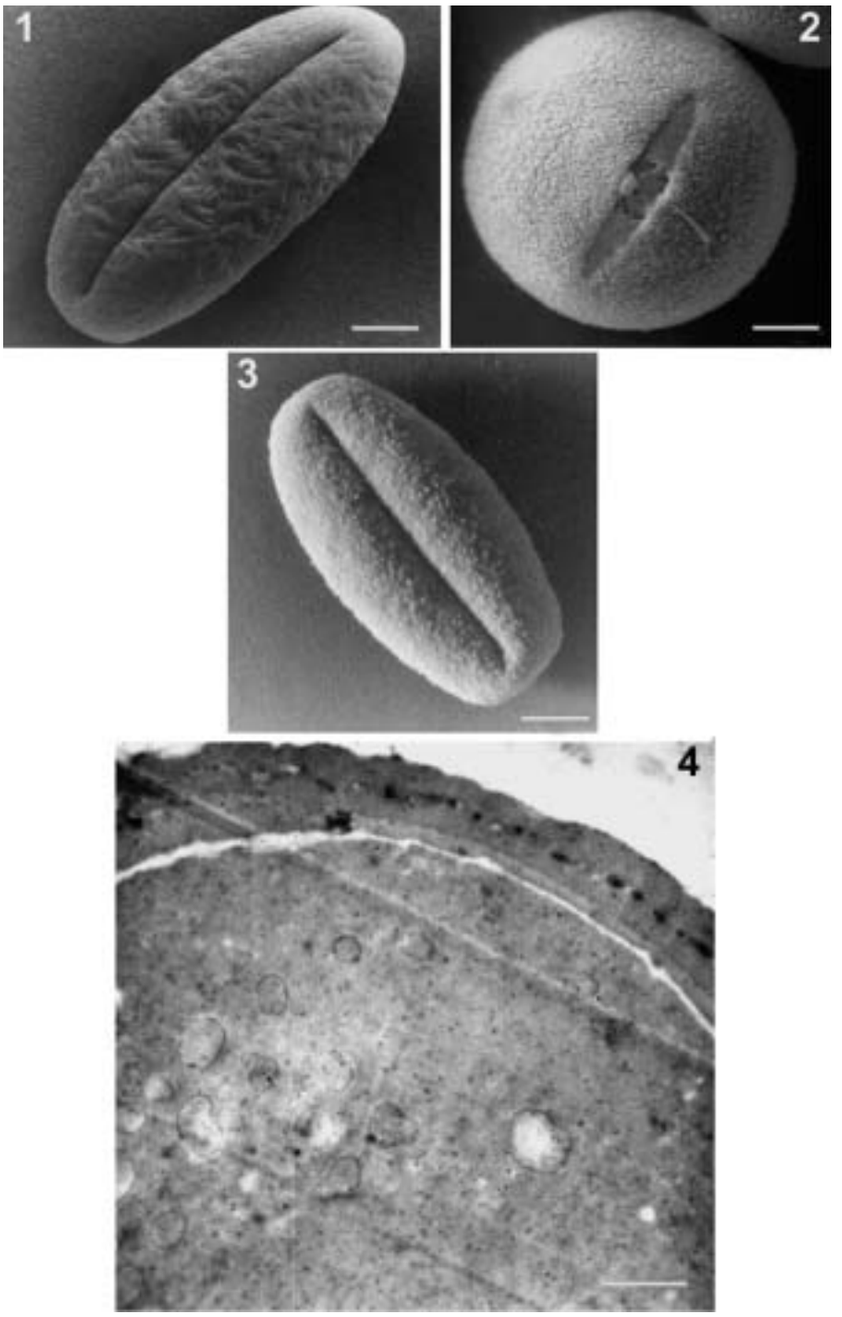
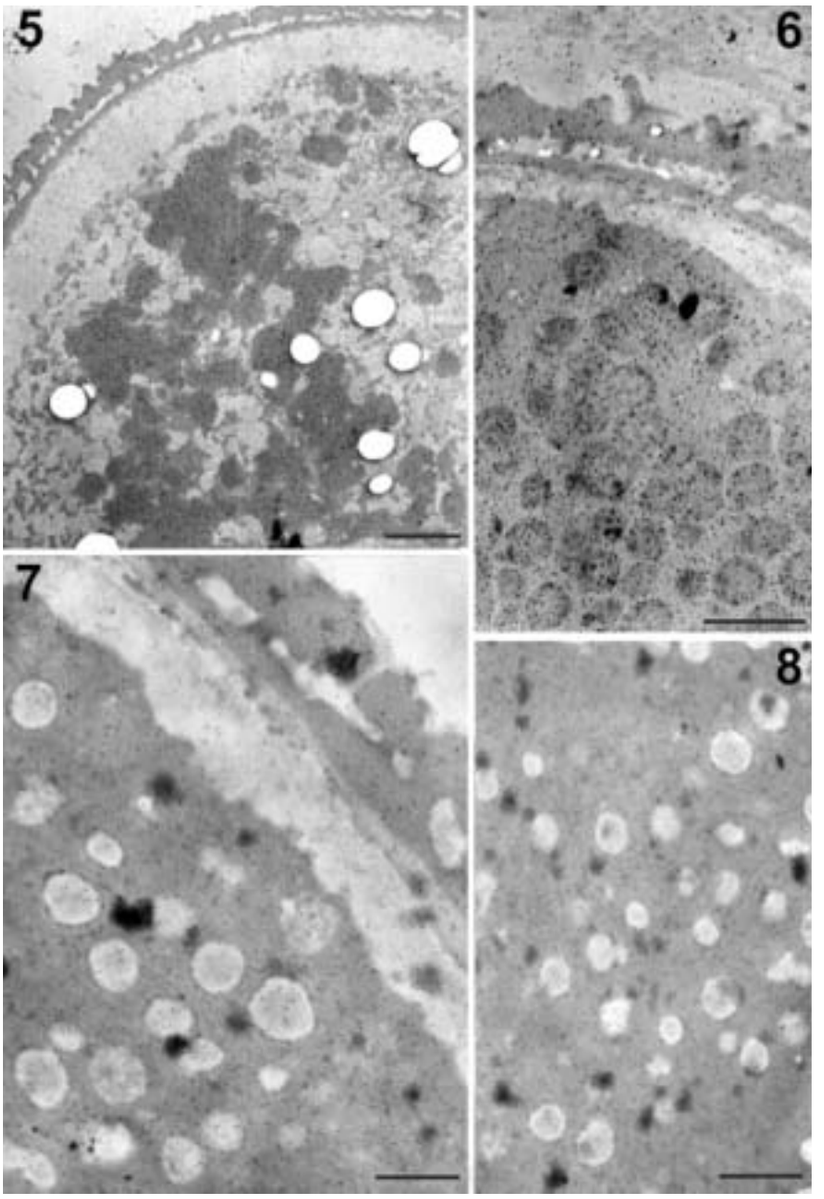


