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Effect of O_3 and NO_2 atmospheric pollutants on *Platanus x acerifolia* pollen: Immunochemical and spectroscopic analysis

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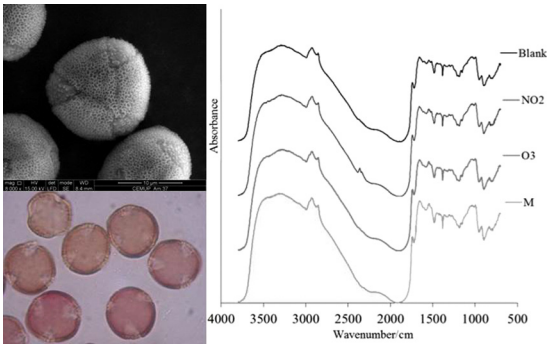
HIGHLIGHTS

- Airborne pollen and pollutants interaction may modify pollen composition.
- Immunoblotting, infrared and X-ray photoelectron spectroscopy techniques were used.
- Modifications occurred on pollen' allergenicity and surface elemental composition.
- Changes are pollutant dependent and occur at levels below the regulated thresholds.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, the effects of two important oxidizing atmospheric pollutants (O_3 and NO_2) on the allergenic properties and chemical composition of *Platanus x acerifolia* pollen were studied. Pollen samples were subjected to O_3 and/or NO_2 under in vitro conditions for 6 h at atmospheric concentration levels (O_3: 0.061 ppm; NO_2: 0.025 ppm and the mixture of O_3 and NO_2: 0.060 and 0.031 ppm respectively). Immunoblotting (using Pla a 1 and Pla a 2 antibodies), infrared and X-ray photoelectron spectroscopy techniques were used. Immunochemical analysis showed that pollen allergenicity changes were different according to the pollutant tested (gas or mixture of gasses) and that the same pollutant gas may interact in a different manner with each specific allergen. The spectroscopy results showed modifications in the FTIR spectral features of bands assigned to proteins, lipids, and polysaccharides of the pollen exposed to the pollutants, as well as in the XPS spectra high-resolution components C 1s, N 1s, and O 1s. This indicates that while airborne, the pollen wall suffers further modifications of its components induced by air pollution, which can compromise the pollen function.

1. Introduction

Air pollutants emission increase in the past decades, particularly related to industrial growth, led to a worldwide environmental problem. In industrialized countries nitrogen dioxide (NO_2), ozone (O_3) and particulate matter (PM) are the most abundant aerosol pollutants,

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with a marked impact on human health such as in increased airway responsiveness and airway inflammation or changes in lung function (D'Amato et al., 2015; WHO, 2013).

As a consequence of anemophilous plants pollination process, large amounts of pollen grains are released into the atmosphere, becoming a seasonal biogenic constituent of the particulate matter fraction of the aerosol (PM). Allergenic airborne pollen size varies between 10 and 58 μm with their outermost layer, the exine, ranging between 1 and 5 μm (Erdtman, 1969).

Pollen grains are allergen-carriers since they contain in their composition many allergen molecules, found in the outer wall as well as in the cytoplasm that in susceptible individuals can trigger respiratory allergic diseases when entering people's airways (D'Amato et al., 2015; Linskens and Cresti, 2000; Sun et al., 2016). Pollen is mainly deposited in the upper respiratory airways, but while in contact with the respiratory mucosa releases allergens that elicit IgE-mediated allergenic reactions (Rouvinen et al., 2010; Traidl-Hoffmann et al., 2009).

In Europe, airborne pollen concentration tends to be lower in urban areas than in rural ones (D'Amato et al., 2013), nevertheless, people living in more urbanized environments are the most affected by pollen-induced respiratory allergies (D'Amato et al., 2013). Typically airborne pollen monitoring and quantification are performed by roof-level samplers to avoid distortion due to local emissions, reflecting a regional trend, and architectonical barriers such as high buildings (Maya-Manzano et al., 2016; Oteros et al., 2017; Rojo et al., 2015; Scheifinger et al., 2013). In fact, Oteros et al. (2015) using a concentric ring method (CRM) to model the relationship between the emission source and the total amount of airborne pollen showed that most of the pollen could come from long and regional distances transport just because most of the sources are located at these distances. Thus, the airborne pollen samplers are less able to detect important intra-urban concentration variations which are significant, within an urbanized environment, to determine the real human exposure risk to allergenic pollen at the breathing level (Davies et al., 2015; García-Mozo et al., 2016; Hjort et al., 2016; Rojo et al., 2015). Furthermore, the concomitant human exposure to airborne pollen and mixtures of primary and secondary air pollutants greatly enhance the occurrence of allergenic respiratory disorders (D'Amato et al., 2015).

