

Presence of esterase in *Pinaceae* pollen

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Received 30 January 1996; accepted 30 April 1997

Abstract

General esterases, like wall heterologous enzymes, may be implicated in allergenic mechanisms. We cytochemically examined the presence of these enzymes, bearing in mind that *Pinus* spp. is often considered a possible cause of allergies. Positive esterase results in fungus spores were found on the strip of a volumetric sampler. Data are reported here, together with the hypothesis of a possible esterase-allergenic link regarding fungus spores. © 1997 Elsevier Science Ireland Ltd.

Keywords: Pollen; Esterase; Blue deposits; Cytochemical evidence; Pinace

1. Introduction

In our urban areas, conifers, pines, cypresses and yews are considered ornamental trees. These trees, which produce a considerable quantity of pollen, dispersing it in the air (Accorsi et al., 1994), have been reassessed as possible producers of allergies.

In previous studies a high percentage of esterase activity was found in cypress pollen, studied as passive subjects of urban pollution (Alaimo et al., 1996; Melati et al., 1996a,b). In these studies the presence of high quantities of esterases was correlated to a hypothetical allergenic mechanism. Inhaled pollen may release these proteins when they burst, and activate the allergopathic drop which, due also to the presence of polluting particles (Alaimo et al., 1994; Alaimo and Melati, 1995; Alaimo et al., 1996) induces 'super-reactivity'. In the same way, we examined the *Pinus* spp (Owens et al., 1985). Pollen esterases have been analysed in various vegetable species, for example in *Annona squamosa* (Vithanage, 1984), *Citrus medica* (Protopapadakis, 1987) and *Citrus reticulata* (Chen et al., 1992), *Cucurbita pepo* (Yang and Tsao, 1981), date palm (Al-Jibouri,

1988), *Luffa cylindrica* (Yang and Tsao, 1981), *Malus* (Babaevskaia et al., 1981), the oil palm (Santos et al., 1993) the olive (Trujillo et al., 1990, 1995), *Petunia hybrida* (Nave and Sawhney, 1986), *Portulaca grandiflora* (Mehan and Malik, 1974; Sood, 1974), the tomato (Zamir et al., 1982), and in *Tulipa* (Baumker et al., 1988).

Nevertheless, there are some plant pollens which do not provoke allergies and which contain such low levels of esterase that they do not show any cytochemical reaction (<0.03 units of absorbance/ μm^2) (e.g. rose, personal data).

2. Materials and methods

Pollen was taken from some male inflorescences (pollen cones with dehiscing microsporangia) of pines situated on streets with heavy traffic, and divided into three groups: two for structural microscopic observations (o.m. Orthoplan, Leica and SEM, Cambridge Stereoscan 360), the other for cytochemical observations (Vithanage et al., 1992). The latter were carried out on portions of the cellulose strip of the volumetric sampler VPPS 2000 (Lanzoni) (Fig. 1a and 1b) and on cryosections (Criostato, Leica) of unfixed microsporangia.

