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11, C11425–C11435, 2011

Interactive Comment

Interactive comment on "Birch and conifer pollen are efficient atmospheric ice nuclei" *by* B. G. Pummer et al.

B. G. Pummer et al.

grothe@tuwien.ac.at

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We thank referee Dr. C. Morris for her constructive comments and suggestions and try to answer all discussion points:

1) I was not sure where the Materials and Methods were presented in the manuscript. They seem to be dispersed throughout the manuscript and interwoven with the Results. A more classical presentation would be very useful.

ANSWER: We have restructured the manuscript and present now: 1. Introduction, 2. Methods: Nucleation measurements, 3. Results: Ice nuclei analysis, 4. Discussion: Nucleation rates and 5. Conclusion. Parts of the former chapter 5. Experimental section were assigned to the chapters, where we mentioned the methods first. Furthermore we split chapter 2. into the sub-chapters 2.1. Oil immersion measurements and 2.2. Simulation chamber measurements, and chapter 3 into 3.1 Pollen surface topology (describing the SEM measurements) and 3.2 Chemical analysis for the rest. Former chapter 5.1 is now part of chapter 2.1, 5.2 of chapter 2.2, and 5.3 of 3.2. The section of chapter 2 describing the differences between oil immersion and simulation chamber setup (p.27223, line 16 - p.27224, line 10) was moved to the last chapter Conclusions (p.27231, between line 6 and 7). The last paragraph of former chapter 2 (p.27224, line 1-10) is now 3.1. Pollen surface topology. The first sentence was additionally extended: "In the past the ice nucleation activity of pollen has been explained by the general roughness and porosity of the pollen surface (Diehl et al., 2001), thus generating possibly active sites for ice nucleation." Chapter 4 is now called "Discussion: Nucleation rates". The separation of experimental description and results in chapter 3 is far more difficult, as the cause for carrying out the experiments is in some cases the result of the measurements described before. Therefore, we would prefer to keep it the way it is.

2) There is also not a clear statement of the objectives. In the Introduction section the authors state that no research has been carried out to describe the nature of pollen ice nucleation activity. This leads the reader to suspect that the objective targets describing this nature. However, the paper deviates quite a bit towards efficiency of pollen as ice nuclei (and this is re-enforced by explicit use of the word “efficient” in the title), but the data presented in the manuscript in its current state are not sufficient to address the question of efficiency.

ANSWER: Our paper principally shows two main results: 1) A comparison of the IN activity of different species, where we have covered a broad spectrum of species, giving a general idea of the IN potential of pollen. 2) We have done investigations on the nature of IN, and we can partly, but not fully describe them yet (e. g. mass range, chemical properties). Before that, nobody seems to have shown interest at all to identify pollen IN, probably because pollen IN activity used to be explained with the surface

ACPD

11, C11425–C11435, 2011

Interactive Comment

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Interactive Discussion

Discussion Paper

topology [Diehl et al., 2001] and because pollen in general were considered to be negligible [Hoose et al., 2010]. We intend to publish both results and have changed our title to "Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen" to move the focus on the second part as well. Concerning efficiency we have extended the discussion in the publication to make our results better comparable: At first, we want to state that the comparability between publications is difficult, as methodologies severely differ from each other (starting with the definition of "nucleation temperature", which is not always the median freezing temperature; or with the numerical value of homogenous freezing temperatures, which is sometimes substantially higher than ours). Additionally, many publications do not give essential information (e. g. concentrations, droplet sizes). Although the pollen concentration of 50 mg/ml (5 wt.%) in the washing water seems to be high, it has to be considered, that a lot of the mass is pollen bulk. When drying up washing water we found that the upper threshold for total material is about 2.4 wt% of the washing water, but it is generally lower. If we consider that this is the total suspended and dissolved material, which contains also IN inactive or even freeze hindering compounds (e. g. salts, low-molecular sugars), the IN concentration is far lower. For example Arizona test dust, which is accepted as a good reference nucleus, shows lower IN activity in comparable studies [Marcolli et al. 2007]. The maximum heat flow lies below 250 K or at 246 K (depending on the particle size) for concentrations of 2.5 wt%. To get 100% comparable data we have done measurements with kaolin (T50=250 K) and Arizona test dust (T50=252 K). We have chosen concentrations of 2.4 wt%, which is the upper limit for concentration of material dissolved from pollen in our washing water. If we consider that 2.4 wt.% is an upper limit, and that only a small or even diminishing fraction of this material is IN active, concentrations are far lower. We inserted the measurement description into p.27225, line 8: "By drying the washing water we were able to show, that the upper threshold for residue content in the water is about 2.4 wt.%. The hypothesis of macromolecular IN could be verified by analyzing the residue with transmission electron microscopy (TEM)." In some former publications (Ariya et al. 2009 gives an