The airborne pollen and air pollutants interaction is complex and many hypotheses have been formulated how this interplay may modify pollen allergenicity. It has been described that gaseous pollutants can alter airborne pollen properties, particularly at the biochemical level, which can enhance the risk of atopic sensitization and lead to an exacerbation of allergic symptoms in susceptible individuals (D'Amato et al., 2015; Frank and Ernst, 2016; Kanter et al., 2013; Ribeiro et al., 2014; Zhao et al., 2016). It has been shown that NO_2 and O_3 can promote the nitration of pollen proteins, with post-translational modification leading to increased or decreased allergenicity (Gruijthuijsen et al., 2006; Karle et al., 2012; Shiraiwa et al., 2012).

Also, airborne pollen and air pollutants interaction can lead to changes in pollen general chemistry that can have an important impact in pollen' primary task that is the reproduction (Senechal et al., 2015; Sousa et al., 2012). Variations in the pollen lipids, proteins, carbohydrates and sporopollenin surface components have been reported after pollen exposure to different gaseous pollutants (Kanter et al., 2013; Zhao et al., 2016).

Therefore, in order to study the effects of air pollutants on pollen after being released into the atmosphere, pollen from randomize samples collected from a batch of *P. x acerifolia* trees located in an urbanized environment was in vitro subjected to gaseous pollutants using concentrations around the atmospheric levels. The effects of O_3, NO_2 and the mixture of both pollutants in *Platanus* pollen allergenic properties using Pla a 1 and Pla a 2 antibodies were evaluated. Also, near surface and surface pollen modifications were studied by infrared and X-ray photoelectron spectroscopy.

2. Material and methods

2.1. Pollen samples

Platanus x acerifolia belongs to the Platanaceae family and is widely planted as an ornamental tree across the Porto's urban area (41°11′ N, 8°39′ W). The pollen collection was performed from trees in a public garden, situated in a residential area, near the Douro River and Atlantic ocean and near to a highway road with high road traffic. The sampling was conducted according to the methodology previously described in Ribeiro et al. (2013). Afterwards, the material collected was brought to the laboratory, dried (24 °C) in a laboratory stove and the pollen was separated using sieves and stored at −20 °C as a single sample.

2.2. Pollen exposure to the gaseous pollutants

Pollen was exposed to O_3, NO_2 and to both gasses simultaneously under in vitro conditions (Table 1), during 6 h, in an environmental chamber system, already detailed in Sousa et al. (2012). The chamber is composed by a Solar Simulator (Newport Oriel 96,000 150 W) to simulate the daylight, fans (SUNON SF23080AF) to homogenize the air, temperature and relative humidity sensors (EBI20 sensor) and gas sensors (AEROQUAL Series 500 sensors; O_3 sensor: 0–0.5 ppm, NO_2 sensor: 0–0.2 ppm). The gas concentration and meteorological conditions were not constant inside the environmental chamber in order to imitate what happens in the atmosphere along the diurnal cycle.

Individual pollen samples (150 mg) were placed in Falcon tubes (50 ml) with both edges open but coated by a mesh with a pore opening size of 23 μm (SEFAR PET 1000). The pollen formed a very thin layer at the bottom covering the mesh. The tube was then positioned over a fan that enables pollen dispersion inside, permitting a homogeneous contact between the pollen grains and the gas and also mimics what occurs in the atmosphere with airborne pollen.

Exposure conditions reproducibility has been demonstrated in Ribeiro et al. (2013).

A A2ZS-1GLAB ozone system connected to a timer (OMRON H3DK-S1) to control the gas injection was used to generate ozone. The NO_2 was obtained in a sealed bottle through the chemical reaction between concentrated nitric acid (to ensure the production of NO_2, otherwise we would have a mixture of NO and NO_2) and solid copper mixed, at stoichiometric amounts.

A blank pollen sample (150 mg), not exposed to the gasses but subjected to the same procedure, was used as the control.

2.3. Protein extraction and estimation

To conduct the immunoblots experiments, pollen protein extracts were obtained by suspending, with continuous stirring, the pollen (50 mg) in phosphate buffered saline (1:20 w/v) at pH 7.4 for 4 h at 4 °C. Afterward, the suspension was centrifuged (16,100 g for 30 min at 4 °C) and the supernatant filtered (0.45 μm Millipore filter) and centrifuged again. The Bradford's method (Bradford, 1976) was used to estimate the soluble protein content of all pollen extracts by means of a colorimetrical reaction with the Coomassie Protein Assay Reagent

Table 1
– Average and standard deviation of gas concentration, temperature and relative humidity registered along the exposure of *Platanus x acerifolia* pollen to O_3, NO_2 and to both gasses simultaneously under in vitro conditions during 6 h in an environmental chamber system.

	[Gas] ppm	Temperature °C	Relative humidity %
Blank	–	25.2 ± 1.5	44.6 ± 0.8
O_3	0.061 ± 0.018	22.8 ± 1.5	65.5 ± 0.8
NO_2	0.025 ± 0.004	24.1 ± 1.3	56.1 ± 1.3
Mixture (O_3 + NO_2)	0.060 ± 0.009 0.031 ± 0.007	26.8 ± 0.7	58.3 ± 1.0