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# Immersion freezing of birch pollen washing water

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## Abstract

In the present study, the immersion freezing behavior of birch pollen, i.e. its ice nucleating active (INA) macromolecules, was investigated at the Leipzig Aerosol Cloud Interaction Simulator (LACIS). For that, washing water of two different birch pollen samples with different regional origin (Northern birch and Southern birch) were used. The immersion freezing of droplets generated from the pollen washing water was already observed at temperatures higher than −20 °C, for both samples. Main differences between the Northern birch pollen and the Southern birch pollen were obvious in a temperature range, between −18 °C and −24 °C, where the ice fraction increased with decreasing temperature. There, the Northern birch pollen washing water featured two different slopes, with one being steeper and one being similar to the slope of the Southern birch pollen washing water. As we assume single INA macromolecules being the reason for the ice nucleation, we concluded that the Northern birch pollen are able to produce at least two different types of INA macromolecules. We were able to determine the heterogeneous nucleation rates for both INA macromolecule types and so could explain the ice nucleation behavior of both, the Southern and the Northern birch pollen washing water.

## 1 Introduction

As ice nucleation in clouds influences precipitation initiation and radiative forcing (Pruppacher and Klett, 1997; Lohmann et al., 2002; Storelvmo et al., 2011) it plays an important role for both, climate and weather. Ice formation in clouds occurs either through homogeneous or heterogeneous ice nucleation. For the latter case an insoluble particle called ice nucleus (IN) lowers the energy barrier for the phase transition from liquid water to ice, causing freezing at higher temperatures than homogeneous ice nucleation. To understand the glaciation of clouds and to be able to model clouds, the knowledge about the ice nucleation ability and thus the heterogeneous nucleation rate





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of the IN, which are present in the atmosphere are necessary. Investigations of ice crystal residues in an air mass sampled in the western USA showed that in this particular case about one third of the atmospheric IN were of biological origin (Pratt et al., 2009). Additionally, Pratt et al. (2009) state that 60 % of the dust particles likely were internally mixed with biological material and also Schnell and Vali (1976) showed that mineral samples, which also contain organic material, froze at higher temperatures than pure mineral samples. So it is likely that mineral dust could carry organic compounds like biological fragments, which are the reasons for the higher freezing temperatures. Biological particles, which include bacteria, viruses, spores, plant and insect fragments, algae and pollen are known to nucleate ice at much higher temperatures than non-biological particles, e.g. mineral dust (Szyrmer and Zawadzki, 1997; Murray et al., 2012). In the case of bacteria, ice nucleating proteins (INP) are responsible for the ice nucleation (e.g. Wolber et al., 1986; Govindarajan and Lindow, 1988). They are thought to act as templates for further water molecules to arrange in an ice-like manner (Graether and Jia, 2001) and so generate water molecule clusters large enough to initiate freezing at temperatures far above the homogeneous freezing point (up to −2°C, see Maki et al., 1974).

In the case of pollen only a few experimental investigations do exist. The high hygroscopicity of pollen was already shown by Durham (1941) and Dingle (1966) but still little is known about their ice nucleating ability. Diehl et al. (2001, 2002) performed some measurements with different pollen types in a wind tunnel. They found that pollen are active ice nuclei in the immersion and condensation mode but not in the deposition mode. Median freezing temperatures of −13.8°C, −15.8°C and −16.2°C were found for birch pollen, oak pollen and grass and pine pollen, respectively. Also Pummer et al. (2012) identified birch pollen as the most ice active pollen type. In that study about 15 different pollen species were investigated in an oil emulsion on an cryo-stage microscope. Birch, Pine and Juniper pollen showed the highest median freezing temperatures with −19°C, −20°C and −21°C, respectively. As these plants are usually





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occurring up to the Northern timberline, it was assumed that this mechanism protects the plant from frost damage (Pummer et al., 2012).

Pollen are the male gametes (sexual reproduction cells) of spermatophytes (seed-producing plants). In order to fertilize a female pistil, pollen have to be transported, either by flying animals, by water or wind. Wind-distributed pollen are emitted by the plants in high numbers into the air. Consequently, they are present in the atmospheric aerosol. However, as generally pollen have sizes between 10 and 100 µm in diameter, they are considered to be too large to remain in the atmosphere for a long time as, e.g. reported by Phillips et al. (2007). They also mentioned that in the case of broken pollen, their small submicron fragments could be numerous and so play a more important role in the atmosphere.

Pummer et al. (2012) found that the pollen bodies themselves are not necessary for the ice nucleation and that suspendable substances, which exist on the surface of the pollen grains and can be easily separated by washing the pollen grains, may induce ice nucleation. Furthermore it has been shown that these substances are not of proteinaceous origin and are most likely sugar-like macromolecules. In literature the occurrence of independent allergens and sugars, originating from pollen, in the atmosphere was already reported (Yttri et al., 2007; Schäppi et al., 1999). There, bursting of the pollen grains by rainfall is thought to be the most probable source. Schäppi et al. (1999) observed that the concentration of allergens and sugars in the atmosphere are ten times higher on rainy than on dry days. Consequentley, fragements of pollen and/or substances washed of from pollen could play an important role in atmospheric ice nucleation as both fragmentation and washing processes may significantly increase the number and life time of ice nuclei compared to the pure pollen.

Therefore, in the present study, the ice nucleation ability of particles, generated from pollen washing water, containing INA macromolecules was investigated at the Leipzig Aerosol Cloud Interaction Simulator (LACIS, Stratmann et al., 2004; Hartmann et al., 2011) and quantified as a function of temperature. In the course of these investigations two different birch pollen samples were considered. In contrast to the existing studies,





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we investigated droplets, containing only one size-segregated particle. We were able to produce these particles so that they contained only a few INA macromolecules, on average. Due to this, the newly developed CHESS-model (Hartmann et al., 2012) could be used for the determination of the heterogeneous ice nucleation rate of single ice nucleating active macromolecules.

## 2 Material and measurement method

### 2.1 Material preperation and particle generation

For the measurements we used two different birch pollen samples from different origin. One sample was ordered from Pharmallerga® and originated from the Czech Republic (Southern birch). The other sample contained pollen from Sweden (Northern birch) and was obtained from AllergonAB®. The measurement preparations, procedures, and conditions were identical for both samples. For preparing pollen washing water, we first suspended 1 g of pollen in 20 mL deionized water. After shaking the suspension, it was placed in the refrigerator over night. The next day, the suspension was shaken again before the pollen were removed by filtering (roundfilter, Schleicher and Schüll Selecta 595). The outer shell of pollen consists of sporopollenin, which is a heterogeneous, tough, rather hydrophobic organopolymer. On the surface of this polymer a lot of different biomolecules are located, like proteins, sugars, polysaccharides, fats and volatile organic compounds (e.g. Clarke et al., 1979; Breiteneder et al., 1989), which were suspended in the washing water. An atomizer (following the design of TSI 3075) was used to generate droplets from the pollen washing water and afterwards these droplets were dried by passing them through a silica gel diffusion dryer. As the pollen washing water consists of a mixture of several substances, the resulting particles also consists of these different biomolecules, including the INA macromolecules. Subsequent, to particle generation, the dry particles were size selected using a Differential





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Mobility Analyzer (DMA, Knutson and Whitby, 1975, type "Vienna medium") in a range between 100 and 800 nm (mobility diameter) and then fed into LACIS.

