ICE NUCLEATION BY MINERAL DUSTS, FUNGAL SPORES, AND BACTERIA: IMPLICATIONS FOR CLIMATE AND THE LONG-DISTANCE TRANSPORT OF THESE AEROSOLS IN THE ATMOSPHERE

by

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Abstract

In the atmosphere, ice can form on solid aerosol particles called ice nuclei. This research focuses on the ice nucleation properties of mineral dusts and biological particles. The motivation for this research is two-fold. First, ice nucleation on these aerosols may influence cloud formation, cloud reflectivity and precipitation patterns in what is an indirect climate effect. This effect is one of the largest uncertainties in current climate models. Second, ice nucleation may be an important removal mechanism for these particles from the atmosphere and may influence their long-distance transport. Currently, ice nucleation is represented in a simplistic manner or not at all in models used to predict the long-distance transport of aerosols.

A temperature and humidity controlled flow cell coupled to an optical microscope was used to study the ice nucleation properties of four mineral dusts, eighteen fungal spores, and six bacteria. It was found that acidic coatings reduce the ice nucleating ability of the mineral dusts. The fungal spores showed a wide range of ice nucleating properties and there was no inherent difference in the ice nucleation ability of spores belonging to different taxonomic groups. Four of the bacteria studied were very poor ice nuclei and the fifth bacterium was an excellent ice nucleus.

The results from the flow cell experiments on fungal spores were used to describe ice nucleation in two modeling studies that simulated atmospheric transport. One study found that a significant fraction of large fungal spores (20 micrometers in diameter) can reach high altitudes where they could act as ice nuclei. The other study focused on smaller spores (3 to 8 micrometers) and found that ice nucleation on these spores effects their long-distance transport to polar and marine regions.
The laboratory results were used to show that mineral dusts are more important than the fungal spores or bacteria that were studied on a global annual scale. These results can be used to improve parameterizations of ice nucleation on mineral dusts and biological particles in future modeling studies investigating the indirect effect of aerosols on climate or the long-distance transport of aerosols in the atmosphere.
Preface

Chapter 2, Chapter 3, and Chapter 4 are co-authored peer-reviewed journal articles and the results from Chapter 5 and Chapter 6 are being prepared for submission to peer-reviewed journals as co-authored journal articles. The details of my contributions to each research chapter mentioned above is provided below.


- Formulated research questions and designed research project with my supervisor.
- Performed all of the ice nucleation experiments.
- Performed all of the data analysis.
- Prepared all of the figures in this publication.
- Writing of the text for this publication was shared with my supervisor.

Chapter 3 (second author on published journal article): Iannone, R., D. I. Chernoff\textsuperscript{a}, A. Pringle, S. T. Martin, and A. K. Bertram (2011), The ice nucleation ability of one of the most abundant types of fungal spores found in the atmosphere, \textit{Atmos. Chem. Phys.}, 11, 1191-1201, doi:10.5194/acp-11-1191-2011.

- Formulated research questions and designed research project with my supervisor and Dr. Richard Iannone.
- Designed and built the spore dispenser used to deposit \textit{Cladosporium} spores on hydrophobic glass slides, later used in ice nucleation experiments, with Dr. R. Iannone.

\textsuperscript{a} Note the candidate’s previous surname was Chernoff.
• Performed the ice nucleation experiments with Dr. R. Iannone.
• Completed the data analysis with Dr. R. Iannone.
• Shared figure preparation for the publication with Dr. R. Iannone.
• Shared writing of the text in the publication with Dr. R. Iannone and my supervisor.
• Dr. A. Pringle and Dr. S. Martin helped formulate research questions and helped design research projects.


• Formulated research questions and designed research project with my supervisor.
• Designed and built the instrumentation used to deposit rust and bunt fungal spores on hydrophobic glass slides for later use in ice nucleation experiments.
• Performed ice nucleation experiments with Dr. R. Iannone, M. J. Wheeler, and R. Mason.
• Performed particle dispersion modelling simulations.
• Completed data analysis with Dr. R. Iannone, M. J. Wheeler, and R. Mason.
• Prepared all of the figures in this publication.
• Writing of the text for this publication was shared mainly with my supervisor, with additional contributions from Dr. E. A. Polishchuk and Dr. I. G. McKendry.
• Additional contributions from co-authors:
  o Dr. T. Fetch Jr. provided rust spores for ice nucleation experiments, advised on the selection of the emission sites chosen for the particle dispersion modelling study, and
helped interpret the ice nucleation results for implications on the spread of rust fungi (agricultural pathogens) and their long-distance transport in the atmosphere.

- Dr. B. J. van der Kamp harvested *Endocronartium harknessii* from the field and consulted on the method used to isolate these spores for use in ice nucleation experiments.
- Dr. E. A. Polishchuk designed the experimental method used to test for bacterial contamination of the rust and bunt spores, and provided interpretation of the results from the contamination tests.


- Formulated research questions and designed research project with my supervisor, Dr. S. Burrows, Dr. R. Iannone, and M. J. Wheeler.
- Designed and built the instrumentation used to deposit fungal spores on hydrophobic glass slides for later use in ice nucleation experiments with Dr. R. Iannone and M. J. Wheeler.
- Performed ice nucleation experiments with Dr. R. Iannone, M. J. Wheeler, and R. Mason.
- Completed data analysis with Dr. R. Iannone, M. J. Wheeler, and R. Mason.
- Prepared most of the figures for this publication; Dr. S. Burrows prepared figures showing results from the EMAC global chemistry-climate transport model.
• Writing of the text for this publication was shared mainly with my supervisor; Dr. S. Burrows wrote the section describing the simulations using the EMAC global chemistry-climate transport model.

• Additional contributions from co-authors:
  o Dr. S. Burrows completed the simulations using the EMAC global chemistry-climate transport model, performed the data analysis on the model results, and provided advice on the discussion of the modelling results.
  o J. Chen prepared Eurotiomycetes cultures for experiments.
  o Dr. E. A. Polishchuk designed the experimental method used to test for bacterial contamination of Agaricomycetes and Ustilagomycetes spores, and provided interpretation of the results from the contamination tests.


• Formulated research questions and designed research project with my supervisor.

• Designed and built the instrumentation used to deposit bacteria on hydrophobic glass slides, later used in ice nucleation experiments.

• Performed the ice nucleation experiments with M. Wagstaff, R. Mason, and M. J. Wheeler.

• Completed the data analysis with M. Wagstaff.

• Prepared all of the figures for this publication.
• Shared writing of the text for this publication with my supervisor.

• Additional contributions from co-authors:
  o J. Chen prepared the bacteria cell suspensions for ice nucleation experiments.
  o N. DeLeon-Rodriguez, K. T. Konstantinidis, T. L. Latham, B. E. Anderson, and A. Nenes provided the sample of *Bacillus subtilis* that was isolated from the atmosphere in a field study [DeLeon-Rodriguez et al., 2013] and, in addition, performed the DNA analysis for species level identification.
  o D. Horne performed the scanning electron microscopy (SEM) experiments and contributed to the discussion on the appearance of the bacteria in the SEM images.
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cloud removal (scavenging coefficient = \( 5.0 \times 10^{-5} \) s\(^{-1} \)) (squares) (i.e. same data as shown
in Figure 4.6); (2) using the default in-cloud removal value and a below-cloud removal
value equal to the default value multiplied by 10 (below-cloud default \( \times 10 \), circles); (3)
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Figure A.1 Taxonomy for the Ascomycota species of fungi studied in Chapter 3 (Cladosporium sp.) and Chapter 5 (P. chrysogenum, Penicillium sp., P. brevcompactum) [Benson et al., 2009; Kirk et al., 2008; Sayers et al., 2009].

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U. avenae, L. nuda, A. bisporus, A. muscaria, B. zelleri, T. abietinum) [Benson et al., 2009; Kirk et al., 2008; Sayers et al., 2009]. .................................................................195

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<table>
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<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>$A$</td>
<td>pre-exponential factor for calculating $J_{het,imm}$</td>
</tr>
<tr>
<td>$A_{aer}$</td>
<td>surface area of an individual particle</td>
</tr>
<tr>
<td>$A_s$</td>
<td>total surface area of particles available for heterogeneous nucleation</td>
</tr>
<tr>
<td>$a_w$</td>
<td>activity of water</td>
</tr>
<tr>
<td>$D_{volume}$</td>
<td>volume equivalent diameter</td>
</tr>
<tr>
<td>$f^{cl}$</td>
<td>grid-box mean cloud fraction</td>
</tr>
<tr>
<td>$f(m,x)$</td>
<td>geometric factor</td>
</tr>
<tr>
<td>$F_{rain}$</td>
<td>flux of rain</td>
</tr>
<tr>
<td>$F_{snow}$</td>
<td>flux of snow</td>
</tr>
<tr>
<td>$\Delta F_{g,het}$</td>
<td>free energy of formation of the critical ice embryo on the surface of an ice nuclei</td>
</tr>
<tr>
<td>$G$</td>
<td>dimensionless parameter equal to $RT/L_{eq}M_w$</td>
</tr>
<tr>
<td>$\Delta g$</td>
<td>activation energy for the diffusion of a water molecule across the ice-water interface</td>
</tr>
<tr>
<td>$INperCell$</td>
<td>cumulative number of ice nuclei per bacteria cell</td>
</tr>
<tr>
<td>$INperParticle$</td>
<td>cumulative number of ice nuclei per dust particle</td>
</tr>
<tr>
<td>$INperSpore$</td>
<td>cumulative number of ice nuclei per spore</td>
</tr>
<tr>
<td>$iwc$</td>
<td>ice water content of a cloud</td>
</tr>
<tr>
<td>$J_{het}$</td>
<td>heterogeneous nucleation rate of ice nucleation</td>
</tr>
<tr>
<td>$J_{het,imm}$</td>
<td>heterogeneous nucleation rate of ice nucleation in the immersion mode</td>
</tr>
<tr>
<td>$j$</td>
<td>bin number</td>
</tr>
</tbody>
</table>
\( k \)  
Boltzmann constant

\( L \)  
length of fungal spore

\( L_{ef} \)  
effective latent heat of formation

\( lwc \)  
liquid water content of a cloud

\( m \)  
compatibility parameter for ice on a solid substrate

\( M_w \)  
molecular weight of water

\( N_{total,j} \)  
total number of drops in bin \( j \)

\( N_{unfrozen}(T) \)  
number of unfrozen drops in bin \( j \) at temperature \( T \)

\( n \)  
total number of bins

\( n_s \)  
ice nucleation active surface site density

\( R \)  
gas constant

\( R_{nuc,ice} \)  
fraction of particles embedded in cloud ice crystals

\( R_{nuc,liq} \)  
fraction of particles embedded in liquid cloud droplets

\( r_g \)  
radius of the ice embryo

\( SPD_j \)  
number of spores or bacteria per drop in bin \( j \)

\( T \)  
temperature

\( T_0 \)  
triple point of water

\( Total\#Spores \)  
total number of spores available to act as ice nuclei in all experiments

for a given spore or bacteria

\( t \)  
observation time

\( \Delta t \)  
length of EMAC model time step

\( x \)  
ratio of the radius of the substrate to the radius of the spherical ice germ
\( X_i \) in-cloud mixing ratio due to nucleation scavenging and subsequent precipitation

\( W \) width of a fungal spore

\( \rho_i \) temperature dependent density of ice

\( \omega \) number of nucleated ice crystals

\( \sigma_{i/s} \) surface tension at the ice-sulfuric acid solution interface

\( \theta \) contact angle between an ice nucleus and the solid surface
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>a.g.l.</td>
<td>above ground level</td>
</tr>
<tr>
<td>a.s.l.</td>
<td>above sea level</td>
</tr>
<tr>
<td>B.C.</td>
<td>British Columbia</td>
</tr>
<tr>
<td>CAPE</td>
<td>convective available potential energy</td>
</tr>
<tr>
<td>CFCAS</td>
<td>Canadian Foundation for Climate and Atmospheric Science</td>
</tr>
<tr>
<td>CCN</td>
<td>cloud condensation nuclei</td>
</tr>
<tr>
<td>ECHAM GCM</td>
<td>European Centre Hamburg General Circulation Model</td>
</tr>
<tr>
<td>EMAC</td>
<td>ECHAM/MESSy</td>
</tr>
<tr>
<td>GDAS1</td>
<td>Global Data Assimilation System</td>
</tr>
<tr>
<td>HYSPLIT</td>
<td>Hybrid Single-Particle Lagrangian Integrated Trajectory</td>
</tr>
<tr>
<td>IN</td>
<td>ice nuclei</td>
</tr>
<tr>
<td>LCL</td>
<td>lifting condensation level</td>
</tr>
<tr>
<td>MESSy</td>
<td>Modular Earth Submodel System</td>
</tr>
<tr>
<td>NSERC</td>
<td>National Sciences and Engineering Research Council of Canada</td>
</tr>
<tr>
<td>S.D.</td>
<td>standard deviation</td>
</tr>
<tr>
<td>sp.</td>
<td>single species</td>
</tr>
<tr>
<td>spp.</td>
<td>multiple species</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>RH&lt;sub&gt;i&lt;/sub&gt;</td>
<td>relative humidity with respect to ice</td>
</tr>
<tr>
<td>RH&lt;sub&gt;w&lt;/sub&gt;</td>
<td>relative humidity with respect to water</td>
</tr>
<tr>
<td>S.D.</td>
<td>standard deviation</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>UBC</td>
<td>University of British Columbia</td>
</tr>
<tr>
<td>U.S.</td>
<td>United States of America</td>
</tr>
</tbody>
</table>
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To my parents, Helen & Bill Chernoff, and my husband, Brad Haga
Chapter 1. Introduction

1.1 Atmospheric Aerosols

Aerosols are suspensions of liquid or solid particles in a gaseous medium [Seinfeld and Pandis, 1998]. In the atmospheric science community, the terms aerosol, particle, and particulate matter are often used interchangeably [Finlayson-Pitts and Pitts Jr., 2000] and these terms are often used to describe the particles alone exclusive of the gaseous medium (i.e. air)[Seinfeld and Pandis, 1998]. Aerosols can be emitted directly into the atmosphere or produced through secondary reactions from gaseous precursors, and they are prevalent in the atmosphere with number concentrations ranging from $10^2$ to $10^8$ cm$^{-3}$ [Finlayson-Pitts and Pitts Jr., 2000; Seinfeld and Pandis, 1998]. Remote regions (such as marine environments) have low aerosol number concentrations, around $10^2$ cm$^{-3}$, rural areas have aerosol number concentrations on the order of $10^3$-$10^4$ cm$^{-3}$, and polluted urban areas are characterized by aerosol number concentrations of at least $10^5$ cm$^{-3}$ and as high as $10^8$ cm$^{-3}$ [Finlayson-Pitts and Pitts Jr., 2000; Seinfeld and Pandis, 1998]. Atmospheric aerosol particles range in size from approximately 2 nm, the size of a cluster of molecules, to around 100 μm, the size of fine drizzle. However, aerosols approximately 2 nm to 10 μm in size are the most important for atmospheric chemistry and physics [Finlayson-Pitts and Pitts Jr., 2000].

Atmospheric aerosol particles come from both natural and anthropogenic sources. Shown in Table 1.1 are global annual mass emissions (Tg year$^{-1}$, where 1 Tg = $10^{12}$ g) for natural and anthropogenic aerosol sources produced by both primary emissions and secondary formation reactions [Andreae and Rosenfeld, 2008; Gieré and Querol, 2010]. Approximately 98 % of aerosol mass emissions come from natural sources, compared to 2 % from anthropogenic sources. Within the natural aerosol component, mineral dust and sea salt have the largest
emissions, together making up approximately 97% of natural aerosol flux. This research focuses on mineral dusts and natural primary biological particles. The largest emission sources for anthropogenic aerosols include industrial dust (~42% of total anthropogenic) and secondary sulfate production (~51% of total anthropogenic aerosol emissions); the later of these major anthropogenic sources is a focus of the current research. Although anthropogenic aerosols make up a small fraction of the total mass emissions (Table 1.1), their number concentrations can dominate certain sizes of aerosols in the atmosphere and in certain locations. For example, some recent modeling studies [Andreae, 2007; Stier et al., 2006] estimated that the contribution from anthropogenic sulfate aerosols was approximately 25-30% of the aerosol number burden for sizes 0.01 to 1 μm in the northern hemisphere at latitudes between 0 °N and 60 °N [Andreae and Rosenfeld, 2008].

Table 1.1 Global mass emissions for major aerosol sources (adapted from Gieré and Querol [2010] and Andreae and Rosenfeld [2008]. Estimates were not readily available for entries with a “-“.

<table>
<thead>
<tr>
<th>Aerosol Type</th>
<th>Aerosol Source</th>
<th>Mass Emission (Tg yr(^{-1}))</th>
<th>Number Production (yr(^{-1}))</th>
<th>Number Burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Primary</td>
<td>Volcanic ash</td>
<td>200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Biological (fungal spores, bacteria, etc.)</td>
<td>35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sea salt</td>
<td>10 013</td>
<td>(1.2 \times 10^{27})</td>
<td>(2.7 \times 10^{25})</td>
</tr>
<tr>
<td></td>
<td>Mineral dust</td>
<td>1 653</td>
<td>(1.4 \times 10^{26})</td>
<td>(1.1 \times 10^{25})</td>
</tr>
<tr>
<td>Natural Secondary</td>
<td>Biological (organic)</td>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sulfate (biological + volcanic)</td>
<td>78</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Natural Aerosol (% of total)</td>
<td></td>
<td>12 004 (98 %)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthropogenic Primary</td>
<td>Fossil fuel (organic)</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Black carbon (open burning + biofuel + fossil fuel)</td>
<td>10.5</td>
<td>-</td>
<td>(2.7 \times 10^{26})</td>
</tr>
<tr>
<td></td>
<td>Industrial dust</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthropogenic Secondary</td>
<td>Organic</td>
<td>3.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sulfate</td>
<td>122</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Anthropogenic Aerosol (% of total)</td>
<td></td>
<td>240 (2 %)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Also shown in Table 1.1 are values for the number production and the number burden of some aerosol types. The number production corresponds to the total number of a certain type of aerosol particle emitted from the surface and the number burden is the expected number of these particles in the atmosphere at a given time. The limited numbers shown in the table illustrate that even though mineral dust and sea salt dominate the mass emissions, they don’t necessarily dominate the number burden.

The composition of atmospheric aerosols varies depending on the size of the particles. Fine particles include those having diameters ≤ 2.5 μm and consist mostly of nitrates, sulfates, ammonium, organic and elemental carbon, and some transitions metals [Seinfeld and Pandis, 1998]. Coarse particles have diameters ≥ 2.5 μm and include mostly crustal materials (including mineral dust), nitrates and biological particles [Seinfeld and Pandis, 1998].

1.1.1 Mineral Dust Aerosols

Mineral dust aerosols become suspended in the atmosphere by strong wind currents and turbulence and consist mostly of eroded and weathered crustal rock components [Murray et al., 2012; Usher et al., 2003]. The main sources for aerosolized mineral dusts are from the Northern Hemisphere “dust belt” that extends from North Africa, across the Middle East, Central and South Asia, to China, in arid and semiarid regions characterized by topographical lows (i.e. areas with low elevation relative to the surrounding terrain) and low rainfall amounts [Prospero et al., 2002; Usher et al., 2003]. Transitory rivers, streams or lakes characterize many of the major dust sources [Prospero et al., 2002]. Although seemingly contradictory, the connection between the presence of water and dust sources to the atmosphere can be rationalized considering the weathering process. The flow of water over crustal rock is much more efficient at producing fine particles (i.e. <10 μm) compared to dry mechanical processes [Prospero et al., 2002].
Additionally, water transports the mineral dust particles to depositional basins, located in topographical lows, where they can become suspended in the atmosphere by wind.

Mineral dusts are omnipresent in the atmosphere, with estimates for global emissions ranging from approximately 1000 to 3000 Tg year\(^{-1}\) (Table 1.1 and Murray et al. [2012], Usher et al. [2003], and Zender et al. [2004]). Modelling studies have estimated their number concentrations to be between 10-50 cm\(^{-3}\) near the surface and 10\(^{-4}\)-10\(^{-1}\) cm\(^{-3}\) in the free troposphere [Hoose et al., 2010b; Sesartic et al., 2013]. The major constituents of the mineral dust aerosol are presented in Table 1.2 along with their chemical formulae [Murray et al., 2012; Usher et al., 2003].

Table 1.2. Major groups of mineral dusts present in the atmosphere [Murray et al., 2012; Usher et al., 2003].

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>SiO(_2)</td>
</tr>
<tr>
<td>Feldspar</td>
<td>WX(_4)O(_8)(^b)</td>
</tr>
<tr>
<td>Calcite</td>
<td>CaCO(_3)</td>
</tr>
<tr>
<td>Illite(^c)</td>
<td>(K,(\text{H}_2)(\text{O}))(Al,Mg,Fe)(_2)(Si,Al)(<em>4)O(</em>{10})[(OH)(_2),H(_2)O]</td>
</tr>
<tr>
<td>Kaolinite(^c)</td>
<td>Al(_4)Si(<em>4)O(</em>{10})(OH)(_8)</td>
</tr>
<tr>
<td>Chlorite(^c)</td>
<td>A(_{5.6})Z(<em>4)O(</em>{10})(OH)(_8)(^d)</td>
</tr>
<tr>
<td>Montmorillonite(^c)</td>
<td>(Na,Ca)(_{0.33})(Al,Mg)(_2)Si(<em>4)O(</em>{10})(OH)(_2)(\cdot)nH(_2)O</td>
</tr>
</tbody>
</table>

Shown in Figure 1.1 is the average atmospheric mineral dust composition from numerous field studies (adapted from Murray et al. [2012], Figure 6). In this dissertation, the clay minerals illite, kaolinite, and montmorillonite as well as quartz are studied: together, these mineral dusts make up approximately 72.5 % of the total aerosolized mineral dusts shown in Figure 1.1.

\(^b\) W: Na, K, Ca and/or Ba; X: Si and/or Al

\(^c\) Member of the clay minerals

\(^d\) A: Al, Fe, Li, Mg, Mn and/or Ni; Z: Al, B, Si and/or Fe
Figure 1.1 Average mineral dust aerosol composition from multiple field studies. “Others” include gypsum, goethite, haematite, palygorskite, and halite. Adapted from Murray et al. [2012].

1.1.2 Biological Aerosols

Biological aerosols are emitted directly into the atmosphere and include microorganisms, dispersal and reproductive units, fragments of animal and plant matter [Després et al., 2012], and are one of the focuses of this dissertation.

The sources of primary biological aerosols are extremely diverse and cosmopolitan, with particles being emitted by all regions of the globe. Plants are sources of debris fragments, decay products, pollen, reproductive spores, fungal spores, and bacteria [Després et al., 2012]. Soil and rock surfaces, agricultural regions, and oceans host a wide variety of bacteria and fungi groups [Després et al., 2012]. Due to the complex nature of the sources and categories of primary biological aerosols, it has been difficult to estimate their global emissions with previous estimates spanning at least two orders of magnitude from <10 Tg year\(^{-1}\) to approximately 1000 Tg year\(^{-1}\) (Table 3, [Després et al., 2012]).

