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Birch and conifer pollen are efficient atmospheric ice nuclei

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Abstract

The ice nucleation of bioaerosols (bacteria, pollen, spores, etc.) is a topic of growing interest, since their impact on ice cloud formation and thus on radiative forcing, an important parameter in global climate, is not yet fully understood. Here we show that pollen of different species strongly differ in their ice nucleation behaviour. The average freezing temperatures in laboratory experiments range from 240 K to 255 K. As the most efficient nuclei (silver birch, Scots pine and common juniper pollen) have a distribution area up to the Northern timberline, their ice nucleation activity may be a cryoprotective mechanism. Far more intriguingly, it has turned out that water, which has been in contact with pollen and then been separated from the bodies, nucleates as good as the pollen grains themselves. So the ice nuclei have to be easily-suspendable macromolecules located on the pollen surface. Once extracted, they can be distributed further through the atmosphere than the heavy pollen grains and so augment the impact of pollen on ice cloud formation even in the upper Troposphere. Our experiments lead to the conclusion that pollen ice nuclei, in contrast to bacterial and fungal ice nucleating proteins, are non-proteinaceous compounds.

1 Introduction

As the phase transition from liquid to frozen water at subzero temperatures is kinetically hindered, droplets of ultrapure water can remain liquid down to temperatures of 235 K. Only structures, which ease the cluster formation in the water, can cause freezing at temperatures as high as the thermodynamic freezing point. These particles are called ice nuclei (IN). Atmospheric IN can trigger cloud glaciations and precipitation, having an impact on both the global radiation balance and the water cycle. The impact on the balance is still debated, as on the one hand ice clouds have a higher albedo than liquid water, so they should cool the climate (Mishchenko, 1996), but on the other they are more likely to precipitate and so reduce total cloud albedo (Lohmann, 2002).

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Atmospheric IN can be of non-biological or biological origin. Among the latter are the ice-nucleating proteins discovered in some bacterial and fungal species, like Pseudomonas syringae (Schnell, 1972; Wolber, 1986), Fusarium avenaceum (Pouleur, 1992), and Rhizoplaca chrysoleuca (Kieft, 1990). Some biogenic ice nuclei, however, are non-protein compounds (Gross, 1988; Mugnano, 1996). But in all cases ice nucleation is not caused by the total surface, but only by definite active sites located on the surface. In-situ measurements of ice clouds at around 8 km height have shown that 33% of all ice-crystal residues are of biological origin (Pratt, 2009).

In the past a few pollen species have been studied in all four primary nucleation modes (Diehl, 2001; Diehl, 2002; von Blohn, 2005). While pollen are IN inactive in the deposition freezing mode, they nucleate in condensation, immersion and contact modes. The measured median freezing temperatures are in the range from 251 K to 265 K. However, no research has been carried out to analyze the nature of pollen IN, although it has been suspected that pollen IN activity can be derived from their rough, porous surface structure, since pollen are known to have nano-sized pores in their exine (Kovacik, 2009). The exine is the outer wall of the pollen, consisting of a robust, very inert biopolymer called sporopollenin, which is composed of aromatic and aliphatic hydrocarbons.

2 Nucleation measurements

15 different pollen species have been investigated: silver birch (Betula pendula), common hazel (Corylus avellana), goat willow (Salix caprea), plane tree (Platanus orientalis), ragweed (Ambrosia artemisiifolia), wormwood (Artemisia absinthium), redtop (Agrostis gigantea), corn (Zea mays), Scots pine (Pinus sylvestris), Irish yew (Taxus baccata), common juniper (Juniperus communis), Pfitzer juniper (Juniperus chinensis pfitzeriana), Northern whitecedar (Thuja occidentalis), Chinese arborvitae (Thuja orientalis), stinging nettle (Urtica dioica). To distinguish between the junper species, the common juniper is referred to as “juniper I”, the Pfitzer juniper as “juniper II”, the

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Northern whitecedar as “thuja I” and the Chinese arborvitae as “thuja II”. This way a broad spectrum of different samples from all over the seed plant taxon is covered: pine, yew, thuja and juniper belong to the conifer phylum. Birch, hazel, willow and plane tree are typical broadleaf trees, which are dicotyledons (they have two seed leafs after germination). Redtop and corn are, like all the grasses, monocotyledons (they have one seed leaf after germination), while ragweed, wormwood and nettle are dicotyl herbs.