## 2.2 LACIS

In this section, the LACIS setup and operating principle are described briefly. For more detailed informations see Niedermeier et al. (2010) and Hartmann et al. (2011, 2012). In Fig. 1, the schematic setup of the LACIS flow tube is shown. LACIS itself consists of seven connected one-meter tube sections with an inner diameter of 15 mm. The wall temperature of each tube section is set separately by different thermostats (TH). In the inlet section of LACIS, the aerosol flow is combined isokinetically with a humidified sheath air flow, such that the aerosol forms a beam of approximately 2 mm in diameter along the center-line of the flow tube. By cooling the tube walls, well-defined temperature and saturation profiles with respect to water and ice can be achieved along the center of the flow tube, where the aerosol beam is placed. When passing through the flow tube, the cooling leads to the activation of the dry particles to droplets and due to further cooling the droplets may freeze. It should be noted that inside LACIS each droplet contains only one single particle and, in case of the size segregated measurements performed in the framework of the present paper, all immersed particles feature the same mobility diameter. At the outlet of LACIS, the Thermally Stabilized Optical Particle Spectrometer (TOPS-ICE, developed and built at TROPOS, Clauss et al., 2012) is used to determine both, the number and the phase state of the hydrometeors. By means of TOPS-ICE, the ice fraction (number of frozen droplets divided by the total number of frozen and unfrozen droplets) and the size of the droplets and ice particles can be analyzed. For the investigation of the immersion freezing behavior of birch pollen washing water, ice fractions in the temperature range between −18 °C to −35 °C were determined. For more detailed informations about the operation mode of LACIS see Hartmann et al. (2011, 2012).





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## 3 Results

### 3.1 Southern birch pollen washing water

In Fig. 2, the results gained concerning the immersion freezing behavior of particles generated from Southern birch pollen washing water are shown. Ice fractions are plotted as function of temperature for 5 different particle sizes (mobility diameter $D_p$: 150, 300, 500, 650, 800 nm). Especially for 500 nm and 800 nm particles, the ice fraction was analyzed as a function of temperature in a range from −18 °C to −35 °C. First, the logarithm of the ice fractions grows linearly with decreasing temperature in the temperature range between −19 °C and −23 °C. With further cooling, the ice fraction curves level off to constant values, suggesting a saturation behavior of the immersion freezing process. The ice fraction values at the saturation level ($f^{\star}_{ice}$) (see ice fractions at −35 °C) are size dependent, e.g., 0.03 and 0.7, for 150 nm and 800 nm particles, respectively. In the left panel of Fig. 3, $f^{\star}_{ice}$ is plotted as function of particle diameter. In general, $f^{\star}_{ice}$ increases with increasing mobility diameter $D_p$.

In the case of pollen, Pummer et al. (2012), suggested that single sugar-like macromolecules, possibly polysaccharides, are responsible for the ice nucleating ability of pollen. Due to washing the pollen grains, different biomolecules, including these INA macromolecules, can be seperated from the pollen bodies (see Sect. 2.1) and become suspended in the washing water. The atomization of the washing water leads to a distribution of the INA macromolecules to the generated droplets and after drying these droplets, particles with or without INA macromolecules are formed. We assume that in the present experiments ice nucleation can only be induced by these single INA macromolecules. Also from the fact that we find a plateau at $f^{\star}_{ice}$, we conclude that not all particles contain such an INA macromolecule, with the fraction of particles without one being $1 - f^{\star}_{ice}$. From this, as we will show now, we are able to determine the ice nucleation rate of a single pollen INA macromolecule. Following the approach described in Hartmann et al. (2012), the average number of INA macromolecules per particle is determined by assuming a Poisson distribution of these molecules over the





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particle population with the expected value $\lambda$ and the stochastic variable $X$ varying in a range of $k = 0, \ldots, n$. The probability that a pollen washing water particle contains $k$ INA macromolecules then is:

$$P_\lambda(X = k) = \frac{\lambda^k}{k!} e^{-\lambda}. \quad (1)$$

Through the fact that the ice fraction $f_{\text{ice}}$, reaches a saturation range with $f^\star_{\text{ice}} < 1$ we can determine the probability that a particle does not contain an INA macromolecule:

$$P(X = 0) = \frac{\lambda^0}{0!} e^{-\lambda} = 1 - f^\star_{\text{ice}} \quad (2)$$

Consequently,

$$\lambda = -\ln(1 - f^\star_{\text{ice}}). \quad (3)$$

The parameter $\lambda$ describes the average number of the INA macromolecules per particle at a given particle size. The respective $\lambda$ values are shown in the right panel of Fig. 3 for different particle surfaces (upper panel) and particle volumes (lower panel). As can be seen, $\lambda$ can be described by both, a linear surface area dependence ($D_{\text{p}}^2$) and a linear volume dependence ($D_{\text{p}}^3$), illustrated by the linear fits (gray lines). Assuming that the particle activated in LACIS are fully soluble, we would have expected a dependence of lambda only on particle volume. A possible explanation for the surface area dependence could be, that in our experiments the particles produced from the pollen washing water dissolve only partly when being activated inside LACIS and only the INA macromolecules, which were present in the dissolved material and on the particle surface induce freezing. When drying pollen washing water, we found that the remnants were a somewhat sticky and sweet smelling substance, resembling honey. This suggest that possibly slowly dissolving substances are contained in the material that is washed off from the pollen and therefore it could be that the generated particles need more





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time than the few seconds they have in LACIS to dissolve completely. As our goal is to calculate heterogeneous nucleation rates of single INA macromolecules, their overall number in the produced particle is not of interest, but the exact average number of the active INA macromolecules, which is given by $\lambda$. For the following calculations we decided to use the surface dependent parameterization for $\lambda$ ($\lambda = 1.777 \times 10^{-6}\,\mathrm{nm}^{-2} \cdot D_p^2$), as the correlation factor ($R^2 = 0.987$) is slightly better than the correlation factor of the linear volume dependence ($R^2 = 0.968$). From that we can derive the heterogeneous ice nucleation rate ($J_{het} = S_{site} j_{het}$) for one single INA macromolecule from the experimentally determined ice fractions, using the CHESS model described in Hartmann et al. (2012). It should be noted that this nucleation rate represents an average over the individual nucleation rates of all macromolecules, present in the particle population

$$f_{ice}(T) = 1 - \exp(-\lambda(1 - \exp(-S_{site} j_{het}(T) t))). \quad (4)$$

Here $S_{site}$ is defined as the ice nucleating surface area of a single INA macromolecule, which is independent of the selected particle mobility diameter. $j_{het}$ is the heterogeneous ice nucleation rate coefficient. $t$ describes the nucleation time, which is known for LACIS measurements from CFD simulations (see Hartmann et al., 2012). With Eq. (4), the heterogeneous ice nucleation rate $J_{het}$ with the unit, numbers per second ($\#\cdot s^{-1}$) can be derived. In Fig. 4, the values for $J_{het}$ for 500 nm and 800 nm are shown for the temperature range, in which the natural logarithm of the ice fraction increases linearly with temperature ($-19 \geq T \geq -23\,^{\circ}\mathrm{C}$).

As we assumed similar INA macromolecules being present in the particle population, $J_{het}$ should be no function of particle diameter, which is the case as can be seen in Fig. 4.

With an exponential fit through the $J_{het}$ values for 500 and 800 nm we get the following fit function:

$$J_{het} = A \cdot \exp(B \cdot T) \quad (5)$$





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with the coefficients $A = 2.320 \times 10^{-8}\,s^{-1}$ and $B = -0.835\,°C^{-1}$. The nucleation rate ranges between $2 \times 10^{-1}\,s^{-1}$ at −19 °C and $4 \times 10^{0}\,s^{-1}$ at −23 °C and is depicted by the red line in Fig. 4. With the calculated average number of INA macromolecules $\lambda$ and the parameterized heterogeneous ice nucleation rate $J_{het}$, we can now determine the ice fraction $f_{ice}$ as a function of temperature and particle size. In Fig. 5, the results for 300 nm, 500 nm and 800 nm particles are shown as straight lines. Both, the increase of the ice fraction with decreasing temperature and the different saturation ranges of the ice fractions $f^{\star}_{ice}$ of the differently sized particles are simulated almost perfectly. In other words, we are able to consistently explain the ice nucleation behavior of the Southern birch pollen washing water by assuming the number of INA macromolecules to be a function of initial particles size and by an ice nucleation rate, that is a function of temperature but that is independent of the initial size of the particle.