Part of this dissertation focuses on two types of primary biological particles, bacteria and fungal spores. Shown in Figure 1.2 are the relative contributions of bacteria and fungal spores to
the total primary biological aerosol composition, given as percentages of number and mass concentrations of air over vegetated areas (i.e. land surfaces covered by plant matter) measured during several field campaigns (adapted from Table 4, [Després et al., 2012]). Number and mass concentrations for bacteria are approximately $10^4$ m$^{-3}$ and 0.1 µg m$^{-3}$; and for fungal spores are approximately $10^3$-$10^4$ m$^{-3}$ and 0.1-1 µg m$^{-3}$, respectively [Després et al., 2012]. Also included are other types of primary biological particles: fungal hyphal fragments, plant debris, viral particles, pollen, algae, and fern spores. Primary biological aerosols have a wide range of sizes, from tens of nanometers (viral particles) to hundreds of microns (pollen and plant debris) [Després et al., 2012], and for this reason the relative contribution from each particle type in Figure 1.2 can be very different when comparing number and mass concentrations. Bacteria cells are usually a few micrometers in size and fungal spores range in size between a few to tens of micrometers [Després et al., 2012] and together they make up approximately 57 % (number concentration) and 19 % (mass concentration) of all the primary biological particles.
1.2 Heterogeneous and Homogeneous Ice Nucleation in the Atmosphere

Ice formation proceeds by one of two nucleation mechanisms. Homogeneous ice nucleation involves the spontaneous freezing of liquid droplets and heterogeneous ice nucleation involves ice formation on an insoluble or partially soluble aerosol particle [Pruppacher and Klett, 1997]. In the atmosphere, homogeneous ice nucleation occurs at temperatures below approximately -37 °C and any ice nucleation occurring at warmer temperatures occurs by heterogeneous ice nucleation [Pruppacher and Klett, 1997]. Those aerosol particles that cause heterogeneous freezing in the atmosphere are referred to as ice nuclei (IN), and they make up a very small subset of the total aerosol population, with the ratio of IN to total aerosol particles generally on the order of 10^{-4}-10^{-6} (e.g. Baustian et al. [2012], Pratt et al. [2011], Prenni et al.}
This dissertation focuses on the heterogeneous nucleation of mineral dust (sulfuric acid coated and uncoated), fungal spores, and bacterial IN. Concentrations of ice nuclei range from less than 1 to 10’s of IN per liter [DeMott et al., 2010; Eidhammer et al., 2010; Grosvenor et al., 2012; Morales Betancourt et al., 2012; Pratt et al., 2009; Pratt et al., 2010; Prenni et al., 2009a]. Despite their low abundance, IN can be significant modulators of cloud processes [Bangert et al., 2012; Lohmann and Feichter, 2005; Lohmann and Diehl, 2006].

1.3 The Effect of Atmospheric Ice Nuclei on Climate

The presence of aerosol particles in the atmosphere has important influences on climate by altering the amount of incoming (solar) or outgoing (terrestrial) energy in the Earth-atmosphere system [IPCC, 2007; Seinfeld and Pandis, 1998]. Radiative forcing is used to quantify the importance of a given factor on climate change and it is defined by the Intergovernmental Panel on Climate Change (IPCC) as the difference in energy supplied to the Earth-atmosphere system between present day (circa (ca.) 2005) and pre-industrial conditions (ca. 1750), given in watts per square meter (W m$^{-2}$) [IPCC, 2007]. A positive radiative forcing corresponds to a warming effect and a negative radiative forcing corresponds to a cooling effect.

Radiative forcing from aerosols is categorized into direct and indirect climate effects. In the direct effect, aerosols alter the radiative balance of the Earth-atmosphere system by scattering and absorbing radiation [Forster et al., 2007]. In the indirect effect, aerosols change the radiative balance by influencing cloud formation; cloud reflectivity; cloud absorption and emission properties; cloud lifetime; and precipitation patterns by acting as nuclei for liquid cloud droplet and ice crystal formation [Forster et al., 2007]. The results presented in this dissertation have implications for predictions of the indirect climate effects of atmospheric ice nuclei.
Clouds can interact with both the incoming solar radiation from the Sun and outgoing terrestrial radiation from the Earth’s surface. Solar radiation includes both the visible and ultraviolet regions of the electromagnetic spectrum and terrestrial radiation includes infrared regions of the electromagnetic spectrum [Seinfeld and Pandis, 1998]. Clouds have a cooling effect by scattering solar radiation away from the Earth’s surface and a warming effect resulting from the absorption of infrared terrestrial radiation by cloud water and ice (see, for example: Smith et al. [1998], Turner [2005], and [Yang et al., 2003]). It is the balance between these interactions that determine whether a particular cloud type will have a net positive (i.e. warming) or negative (i.e. cooling) radiative forcing [IPCC, 2007]. Low altitude clouds (below approximately 7 km) reflect significant amounts of solar radiation and absorb very little infrared radiation and, therefore, have an overall cooling effect. High altitude clouds (7-18 km) absorb significant infrared radiation and reflect very little solar radiation and these clouds have an overall warming effect.

Clouds containing the ice phase include (1) mixed-phase clouds, which contain liquid droplets and ice crystals and exist in the atmosphere at altitudes between 2-7 km; and (2) ice clouds, which contain only ice crystals and are present at altitudes of 7-18 km [Seinfeld and Pandis, 1998]. Ice is formed by heterogeneous nucleation in mixed-phase clouds and by both homogeneous and heterogeneous nucleation in ice clouds; the results presented in this dissertation are mainly related to mixed-phase cloud processes. Human activities have increased the atmospheric aerosol burden [Andreae and Rosenfeld, 2008], and consequently, the number of ice nuclei in the atmosphere may have increased since pre-industrial times. The indirect effect of aerosols acting as ice nuclei in mixed-phase clouds are depicted in Figure 1.3, modified from DeMott et al. [2010].
Figure 1.3. Indirect aerosol effect from atmospheric ice nucleation on mixed-phase clouds in (a) pre-industrial times and (b) the present day (adapted from DeMott et al. [2010]).

Mixed-phase clouds are believed to have a significant cooling effect on the earth-atmospheric system by reflecting incoming solar radiation at the cloud tops [Hartmann et al., 1992; IPCC, 2007]. This cooling effect is partially offset by the absorption of infrared terrestrial radiation by cloud water and ice, but cooling still dominates.

An increase in IN is believed to decrease the cooling from mixed-phase clouds. An increase in the number of ice nuclei in mixed-phase clouds would result in more ice crystals existing in the cloud. Because the vapor pressure over liquid water is greater than over ice, the ice crystals would grow by water vapor transfer from the liquid droplets to the ice crystals. The growth of ice crystals at the expense of liquid water droplets in mixed-phase clouds is known as the Wegener-Bergeron-Findeisen process, and can lead to rapid glaciation of these types of
clouds. This process leads to increased amounts of precipitation, decreased cloud lifetimes from the deposition of the large ice crystals, and less solar radiation being reflected at the cloud tops [DeMott et al., 2010]. As a result of this overall process, an increase in IN causes a positive radiative forcing via mixed-phase clouds (compared to the radiative forcing of mixed-phase clouds with low IN concentrations).

The indirect effect of aerosol particles represents some of the largest uncertainties in climate modeling and prediction [IPCC, 2007]. Furthermore, the indirect climate effect from ice nucleation was deemed so uncertain that it was not included in the most recent IPCC report [IPCC, 2007]. From the discussion above, the presence of ice nuclei can have profound impacts on cloud formation properties, precipitation patterns, and the radiative properties of the atmosphere. Ice nucleation is often represented in a simplistic manner in current climate models (for example, see: Betancourt et al. [2012], Curry and Khvorostyanov [2012], Ervens and Feingold [2012], Lohmann et al. [2007], Lohmann and Hoose [2009], Storelvmo et al. [2011], and Yun and Penner [2012]), and this is due in part to the low level of scientific understanding of this process [Cantrell and Heymsfield, 2005; Forster et al., 2007; Lohmann and Feichter, 2005]. It is, therefore, important to better quantify the ice nucleation properties of atmospheric particles so that improved models for climate prediction can be developed, which should result in improved predictions of the magnitude of the indirect effect of IN on climate. Here, the ice nucleation properties of two important types of ice nuclei have been studied, mineral dusts, both uncoated and acid coated, and biological particles. These results can be used to improve parameterizations for ice nucleation that are currently used in climate models.
1.4 The Effect of Ice Nucleation on the Long-Distance Transport of Aerosols

In addition to the indirect climate effect, another reason for studying the ice nucleation properties of aerosol particles is because ice nucleation, followed by precipitation, might be an important removal mechanism for particles from the atmosphere and could therefore affect their long-distance transport. The long-distance transport of aerosols has important implications for pollutant dispersion and air quality, the spread of human and plant diseases and the fertilization of oceans. In models used to predict the long-distance transport of aerosols, ice nucleation is either not included or, if so, only in a very simplistic manner (for example: see Devenish et al. [2012]; von Hardenberg et al. [2012]; Zhang et al. [2012]; and Zhao et al. [2012]). The ice nucleation properties of mineral dusts and biological particles was studied here, and this information can be used to improve the parameterizations for ice nucleation in models used to predict the long-distance transport of these important aerosol species.

1.5 Motivation for Studying Ice Nucleation on Mineral Dusts

Mineral dusts have been shown to be effective ice nuclei in laboratory experiments [Hoose and Möhler, 2012; Murray et al., 2012], and field studies have indicated that mineral dusts can influence cloud formation and properties [DeMott et al., 2003; Sassen, 2002; Sassen et al., 2003]. Mineral dusts can become coated with inorganic or organic material while in the atmosphere [Hinz et al., 2005; McNaughton et al., 2009; Sullivan et al., 2007; Usher et al., 2003; Wiacek and Peter, 2009], and these coatings are expected to originate from several sources, including pollution, (e.g., SO$_2$). Coated mineral dust particles may have different ice nucleation properties compared to pure mineral dusts, possibly causing changes in cloud formation conditions, cloud properties (e.g. thickness), cloud optical properties (e.g. absorption and reflectivity), and precipitation patterns. A recent modelling study by Girard et al. [2013]
investigated the effects of acid coated dust aerosols on the properties of Arctic clouds using laboratory results on uncoated and sulfuric acid coated kaolinite from Eastwood et al. [2008] and Eastwood et al. [2009] to parameterize ice nucleation in their model. Compared to simulations for uncoated dust particles, Girard et al. [2013] found that the inclusion of acid-coated dust particles significantly changed the microstructure of ice and mixed-phase clouds in the Arctic, causing an overall cooling effect. To expand on the previous work by Eastwood et al. [2008] and Eastwood et al. [2009] that studied uncoated and sulfuric acid coated kaolinite, the ice nucleation properties of a range of atmospherically relevant mineral dusts (kaolinite, illite, montmorillonite, quartz, see Figure 1.1) without coatings, and with acid coatings were investigated. The results from these experiments can be used to improve parameterizations for ice nucleation in climate models and to study how anthropogenic pollutants may impact the indirect aerosol effect caused by mineral dusts.

Mineral dusts have been shown to travel large distances in the atmosphere [Chiapello et al., 2005; Duce et al., 1980; Glaccum and Prospero, 1980; Prospero, 1999; Prospero et al., 1981], and ice nucleation on these aerosols may effect their long-distance transport. One reason it is important to understand the long-distance transport of mineral dusts is because they are an important source of iron to the oceans, an essential nutrient needed for ocean productivity [Mahowald et al., 2009; Schulz et al., 2012]. Mineral dust provides approximately 95 % of the global iron budget [Mahowald et al., 2009] and approximately 25 % of the global annual dust emissions are deposited in the ocean [Shao et al., 2011]. Also, several studies have shown increased levels of biological organisms during dust storms [Grishkan et al., 2012; Hara and Zhang, 2012; Hua et al., 2007; Jeon et al., 2011; Maki et al., 2010], indicating that mineral dusts may be an important mechanism for the spread of disease-causing microorganisms [Molesworth
et al., 2002; Polymenakou et al., 2008]. Lastly, the long-distance transport of mineral dusts has been associated with poor air quality and adverse health effect at locations far from the source [Cottle et al., 2013; De Longueville et al., 2010; Prospero, 1999; Prospero et al., 2008; Remoundaki et al., 2011]. For the reasons listed above, it is important to develop accurate models to predict the long-distance transport of mineral dusts in the atmosphere. The results presented in this dissertation for uncoated and acid coated mineral dusts (Chapter 2) may help to improve the description of ice nucleation on mineral dusts as a removal mechanism of these aerosols from the atmosphere.

1.6 Motivation for Studying Ice Nucleation on Biological Particles

Several field studies have identified biological particles as a potentially important component of the atmospheric ice nuclei population [Bowers et al., 2009; Christner et al., 2008a; Christner et al., 2008b; Conen et al., 2011; Creamean et al., 2013; Garcia et al., 2012; Jayaweera and Flanagan, 1982; Pratt et al., 2009; Prenni et al., 2012; Prenni et al., 2009b; Prenni et al., 2013]. The findings from these field studies have motivated a resurgence in laboratory research aimed at identifying and quantifying the ice nucleation properties of important biological ice nuclei that have been identified in the atmosphere (for example, see: Attard et al. [2012], Joly et al. [2013], Attard et al. [2012], Pummer et al. [2012], and the review by Després et al. [2012]). Using the results from these field and laboratory studies, some recent modeling studies have focused on the importance of biological ice nucleation for the climate indirect effect [Diehl and Wurzler, 2010; Diehl et al., 2006; Grützun et al., 2008; Hoose et al., 2010a; Levin et al., 1987; Phillips et al., 2009; Sesartic et al., 2012, 2013]. However, due to the limited knowledge on the numbers and sources of biological particles in the atmosphere, as well as their ice nucleation properties at atmospherically relevant conditions, the role of biological ice
nucleation in atmospheric processes remains uncertain and several recent reviews have called for continued research in this area [DeMott and Prenni, 2010; Després et al., 2012; Möhler et al., 2007; Morris et al., 2011].

Ice nucleation on biological particles, followed by precipitation, might be an important removal mechanism for these aerosols and could affect their long-distance transport. This is particularly important for biological allergens and disease-causing microorganisms that have adverse health impacts, and also for biological pathogens that can cause economic losses to agricultural crops. Current models that are used to predict the long-distance transport of biological particles include ice nucleation in a very simplistic manner, or not at all (see, for example: Andrade et al. [2009], Aylor [1986], Aylor [2003], Fröhlich-Nowoisky et al. [2012], Helbig et al. [2004], Isard et al. [2005], Kim and Beresford [2008], Magarey et al. [2007], Pan et al. [2006], Pasken and Pietrowicz [2005], Pfender et al. [2006], Skelsey et al. [2008], Wang et al. [2010], and Wilkinson et al. [2012]). A recent study by Burrows et al. [2013] showed that the long-distance transport of biological particles can be sensitive to the representation of ice nucleation in mixed-phase clouds. Here, the ice nucleation properties of fungal spores and bacteria, important components of the biological aerosol population (see Figure 1.2 and Section 1.1.2), have been determined from laboratory experiments. In Chapter 4 and Chapter 5, the laboratory results are used in two modelling studies to investigate the influence of ice nucleation on fungal spores on their long-distance transport. The modelling results suggest that ice nucleation can play an important role in the long-distance transport of biological particles.
1.7 Atmospheric Conditions Studied

1.7.1 Mechanisms for Heterogeneous Ice Nucleation

Four mechanisms for heterogeneous ice nucleation have been identified: deposition, immersion, condensation and contact ice nucleation. Of these mechanisms, deposition and immersion nucleation are studied in this dissertation and are depicted in Figure 1.4. In deposition nucleation, ice nucleates when water vapor adsorbs directly onto the IN surface and immersion nucleation involves the formation of ice on an IN contained within a liquid droplet.

Figure 1.4. Deposition and immersion heterogeneous ice nucleation. Squares represent solid particles (i.e. ice nuclei); a circle represents a liquid droplet; hexagons represent the formation of an ice crystal.

1.7.2 Temperature Range Studied

The vertical structure of the atmosphere consists of several layers that are characterized by reversals in the temperature gradient, as shown in Figure 1.5. The troposphere is the lowest layer and contains approximately 85 % of the total mass of the atmosphere; it extends from the Earth’s surface to where the temperature stops getting colder with increasing altitude, around 11
km [Jacob, 1999]. The troposphere contains all of the weather patterns (clouds, precipitation, etc.) we are familiar with on Earth [Ahrens, 2001]. All of the research presented in this dissertation was done at temperatures relevant for the troposphere, from 0 °C down to -40 °C.

Figure 1.5. Vertical structure of the atmosphere (temperature and pressure adapted from Wallace [2006] and Jacob [1999], respectively).

1.8 Overview of Dissertation

This research focuses on the heterogeneous ice nucleation properties of mineral dusts (uncoated and acid coated), fungal spores, and bacteria. Chapter 1 (this chapter) gives an introduction to atmospheric aerosol particles and highlights the motivation for studying their ice
nucleation properties; Chapter 2-Chapter 6 are the research chapters; and Chapter 7 summarizes the main conclusions and provides recommendations for future research.

Chapter 2 is a study on the deposition ice nucleation properties of uncoated and acid coated mineral dusts (kaolinite, illite, montmorillonite, and quartz) as well as a biological IN (SNOMAX). In this study the ice nucleating ability of an IN is described using the onset conditions, as is often done in deposition ice nucleation studies. The onset conditions correspond to the temperature and relative humidity over ice (RH$_i$) at which the first ice nucleation event is observed for a sample of particles (mineral dust or SNOMAX) in a given experiment.

Immersion ice nucleation of biological particles is studied in Chapter 3-Chapter 6. In these experiments, ice nucleation ability is described using the freezing temperatures of a population of droplets containing a particular species of biological particle (fungal spores or bacteria). Chapter 3 describes a new experimental method that was developed to aerosolize fungal spores from cultures, and provides immersion ice nucleation results for the fungus *Cladosporium*, one of the most abundant fungal spores in the atmosphere. In Chapter 4, the immersion ice nucleation properties of rust and bunt fungal spores that are pathogenic to agricultural crops are presented, and used together with particle dispersion modeling results to discuss the likelihood that these spores reach freezing altitudes in the atmosphere and how immersion ice nucleation might affect their long-distance transport. The rust species studied were *Puccinia graminis*, *Puccinia triticina*, *Puccinia allii*; and the bunt species studied include *Tilletia laevis* and *Tilletia tritici*. Chapter 5 is a study on the immersion ice nucleation properties of 11 species of fungal spores from the classes *Agaricomycetes* (*Lepista nuda*, *Agaricus bisporus*, *Amanita muscaria*, *Boletus zelleri*, *Trichaptum abietinum*), *Ustilagomycetes* (*Ustilago nuda*, *Ustilago nigra*, *Ustilago avenae*), and *Eurotiomycetes* (*Penicillium* sp., *Penicillium*
*chrysogenum, Penicillium brevicompactum*). Using the immersion ice nucleation results on these fungal spores as input into a global chemistry-climate transport model, we discuss the implications of immersion ice nucleation on fungal spores for their long-distance transport in the atmosphere. Finally, the results from this chapter are combined with previous immersion ice nucleation studies on fungal spores to investigate whether there might be a systematic difference in the immersion ice nucleation properties of fungal spores belonging to the two main fungal phyla, *Ascomycota* and the *Basidiomycota*. Chapter 6 is a study on the immersion ice nucleation characteristics of five species of bacteria (*Pseudomonas syringae, Bacillus subtilis, Comamonas testosteroni, Pseudomonas putida*, and *Micrococcus luteus*) that have been identified in the atmosphere, and discusses the likelihood that these bacteria might be important atmospheric IN.
Chapter 2. Effects of Sulfate Coatings on the Ice Nucleation Properties of a Biological Ice Nucleus and Several Types of Minerals

2.1 Introduction

Mineral dust particles are abundant in the atmosphere and both laboratory and field studies have shown that mineral dust particles are effective heterogeneous IN. Laboratory studies indicate that mineral dust particles can lower the supersaturation required for ice formation compared to homogeneous nucleation. Field measurements have shown that these particles can have a significant effect on cloud formation and cloud properties [DeMott et al., 2003; Sassen, 2002; Sassen et al., 2003]. Measurements have also shown that the cores of ice crystals often contain mineral dust inclusions, indicating these particles play an important role in atmospheric ice formation [Cziczo et al., 2004; Heintzenberg et al., 1996; Twohy and Poellot, 2005].

While in the atmosphere, mineral dust particles can be coated with organic and inorganic material [Hinz et al., 2005; McNaughton et al., 2009; Sullivan et al., 2007; Usher et al., 2003; Wiacek and Peter, 2009]. These coatings may impact the ice nucleation properties of mineral dust [Gallavardin et al., 2008; Phillips et al., 2008]. Nevertheless, there have only been a few studies that have directly compared the ice nucleating ability of uncoated and coated mineral dust particles at atmospherically relevant conditions [Archuleta et al., 2005; Cziczo et al., 2009; Eastwood et al., 2009; Gallavardin et al., 2008; Kanji et al., 2008; Knopf and Koop, 2006; Möhler et al., 2008a; Möhler et al., 2008b; Salam et al., 2007]. A few other studies have measured the freezing properties of aqueous inorganic or organic solution droplets containing mineral dust particles [Ettner et al., 2004; Hung et al., 2003; Koop and Zobrist, 2009; Zobrist et al., 2008; Zuberi et al., 2002].
Recently, Eastwood et al. [2009] showed that sulfuric acid coatings (ammonium-to-sulfate ratio (ASR) = 0) can have a significant effect on the ice nucleation properties of kaolinite particles. Kaolinite particles can make up approximately 5-10 % of mineral dust mass [Glaccum and Prospero, 1980]. In this previous study, the authors showed that a sulfuric acid coating on kaolinite particles increased the relative humidity over ice (RH_i) required for ice nucleation compared to uncoated particles by approximately 30 %, consistent with recent results on Arizona test dust (ATD) and illite [Möhler et al., 2008a]. These authors also looked at the effect of ammonium sulfate coatings (ASR =2) on kaolinite. These coatings had a different effect than sulfuric acid coatings. At the coldest temperature studied (-37.1 °C), the ammonium sulfate coated particles were as efficient as the uncoated kaolinite particles, while at the warmer temperatures (-33.4 °C and -29.2 °C), the onset RH_i values were significantly higher than the uncoated case. These results suggest that the ammonium-to-sulfate ratio of the coating is important, at least for certain sizes.

In addition to mineral dust, biological particles may play an important role in the formation of ice clouds in the atmosphere [Ariya et al., 2009; Christner et al., 2008a; Christner et al., 2008b; Möhler et al., 2007; Phillips et al., 2009; Szyrmer and Zawadzki, 1997]. In particular, the bacteria Pseudomonas syringae (P. syringae) has been identified as an extremely efficient IN, demonstrating ice nucleating activity at temperatures as warm as -2 °C in purified samples [Möhler et al., 2007]. However, the abundance of biological particles in the atmosphere and their activity under atmospheric conditions remain poorly understood. The potential role of biological particles in atmospheric ice formation has recently been emphasized in field studies done in Wyoming and the Amazon basin [Pratt et al., 2009; Prenni et al., 2009b]. In both studies, the ice nuclei composition was dominated by mineral dust and biological particles.
Similar to mineral dust, biological particles can also be coated with inorganic and organic material in the atmosphere [DeMott et al., 2003; Lammel et al., 2005]. We are not aware of any studies that directly compare the ice nucleation properties of uncoated and coated biological particles. Studies have investigated the freezing of dilute aqueous solutions [Chen et al., 2002; Kawahara et al., 1995; Kawahara et al., 1996; Obata et al., 1993; Pouleur et al., 1992; Yin et al., 2005] and concentrated aqueous solutions [Koop and Zobrist, 2009] containing biological particles.

In the following, we expand on the previous work by Eastwood et al. [2009] and consider other mineral dusts which have been shown to be abundant in the atmosphere [Chester et al., 1972; Glaccum and Prospero, 1980; Usher et al., 2003], as well as a more complete range of sulfate coatings. The ice nucleation properties of SNOMAX, a proxy for biological ice nucleators, are also investigated. SNOMAX is produced from cells of Pseudomonas syringae that have been grown under optimal conditions to maximize ice nucleation. We interpret our results using classical nucleation theory. The results from this study should prove useful for understanding the effects of anthropogenic emissions of SO$_2$ and NH$_3$ on climate by influencing the ice nucleating properties of mineral dust and biological particles.