As a reference, Snomax has been used, which is an IN protein extracted from Pseudomonas syringae and nucleates at temperatures about 270 K. Due to its high IN efficiency it is commercially available as an additive for snowguns (Wolber, 1986).

Measurements have been performed by cryo-microscopy: the core of the experimental setup is a cryo cell consisting of a Peltier element placed in a Teflon box. In the cap a glass window allows inspection of the cell inside. The cryo cell is placed on a light microscope desk with the glass window below the objective. The Peltier element can be cooled to temperatures below 220 K with a measurement error smaller than ±1 K. The accuracy of the temperature measurement has been measured by observing the melting process of pure water droplets, which is supposed to take place at 273 K at pressures around 1 atm.

The samples have been measured in oil immersion mode, where small water droplets are dispersed in an oil matrix (Marcolli, 2007). The sample pollen are suspended in the emulsion and distribute between the two phases. The emulsion consists of 45 wt. % MilliQ water and 55 wt. % oil, which itself is composed of 90 wt.% paraffin and 10 wt. % lanolin (water-free grade). The prepared samples are placed on a glass slide and set onto the Peltier stage, where they are chilled with a cooling rate of 1–2 $K \cdot min^{-1}$. Frozen droplets appear dark due to increased light scattering and contain visible internal structures, like edges or cubes. Finally, the fraction of frozen droplets is plotted against the current temperature. Only droplets with diameters of 10 µm–200 µm have been accounted for, because smaller droplets are more difficult to observe, and larger droplets behave more unpredictable and do not appear in the real upper atmosphere. Moreover, larger droplets demand more space, so fewer droplets

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can be observed in the display window of the microscope camera during one nucleation experiment, what worsens the statistics.

The different samples are compared by a characteristic number, the median freezing temperature, which is the temperature with 50 % of all droplets frozen. This value is more reliable than the initial freezing temperature, which is the temperature where the first droplet freezes, because the latter one may be manipulated by outliers and is less reproducible. The different freezing median temperatures of pollen species are shown in Fig. 1 and Table 1.

To verify the measurements of the oil immersion mode pollen IN activity of some species was additionally measured in a smog chamber, which is closer to reality and eliminates the possible influence of the oil matrix. Therefore droplets of an aqueous pollen suspension were nebulized into the chamber with an ultrasonic nebulizer. Then the chamber was cooled adiabatically by partial evacuation. Samples of different IN activities have been chosen to check, if the chamber measurements show the same pattern as the oil immersion method.

Although the median freezing temperatures differ for some Kelvin from the oil immersion mode, they are principally within the same range. Differences can be explained by following deviations from our oil immersion measurements: (i) the pollen have to be crushed, or else they, respectively the droplets are too big and deposit before they can be measured, (ii) artificial rainwater has been used as a solvent instead of MilliQ water to model real conditions in clouds, (iii) although the initial droplet size lies in the range of 300 nm–700 nm, the exact droplet size in the chamber is not known, as the adiabatic cooling enlarges the droplets, (iv) the droplets in the oil immersion are static, while the droplets in the chamber are dynamic and can collide, easing nucleation events, (v) the temperature in the innermost region of the chamber might be slightly lower than measured by the temperature sensor, which is placed close to the chamber wall, (vi) a slight change in the surface tension of the droplets caused by the oil matrix might change nucleation temperatures. All these considerations together might explain the small difference between nucleation temperatures. The results are listed in Table 1.

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In the past the ice nucleation activity of pollen has been explained by the general roughness and porosity of the pollen surface (Diehl, 2001). Measurements with electron microscopy have shown that all pollen have a rich surface topology (see Fig. 4). Although pollen from different species exhibit difference on the microscale, their different ice nucleation activities cannot be directly correlated with their microtexture, respectively microstructural elements. Even species with a similar microtexture, which results from close relatedness, can show different nucleation behaviour. For example, birch and hazel are in the same family and their pollen look rather the same, but their median freezing temperature differs by 6 K–8 K (see Fig. 4 and Table 1). The same can be said about the thuja and the juniper species, which are all closely related.

