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Effect of water absorption on pollen adhesion

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ABSTRACT

Pollens possess a thin liquid coating, pollenkitt, which plays a major role in adhesion by forming capillary menisci at interfaces. Unfortunately, the influence of humidity on pollenkitt properties and capillary adhesion is unknown. Because humidity varies widely in the environment, the answers have important implications for better understanding plant reproduction, allergy and asthma, and pollen as atmospheric condensation nuclei. Here, pollenkitt-mediated adhesion of sunflower pollen to hydrophilic and hydrophobic surfaces was measured as a function of humidity. The results quantify for the first time the significant water absorption of pollenkitt and the resulting complex dependence of adhesion on humidity. On hydrophilic Si, adhesion increased with increasing RH for pollens with or without pollenkitt, up to 200 nN at 70% RH. In contrast, on hydrophobic PS, adhesion of pollenkitt-free pollen is independent of RH. Surprisingly, when pollenkitt was present adhesion forces on hydrophobic PS first increased with RH up to a maximum value at 35% RH (~160 nN), and then decreased with further increases in RH. Independent measurement of pollenkitt properties is used with models of capillary adhesion to show that humidity-dependent changes in pollenkitt wetting and viscosity are responsible for this complex adhesion behavior.

1. Introduction

Pollen particles, carrying the male gamete of plants, possess a range of unique ornamentations with various morphologies and feature sizes. They can effectively disperse in air or entangle with hairs on animals allowing for their distribution over large areas. Understanding the adhesion mechanisms of pollen particles is of scientific interest due to their role in plant reproduction [1], the health of pollinators [2,3], human environmental health [4], and ice and water formation in the atmosphere [5,6]. For example, consider that the maintenance of healthy bee communities, which has a complex interdependent relationship with pollination ecology, is of current worldwide concern [2,3]. In addition, proteins carried on pollens contribute to asthma and allergies in humans and the pollen grain's adhesive capabilities are significant factors in pollen environmental distribution [7]. Finally, pollen grains are known to be carried into the atmosphere where they can absorb organic pollutants, age under UV exposure, and act as nucleation sites for water condensation or ice formation [8]. Therefore, their surface properties and sensitivity to humidity are of interest due to their potential role in climate and weather. Due to their unique geometrical features that enable fine-tuning of adhesion, pollens have been recently explored as a bio-organic template for the synthesis of biomimetic functional materials in a variety of applications [9–12], including adhesives, composites, paints and pigments, drug delivery, and porous media for sensors, adsorbents, catalyst supports and filtration [13,14]. Despite the wide range of fields influenced by pollen properties, there still is a lack of quantitative understanding of pollen surface properties and how these affect adhesion and release characteristics. In particular, one of the compelling unresolved questions is the role of the liquid pollenkitt coating in carrying water and mediating the sensitivity of pollen adhesion to the effects of humidity.

The pollen exine shell is composed predominantly of sporopollenin [15–18], a crosslinked polymer which is one of the most chemically-stable naturally-occurring materials, and has good thermal and mechanical stability [19,20]. Pollen grains are additionally coated with a liquid that resides on or within cavities in the exine wall [21]. This coating material (named pollenkitt by Knoll [21]) is especially prevalent in pollen from *entomophilous* plants, and is a mixture composed of saturated and unsaturated lipids, and lesser amounts of carotenoids, flavonoids, proteins and carbohydrates, and is of great importance in pollination ecology [4,15]. It is generally thought to occur as a water-in-oil

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emulsion in nature, i.e., there exists an aqueous phase of dispersed water droplets that may also contain polar organics. The dispersal of entomophilous pollens is thought to be facilitated by pollenkitt's ability to keep pollen grains together during transport and to promote adhesion to animals. It is known that these pollinators eat pollenkitt and that pollenkitt is an important source of nutrients. Pollenkitt is also thought to play a role in adhesion of pollen to the stigma, an important prerequisite for cell recognition and pollen germination [1].

In previous work, we described how pollen adhesion is quantitatively dependent on both a dry mechanism controlled by van der Waals forces and the size of ornamentations, and a 'wet' mechanism controlled by the capillary forces of pollenkitt liquid bridges between pollen and a substrate surface [22]. However, despite the wide variation of humidity in nature, surprisingly little is known about the water content of pollenkitt as a function of humidity, or the effect that water uptake has on capillary-driven adhesion mediated by pollenkitt. In the present work, the adhesion forces between sunflower pollen grains with and without pollenkitt were studied on both hydrophilic silica-coated silicon (Si) and hydrophobic polystyrene (PS) surfaces were studied as a function of relative humidity (RH) by using atomic force microscopy (AFM). We describe below our finding that pollenkitt indeed absorbs a significant amount of water and that this water uptake has a marked effect on the adhesion of pollen to hydrophilic and hydrophobic surfaces. The adhesion forces are modeled and the wetting properties and viscosity of pollenkitt are also determined independently, in order to elucidate their role in RH-dependent adhesion of pollenkitt.