2.2 Experimental

2.2.1 Ice Nucleation Measurements

The apparatus used in these studies is shown in Figure 2.1 and has been used in several previous ice nucleation studies [Dymarska et al., 2006; Eastwood et al., 2008; Eastwood et al., 2009; Parsons et al., 2004; Wheeler and Bertram, 2012]. An optical microscope (Zeiss Axiolab A equipped with a 10× objective) coupled to a flow cell, with humidity (RH) and temperature control, was used to study the ice nucleation properties of mineral dust particles. Relative
humidity is the ratio of the water vapor partial pressure to the saturation vapor pressure multiplied by 100 and can be measured with respect to liquid water (RH_w) or ice (RH_i). The bottom surface of the flow cell supported the (coated or uncoated) mineral dust or SNOMAX particles. It consisted of a glass cover slide which had been treated with dichlorodimethylsilane to make a hydrophobic layer that reduced the probability of ice nucleation directly on the surface of the glass slide. A humidified flow of ultrapure helium (He) was passed over the particles, and the frost point temperature of the gas was measured downstream of the flow cell using a hygrometer. The frost point temperature corresponds to the temperature at which RH_i = 100 % and was used to calculate the water vapor pressure of the humidified gas stream. During an ice nucleation experiment, the RH_i was adjusted by keeping the frost point, and therefore the water vapor pressure, constant and adjusting the temperature of the flow cell. The temperature of the flow cell was controlled using a combination of a refrigerating circulator (ULT-95, Thermo Neslab) and an electrical heater supported by a temperature controller. The flow cell temperature was measured using a Pt-100 resistance temperature detector (RTD) and the particles were monitored throughout each experiment with a digital camera (Sony XC-ST50 equipped with a 0.4× reduction lens) connected to an optical microscope.
Figure 2.1 Schematic of the flow cell apparatus used to study ice nucleation on mineral dust (Chapter 2 i.e. this chapter) and biological particles (Chapter 3-Chapter 6). Shown in (a) is a general overview of the apparatus, including flow of humidified He gas, flow cell, microscope, digital camera and computer, and hygrometer used to measure RH. Shown in (b) is a detailed view of the flow cell.

Shown in Figure 2.2(a) is a pictorial representation of a typical deposition ice nucleation experiment. In the first step of an experiment, particles were deposited on the bottom surface of the flow cell. To begin an ice nucleation experiment, the temperature of the flow cell was set to room temperature to achieve a relative humidity of approximately 0 %. Next, the temperature was decreased rapidly until a RH$_i$ of ~80 % was reached. Next, to observe ice nucleation, the temperature of the flow cell was further decreased at a rate of 0.1 °C min$^{-1}$, corresponding to a RH$_i$ rate of approximately 1 % min$^{-1}$, until the first ice nucleation event was observed. The temperature and relative humidity at which the first nucleation event was observed are referred to as the onset conditions. Finally, the temperature of the flow cell was returned to room
temperature, causing the ice crystals on the slide to evaporate and allowing for the particle surface area to be determined. In Figure 2.2(b) are images from a deposition ice nucleation experiment on kaolinite particles, the first showing the particles before ice nucleation has occurred, and the second image was taken after ice had nucleated on a kaolinite particle and grown into a large ice crystal.

**Figure 2.2** Pictorial representation of deposition ice nucleation experiments on (coated and uncoated) mineral dust and SNOMAX particles. Shown in (a) are the temperature and RH profiles for a typical deposition ice nucleation experiment. Black circles represent aerosol particles and the blue hexagon represents an ice crystal. Shown in (b) are two images from a deposition ice nucleation experiment on kaolinite particles, one image before ice nucleation has occurred and one after ice has nucleated on a kaolinite particle and grown into a large ice crystal.
Typical experimental RH trajectories used in our experiments are illustrated in Figure 2.3. The three trajectories correspond to ice frost points of -26.5 °C, -31.5 °C, and -36.5 °C. Included in this figure is the threshold for homogeneous freezing of sulfuric acid droplets 8 μm in diameter at a freezing rate of 10 s⁻¹ [Koop et al., 2000]. The RH ramp rate was approximately 1 % min⁻¹. In a previous study in our group, experiments were carried out using a ramp rate of approximately 0.5 % min⁻¹. No difference in results was obtained, suggesting the aqueous coatings were in equilibrium with the water vapor [Eastwood et al., 2009]. Growth rate calculations of aqueous solution droplets have also been done in our group using the equations presented by Pruppacher and Klett [1997] to further confirm that the sulfuric coatings were in equilibrium with the gas-phase water vapor in our experiments. The uncertainty in our measurements due to non-equilibrium conditions is at most 3 % RH [Eastwood et al., 2009].
Figure 2.3 Typical experimental trajectories. The temperature was reduced at a rate of 0.1 °C min⁻¹ while the water vapor partial pressure was held constant. The trajectories correspond to ice frost points of -26.5 °C, -31.5 °C, and -36.5 °C, where the ice frost point is defined as the temperature at which RHᵢ = 100 %.

Trajectories were calculated using the saturation vapor pressures of water and ice from the parameterization of Murphy and Koop, [2005]. The dashed line represents the threshold for homogeneous freezing of sulfuric acid droplets 8 μm in diameter at a freezing rate of 10 s⁻¹[Koop et al., 2000].

2.2.2 Sample Preparation and Thickness of the Coatings

The mineral dusts used in this study are listed in Table 2.1 along with their chemical formulae.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolinite</td>
<td>Al₄Si₄O₁₀(OH)₈</td>
</tr>
<tr>
<td>Illite</td>
<td>(K,H₃O)(Al,Mg,Fe)₂(Si,Al)₄O₁₀[(OH)₂,H₂O]</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>(Na,Ca)₀.₃₃(Al,Mg)₂Si₄O₁₀(OH)₂•nH₂O</td>
</tr>
<tr>
<td>Quartz</td>
<td>SiO₂</td>
</tr>
</tbody>
</table>
Kaolinite and montmorillonite were purchased from Fluka; illite was provided by the Clay Minerals Society; and quartz was obtained from U.S. Silica. SNOMAX was purchased from York Snow, Inc. As mentioned, SNOMAX is produced from cells of *Pseudomonas syringae* that have been grown under optimal conditions to maximize ice nucleation frequency. The cells are then concentrated using ultrafiltration and frozen into pellets. Next, the pellets are freeze-dried and exposed to Beta irradiation to make a sterile product [*Lee et al., 1995*]. Note that although SNOMAX is made from cells of *Pseudomonas syringae*, it does not display the same freezing spectrum (fraction frozen vs. temperature) as some naturally occurring strains of *P. syringae*. Also, note that the freezing spectrum of naturally occurring *P. syringae* can vary significantly from strain to strain [*Gross et al., 1983; Hirano et al., 1985; Möhler et al., 2008c; Ward and DeMott, 1989*].

Coated particles were prepared by mixing the minerals or SNOMAX with the coating material in high-purity water to create a suspension. The composition of the suspension was 1 weight % (wt%) mineral or SNOMAX and 0.2 wt% coating material. This suspension was then placed in an ultrasonic bath for 10 minutes and then stirred for approximately two to four days to ensure the coating material had adequate time to interact with the heterogeneous IN. To deposit the particles on the glass slide, the suspension was passed through a nebulizer using high-purity nitrogen (N₂) as a carrier gas. The flow from the nebulizer was directed at a hydrophobic glass slide and droplets containing the particles were deposited on the surface of the slide upon impaction. Water then evaporated, leaving behind the coated particles. Coated particles produced by this method had an average weight fraction of H₂SO₄ or NH₄HSO₄ of 0.167 under dry conditions.
Uncoated illite, quartz and SNOMAX particles were prepared using a procedure similar to the above. First, these minerals or SNOMAX particles were mixed in high-purity water (composition was 1 wt% mineral or SNOMAX) to create a suspension. The suspension was then placed in an ultrasonic bath for 10 minutes and then stirred for two to four days to be consistent with the experimental procedure for the coating experiments. These suspensions were then nebulized, creating droplets on the hydrophobic slides. Water then evaporated, leaving behind the uncoated particles.

For uncoated kaolinite and montmorillonite, we use the results previously published by our group [Eastwood et al., 2008; Eastwood et al., 2009]. In these previous experiments, uncoated kaolinite was suspended in water and then nebulized as described above. For montmorillonite, the particles were produced by dry dispersion. This involved placing the dry particles in a glass vessel immersed in an ultrasonic bath and a flow of ultra-high-purity N₂ was passed through the glass vessel to entrain the particles. The flow was then directed at the hydrophobic glass slide, and the particles were deposited on the slide by impaction.

The thickness of the coatings in our experiments was estimated based on the compositions of the starting suspensions and assuming a spherical core shell model (e.g. a kaolinite core surrounded by a uniform H₂SO₄ or NH₄HSO₄ coating). According to our calculations, under dry conditions (<1 % RHᵢ) a kaolinite core with a diameter of 15 µm will have a 0.7 µm thick coating, and a 5 µm core will have a coating of 0.2 µm. A coating of 0.2 µm represents at least a few hundred sulfate layers covering the surface of the particle.

Previously in our group, the thickness of the coatings on kaolinite particles (using the same technique for particle production as discussed above) was further characterized by monitoring the change in particle size as the relative humidity with respect to water (RHᵢ) was
increased from < 1 % to 95 % [Eastwood et al., 2009]. From the change in size, we estimated the total amount of water adsorbed when cycling between < 1 % and 95 % RH\textsubscript{w} using the thermodynamic model of Clegg et al. [1998]. From this, we estimated the amount of H\textsubscript{2}SO\textsubscript{4} on each particle and, in turn, the thickness of the H\textsubscript{2}SO\textsubscript{4} coating under dry conditions. Measurements made for 15 individual particles yielded an average weight fraction for the coating of 0.12 ± 0.07, under dry conditions. The uncertainty in this value derives from the uncertainty in the relative humidity measurements.

Two different types of nebulizers were used in our studies. For the minerals, we used an in-house design. This resulted in average particle sizes ranging from 6-10 \textmu m. For the SNOMAX particles, we also used this in-house design, with average sizes ranging from approximately 16-23 \textmu m. To generate smaller SNOMAX particles, we used a commercially available design (Meinhard Glass Products, Model Number TR-30-A1). The commercial nebulizer generated SNOMAX particles with average sizes of approximately 6-7 \textmu m. Note the SNOMAX particles considered in our studies are most likely agglomerates of several SNOMAX cells, which are typically 1-2 \textmu m, and/or cell fragments. See below for more details on particle size distributions used in the experiments.
2.2.3 **Particle Number, Particle Sizes, and Total Surface Area**

In typical freezing experiments, a sample held between 100 and 1000 individual particles. Hence, our results correspond to when 0.1 to 1% of the particles nucleated ice. The total surface area of mineral dust or SNOMAX deposited in any particular experiment ranged from $2 \times 10^{-5}$ to $3 \times 10^{-3}$ cm$^2$. The mean diameters and standard deviations for all particle types considered in this study are presented in Table 2.2.

**Table 2.2 Particle sizes and standard deviations for the mineral dust and SNOMAX particles studied.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Coating</th>
<th>Size (μm)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolinite</td>
<td>None</td>
<td>7.7</td>
<td>5.3</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>H$_2$SO$_4$</td>
<td>7.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>NH$_4$HSO$_4$</td>
<td>10.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>6.9</td>
<td>5.0</td>
</tr>
<tr>
<td>Illite</td>
<td>None</td>
<td>5.9</td>
<td>3.8</td>
</tr>
<tr>
<td>Illite</td>
<td>H$_2$SO$_4$</td>
<td>7.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>None</td>
<td>8.1</td>
<td>n.a.$^c$</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>H$_2$SO$_4$</td>
<td>7.7</td>
<td>3.8</td>
</tr>
<tr>
<td>Quartz</td>
<td>None</td>
<td>8.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Quartz</td>
<td>H$_2$SO$_4$</td>
<td>10.1</td>
<td>6.5</td>
</tr>
<tr>
<td>SNOMAX$^f$</td>
<td>None</td>
<td>5.8</td>
<td>3.7</td>
</tr>
<tr>
<td>SNOMAX$^f$</td>
<td>H$_2$SO$_4$</td>
<td>6.6</td>
<td>5.1</td>
</tr>
<tr>
<td>SNOMAX</td>
<td>None</td>
<td>15.9</td>
<td>8.9</td>
</tr>
<tr>
<td>SNOMAX</td>
<td>H$_2$SO$_4$</td>
<td>22.8</td>
<td>15.6</td>
</tr>
</tbody>
</table>

---

$^c$ n.a. (not available)  
$f$ These particles were deposited using a commercially available nebulizer, all other particles were deposited using an in-house made nebulizer.
2.3 Results and Discussion

2.3.1 Effect of Sulfuric Acid Coatings on the Ice Nucleation Properties of Different Minerals

Shown in Figure 2.4 are the onset RH\textsubscript{i} values for uncoated and sulfuric acid coated kaolinite, illite, montmorillonite, and quartz particles plotted as a function of surface area. A logarithmic scale for surface area was used to reduce the spread in the data and to allow for a comparison to be made between the onset RH\textsubscript{i} values for the different mineral dusts studied.

![Figure 2.4 Ice nucleation measurements on uncoated (closed symbols) and H\textsubscript{2}SO\textsubscript{4} coated (open symbols) kaolinite, illite, montmorillonite and quartz particles. Data are plotted as onset RH\textsubscript{i} against surface area (cm\textsuperscript{2}). Surface area is plotted on a logarithmic scale to reduce spread in the data. Each data point represents a single freezing event and the error bars arise from experimental uncertainty. All experiments were done at an ice frost point of -36.5 °C. Kaolinite results are taken from Eastwood et al. [2009]; uncoated montmorillonite results are taken from Eastwood et al. [2008]. The dashed line represents the threshold for homogeneous freezing of sulfuric acid droplets 8 μm in diameter at a freezing rate of 10 s\textsuperscript{-1}[Koop et al., 2000].}
Each data point corresponds to one freezing event, and the error bars represent the uncertainty in measuring the frost point and temperature at the onset. All experiments were carried out with an ice frost point of -36.5 °C. Included is the RH, at which liquid water saturation occurs for this experimental trajectory. The RH, necessary for water saturation was determined by calculating the RH, at which the experimental trajectory crosses the water saturation line in Figure 2.3. Also included in Figure 2.4 is the threshold for homogeneous freezing of sulfuric acid droplets. This threshold was determined by calculating the RH, at which the experimental trajectory crosses the homogeneous freezing line for sulfuric acid in Figure 2.3. As mentioned above, the data for kaolinite (uncoated and coated) and montmorillonite (uncoated) are taken from Eastwood et al. [2009] and Eastwood et al. [2008], respectively, and are plotted here again for comparison purposes. All other data were obtained during this study. The total surface area in any particular experiment ranged from approximately \(2\times10^{-3}\) to \(3\times10^{-3}\) cm\(^2\). Over this relatively narrow range, the onset results did not depend strongly on the surface area, so we combined the data and compared the averages and the 95 % confidence intervals for the coated and uncoated cases in Figure 2.5.
Figure 2.5 Average onset values for uncoated and sulfuric acid coated kaolinite, illite, montmorillonite and quartz particles studied using a frost point of -36.5 °C. Error bars represent the 95 % confidence intervals. The dashed line represents the threshold for homogeneous freezing of sulfuric acid droplets 8 μm in diameter at a freezing rate of 10 s⁻¹ [Koop et al., 2000].

The data show that, for kaolinite and illite, the coatings have a major impact on the RH_i required for ice nucleation; the coating increased the onset RH_i value by about 30 %. The effect for montmorillonite and quartz is smaller; in this case, the increase in onset RH_i is about 20 %, but there is still a statistically significant effect. It is interesting to note that for kaolinite, montmorillonite and quartz, the average onset values fall below water saturation and the conditions necessary for homogeneous freezing of the H_2SO_4 coating. As a result, heterogeneous freezing on these mineral cores is most likely still the dominant mechanism for nucleation in our experiments. For the case of illite, the average onset RH_i overlaps with the conditions necessary for homogeneous nucleation of the H_2SO_4 coating if one considers the confidence intervals. This
suggests that the coatings on illite may be “shutting off” heterogeneous freezing and favoring homogeneous nucleation of the aqueous coating.

Recently, there have been several studies of the effect of sulfuric acid coatings on the ice nucleation properties of mineral dust particles. In a majority of the studies, a significant reduction in the ice nucleation efficiency after sulfuric acid coating was observed [Archuleta et al., 2005; Cziczo et al., 2009; Gallavardin et al., 2008; Möhler et al., 2008a]. Specifically, the coatings lead to an increase in the RH\textsubscript{i} required for ice nucleation [Archuleta et al., 2005; Cziczo et al., 2009; Gallavardin et al., 2008; Möhler et al., 2008a], and the coated particles often required saturations approaching those for homogeneous freezing of aqueous solutions, as observed here [Cziczo et al., 2009; Möhler et al., 2008a]. However, in some cases, the impact of the coating was much less significant [Archuleta et al., 2005; Cziczo et al., 2009] or was not significant at all [Knopf and Koop, 2006], and for some minerals a decrease in RH\textsubscript{i} necessary for ice nucleation was observed [Archuleta et al., 2005]. Differences in particle size, particle type, coating thickness, and temperature range studied may account for the variation between these results.

Field measurements also support the hypothesis that atmospheric processing of mineral dust leads to a reduced ice nucleation ability. Phillips et al. [2008] compared field and laboratory data and concluded that atmospheric processing leads to a reduced ice nucleation efficiency. Prenni et al. [2009b] noted the near absence of ice nuclei composed of mixed dust and sulphate, suggesting that coatings may affect the ability of these particles to act as ice nuclei. DeMott et al. [2003] noted that mineral dust particles that acted as good ice nuclei were relatively pure in form.
The mechanism responsible for the deactivation of the ice nucleation ability of mineral dusts may be related to the mineral surface and how this surface interacts with the sulfuric acid coatings. Kaolinite, illite and montmorillonite are clay minerals composed of layers of aluminosilicate sheets. The structure of quartz consists of silicon-oxygen tetrahedrons linked by shared oxygen atoms [Gualtieri, 2000; Viani et al., 2002].

The composition of the sulfuric acid coating at the beginning of each experiment was very acidic; the starting pH of the coating was below zero. The Point of Zero Charge (PZC) is defined as the pH at which the net surface charge is zero for a particular material [Stumm, 1992], and there is a PZC associated with the surfaces of each mineral dust studied. Kaolinite surfaces have PZC’s at pH 6 or above depending on the crystalline face [Stumm, 1992]; the surfaces of illite and montmorillonite have PZC’s at 2.2 or higher [Kriaa et al., 2009; Parks, 1965; Rozalen et al., 2009]; and quartz has a PZC of 2.2 [Parks, 1965]. Accordingly, the exposed areas of the mineral particles would be protonated under the acidic conditions in our experiments. The positively charged, protonated environment should facilitate strong adsorption of sulfate anions to the mineral surface, changing the chemical and physical properties of the surfaces [Eastwood et al., 2009]. It is therefore hypothesized that the ice nucleation properties of the mineral dusts would change when coated with sulfuric acid. Molecular simulations, similar to recent simulations on uncoated mineral surfaces, would be useful to better understand these processes [Croteau et al., 2008; Hu and Michaelides, 2007, 2008].

2.3.2 Effect of Ammonium-to-Sulfate Ratio on the Ice Nucleation Properties of Kaolinite

Shown in Figure 2.6 are the results for kaolinite coated with ammonium bisulfate. Also included for comparison are the results for uncoated, sulfuric acid coated and ammonium sulfate coated kaolinite particles.
Figure 2.6 Onset results for NH$_4$HSO$_4$ coated kaolinite particles (open triangles). Included for comparison are previous results for uncoated kaolinite, H$_2$SO$_4$ coated and (NH$_4$)$_2$SO$_4$ coated kaolinite particles (filled symbols) [Eastwood et al., 2009]. Error bars are given as 95 % confidence intervals. Results shown are an average of at least 6 separate measurements. The dotted line represents the deliquescence relative humidity (DRH) for ammonium sulfate; the DRH line for NH$_4$HSO$_4$ lies below 70 % RH over the temperature range shown. The dashed line represents the threshold for homogeneous freezing of sulfuric acid droplets 8 µm in diameter at a freezing rate of 10 s$^{-1}$ [Koop et al., 2000].

Each data point in this figure is the average from at least six separate measurements done for each particle type and temperature. The error bars represent 95 % confidence intervals of the onset RH$_i$ values.

From Figure 2.6, one can conclude that kaolinite particles coated with ammonium bisulfate are less efficient ice nuclei than uncoated kaolinite particles; the coating increased the onset RH$_i$ by approximately 18 to 26 % compared to uncoated kaolinite particles. Also, in general, it appears that sulfuric acid coatings (ASR=0) have the largest effect on the nucleation
properties of kaolinite. This is most clear at the lowest temperatures studied. Ammonium bisulfate coatings (ASR=1) appear to be intermediate between sulfuric acid and ammonium sulfate. Again, these differences are clearest at the lowest temperatures.

As suggested above, the sulfuric acid coating may hinder ice nucleation by protonation of the kaolinite surface and by adsorption of sulfate anions to the protonated surface. The pH of the ammonium bisulfate coating at the beginning of each experiment was 0.87. Hence, a similar effect can occur for ammonium bisulfate, which would explain why ammonium bisulfate also changes significantly the ice nucleation properties of kaolinite particles. It is also possible that the acid solutions irreversibly react with the mineral surfaces. For example, recently it was shown that when mineral surfaces are exposed to pH values less than 1.0, an increase in dissolution of aluminosilicates with decreasing pH was observed as well as precipitation of an amorphous silica phase [Shaw et al., 2009]. These processes could be occurring in our experiments (and in the atmosphere) and potentially could explain the difference between the sulfuric acid and ammonium bisulfate coatings, since the sulfuric acid solutions will have lower pH values and lead to increased dissolution rates.

Figure 2.6 also shows that ammonium sulfate coatings (ASR = 2) are most sensitive to temperature. This is most likely related to the phase of the coatings. H$_2$SO$_4$ does not undergo deliquescence and efflorescence and NH$_4$HSO$_4$ deliquesces at < 70 % RH over the temperature range studied here. As a result, H$_2$SO$_4$ and NH$_4$HSO$_4$ coatings will remain liquid during our experiments. In contrast, (NH$_4$)$_2$SO$_4$ coatings can remain solid for some of the conditions used in our experiments. The deliquescence relative humidity for (NH$_4$)$_2$SO$_4$ is shown in Figure 2.6 as a dotted line. If the (NH$_4$)$_2$SO$_4$ coatings are crystalline, this solid can also act as a heterogeneous ice nuclei (at least for bigger particles) [Abbatt et al., 2006; Baustian et al., 2010;
Shilling et al., 2006; Zuberi et al., 2002. For a further discussion of the \((\text{NH}_4)_2\text{SO}_4\) results as well as an explanation of the temperature trend observed for \((\text{NH}_4)_2\text{SO}_4\) coatings, see Eastwood et al. [2009].

2.3.3 Ice Nucleation on Uncoated SNOMAX

The onset results for uncoated and sulfuric acid coated SNOMAX particles are presented as a function of surface area in Figure 2.7. Each data point represents one freezing event and the error bars represent the uncertainty in RH_i.

![Figure 2.7 Ice nucleation measurements on uncoated (closed symbols) and H_2SO_4 coated (open symbols) SNOMAX particles. Data are plotted as onset RH_i against surface area (cm$^2$). Squares and circles represent particles made using a commercially available and in-house built nebulizer, respectively. The dashed line in the left-hand panel represents the threshold for homogeneous freezing of sulfuric acid droplets 8 µm in diameter at a freezing rate of 10 s$^{-1}$[Koop et al., 2000]; this line lies above water saturation for the middle and right-hand panels.](image-url)

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The onset data for SNOMAX has been summarized as a function of temperature in Figure 2.8. The results for sulfuric acid coated SNOMAX will be discussed in the next section. The results for the uncoated case, shown in Figure 2.7 and Figure 2.8, show that ice nucleation occurs at approximately 110-120 % RH, independent of temperature. This indicates that SNOMAX is a reasonably good ice nucleus at atmospherically relevant conditions.

