



Wind-induced mechanical rupture of birch pollen: Potential implications for allergen dispersal



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ABSTRACT

Mechanical rupture of pollen grains after a mechanical shock against a solid surface was tested as a new approach to one of the mechanisms related to the release of allergen loaded particles from pollen. Birch pollen was aerosolized and sent into an impactor to mimic a shock against a surface (trees, soil, walls, etc.) at wind speeds of atmospheric-relevance. Small particles in the range of 1–2.5 μm were released subsequently to pollen impaction. The number of particles released increased with the velocity of impaction and with the water content of pollen. Damaged grains were observed by transmission electron microscopy and released particles are most likely cytoplasmic granules. This mechanism of pollen rupture and/or discharge may be of great importance in urban environment and may significantly contribute to thunderstorm-related allergic asthma and other associated pollen subparticles IgE mediated diseases.

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1. Introduction

Detection of pollen allergens in the PM_{10} fraction of the atmospheric aerosol have been widely reported for different pollen species, environments and sampling conditions (Busse, Reed, & Hoehne, 1972; Miguel, Cass, Glovsky, & Weiss, 1999; Riediker, Koller, & Monn, 2000a; Schäppi, Taylor, Suphioglu, & Knox, 1997; Spiekma et al., 1990; Spiekma & Nikkels, 1999; Suphioglu et al., 1992; Traidl-Hoffmann et al., 2003). Several hypotheses are proposed to explain the presence of pollen allergens in small-sized atmospheric particles (Duhoux, 1982; Grundstein & Sarnat, 2009; Liu & Zhang, 2004; Sofiev & Bergmann, 2012; Solomon, 2002; Taylor, Jacobson, House, & Glovsky, 2007). A first suggested mechanism can be the dispersion of pollen parts, either outer subparticles as orbicules (Vinckier, Cadot, Grote, Ceuppens, & Smets, 2006; Vinckier, Cadot, & Smets, 2005) or inner pollen subparticles including pollen cytoplasmic granules (Motta, Marliere, Peltre, Sterenberg, & Lacroix, 2006). Orbicules dispersion is greatly dependent on the species: birch pollen orbicules are for example uncommon when compared

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at least with cypress ones. Secondly, free allergens may be associated to small inorganic particles following a contact between pollen grains and such particles including pollutants (Knox et al., 1997; Solomon, 2002). The biology and chemistry of this specific mechanism is difficult to explore in atmospheric conditions but it has been demonstrated under laboratory conditions (Knox et al., 1997; Ormstad, Johansen, & Gaarder, 1998; Taylor, Flagan, Miguel, Valenta, & Glovsky, 2004).

In some potential, already experimentally explored, allergen dispersal mechanism, pollen cytoplasm is released subsequently to a contact of pollen grain with liquid water or in high relative humidity conditions (Grote, Valenta, & Reichelt, 2003; Miguel, Taylor, House, Glovsky, & Flagan, 2006; Taylor et al., 2004). Rupture of pollen envelope occurs then via osmotic shock for grass species or mainly via abortive germination for birch. It permits in both cases the dispersion of pollen granules (Taylor et al., 2004; Taylor, Flagan, Valenta, & Glovsky, 2002). Hundreds of these particles are present in each pollen grain with a size comprised between 0.5 and 4.5 μm (Bacsi, Choudhury, Dharajiya, Sur, & Boldogh, 2006). Different authors speak at the same time of the starch granules (Schäppi, Suphioglu, Taylor, & Knox, 1997), polysaccharide particles (Heslop-Harrison & Heslop-Harrison, 1982), and other paucimicronic elements. The allergenic potential of these granules have already been reported for several pollens (Abou Chakra et al., 2011, 2012, 2009; Grote, Vrtala, Niederberger, Valenta, & Reichelt, 2000; Schäppi, Taylor, Staff, Suphioglu, & Knox, 1997; Schäppi et al., 1999). Allergens are indeed mainly located into pollen cytoplasm where pollen granules are stored (Emilsson, Berggren, Svensson, Takahashi, & Scheynius, 1996; Grote, 1999; Grote et al., 2003). Such small particles, disseminated in the atmosphere, have a clear potential for deep penetration in the human respiratory tract where they could effectively be determinant for triggering asthma (Abou Chakra et al., 2012; Beggs, 1998). The mechanical properties of pollen have already been studied with the aim of optimizing the extraction of nutrients or active substances located inside the grains (Liu & Zhang, 2004). Mechanical destruction of pollen is also studied by palynologists for questions regarding the preservation of pollen through the ages (Campbell, 1991; Campbell & Campbell, 1994). Degradation of pollen has already been observed in atmospheric conditions with pollen being torn, with cracks or empty of their content (Shahali, Pourpak, Moin, Mari, & Majd, 2009; Shahali, Pourpak, Moin, Zare, & Majd, 2009; Taylor et al., 2002, 2004). Nevertheless and to the best of our knowledge, the mechanical rupture of pollen grains has never been directly experimented for atmospheric-relevant conditions, even if, by instance, works on rebound have already been proposed (Aylor & Ferrandino, 1967; Paw, 1983).

