



Evaluation of hirst-type sampler and PM₁₀ impactor for investigating adhesion of atmospheric particles onto allergenic pollen grains

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Abstract Hirst-type sampler (HTS) is the standard equipment for pollen sampling while cascade impactors are commonly used for particulate matter (PM) samplings. In this work, we investigated the feasibility of using such devices to reliably assess the atmospheric PM adhesion onto pollen grains (PGs). Birch PGs were deposited on sampling substrates and then exposed to ambient PM in each type of sampling device operating simultaneously. In the HTS, the surface of PGs was significantly polluted with PM

during sampling (93.5% of polluted PGs with on average 4 particles per PG with a mean diameter of 2.2 µm). A modified entrance slit was tested and proved to significantly reduce the PM sampling artifact (28% of polluted PGs), although not completely canceling it. In contrast, in the PM₁₀ impactor, all PGs remained free from particulate pollutants. We concluded that the PM pollution of PGs reported in previous studies using a HTS may be overestimated. While PG samplings using a HTS might provide insights into the co-exposure to allergenic pollen and other ambient PM, a size-selective aerosol sampler proves to be more appropriate for a reliable assessment of the extent of PM pollution of airborne pollen in order to give a realistic depiction of the physical and chemical state of ambient PGs that could be inhaled.

Marie Choël and Anastasia Ivanovsky have contributed equally to the work.

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1 Introduction

Alterations of pollen grains (PGs) by gaseous pollutants are well documented (Sénéchal et al. 2015). Gaseous pollutants can alter proteins and lipids or interfere with the germination capacity of birch pollen (Ackaert et al. 2014; Beck et al. 2013; Bryce et al. 2010; Cuinica et al. 2013, 2014, 2015; Darbah et al. 2007; Erler et al. 2011; Franze et al. 2005; Gruijthuisen et al. 2006; Helander et al. 1997; Karle et al. 2012; Kozlov and Zvereva 2004; Reinmuth-Selzle et al. 2014; Zhu et al. 2018; Žiarovská et al. 2013). Furthermore, air pollutants can trigger serious exacerbations of allergic diseases (Heinrich 2019; Orellano et al. 2017). People suffering from rhinitis and living in urban areas with high levels of air pollution are more prone to report severe nasal symptoms (Burte et al. 2020). In particular, particulate matter deposited onto allergenic PGs could play a proinflammatory role contributing to the enhancement of pollinosis symptoms (D'Amato 2002). For instance, PGs with atmospheric particles accumulated on their surface are more effective at eliciting allergic symptoms in mice (Majd et al. 2004). Furthermore, a high exposure to PM₁₀ (particulate matter < 10 µm) and PM_{2.5} (particulate matter < 2.5 µm) is associated with an increase in rhinitis severity (Burte et al. 2020). High exposure to PM_{2.5} is also associated with a poorer asthma control for pediatric patients (Li et al. 2019). PM may stick to PGs during their atmospheric residence time (Guedes et al. 2009). Moreover, pollen allergens could be transferred to particles adsorbed on the surface of PGs, resulting in the release of allergen-loaded inhalable particles in the atmosphere (Behrendt et al. 1992; Namork et al. 2006; Riediker et al. 2000; Schäppi et al. 1996). The mechanisms underlying the association between PM and the severity of pollen-related allergy symptoms are however still not yet completely elucidated. The extent of such pollution of the surface of allergenic PGs by atmospheric PM is not known primarily because of a lack in quantitative studies (Visez et al. 2020). Indeed, most of the observations reported in the literature for particle adhesion to PGs are qualitative and therefore could not allow

estimating the frequency of occurrence of the phenomenon (Visez et al. 2020). Hence, the pollution state of airborne PGs is largely unknown.

Hirst-type sampler (HTS) has previously been employed to investigate the adhesion of PM onto the surface of PGs (Duque et al. 2012, 2013; Ribeiro et al. 2015). The average number of particles per PG was found particularly high: 16 ± 12 for *Acer*, 39 ± 32 for *Pinus*, and 4 ± 4 for *Platanus*, with adhered particle sizes ranging from 0.1 to 25.8 µm (Ribeiro et al. 2015). To our knowledge, this study was the first to provide quantitative data about the particulate pollution of the surface of allergenic pollen. HTS is the prevalent method for monitoring atmospheric concentrations of allergenic PGs with more than 600 devices currently in operation worldwide (Buters et al. 2018; Galán et al. 2014). However, we question whether the use of an HTS is adequate for investigating PM adhesion onto PGs due to the low cut size of this sampling device at 3.7 µm that enables this device to efficiently trap particles and spores down to the size range 2–6 µm (Levetin 2004).

On the other hand, size-fractionated sampling of atmospheric PM is commonly carried out using cascade impactors (Marple 2004). These devices are designed as a substitute for the human respiratory tract to collect and separate atmospheric particles according to their degree of penetration in the human respiratory system. Typically, multistage cascade impactors consist of a pre-separator size selective inlet/stage designed to remove all large particles and consecutive stages which fractionate particles in lower size ranges. For instance, the PM₁₀ impactor (Dekati Ltd.) is a three-stage cascade impactor with 50% cut-points (i.e., particle size with 50% collection efficiency) of 10 µm (first stage), 2.5 µm (second stage) and 1 µm (third stage). PM_{10-2.5} and PM_{2.5-1} size fractions of atmospheric particles collected, respectively, on the second and third stages of the PM₁₀ impactor can be investigated by scanning electron microscopy (SEM) combined with energy-dispersive X-ray spectrometry (EDX) so as to study the morphology and elemental composition of single atmospheric particles (Bradley 1958; Choël et al. 2010; Mogo et al. 2005). As the size of most windborne PGs ranges between 20 and 40 µm (ANSES 2014), the use of the first stage (PM_{>10}) would be appropriate to sample PGs, while the second and third stages sample PM in the size range 1–10 µm.