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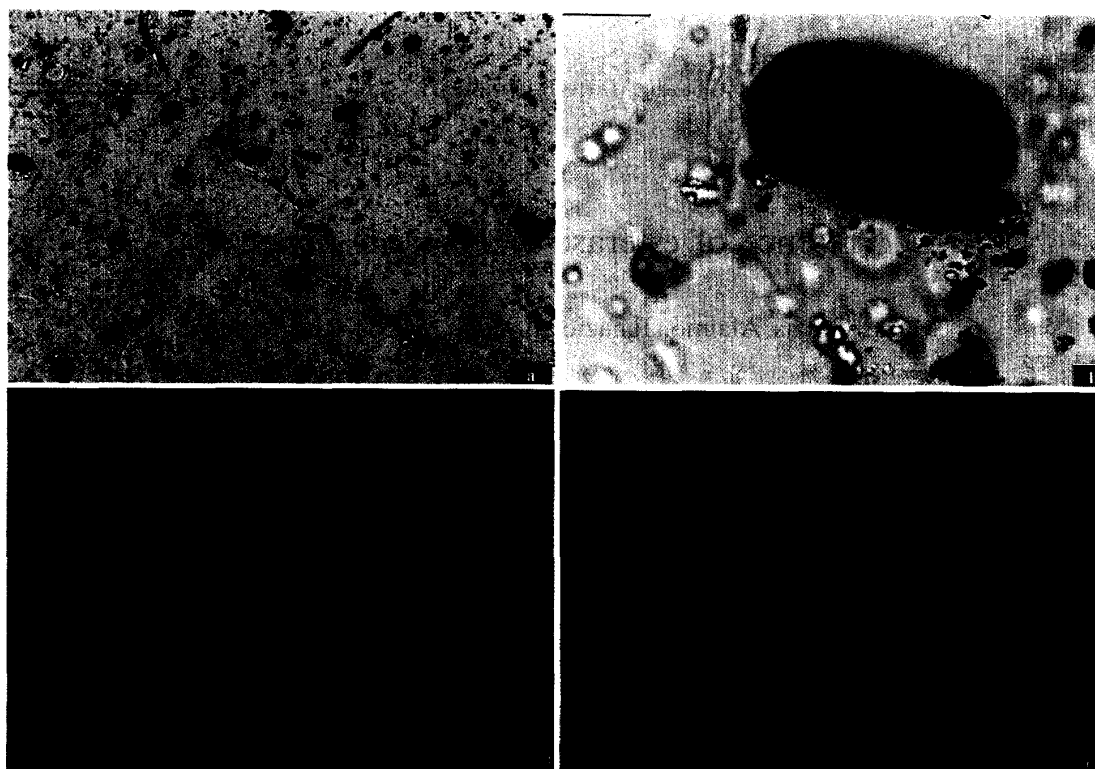


Fig. 1. *Pinaceae* pollen is disseminated by air (anemofilia) and high concentrations of their pollen grain can be monitored in Palermo (a) (May 1994: $\cong 40 \text{ g/m}^3$), often there are aggregate components of polluting particles on the pollen (b), only ph comparison shows the aerodynamic structure of the seed (c) and wing of the seed (d), by anemochorous dispersal (anemochory) (SEM).

Materials were tested with naphthol ASD acetate as substrate and fast blue BB, as diazonium salt, at pH 6.5 in 0.2 M Tris buffer; they were incubated, in light, at 37°C for 1 h.

Blue deposits indicate the sites of general esterase activity (Gahan, 1984), which are absent in control materials where the substrate was omitted.

Cytochemical observations were also directly carried out on portions of the sampler strip VPPS to avoid the possibility that elution manipulations might modify enzymatic activity, the silicone of the strip being inert compared with the vitality of the pollen.

3. Results and discussion

Our observations on pine pollen (Fig. 2a) reveal esterase activity in the pollen grain walls, in the form of green–blue fibrillose masses situated in the central body of the grain and not in the lateral sacchi (Fig. 2c).

Enzyme activity was higher in the walls than in the protoplasts, and more so in the split grains. Local accumulations of fibrillar material are seen in the in-

ner hemicellulosic layer (intine) and, subsequently, incorporated in the outer sporopollenin layer (exine). The uncoloured controls were used to evaluate specificity of colouring (Fig. 2b).

On the strip of the volumetric sampler, *Alfernaria* spp. conidia and *Cladosporium* spp. spores also occasionally presented blue-coloured deposits, which indicate general esterase activity after cytochemical reaction. (Fig. 2d).

The presence of esterase activity in pollen, which in the past was not considered to be significant in the provocation of allergic reactions (Zawisza and Samolinska-Zawisza, 1991), but which was then re-evaluated (Fountain and Cornford, 1991; Knox and Heslop-Harrison, 1975), also by allergists (Passali, 1997, personal communication), may explain this role.

The wall proteins, expelled after inhalation, can be identified as antigens and activate allergenic reaction.

Our proposal is to use the esterases, already identified as species-specific markers in vegetable typification, as test substances in allergometry, extracting and purifying them. Experts could then evaluate the possibility of using the esterases extracted for the preparation of monoclonal vaccines.