![Figure 2.8 Onset results for uncoated (filled symbols) and H$_2$SO$_4$ coated (open symbols) SNOMAX. Open stars represent freezing of aqueous acid solution drops containing SNOMAX inclusions from Koop and Zobrist, [2009]. Error bars represent the 95 % confidence intervals. Results shown are an average of at least 3 separate measurements. Squares and circles represent particles made using a commercially available and in-house built nebulizer, respectively. The dashed line represents the threshold for homogeneous freezing of sulfuric acid droplets 8 µm in diameter at a freezing rate of 10 s$^{-1}$[Koop et al., 2000].](image-url)
The ice nucleation properties of SNOMAX have been investigated in other studies [Möhler et al., 2008c; Ward and DeMott, 1989; Wood et al., 2002], but these measurements were done at -15 °C and above. In addition to the current study, there has only been one previous study to look at freezing of these particles at lower temperatures [Kanji et al., 2011]. There have also been numerous studies on the ice nucleation properties of unaltered *P. syringae*. These studies have also focused on warmer temperatures than employed in the current study, so the results are not directly comparable [Lindow et al., 1978; Lindow et al., 1989; Möhler et al., 2008c; Vali, 1971; Wolber et al., 1986].

2.3.4 Effect of Sulfuric Acid Coatings on the Ice Nucleation Properties of SNOMAX

The ice nucleation results for uncoated and sulfuric acid coated SNOMAX particles are compared in Figure 2.7 and Figure 2.8. Unlike the mineral dust results, the sulfuric acid coatings did not hinder the ice nucleating ability of SNOMAX particles.

The fact that the uncoated and coated results were similar was somewhat surprising in light of the mineral dust results presented earlier in this paper. We offer here a few different explanations. First, it is possible that a few SNOMAX particles were not completely covered with the acid solution, providing a site for ice nucleation. This did not occur in the mineral dust studies, which had identical experimental conditions. Nevertheless, it may have occurred in the SNOMAX studies. Unfortunately, it is not possible to verify that every particle is completely covered in our experiments. Even if some SNOMAX particles were not covered completely, these particles were still exposed to a dilute acid solution (2×10⁻² M H₂SO₄, pH 1.6) for 2-4 days prior to nebulization. At a minimum, our results show that long exposure to dilute acid solutions does not modify the ice nucleation properties of SNOMAX particles at the temperatures and relative humidities studied.
Another possible explanation for the coated SNOMAX results is that the acid solution does not significantly modify active ice nucleation sites present on SNOMAX. It is thought that the reason SNOMAX and unaltered *P. syringae* are good IN is due to a certain protein located in the outer cell membrane. Experimental evidence has shown that the protein forms aggregates on the outer membrane in such a manner that the hydrophilic repetitive region of the protein provides a lattice match for the hydrogen bonding requirements of ice [Green and Warren, 1985; Gurian-Sherman and Lindow, 1993; Lee et al., 1995; Morris et al., 2004; Szyrmer and Zawadzki, 1997]. Our current results may suggest that sulfuric acid solutions do not modify this environment significantly enough to influence ice nucleation for the temperatures and RH values explored.

Recently, the freezing properties of dilute and concentrated acid solution droplets containing SNOMAX were studied using differential scanning calorimetry [Koop and Zobrist, 2009]. These authors reported results in terms of freezing temperatures and water activities. Shown in Figure 2.8 are the freezing conditions predicted by the Koop and Zobrist [2009] data for the temperatures used in our experiments. These predictions are in good agreement with the results we obtained for coated particles. This agreement provides some support that the particles in our experiments are completely coated and, also, that coatings by acids have relatively little effect on the freezing conditions, at least for the temperature range we studied.

Several studies also investigated the freezing properties of dilute acid solutions containing other species of bacteria, many in the *Pseudomonas* genera, known to be effective IN. These studies were done at warm temperatures, and as a result, are not directly comparable to our studies. Nevertheless, it is interesting to note that in the experiments where an acid effect was observed, the freezing temperature, even in the acidic solutions, was still above -11 °C; which is
above the temperature range employed in our studies [Chen et al., 2002; Kawahara et al., 1996; Obata et al., 1993; Pouleur et al., 1992; Yin et al., 2005]. Also, one study noted that the freezing temperature of one species of *Pseudomonas* was not sensitive to the pH range studied (3.5 to 5.0) [Kawahara et al., 1995].

### 2.3.5 Nucleation Rate, $J_{het}$

The heterogeneous nucleation rate, $J_{het}$, is defined as the number of nucleation events per unit surface area of solid material per unit time. Note that $J_{het}$ is referred to as both a rate [Archuleta et al., 2005; Hung et al., 2003; Pruppacher and Klett, 1997] and a rate coefficient [Dymarska et al., 2006; Marcolli et al., 2007] in the literature. The heterogeneous nucleation rate is related to the onset data through equation (2.1):

$$J_{het} = \frac{\omega}{A_s t},$$

(2.1)

where $\omega$ is the number of ice crystals nucleated, $A_s$ is the total mineral dust/SNOMAX surface area available for heterogeneous nucleation, and $t$ is the observation time. At the onset of ice nucleation, $\omega$ was equal to one. Table 2.3 lists the nucleation rates determined in our experiments.
Table 2.3 $J_{\text{het}}$ values and contact angles ($\theta$) for uncoated and sulfuric acid coated mineral dusts and uncoated SNOMAX.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Type</th>
<th>Onset Temperature ($^\circ$C)</th>
<th>RH$_1$ (%)</th>
<th>$J_{\text{het}}$ (cm$^{-2}$s$^{-1}$)</th>
<th>$J_{\text{het, upper}}$ (cm$^{-2}$s$^{-1}$)</th>
<th>$J_{\text{het, lower}}$ (cm$^{-2}$s$^{-1}$)</th>
<th>$\theta_{\text{lower}}$ ($^\circ$)</th>
<th>$\theta$ ($^\circ$)</th>
<th>$\theta_{\text{upper}}$ ($^\circ$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaolinite</td>
<td>Pure</td>
<td>-37.0</td>
<td>104±2</td>
<td>281</td>
<td>2814</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>Coated</td>
<td>-39.2</td>
<td>134±5</td>
<td>224</td>
<td>2237</td>
<td>2</td>
<td>58</td>
<td>72</td>
<td>100</td>
</tr>
<tr>
<td>Illite</td>
<td>Pure</td>
<td>-36.5</td>
<td>112±5</td>
<td>1292</td>
<td>12921</td>
<td>13</td>
<td>11</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Illite</td>
<td>Coated</td>
<td>-38.9</td>
<td>142±6</td>
<td>1000</td>
<td>10000</td>
<td>10</td>
<td>64</td>
<td>79</td>
<td>109</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>Pure</td>
<td>-37.3</td>
<td>108±4</td>
<td>931</td>
<td>9307</td>
<td>9</td>
<td>8</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Montmorillonite</td>
<td>Coated</td>
<td>-38.0</td>
<td>125±5</td>
<td>1281</td>
<td>12808</td>
<td>13</td>
<td>48</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Quartz</td>
<td>Pure</td>
<td>-37.3</td>
<td>120±5</td>
<td>606</td>
<td>6057</td>
<td>6</td>
<td>17</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>Quartz</td>
<td>Coated</td>
<td>-38.7</td>
<td>140±4</td>
<td>805</td>
<td>8046</td>
<td>8</td>
<td>63</td>
<td>78</td>
<td>108</td>
</tr>
<tr>
<td>SNOMAX</td>
<td>Pure</td>
<td>-37.1</td>
<td>116±6</td>
<td>127</td>
<td>1271</td>
<td>1</td>
<td>15</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>SNOMAX</td>
<td>Pure</td>
<td>-31.7</td>
<td>112±2</td>
<td>94</td>
<td>936</td>
<td>1</td>
<td>13</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>SNOMAX</td>
<td>Pure</td>
<td>-26.8</td>
<td>116±1</td>
<td>137</td>
<td>1370</td>
<td>1</td>
<td>16</td>
<td>19</td>
<td>22</td>
</tr>
</tbody>
</table>
The uncertainty in $J_{het}$ was determined by considering the uncertainties in $A_s$ and $t$. We used 10 s for the observation time with an upper limit of 20 s (the time between image captures) and a lower limit of one second. Note, however, that nucleation may have happened at a shorter time than one second. If this is the case, the calculated nucleation rates will be lower limits to the true nucleation rates. For the surface area, we used the geometric surface area of the particles determined directly from the optical microscope. For an upper limit to the surface area, we multiplied the geometric surface area of the particles by a factor of 50, based on scanning electron microscope measurements of kaolinite particles [Eastwood et al., 2008]. The data shown in Table 2.3 suggest that our experiments are typically sensitive to values of $J_{het}$ ranging from 2 to 13000 cm$^{-2}$s$^{-1}$.

### 2.3.6 Classical Nucleation Theory Parameters from $J_{het}$

The applicability of standard classical nucleation theory to heterogeneous nucleation on minerals and biological particles remains to be determined. In fact, some measurements show that, for precise predictions, active site theory is required [Archuleta et al., 2005; Hung et al., 2003; Marcolli et al., 2007]. Nevertheless, classical nucleation theory is a relatively convenient and simple way to parameterize laboratory data. Hence, classical nucleation theory is a reasonable starting point for analyzing our experimental data. Below, we analyze the nucleation rates using classical nucleation theory. From this analysis, we determined the contact angle between an ice embryo and the mineral surface.

In this analysis, we focus on the results for uncoated minerals, uncoated SNOMAX and sulfuric acid coated minerals studied at an ice frost point of -36.5 °C. We did not do a similar analysis for the ammonium bisulfate coated studies since the thermodynamic parameters of the ammonium bisulfate solution needed for the calculations are not readily available. Also, we...
excluded the sulfuric acid coated SNOMAX results since the coated results were not statistically different from the uncoated results.

All of the uncoated results that we focused on for the classical nucleation theory analysis corresponds to deposition freezing. To convert the nucleation rates for these deposition freezing results to contact angles, we used the procedure outlined in our previous paper [Eastwood et al., 2008]. For details on the calculations, please see this previous publication.

All the sulfuric acid coated results that were analyzed using classical nucleation theory correspond to immersion freezing. According to standard classical nucleation theory, the rate of heterogeneous nucleation ($J_{het,imm}$) by immersion freezing is defined as [Archuleta et al., 2005; Pruppacher and Klett, 1997; Tabazadeh et al., 1997; Tabazadeh et al., 2000]:

$$J_{het,imm} = A \exp \left( \frac{-\Delta F_{g,het} - \Delta g}{kT} \right),$$  \hspace{1cm} (2.2)

where $A$ is the pre-exponential factor in units of cm$^{-2}$ s$^{-1}$, $\Delta F_{g,het}$ is the free energy of formation of the critical embryo on the surface in units of J, $k$ is the Boltzmann constant in J K$^{-1}$, $T$ is the onset temperature in K, and $\Delta g$ is the activation energy for the diffusion of a water molecule across the ice-water interface in J.

The critical embryo is approximated as a spherical cap on a curved surface. The free energy of formation of the critical embryo for immersion freezing can be described by the following equation [Pruppacher and Klett, 1997]:

$$\Delta F_{g,het} = \frac{4}{3} \pi r_g^2 \sigma_{ij} f(m,x),$$  \hspace{1cm} (2.3)
where \( r_g \) is the radius of the ice embryo; \( \sigma_{i/s} \) is the surface tension at the ice-sulfuric acid solution interface; \( f(m,x) \) is the geometric factor; \( m \) is the compatibility parameter for ice on a solid substrate; and \( x \) is the ratio of the radius of the substrate to the radius of spherical ice germ.

Assuming the radius of the substrate to be much larger than the radius of the ice germ (a good approximation under our experimental conditions), \( f(m,x) \) is defined as follows:

\[
f(m,x) = \frac{m^3 - 3m + 2}{4}. \tag{2.4}
\]

The compatibility parameter, \( m \), is equal to \( \cos \theta \), where \( \theta \) is the contact angle between an ice nucleus and the solid surface.

Also, the radius of the ice embryo is given (in cm) as \([Archuleta et al., 2005; DeMott and Lynch, 2002; Khvorostyanov and Sassen, 1998]\):

\[
r_g = \frac{2\sigma_{i/s}}{\rho_i L_{ef} \left[ \ln \left( \frac{T_0}{T} \right) a_w \right]}. \tag{2.5}
\]

where \( \rho_i \) (g cm\(^{-3}\)) is the temperature dependent density of ice, \( \sigma_{i/s} \) (erg cm\(^{-2}\)) is as defined above, \( L_{ef} \) (cal g\(^{-1}\)) is the effective latent heat of formation, \( T_0 \) is the triple point of water, \( a_w \) is the water activity, and \( G \) is a dimensionless parameter equal to \( RT/L_{ef} M_w \) \([Khvorostyanov and Sassen, 1998]\), where \( R \) is the gas constant and \( M_w \) is the molecular weight of water.

We calculated \( \theta \) according to the method used by Archuleta et al. [2005]. First, equation (2.2) was used to obtain \( \Delta F_{g,het} \) using the temperature dependent expression for \( \Delta g \) (ergs \( \times 10^{13} \)) from Tabazadeh et al. [1997], the experimentally determined \( J_{het,imm} \) values from equation (2.1), and a pre-exponential factor, \( A \), equal to \( 10^{20} \) cm\(^{-2}\) s\(^{-1}\) [Fletcher, 1969; Hung et al., 2003]. Second, equation (2.5) was used to find \( r_g \). In this calculation, we used \( \rho_i \) from Pruppacher and Klett.
[1997], $\sigma_{\theta}$ from Tabazadeh et al. [2000], and $L_{ef}$ from Khvorostyanov and Sassen [1998]. Third, equation (2.3) was used to find $f(m,x)$. Fourth, equation (2.4) can be evaluated for $m$, and finally, the compatibility parameter can be used to find the contact angle, $\theta$.

In Table 2.3, the nucleation rates and contact angles calculated using the procedures discussed above are listed for uncoated and sulfuric acid coated mineral dust particles, as well as the uncoated SNOMAX results. The upper and lower limits for $J_{het}$ (and hence $\theta$) were determined using the upper and lower limits to the observation times in our experiments and the upper limits to the surface area estimated from scanning electron microscopy (see Section 2.3.5 for more details). The contact angle values for measurements done at an ice frost point of -36.5 °C are also illustrated in Figure 2.9.

![Figure 2.9](image)

**Figure 2.9** Average contact angles for uncoated and sulfuric acid coated mineral dust particles and uncoated SNOMAX particles studied at a frost point of -36.5 °C. Error bars are given as 95 % confidence intervals. The sulfuric acid coated SNOMAX results were excluded since the coated results were not statistically different from the uncoated results.
The data show that for uncoated ice nuclei the contact angles are small (below approximately 20°). For mineral dust particles coated with sulfuric acid, the contact angles are larger (above approximately 60°). These values may be useful for future modeling studies of ice nucleation in the atmosphere and for comparing results between different laboratories. However, keep in mind that our calculations assume one contact angle for a given sample type. In reality, particles within a given sample type may have a range of ice nucleation efficiencies and hence a range of contact angles [Archuleta et al., 2005; Hung et al., 2003; Marcolli et al., 2007]. If this is the case, contact angles determined from onset conditions (as done in the current study) may overestimate the nucleation rate on the same sample exposed to longer nucleation times or RH values above the onset values. For these reasons, extrapolation outside our experimental conditions should be done with caution. This has been addressed in more detail by Wheeler and Bertram [2012].

2.4 Conclusions

An optical microscope coupled to a flow cell was used to study the heterogeneous ice nucleation properties of uncoated and coated mineral dust and SNOMAX particles at temperatures ranging from approximately -26.5 to -39 °C. The results show that H₂SO₄ coatings significantly modified the heterogeneous ice nucleation properties of all the minerals studied. For kaolinite and illite, the acid coatings increased the onset RHₖ by approximately 30 %; for montmorillonite and quartz, the acid coatings increased the onset RHₖ by about 20 %. Our studies also show that NH₄HSO₄ coatings influence the heterogeneous ice nucleation properties of kaolinite particles. The coated particles are less effective at nucleating ice than uncoated particles, with the onset RHₖ increasing by approximately 18 to 26 %, depending on temperature.
Onset results indicate that uncoated SNOMAX, a biological IN made from cells of *P. syringae*, is a reasonably good ice nucleus, having onset values between 110 to 120 % RH. Unlike the mineral dust results, the sulfuric acid coatings did not hinder the heterogeneous ice nucleating ability of SNOMAX particles within experimental uncertainty. One possible explanation is that a few SNOMAX particles were not completely covered with the acid solution, providing a bare site for ice nucleation. However, the agreement between our coated results and the recent results by *Koop and Zobrist* [2009] for sulfuric acid solutions containing SNOMAX provides some support that the particles in our experiments are completely coated. Another possible explanation for the coated SNOMAX results is that the acid solution does not significantly modify the active ice nucleation sites for SNOMAX, which are believed to be certain proteins located in the outer cell membrane.

The heterogeneous nucleation rates ($J_{het}$) and contact angles ($\theta$) were determined according to classical nucleation theory for all uncoated and sulfuric acid coated mineral dusts studied, and for uncoated SNOMAX particles. The data show that, for all uncoated ice nuclei, the contact angles are small (below approximately 20°). For mineral dust particles coated with sulfuric acid, the contact angles are larger (above approximately 60°). The contact angles presented here are average values, however, previous work has indicated that it is the probability distribution function (PDF) of contact angles that will be important to measure in future work [*Eidhammer et al., 2009; Marcolli et al., 2007; Phillips et al., 2008*]. *Marcolli et al.* [2007] showed that the most active ice nucleation sites are rare in that they lie at the tail of the PDF, and furthermore, that it is these sites that are involved in ice nucleation in a population of particles of a given type.
Combined, our results support the idea that anthropogenic emissions of \( \text{SO}_2 \) and \( \text{NH}_3 \) may influence the heterogeneous ice nucleating properties of mineral dust particles by increasing the relative humidity required for ice nucleation.
Chapter 3. The Ice Nucleation Ability of One of the Most Abundant Types of Fungal Spores Found in the Atmosphere

3.1 Introduction

Several recent reviews have highlighted the need to quantify the number and source of biological ice nuclei in the atmosphere [Ariya et al., 2009; DeMott and Prenni, 2010; Martin et al., 2010; Möhler et al., 2007; Szyrmer and Zawadzki, 1997]. In addition, it has been suggested that the most important carbonaceous particles that may be acting as ice nuclei above -15 °C may be biological particles [DeMott and Prenni, 2010]. If biological ice nuclei are abundant in the atmosphere, they can influence the hydrological cycle and may play an important role in precipitation [Morris et al., 2004; Sands et al., 1982].

Recent field measurements of IN have highlighted the potential importance of biological particles in ice cloud formation in the atmosphere [Pratt et al., 2009; Prenni et al., 2009b]. Pratt et al. [2009] observed that ice residuals collected in situ from cloud particles (from -31 °C to -34 °C) contain a significant fraction of biological material. Another recent study concerning biological IN has demonstrated that, for pristine conditions over the Amazon rainforest, the level of IN can be predicted through measurements of a combination of mineral dust and biological particles [Prenni et al., 2009b]. To account for their data, they had assumed that the sampled biological particles could induce ice nucleation with an efficiency of ~0.2 for temperatures between -18 °C and -31 °C. At temperatures above approximately -25 °C, biological IN appeared to dominate over mineral dust IN.

There has also been a renewed interest in laboratory studies on the ice nucleation properties of biological particles, with studies focusing on bacteria, fungal spores and pollen
found in the atmosphere (see for example Chernoff and Bertram [2010], Conen et al. [2011], Després et al. [2012], Gonçalves and Massambani [2012], Hartmann et al. [2012], Iannone et al. [2011], Koop and Zobrist [2009], Möhler et al. [2007], Möhler et al. [2008c], Morris et al. [2013], Pummer et al. [2012], and references therein). The most well characterized biological IN species is the bacterium *Pseudomonas syringae* (*P. syringae*). This bacterium is an efficient IN species that is associated with onsets for ice formation as high as -2 °C [Lindow et al., 1989; Maki et al., 1974; Möhler et al., 2007; Morris et al., 2004; Vali et al., 1976]. The ice nucleating ability of pollen, relatively large bioaerosol particles with a diameter range of 10-100 µm, has been addressed in several studies [Diehl et al., 2002; Diehl et al., 2001; von Blohn et al., 2005]. Freezing temperatures for 4 types of pollen particles in the immersion freezing mode ranged from -13.5 °C to -21.5 °C [von Blohn et al., 2005]. However, the fraction of pollen grains that are effective IN has not yet been determined. Concerning the IN ability of fungi, only a small number of fungal species have been studied [Jayaweera and Flanagan, 1982; Morris et al., 2013; Pouleur et al., 1992], although, for example, more than 1000 fungal species were identified in atmospheric samples collected over Mainz, Germany [Fröhlich-Nowoisky et al., 2009].

Recent modelling studies have begun to explore the effect of biological ice nucleation on cloud microphysics, dynamics and precipitation [Ariya et al., 2009; Diehl and Wurzler, 2010; Diehl et al., 2006; Grützun et al., 2008; Hoose et al., 2010a; Levin et al., 1987; Phillips et al., 2009; Sesartic et al., 2012, 2013]. However, due to the limited understanding of the ice nucleation properties of biological particles, such modelling studies often have significant uncertainties. More studies on the ice nucleation properties of biological particles are needed, especially focusing on the abundant types of biological particles found in the atmosphere.
Spores are the reproductive units of fungi and they are abundant in the atmosphere [Bauer et al., 2008; Després et al., 2012; Madelin, 1994]. The amount of fungal spores in the atmosphere is subject to the time of day and various meteorological factors [Gilbert and Reynolds, 2005; Hirst, 1953; Kurkela, 1997; Stennett and Beggs, 2004; Stępalska and Wolek, 2009]. Concentrations of fungal spores are on the order of 1 to 10 L⁻¹ in the continental boundary layer [Elbert et al., 2007] with peak concentrations of 20 to 35 L⁻¹ previously observed [Goncalves et al., 2010; Ho et al., 2005; Khattab and Levetin, 2008; Nayar and Jothish, 2013; Oliveira et al., 2005; Quintero et al., 2010]. As a result, they are of sufficient quantity for their consideration in mixed-phase and ice cloud formation. To determine their atmospheric relevance, knowledge of their activation spectrum (i.e., proportion of active IN against temperature and ice supersaturation) is needed. Fungal spores can also be transported large distances and to high altitudes in the troposphere [Amato et al., 2007; Bowers et al., 2009; Ebner et al., 1989; Fulton, 1966; Gregory, 1978; Hirst et al., 1967; Kelly and Pady, 1953; Pady and Kelly, 1953; Pady and Kapica, 1955; Proctor and Parker, 1938] and even into the stratosphere [Griffin, 2004; Imshenetsky et al., 1978; Smith et al., 2010].

This investigation focuses on the ice nucleation properties of one of the most abundant types of fungal spores found in the atmosphere: spores from the genus Cladosporium. Species of Cladosporium are regularly found on living and dead plant material. The spores of Cladosporium are passively launched (i.e, separated from the mycelium via wind currents) and they have mean aerodynamic diameters between roughly 2-4 μm [Fröhlich-Nowoisky et al., 2009; Hameed and Khodr, 2001; Jung et al., 2009a; Reponen et al., 2001]. Cladosporium spores have been frequently observed as the dominant spore near the ground in sampling studies, where they often comprise at least 35 % of the total count as a yearly average [Al-Subai, 2002; Herrero et al.,
2006; Li and Kendrick, 1995; Lim et al., 1998; Mallo et al., 2011; Mitakakis and Guest, 2001; Pyrri and Kapsanaki-Gotsi, 2007].

In these studies, an optical microscope coupled with a temperature-controlled flow cell and a digital video camera were used to observe ice nucleation events for spores immersed in droplets of ultrapure water. In other words, this study focuses on heterogeneous freezing by spores in the immersion mode. These measurements were then used to assess the importance of ice nucleation on Cladosporium spores in the atmosphere.