### 3.2 Northern birch pollen washing water

The results gained during the experiments examining the immersion freezing behavior of particles from the Northern birch pollen washing water are shown in Fig. 6. Again, different mobility diameters (100, 200, 300, 500, 650, 800 nm) were considered, with the 300 nm, 500 nm and 800 nm particles being analyzed in more detail. In the temperature range between −17 °C and −20 °C the ice fraction of the particles generated from the Northern birch pollen washing water steeply increased with decreasing temperature. Subsequent to this steep increase, the ice fractions rise less steeply and finally reach the saturation range. In the left panel of Fig. 7, the ice fraction in the saturation range $f^{\star}_{ice}$ for the Northern birch pollen washing water is plotted against the mobility diameter $D_p$. As for the Southern birch pollen washing water, $f^{\star}_{ice}$ increases with increasing $D_p$, but also not linearly. For $\lambda$ (right panels of Fig. 7), we again found a better agreement between $\lambda$ and $D_p^2$ ($R^2 = 0.992$) than between $\lambda$ and $D_p^3$ ($R^2 = 0.928$), which shows that also for the Northern birch pollen washing water, the produced particles dissolve only partly, when being activated inside LACIS. We





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calculated the heterogeneous nucleation rate of the Northern birch pollen sample in the same way as for the Southern birch pollen sample by assuming that only one type of INA macromolecules is responsible for freezing. Therefore we used the ice fraction measurements in the temperature range in which the natural logarithm of the ice fraction increases linearly with temperature between −17°C and −20°C. After calculating the ice fraction by using the exponential fit parameterization of the heterogeneous nucleation rate ($J_{het} = 2.684 \times 10^{-16} \exp(-1.893\,^{\circ}C^{-1} \cdot T)\,s^{-1}$) and the determined $\lambda$ values we noticed that a model including only one INA macromolecule could not fully represent our data as can been seen in Fig. 8. The mean square errors between the measured and the calculated ice fraction for the 300 nm, 500 nm and 800 nm particles showed values of 7.22, 14.47 and 2.58, respectively.

A possible explanation for this discrepancy could be that there is more than one INA macromolecule being present in the Northern birch pollen washing water. To clarify this, we used a method (the so called differential spectra), which was already used by Vali (1971) in a similar way to quantify IN which are active at a specific temperature. Therefore the fraction of droplets which froze per temperature interval was calculated for the 500 nm and 800 nm particles, i.e. $f_{ice}(T_2) - f_{ice}(T_1)/(T_2 - T_1)$ (see Fig. 9). In our case we observe that the particles of the Southern birch pollen washing water show one mode, and it can be seen that all the IN become active in the temperature range from roughly −20°C to −25°C. The particles of the Northern birch pollen washing water, however, show two modes in separate temperature ranges, where one mode occurs roughly in the same temperature range as found for the Southern birch sample. This result for the Northern birch sample can be interpreted as two different kinds of INA macromolecules being active, one similar to the one found for the Southern birch sample and an additional one being ice active already in the temperature range from −17°C to −20°C. Despite the small differences between the two peaks from the two different samples in the temperature ranges from −20°C to −25°C we assume that the respective INA macromolecules are of the same kind. A detailed discussion of these assumptions will be done in Sect. 4.





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In the following these two different INA macromolecules will be called INA North and INA South, with INA North being the more IN active molecule that is assumed to be present only in the sample of the Northern birch pollen and INA South being the less IN active molecule, present in both birch pollen samples. For the further calculations we assume, that the ice nucleation rate of the INA South $(J_{het})_S$ is similar to the one determined for the Southern pollen washing water. Additionally, we assume that the average number of INA macromolecules present in the particles is equal to the sum of both, the average number of INA North ($\lambda_N$) and INA South ($\lambda_S$).

$$\lambda_{ges} = \lambda_N + \lambda_S \tag{6}$$

Thus, assuming a surface area dependence of the $\lambda$ values for the individual INAs, $\lambda_N$ and $\lambda_S$, is reasonable. To calculate the heterogeneous nucleation rate of the INA North $(J_{het})_N$ we again use the CHESS model assuming a combination of INA South and INA North:

$$f_{ice}(T) = 1 - \exp(-\lambda_S(1 - \exp(-(J_{het}(T))_S t))) \times \exp(-\lambda_N(1 - \exp(-(J_{het}(T))_N t))). \tag{7}$$

Although, $(J_{het})_S$ is known from the Southern birch pollen washing water measurements and $\lambda_S$ can be derived from Eq. (6) with $\lambda_S = \lambda_{ges} - \lambda_N$, we still have two unknown parameters ($\lambda_N$ and $(J_{het})_N$). These parameters were determined by fitting Eq. (7) to the whole 500 nm and 800 nm dataset in an iterative manner. The 300 nm particles were excluded from the fitting procedure as the data set is considered too sparse.

The results are shown in Fig. 10. Both, the two different slopes and the final saturation range ($f^*_{ice}$) are represented very well for the two fitted curves, i.e. 500 nm and 800 nm. The parameters obtained from the fitting procedure were then used to model $f_{ice}$ for the 300 nm particles, and, as can be seen in Fig. 10 the resulting curve reproduces the measured data well, too. The mean square errors between the measured and the calculated ice fraction for the 300 nm, 500 nm and 800 nm particles showed





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values of 0.44, 0.51 and 1.3, respectively, which are considerably smaller as when we assumed only one type of INA macromolecule being present in the Northern birch pollen sample like shown above. This supports the assumption of two differently behaving INA macromolecules being internally mixed in the Northern birch sample, and one of them being similar to the INA macromolecule found in the Southern pollen washing water.

In Fig. 11, the parameterizations for the heterogeneous nucleation rates for the INA North and the INA South (left panel) as well as the different $\lambda$ parameterizations (right panel) are shown. It is obvious from the $\lambda$ values that the INA South macromolecules are much more abundant in the Northern birch pollen washing water compared INA North macromolecules. The heterogeneous nucleation rate for the INA North (blue line) is much steeper than the heterogeneous nucleation rate for the INA South (red line) and the values lie between $5 \times 10^{-2}\,s^{-1}$ at $-17\,^{\circ}C$ and $2 \times 10^{1}\,s^{-1}$ at $-19\,^{\circ}C$. The two different nucleation rates intersect at about $-16.5\,^{\circ}C$. Further discussions of this different behavior of the two INA macromolecules will follow in the next section.

## 4 Discussion

The investigation of particles generated from the washing water of two different birch pollen samples (Northern birch and Southern birch) shows differences between the INA macromolecules present in the two samples. As the ice fraction of the particles generated from the Southern birch washing water showed a logarithmically linear increase with decreasing temperature (see Fig. 2), we assume that there is only one type of INA macromolecule. In contrast to that the ice fraction of the particles generated from the Northern birch pollen washing water shows two different slopes (see Fig. 6), with one being much steeper and one being similar to the slope of the Southern birch pollen washing water. This could also be shown by the fraction of droplets which froze per temperature interval (Fig. 9). So it seems that the Northern birch pollen can express a second, more ice active type of INA macromolecule. Considering the heterogeneous





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nucleation rates of these two different macromolecules (see left panel of Fig. 11), one notices the big differences in the slope. The heterogeneous nucleation rate for the INA North (blue line) is much steeper than the heterogeneous nucleation rate for the INA South. Based on the findings from Niedermeier et al. (2011a) it can be concluded that the slope of the nucleation rate is connected with a distribution of the ice nucleation related properties (e.g. contact angles) of the whole INA macromolecule population. The steeper the slope of the average nucleation rate, the narrower is the distribution of the ice nucleating related properties of the whole INA macromolecule population, which indicate a more homogeneous INA macromolecule. So it can be said that the INA North is more homogeneous with respect to the ice nucleating related properties than the INA South. The reason for that as well as for the seeming existence of two different types of INA macromolecules may lie in the possible different chemical structures of the macromolecules. To clarify this, we have to consider the biological point of view. Biomolecules, like the INA macromolecules we examined here, are usually built up by a sequence of small building blocks, e.g. proteins by amino acids, and polysaccharides by monosaccarides. Differences in the structures of these linear or branched chains of units usually lead to changes in the biological function and the biochemical properties of the macromolecule.