3 Ice nuclei analysis

It has been shown that water, which has been in contact with pollen for some hours, adopts the ice nucleation activity and keeps it after separation from the pollen grains (see Fig. 1 and Table 1). Therefore pollen and water were mixed and left for some hours at room temperature, being occasionally shaken up again. Then the water fraction is decanted or, if the pollen do not sediment well, filtrated. The applied pollen concentration for preparing the washing water is 50 mg ml^{-1}. Both the washing water and the washed pollen bodies show the same IN activity in cryo-microscopic measurements as the untreated pollen. Nucleation data show two steps for most species: one at approximately the same temperature as the whole pollen grains, and one at the homogenous nucleation temperature at 237 K (see Fig. 2). The most important cognition of these data is the fact that the active sites of the pollen surface are suspendable nano-structures on the pollen surface, which are too small to be recognized in the electron microscope, but easily leave the pollen bulk. Furthermore, the relative amount of IN on the pollen surface can be correlated with the ratio of the two steps in the nucleation curve. While the birch sample is fully frozen (100 %) before reaching the homogenous nucleation temperature, others show far lower heterogenic nucleation

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behaviour (see Table 1). This finding can be explained by the fact that the IN on some pollen are scarce or inefficient. This is most likely the reason for the broad steps in the nucleation spectra of some species, as individual pollen show individually different ice nucleation behaviour, if the IN concentration on the surface varies on a low level. For example, willow and hazel pollen grains show a large freezing temperature span, a discrepancy of some Kelvin between whole pollen grain and washing water nucleation, and a high fraction of homogenous nucleation events.

The hypothesis of macromolecular IN can be verified by drying up washing waters and analyzing them with transmission electron microscopy (TEM). Therefore a droplet of washing water is placed on a carbon-coated copper grid and left to dry up. After that the residues are investigated. These residues lack structures down to the level of about 10 nm, so the IN cannot be much bigger than that. To estimate the mass range of the IN, washing water has been filtered with Vivaspin tubes of different mass exclusion limits. According to these measurements, the pollen IN are in the mass range of 100 kDa–300 kDa.

To characterize and identify the IN of the pollen surface, some stress tests have been performed with the washing waters of selected pollen species: birch, pine, yew, juniper I, willow, hazel. For testing the thermal stability, which, together with other data can assist the determination of the chemical nature of the IN, each sample was heated to the demanded temperature (preparations have been performed in a range from 355 K–460 K) for 1 h. Then the water-free residue was re-suspended in water again and used for oil immersion measurements. The species differ in their thermal stability, with a stability range up to 445 K for birch and pine (see Table 2).

Furthermore our measurements indicate that pollen IN are non-proteinaceous compounds, as they are insensitive towards treatment with 6M guanindinium chloride, which has been added to washing water and destroys the structure of proteins (Breslow, 1991), including the Snomax IN protein. A reduction of the nucleation temperature for some Kelvin can be explained by the increase of electrolyte concentration, which appears also at the pure water reference (see Table 2). The pollen nuclei are also

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insensitive towards protein-breakdown by the enzymes pronase E and papain. Therefore 5 mg ml^{-1} pronase E was added to the washing water, which was then stored for 4.5 hours at 310 K. The same procedure was carried out with 2 mg ml^{-1} papain and incubation for 5 h at 340 K. Papain treatment has not been used for Snomax, as it already decomposes thermally at the demanded temperature optimum. As the pH of the used MilliQ water is about 6, no pH adjustment, which could manipulate nucleation temperatures, was necessary. The same procedure was performed with pure MilliQ water to proof that the procedure itself has no influence on the nucleation temperature and Snomax as reference, which is harmed by this procedure. The same procedure was carried out with pancreas lipase, which breaks down fats: Washing water was spiked with 2 mg ml^{-1} lipase and then heated to 308 K for 3 h . Again the IN keep their activity. Digestion with 5 mg ml^{-1} cellulase Onozuka (breaks down cellulose) for 4.5 h at 310 K and with alpha-amylase (breaks down many, but not all alpha-glycosidic sugar bonds) for 3 h at 313 K show no effect on nucleation temperatures either (see Table 1).

As in agreement with literature, the denaturation of the bacterial ice nucleating protein with a protease or by moderate heat does not totally eliminate ice nucleation, but decreases temperatures for about 5 K. This is consistent with measurements of IN proteins in the past (Kieft, 1990, Tsumuki, 1994). Only under severe stress (e.g. temperatures above 400 K or treatment with guanidinium chloride) Snomax fully loses its ice nucleation activity. To test the stability in acidic conditions pollen were incubated at different sulphuric acid concentrations (0.5 M, 5 M, 10 M) and different temperatures (293 K, 333 K, 358 K) for 1 h. After that all samples were diluted to a concentration of 0.5 M sulphuric acid, otherwise the nucleation point decreases too much, simply due to the high presence of an electrolyte. The result is that it needs at least 5M sulphuric acid to destroy the IN. As a side effect, the sample turns dark orange, which might be the result of sample oxidation. With 10M sulphuric acid the sample turns immediately red – and when heating it blackens due to carbonization.