3.2 Experimental

3.2.1 Freezing Experiments and Apparatus

The apparatus used in the freezing measurements was described in detail in Section 2.2.1 of the previous chapter. It consisted of an optical microscope coupled to a flow cell wherein the relative humidity (RH) could be accurately controlled and the temperature could be decreased or increased at a fixed rate ranging from 0.1-5.0 °C min⁻¹. A schematic and details of the flow cell is shown in Figure 2.1

A pictorial representation of the temperature profile in the current immersion freezing experiments is shown in Figure 3.1(a). The experimental protocol for the freezing experiments consisted of the following steps. Cladosporium spores were deposited on the bottom surface of the flow cell using the procedure described in Section 3.2.3. Next, the temperature of the flow cell was decreased to 2 °C and the dew point in the flow cell was set to approximately 3-5 °C using a humidified flow of ultra high purity (UHP) He (99.999 %, Praxair). This resulted in the nucleation and growth of water droplets on the bottom surface of the flow cell and on the spores. Droplets were allowed to grow to 100–200 µm in diameter. The gas flow was then stopped and the flow cell was isolated by closing the valves immediately upstream and downstream of the
cell. Next, the temperature in the flow cell was decreased at a rate of 5 °C min⁻¹ until a temperature of -40 °C was reached. During this process, digital video was captured at a frame rate of 30 fps (frames per second) by use of the digital video camera. Images were acquired from the digital video and analyzed to determine the freezing spectrum, defined as the fraction of droplets frozen as a function of temperature. After freezing, the temperature was increased to room temperature and the droplets were exposed to a flow of dry He gas to fully evaporate the water, leaving only the spore inclusions on the slide. Images were also acquired during this procedure to determine the amount of fungal material contained in each droplet. This experimental protocol was used to study Cladosporium spores in this chapter (Chapter 3), and also for additional studies on fungal spores (Chapter 4 and Chapter 5) and bacteria (Chapter 6).

As an example from a typical immersion ice nucleation experiment, shown in Figure 3.1(b) are three images from a freezing experiment on fungal spores (Boletus zelleri, studied in Chapter 5): the first image shows a liquid droplet containing fungal spores before freezing had occurred; the second image shows the same droplet, now frozen; and the third image shows the fungal spores after evaporation of the frozen droplet.
Figure 3.1 Pictorial representation of immersion ice nucleation experiments studying biological particles (Chapter 3-Chapter 6). Shown in (a) is the temperature profile for a typical immersion ice nucleation experiment. Black circles represent aerosol particles and the blue hexagons represent ice crystal. Shown in (b) are three images from an immersion ice nucleation experiment on fungal spores (*Boletus zelleri*, studied in Chapter 5): the first image shows a liquid droplet containing mushroom spores before freezing had occurred; the second image shows the same droplet, now frozen; and the third image shows the mushroom spores after evaporation of the frozen droplet.

Experiments were also performed using ultrapure water droplets without spores. This provided a means to determine whether the hydrophobic substrate supporting the particles influenced the freezing results. In these experiments, pure water droplets were condensed from the vapour phase onto the hydrophobic surface and their freezing temperatures were determined using the same procedure as outlined above for heterogeneous freezing.
3.2.2 Temperature and Cooling Rate Calibration

The RTD was calibrated against the melting points of ultrapure water (0.0 °C) and \( n \)-decane (-29.7 °C). Melting point determinations indicated a +0.16 °C offset for \( n \)-decane and a +0.07 °C offset for water. The reported freezing temperatures were corrected for bias using a linear fit function based on this offset data.

3.2.3 Preparation and Collection of Spores of \textit{Cladosporium} sp.

\textit{Cladosporium} was obtained from existing stock at the Canadian Centre for the Culture of Microorganisms (Department of Botany, University of British Columbia, Canada). Colonies of \textit{Cladosporium} were grown on potato dextrose agar in plastic Petri dishes. The cultures were incubated for a minimum of 3 weeks at 26 °C before any experiments were carried out. Preparation and collection of spores were conducted in a Class II biological safety cabinet to avoid contamination of the samples by dust and to prevent the release of spores into the laboratory environment.

Spores were harvested from fungal colonies and deposited on hydrophobic slides using a spore dispenser (i.e., an RH meter and a flow cell) and an impactor (Figure 3.2).
Figure 3.2 Schematics for the collection of fungal spores using a spore dispenser and an impactor. Panel (a) depicts the arrangement of the spore dispenser and impactor. Panel (b) provides a detailed view of the impactor, where the impactor tube’s aperture size and the distance from glass slide are given. Excised portions of *Cladosporium* cultures were placed inside the borosilicate glass flow tube. The tube was sealed inside the flow cell where a \( \frac{1}{4} \)" outer diameter stainless steel tube with bent tip directed a flow of \( N_2 \) (~5×10³ cm³ min⁻¹ for 1 h) at the cultures. Experiments either used dry or humidified (~35 % relative humidity (RH)) \( N_2 \) gas.

A scalpel was used to excise portions of the *Cladosporium* cultures from the Petri dish, which were then transferred to a sterilized borosilicate glass flow tube. The tube was placed inside the flow cell and the spore dispenser was then hermetically sealed.

Inside the flow cell, UHP \( N_2 \) gas (99.999 %, Praxair) was passed over the fungal cultures to dislodge and aerosolize the spores. A flow rate of 5×10³ cm³ min⁻¹ was maintained during a one hour collection time. A 120 cm³ Supelcarb HC Hydrocarbon Trap (Sigma-Aldrich) minimized organic contaminants within the 300 L of \( N_2 \) admitted to the system during spore collection. Experiments used either dry \( N_2 \) or a humidified flow of \( N_2 \) (RH ~35 %). Impaction of the spores onto the hydrophobic glass substrate occurred as the \( N_2 \) carrier gas exited a borosilicate glass impactor tube.

An Olympus IX70 inverted microscope equipped with a 40× objective was used to determine the morphology of the particles, and a 20× objective was used to determine the size distributions of the collected spores.
3.3 Results and Discussion

3.3.1 Morphology and Size Distribution of Collected Spores

Individual spores often appeared to be either roughly circular or, alternatively, lemon-shaped. Figure 3.3 provides several images of Cladosporium sp. spores at 40× magnification using the Olympus IX70 microscope.

![Images of spores](image)

**One Spore Unit**

- Panel **a** shows an area of $8.6 \mu m^2$, length of $4.4 \mu m$, and width of $2.3 \mu m$.
- Panel **b** shows an area of $14.4 \mu m^2$, length of $6.1 \mu m$, and width of $3.0 \mu m$.
- Panel **c** shows an area of $12.0 \mu m^2$, length of $4.4 \mu m$, and width of $2.9 \mu m$.

**Two Spore Units**

- Panel **d** shows an area of $18.9 \mu m^2$, length of $7.7 \mu m$, and width of $2.2 \mu m$.
- Panel **e** shows an area of $26.4 \mu m^2$, length of $9.3 \mu m$, and width of $2.5 \mu m$.

Figure 3.3 Images of spores of Cladosporium collected on a glass slide and observed with the Olympus IX70 microscope using a 40× objective. Panels (a–c) show dispersal units for Cladosporium containing one spore, whereas panels (d and e) depict observations of two or more spores (comprising 10 % of the total observations during size studies using a 20× objective). Measurement values for area ($A$), length ($L$), and width ($W$) are provided in each panel (from images taken with a 20× objective). Each image measures $20 \mu m \times 20 \mu m$. 
The morphology of these spores and their aspect ratios are consistent with results reported in the literature [Reponen et al., 1997; Schubert et al., 2007]. Approximately 90% of the spore dispersal units (i.e., particles dislodged from the fungal mycelium) contained 1 spore (Figure 3.3 a–c), and the remaining 10% contained 2 or more spores (Figure 3.3 d,e). Observations in the atmosphere at ground level have shown that a significant fraction of the collected spore dispersal units contain 2 or more spores [Davies, 1957; Harvey, 1967; Hyde and Williams, 1953].

In addition to characterizing the morphology of the spores, we also determined the size distribution of individual spores to further confirm that the collected material was actually spores and also to provide an estimate of the size of spores used in our studies. The volume equivalent diameter ($D_{volume}$) for each individual spore was calculated using images recorded with the Olympus IX70 microscope (20× objective) and the formula $D_{volume} = \sqrt{W^2L}$, where $W$ represents the maximum width orthogonal to the length $L$ [Reponen et al., 2001]. Any dispersal unit that contained 2 or more spores (i.e., Figure 3.3 d,e) was not included in these calculations. The results from these calculations are shown in Figure 3.4.

Figure 3.4 Distribution of volume equivalent diameters ($D_{volume}$) for spores of Cladosporium observed with the Olympus IX70 microscope at 20× magnification. Only spore areas for one spore unit were included in calculations of $D_{volume}$. Data are centered on integer values for $D_{volume}$. The mean volume equivalent diameter is 3.2±1.0 μm (1σ S.D.).
Individual spores had a mean volume equivalent diameter of 3.2±1.0 μm (1σ standard deviation (S.D.)). This mean diameter is consistent with reported diameters for spores of *Cladosporium* [Carlile et al., 2001; Fröhlich-Nowoisky et al., 2009; Hameed and Khodr, 2001; Jung et al., 2009b; Reponen et al., 1997]. The mean diameter may be an upper limit to the true mean size, because in our experiments particles having diameters less than approximately 1 μm were not resolved with the microscope. The mean size corresponds to single spores collected with the impactor. In the freezing experiments, on average 4.8 spores (i.e., spore units) were contained in each droplet (see Section 3.3.3).

### 3.3.2 Pure Water Droplets

A total of 113 individual freezing events were observed for pure water droplets without spores. Figure 3.5 provides a summary of these freezing results.
Figure 3.5 Combined data for homogeneous nucleation experiments on pure water droplets (113 freezing events) and heterogeneous nucleation experiments on water droplets containing *Cladosporium* spores (292 freezing events). Both graphs show the same data, but the bottom graph has been plotted on a logarithmic $y$-axis.
As shown, 50% of the droplets had frozen at approximately -37 °C, which is within the range of temperatures expected when considering classical homogeneous nucleation theory. In Figure 3.6, the mean homogeneous freezing data are plotted as a function of droplet size (binned in 20 μm diameter bins).

Figure 3.6 Combined data for mean freezing temperatures against binned droplet diameters (20 μm wide intervals for each droplet diameter size bin) from homogeneous (○) and heterogeneous (×) nucleation experiments. Horizontal lines provide ranges for each of the 20 μm diameter size bins. Vertical lines represent bounds for the 95% confidence interval about each mean.

From the plotted data, a small dependence of freezing temperature on droplet size was observed as expected from classical nucleation theory [Pruppacher and Klett, 1997]. The mean freezing temperatures for all the size ranges studied are within 0.5-2.0 °C of the predicted homogeneous freezing temperatures using homogeneous nucleation rates and equations presented in Pruppacher [1995] and Pruppacher and Klett [1997]. In addition, the measured freezing temperatures are in good agreement with freezing temperatures reported in other studies for which suspended droplets (i.e., aerosol droplets) were used. Wood et al. [2002], for example,
reported average freezing temperatures of -37.0 ºC to -37.3 ºC for droplet diameters of 40–66 µm. The reasonable agreement between the predicted homogeneous freezing temperatures, previous homogeneous freezing experiments, and the results of this study illustrates that the hydrophobic surface supporting the particles does not significantly influence the freezing temperatures. This observation is consistent with previous measurements using similar supports [Bertram et al., 2000; Koop et al., 1998].

3.3.3 Heterogeneous Nucleation

Nine freezing experiments were carried out for water droplets containing fungal spores of Cladosporium, and 16-43 droplets were analyzed for freezing in each experiment. Spores were encompassed in each droplet, existing either as single spores or as small chains of multiple spores (Figure 3.3). Only droplets that presented clear visual evidence for freezing and that contained spores were considered for the heterogeneous nucleation analyses. A total of 292 individual heterogeneous freezing events were observed. In these experiments, the average surface area for the spore inclusions was 217 µm² (σ = 172 µm²). Assuming that one spore corresponds to a diameter of 3.2 µm (as presented in Section 3.3.1) then, on average, each droplet in these experiments contained 4.8 spores.

The results from the heterogeneous freezing experiments are summarized in Figure 3.5. As seen, the heterogeneous freezing results are warmer than the pure water freezing experiments, where 1 % of the droplets heterogeneously froze at -28.5 ºC and 10 % froze at -30.1 ºC. It is interesting to note that the ice nucleation ability of Cladosporium is similar to kaolinite in the immersion mode. Kaolinite, an abundant mineral in the atmosphere, is often considered a good ice nucleus [Lüönd et al., 2010; Murray et al., 2011].
In Figure 3.6, the heterogeneous freezing results are plotted as a function of droplet diameter in the same manner as for the freezing experiments of pure water droplets. This plot illustrates that the heterogeneous freezing results are statistically different from the pure water case and, hence, the fungal spores are acting as ice nuclei. The difference between homogeneous and heterogeneous freezing ranges from ~3 °C to ~5 °C.

Another way to analyze and present the heterogeneous freezing data is to do so as a function of inclusion size (i.e., surface area) contained within each droplet undergoing heterogeneous nucleation. A summary of the heterogeneous data binned according to inclusion area is provided in Figure 3.7.

![Figure 3.7 Box plot showing a five-number statistical summary for homogeneous nucleation experiments (leftmost box) and binned data from all heterogeneous nucleation experiments. Each box represents temperatures as maximum and minimum values, and the 1st, 2nd (median), and 3rd quartiles. Freezing data are distributed into 200 μm² bins that represent the total area of all observable inclusions per frozen droplet (bottom x-axis). The average uncertainty in the inclusion area is ±28 μm². Assuming that all inclusions are spores with a mean volume equivalent diameter of 3.2 μm, the top x-axis provides the numbers of Cladosporium spores corresponding to each area bin.](image-url)
Included for comparison are the homogeneous data (zero surface area). Included as a secondary x-axis is the number of spores in each droplet, assuming an average spore diameter of 3.2 μm for the size of an individual spore. The general trend observed in Figure 3.7 is an increase in the median freezing temperature as the surface area of the inclusion increases per droplet (i.e., as the number of spores per droplet increases). This trend is consistent with previous heterogeneous freezing experiments in which the freezing temperature depended on the surface area of the heterogeneous ice nuclei [Archuleta et al., 2005; Hung et al., 2003; Kanji et al., 2008; Marcolli et al., 2007; Phillips et al., 2008]. The median heterogeneous freezing temperature for the smallest size bin (10–200 μm²) is approximately 3 ºC warmer compared to the homogeneous nucleation data. The dependence of spore inclusions on freezing temperatures has a pronounced effect, especially for >8 spores per droplet at which point the average difference in supercooling between homogeneous nucleation and heterogeneous nucleation is 8 ºC.

There is a paucity of studies on the heterogeneous freezing of fungal spores for which comparisons may be made to this study. One study examined the IN ability of a wide variety of fungal genera, including 14 species of the genus Fusarium [Pouleur et al., 1992]. That study reported high freezing temperatures for the genus Fusarium (up to -2.5 ºC). A caveat, however, is that the experiments were performed on droplets containing a suspension of fungal material and this does not provide specific results on the ice nucleating ability of fungal spores. Furthermore, the dependence on surface area was not addressed. Similarly, several ice nucleation experiments were carried out with species of lichens [Ashworth and Kieft, 1992; Kieft, 1988; Kieft and Ahmadjian, 1989; Kieft and Ruscetti, 1990], which are symbiotic organisms wherein fungi and algae and/or cyanobacteria form a single biological entity [Nash III, 2008]. In one particular study, Rhizoplaca chryssoleuca, a species of lichen, was found to induce freezing at
approximately -4 °C [Kieft and Ruscetti, 1990]. However, the ice nucleation properties of spores from lichens were not addressed.

Compared to biological particles such as the bacterium P. syringae, Cladosporium is not an effective IN. For example, strains of P. syringae induce freezing at temperatures as high as -2 °C [Lindow et al., 1989; Maki et al., 1974; Morris et al., 2004] and for some strains a freezing fraction of 10^{-2} (i.e., the fraction of particles that are good IN) is reached at a supercooling of only 10-12 °C (see Figure 1 in Phillips et al. [2009]). There is evidence that the high effectiveness of these IN species is due to a protein located in the outer cell membrane that provides a suitable crystallographic match for water clusters [Green and Warren, 1985; Gurian-Sherman and Lindow, 1993; Kajava and Lindow, 1993; Lee et al., 1995; Morris et al., 2004; Szyrmer and Zawadzki, 1997]. The poor ice nucleation ability of Cladosporium spores compared to P. syringae is believed to be related to the composition of the spore surfaces.

Spores from several species within the genus Cladosporium have been shown to be coated with a proteinaceous, hydrophobic rodlet layer [Amanianda et al., 2009; Latgé et al., 1988]. The rodlet layer is largely composed of proteins called hydrophobins, which occur uniquely in mycelial fungi and have been identified in fungi from the phyla Basidiomycota and Ascomycota [Linder, 2009; Linder et al., 2005; Wessels, 1997; Whiteford and Spanu, 2002; Wösthen, 2001]. These hydrophobic rodlet layers are generally found on the outer surfaces of spores and cause them to have a hydrophobic interface. Since it is thought that a major requirement for adequate IN ability is a surface that can undergo hydrogen bonding [Pruppachter and Klett, 1997], it is not surprising that the studied Cladosporium spores appear to be poor IN.
3.3.4 Atmospheric Implications

Above -15 °C, many mineral dusts become ineffective IN, although some field experiments show that some mineral dust particles are effective ice nuclei as warm as -5.2 to -8.8 °C [Sassen et al., 2003]. It has been suggested that the most important carbonaceous particles acting as ice nuclei above -15 °C may be biological particles [DeMott and Prenni, 2010]. Our data suggests that, at temperatures above -15 °C, Cladosporium spores are not likely an important IN species in the atmosphere. Over this temperature range, we can expect that less than 0.5 % will nucleate ice as a very conservative estimate from our data. Assuming that concentrations of fungal spores are on the order of ~10 L⁻¹ in the atmosphere [Elbert et al., 2007], based on measurements in the boundary layer, and as an upper limit we assume that 50 % of all spores are from the genus Cladosporium, it is estimated that the number of IN from Cladosporium spores is significantly less than ~0.025 L⁻¹. This value is a factor of approximately 4 to 800 smaller than the number of IN observed in the atmosphere at temperatures around -15 °C [DeMott et al., 2010].

At low temperatures (i.e., -25 °C to -35 °C), spores of Cladosporium and other similar spores may compete with other active IN (e.g., mineral dust). Modelling studies are required to assess the importance of Cladosporium in this temperature range. As mentioned above, Cladosporium has a similar ice nucleation ability in the immersion mode to kaolinite, which is an abundant mineral dust in the atmosphere [Lüönd et al., 2010; Murray et al., 2011].

A recent study concerning biological IN in the wet season over the Amazon rainforest has demonstrated that the level of atmospheric IN can be predicted through measurements of a combination of mineral dust and biological particles [Prenni et al., 2009b]. To explain their data, Prenni et al. had assumed that the sampled biological particles could induce ice nucleation with
an efficiency of ~0.2 for temperatures between -18 and -31 ºC. At temperatures above approximately -25 ºC, biological particles appeared to dominate. The size range of IN measured by Prenni et al. was \( \leq 1.3 \) µm in aerodynamic diameter. Some species of fungi can produce spores in this size range, but the fraction of *Cladosporium* spores in this size range is very small. In addition, for *Cladosporium*, less than 0.5 % of the droplets were observed to freeze at temperatures above \(-25^\circ C\) according to Figure 3.5. Hence, *Cladosporium* spores cannot explain the observations by Prenni et al.. Some other type of biological material must have been active as ice nuclei in these studies.

In a recent study by Pratt et al. [2009], ice residuals collected *in situ* from cloud particles at -31 ºC to -34 ºC contained a significant fraction of biological material. There was, however, a notable size cutoff: ice residual particles >700 nm were not admitted to their MS (mass spectrometry) instrument (for identification of biological markers within individual particles; a total of 46 particles were examined). The number of intact spores with aerodynamic diameters less than 700 nm in the atmosphere is likely small [Fröhlich-Nowoisky et al., 2009; Hameed and Khodr, 2001; Jung et al., 2009b; Reponen et al., 2001]. Hence, some other biological material, besides intact fungal spores, must have been responsible for the observations by Pratt et al. [2009].

### 3.4 Summary and Conclusions

Given the lack of published studies on the heterogeneous freezing of fungal spores, cloud modelling calculations incorporating the effect of fungal spores have relied on assumptions. We focused on one of the most abundant types of fungal spores found in the atmosphere: spores from the genus *Cladosporium*. The onset for heterogeneous freezing of pure water droplets containing spores of *Cladosporium* occurred as high as -28.4 ºC. However, there was a strong dependence
between the freezing temperature and the total spore surface area of *Cladosporium* for a given droplet. As such, mean freezing temperatures for droplets containing 1–5 spores are expected to be approximately -35.1±2.3 °C. Our result suggests that fungal spores are ineffective IN at temperatures warmer than -15 °C. Assuming that the concentration of all types of fungal spores in the atmosphere is ~10 L⁻¹, and that 50 % of these spores are of *Cladosporium*, the number of IN from *Cladosporium* spores is estimated as ~0.025 L⁻¹. The poor ice nucleation ability of *Cladosporium* spores compared to the bacterial IN *P. syringae* can be rationalized on the basis of the spore surface of *Cladosporium*, which is coated with hydrophobins (a class of hydrophobic proteins that appear to be widespread in filamentous fungi). By comparison, the surface of *P. syringae* is believed to contain a protein that provides a hydrogen-bonding lattice match to ice.

Spores of *Cladosporium* spp. may, nevertheless, compete with other active IN such as mineral dust at temperatures from -25 °C to -35 °C. A detailed modelling study is required to examine their impacts over this temperature range. The conclusions in this paper are based on fungal spores obtained from one species of fungi. For further generalization, studies on other types of fungal spores are required. However, it is interesting to note that hydrophobins are thought to be ubiquitous in filamentous fungi, rendering the bulk of species as unlikely candidates for effective IN based on our current understanding of heterogeneous ice nucleation.
Chapter 4. Ice Nucleation Properties of Rust and Bunt Fungal Spores and Their Transport to High Altitudes Where They Can Cause Heterogeneous Freezing

4.1 Introduction

Rusts are fungi from the class Pucciniomycetes, while bunts are fungi from the class Exobasidiomycetes [Kirk et al., 2008] (see Appendix A, Figure A.2 for details on the taxonomy of these fungi). Both classes of fungi are pathogenic and infect a broad range of hosts, including agricultural crops [Bockus et al., 2010; Hoffmann, 1982; Littlefield, 1981; Staples, 2000; Ziller, 1974]. The spread of these fungi has led to epidemics on large scales and to major economic losses [Dean et al., 2012; Littlefield, 1981; Matanguihan et al., 2011; Singh et al., 2011]. The main mechanism for the spread of rust and bunt fungi is by wind-dissemination of spores [Eversmeyer and Kramer, 2000; Gregory, 1973; Madelin, 1994; Staples, 2000; Webster and Weber, 2007].

Here, we focus on the ice nucleation properties of spores from rust and bunt fungi. There have not been any previous measurements of the ice nucleation properties of bunt spores, and ice nucleation studies of rust spores have been limited to warm temperatures (≥-10 °C) [Gonçalves and Massambani, 2012; Morris et al., 2013]. One reason we focused on the ice nucleation properties of rust and bunt spores is because their concentrations in the boundary layer can be large enough for consideration as an IN source over regions infected with these fungi. For example, rust spore concentrations of about 2-12 L⁻¹ at heights of 1-14 m above ground level (a.g.l.) have been measured above wheat fields [Eversmeyer and Kramer, 1975; Eversmeyer et al., 1973; Roelfs, 1972]. If these spores cause ice nucleation at warm temperatures, and a large
fraction reach cloud heights, they can potentially change the formation characteristics as well as the properties of clouds containing the ice phase [Delort et al., 2010; DeMott and Prenni, 2010; Möhler et al., 2007; Morris et al., 2004; Morris et al., 2011; Pöschl et al., 2010; Sands et al., 1982]. Another reason we focused on the ice nucleation properties of these spores is because ice nucleation, followed by precipitation, may be an important removal mechanism of these spores from the atmosphere. If this process is important, it should be incorporated into atmospheric models used to predict the long-distance transport of these spores [Andrade et al., 2009; Aylor, 1986, 2003; Fröhlich-Nowoisky et al., 2012; Isard et al., 2005; Kim and Beresford, 2008; Magarey et al., 2007; Pan et al., 2006; Pfender et al., 2006; Skelsey et al., 2008; Wang et al., 2010; Wilkinson et al., 2012].