2. Materials and methods

2.1. Pollen and substrate preparation

Piranha-etched Si wafers were used as hydrophilic reference surfaces, since they are coated with a thin layer of silicon oxide. Hydrophobic PS (M_w = 230,000, Sigma–Aldrich) surfaces were prepared by knife-edge coating 10% m/m in PS/toluene solutions on Piranha-etched silicon substrates [23], followed by drying at room temperature for 24 h and annealing at 60 °C under vacuum for 2 h. Film thickness, measured with interferometry, was approximately 1–2 μm, which far exceeds the range of van der Waals interactions (~20 nm) of the underlying silicon substrate. The mean (R_a) and root-mean-square (rms) roughnesses of Si and PS surfaces were 0.2 ± 0.1 and 0.3 ± 0.1 nm, 2.2 ± 0.2 and 2.7 ± 0.2 nm, respectively, obtained from topography scans of three random 10 μm × 10 μm areas using AFM (Veeco Dimension 3100).

Sunflower, a widespread flowering plant, produces pollen with uniform size and morphology consisting of well-organized nanoscale-spiny features. Sunflower pollen is entomophilous and carries a liquid pollenkitt coating whose composition has been characterized previously [24], and represents an ideal model for this study [8,25]. Non-defatted sunflower (*Helianthus annuus*) pollen, containing pollenkitt (PK+, Greer Laboratories, Lenoir, NC) was stored at 0 °C. To provide a pollenkitt-free control (PK−), we washed PK+ with chloroform:methanol (3:1) [26] solution for 24 h (a solvent for external pollenkitt, but non-solvent for sporopollenin) prior to depositing on filter paper (P5, Fisher Scientific, Pittsburgh, PA) and drying in air.

2.2. Force measurements

Adhesion was measured by using a NanoScope IIIA Multimode AFM (Veeco Metrology, Santa Barbara, CA) with tipless rectangular cantilevers (Applied NanoStructures, Inc., Santa Clara, CA, nominal spring constants of 1.2–6.4 N/m). Single pollen grains were glued to tipless cantilevers with epoxy resin using a procedure described elsewhere [27]. The spring constants for cantilevers with attached pollen, determined by methods described elsewhere [28,29], were 2.1–2.3 N/m for PK+ and 1.8–1.9 N/m for PK−. A series of ten force-distance curves were measured for each combination of pollen tip (PK+ or PK−)-substrate (PS or Si), on three separate substrates within three randomly chosen 10 μm × 10 μm areas, under a nitrogen/water atmosphere (20 °C) with controlled relative humidity. Each experiment commenced by cycling the scanner in z direction for ~1 h to allow the scanner to reach thermal equilibrium. Adhesion measurements were performed by extending the scanner over a ~500 nm distance at 10 nm/s until contact with the surface, at which point scanner extension was continued until a loading force of 200 nN was reached, followed by scanner retraction at 10 nm/s. Force versus distance curves were generated by multiplying the measured downward deflection of the cantilever times the spring constant of the cantilever. The force-distance data indicated that only one spine makes contact with the substrate [22].

2.3. Mass uptake measurements in humid air

Thermal resonance data was acquired simultaneously with force data by taking raw (prior to analog filtering) vertical deflection, horizontal deflection and summed signals directly from the AFM and routing them into a PCI-6120 data acquisition card through a BNC-2110 interface (National Instruments, Austin, TX). The pollen-loaded cantilever resonated at its natural thermal resonance frequency; no external oscillation was applied to the cantilever holder. To correlate these signals with scanner movement in the z dimension, the z scanner voltage was taken from the signal access module and sent to the data acquisition card. Data acquisition software written in LabVIEW (National Instruments, Austin, TX) converted the deflection data into the frequency domain (i.e., power spectral density) using the discrete Fourier transform with a Blackman–Harris window. Cantilever resonance data was acquired with a sampling rate of 800 kHz with a 50 Hz resolution along the frequency axis of the power spectral density. To improve the signal-to-noise ratio, an average of fifteen FFTs was taken. Frequency shifts, Δf, are measured at all RH values relative to the lowest RH of 17%. The relationship between mass increase (Δm) and frequency shift, Δf, as water is taken up by the pollen sample (by condensation, adsorption or absorption) can be determined from a single-degree-of-freedom (SDOF) approximation, as described in detail in supplementary material (see Fig. S1 and corresponding discussion in supplementary material). In this manner, all mass changes, Δm, are relative to the lowest RH of 17%.

2.4. Control and variation of relative humidity

The AFM was enclosed in a plastic chamber into which a stream of N_2 gas with controlled RH is introduced, allowing investigation of the effect of humidity on the AFM force measurements. Relative humidities between 15% and 70% were achieved by adjusting the flow rate ratio of as-received dry N_2 gas (UHP300, Airgas USA, LLC.) and N_2 gas bubbled through water.

2.5. Scanning electron microscopy (SEM)

The pollen AFM probes were characterized by scanning electron microscopy (Zeiss Ultra60 FE-SEM) after all force measurements were finished, at an accelerating potential of 10.0 kV. Probe tips were sputtered with gold and then mounted on metal stubs using carbon tapes.