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4 Nucleation rates

Strictly speaking freezing is a time-dependent statistical process, so in fact the definition of the median freezing temperature may be questionable. However, measurements like described in Sect. 2 have also been carried out at different constant temperatures. By correlating the fraction of frozen particles with the measuring time, one can calculate nucleation rate with the formula (Murray, 2010):

$$\ln\left(\frac{n_{\mathrm{tot}} - n_{\mathrm{nuc}}}{n_{\mathrm{tot}}}\right) = -J \cdot V \cdot t \tag{1}$$

n_{tot} is the total droplet number, n_{nuc} the number of frozen droplets, t the time in seconds, V the average droplet volume in cm^3 and J the nucleation rate in $\mathrm{s}^{-1}\,\mathrm{cm}^{-3}$. Formally the droplet freezing is treated like a first-order decay reaction with liquid droplets as educt and J as speed constant.

The nucleation rate measurements are isothermic. With increasing time more and more droplets freeze. But measurements in this study, as well as others have shown (Murray, 2010), that J strongly depends on the temperature, so that laboratory measurements can only be performed in a temperature interval of few Kelvin (see Fig. 5). Due to this steep slope median freezing is reached within milliseconds some degrees below, or years some degrees above this interval. For this reason it is legitimate to concentrate on this narrow window with nucleation rates of about $10^5\,\mathrm{cm}^{-3}\,\mathrm{s}^{-1}$ and define the corresponding temperature as median freezing temperature. As a consequence nucleation spectra can be constructed, where the fraction of frozen particles is plotted against T without any time dependence.

In this study the homogenous nucleation rate J_{hom} has been determined with MilliQ water droplets in the temperature range 236.5 K–240.5 K. Emulsions with birch washing water have been measured to calculate the heterogenous nucleation rate J_{het} for this sample at 254 K – 258 K.

Concerning nucleation rate calculations it has to be admitted that the suggested volume dependence is only exact for homogenous freezing, while at heterogenous

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freezing it is overcompensated by the amount of IN per droplet (Iannone, 2011). However, if the IN are homogenously distributed in the aqueous phase, larger droplets should contain a higher number of IN, leading to a pseudo-dependence on the droplet size. As a consequence droplets of similar size behave more or less the same way, but if the droplets differ much in size (e.g. more than one magnitude), the size dependence becomes more impressive.

Our measurements proof, that many pollen species produce far more efficient ice nuclei than all studied mineral dust species, as they have far higher nucleation rates at given temperature (Eastwood, 2008).

5 Experimental section

The Juniper II pollen have been collected in the tree nursery of Kagran in Vienna. The other conifer pollen as well as nettle and wormwood pollen have been purchased from Pharmallerga, while all other pollen have been purchased from AllergonAB.

5.1 Microscope Set-up

The microscopes used are a Zeiss Axio ScopeA1 and an Olympus BX51. The cryo cell (see Fig. 3) has been constructed in the Institute of Material Chemistry at Vienna University of Technology. It contains a three-stage Peltier element of the type UEPT-330-119-045C200, which is cooled with water (about 290 K). Photos are taken with a MDC-200 microscope camera. While and after opening the cell, e.g. when changing the sample, it has to be flushed with nitrogen or synthetic air to keep out air humidity, which would disturb the measurements.

Scanning electron microscopy pictures (see Fig. 4) have been captured with a FEI Quanta™ 200 FEGSEM, transmission electron microscopy pictures with a FEI Tecnai F20.

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5.2 Chamber Set-up

The smog chamber is the LOTASC at the University of Bayreuth, which is a 3.5 m^3 glass chamber (Behnke, 1988). The initial air humidity at the beginning of measurements is 85 %. By pumping down to 400 mbar the temperature drops by 10 K – so the system has to be pre-cooled for reaching low temperatures.

The principle of ice particle detection in the chamber is the change of the Stokes vector of backscattered light at planar surfaces like ice, in contrast to the preservation of the Stokes vector on spherical surfaces like droplets. The freezing process is observed by depolarization of a diode laser (Acculase lc, 635 nm wavelength, 5 mW power) going through the chamber. When aerosol droplets freeze, they increase the depolarization degree of the Mie-scattered light. The backscattered light is measured by a photomultiplier (Hamamatsu R374) in 175° setup relative to the direction of the laser beam. An essential component to measure the depolarization degree is the rotatable linear polarity filter at the detector window, which oscillates between transmitting and absorbing the different planes of the scattered laser light. This simplification compared to a setup with two photomultipliers demands a more sophisticated data analysis.