Below, we first quantify the freezing properties of spores from four different rust species and two different bunt species in the immersion mode, which here refers to freezing of liquid water droplets with spores immersed in the droplets. See Table 4.1 for a list of the species studied, their hosts, the spore dimensions, and the spore types. Second, we used a particle dispersion model to estimate if the rust and bunt spores studied, which are 15-19 µm in diameter and hence have a significant terminal settling velocity, will reach high altitudes in the atmosphere where they can cause heterogeneous freezing. Third, we use the combined results to discuss the atmospheric implications of ice nucleation on rust and bunt fungal spores.
Table 4.1 Description of the rust and bunt spores studied.

<table>
<thead>
<tr>
<th>Class</th>
<th>Common Name</th>
<th>Species</th>
<th>Host</th>
<th>Average Spore Size(^{g,h,i,j}) (µm)</th>
<th>Spore Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pucciniomycetes</em></td>
<td>Rust fungi</td>
<td><em>Puccinia graminis</em></td>
<td>wheat</td>
<td>21(±3)×13(±2)</td>
<td>uredospore</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Puccinia triticina</em></td>
<td>wheat</td>
<td>22(±2)×18(±2)</td>
<td>uredospore</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Puccinia allii</em></td>
<td>leek</td>
<td>23(±4)×18(±3)</td>
<td>uredospore</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Endocronartium harknessii</em></td>
<td>pine</td>
<td>24(±3)×17(±2)</td>
<td>aeciospore</td>
</tr>
<tr>
<td><em>Exobasidiomycetes</em></td>
<td>Bunt fungi</td>
<td><em>Tilletia laevis</em></td>
<td>wheat</td>
<td>18(±2)×15(±2)</td>
<td>teliospore</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Tilletia tritici</em></td>
<td>wheat</td>
<td>18(±2)×16(±2)</td>
<td>teliospore</td>
</tr>
</tbody>
</table>

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\(^g\) Standard deviation.

\(^h\) Length and width determined from microscope images of single spores.

\(^i\) Number of spores used for size analysis: 209 (*P. graminis*), 188 (*P. triticina*), 268 (*P. allii*), 50 (*E. harknessii*, *T. laevis*, *T. tritici*).

\(^j\) The expected uncertainty due to the size measurements is approximately 2 µm, the resolution of the Olympus IX70 microscope.
4.2 Experimental

4.2.1 Freezing Experiments

The apparatus used to study freezing was described in detail in Chapter 2 (Section 2.2.1, Figure 2.1), and the experimental protocol for the immersion freezing experiments was described in Chapter 3 (Section 3.2.1, Figure 3.1). In each experiment, a new (i.e. fresh) sample of spores was used. Images extracted from the digital video were then used to determine: (1) the freezing temperature of each droplet and (2) the 2-dimensional (2-D) projected surface area of fungal spores within each droplet. During the freezing experiments, rust spores were often located on the surface of the droplets and bunt spores were often contained within the droplets. Although freezing experiments utilized droplets with diameters of 100-150 µm, the freezing results are not expected to change significantly with the size of the droplets, since the important property for immersion freezing is the surface area of the spores in contact with water.

4.2.2 Spore Properties and Number of Spores Per Drop

The average projected 2-D surface area per spore for individual spores was determined in separate experiments using images recorded with an Olympus IX70 microscope (10× objective). From the average projected 2-D surface area per spore and the total projected 2-D surface area of spores in each droplet (determined from images of spores after the droplets had been evaporated), the number of spores in each droplet in the freezing experiments was estimated. Note that this method for determining the number of spores per droplet gave the same results (within experimental uncertainty) as manually counting the number of individual spores (based on tests with several rust species). Images recorded with the Olympus IX70 microscope (10× objective) were also used to determine the average length and width of the spores.
The morphology and surface features of the spores were investigated using scanning electron microscopy (SEM). With the exception of \textit{E. harknessii}, which was imaged using field emission SEM (Hitachi S4700 FE-SEM, UBC BioImaging Facility), all spores were studied with variable pressure SEM (Hitachi S2600 VP-SEM, University of British Columbia (UBC) BioImaging Facility). Loose spores were deposited onto double-sided glue tabs affixed to aluminum SEM stubs and sputter-coated with either gold (\textit{E. harknessii}, \textit{T. tritici}) or gold/palladium (\textit{P. graminis}, \textit{P. triticina}, \textit{P. allii}, \textit{T. laevis}). After deposition, the stubs were mounted in the SEM instrument. The working distance used in the SEM experiments was between 7.6 and 20.9 mm, and the beam accelerating voltage used was between 3-10 kV. Images were collected using secondary electrons.

4.2.3 Spore Samples and Slide Preparation

Spores of \textit{Puccinia graminis} and \textit{Puccinia triticina} were provided by the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba. To prepare slides containing spores of \textit{P. graminis} and \textit{P. triticina}, the spores were placed inside a sealed glass vessel, which also contained a small fan and a slide suspended above the spores with a wire mesh. The vessel was then immersed in a sonicator bath to produce vibrations and when both the sonicator and the fan were turned on, the spores were suspended in the gas phase and mixed throughout the vessel. This resulted in spores being deposited on the slides suspended in the vessel.

\textit{Endocronartium harknessii} (sample held at the UBC Herbarium under the label AKB 5) was obtained from a pine tree in Terrace, British Columbia, Canada. Spores were harvested by tapping swollen, spore-filled galls on one of the infected pine branches over a clean surface. The spores were transferred to hydrophobic slides using a paintbrush; the paintbrush was lightly touched to the spores and then tapped while located over the glass slide.
*Tilletia laevis* and *Tilletia tritici* infected wheat heads were acquired from the Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta. Small bunt balls containing spores were removed from infected wheat heads and a clean razor blade was used to make an incision to release the spores onto a clean surface. As with *E. harknessii*, a paintbrush was used to transfer the spores to a hydrophobic glass slide.

*Puccinia allii* (UBC Herbarium, AKB 4) was harvested from leek plants in Vancouver, British Columbia, Canada. Slides containing *P. allii* spores were prepared by shaking and tapping (with a clean spatula) the infected leek leaves over the hydrophobic glass slides.

### 4.2.4 Particle Dispersion Modelling

The Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model, version 4, developed by the National Oceanographic and Atmospheric Administration, was used to estimate if rust and bunt spores with sizes similar to the sizes used in our experiments will reach high altitudes, where the temperatures are cold enough for immersion freezing. Operated in the three-dimensional particle mode, the model simulates the release, dispersion, transport, and deposition of particles [Draxler and Hess, 1997, 1998; Draxler et al., 2013]. A point source was simulated by releasing many (i.e. 3000) particles at a single time and location. After release, the motion of each particle in space and time (i.e. its dispersion) was determined from an advection component, dependent on wind velocities, and a random component to take turbulence into account [Draxler and Hess, 1997].

Four North American wheat-producing agricultural sites, two in the United States of America (U.S.) and two on the Canadian prairies, were chosen as the emission sites for the simulations. The U.S. sites are located in Kansas (+37° 18' 3.94", -97° 20' 39.70") and North Dakota (+48° 38' 37.67", -101° 24' 13.71"), the top two wheat-producing states in the U.S.
Figure 4.1 HYSPLIT source emission sites used for particle dispersion calculations for agricultural sites in Kansas, U.S. (+37° 18' 3.94", -97° 20' 39.70") ; North Dakota, U.S. (+48° 38' 37.67", -101° 24' 13.71") ; Saskatchewan, Canada (+51° 36' 21.69", -102° 0' 53.10") ; and Manitoba, Canada (+49° 29' 49.23", -98° 2' 11.09"). Three thousand particles were released every three days during periods of expected high concentrations of fungal spores at each location. Kansas: 01 February-28 June 2010; North Dakota, Manitoba, and Saskatchewan: 01 May-28 September 2010.

The “Puccinia pathway” describes the long-distance transport of wheat rust spores from Mexico and Texas to the Canadian prairies in the spring, followed by the southward return of the spores during the fall months [Eversmeyer and Kramer, 2000; Hamilton and Stakman, 1967;
Nagarajan and Singh, 1990]. The four emission sites were chosen, in part, because they lie directly within this pathway and, as such, are susceptible to wheat rust outbreaks during the growing seasons [Eversmeyer and Kramer, 2000].

Emissions were simulated during periods that overlapped with the growing season and harvesting of wheat in the different regions. The greatest risk for the transmission of rust and bunt pathogens is during the growing season and the highest spore emissions are expected during harvest. In Kansas, the main wheat crop is winter wheat (Kansas Wheat, available at http://www.kswheat.com), which is cultivated in the fall, remains dormant throughout the winter months, and is harvested mid-summer (late June-early July). To overlap with the main growing period of winter wheat, spore emissions were simulated from 01 February to 28 June 2010 for Kansas. In North Dakota and a majority of the wheat producing regions of Canada, spring wheat is the major wheat crop (North Dakota Wheat Commission, available at http://www.ndwheat.com; Natural Resources Canada, available at http://atlas.nrcan.gc.ca). Spring wheat is cultivated in the spring (late April-early May) and harvested in the fall (late September). Spore emissions in North Dakota, Saskatchewan, and Manitoba were simulated from 01 May to 28 September 2010 to overlap with the growing season of spring wheat.

A release height of 2 m a.g.l. was used for all simulations, which is approximately 1 m above the typical height of a wheat canopy [Eversmeyer and Kramer, 1975, 2000; Eversmeyer et al., 1973]. Three thousand particles were injected into the atmosphere every three days from each of the four locations throughout the simulation periods. Emissions every third day were chosen to optimize computation time while providing a good representation of the meteorological conditions during the simulation period. Rust and bunt spores are released from their host surface by a passive release mechanism, with the greatest likelihood of release
occurring in windy, turbulent and dry conditions [Gregory, 1973]. As such, these spores typically show a diurnal pattern of spore release with a peak occurring in the middle of the day [Gregory, 1973; Pfender et al., 2006]. For these reasons, we released the particles at noon from all of the locations studied. Model calculations were carried out for spherical particles having diameters of 15 and 19 μm, which reflects the size range of the studied spores (see Table 4.1).

Meteorological data such as horizontal wind speed and direction as well as precipitation were obtained from the Global Data Assimilation System (GDAS1) data archives (at 1° latitude-longitude resolution), provided by the National Weather Service’s National Center for Environmental Prediction. Default parameters were used to calculate the vertical and horizontal turbulence, boundary layer stability, vertical mixing profile, and the mixed layer depth [Draxler et al., 2013].

The gravitational settling velocity of the particles was calculated from the particle diameter, particle density and air density [Draxler and Hess, 1997, 1998]. For the particle density, a value of 1 g cm\(^{-3}\) was used based on previous measurements of fungal spore densities [Gregory, 1973]. Dry deposition was calculated from the deposition velocity, which was set equal to the gravitational settling velocity.

In most simulations, the default parameters for wet deposition were used. Specifically, in-cloud removal was represented by an in-cloud scavenging ratio of \(3.2 \times 10^5\). This parameter is unitless and is defined as the ratio of the particle concentration in liquid water (units of g L\(^{-1}\)) to the particle concentration in the air (units of g L\(^{-1}\)) [Draxler et al., 2013]. The below-cloud removal was represented using a scavenging coefficient of \(5.0 \times 10^{-5} \text{ s}^{-1}\) [Draxler et al., 2013]. To estimate the sensitivity of the results to the wet deposition parameters, two sets of sensitivity simulations were run for the Kansas site (15 μm particles) using different wet deposition
parameters. In the first set of sensitivity simulations, a value of $3.2 \times 10^6$ was used for the in-cloud scavenging ratio and the default value was used for the below-cloud scavenging ratio. In the second set of sensitivity simulations, the default value was used for the in-cloud scavenging ratio and a value of $5.0 \times 10^{-4}$ $\text{s}^{-1}$ was used for the below-cloud scavenging coefficient.

In our freezing experiments, the cumulative number of ice nuclei per spore was approximately 0.01 at temperatures around -25 °C (see Section 4.3.3, Figure 4.4). Assuming an atmospheric lapse rate of 7 °C km$^{-1}$ [Wallace and Hobbs, 2006] and a surface temperature of 20 °C, particles released at the surface would have to be lofted to an altitude of 6.4 km to reach a temperature of -25 °C. From the simulations, we calculated the fraction of particles released from the surface that reach altitudes of $\geq 6$ km a.g.l.

A caveat to the HYSPLIT results is that wet deposition is treated in a simple way in the model (i.e. two parameters). In reality, wet deposition can involve several microphysical processes including droplet activation (i.e. the particles act as cloud condensation nuclei), collision-coalescence, homogeneous and heterogeneous freezing, and the Wegener-Bergeron-Findeisen process. To treat these processes explicitly, a cloud-resolving model is needed. However, simulations with a cloud-resolving model are beyond the scope of the current manuscript.

4.3 Results and Discussion

4.3.1 Spore Properties

Shown in Figure 4.2 are SEM images for the rust and bunt spores investigated. *Puccinia* spores (*P. graminis*, *P. triticina*, *P. allii*) have echinulate surfaces, *E. harknessii* spores have verrucose surfaces, *T. laevis* spores have smooth surfaces, and *T. tritici* spores have reticulate surfaces. Listed in Table 4.1 are the average dimensions (length and width) of the spores.
investigated. These dimensions lie within the range of values reported in the literature [Anikster et al., 2005; Anikster et al., 2004; Bockus et al., 2010; Bushnell and Roelfs, 1984; Ziller, 1974].

Figure 4.2 SEM images of rust (P. graminis, P. triticina, P. allii, E. harknessii) and bunt (T. laevis, T. tritici) spores studied.

4.3.2 Fraction of Droplets Frozen as a Function of Temperature

Shown in Figure 4.3 is the fraction of droplets frozen as a function of temperature for the different rust and bunt spore species. The freezing results presented in Figure 4.3 (as well as Figure 4.4 and Figure 4.5) include all the experiments on all spore types. The total number of observed freezing events for each spore type was 168, 108, 77, 118, 157, and 137 for P. graminis, P. triticina, P. allii, E. harknessii, T. laevis, and T. tritici, respectively. Rust spores are shown in red (open symbols) and bunt spores are shown in blue (closed symbols). The average number of spores per droplet was 12, 5, 5, 6, 10, and 12 for the experiments with spores of P. graminis, P. triticina, P. allii, E. harknessii, T. laevis, and T. tritici, respectively. Also included
for comparison are results for homogeneous freezing (× symbols), determined with the same experimental apparatus and using similar droplet sizes as the current study [Iannone et al., 2011].

Figure 4.3 Immersion freezing results for rust and bunt spores, shown as the temperature-dependent fraction of frozen droplets. Rust spores (P. graminis, P. triticina, P. allii, E. harknessii) are in red (open symbols); bunt spores (T. laevis, T. tritici) are in blue (closed symbols). Included are previous homogeneous nucleation results (×) from Iannone et al. [2011], acquired using the same experimental setup as the current study. Because the freezing temperatures for the droplets containing spores are significantly warmer than homogeneous freezing temperatures, Figure 4.3 illustrates that the spores act as heterogeneous ice nuclei. Since the number of spores per droplet is different for the fungal species studied, conclusions about the relative ice nucleating ability of these spores should not be made based on Figure 4.3.
4.3.3 Cumulative Number of Ice Nuclei per Spore as a Function of Temperature, \( INperSpore \)

From the measurements of the fraction of droplets frozen vs. temperature (Figure 4.3) and information on the number of spores contained in each droplet, the cumulative number of ice nuclei per spore as a function of temperature (\( INperSpore(T) \)) was calculated following the method outlined by Vali [1971]. First, the droplets were binned by the number of spores per drop. Then, from the binned data, the cumulative number of ice nuclei per spore as a function of temperature was calculated with the following equation (note that only those bins containing at least 5 droplets were used in the calculation in order to have reasonable statistics in each bin):

\[
INperSpore(T) = \frac{\sum_{j=1}^{n} \left(-\ln \frac{N_{unfrozen,j}(T)}{N_{total,j}}\right) \times N_{total,j}}{\sum_{j=1}^{n} SPD_j \times N_{total,j}},
\]  

(4.1)

where \( j \) is the bin number; \( n \) is the total number of bins; \( N_{unfrozen,j}(T) \) is the number of unfrozen drops in bin \( j \) at temperature \( T \); \( N_{total,j} \) is the total number of drops in bin \( j \); and \( SPD_j \) is the number of spores per drop in bin \( j \).

We also calculated \( INperSpore(T) \) at the warmest observed freezing temperature (onset of freezing) using the following equation:

\[
INperSpore(T) = \frac{1}{Total \# \text{ Spores}}.
\]  

(4.2)

The numerator in Equation (4.2) represents the number of freezing events observed (i.e. 1) and the denominator represents the total number of spores available to act as ice nuclei in all the experiments. Equation (4.2) assumes the probability of multiple nucleation events in the same droplet is unlikely, a valid assumption at the warmest freezing temperature.
Shown in Figure 4.4 is the overall cumulative number of ice nuclei per spore for the rust and bunt spores. Rust spores are shown in red (open symbols), and bunt spores are shown in blue (closed symbols). For the rust and bunt spores, the cumulative number of ice nuclei per spore was $5 \times 10^{-3}$ at temperatures between approximately -20.5 °C and -27.0 °C, 0.01 between approximately -21.5 °C and -28.0 °C, and 0.1 between approximately -23.5 °C and -31.5 °C. Figure 4.4 also shows that, on average, the rust spores are better ice nuclei than the bunt spores, at least for the species studied here and when comparing the cumulative number of ice nuclei per spore.

Figure 4.4 Cumulative number of ice nuclei per spore or particle for the rust and bunt spores and submicron Asian mineral dust. Rust spores from this study (P. graminis, P. triticina, P. allii, E. harknessii) are in red (open symbols); bunt spores from this study (T. laevis, T. tritici) are in blue (closed symbols); submicron Asian mineral dust (experiments ACI04_16 and ACI04_19) from Niemand et al. [2012] are in black (filled diamonds); and rust spores from Morris et al. [2013] (P. graminis, P. triticina, P. allii) are in red (open symbols).
It is interesting to discuss these freezing results in light of the spore surface features, especially for the bunt spores. Based on Figure 4.4, the bunt spores, *T. laevis* and *T. tritici*, have nearly identical $IN_{perSpore}(T)$ values. However, the differences in surface features for these spores are very apparent (see Figure 4.2), with *T. laevis* having a smooth surface and *T. tritici* having a reticulate surface. This suggests that the different surface features between *T. laevis* and *T. tritici* do not translate to a large difference in $IN_{perSpore}(T)$.

Two previous studies investigated the immersion freezing of rust fungal spores [Gonçalves and Massambani, 2012; Morris et al., 2013]. Gonçalves and Massambani [2012] studied the freezing of droplets containing rust spores that grow on Coffee, *Hemileia vastatrix*, and found an average freezing temperature around -4 °C. However, $IN_{perSpore}(T)$ values were not reported. Morris et al. [2013] studied the immersion freezing properties of *Puccinia* spores from a variety of different sources, and observed onset temperatures as warm as -4 °C. In contrast to our freezing experiments, the experiments by Morris et al. [2013] focused on $IN_{perSpore}(T)$ values $< 0.001$. Shown in Figure 4.4 are results from Morris et al. [2013] for *P. graminis*, *P. triticina*, and *P. allii* (the species that both our group and Morris et al. [2013] studied). If we extrapolate our $IN_{perSpore}(T)$ results to warmer temperatures, our results are roughly consistent with the results by Morris et al. [2013].

There have only been a few other studies investigating the immersion freezing temperatures of fungal spores from different taxonomic classes compared to the classes studied here. Iannone et al. [2011] (Chapter 3) studied *Cladosporium* spores and Jayaweera and Flanagan [1982] studied *Penicillium digitatum*, *Cladosporium herbarum*, *Penicillium notatum*, *Penicillium frequentens*, and *Rhizopus stolonifera* spores. Table 4.2 lists the temperature for which the cumulative number of ice nuclei per spore was equal to 0.01 in these previous studies.
Also included are results from the current study. Comparing the data in Table 4.2, the rust and bunt spores studied here are better ice nuclei than Cladosporium sp. studied by Iannone et al. [2011]. However, the rust and bunt spores studied here are less efficient ice nuclei than some of the fungal spores studied by Jayaweera and Flanagan [1982], with P. allii and E. harknessii having comparable freezing temperatures (for INperSpore = 0.01) as Penicillum notatum, Penicillium frequentens, and Rhizopus stolonifera. Table 4.2 shows that the freezing results from one type of fungal spore should not be generalized to other types of fungal spores without additional information.

Table 4.2 Temperatures at which the cumulative number of ice nuclei per spore equals 0.01.

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Spore Size(^k) (μm)</th>
<th>T(INperParticle = 0.01) (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladosporium sp.</td>
<td>5×3</td>
<td>-29.2</td>
<td>Iannone et al. [2011]</td>
</tr>
<tr>
<td>Penicillium digitatum</td>
<td>not given</td>
<td>-10.0</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>not given</td>
<td>-15.0</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td>Penicillium notatum</td>
<td>not given</td>
<td>-22.0</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td>Penicillium frequentens</td>
<td>not given</td>
<td>-22.5</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td>Rhizopus stolonifera</td>
<td>not given</td>
<td>-23.0</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td>Puccinia graminis</td>
<td>21×13</td>
<td>-24.9</td>
<td>this study</td>
</tr>
<tr>
<td>Puccinia triticina</td>
<td>22×18</td>
<td>-26.0</td>
<td>this study</td>
</tr>
<tr>
<td>Puccinia allii</td>
<td>23×18</td>
<td>-22.4</td>
<td>this study</td>
</tr>
<tr>
<td>Endocronartium harknessii</td>
<td>24×17</td>
<td>-21.4</td>
<td>this study</td>
</tr>
<tr>
<td>Tilletia laevis</td>
<td>18×15</td>
<td>-27.8</td>
<td>this study</td>
</tr>
<tr>
<td>Tilletia tritici</td>
<td>18×16</td>
<td>-27.6</td>
<td>this study</td>
</tr>
</tbody>
</table>

\(^k\) Length and width determined from microscope images of single spores.
In addition to the fungal spore results discussed above, Pouleur et al. [1992] performed freezing experiments on droplets containing suspensions of fungal material (a combination of fungal spores and the mycelium). Since these studies did not isolate and report the freezing properties of fungal spores, a direct comparison with the current results is not possible.

Harvested rust spores can contain bacteria cells, which may also cause ice nucleation. Morris et al. [2013] previously showed that bacteria associated with washed *Puccinia* spores only accounted for a small fraction of ice nuclei in spore suspensions active at warmer temperatures. Based on SEM images (e.g. Figure 4.2), most spores in our experiments did not have bacteria cells attached to their surface (personal communication, D. Horne, UBC BioImaging Facility). A possible exception was *T. tritici* spores, which had circular features approximately 1 µm in size that could be assigned to bacteria cells. Alternatively, these circular features may have been small pieces of protective casing that enclosed the spores as they grew within the wheat plants. A qualitative test for the presence of bacteria on the surface of the rust and bunt spores was performed by placing the spores on nutrient agar and tryptic soy agar, two types of rich media that would support bacterial growth. The cultures were checked after 2 days and after 2 weeks, and in both cases bacterial growth was observed on plates containing spores of *P. allii* and *P. triticina*, with the level of growth indicative of low levels of bacterial contamination associated with the spores. The remaining plates, containing spores of *P. graminis*, *E. harknessii*, *T. laevis*, *T. tritici*, had no detectable bacterial growth on these media. Based on the SEM images and culture tests it is unlikely that the spores contained high concentrations of bacteria cells. In cases where the spores contained low levels of bacteria cells, and if these bacteria cells are good ice nuclei, they could be responsible for some of the freezing
at the low \( INperSpore(T) \) values reported here. In these cases the \( INperSpore(T) \) values should be considered as upper limits.