To our knowledge, no study reports the investigation of PM pollution of PGs using a PM₁₀ impactor.

In this work, we investigated the feasibility of sampling PGs in an unbiased manner regarding the PM pollution of their surfaces. We determined the amount and nature of atmospheric particles adhering onto PGs after exposure to outdoor air in an HTS and a PM₁₀ impactor. Furthermore, to evaluate the effect of the HTS inlet size on the cut size of the collected PM, a modified HTS fitted with a larger orifice was tested. The evaluation of these devices for the sampling of pollen without artifact is critical to correctly assess the particulate pollution state of allergenic PGs.

2 Materials and methods

Birch (*Betula pendula*) pollen was harvested on trees at the campus of the University of Lille during the flowering season in 2017. Birch catkins were collected just before dehiscence to prevent PGs from coming into contact with atmospheric PM. Catkins were stored for 24 h in a laboratory hood to allow drying. PGs were extracted from opened catkins by sieving at 40 µm, placed in a dry atmosphere for 24 h and then hermetically sealed in glass vials stored at 4 °C until use.

In order to evaluate the sampling artifact of HTS and PM₁₀ impactor, the collection surfaces of both samplers were sprinkled with birch PGs and then exposed inside the samplers to outdoor air flow for a specified duration. The following procedure was applied for the evaluation of the sampling artifact for both HTS and PM₁₀ impactor (see Figure S1 in Supplementary Material). Birch PGs were deposited by sieving on top of two pieces of conductive double-sided adhesive carbon tape (one piece for each sampler type). Prior to exposure to outdoor air, each of the two carbon tapes covered with birch PGs were examined directly (without metal coating) in a variable-pressure scanning electron microscope (VP-SEM) (JEOL JSM-7800F LV) fitted with an energy-dispersive X-ray (EDX) analysis system (80 mm² SSD, Aztec Oxford Instrument). The chamber pressure was 100 Pa. The accelerating voltage was 10 kV for electron imaging and 15 kV for X-ray elemental mapping. On each sample, several areas of the carbon tape with deposited PGs were imaged and their position coordinates were recorded. After this initial step of PG mappings,

one carbon tape covered with birch PGs was placed in a HTS (VPPS 2000, Lanzoni s.r.l.) and the other carbon tape was mounted on the first stage (PM_{>10}) of a PM₁₀ impactor (Dekati Ltd.).

In a first experiment, exposure of PGs to outdoor air was carried out in the two samplers installed approximately 3 m apart on the rooftop of a faculty building at 20 m above ground level (see Figure S2A in Supplementary Material). The exposure to ambient air was conducted on the University of Lille campus, France (50° 36' N, 3° 8' E), on 22 June 2017 during 9 h from 9:00 a.m. to 6:00 p.m. local time. In the HTS equipped with a daily sampling head, airborne particles with sufficient inertia are impacted onto the sticky surface of a glass slide placed behind the orifice, which moves past the orifice at 2 mm h⁻¹ (see Figures S2B and S2.C in Supplementary Material). Thus, over the course of 9 h, an 18 mm long trace is obtained with the deposit at any point representing the mean concentration over a 1-h sampling period (Levetin 2004; Mullins and Emberlin 1997). Field exposure was conducted several weeks after the birch pollination season. Meteorological conditions were measured by a weather station (Vantage Pro 2, Davis Instrument) installed on the sampling site (data available at <https://www-loa.univ-lille1.fr>, accessed June 6, 2019). The mean temperature and relative humidity were 24.4 ± 3.0 °C and 64 ± 5%, respectively. During the sampling period, the prevailing winds originated from the west with an average wind speed of 4.2 ± 0.8 m s⁻¹. The average PM mass concentrations measured at the closest monitoring station located about 2 km west of the sampling site were 30 ± 4 µg m⁻³ and 19 ± 2 µg m⁻³ for PM₁₀ and PM_{2.5}, respectively (data available at <https://www.atmo-hdf.fr>, accessed June 6, 2019).

The conventional inlet of HTS is a rectangular opening of 14 mm × 2 mm with an aerodynamic cut size reported as 3.7 µm (Levetin 2004). An alternate orifice of 14 mm × 0.5 mm with a cut size of 2.17 µm was designed to efficiently trap small spores (Khattab and Levetin 2008). Reversely, in an attempt to avoid collecting small PM, a larger inlet of 14 mm × 5.3 mm was precision machined in a brass block for this study to obtain an aerodynamic cutoff diameter ($D_{p,50}$) of 10 µm with 50% collection efficiency. Thus, as a complement, a modified HTS fitted with this larger orifice was tested to evaluate the effect of the HTS inlet size on the cut size of the collected PM (see

Figure S2D in Supplementary Material). For this second experiment, birch PGs were exposed to outdoor air in the modified HTS, on 17 May 2019 during 5 h (from 2:00 p.m. to 6:00 p.m. local time) at the same location of the previous experiment. PM_{10} and $PM_{2.5}$ mass concentrations measured at the closest monitoring station were, respectively, $28 \pm 2 \mu\text{g m}^{-3}$ and $24 \pm 2 \mu\text{g m}^{-3}$. Prevailing winds originated from the east with an average wind speed of $1.0 \pm 0.5 \text{ m s}^{-1}$.