Figs. 1–3. SEM photographs. **Fig. 1.** *Castanea sativa* pollen grain; rugulate exine is visible. Bar = 2 µm. **Fig. 2.** *Fagus sylvatica* pollen grain; rugulate exine is visible. Bar = 7 µm. **Fig. 3.** *Quercus ilex* pollen grain; scabrate-rugulate exine is visible. Bar = 5 µm. **Fig 4.** Ultrathin section of *Castanea sativa* pollen grain labelled with human IgE anti-*Cupressus arizonica* revealed with goat anti-rabbit IgG coupled with 5 nm gold particles. Gold markers are in cytoplasm (on organelles and cytosol) and on cell wall. Bar = 1.2 µm.

naires administered to personnel of Tor Vergata University (students, professors, researchers, technicians), 3% of the allergic subjects report sensitivity to Fagaceae pollen (Grilli et al., 2002). However, the literature contains very scant and often contradictory data on the allergenic activity of Fagaceae pol-



Figs. 5–8. Ultrathin sections of pollen grains. **Fig. 5.** *Fagus sylvatica* pollen grain labelled with human IgE anti-*Cupressus arizonica* revealed with goat anti-rabbit IgG coupled with 10 nm gold particles. Gold markers are in cytoplasm, on organelles and on nucleus. Less labelling is present on cell wall. Bar = 2.5 µm. **Fig. 6.** Detail of cytoplasm of *Fagus sylvatica* with gold labelling. Bar = 1.7 µm. **Fig. 7.** *Quercus ilex* pollen grain labelled with human IgE anti-*Cupressus arizonica* revealed with goat anti-rabbit IgG coupled with 5 nm gold particles. Gold markers are on wall, and less labelling is on cytoplasm. Bar = 1 µm. **Fig. 8.** Detail of cytoplasm of *Quercus ilex* with gold labelling. Bar = 1 µm.

len (Negrini et al., 1992; Prados et al., 1995; Subiza et al., 1995).

This study investigated cross-reactivity between *Cupressus arizonica* pollen and three Fagaceae species: *Quercus ilex, Castanea sativa, Fagus sylvatica*. Using an immunocytochemical method with human IgE raised against extracts of *Cupressus arizonica* pollen on ultrathin sections of Fagaceae pollen, the presence of similar molecules in the two pollen families was checked. Human IgE raised

against *C. arizonica* pollen recognizes a glucidic epitope of a glycoprotein formed by 7 residues (Iacovacci et al., 2001).