Recently Niemand et al. [2012] investigated the immersion freezing of submicron Asian mineral dust particles. From their measurements of the ice nucleation active surface site density, \( (n_s) \), we calculated the cumulative number of ice nuclei per particle, \( INperParticle(T) \), using the following equation [Connolly et al., 2009; Hoose and Möhler, 2012]:

\[
INperParticle(T) = n_s \times A_{aer},
\]

where \( A_{aer} \) is the surface area of an individual dust particle, given in \( m^2 \). To determine \( A_{aer} \), we assumed the particles were spherical and used the median diameters reported by Niemand et al. [2012]. In Figure 4.4, \( INperParticle(T) \) values calculated from the measurements of \( n_s \) by Niemand et al. [2012] are shown as filled black diamonds for comparison with the \( INperSpore(T) \) results for the rust and bunt spores. On a per particle basis, Figure 4.4 indicates that the rust spores, on average, are better ice nuclei than the submicron Asian mineral dust particles studied by Niemand et al. [2012]. This supports previous findings that many biological particles are more efficient ice nuclei than mineral dusts on a per particle basis [Hoose and Möhler, 2012; Murray et al., 2012].

### 4.3.4 Ice Nucleation Active Surface Site Density, \( n_s \)

The greater ice nucleating activity of rust spores on a per particle basis compared to submicron Asian mineral dust particles may be mainly due to the difference in size of the particles (i.e. the rust spores have a larger surface area available for ice nucleation than the mineral dust particles studied by Niemand et al. [2012]). To explore this further, we compared the ice nucleation active surface site density, \( n_s \), which represents the number of ice nucleation sites per unit area [Connolly et al., 2009].
The ice nucleation active surface site density for the spores was determined by dividing $IN_{perSpore}(T)$ by the surface area of an individual spore. The surface area of each individual spore was determined by assuming its shape as a prolate spheroid with the average dimensions of length and width given in Table 4.1. The ice nucleation active surface site density for Asian mineral dust was taken directly from Niemand et al. [2012]. To calculate $n_s$ for the mineral dust particles, Niemand et al. [2012] used the volume-equivalent spherical diameters from measurements with a scanning mobility particle sizer and an aerodynamic particle sizer.

Figure 4.5 shows $n_s$ as a function of temperature for the rust and bunt spores investigated in the current study, and also for Asian mineral dust particles investigated by Niemand et al. [2012] (filled black diamonds). Rust spores are shown in red (open symbols), and bunt spores are shown in blue (closed symbols). Figure 4.5 shows that, once normalized to surface area, all of the fungal spores studied here are less efficient ice nuclei compared to Asian mineral dust.
Figure 4.5 Ice nucleation active surface site density, $n_s$ (m$^{-2}$), for rust and bunt spores. Rust spores ($P. graminis$, $P. triticina$, $P. allii$, $E. harknessii$) are in red (open symbols); bunt spores ($T. laevis$, $T. tritici$) are in blue (closed symbols). Included for comparison are experimental results for submicron Asian mineral dust (experiments AC104_16 and AC104_19, Niemand et al. [2012]).

Most of the spores studied here have rough surfaces. We estimate, based on the SEM images shown in Figure 4.2 that the surface area of some of the spores may be up to a factor of 10 higher than the geometric surface areas used when calculating $n_s$. As a result, the $n_s$ values reported here should be considered as upper limits to the true $n_s$ values. A similar argument can be applied to the mineral dust $n_s$ values shown in Figure 4.5.

4.3.5 Atmospheric Dispersion Modelling Results

Shown in Figure 4.6 are the average fraction of particles, originally released from each site (see Figure 4.1) at altitudes of $\geq 6$ km. For these simulations, the default parameters for wet
deposition were used. The fraction of particles $\geq 6$ km is highest three days after release from the sites, suggesting that atmospheric residence time of these particles is on the order of days. The fraction of particles $\geq 6$ km varied with the particle size (compare Figure 4.6(a) with Figure 4.6(b)) and the site location (compare the different symbols in Figure 4.6). Nevertheless, in all cases, a significant fraction of the particles reached altitudes of at least 6 km. For example, after three days, between 6-9 % of 15 $\mu$m particles and between 3-6 % of 19 $\mu$m particles are at altitudes of at least 6 km.

![Figure 4.6: Average fraction of the total spores emitted from four North American locations observed at altitudes $\geq 6$ km, calculated with the HYSPLIT particle dispersion model and assuming spherical particles of unit density: (a) 15 $\mu$m and (b) 19 $\mu$m particles.](image)

Shown in Figure 4.7 are results for the Kansas location (15 $\mu$m particles) calculated using different parameters for wet deposition to investigate the sensitivity of the results to these parameters. When the below-cloud scavenging coefficient was increased by an order of magnitude over the default value, there was no difference in the fraction of particles reaching altitudes $\geq 6$ km (compare squares and circles in Figure 4.7). When the in-cloud scavenging ratio was increased by an order of magnitude over the default value, the fraction of spores reaching
altitudes \geq 6 \text{ km} \text{ decreased by a few percent (compare squares and triangles in Figure 4.7). These results suggest that our simulations are only slightly sensitive to the in-cloud removal parameter and not sensitive to the below-cloud removal parameter. Nevertheless, the results in Figure 4.7 should be considered as upper limits for the fraction of spores that are expected to reach altitudes \geq 6 \text{ km}.}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.7.png}
\caption{HYSPLIT wet deposition sensitivity study for the Kansas emission site, 15 \text{ m particles. The average fraction of the total spores emitted observed at altitudes \geq 6 \text{ km} for three cases: (1) default for in-cloud removal (scavenging ratio = 3.2 \times 10^5) and below-cloud removal (scavenging coefficient = 5.0 \times 10^{-5} s^{-1}) (squares) (i.e. same data as shown in Figure 4.6); (2) using the default in-cloud removal value and a below-cloud removal value equal to the default value multiplied by 10 (below-cloud default \times 10, circles); (3) using the default below-cloud removal value and an in-cloud removal value equal to the default value multiplied by 10 (in-cloud default \times 10, triangles).}}
\end{figure}
The data shown in Figure 4.6 and Figure 4.7 are averages based on simulations for many different days. The fraction of spores ≥ 6 km was also found to vary significantly from day to day. As an example, Figure 4.8 (a)-(e) shows results from individual simulations for the Kansas site (15 µm particles) with emission dates ranging from 01 February 2010 to 28 June 2010. For some emission dates, the fraction of 15 µm particles at an altitude of at least 6 km was > 50 % (e.g. 01 June 2010), while on other days, the fraction was < 10 %. This variability is expected as the lofting of spores into the atmosphere should vary with meteorological conditions, particularly vertical motion as influenced by atmospheric stability, which varies from day to day.

To explore further the meteorological conditions that lead to a large fraction of spores ≥ 6 km, the convective available potential energy (CAPE) at the Kansas site is included in Figure 4.8(f). CAPE provides an indication of the likelihood, intensity and depth of convection in the troposphere: CAPE values below 1000 support weak (and likely shallow) convection; values between 1000-2500 indicate moderate convection; values between 2500-4000 indicate strong convection, and CAPE above 4000 indicates extreme convection that likely extends through the full depth of the troposphere [Wallace and Hobbs, 2006]. CAPE values were determined from the GDAS1 meteorological data. Figure 4.8 illustrates that the fraction of spores ≥ 6 km correlates with CAPE values, suggesting that convection is important for transporting rust and bunt spores to high altitudes, at least for the Kansas emission site studied here [Wallace and Hobbs, 2006].
Figure 4.8 HYSPLIT results for 15 µm particles released from the Kansas emission site. Shown is the fraction of total spores emitted in the atmosphere ≥ 6 km (a) 1 day, (b) 2 days, (c) 3 days, (d) 4 days, and (e) 7 days after emission over the entire simulation period (01 February-28 June 2010). Shown in (f) is the convective available potential energy (CAPE) as an indication of convection during the simulation period. Shown in (g) is the lifting condensation level (LCL) to indicate the altitude needed for condensation of liquid water.
The HYSPLIT simulations are roughly consistent with the limited field data on rust and bunt spores at high altitudes in the atmosphere [Eversmeyer and Kramer, 2000; Gregory, 1973]. For example, Craigie [1945] observed rust spores in the Canadian prairies at an altitude of 4.6 km. Other examples of studies that have identified fungal spores at high altitudes include: Amato et al. [2007] at 1.5 km above sea level (a.s.l.); Bauer et al. [2002] at 1.6 km a.s.l.; Bowers et al. [2009] at 3.2 km a.s.l.; Imshenetsky et al. [1978] at altitudes up to 77 km a.g.l.; Jayaweera and Flanagan [1982] at altitudes up to 7 km a.g.l.; and Pady and Kelly [1954] at 2.4 km a.s.l.

The HYSPLIT simulations show that a fraction of the spores reach high enough altitudes, and hence low enough temperatures, for freezing in the immersion mode. Another prerequisite for immersion freezing, in addition to low temperatures, is exposure to water supersaturations followed by condensation of water on the particles. To investigate this further, for the Kansas site we calculated the lifting condensation level (LCL) (Figure 4.8(g)). The LCL is the altitude at which an air parcel becomes saturated (i.e. relative humidity = 100 %) when lifted adiabatically [Wallace and Hobbs, 2006]. The LCL was calculated according to Lawrence [2005] using meteorological data from GDAS1. Figure 4.8(g) shows that the LCL at the Kansas site over the simulation period varied between approximately 20-2400 m. These results indicate that the spores that reach 6 km in altitude would have been exposed to supersaturated conditions, at least for the Kansas emission site during the simulation period of 01 February to 28 June 2010.

4.4 Implications and Conclusions

4.4.1 Implications for Predicting the Long-Distance Transport of Rust and Bunt Fungal Spores

For the spores studied here, the freezing results showed that the cumulative number of ice nuclei per spore was $5 \times 10^{-3}$, 0.01 and 0.1 at temperatures of roughly -24 °C, -25 °C and -28 °C,
respectively. In addition, the particle dispersion modelling results suggests that after three days and during periods of high spore production, a relatively large fraction of spores released over agricultural regions in Kansas (U.S.), North Dakota (U.S.), Saskatchewan (Canada), and Manitoba (Canada) can reach at least 6 km in altitude. Furthermore, strong convection is shown to be an important mechanism leading to the lofting of spores into the middle and upper troposphere. Taken together these results are consistent with the idea that ice nucleation by these fungal spores may play a role in the removal of these particles from the atmosphere.

An aspect not considered in this study is the viability of rust and bunt spores after being exposed to low temperatures. Eversmeyer and Kramer [1995] showed that the viability of spores from the rust species *Puccinia graminis* and *Puccinia recondita* decreased significantly when exposed to temperatures between 0 °C and -4 °C for several hours. Additional studies on the viability of rust and bunt spores for the range of conditions used in our experiments would be useful and complementary to the freezing studies. Together, these two results could be used to determine if ice nucleation followed by precipitation plays a role in the spread of these plant pathogens.

**4.4.2 Implications for the Formation Conditions and Properties of Ice and Mixed-Phase Clouds in the Atmosphere**

In order for rust or bunt spores to influence mixed-phase or ice cloud properties, the concentration of IN from these spores need to be comparable to IN concentrations from other atmospheric particles that are known to be good ice nuclei, such as mineral dust particles. In the following, we compare potential IN concentrations from rust and bunt spores with potential IN concentrations from mineral dust. In this comparison, we focus on a height of 6 km to be consistent with the HYSPLIT simulations. In addition, we focus on global annual mean
concentrations of IN, as done previously [Murray et al., 2012]. To estimate the global annual mean concentrations of IN from fungal spores at 6 km, we use the following equation [Hoos and Möhler, 2012; Murray et al., 2012; Vali, 1971]:

\[ [IN(T)] = [Spores] \times \{ 1 - \exp(-INperSpore(T)) \}, \]

where \([IN(T)]\) is the concentration of ice nuclei and \([Spores]\) is the concentration of fungal spores. To estimate the global annual mean concentrations of IN from Asian mineral dust, a comparable equation is used:

\[ [IN(T)] = [particles] \times \{ 1 - \exp(-INperParticle(T)) \} \]

where \([particles]\) is the concentration of Asian mineral dust.

To estimate a conservative upper limit to the global annual mean concentrations of IN from rust and bunt spores at 6 km, we used the \(INperSpore(T)\) values reported in Figure 4.4 and 1 L\(^{-1}\) for \([Spores]\). Shown in Figure 4.9 are the \([IN(T)]\) values as a function of temperature calculated with these assumptions. These values should be considered as conservative upper limits to the global annual mean concentrations since (a) rust spore concentrations of 2-12 L\(^{-1}\) have been measured at 1-14 m above wheat fields [Eversmeyer and Kramer, 1975; Eversmeyer et al., 1973; Roelfs, 1972], (b) the HYSPLIT simulations for four wheat producing regions show that 6-9 % of 15 µm particles and between 3-6 % of 19 µm are at an altitude ≥ 6 km after 3 days and (c) simulations by Hoos et al. [2010b] showed the global annual mean concentrations of fungal spores at 6 km can be 1 to 2 orders of magnitude less than surface concentrations.
Figure 4.9 Estimated global annual mean concentrations of IN at 6 km for rust and bunt fungal spores as well as for Asian mineral dust. The values for rust and bunt spores are conservative upper limits.

To estimate a global annual mean concentration of IN from Asian mineral dust at 6 km, we used the $IN_{\text{perParticle}}(T)$ values shown in Figure 4.4 (which were calculated from data in Niemand et al. [2012]) and $[\text{particles}] = 0.1 \text{ cm}^{-3}$ to $10 \text{ cm}^{-3}$, based on simulations with a global climate model [Hoose et al., 2010b]. This approach follows the method used by Murray et al. [2012]. Shown in Figure 4.9 are the $[IN(T)]$ values as a function of temperature calculated with these assumptions. Even using a conservative upper limit to the global mean concentrations of IN from rust and bunt spores, the concentration of IN from rust and bunt spores are significantly less than from mineral dust. This suggests that ice nucleation from rust and bunt spores is unlikely to compete with mineral dust on a global and annual scale. The main reason is that on a
global scale mineral dusts have much higher concentrations than rust and bunt spores. The results from this simple analysis are consistent with recent global modeling studies showing that fungal spores are not likely to be important ice nuclei on the global scale [Hoose et al., 2010a; Sesartic et al., 2013].

On the other hand, ice nucleation on rust and bunt spores may impact ice and mixed-phase clouds regionally. Conditions when rust and bunt spores may impact ice and mixed-phase clouds may include when combine harvesting of infected crops takes place [Garcia et al., 2012; Lighthart, 1984], and also during periods when the concentrations of mineral dust particles are low. The ice nucleation results presented here should be useful in future modelling studies that explore the effect of rust and bunt spores on ice and mixed-phase clouds on the regional scale.

Fungal spores that remain in the atmosphere long enough may become coated with inorganic or organic materials. Such coatings could alter the ice nucleation characteristics of the fungal spores, similar to the effect of coatings on mineral dust [Archuleta et al., 2005; Attard et al., 2012; Chernoff and Bertram, 2010; Cziczo et al., 2009; Gallavardin et al., 2008; Kanji et al., 2008; Knopf and Koop, 2006; Koehler et al., 2010; Koop and Zobrist, 2009; Möhler et al., 2008b; Niedermeier et al., 2010; Niedermeier et al., 2011; Reitz et al., 2011; Salam et al., 2007; Sullivan et al., 2010; Yang et al., 2011; Zobrist et al., 2008]. However, additional studies on the effects of coatings on the ice nucleation properties of fungal spores are needed.

The current analysis focused on immersion freezing. Rust and bunt spores could also influence cold clouds by acting as ice nuclei in the deposition mode, where deposition nucleation refers to ice nucleation directly on the solid IN surface in the absence of liquid water. Rust and bunt spores may also impact warm clouds by acting as giant cloud condensation nuclei [Möhler et al., 2007]. Research on these topics would be interesting avenues for further studies.
Chapter 5. Ice Nucleation of Fungal Spores From the Classes *Agaricomycetes*, *Ustilagomycetes*, and *Eurotiomycetes*, and the Importance of Ice Nucleation on These Spores to Their Transport in the Atmosphere.

### 5.1 Introduction

Fungi can be classified into 35 different classes [Hibbett et al., 2007]. So far, the ice nucleation properties of spores from only 5 classes (*Dothideomycetes*, *Exobasidiomycetes*, *Eurotiomycetes*, *Pucciniomycetes*, *Sordariomycetes*) have been investigated [Haga et al., 2013; Iannone et al., 2011; Jayaweera and Flanagan, 1982; Morris et al., 2013; Pouleur et al., 1992]. Listed in Table 5.1 are details on the fungal species previously investigated in ice nucleation studies.

To add to the limited amount of data on the ice nucleation properties of fungal spores, we studied the ice nucleation properties of fungal spores from the classes *Agaricomycetes*, *Ustilagomycetes*, and *Eurotiomycetes*. A list of the fungal species studied is given in Table 5.2 and further details on their taxonomy is given in Appendix A, Figure A.1 and Figure A.2. We focused on these classes since they are thought to be abundant in the atmosphere.
Table 5.1 Previous ice nucleation studies on fungal spores.

<table>
<thead>
<tr>
<th>Class</th>
<th>Fungal Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dothideomycetes</td>
<td>Cladosporium sp.(^1)</td>
<td>Iannone et al. [2011]</td>
</tr>
<tr>
<td></td>
<td>Cladosporium herbarum</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td>Exobasidiomycetes</td>
<td>Tilletia laevis</td>
<td>Haga et al. [2013]</td>
</tr>
<tr>
<td></td>
<td>Tilletia tritici</td>
<td>Haga et al. [2013]</td>
</tr>
<tr>
<td>Eurotiomycetes</td>
<td>Penicillium digitatum</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td></td>
<td>Penicillium notatum</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td></td>
<td>Penicillium frequentens</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
<tr>
<td>Pucciniomycetes</td>
<td>Puccinia graminis</td>
<td>Haga et al. [2013], Morris et al. [2013]</td>
</tr>
<tr>
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<td>Puccinia triticina</td>
<td>Haga et al. [2013], Morris et al. [2013]</td>
</tr>
<tr>
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<td>Puccinia allii</td>
<td>Haga et al. [2013], Morris et al. [2013]</td>
</tr>
<tr>
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<td>Haga et al. [2013]</td>
</tr>
<tr>
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<td>Morris et al. [2013]</td>
</tr>
<tr>
<td></td>
<td>Puccinia lagenophorae</td>
<td>Morris et al. [2013]</td>
</tr>
<tr>
<td></td>
<td>Puccinia sp.</td>
<td>Morris et al. [2013]</td>
</tr>
<tr>
<td></td>
<td>Puccinia striiformis</td>
<td>Morris et al. [2013]</td>
</tr>
<tr>
<td></td>
<td>Hemileia vastratrix</td>
<td>Morris et al. [2013]</td>
</tr>
<tr>
<td>Sordariomycetes</td>
<td>Fusarium acuminatum</td>
<td>Pouleur et al. [1992]</td>
</tr>
<tr>
<td></td>
<td>Fusarium avenaceum</td>
<td>Pouleur et al. [1992]</td>
</tr>
<tr>
<td>Unidentified class</td>
<td>Rhizopus stolonifera</td>
<td>Jayaweera and Flanagan [1982]</td>
</tr>
</tbody>
</table>

\(^1\) sp. = unidentified species.
Table 5.2 Description of the fungal spores investigated in the current study.

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Spore Size(\mu m)</th>
<th>Spore Type</th>
<th>Median Spores/Drop</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agaricomycetes</strong></td>
<td><em>Agaricus bisporus</em></td>
<td>8(±2)(\times)7(±2)</td>
<td>basidiospore</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>Amanita muscaria</em></td>
<td>10(±2)(\times)7(±2)</td>
<td>basidiospore</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td><em>Boletus zelleri</em></td>
<td>13(±2)(\times)6(±2)</td>
<td>basidiospore</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>Lepista nuda</em></td>
<td>8(±2)(\times)5(±2)</td>
<td>basidiospore</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>Trichaptum abietinum</em></td>
<td>7(±2)(\times)4(±2)</td>
<td>basidiospore</td>
<td>15</td>
</tr>
<tr>
<td><strong>Ustilagomycetes</strong></td>
<td><em>Ustilago nuda</em></td>
<td>7(±2)(\times)6(±2)</td>
<td>teliospore</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><em>Ustilago nigra</em></td>
<td>9(±2)(\times)7(±2)</td>
<td>teliospore</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>Ustilago avenae</em></td>
<td>8(±2)(\times)6(±2)</td>
<td>teliospore</td>
<td>15</td>
</tr>
<tr>
<td><strong>Eurotiomycetes</strong></td>
<td><em>Penicillium chrysogenum</em></td>
<td>4(±1)(\times)3(±1)</td>
<td>conidiospore</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium sp.</em></td>
<td>4(±1)(\times)3(±1)</td>
<td>conidiospore</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium brevicompactum</em></td>
<td>4(±1)(\times)3(±1)</td>
<td>conidiospore</td>
<td>6</td>
</tr>
</tbody>
</table>

\(m\)The uncertainty in the size measurements is the resolution limit of the Olympus IX70 microscope. The 20\(\times\) objective was used for the *Agaricomycetes* and the *Ustilagomycetes* spores with an uncertainty of approximately 2 \(\mu m\), and the 30\(\times\) and 40\(\times\) objectives were used for the *Eurotiomycetes*, both having uncertainties of approximately 1 \(\mu m\).
Agaricomycetes are cosmopolitan all over the globe [Webster and Weber, 2007]. Spores from this class have been found to be important components of the air-borne fungal spore population both near the surface [Fröhlich-Nowoisky et al., 2009; Fröhlich-Nowoisky et al., 2012; Herrero et al., 2006; Magyar et al., 2009; Mallo et al., 2011; Morales et al., 2006; Oliveira et al., 2009a, b; Yamamoto et al., 2012; Zoppas et al., 2006], and also at high altitudes [Amato et al., 2007; Bowers et al., 2009]. For example, over half of the fungal species identified from continental air by Fröhlich-Nowoisky et al. [2012] were from the class Agaricomycetes. The few studies that have quantified Agaricomycetes spore concentrations to the genus level have reported surface concentrations of roughly $10^{-3}$ to 1 L$^{-1}$ [Li, 2005; Magyar et al., 2009; Morales et al., 2006].