Dimensions of the orifice for the modified HTS inlet were calculated according to the theoretical equations applying for slit impactors (Marple and Liu 1974) and in such a way that the Reynolds number defined by Eq. 1 is identical for the two slits (conventional and modified entrance slits).

$$Re = \frac{2VW}{\mu} \quad (1)$$

where V is the particle velocity, W is the slit width and μ is the dynamic viscosity of the gas.

So, given that the ratio W/S is larger than 2 (where S is the distance between the acceleration plate and the impaction plate) for both slits, we can assume that the Stokes number with 50% efficiency (St_{50}) is also identical for both slits and is expressed by Eq. 2.

$$St_{50} = \frac{\rho_p CVD_{p,50}^2}{9\mu W} \quad (2)$$

where ρ_p is the density of the particle and C is the Cunningham coefficient.

It follows:

$$\frac{{}^1C^1V^1D_{p,50}^2}{{}^1W} = \frac{{}^2C^2V^2D_{p,50}^2}{{}^2W} \quad (3)$$

where 1 refers to the conventional Hirst slit and 2 designates the modified Hirst slit so that ${}^1D_{p,50}$ and ${}^2D_{p,50}$ equal $3.7 \mu\text{m}$ and $10 \mu\text{m}$, respectively.

The pressure loss in the sampler being low, the pressure was considered constant. The Cunningham coefficient C can then be written only as a function of $D_{p,50}$ as follows:

$$C = 1 + \frac{0.16}{iD_{p,50}} + \frac{0.055}{iD_{p,50}} e^{-iD_{p,50}/0.15} \quad (4)$$

A slit width ${}^2W$ of 5.33 mm was calculated by solving these equations.

After outdoor exposure, the exact same areas of the carbon tapes imaged prior exposure to ambient air were

relocated and images were recorded using the same magnification for comparison. Backscattered electron images (BSE) were processed using ImageJ (Fiji open source software) to count the number of particles adhered onto the carbon tape and on PGs and also to obtain the projected area of particles (Schindelin et al. 2012). Using the projected area, a circle equivalent diameter (D_p) of particles was calculated using Eq. 5.

$$D_p = 2 \times \sqrt{\frac{\text{Area}}{\pi}} \quad (5)$$

The aerodynamic equivalent diameter (D_{aer}) was calculated with Eq. 6 from the circle equivalent diameter (D_p) for comparison with the aerodynamic cut size of samplers defined for a standard density of particles ρ_0 (1 g cm^{-3}).

$$D_p = D_{\text{aer}} \sqrt{\frac{\rho_0}{\rho}} \quad (6)$$

where ρ is the atmospheric particle density.

The atmospheric particle density depends on the physical particle properties and is also related to the chemical composition of the sampled particle. In our study, ρ was taken to 2.65 g cm^{-3} for mineral particles, which is the commonly assumed density for mineral dust or 1.65 g cm^{-3} for other types of particles, which is the mean apparent particle density in urban air (Mahowald et al. 2014; Pitz et al. 2003).

3 Results

Figures 1a and 2a depict birch PGs deposited on the sticky carbon tape and directly observed uncoated by VP-SEM. These control observations show that both the substrate and the PGs were initially free from particles of unknown origins and from orbicules (Ruggiero and Bedini 2018). Besides verifying the absence of contamination prior to exposure to atmospheric PM, these microscopic examinations aimed at recording the position coordinates so as to observe the exact same PGs after simultaneous exposure to atmospheric PM in a conventional HTS (Fig. 1b) and a PM_{10} impactor (Fig. 2b). All PGs priorly observed by SEM were recovered after exposure, indicating that no re-entrainment of PGs occurred during exposure. By comparison to unexposed PGs, it became readily apparent that both the substrate and

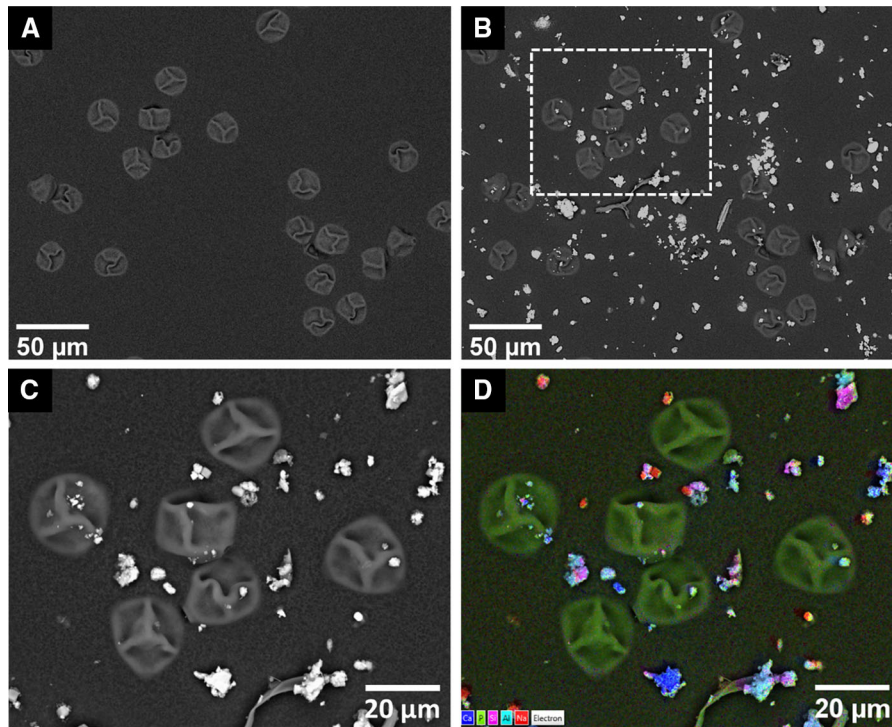


Fig. 1 Backscattered electron images of (a) birch PGs observed prior exposure to outdoor air and (b) the exact same PGs observed after exposure to atmospheric PM in HTS during 9 h. c Enlarged view (corresponding to the dashed rectangle in