ACPD
11, C11425–C11435, 2011

overview) high nucleation temperatures were achieved by large droplets (in the millimetre range), increasing the total IN number per droplet at a given particle concentration. Field et al. (2006) and Möhler et al. (2006, 2008) carried out detailed measurement campaigns with accurate description, but these studies dealt with the deposition freezing mode at different air humidities and far lower temperatures. So unfortunately we cannot compare them directly with our findings. Our simulation chamber measurements showed a higher median freezing temperature for birch pollen, namely 261 K (in accordance with 259 K found by Diehl et al. 2002), which is higher than the freezing temperatures of 251-260 K for the most efficient mineral dusts in the immersion mode [Ariya et al. 2009]. We added this discussion at the end of our paper before the acknowledgements, starting from p.27232, line 11: "We think that some pollen are more potent ice nuclei than fresh soot and dust, as it has been suggested before (von Blohn et al., 2005). Although the mass concentrations of 2.4 wt.% in the washing water in our study seem to be high, it has to be considered, that this was the upper threshold for total extractable material. As there are many different substances on the pollen surface, and most of them are inactive or even freeze depressing (e. g. low-molecular sugars and salts), the concentration of active sites has to be far lower, most likely vanishing low compared to the non-active material. If we compare our results with Arizona test dust (Marcolli et al. 2007), which is one of the most active mineral dusts, we see that the maximum nucleation intensity occurs at temperatures of 246 K to 250 K depending on the particle size for concentrations of 2.5 wt.%. T50 of birch measured in this study was 254 K in the oil immersion mode and 261 K in the simulation chamber. Fresh soot shows median freezing temperatures below 245 K for comparable droplet sizes (Diehl and Mitra, 1998). Other publications are difficult to compare (an overview is given by Ariya et al., 2009), as some of them lack essential information, like IN concentrations. Another problem are the large droplet radii, causing a huge number of IN per droplet, even if concentrations are low. For doubtless comparability we have carried out measurements with two of the most efficient known mineral dusts with concentrations of 2.4 wt.%. The determined median freezing temperatures were 252 K for Arizona test

ACPD

11, C11425–C11435, 2011

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Interactive Discussion

Discussion Paper

dust and 250 K for Kaolin. As the IN of the most active pollen species show higher median freezing temperatures at – most likely far – lower concentrations, we conclude that pollen IN have to be relative efficient. Of course pollen ice nuclei cannot reach the very high IN activity of IN-positive bacteria and fungi, but are nevertheless more efficient than many other atmospheric aerosols." We agree with von Blohn et al. 2005, who wrote “that the ice nucleating efficiency of pollen is lower than that of other biological particles such as bacteria or fungi, but it is still higher than that of soot particles and higher or at least in the range of the ice nucleating ability of mineral particles as montmorillonite and kaolinite”. The fact that they generalize and do not discriminate between pollen species as we do might be excused by the narrower spectrum of species, which produce relatively better ice nuclei in comparison with our study (e. g. birch, pine). Fresh soot, to show our point, showed a median freezing temperature below 245 K for comparable droplet sizes [Diehl and Mitra 1998].

Ariya, P. A., Sun, J., Eltouny, N. A., Hudson, E. D., Hayes, C. T., and Kos, G.: Physical and chemical characterization of bioaerosols – implications for nucleation processes, Internat. Rev. Phys. Chem., 28, 1-32, 2009.

Diehl, K., and Mitra, S. K.: A laboratory study of the effects of a kerosene-burner exhaust on ice nucleation and the evaporation rate of ice crystals, Atmos. Environ., 32, 3145-3151, 1998.

Field, P. R., Möhler, O., Connolly, P., Krämer, M., Cotton, R., Heymsfield, A. J., Saathoff, H., and Schnaiter, M.: Some ice nucleation characteristics of Asian and Saharan desert dust, Atmos. Chem. Phys., 6, 2991-3006, doi:10.5194/acp-6-2991-2006, 2006.

Möhler, O., Field, P. R., Connolly, P., Benz, S., Saathoff, H., Schnaiter, M., Wagner, R., Cotton, R., Krämer, M., Mangold, A., and Heymsfield, A. J.: Efficiency of the deposition mode ice nucleation on mineral dust particles, Atmos. Chem. Phys., 6, 3007-3021, doi:10.5194/acp-6-3007-2006, 2006.

Möhler, O., Benz, S., Saathoff, H., Schnaiter, M., Wagner, R., Schneider, J., Wal-

ACPD

11, C11425–C11435, 2011

Interactive Comment

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Interactive Discussion

Discussion Paper

ter, S., Ebert, V., Wagner, S.: The effect of organic coating on the heterogeneous ice nucleation efficiency of mineral dust aerosols, Environ. Res. Lett., 3, 025007, doi:10.1088/1748-9326/3/2/025007, 2008.