MATERIALS AND METHODS

Pollen from ripe anthers was collected from May to June 2001 at Tor Vergata University (*Quercus ilex*), Arcinazzo Romano, Rome (*Castanea sativa*) and Ovindoli, Aquila (*Fagus sylvatica*). The pollen was fixed in 2.5% glutaraldehyde in phosphate buffer, dehydrated and embedded in Epon resin.

For labelling, ultrathin sections of embedded pollen were incubated with human IgE purified from the blood of a subject sensitive to *Cupressus arizonica* pollen. The first antibody was revealed with a secondary antibody (rabbit anti-human IgE) and a tertiary gold-conjugated antibody (goat anti-rabbit IgG). Labelling was observed with a Zeiss CEM 902 transmission electron microscope at 80 KV.

For SEM observations, pollen was dehydrated in ethanol, critical-point dried, mounted on aluminum stubs, sputtered and observed with a Zeiss DSM 950 scanning electron microscope.

RESULTS

Castanea sativa pollen is very small, with a major diameter of 10–12 µm. It is tricolporate with long colpi and small pori. The exine is rugulate, with soft sculpture (Fig. 1). *Fagus sylvatica* pollen is large (40–45 µm of diameter) and tricolporate with short colpi and big pori. The exine is thin and rugulate with heavy sculpture (Fig. 2). *Quercus ilex* pollen is of intermediate size (20–25 µm in diameter). It is tricolporate with large colpi. The exine is verrucate-rugulate (Fig. 3).

Immunogold labelling indicated that human IgE raised against *Cupressus* recognizes glucidic epitopes of proteins on the Fagaceae pollen tested. *Castanea sativa* pollen was labelled on the wall and also in the cytoplasm (Fig. 4). *Fagus sylvatica* pollen was labelled in particular on cytoplasmic organelles and on the nucleus (Figs. 5, 6). *Quercus ilex* pollen shows intense labelling over the grain, in particular on the exine and intine (Figs. 7, 8).

DISCUSSION

IgE raised against *Cupressus* allergen recognizes the glucidic epitope of proteins present in Fagaceae pollen. Although this epitope can be widely diffused, we can surmise that there is cross-reactivity between the allergens of the pollen of these two families of plants. The allergy caused by Cupressaceae pollen is well identified, because *Cupressus*, the genus that produces the major amount of pollen, blossoms in winter, so the allergic symptoms can be recognized and attributed to it. On the other hand, Fagaceae blossom in spring and early summer, together with several well-known allergenic plants such as Gramineae, olive, Parietaria and others, so allergic symptoms due to Fagaceae pollen can be confused with other types of pollinosis. Moreover, Fagaceae pollen elicits only polysensitization, so it is very difficult to discriminate its pollinosis from others. For this reason it is important to investigate this family of plants to clarify the allergenic activity of its pollen.

REFERENCES

EUROPEAN ALLERGY WHITE PAPER, 1997. *Allergic disease as a public health problem*. UCB Institute of Allergy, Belgium.

GRILLI CAIOLA M, MAZZITELLI A, CAPUCCI E, and TRAVAGLINI A. 2002. Monitoring of pollinosis and airborne pollen in a University of Rome. *Aerobiologia* (in press).

GROTE M. 1999. In situ localization of pollen allergens by immunogold electron microscopy: allergens at unexpected sites. *International Archives of Allergy and Immunology* 118: 1–6.

IACOVACCI P, PINI C, AFFERNI C, BARLETTA B, TINGHINO R, SCHININA E, FEDERICO R, MARI A, and DI FELICE G. 2001. A monoclonal antibody specific for a carbohydrate epitope recognizes an IgE-binding determinant shared by taxonomically unrelated allergenic pollens. *Clinical and Experimental Allergy* 31: 458–465.

NEGRINI AC, and ARROBBA D, 1992. Allergenic pollens and pollinosis in Italy: recent advances. *Allergy* 47: 371–379.

KNOX B, and SUPHIOGLU C. 1996. Environmental and molecular biology of pollen allergens. *Trends in Plant Science* 1: 156–164.

PRADOS M, ARAGON R, CARRANCO MI, and MARTINEZ J. 1995. Assessment of sensitization to holm oak (*Quercus ilex*) pollen in the Mérida area (Spain). *Allergy* 50: 456–459.

SUBIZA J, JEREZ M, JIMONEZ JA, NARGANES MJ, CABRERA M, VARELA S, and SUBIZA E. 1995. Allergenic pollen and pollinosis in Madrid. *Journal of Allergy and Clinical Immunology* 96: 15–23.

TRAVAGLINI A, MAZZITELLI A, LEONARDI D, and GRILLI CAIOLA M. 2002. Monitoraggio di polline urbano e allergie. In: Atti dell'Accademia dei Lincei. Conferenza annuale della Ricerca: Ecosistemi Urbani, 22–24/10/2001.