Fig. 1b) of birch PGs after exposure to ambient PM. d X-ray elemental mapping of the same area showing calcium (blue), phosphorus (green), silicon (pink), aluminum (cyan) and sodium (red)

the PGs were covered in particles after exposure in the HTS. By contrast, very few particles, exclusively found on the carbon tape, were observed in the case of PGs exposed to outdoor air in the PM₁₀ impactor (Fig. 1d). As can be seen from the magnified views (Figs. 1c and 2c), PGs exposed in the HTS were heavily polluted by particles while PGs exposed in the PM₁₀ impactor remained free from particles, although the volume of air sampled was the same for both samplers (about 5.4 m³).

Out of 535 birch PGs observed after exposure in the HTS, 93.5% got polluted with an average of 4.3 particles per PG (Table 1). The average number of particles per PG falls to 4.0 when considering the total number of birch PGs observed (polluted and unpolluted) after exposure in the HTS. The size of particles sampled using the HTS was on average 2.2 µm while that of particles collected using the PM₁₀ impactor was significantly higher (12.5 µm). By replacing the standard orifice of the HTS with a larger orifice (air entrance orifice of 14 × 5.3 mm instead of 14 × 2 mm), the proportion of polluted PGs

drastically decreased to 28% (Table 1). As the PM₁₀ concentration during both experiments were on the same order (30 µg m⁻³ and 28 µg m⁻³ for the first and second experiment, respectively), this decrease may be partly related to the shorter exposure time (5 h instead of 9 h). However, the average size of particles adhered to PGs increased from 2.2 µm for the conventional HTS to 4.4 µm for the modified HTS. Considering an average particle size (D_p) of 2.2 µm and a particle density of 2.65 g cm⁻³ in the case of mineral dust and 1.65 g cm⁻³ in the case of urban dust, the calculated aerodynamic diameter (D_{aer}) of particles trapped in the conventional HTS is 3.6 µm and 2.8 µm, respectively. Although most particles were irregularly shaped and not spherical, our calculation gives an estimate of D_{aer} in good agreement with a cutoff size of 3.7 µm reported in the literature for the HTS (Khattab and Levetin 2008; Levetin 2004). Similarly, considering an average particle size (D_p) of 4.4 µm and mean particle densities of 2.65 g cm⁻³ and 1.65 g cm⁻³, the calculated aerodynamic diameter (D_{aer}) of particles trapped in the modified HTS is

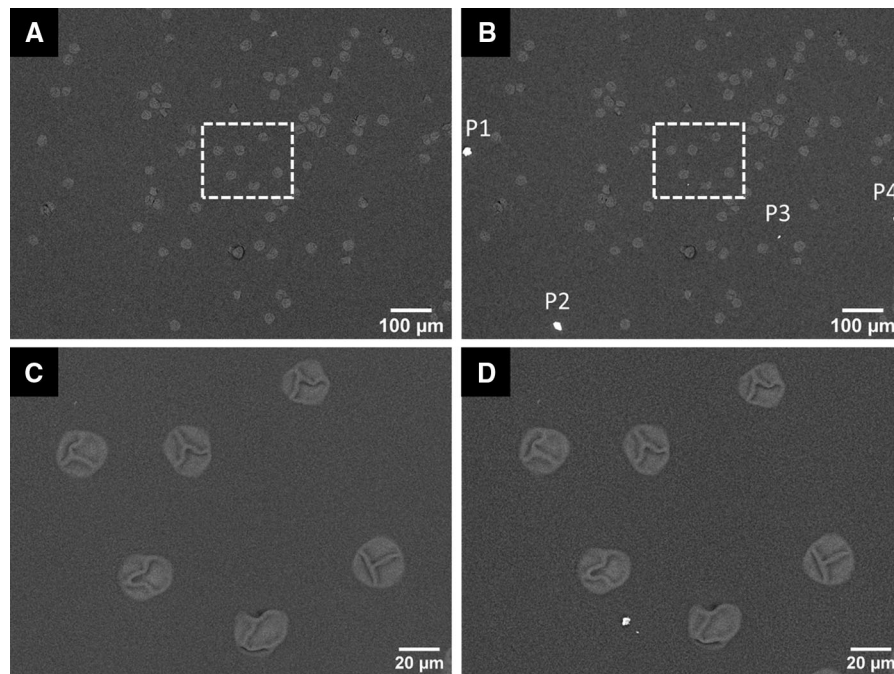


Fig. 2 Backscattered electron images of (a) birch PGs observed prior exposure to atmospheric PM and (b) the exact same PGs observed after exposure to outdoor air in a PM₁₀ impactor during 9 h. Enlarged views (corresponding to the dashed

rectangles in the top panel) of birch PGs observed (c) prior and (d) after exposure to ambient PM. P1, P2, P3 and P4 are particles observed on the carbon tape

Table 1 Characteristics of the three tested samplers, number of PGs and particles observed, size parameters of observed particles