3) The bulk of the methods and results are written in the present or present perfect tenses. This makes it very difficult to differentiate accepted fact or regular practice from the methods and results specifically contributed by this work. For methods and results, the verb tense should be simple past tense. Present tense is used for accepted facts or to indicate a regular practice. Overall, the manuscript needs numerous corrections for proper English grammar and vocabulary, and verification that sentences are complete. In addition the authors should note that all Latin names should be in italics.

ANSWER: We changed the tenses to past tense when describing the experiments (with exception of the description of the microscope and simulationchamber setup), and the formatting of the Latin names was changed to italic. Some smaller errors (e. g. typos) were corrected.

4) How do the authors define efficiency of ice nucleation activity? The ice nucleation activity reported here occurs well below -15°C. Admittedly it occurs at temperatures above the homogenous freezing temperature of water, but it does not seem to be effective at temperatures remarkably warmer than those for mineral dust. And clearly it does not approximate the activity of certain ice nucleation active bacteria. For the ice nucleation activity characterized for intact pollen grains in suspension, the authors do not give any information about the activity per pollen grain or per mass of pollen. Is it comparable to that of mineral dust on a per-weight or a per-particle basis? How do the different pollen species compare to each other on a per-pollen grain basis in terms of INA? Overall, it was very surprising that there was no information about the total numbers of pollen grains tested in the freezing tests and if the numbers tested were comparable among the different species of pollen.

ANSWER: In our sense “efficiency” means that nucleation rates at given temperatures

ACPD

11, C11425–C11435, 2011

Interactive Comment

are very high compared to most non-biological IN sources. We do not question the high importance of some bacteria and fungi (we confirm this known fact with our measurements of Snomax as a reference), but in order to fully describe the biosphere-atmosphere interaction all bioaerosols have to be investigated. Although pollen of many species are weak ice nuclei and comparable to mineral dust and soot, some (birch, pine, juniper I) are better. Definitely, we do not claim that all pollen are efficient ice nuclei, as e.g. ragweed and corn are not at all. The pollen concentrations were a result of optimization by trial and error. The intention was that as many droplets as possible should at least contain one pollen, but not many more. The optimum pollen concentration for whole pollen grains was about 50 mg/ml water fraction, so we chose this concentration also for our washing water. But to give an accurate concentration is difficult, as the distribution of pollen was never totally homogenous throughout the whole emulsion. The advantage of the microscope setup is that you can focus on a segment with the conditions you want and neglect segments with non-fitting conditions (e.g. too large or small droplets, too few droplets containing pollen, large agglomerations of pollen, bad optical properties hindering observation, etc.). Another problem is the distribution between the water and the oil phase, which causes a loss of pollen grains. The extraction of the pollen ice nuclei with water made the experimental work far easier, as all the problems described above simply do not manifest. The number of IN per pollen is not known, as the IN themselves are not known, but as one pollen grain surely carries more than one IN active site, a multiplication effect can take place in the atmosphere after IN release, so one pollen grain can cause a lot of freezing events. In the experiment pollen concentrations were the same for all investigated pollen species.

5) Did the authors check the purity of their pollen to verify that it was not transporting contaminants such as bacteria or fungi? Pollen must be collected from plants (probably field-grown plants to assure sufficient production) and cannot be propagated under aseptic conditions as can certain micro-organisms. One way to verify this might be to place a given number of pollen grains on sterile microbiological media (for cultivating bacteria or fungi) to see if any microbial colonies develop.

ACPD

11, C11425–C11435, 2011

ANSWER: In the beginning we had this idea too, but there are good reasons why we do not think so any more: By now we have done measurements with birch pollen from three different sources, and they all show the same results (AllergonAB®, Pharmallerga® and Sigma Aldrich®). Contamination should either not discriminate at all between species (so all pollen should nucleate alike), or it should discriminate between individual samples. Neither is the case. The second reason is that bacteria do not fit through the Vivaspin tubes, and bacterial IN are in contrast to fungal and pollen IN tightened stronger to the cell wall. Then our chemical analysis has shown differences between pollen and bacterial IN (especially the heat degeneration curve of birch strongly contradicts the hypothesis of contamination with IN active bacteria). We added a paragraph in the Conclusions chapter, p.27232, between line 20 and 21: "As pollen are not produced under sterile laboratory conditions like microorganisms, the question arises, if pollen IN activity might be caused by bacteria growing on the material, as it is the case on decaying leaf litter (Vali et al. 1976). But the different biochemical and thermal properties make this hypothesis very unlikely, so we are convinced that the IN have to originate from the pollen itself." The suggestion to add pollen to sterile nutrient media is in fact a helpful advice, which we intend to realize as soon as possible.

Vali, G., Christensen, M., Fresh, R. W., Galyan, E. L., Maki, R., and Schnell, R. C.: Biogenic ice nuclei part II: bacterial sources, J. Atmos. Sci., 33, 1565-1570, 1976.