To inject the pollen they are crushed with a rocker mill and suspended in artificial rainwater, which contains the following ingredients: 0.04 mM Ca^{2+}, 0.02 mM Mg^{2+}, 0.04 mM Na^{+}, 0.10 mM K^{+}, 0.10 mM NH_4^{+}, 0.06 mM SO_4^{2-}, 0.09 mM NO_3^{-}, 0.04 mM Cl^{-}. The suspension is put into a plastic flask with an ultrasonic nebulizer (type QUV-HEV FT25/16-A), which generates small droplets, that are blown into the chamber by a nitrogen stream.

5.3 Size exclusion

Vivaspin tubes are commercially available plastic tubes horizontally separated into two compartments by a polyethersulfone membrane. The upper compartment is filled with the solvent to filtrate. When the tube is centrifuged at 4000 rpm, the solvent is pressed through the membrane. Only molecules below a given mass may pass the membrane,

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while larger molecules are retained. This way the solvent is free of molecules larger than the mass limit of the membrane. Vivaspin tubes can be purchased with different upper mass limits: We have used tubes with 5, 10, 30, 50, 100 and 300 kDa. Only filtrates of the 300 kDa tubes keep their IN acitivity, while all others become IN-negative – leading to the conclusion, that the pollen IN have to be smaller than about 300 kDa, but larger than about 100 kDa.

6 Conclusions

As a whole, it has been shown that the ice nucleating activity of pollen can be derived from surface macromolecules, which cause different ice nucleation behaviour of different pollen species. Although these IN have not been fully characterized yet, several substance classes can be excluded. For example, the structure of proteins is destroyed by 6 M guanidinium chloride, as we have shown with the ice nucleating protein in Snomax. Our pollen ice nuclei, however, are not affected at all. The IN could be polysaccharides, which are common substances on the pollen surface (Clarke, 1979; Grote, 1989). This hypothesis is consistent with the behaviour in sulphuric acid, as the cooking of food samples in concentrated sulphuric acid is a standard method to break down and measure the carbohydrates content (Dubois, 1956). Biological saccharides can be either free or bound to proteins to form so-called glycoproteins. If the pollen ice nuclei are the latter, then their activity have to be caused by the sugar side chains alone and not the protein core. Alternatively, the IN might be oxidized organic polymers.

The heat degradation curves of Snomax (see Table 2) showing two steps can be explained by different levels of destruction: Biomolecules have several levels of structuration. The primary structure, which is the sequence of monomers, is the most stable structure, but is by far not enough for biomolecules to fulfil their purpose. The tertiary structure is the general arrangement of the protein and is the most fragile. For a full-working biomolecule all structures have to be intact – the more the structures are damaged the lower is the biological activity. A loss of the tertiary structure alone

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can totally or partly deactivate the function of a biomolecule. Derivated from that we believe that the steps in the heat degradation curve of Snomax are caused from the stepwise reduction of structure and so nucleation activity, while the most robust pollen samples (birch, pine) keep their IN activity up to about 400 K and do not fully lose their activity at temperatures up to about 450 K. This higher thermal stability points rather at polysaccharides than at the tendencially more sensitive proteins.

In the past, pollen have been rejected as important atmospheric IN, as they are not as abundant in the atmosphere as bacteria or mineral dust and are too heavy to reach higher altitudes. According to recent model calculations (Hoose, 2010) average particle concentrations are below 100 m^{-3} above the continental surface and nearly zero above the oceanic surface. But as experimental data are scarce, the real concentrations could be much higher than expected. Another study (Jacobson, 2009) estimates global pollen emissions to be nearly twice as high. Locally and temporarily (e.g. in a forest during pollen season) pollen can reach concentrations of thousands m^{-3} and cause intensive Mie scattering of the sunlight, which leads to the so-called pollen corona (Mims, 1998). Even the annual global emission is not constant, as there are so-called mast-years, in which pollen concentrations in the atmosphere can be much higher than average (Kelly, 1994). Pollen concentrations strongly decrease with the altitude, so that there are nearly no pollen at 5 km height. However, as we show in this study, it does not require the pollen body to cause ice nucleation, but just some macromolecules, which can be separated very easily from the pollen, and can principally reach higher altitudes. It is known, that material from the pollen surface, like allergens, can indeed leave the pollen body and be distributed independently (Solomon, 1983; Schäppi, 1999). We conclude that the impact of pollen on the global atmosphere might have been underestimated.