	Conventional HTS	PM ₁₀ impactor	Modified HTS
Day of experiment	22 June 2017		17 May 2019
PM ₁₀ (μg m ⁻³)	30 ± 4		28 ± 2
PM _{2.5} (μg m ⁻³)	19 ± 2		24 ± 2
Inlet shape and size	Rectangular orifice 14 × 2 mm	Circular orifice 8 mm	Rectangular orifice 5.3 × 2 mm
Flow rate	10 L/min	10 L/min	10 L/min
Total number of observed PGs	535	304	87
Percentage of polluted PGs	93.5%	0%	28%
Number of particles ($D_p > 0.1 \mu\text{m}$) counted on PGs	2161	0	29
Number of particles detected on the substrate	16,285	15	128
Average number of particles per PG	4.0	0	0.3
Average diameter of particles adhered to PGs (D_p , μm)	2.2 ± 2.4	12.5 ± 9.3*	4.4 ± 5.1
Size range of particles adhered to PGs (μm)	0.1–20.7	1.1–27.9	0.4–24.8
Average aerodynamic diameter of particles adhered to PGs (D_{aer} , μm)	2.8–3.6	16.1–20.2*	5.6–7.2

*For 15 particles found on the carbon tape

7.2 μm and 5.6 μm, respectively. There is a shift to higher particle sizes in the modified HTS, consistent with the increase in the entrance slit width.

Figure 1d represents X-ray elemental mappings of PGs and particles. In addition to carbon, nitrogen and oxygen, which are constituent elements of biological

particles, phosphorus was the main element detected in birch PGs (see the green colored PGs in the X-ray elemental mapping in Fig. 1d). X-ray microanalysis of *Brassica* pollen in a variable-pressure scanning electron microscope (VP-SEM) without metal coating revealed that the intensity of the X-ray emission of P was greater than that of S, K and Ca, similar to our elemental analysis of uncoated birch pollen in low-vacuum SEM (Iwano et al. 1999). The types of particles adhered to PGs were sea-salts containing N, O, Na, Cl, S, Ca and Mg, mineral dust containing aluminosilicates (Si, Al), metallic oxides (Mn, Fe, Ti), carbonates (Ca, Mg), and biogenic particles containing C, O, K, P, S, Cl, Ca and Si. The dominant particles were composed of silicon, calcium, aluminum and iron. Noticeably, soot aggregates were also found lying both on the carbon tape and on the surface of PGs (Fig. 3). These soot agglomerates have a characteristic morphology with hundreds of carbon nanoparticles arranged in a chain-like structure (Fruhstorfer and Niessner 1994; Wittmaack 2004).

4 Discussion

Personal exposure to allergenic pollen and fine PM enhances the prevalence and the severity of respiratory allergic diseases (D'amato 2000). The quantitative assessment of co-exposure to pollen and PM is,



Fig. 3 Secondary electron image of a birch PG observed after exposure to outdoor air in the conventional HTS. The exine is partially covered with mineral dust particles (bright irregularly shaped supermicron particles) and soot aggregates (arrows point to clusters of carbon nanospheres arranged in a chain-like structure lying on the substrate and on the pollen wall)

however, based on relating pollen counts and total suspended particulate (TSP) or PM_{10} and $PM_{2.5}$ mass concentrations without specific analysis of the pollution state of PG surfaces (Grewling et al. 2019a, b). The pollen lifetime in the atmosphere can vary from half a day up to a couple of days and pollen can be transported up to 1000 km (Sofiev et al. 2006). Thus, while airborne, pollen may scavenge and carry other PM found in the atmosphere. Whether or not the symptoms of pollen allergy and allergic asthma are aggravated upon inhalation of actually polluted PGs (excluding conclusions made with animal models) is an unresolved issue. As confirmed by our exposure of birch PGs to outdoor air, pollen is a suitable surface for the adhesion of atmospheric particles of a large diversity in size and chemical composition. The chemical composition of adhered particles may confer modified inflammatory properties to pollen (Davies et al. 1998; Knox et al. 1997; Mazzarella et al. 2012). In our study, both supermicron and submicron soot aggregates were trapped on the collecting surface of the HTS. Laboratory studies have reported, for example, that soot particles from diesel exhaust can increase the allergy potency of PGs (Chehregani and Kouhkan 2008).

Moreover, there is a major difference between being exposed simultaneously to PGs and PM and being exposed to PGs with adhered particles. Indeed, PGs and smaller particles will not deposit upon inhalation on the same regions of the respiratory system. PGs are more likely to deposit in the upper airways due to inertial impaction whereas smaller particles likely deposit in lower airways due to sedimentation and diffusion (Carvalho et al. 2011). The Deposition of PGs and smaller particles is unlikely to occur in the exact same spot in the respiratory system unlike when particles are already attached to the pollen surface. As a result, besides pollen and spores monitoring, the HTS can only be used for temporal tracking of co-exposure to other PM. Noticeably, the ability of the HTS to simultaneously trap non-biological particles (dust, soot) along with pollen and spores was put forward as a time-resolved sampling method to evaluate the air quality (Leuschner and Boehm 1981). It has been already taken advantage of the ability of the HTS to collect biological and non-biological particles in the size range between 2 and 100 μm. In these works, the concentrations of solid particles in the atmosphere