Differences could be explained in several ways:

- It is possible, but unlikely, that birch expresses in fact two completely different ice nucleating molecules, that could even belong to different substance classes.
- For the proper function of a biological macromolecule, the folding is of crucial importance. For example, the misfolding of common prions can make them infectious and cause severe illnesses (Pan et al., 1993). Analogously, the folding of the INA macromolecules in the samples could differ from each other. Consequently, the structuring effect on the water could be different.
- The exchange of one building block in the sequence of a biological macromolecule can fundamentally change its properties – a phenomenon that is called point





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mutation and can easily cause differences between different individuals of one species (Takahashi et al., 1994).

– Posttranslational modification of biological macromolecules can be caused by environmental stress, such as exposure to reactive atmospheric trace gases (Franze et al., 2005). As the exposure to stresses depends on location and time, samples taken from different sites are likely to show different behaviour.

From that, one can make the assumption that in case of the INA South, the heterogeneities in the ice nucleating related properties could be caused e.g. due to slightly different foldings of the macromolecules and in the case of INA North, which can be found only in the Northern birch pollen sample, the different climatic conditions may have caused mutations and modification of the macromolecules. So it seems that the region where the plant occurs may play an important role for the formation of more or less ice nucleating active macromolecules. So in colder areas, where frost damage during the pollen flight times is more likely the plant produces INA macromolecules, which are active at higher temperatures. But these are only speculations and to clarify this completely more biochemical investigations will be necessary to identify the pollen ice nuclei.

Finally, we will have a short look on the atmospheric application of our measurements. Schäppi et al. (1999) found, that concentrations of allergens after rain shows an significant increase, which is due to bursting of pollen grains by osmotic shock. In that way, also the INA macromolecules could be separated from the pollen grains. A preliminary estimate shows, that it is likely that each birch pollen carries several thousand macromolecules (see Appendix A). So if they are separated from the pollen grains their concentration in the atmosphere would increase extremely. Due to this large increase of sources for INA macromolecules and due to the long residence times of these nano-sized particles in the atmosphere, ice nucleation due to pollen might play a larger role than e.g. reported in Hoose et al. (2010).





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## 5 Summary and conclusion

In this study the immersion freezing behavior of birch pollen washing water was investigated at the Leipzig Aerosol Cloud Interaction Simulator (LACIS). Particles generated from washing water from two different birch pollen samples, one from the Czech Republic (Southern birch pollen) and one from Sweden (Northern birch pollen), were used to quantify their freezing behavior as function of temperature. Particles from Southern birch pollen washing water showed a logarithmically linear increase of the ice fraction with decreasing temperature in a temperature range between −19°C and −23°C. With further cooling the ice fraction leveled off to a constant value. However, particles from the Northern birch pollen washing water showed a somewhat different behavior. A saturation range was also found for the lower temperatures, but for the increase of the ice fraction two different slopes were observed. In the temperature range between −17°C and −20°C, the ice fraction increased much steeper than for the Southern birch pollen washing water but with further cooling the increase became shallower and showed a slope similar to the Southern birch pollen washing water. Assuming that immersion freezing is caused by an INA macromolecule (Pummer et al., 2012) we conclude first, based on the existence of a plateau region, that not all droplets contain one or more INA macromolecules, second that this INA macromolecule is able to show at least two different types and third that one of these types is present in both, Northern and Southern birch pollen washing water. Following the approach of Hartmann et al. (2012), we calculated the average number $\lambda$ of INA macromolecules in one particle by assuming the INA macromolecules to be Poisson distributed over the whole particle population. Through measuring differently sized particles generated from the pollen washing water, we found that $\lambda$ depend slightly better on particle surface than on particle volume for both, the Northern and Southern birch pollen washing water. From that, it is suggestive that the produced particles from the pollen washing water only partly dissolve when being activated inside LACIS. Furthermore, by using the newly developed CHESS model (Hartmann et al., 2012), the heterogeneous ice nucleation rate $J_{het}$ for





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the two different INA macromolecule types (INA North, INA South) was parameterized as function of temperature. $J_{het}$ From the INA North showed a much steeper increase with decreasing temperature than the $J_{het}$ from the INA South, which was present in both birch samples. From that we concluded that the INA North is more homogeneous with respect to the ice nucleating related properties than the INA South. Summarizing it can be said that the INA macromolecules which can be found on birch pollen grains are efficient ice nuclei. They nucleate ice already at temperatures higher than −20°C and are more efficient than most of the non-biological particles (e.g. mineral dust, see e.g. Murray et al., 2012; Hoose and Möhler, 2012; Hartmann et al., 2011; Niedermeier et al., 2010). There abundance in the atmosphere is indeed unclear, but the possibility of multiplication of pollen fragments by rain bursting was already shown (Schäppi et al., 1999). Furthermore, it seems that the source region of the pollen plays an important role for there being more or less active IN. So it is possible that the importance of pollen for the ice nucleation in the atmosphere has been underestimated up to now.

## Appendix A

### The number of INA macromolecules per pollen

The number of INA macromolecules per pollen was estimated as follows:

a birch pollen is known to have an average size of about 25 µm (Diehl et al., 2001) and it can be assumed to be spherical (see e.g. SEM pictures by Pummer et al., 2012; Grote et al., 1989). A density of $1\,g \cdot cm^{-3}$ was assumed (Niklas, 1985). From this the number of pollen in 1 g of the pollen sample can be estimated to be $1.2 \times 10^8$.

A sample was prepared similar to the procedure described in the main text, and both, the solution/suspension and also the pollen caught in the filter were dried and weighted. The amount of material found in the solution/suspension was 0.3 g, i.e. 30 % of the original pollen mass.





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For a particle of known size, the dry mass can be calculated, again assuming spherical particles and a density of the suspended material of about $1.6\,g \cdot cm^{-3}$ (assumed average density for soluble pollen surface material). The average number of macromolecules in a particle $\lambda$ as a function of particle size is known, and with this the average number of macromolecules per particle mass.

By setting the particle mass in relation to the total suspended mass, we can derive the number of macromolecules per pollen, which results in values of about $2 \times 10^4$. The above described procedure assumes that the particles dissolve completely. If this was not the case and only an outer shell of the particles would go into solution, the number of macromolecules per pollen would even increase, so that it is safe to assume that each birch pollen carries several thousands of macromolecules.