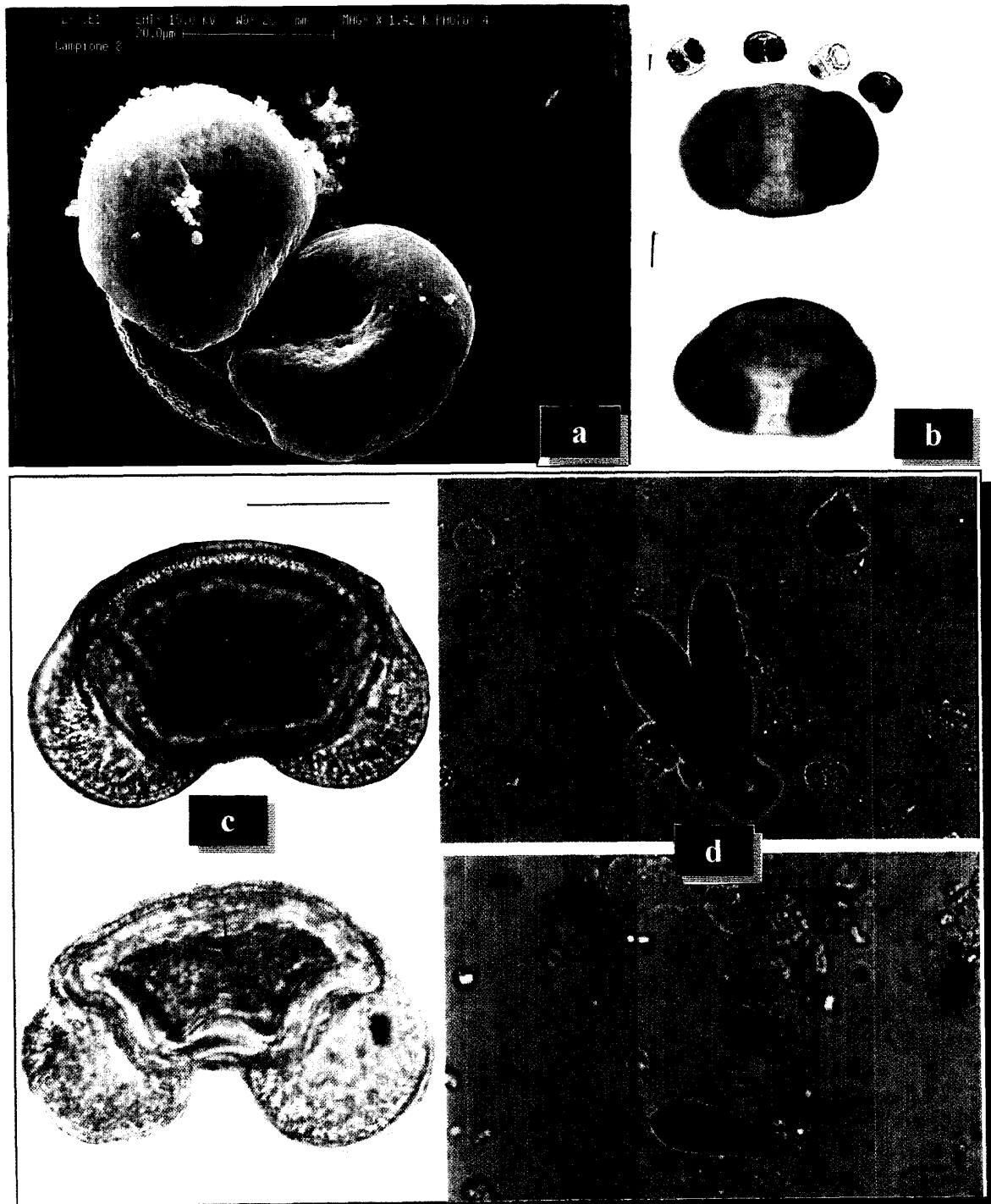


Fig. 2. Structure of pollen, saccate, monopening of *Pinus maritima* L. with tectate, alveolar exine and a thick intine (a) (SEM). Cytochemical reaction tests (b) (o.m.)—Blue-green coloured deposits in central body of pollen which indicate sites of esterase activity (c) (o.m.). There are no fibrillose colour deposits in the lateral sacci: *Alternaria* spp. conidia (1) (2), and *Cladosporium* spp. spores (3) with blue-green coloured deposits, sites of esterase activity (d)) (o.m.) (May–June 1995).

Acknowledgements

This work was supported with funding from the Sicilian Region.

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