differentiated into non-biological material from natural and non-natural sources (e.g., soot, dust, sand, diesel exhaust particles, partially burnt residues) and biological material (e.g., PGs and fungal spores) were determined by light absorption spectrophotometry (Cariñanos et al. 1998, 2000; Domínguez-Vilches et al. 1995). As regards the surface condition of windborne pollen, field experiments with birch PGs exposed to outdoor air inside the conventional HTS have indeed highlighted an artifact causing substantial PM pollution of PG surfaces. This conclusion is consistent with the aerodynamic cut-size of $3.7 \mu\text{m}$ reported for the HTS (Khattab and Levetin 2008; Levetin 2004). Hence, the conventional HTS must not be used to collect PGs for the purpose of observation of adhered atmospheric particles to PG surfaces. The increase of the HTS cut-size by fitting a larger inlet was not sufficient to prevent PGs from being contaminated with PM during the second experiment with the modified HTS. The exposure experiment of birch PGs deposited in the HTS led to the clear conclusion that atmospheric particles with an average size of $2.2 \mu\text{m}$ bombarded the sampling surface and the surface of PGs. While the total sampling time with the conventional HTS was 9 h, PGs were actually exposed to ambient air flow for about 1 h as the collection surface slid at 2 mm h^{-1} past the $14 \times 2 \text{ mm}$ entrance orifice (Mullins and Emberlin 1997). The use of a larger entrance slit allowed for a decrease from 4.0 to 0.3 particles adhered per PG. However, atmospheric sampling of PGs for the purpose of quantifying particle adhesion has already been carried out with the conventional HTS and a significant amount of particles adhering to the surface of PGs was reported in the case of *Acer* (16 ± 12 particles per PG), *Pinus* (39 ± 32 particles per PG) and *Platanus* (4 ± 4 particles per PG) pollen collected in ambient air of Porto (Ribeiro et al. 2015). Figure 4 displays the average number of particles adhered per PG found in this work and other data reported in the literature. The pollution by particles observed on birch PGs exposed inside the HTS was on the same order of magnitude as that of PGs collected in Porto with daily PM_{10} of 14 to $86 \mu\text{g m}^{-3}$ and daily $\text{PM}_{2.5}$ concentrations of 3 to $14 \mu\text{g m}^{-3}$. Figure 4 highlights a correlation between the pollen size and the number of adhered particles on pollen surface. Indeed, PGs may have been contaminated during both atmospheric transport and sampling. Several mechanisms might explain atmospheric

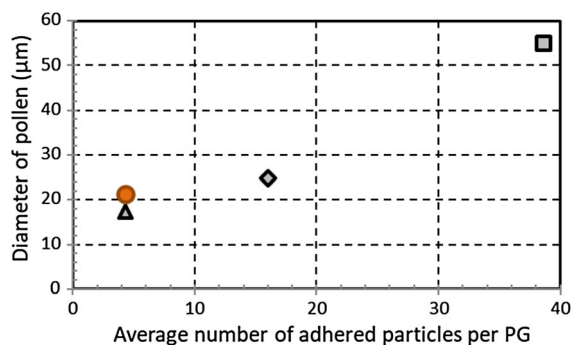


Fig. 4 Average number of particles per PG as a function of the pollen size. Orange circle: *Betula*, this work; triangle: *Platanus*, diamond: *Acer* and square: *Pinus* (Ribeiro et al. 2015). *Betula* PGs were exposed to outdoor air inside a HTS while *Platanus*, *Acer* and *Pinus* were collected from outdoor air using a HTS

particle adhesion to PG surface: Brownian motion, electrostatic interactions, gravitational coagulation and cycles of deposition/resuspension (Seinfeld and Pandis 2016). A large PG size means a small Brownian diffusion and therefore few interactions with surrounding suspended particles. Again, for a similar PG density, a large PG size means a higher weight and settling velocity, resulting in a shorter atmospheric residence time and poorer resuspension compared to a lighter PG. In contrast, the contamination of PGs during sampling should be directly proportional to the exposed surface, that is to the PG size. All these considerations suggest that the sampling artifact in the HTS is at least of the same order of magnitude as the particulate contamination during the atmospheric transport of pollen.

The demonstration of a significant sampling artifact with the HTS for the determination of surface adhered particles on PGs questions our knowledge of the surface condition of windborne PGs. We argue that in the present state of knowledge, it is impossible to know whether the pollution of PG surfaces by atmospheric particles is significant for allergy and asthma or not. Samplings of PGs of different allergenic species under different environmental conditions are necessary to better understand the surface condition of inhaled PGs. Such airborne pollen samplings must be performed with a PM_{10} impactor and not with a HTS. PGs deposited inside a PM_{10} impactor were observed free of particles after outdoor exposure. Their surface remained intact, demonstrating that it can be employed for sampling of airborne

PGs to investigate their surface condition after interaction with PM during atmospheric transport.

Atmospheric PGs may also be collected for the purpose of detecting alterations induced by gaseous air pollutants at the scale of individual PGs (Gong et al. 2019). The use of denuders at least for ozone and nitrogen dioxide is then necessary (Ali et al. 1989). The HTS is very sensitive to any pressure drop at the sampler inlet and is therefore not suitable for the addition of a denuder (Oteros et al. 2016). In contrast, a PM₁₀ impactor can be easily connected to powerful pumps and equipped with denuders for the collection of airborne PGs. Furthermore, no resuspension of PGs was observed with the PM₁₀ impactor during the 9-h field experiment, meaning that PGs were not re-entrained in the air flowing through the device. However, even if the use of a PM₁₀ impactor is strongly encouraged, it must be mentioned that the collection efficiency for PGs is not known for this sampler. This collection efficiency needs to be precisely quantified considering the wind speed and direction in relation with the impactor inlet.

