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Behavior of profilins in the atmosphere and in vitro, and their relationship with the performance of airborne pollen

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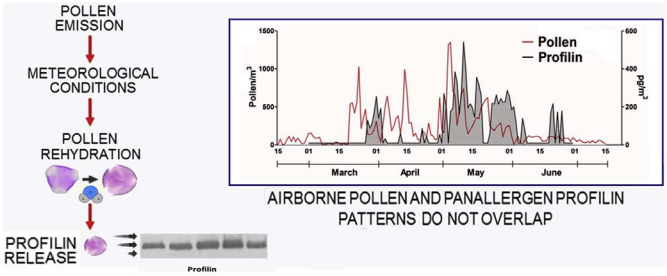
GRAPHICAL ABSTRACT



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ABSTRACT

Most pollen allergens in the air are carried by pollen grains, but the presence of airborne smaller respirable particles containing pollen allergens has also been demonstrated. Meteorological factors drastically affect the occurrence of pollen, allergen release in the air and diffusion of the latest. In order to shed light on this phenomenon, the dynamics of pollen and the pollen panallergen profilin in the air of two European cities (León, Spain and Bologna, Italy) having different weather conditions, were analyzed. Pollen sampling was performed continuously from March to June 2015 using two seven-day recording volumetric trap of Hirst-type, while the particles for aeroallergen quantification were sampled with a Burkard Cyclone sampler and the profilin content in aerosol samples was quantified using an indirect double-antibody sandwich ELISA. In both cities, pollen and profilin concentrations followed a similar trend and showed a significant correlation; however, peaks were often misaligned, with the profilin peaks following those of pollen. Several meteorological parameters, such as relative humidity, significantly influenced pollen and allergen dispersion. In vitro pollen tests were thus performed in order to mimic pollen rehydration, occurring in natural conditions and a massive protein release from allergenic pollen was detected during the early stages of pollen rehydration when profilin was also extruded from the grains. The different timing and protein amounts released from different pollen during hydration might explain, at least in part, the non-synchronous pollen and profilin peaks detected in the atmosphere.

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1. Introduction

Pollen allergy is a major problem for a considerable and steadily increasing percentage of people worldwide (Bousquet et al., 2007; Eder et al., 2006), thus it is of great interest to monitor the allergenic potential of the air. The assessment of airborne pollen concentration is a traditional tool (D'Amato et al., 2007). However, daily pollen concentrations do not always match with the symptoms of pollen allergy sufferers or with allergen content in the air (Bastl et al., 2016). This is reasonably because airborne pollen allergens are also found in non-pollen fractions (De Linares et al., 2010; Fernández-González et al., 2011). From here, the question arises as to whether the methods currently available for measuring atmospheric allergenicity are adequate (Brito et al., 2011; Buters et al., 2012; D'Amato et al., 1998).

Knowledge regarding the influence of weather conditions on allergen release is still poor and debated (D'Amato and Cecchi, 2008). It is nevertheless well-known that meteorological factors significantly affect both pollen and allergen fluctuations. They can either directly affect the amount of airborne pollen or, indirectly, cause its rupture resulting in allergen release.

Allergens can be pollen-specific or, as in the case of panallergens, share sequence and structural conformation thereby leading to cross-reactions between unrelated pollen and often also between pollen and foods. The most clinically relevant plant panallergens are profilins, polcalcins, non-specific lipid transfer proteins and pathogenesis-related protein members of family 10 (Hauser et al., 2010; McKenna et al., 2016). Profilins are small, ubiquitous, and multifunctional actin binding proteins involved in actin cytoskeleton dynamics and this explains their ubiquitous expression and high levels of conservation (McKenna et al., 2016). The conservation of their primary structure, reaching homologies of 86% and 73% in the case of olive (Ole e 2) with *Betula* and pollen grass profilins, respectively (Ledesma et al., 1998; Martinez et al., 2002; Morales et al., 2008) and the conservation of the secondary and 3-dimensional structures causes strong serologic cross-reactivity of the molecules (Santos and Van Ree, 2011).

Profilins have a high allergenic relevance as they can elicit both nasal and bronchial responses in sensitive patients (Ruiz-García et al., 2011). Worldwide, prevalence of profilin sensitization, lies between 5% and 42%, while, for some profilins, the sensitization rates can reach proportions of major allergens (> 50%) (e.g. Pho d 2 from *Phoenix dactylifera* and Fra e 2 of *Fraxinus excelsior*) (Asturias et al., 2005; Mas et al., 2014; McKenna et al., 2016). Given that profilins are strongly allergenic and widespread proteins and that their airborne concentration may be influenced not only by pollen concentration but also by meteorological factors, we determined the daily fluctuations of airborne pollen and aero-profilin concentrations, and, in parallel, examined which meteorological factors most markedly influenced them. For this purpose, two European cities with different weather conditions were chosen: León (Spain) and Bologna (Italy). These cities differ in their geography, topography, climate and the other environmental characteristics (population, industrialization, pollution, etc.), and the differences in environmental conditions affect plant taxa presence and abundance. The two cities were thus selected for a more fulfilling analysis between pollen concentration and meteorological factors.