Our measurements have shown lower median freezing temperatures than the measurements performed by K. Diehl and N. von Blohn. This can be explained by the fact that they used droplets with larger radii in their measurements (256 µm–372 µm), the different setup and by individual differences between the biological samples.

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It is interesting to note that especially those pollen show high ice nucleation activity which are domiciled at higher geographic latitudes. The best ice nuclei, birch, pine and juniper I pollen are domestic at regions as far as the Northern timberline, and birch is additionally one of the first blooming species in the year, namely from February to April. On the other hand, the worst ice nucleators, thuja II, plane tree, corn and ragweed, are late bloomers (May or even later) and originated in regions of lower latitudes. As it has been proved for other organisms that extracellular freezing is a protection mechanism developed by evolution in order to enhance the chance of survival in cold environments (Duman, 2001; Howe, 2003), the IN on the pollen surface could be indeed a cryoprotective adaptation.

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Table 1. Median freezing temperatures from the oil immersion mode and the smog chamber measurements: T_{50} stands for the temperatures, where 50 % of all droplets are frozen (median freezing temperatures), with A for the whole pollen grains in oil immersion, B for the washing waters in oil immersion and C for the measurements in the smog chamber.

sample	d[µm][a]	T_{50}^{A}[K]	T_{50}^{B}[K]	T_{50}^{C}[K]	%pos[b]
Snomax	–	–	268	270	100
Birch	20	254	255	261	100
Pine	40	253	252	–	70
Juniper I	20	252	253	247	100
Yew	20	250	249	–	68
Juniper II	20	248	249	–	100
Redtop	20	248	248	246	58
Willow	20	247	249	–	34
Hazel	25	246	249	244	77
Thuja I	20	–	248	–	48
Nettle	13	–	248	–	62
Wormwood	20	–	247	–	63
Thuja II	20	–	245	–	38
Plane Tree	20	246	244	245	74
Corn	65	242	237	–	100
Ragweed	20	240	237	243	100
blank	–	–	237	< 240	0

[a] Average pollen grain diameters according to SEM measurements.

[b] Percentage of droplets that froze heterogeneously in the immersion measurements with washing waters.

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Table 2. Median freezing temperature dependence (in K) on thermal, chemical and enzymatic treatment.

treatment		Snomax	Birch	Pine	Juniper	blank
none		268	255	252	253	237
355 K[a]		262	254	–	249	–
385 K		263	254	251	249	–
415 K		235	250	249	246	–
445 K		236	247	241	237	–
460 K		–	236	236	234	–
475 K		–	236	–	–	–
Pronase		263	255	251	252	237
Papain		–	254	249	249	237
Lipase		–	253	250	253	238
Amylase		–	255	251	253	235
Cellulase		265	–	251	251	237
H_2SO_4	0.5 M	–	252	244	248	232
	5.0 M	–	240	234	236	232
	10.0 M	–	231	–	–	232
G_*Cl[b]	1 M	261	252	249	249	232
	6 M	227	252	248	249	223

[a] The temperature to which the sample is heated before nucleation measurements.

[b] G_*Cl stands for guanidinium chloride.

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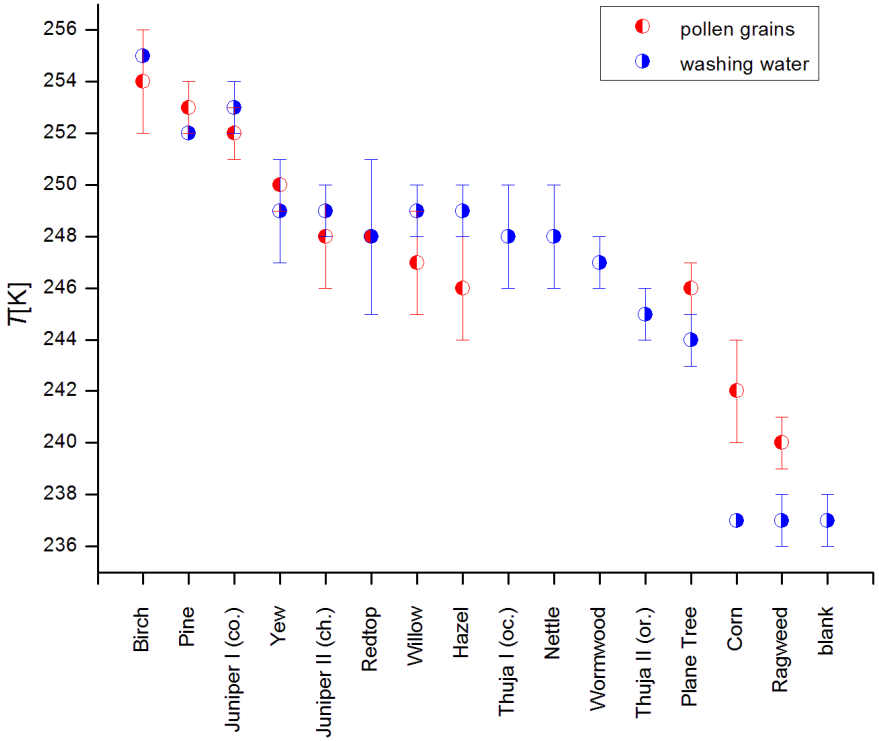