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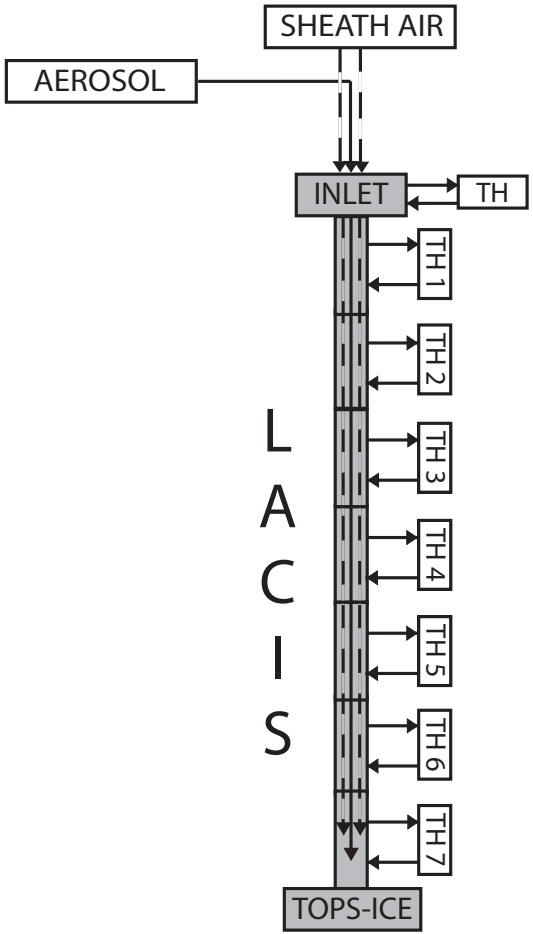


**Fig. 1.** Setup of the LACIS flow tube. The abbreviation TOPS-ICE declares Thermally Stabilized Optical Particle Spectrometer.





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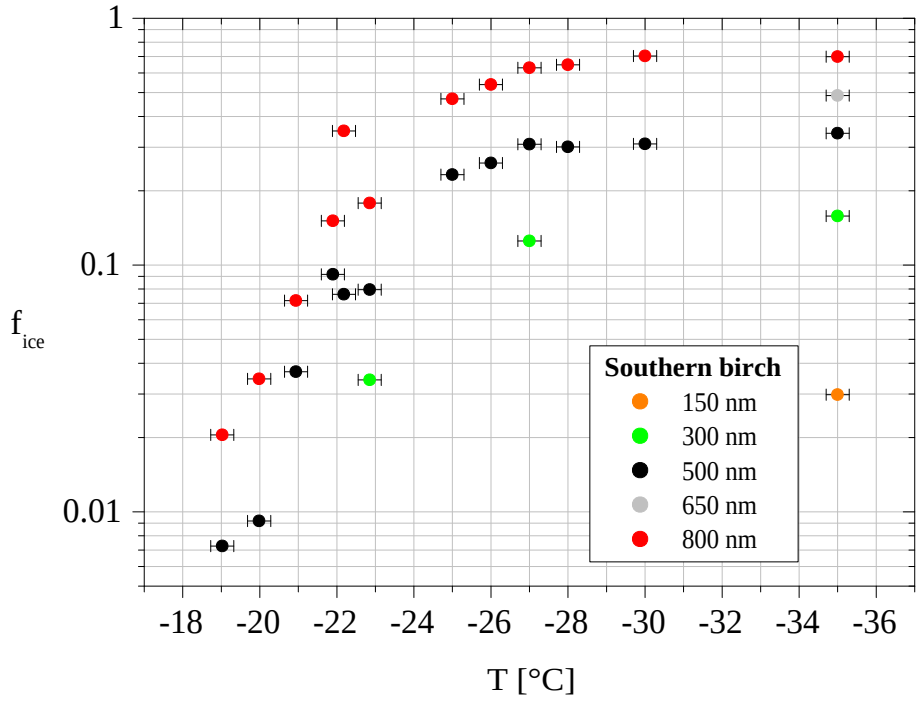


**Fig. 2.** Ice fraction $f_{ice}$ as function of temperature $T$ for 150, 300, 500, 650, 800 nm Southern birch pollen washing water particles.





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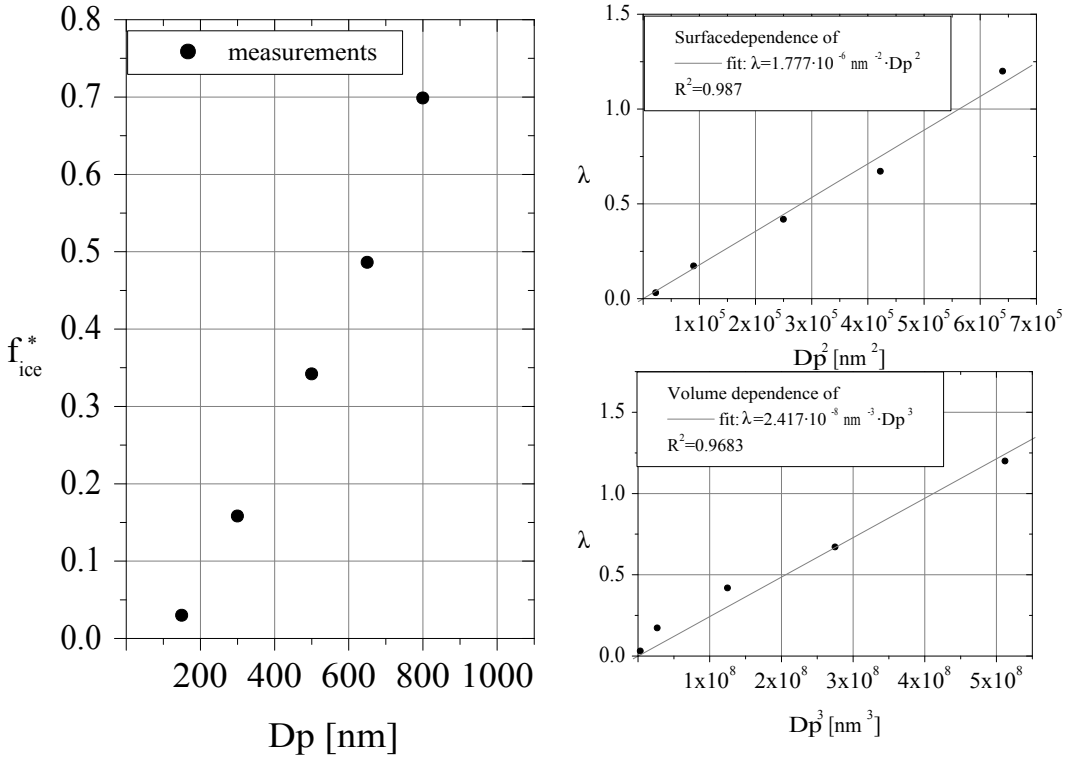


**Fig. 3.** Ice fraction in the saturation range $f^*_{ice}$ of the Southern birch pollen washing water vs. mobility diameter for $T$ = −35°C (left panel). Expected value $\lambda$ of the Poisson distribution describing the average number of INA macromolecules per Southern birch pollen washing water particle as function of particle surface ($D_p^2$) (upper right panel) and as function of particle volume ($D_p^3$) (lower right panel). $R^2$ is the correlation factor.