6) In the Discussion, the authors suggest that the ice nucleation active material from pollen, once separated from the pollen grain, could be transported to altitudes much higher than the pollen grains themselves can be transported. I wondered about the mechanisms that would be involved in this transportation. Under what natural conditions would the ice nucleation active material be removed from the pollen? – I suppose that this would involve free moisture, but would this be in a wet aerosol, or on plants or elsewhere? And then what would be the conditions that would favour the separation of the pollen from this water that would also lead to continued dissemination of the ice nucleation active material?

ACPD

11, C11425–C11435, 2011

ANSWER: Schäppi et al. (1999) suggest, that rain may cause bursting of pollen grains which release then material, like allergenes. Solomon et al. (1983) found allergenic material in the fraction of aerosols smaller than 5 μm, which contained no pollen or larger pollen fragments. So the loss of material from the pollen grains seems to be a common mechanism. Grote et al. (2001) observed the bursting of wet pollen and release of material by electron microscopy. Yttri et al. (2007) also see pollen rupture, and also wood burning as main sources for free pollen sugars in the atmosphere. We have amended our discussion with this information, from p. 27231, line 22: "It is known, that pollen constituents, such as allergens and sugars, can indeed leave the pollen body and be distributed independently. The most probable mechanism is the pollen grain bursting by rain, which releases material, like allergens (Schäppi et al., 1999). As a consequence allergenic material was found in aerosol particles smaller than 5 μm, which contained no pollen or bigger fragments (Solomon et al., 1983). The release of material by bursting of wet pollen has been observed by electron microscopy (Swoboda et al., 2001). Not only allergens, but also sugars originating from pollen can be detected in the atmosphere (Yttri et al., 2007). These authors see pollen rupture and wood burning as their main sources in the atmosphere. The contrast between the hydrophilic properties of many of the surface components and the relative hydrophobia of the sporopollenin boosts the suspension of surface components in water droplets. According to that we conclude that the impact of pollen on the global atmosphere might have been underestimated." The described pollen rupturing process takes place in the atmosphere itself and can explain the high pollen material concentrations in the atmosphere, which are undoubtedly there, in the absence of whole pollen grains. Released material can be either set free (and further distributed) or immersed in the attacking droplet (and cause, if temperatures are low enough, ice nucleation immediately). The fact that on the one hand sporopollenin, which is the bulk material of the pollen exine is mostly hydrophobic, but many of the known surface molecules are hydrophilic (e.g. allergens would not be such an issue, if they were not hydrophilic, what makes them infiltrate human mucosa).

Swoboda, I., Grote, M., Verdino, P., Keller, W., Singh, M., B., DeWeerd, N., Sperr, W. R., Valent, P., Balic, N., Reichelt, R., Suck, R., Fiebig, H., Valenta, R., and Spitzauer, S.: Molecular characterization of polyglacturonases as grass pollen specific marker allergens: expulsion from pollen via submicronic respirable particles, J. Immunol., 172, 6490-6500, 2004.

Yttri, K. E., Dye, C., and Kiss, G.: Ambient aerosol concentrations of sugars and sugar-alcohols at four different sites in Norway, Atmos. Chem. Phys., 7, 4267-4279, doi:10.5194/acp-7-4267-2007, 2007.

7) I appreciated the speculations made in this manuscript particularly about the mechanisms involved in ice nucleation activity and the possible link between life history of the plant species (adaptation to colder climates) and the rate of ice nucleation activity of its pollen. However, I think that the authors should be careful to make a very balanced and objective evaluation of the potential importance of pollen in atmospheric ice nucleation given the relatively cold temperature of its activity.

ANSWER: The hypothesis that pollen IN might be a cryoprotective mechanism evolved by cryophilic species is of course not proven, but it fits for most of the data really well. The idea of cryoprotective adaptation by forming IN or antifreeze proteins is not new, but has been a topic of a load of studies. We did not intend to lose focus on the atmospheric relevance as well by mentioning the hypothesis of adaptation. On the contrary it would be a perfect example for one mechanism having several effects – here a selection advantage of some species and an impact on the atmosphere. We did not perform model calculations, as it is a too early stage right now. We think that in the first step we have to evaluate the IN quality of pollen, before we can apply the resulting parameters in an atmospheric model calculation. We do not claim that we have given all the answers in our paper, but that we have made a big first step, which should be shared with the community to let everybody interested participate in the journey. But we do think that some pollen species are more important than many other (esp. inorganic, fresh soot) aerosols in the atmosphere, as we have explained in points 2 and 4.

ACPD
11, C11425–C11435, 2011

Interactive comment on Atmos. Chem. Phys. Discuss., 11, 27219, 2011.

ACPD

11, C11425–C11435, 2011

Interactive Comment