In this work, pollen grains were sent into an impactor with relatively low wind-speed to simulate a mechanical shock of pollen against a solid surface (tree, soil, walls, etc.). The subsequent release of particles was quantified with an Aerodynamic Particle Sizer. Birch pollen was chosen for its relevance to the current allergy context in Northern and Eastern Europe where the birch tree is largely present in parks, gardens and recreation zones within urban and suburban areas. Birch pollen is among the most allergenic pollen in these regions. In the present work, we suggest that the rupture of pollen grain following a mechanical stress is a mechanism for pollen cytoplasmic granules and pollen allergen atmospheric dispersal.

2. Materials and methods

2.1. Birch pollen material

Birch Pollen (*Betula pendula*) was harvested in semi-rural areas during pollination of birch in March 2014. Fresh pollen was sieved at 40 μm , placed several hours in a dry atmosphere for desiccation and then stored hermetically at 4 °C. All experiments were done within the month following harvesting.

Fresh water content of the pollen was estimated at about 40% by heating at 100 °C for 8 h and subsequent weighing. Mass of pollen harvested per catkin was around 10 mg, corresponding to an estimated number of 1.6 million pollen grains per catkin, given a single grain pollen mass of 6.1–6.7 ng (Ertzman, 1969). For a single *Betula* pollen grain, Schäppi et al. estimated the amount of major allergen Bet v1 at 0.006 ng, meaning at least around 5% of the total pollen protein content (Schäppi, Suphioglu, Taylor, & Knox, 1997; Schäppi, Taylor et al., 1997).

Dried pollen was obtained by flushing the grains during 8 or 15 h with synthetic air. After drying, pollen water content was estimated to respectively 10% and 0% for pollen dried for 8 h and 15 h. Humidified pollen was obtained by flushing during 8 h the pollen with synthetic air at relative humidity of 40%; the humidified pollen has an estimated water content of 40%.

2.2. Impactor device

The experimental set-up is represented schematically in Fig. 1 for total air flows between 5 and 15 L min^{-1} . Prior to an experiment, the set-up was purged several minutes with particle-free synthetic air. During these purges, the absence of particles was checked with an Aerodynamic Particle Sizer (APS 3321 TSI). The APS gives aerodynamic diameter size distribution from 0.5 to 20 μm with a sampling time set to 20 s. PM_{10-20} will refer to the fraction of the particles with an aerodynamic diameter between 10 and 20 μm .

2.3. Experimental procedures

For an experimental run with pollen, 3 mg of pollen were aerosolized into an air stream with flows between 5 and 15 L min^{-1} regulated by mass-flow controllers. Native pollen size-distributions were obtained with Fig. 1 set-up (a): the aerosolized pollen was sent to a buffer volume of 1 L where the APS collects 5 L min^{-1} . Mechanical stress of the pollen was performed with a 3-stage cascade impactor (Dekati PM_{10}) as shown in Fig. 1 set-up (b). The impactor was solely equipped

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