In pollen samples from the two cities, the effect of in vitro pollen rehydration on the release of proteins, amongst which profilin, was investigated. Pollen rehydration could occur according to relative humidity values in the atmosphere thus causing release of proteins from pollen grains. Rapid release of allergenic proteins into aqueous media during pollen rehydration is an event observed in different pollen (de Dios Alche et al., 2004; Grote et al., 1993; Vega-Maray et al., 2006; Vrtala et al., 1993); this condition mimics the interaction between pollen and human mucosa (Morales et al., 2008). Profilins are very abundant in pollen (Staiger and Blanchoin, 2006) and, due to their high solubility, are released in large amounts into the culture medium during in vitro pollen hydration and germination (Morales et al., 2008; Vrtala et al., 1993). In the present paper, this feature has been investigated in vitro on pollen grains from different species and discussed in the light of data on pollen and allergen sample collected in the two cities analyzed.

2. Material and methods

2.1. Air sampling

Pollen sampling was performed continuously starting from March to June 2015 using two Hirst-type seven-day recording volumetric trap (VPPS, 2000 sampler, Lanzoni S.r.l., Bologna, Italy) with a suction flow rate of 10 L/min. Pollen data is expressed as daily mean pollen concentrations, pollen grains per air cubic meter (pollen/m^3), according the recommended terminology for aerobiological studies (Galán et al., 2017).

The atmospheric aerosol for the quantification of the allergenic fraction was sampled with a low-volume sampler, a continuous wind-oriented cyclone sampler with a suction flow rate of 16.5 L/min (Burkard Manufacturing Co. Ltd.). The sampling efficiency of this apparatus was described by Emberlin (1995) and Moreno-Grau (Moreno-Grau et al., 2006). The atmospheric particles were collected dry directly into a 1.5 mL vial every 24 h during the pollen sampling period and from the same sampling stations and then stored at −20 °C.

The Hirst-type trap and the Burkard Cyclone sampler were located in the same sampling site, i.e. on a terrace 15 m a.g.l at León University Campus (Spain). The Burkard Cyclone sampler was located on the roof (25 m a.g.l.) of the ISAC-CNR building in Bologna (Italy) and the Hirst-type trap on the roof (20 m a.g.l.) of the ARPA building in Bologna (http://www.arpae.it/).

2.2. Details of the monitoring stations

The city of León (838 m a.s.l.), is located in the north-western Iberian Peninsula. From biogeographic point of view, the city belongs to the Mediterranean region and has a continental climate. The climatic average of some meteorological parameters is 2 624 sun hours, 78 rain days and 16 storm days. The mean temperature is 10.9 °C with an annual precipitation of 560 mm. In winter, there are meanly 74 frost days and 16 snow days but strong snowfalls are not frequent. The summer is warm, but dry and tempered by the altitude of the city, with a maximum temperature around 27 °C and a wind speed greater than 5 m/s (Castro et al., 2007). The city of Bologna (54 m a.s.l.) is located close to Apennine Mountains south of Po Plain, a large flat area in the northern Italy that belongs biogeographically to the Continental region (Zauli Sajani et al., 2008). This area is characterized by a sub-continental climate. The climatic average of some meteorological parameters is 1950 sun hours, 75 rain days and 8 storm days. The mean temperature is 14 °C with an annual precipitation of 780 mm with a xerothermic period in July and August. In winter, there are meanly 11 frost days and 6 snow days. The summer is warm and highly humid with a maximum temperature around 31 °C, relative humidity 60–84% and a low wind speed less than 3 m/s (ISPRA - National System for Environmental Protection, 2016. Climate Indicators in Italy, 2015-Edition XI, http://www.isprambiente.gov.it/en/publications/state-of-the-environment/climate-indicators-in-italy-2015-edition-xi).

2.3. Protein extraction from Cyclone samples and immunochemical aero-profilin quantification

Extraction of samples was performed as previously reported, with minor modifications (Moreno-Grau et al., 2006; Takahashi et al., 2001). Briefly, the dry Cyclone samples were centrifuged at 18 000 × g for 1 min and then extracted at room temperature for 2 h with 120 µL of phosphate buffer (50 mM pH 7.0) supplemented with 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20, and 125 mM ammonium bicarbonate. The extract was separated by centrifugation at 2000 × g for 10 min and