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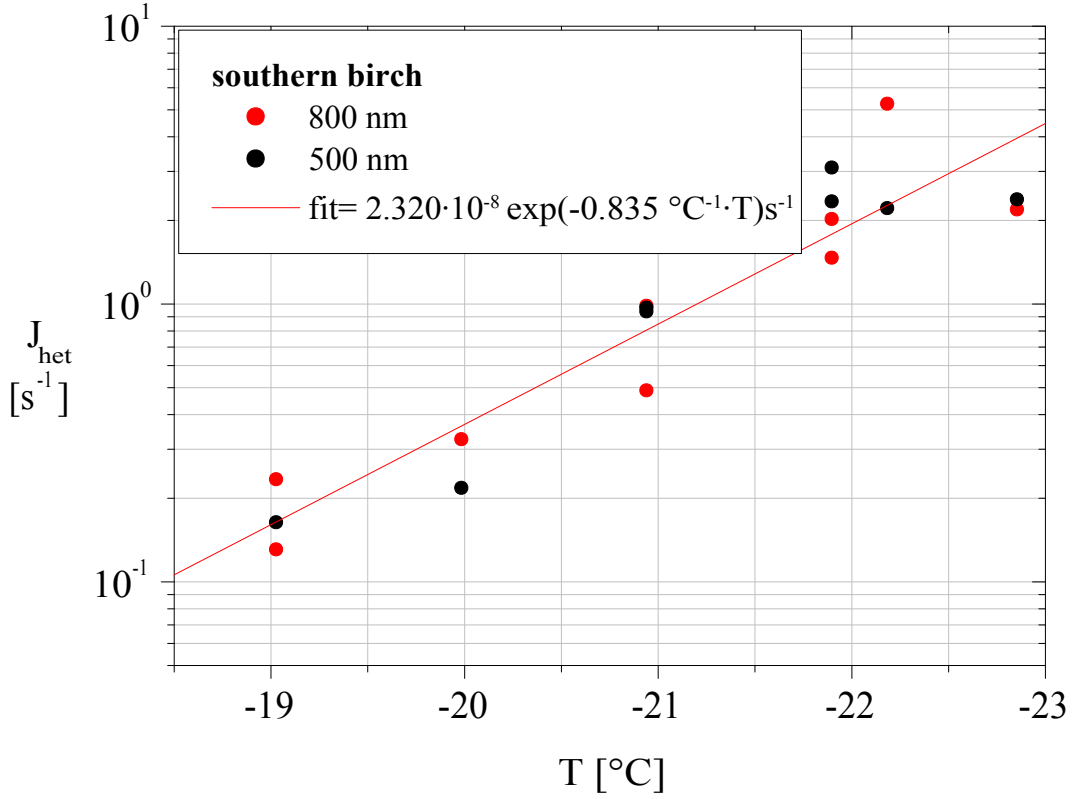


**Fig. 4.** Heterogeneous ice nucleation rate ($J_{het}$) for 500 nm and 800 nm washing water particles of Southern birch pollen as function of temperature $T$.





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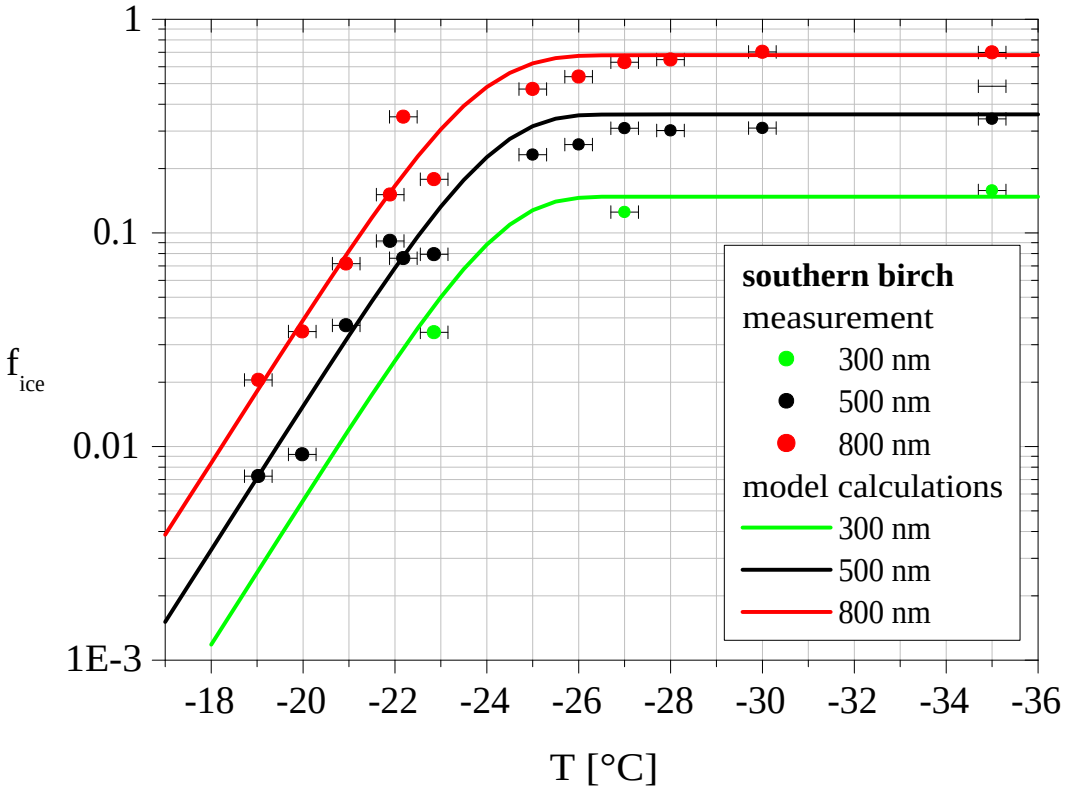


**Fig. 5.** Ice fraction $f_{ice}$ as function of temperature $T$ for 300 nm, 500 nm and 800 nm Southern birch pollen washing water particles (dots) and model calculations for the different sizes (lines).





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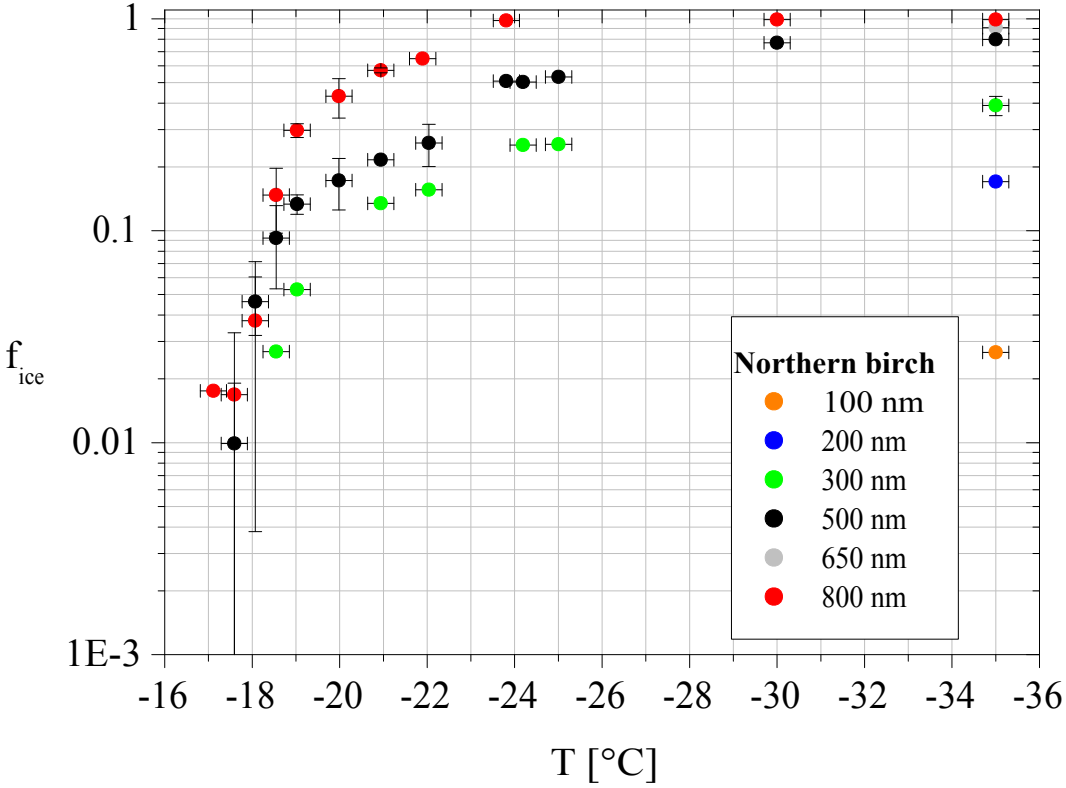


**Fig. 6.** Ice fraction $f_{ice}$ as function of temperature $T$ for 100, 200, 300, 500, 650 and 800 nm Northern birch washing water particles. The error bars results from the single standard deviation which were determined from at least 3 measurements under identical conditions.