Fig. 1. Median freezing temperatures of different pollen samples: whole pollen grains and washing waters. The error bars mark the standard deviation rounded to integer numbers in Kelvin. If the calculated error is smaller than 0.5 K, no error bars are given.

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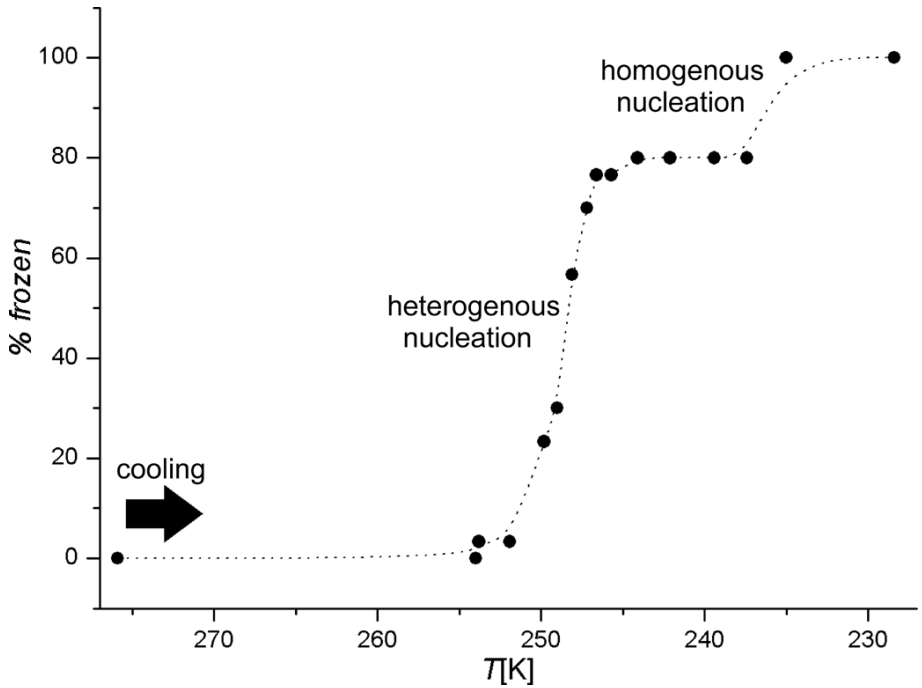

Fig. 2. The curve shows a typical nucleation curve for an oil emulsion with hazel pollen IN. Some droplets nucleate at about 237 K, meaning that they do not contain IN. For the determination of the median freezing point only the heterogenous nucleation step is used. The fraction that nucleates heterogenously in the washing water experiments is given for each species in Table 1.

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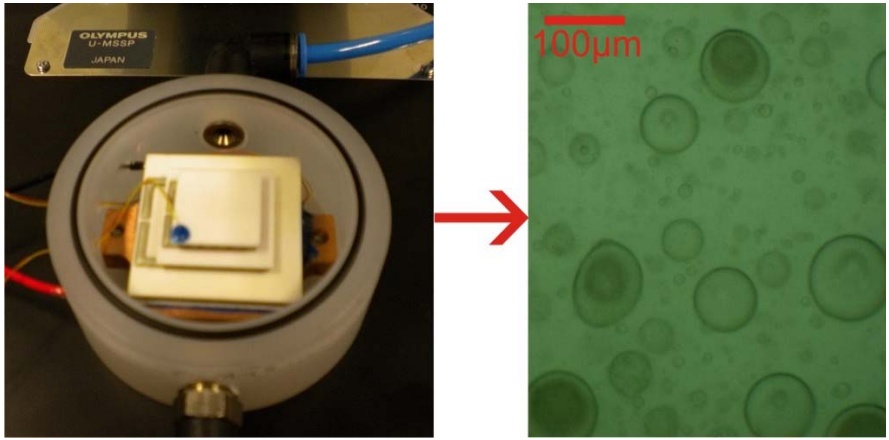

Fig. 3. The cryo cell used for oil immersion measurements, and a partly frozen pure water emulsion photographed at 237 K.