5 Conclusion

So far, the particle adhesion to pollen surfaces during atmospheric transport has not been extensively studied. Such particles-pollen complexes, forming intimate mixtures of pollen-borne allergens and chemicals, could cause a cocktail effect potentially aggravating the symptoms of allergy and asthma. In this work, the HTS was proven not to be a proper device for sampling pollen with the aim to investigate alterations of PGs by air pollutants. Due to the low aerodynamic cut-point of the HTS (3.7 µm), atmospheric particles collide with PG surfaces during sampling resulting in a positive artifact (overestimation of the number of particles adhered to PGs). As a result, previous quantitative studies performed with a HTS have very likely overestimated the number of particles adhered to PGs. By contrast, impactors equipped with a size-selective pre-separator whose intended use is to separate particles larger than 10 µm (typically PGs) from finer particles accurately ensure the collection of airborne pollen free from pollution by particles during sampling. As more studies are required to quantitatively assess the atmospheric particle adhesion onto allergenic PGs, the use of PM₁₀

impactors would be appropriate for future pollen-pollution-related studies.

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SUPPORTING MATERIAL FOR THE MANUSCRIPT:

**EVALUATION OF HIRST-TYPE SAMPLER AND PM₁₀ IMPACTOR FOR
INVESTIGATING ADHESION OF ATMOSPHERIC PARTICLES ONTO ALLERGENIC
POLLEN GRAINS**

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Keywords: airborne pollen, particulate matter, pollen sampling, pollen allergy.

MATERIALS AND METHODS

The installation setup of the artefact experiment is shown in Figure S1. The Dekati[®] PM10 impactor is an inertial multistage cascade impactor which separates suspended particulate matter (PM) into three size fractions. The inertia of a particle is a function of its shape, density, and velocity. As the shape and density of a particle are unknown for ambient PM, particle size at each stage of the impactor is therefore expressed as an aerodynamic diameter. The PM10 impactor has aerodynamic cut-points of 10, 2.5 and 1 μm to separate $\text{PM}_{>10}$, $\text{PM}_{2.5-10}$ and $\text{PM}_{1-2.5}$ size fractions, respectively. The flow rate of the PM10 impactor is 10 L min^{-1} with a gas velocity ($3.3 \text{ m}\cdot\text{s}^{-1}$) at the inlet. The HTS is a one-stage impactor designed to trap airborne biological particles, i.e. pollen grains (PGs) and fungal spores (Hirst 1952). It is equipped with a wind vane which orientates the air inlet opening according to the prevailing wind direction. The built-in vacuum pump sucks air through the inlet at a constant rate of 10 L min^{-1} . The air flows in through a rectangular orifice of $14 \text{ mm} \times 2 \text{ mm}$. The gas velocity at the entrance slit is $6 \text{ m}\cdot\text{s}^{-1}$ enabling efficient impaction of PGs onto an adhesive surface perpendicular to the air stream and moving at $2 \text{ mm}\cdot\text{h}^{-1}$.