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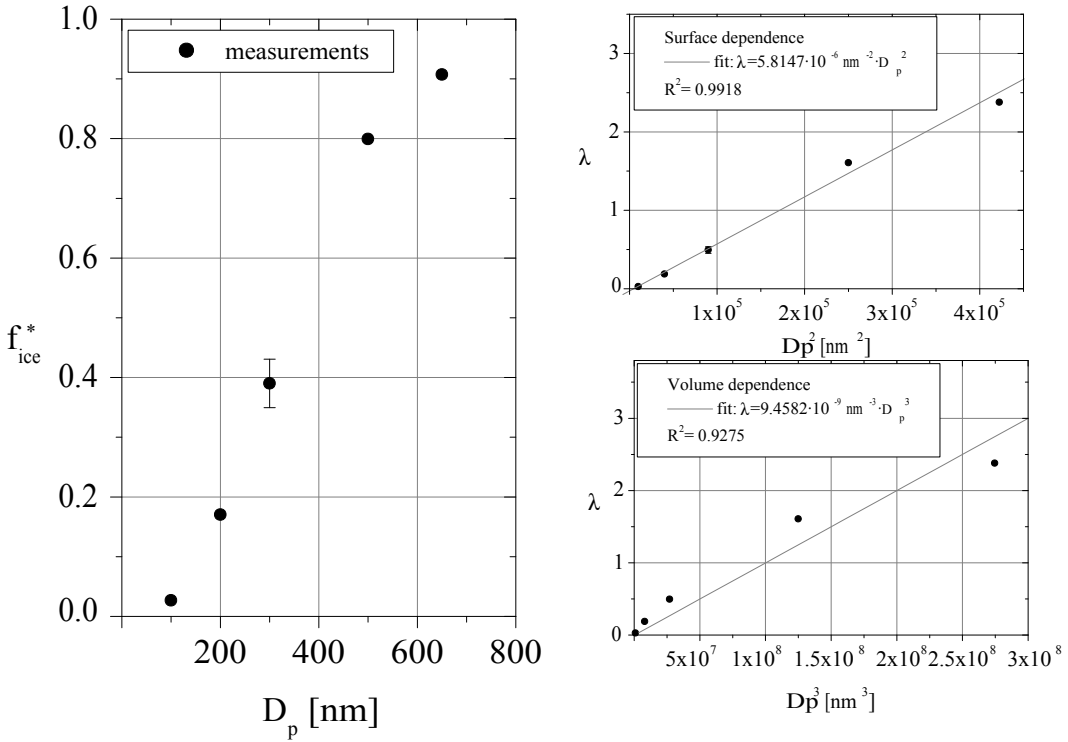


**Fig. 7.** Ice fraction in the saturation range $f^*_{ice}$ of the Northern birch pollen washing water vs. mobility diameter for $T$ = −35 °C (left panel). Expected value $\lambda$ of the Poisson distribution describing the average number of INA macromolecules per Northern birch pollen washing water particle as function of particle surface ($D_p^2$) (upper right panel) and as function of particle volume ($D_p^3$) (lower right panel). $R^2$ is the correlation factor.





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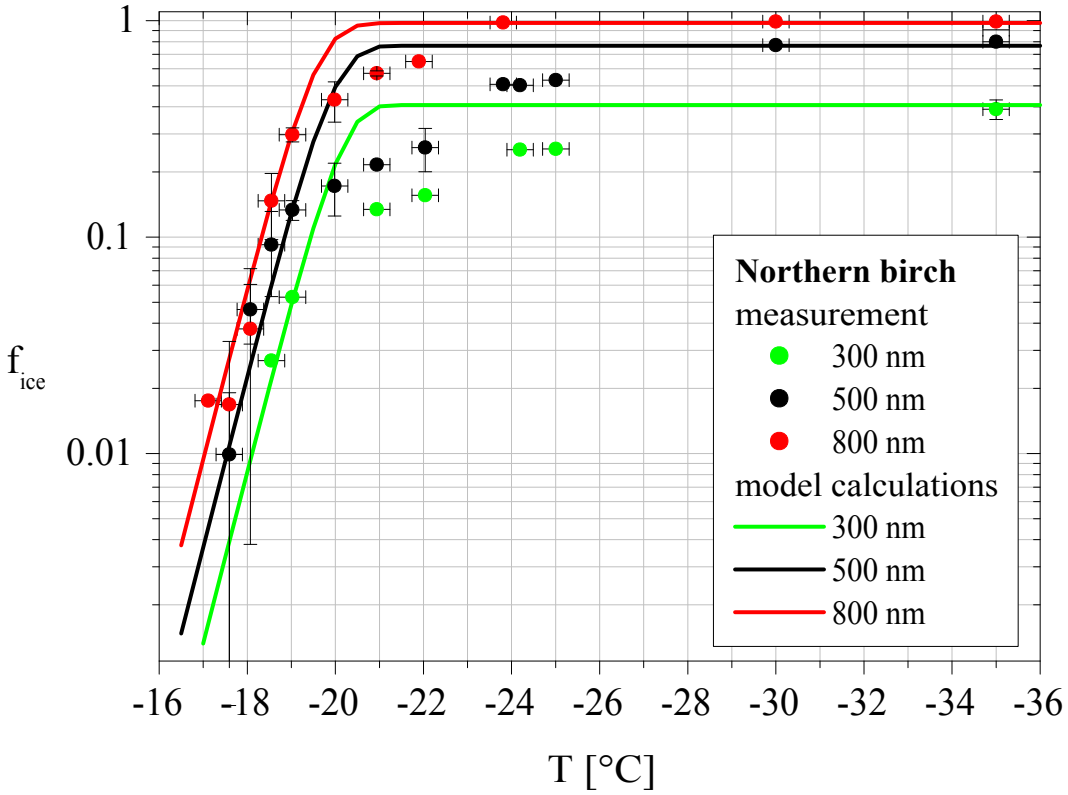


**Fig. 8.** Ice fraction $f_{ice}$ as function of temperature $T$ for 300 nm, 500 nm and 800 nm Northern birch pollen washing water particles (dots) and model calculations for the different sizes (lines) assuming only one INA macromolecules being present in the Northern birch pollen sample. A nucleation rate with the parameterization $J_{het} = 2.684 \times 10^{-16} \exp(-1.893\,°C^{-1} \cdot T)\,s^{-1}$ was used. The mean square errors between the measured and the calculated ice fraction for the 300 nm, 500 nm and 800 nm particles showed values of 7.22, 14.47 and 2.58, respectively.