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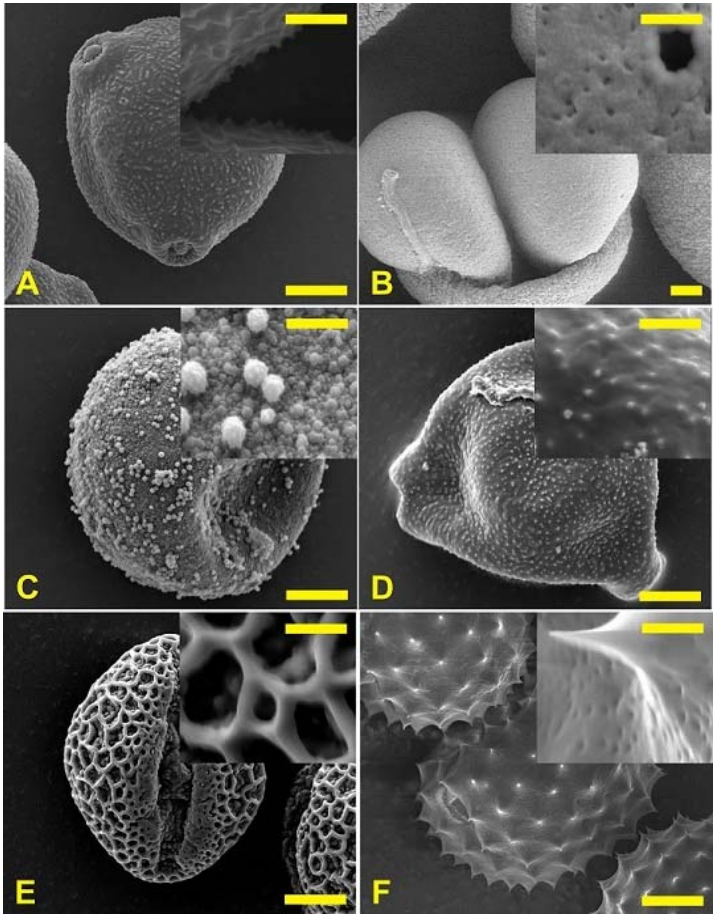

Fig. 4. SEM pictures of birch **(A)**, pine **(B)**, juniper l **(C)**, hazel **(D)**, willow **(E)** and ragweed **(F)**. The bar length is 5 µm in the large and 1 µm in the small pictures.

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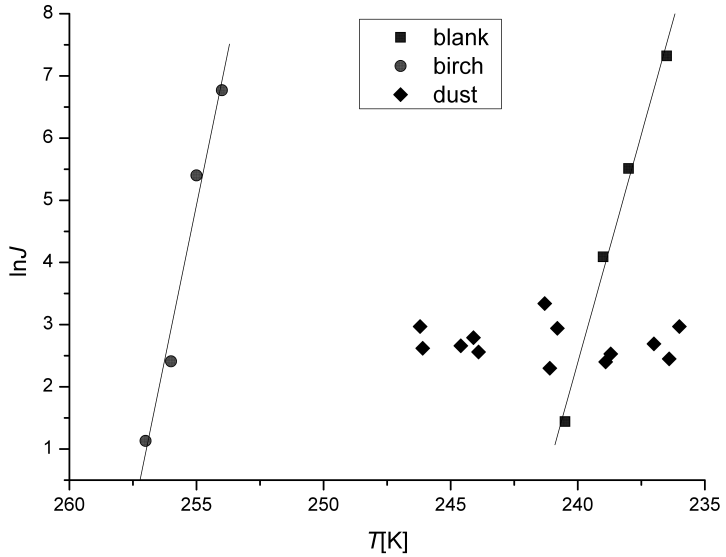

Fig. 5. Plot of the nucleation rates J_{hom}^{blank} and J_{het}^{birch}. The data of mineral dust have been taken from literature (Eastwood, 2008) in order to show the much higher IN potential of biogenic particles in comparison to most inorganic compounds.

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