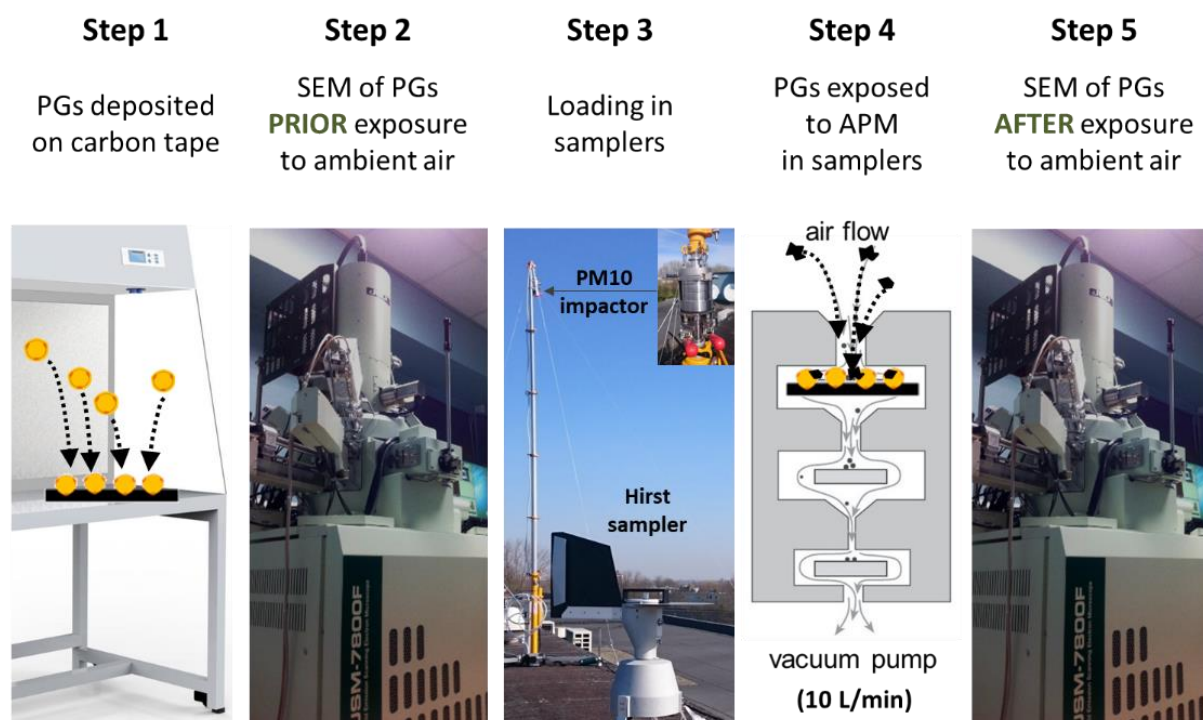


Figure S1. Schematic view of the field experiment. **Step 1:** Birch PGs from mature catkins were deposited by sieving on conductive double sided adhesive carbon tapes in the laboratory. **Step 2:** Each of the two carbon tapes covered in birch PGs was first observed without metal coating using a low-vacuum Scanning Electron Microscope (SEM, JSM-JEOL 7800F-LV). On each tape, several spots were selected to observe native deposited PGs. Positions (x,y) of each spot were recorded automatically by the software of the SEM. **Step 3:** One carbon tape covered in birch PGs was placed into a Hirst-type sampler (VPPS 2000, Lanzoni s.r.l.) and the other one was placed on the first stage ($>10\ \mu\text{m}$) of a PM10 impactor (Dekati Ltd.). **Step 4:** Birch samples were exposed to ambient PM (APM) during 9 hours (from 9:00 a.m. to 6:00 p.m. local time) in both samplers installed at the same location and simultaneously operating with an air flow rate of $10\ \text{L}\cdot\text{min}^{-1}$ (Figure S2). **Step 5:** Exposed birch PGs were analyzed using SEM. The exact same spots of the carbon tape observed prior to exposure to ambient air were relocated. SEM images of birch PGs were compared prior and after exposure to ambient PM.

The first field experiment was conducted after the birch pollination season on 22 June 2017. The step-by-step procedure is described in Figure S2.

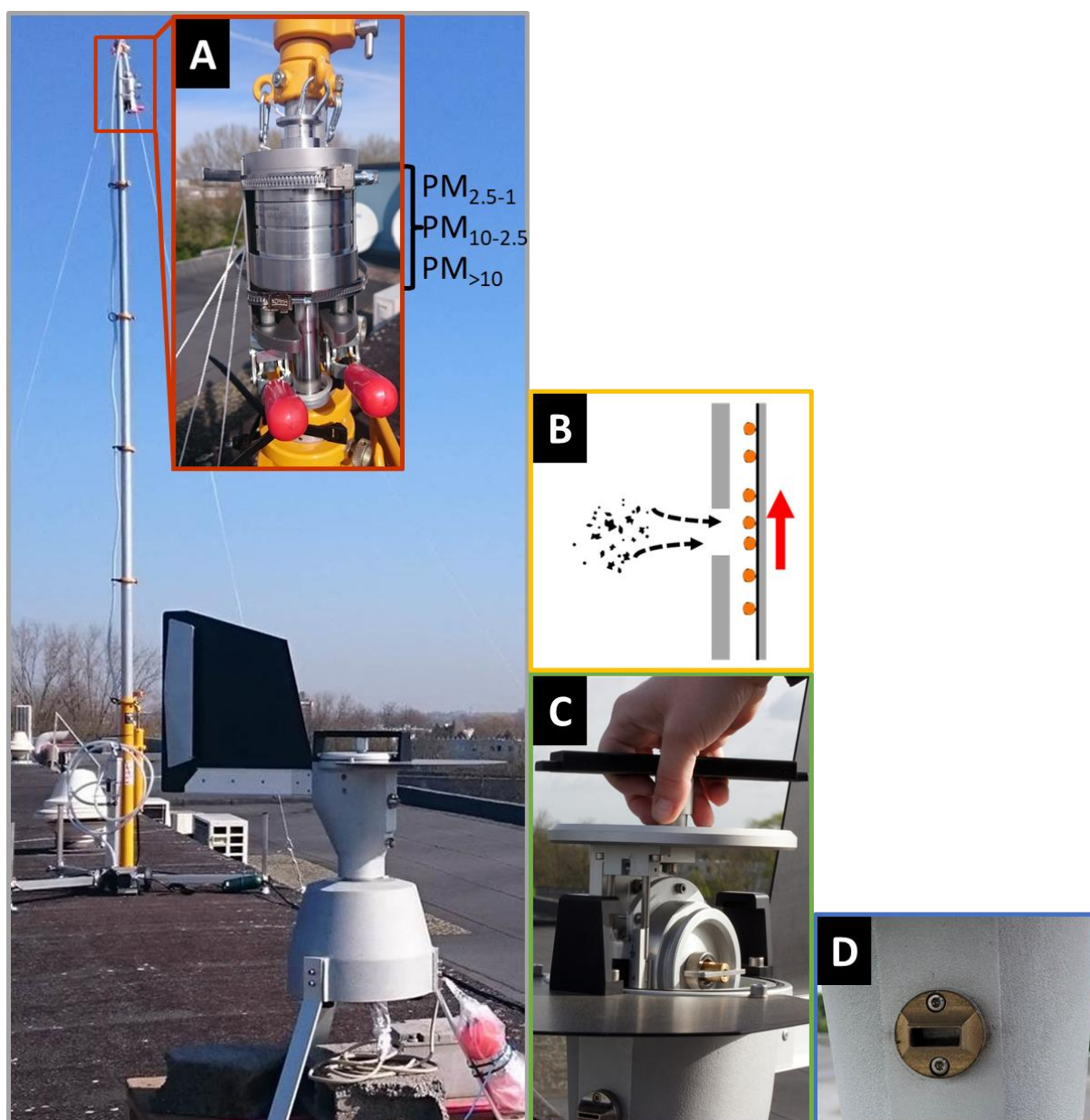


Figure S2. HTS (VPPS 2000, Lanzoni s.r.l.) and PM10 impactor (Dekati Ltd.) installed on a rooftop at 20 meters a.s.l. on the University of Lille campus ($50^{\circ}36'N$, $03^{\circ}08'E$). (A) Size fractionation of PM in Dekati® PM10 impactor. (B) Schematic of the inlet and the collecting substrate sliding at 2 mm h^{-1} in HTS equipped with a daily sampling head. (C) Conventional HTS with an entrance slit of $14 \times 2 \text{ mm}$. (D) Modified Hirst slit of $14 \times 5.3 \text{ mm}$ built in a brass block.

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