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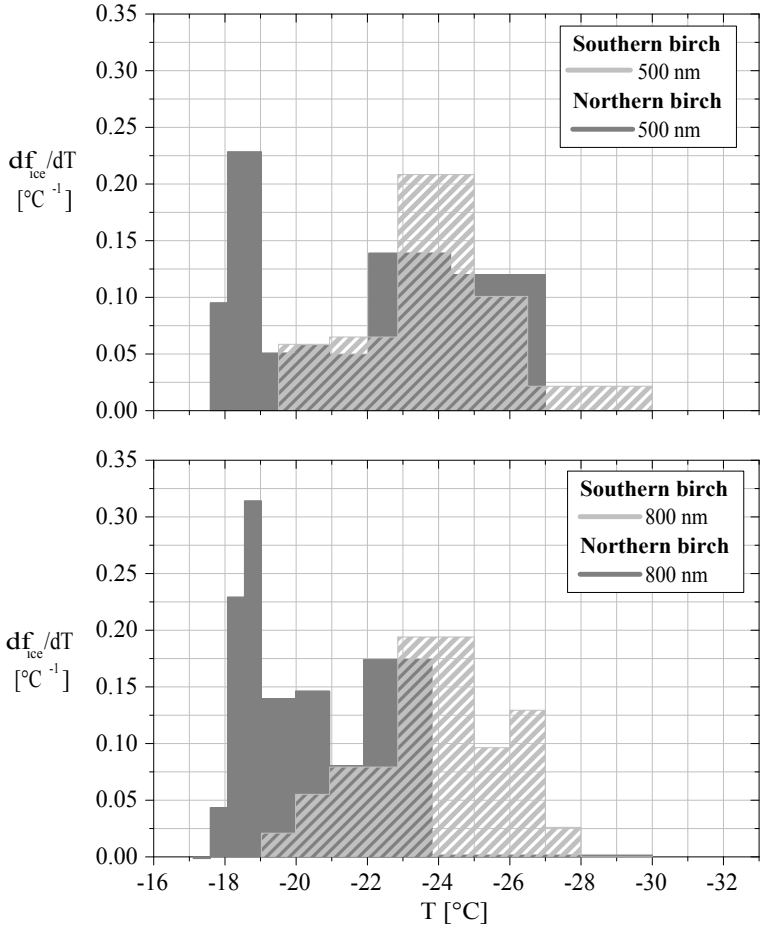


**Fig. 9.** Change of the ice fraction $f_{ice}$ per temperature interval for the 500 nm (upper panel) and 800 nm (lower panel) particles of the Southern birch pollen washing water(light gray) and the Northern birch pollen washing water (dark gray).





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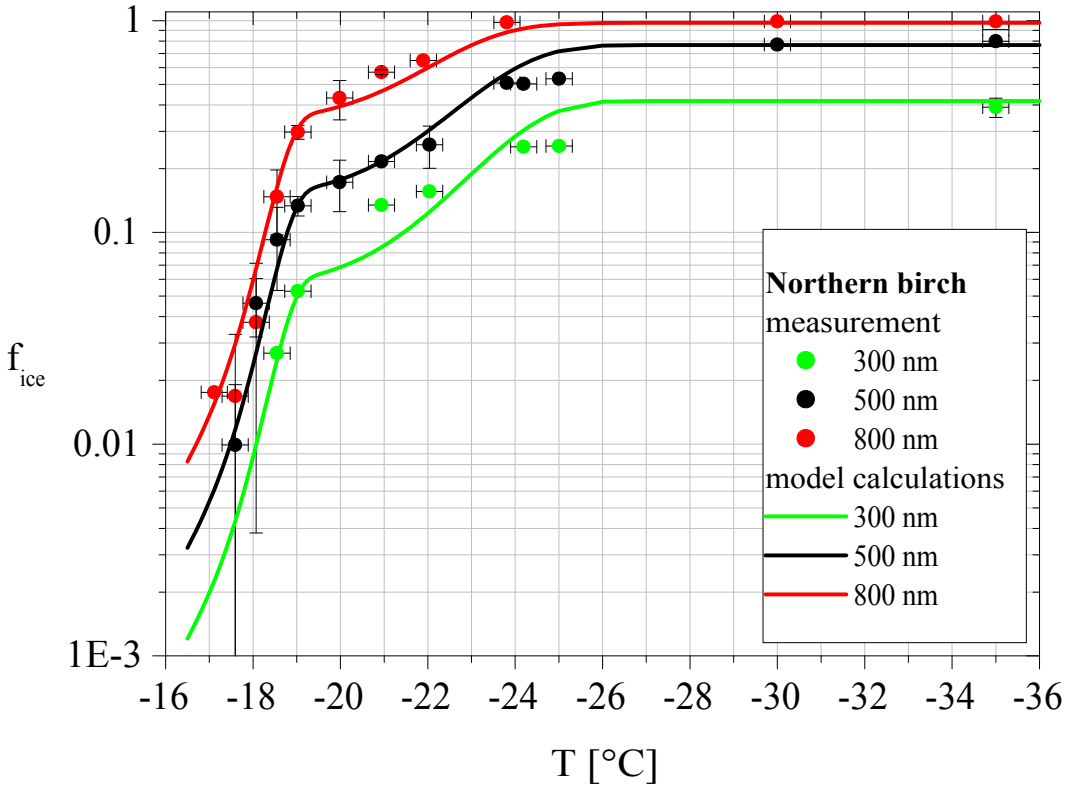


**Fig. 10.** Ice fraction $f_{ice}$ as function of temperature $T$ for 300 nm, 500 nm and 800 nm Northern birch pollen washing water particles (dots) and model calculations for the different sizes (lines). The model calculation results from the combination of the Southern INA molecules and the Northern INA molecules. The mean square errors between the measured and the calculated ice fraction for the 300 nm, 500 nm and 800 nm particles showed values of 0.44, 0.51 and 1.3, respectively.





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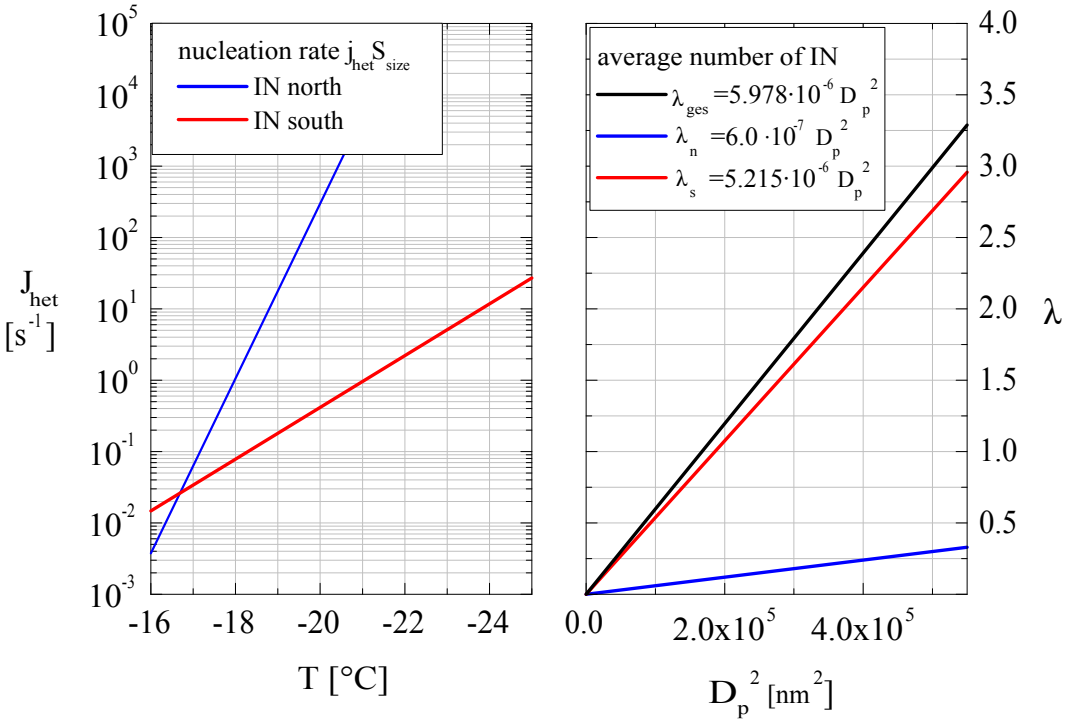


**Fig. 11.** Left panel: fit curves of the heterogeneous nucleation rate of INA North ($(J_{het})_N = 9.186 \times 10^{-23} \exp(-2.822\,°C^{-1} \cdot T)\,s^{-1}$, blue line) and INA South (red line). right panel: $\lambda_N$, $\lambda_S$ and $\lambda_{ges}$ of the Northern birch pollen washing water.





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