Ustilagomycycetes have frequently been identified from surface air samples [Mallo et al., 2011; Pyrri and Kapsanaki-Gotsi, 2007] and have been shown to make up to a third of the total fungal spores in some regions [Hasnain et al., 2005; Herrero et al., 2006; Mitakakis and Guest, 2001; Morales et al., 2006]. Boundary layer concentrations have been measured to be roughly 0.05 to 6 L$^{-1}$ [Calderon et al., 1995; Gregory, 1952; Hasnain et al., 2005; Hirst, 1953; Khattab and Levitin, 2008; Magyar et al., 2009; Morales et al., 2006; Nayar and Jothish, 2013; Pyrri and Kapsanaki-Gotsi, 2007; Sabariego et al., 2000; Troutt and Levitin, 2001; Wu et al., 2004], and Ustilago spores (the genus studied here) were identified at high altitudes in the troposphere by Pady and Kelly [1954]. Ustilagomycycetes are also agricultural pathogens and have caused widespread damage to crops [Webster and Weber, 2007]. Hence, understanding the transport of these spores in the atmosphere (which may involve ice nucleation) is of interest from both economic and health perspectives.
Eurotiomycetes are found on all types of decaying material and are one of the most ubiquitous types of fungi [Webster and Weber, 2007]. Eurotiomycetes have also been identified as important human allergens (see, for example: Horner et al. [1995], and references therein). The specific types of spores from the class Eurotiomycetes we studied here were from the genus Penicillium. These spores are frequently present in surface air [Fröhlich-Nowoisky et al., 2009; Goncalves et al., 2010; Mallo et al., 2011; Pyrri and Kapsanaki-Gotsi, 2007; Pyrri and Kapsanaki-Gotsi, 2012; Shelton et al., 2002], and they often represent a large majority of identified spores (up to 35%) with typical surface concentrations of roughly 0.1 to 5 L⁻¹ [Crawford et al., 2009; Fernández et al., 2012; Khattab and Levetin, 2008; Li and Kendrick, 1995; Nayar and Jothish, 2013; Pyrri and Kapsanaki-Gotsi, 2012; Quintero et al., 2010; Wu et al., 2004]. During periods of high spore productivity, concentrations of Penicillium spores as high as 10 L⁻¹ have been observed [Pyrri and Kapsanaki-Gotsi, 2012]. Penicillium spores have also been identified at high altitudes in the troposphere and stratosphere [Amato et al., 2007; Fulton, 1966; Griffin, 2004; Imshenetsky et al., 1978; Jayaweera and Flanagan, 1982; Kelly and Pady, 1953; Pady and Kelly, 1954; Pady and Kapica, 1955; Proctor and Parker, 1938].

In addition to studying the ice nucleation properties of spores from the classes Agaricomycetes, Ustilagomycetes, and Eurotiomycetes, we also investigated whether ice nucleation on these spores, followed by precipitation, can influence the transport and removal of these spores in the atmosphere. We do this using a global chemistry-climate transport model and by comparing simulations with and without ice nucleation included in the model. These simulations suggest that ice nucleation on the spores studied may be important for the transport of these spores to remote regions (such as the marine boundary layer, polar regions, and the upper troposphere).
Fungal spores from the phyla *Basidiomycota* and *Ascomycota* make up approximately 98% of known fungal species [James et al., 2006]. Recent field measurements observed an increase in concentration of *Ascomycota* compared to *Basidiomycota* in remote marine regions [Fröhlich-Nowoisky et al., 2012]. One possible explanation for this was that *Basidiomycota* spores are more efficient ice nuclei compared to *Ascomycota* spores and that ice nucleation, followed by precipitation, is an important removal mechanism of spores from the atmosphere [Fröhlich-Nowoisky et al., 2012]. We also use our new freezing data, together with freezing data from the literature to assess if fungal spores from the phylum *Basidiomycota* are better ice nuclei that fungal spores from the phylum *Ascomycota*.

5.2 Experimental

5.2.1 Freezing Experiments

The instrument used to study the immersion freezing properties of the fungal spores consists of an optical microscope (Zeiss Axiolab A with a 10× objective) coupled with a flow cell that had temperature and relative humidity control. This apparatus or a similar apparatus has been used in many previous ice nucleation studies (Chapter 2-Chapter 4). For these experiments, the same method as was described in previous chapters was used to perform the immersion ice nucleation experiments on fungal spores (for a detailed description of the protocol for these experiments, see Chapter 3, Section 3.2.1). For each droplet, the freezing temperature was determined and the 2-dimensional (2-D) surface area of fungal spores contained within each droplet was calculated.

5.2.2 Spore Properties and Number of Spores Per Drop

Using microscope images (Olympus IX70: 20× objective for *Ustilagomycetes* and *Agaricomycetes* fungi; 30× and 40× objectives for *Eurotiomycetes* fungi), we calculated the
average projected 2-D surface area per spore and the spore dimensions (length and width) for each species. Using the average 2-D surface area per spore and the 2-D surface area of fungal spore per drop (Section 5.2.1), we determined the number of spores per drop. This methodology was previously used by Haga et al. [2013] (Chapter 4) for rust and bunt fungal spores.

Finally, the surface features of the spores were studied using scanning electron microscopy (SEM) images that were acquired at the University of British Columbia (UBC) Bioimaging Facility. The images for A. muscaria, B. zelleri, L. nuda, T. abietinum, the Ustilagomycetes spores (U. nuda, U. nigra, U. avenae) and P. chrysogenum were acquired using variable pressure SEM (Hitachi S2600 VP-SEM). A. bisporus, Penicillium sp., and P. brevicompactum spores were imaged by field emission SEM (Hitachi S4700 FESEM). Glass slides containing Agaricomycetes spores (A. bisporus, A. muscaria, B. zelleri, L. nuda, T. abietinum) were affixed onto aluminum SEM stubs using double-sided glue tabs, sputter coated with 12 nm gold (Au) palladium (Pd) and mounted in the SEM instrument. Similarly, loose Ustilagomycetes (U. nuda, U. avenae, U. nigra) spores were deposited directly onto double-sided glue tabs, affixed onto aluminum SEM stubs, sputter coated with 8 nm Au and mounted in the SEM instrument. To prepare the Eurotiomycetes (P. chrysogenum, Penicillium sp., P. brevicompactum) samples for imaging, cultures of each fungal species were first fixed using osmium vapor (4 % OsO₄ (aq)), then a portion of the culture was excised and mounted onto an aluminum SEM stub using Aquadag (a graphite based adhesive), and finally the samples were allowed to air dry prior to imaging. For the SEM experiments the working distance varied between 5 and 15 mm, the accelerating voltage was between 3 and 25 kV, and the images were captured using secondary electrons.
5.2.3 Spore Samples and Slide Preparation

*Agaricus bisporus*, the common button mushroom, was purchased from a local grocery store in Vancouver, British Columbia (B.C.), Canada. The remaining *Agaricomycetes* fungi (*Amanita muscaria*, *Boletus zelleri*, *Lepista nuda*, *Trichaptum abietinum*) were harvested from the Pacific Spirit Regional Park surrounding the UBC campus in Vancouver, B.C. To prepare slides containing *Agaricomycetes* spores, sections of the fruiting body were placed on a wire mesh in a sealed and humidified chamber. Hydrophobic glass slides were placed underneath the wire mesh, exposed to the part of the fungus that releases spores under humidified conditions. Over time, spores were naturally released and they were deposited onto the glass slides.

*Ustilagomycetes* spores (*Ustilago nuda*, *Ustilago nigra*, *Ustilago avanae*) were acquired from the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada. Slides containing *Ustilagomycetes* spores were prepared analogously to the method used by Haga *et al.* [2013] for rust (*Pucciniomycetes*) spores. Specifically, a spore cloud was created using a small fan within a sealed glass vessel containing spores, and immersing the vessel in a sonicator bath. Using this set-up, spores were deposited onto glass slides suspended on wire mesh within the vessel.

The *Eurotiomycetes* species studied here were obtained from the UBC Bioservices Laboratory collection, located within the Department of Chemistry: *Penicillium chrysogenum* (collection #177), *Penicillium* sp. (collection #58), *Pencillium brevicompactum* (collection #54). *Pencillium* spores were aerosolized from fungal cultures and deposited on a hydrophobic glass slide using a custom-built flow cell recently developed in our laboratory and previously used on *Cladosporium* [Iannone *et al.*, 2011].
5.2.4 Global Chemistry-Climate Transport Model, EMAC

We simulated the transport and removal of atmospheric aerosol particles in a global chemistry-climate model, ECHAM/MESSy Atmospheric Chemistry (EMAC). The EMAC model is a numerical chemistry and climate simulation system that includes sub-models describing tropospheric and middle atmosphere processes and their interaction with oceans, land and human influences [Jöckel et al., 2005; Jöckel et al., 2006; Kerkweg et al., 2006; Lawrence and Rasch, 2005; Tost et al., 2006]. It uses the first version of the Modular Earth Submodel System (MESSy1) to link multi-institutional computer codes. The core atmospheric model is the 5th generation European Centre Hamburg general circulation model (ECHAM5 GCM) [Roeckner et al., 2003]. For the present study, we applied EMAC (ECHAM5 version 5.3.01, MESSy version 1.9) in the T63L31 resolution, i.e. with a spherical truncation of T63 (corresponding to a quadratic Gaussian grid of approx. 1.9 by 1.9 degrees in latitude and longitude or approximately 140 × 210 km at mid-latitudes) with 31 vertical hybrid pressure levels up to 10 hPa. This model resolution provides a reasonable balance between accuracy and computational expense: tracer transport in the ECHAM5 GCM has been shown to be comparatively insensitive to further increases in model resolution [Aghedo et al., 2010].

Simulations were for five years and an additional year of spin-up time, with the model setup as described in detail in Burrows et al. [2009b], and with modifications to the scavenging scheme [Tost et al., 2010], including slow downward transport of crystal-borne species due to the sedimentation of crystals, and the release and repartitioning of tracers associated with evaporation of droplets and melting of ice crystals. Briefly, aerosols are treated as monodisperse passive aerosol tracers, emitted at the Earth’s surface. Atmospheric transport is simulated (including advection and parameterized convective mass flux), and tracers are removed by dry
and wet deposition to the surface. The EMAC model, used in similar configurations, has been shown to be capable of realistic simulations of aerosol transport and deposition for the transport of African dust to Europe [Gläser et al., 2012] and of radioactive aerosol from the Chernobyl accident [Lelieveld et al., 2012].

Removal processes of particles in the model included sedimentation, dry deposition, impaction scavenging, and nucleation scavenging by liquid, mixed-phase, and ice clouds. We use the methods outlined by Burrows et al. [2009b] to describe sedimentation, dry deposition, and impaction scavenging. Nucleation scavenging was prescribed with a scavenging parameter, \( R \), where \( R_{\text{nuc,liq}} \) and \( R_{\text{nuc,ice}} \) are the fraction of particles embedded in cloud droplets or ice particles within a cloud, respectively. For liquid clouds (i.e. clouds at temperatures > 0 °C), we assume that \( R_{\text{nuc,liq}} \) is unity, as done previously [Heald and Spracklen, 2009]. A value of unity assumes that the particles are efficient cloud condensation nuclei (CCN). Here, we are using the immersion freezing results to assess implications for fungal spore global transport. It follows that in order for the spores to be incorporated into liquid droplets, they would have to be active as CCN. Simulations were also run assuming the spores are inactive as CCN (i.e. \( R_{\text{nuc,liq}} = 0 \)), and these results are included in Appendix B (Figure B.1 and Figure B.2). For mixed-phase clouds (i.e. clouds at temperatures between 0 °C and -35 °C), we carried out simulations with two different values of \( R_{\text{nuc,ice}} \). These two different simulations were carried out to test if ice nucleation can impact the long-distance transport of the particles in the atmosphere. In the first simulation (referred to as IN-Inactive), we used an \( R_{\text{nuc,ice}} \) value of zero at all temperatures relevant for mixed-phase clouds (0 to -35 °C), and in the second simulation (referred to as IN-Active) we assumed \( R_{\text{nuc,ice}} \) is zero for mixed-phase clouds at temperature > -25 °C, but equal to unity for temperatures between -25 and -35 °C. A temperature of -25 °C for the onset of mixed-
phase ice nucleation scavenging was chosen because it represents the approximate temperature where freezing occurs for the spores studied here (see Figure 5.3). For ice clouds (i.e. clouds at temperatures $< -35 \, ^\circ\text{C}$, which is the homogeneous freezing temperature of water droplets), we assume that $R_{\text{nuc,ice}}$ is 0.05 for both simulations mentioned above (IN-Inactive and IN-Active). This value is consistent with measurements of cloud scavenging at low temperatures [Henning et al., 2004; Verheggen et al., 2007]. A summary of the nucleation scavenging coefficients is included in Table 5.3.

Table 5.3 Nucleation scavenging schemes used in EMAC.

<table>
<thead>
<tr>
<th>Scavenging Scheme</th>
<th>Liquid Nucleation Scavenging ($R_{\text{nuc,liq}}$)</th>
<th>Ice Nucleation Scavenging ($R_{\text{nuc,ice}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mixed-Phase Clouds ($0 , ^\circ\text{C} &gt; T &gt; -35 , ^\circ\text{C}$)</td>
<td>Ice Clouds ($T &lt; -35 , ^\circ\text{C}$)</td>
</tr>
<tr>
<td>IN-Inactive</td>
<td>0 for entire temperature range</td>
<td>0.05</td>
</tr>
<tr>
<td>IN-Active</td>
<td>0 for $T &gt; -25$</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>1 for $-25 &gt; T &gt; -35$</td>
<td></td>
</tr>
</tbody>
</table>

The in-cloud local rate of change in the mixing ratio, $X_i$, due to nucleation scavenging and subsequent precipitation is:

$$\frac{\Delta X_i}{\Delta t} = X_i f_{\text{cl}} \left( \frac{R_{\text{nuc,ice}} F_{\text{snow}}}{iwc} + \frac{R_{\text{nuc,liq}} F_{\text{rain}}}{lwc} \right),$$

where $\Delta t$ is the length of the model time step; $f_{\text{cl}}$ is the grid-box mean cloud fraction; $F_{\text{snow}}$ and $F_{\text{rain}}$ are the fluxes of snow and rain, respectively; $iwc$ and $lwc$ are the ice water content and liquid water content of the cloud, respectively; and $R_{\text{nuc,ice}}$ and $R_{\text{nuc,liq}}$ are the nucleation scavenging efficiencies of cloud ice and cloud liquid water, respectively.

For nucleation scavenging, the same scavenging parameterizations were applied to both stratiform clouds and to parameterized convective clouds. Simulations were run for 3 $\mu$m and 8 $\mu$m particles in diameter, which overlaps with the sizes (volume equivalent diameters) of the
spores studied here. In the simulations, fungal spores were emitted globally and continuously for all land surfaces, excluding land ice.

5.3 Results and Discussion

5.3.1 Spore Properties

SEM images of the fungal spores studied here are shown in Figure 5.1. The average dimensions for the spores are given in Table 5.2 and are consistent with other literature values: Bockus et al. [2010], Gilbertson and Ryvarden [1987], Lincoff [1981], Mathre [1982], Mitchell and Walter [1999], Pasanen et al. [1991], Raper et al. [1949], and Vanky [1987]. In many of the images, the spores appear deflated (A. bisporus, U. nuda, U. nigra, U. avenae, P. chrysogenum, Penicillium sp., P. brevicompactum) or as partial spheroids (T. abietinum). These features are likely due to the sample processing prior to imaging (see Section 5.2.2) or from a loss in turgidity of the fungal spores in the vacuum of the SEM instrument.
Figure 5.1 SEM images of the fungal spores studied. *Agaricomycetes* species include *A. bisporus*, *A. muscaria*, *B. zelleri*, *L. nuda*, and *T. abietinum*; *Ustilagomycetes* species include *U. nuda*, *U. nigra*, and *U. avenae*; *Eurotiomycetes* species include *P. chrysogenum*, *Penicillium* sp., *P. brevicompactum*. 
5.3.2 Fraction of Droplets Frozen vs. Temperature

Shown in Figure 5.2 is the fraction of frozen droplets as a function of temperature including all freezing events that were observed during these experiments. Results for droplets containing Agaricomycetes spores are shown in blue (open symbols), Ustilagomycetes fungi are shown in green (open symbols with horizontal line), and Eurotiomycetes spores are in red (red symbols with vertical line).

![Graph showing fraction of droplets frozen vs. temperature for different spore species.]

**Figure 5.2** Fraction of droplets frozen for Agaricomycetes, Ustilagomycetes, and Eurotiomycetes spores. Agaricomycetes spores (A. bisporus, A. muscaria, B. zelleri, L. nuda, T. abietinum) are in blue (open symbols); Ustilagomycetes spores (U. nuda, U. nigra, U. avenae) are in green (open symbols with horizontal line); Eurotiomycetes spores (P. chrysogenum, Penicillium sp., P. brevicipactum) are in red (open symbols with vertical line). Homogeneous nucleation results (∗) [Iannone et al., 2011] obtained using the same apparatus as this study are also included.

The median number of spores per droplet is given for each spore species in Table 5.2. Also included in Figure 5.2 (as ∗’s), are previous results from Iannone et al. [2011] for...
homogeneous nucleation using the same experimental method as used here. Figure 5.2 shows that the fungal spores studied here initiate freezing at warmer freezing temperatures compared to the homogeneous results of Iannone et al. [2011].

5.3.3 Cumulative Number of Ice Nuclei Per Spore as a Function of Temperature, \(IN_{per\text{Spore}}\)

The cumulative number of ice nuclei per spore as a function of temperature (\(IN_{per\text{Spore}(T)}\)) was determined from the number of spores per drop and the fraction of frozen droplets as a function of temperature (Figure 5.2) using the method presented by Vali [1971]. For details, see Haga et al. [2013] (Chapter 4), Section 4.3.3.

The temperature dependent \(IN_{per\text{Spore}(T)}\) spectra for the spores studied here are given in Figure 5.3: Agaricomycetes spores are shown in blue (open blue symbols), Ustilagomycetes spores are shown in green (open green symbols with horizontal line), and Eurotiomycetes spores are in red (open red symbols with vertical line). From Figure 5.3, the cumulative number of ice nuclei per spore was 0.001 between -20°C and -30 °C, 0.01 between -25 °C and -34 °C and 0.1 between -29 °C and -38 °C. Also, on average, the order of ice nucleating ability for these spores is Ustilagomycetes > Agaricomycetes > Eurotiomycetes. To illustrate this point, as an example, the cumulative number of ice nuclei per spore was 0.01 at approximately -27 °C for the Ustilagomycetes spores, 0.01 at approximately -29 °C for the Agaricomycetes spores, and 0.01 at approximately -31 °C for the Eurotiomycetes spores.
Figure 5.3 Cumulative number of ice nuclei per spore for the fungal spores studied calculated using the data shown in Figure 5.2. 

*Agaricomycetes* spores (*A. bisporus, A. muscaria, B. zelleri, L. nuda, T. abietinum*) are in blue (open symbols); *Ustilagomycetes* spores (*U. nuda, U. nigra, U. avenae*) are in green (open symbols with horizontal line); *Eurotiomycetes* spores (*P. chrysogenum, Penicillium sp., P. brevicompactum*) are in red (open symbols with vertical line). Also shown is the cumulative number of ice nuclei per spore for *Eurotiomycetes* spores studied by *Jayaweera and Flanagan* [1982], and the cumulative number of ice nuclei per particle for submicron Asian dust by *Niemand et al.* [2012] (half-filled diamonds).

There has only been one previous study on the ice nucleation ability of *Eurotiomycetes* spores [Jayaweera and Flanagan, 1982] and to our knowledge there have not been any previous studies on the ice nucleation of *Ustilagomycetes* or *Agaricomycetes* spores. The *Eurotiomycetes* species studied by *Jayaweera and Flanagan* [1982] include *Penicillium digitatum, Penicillium notatum*, and *Penicillium frequentens*. The results from these previous studies are included in Figure 5.3.
From Figure 5.3, when comparing the temperatures at which the cumulative number of ice nuclei per spore is equal to 0.01, all of the *Ustilagomycetes*, *Agaricomycetes* and *Eurotiomycetes* spores studied here are poorer IN than those studied by Jayaweera and Flanagan [1982]. Comparing just the *Penicillium* results from the current study and with those studied by Jayaweera and Flanagan [1982] suggests that: (1) *P. chrysogenum*, *Penicillium* sp., and *P. brevicompactum* are poor IN compared to *P. digitatum*, *P. notatum*, and *P. frequentens*, and (2) even within the same genus, the INperSpore values can vary drastically (i.e. over approximately 24 °C when looking at the temperatures at which INperSpore is equal to 0.01 in Figure 5.3).

*Morris et al.* [2013] recently studied the importance of bacteria cells present on the surface of rust fungal spores in contributing to the ice nucleating properties of the spores. It is, therefore, important to consider the possibility that the fungal spores studied here may contain bacterial or fungal contaminants on their surface, and to discuss the likelihood that these contaminants would change the ice nucleating properties of the spores. From the SEM images presented in Figure 5.1, there is no indication of any bacterial or fungal surface contamination on the fungal spores, (personal communication, D. Horne, UBC Bioimaging Facility). Since the *Eurotiomycetes* spores studied here (*P. chrysogenum*, *Penicillium* sp., *P. brevicompactum*) were grown in culture, any bacterial contamination of the spores would have been apparent in the culture stage of these experiments. The remaining spores (with the exception of *A. bisporus*) were qualitatively tested for surface contamination by bacteria and/or fungi using the same method from *Haga et al.* [2013] (Chapter 4) for rust and bunt spores: the spores to be tested were cultured in media (nutrient agar and tryptic soy agar) that would support contaminant growth and the cultures were monitored for two weeks. The test indicated low levels of surface contamination for four of the fungal spores studied here. Specifically, *U. avenae*, *U. nigra*, *U.
nuda cultures indicated surface contamination on the spores after 4 days, and colonies were observed in the T. abietinum culture after 13 days. For the remaining spores, B. zelleri, L. nuda, and A. muscaria, there was no indication of surface contamination. From the discussion above, it seems unlikely that the ice nucleation properties of the spores studied here are appreciably affected by surface contamination by fungi or bacteria. However, we cannot rule out this possibility and, so, the INperSpore spectra presented in the current study should be taken as upper limits.

Mineral dust particles are considered to be important atmospheric ice nuclei. For comparison, in Figure 5.3 we have included recent immersion freezing results by Niemand et al. [2012] for submicron Asian mineral dust (experiments ACI04_16 and ACI04_19). Specifically, INperParticle(T) for the Asian mineral dust was calculated from the ice nucleation active surface site density, ns, values from Niemand et al. [2012] and as described by Haga et al. [2013](Chapter 4, Section 4.3.3), and is shown in Figure 5.3 (half-filled diamonds). With the exception of one of the Ustilagomycetes species, U. nigra, which has similar ice nucleation properties as the Asian mineral dust, all of remaining spores studied here are poorer IN than the Asian mineral dust studied by Niemand et al. [2012] on a per particle basis.

5.3.4 Ice Nucleation Active Surface Site Density, ns

When comparing ice nuclei with different sizes, it is important to consider that larger particles may be more efficient IN on a per particle basis because they have more surface area available for nucleation. We normalized the INperSpore spectra (Figure 5.3) for surface area to calculate the ice nucleation active surface site density, ns, for the fungal spores in the current study. The ice nucleation active surface site density corresponds to the number of ice nucleation
sites per unit surface area of a particular type of IN [Connolly et al., 2009], and was calculated according to:

\[ n_s = \frac{IN\text{perSpore}}{A_{aer}}. \] (5.2)

To calculate the surface area for each fungal spore \( A_{aer} \), we approximated the spores to be prolate spheroids having length and width shown in Table 5.2.

The temperature dependent ice nucleation active surface site density for the fungal spores is shown in Figure 5.4. For comparison, included in Figure 5.4 are \( n_s \) values for Asian mineral dust [Niemand et al., 2012] (experiments ACI04_16 and ACI04_19), and also results from Jayaweera and Flanagan [1982] for Eurotiomycetes spores. The \( n_s \) values for the Eurotiomycetes spores studied by Jayaweera and Flanagan [1982] were calculated using spore dimensions and morphologies from the literature [Raper et al., 1949]. Specifically, \( P. \) digitatum, was approximated to be a prolate spheroid having dimensions 7.75 × 5.5 μm; and both \( P. \) notatum and \( P. \) frequentens spores were assumed to be spheres with diameter 3.25 μm.
Figure 5.4 Ice nucleation active surface site density, $n_s$ (m$^{-2}$), for fungal spores determined using the data in Figure 5.3. Agaricomycetes spores (A. bisporus, A. muscaria, B. zelleri, L. nuda, T. abietinum) are in blue (open symbols); Ustilagomycetes spores (U. nuda, U. nigra, U. avenae) are in green (open symbols with horizontal line); Eurotiomycetes spores (P. chrysogenum, Penicillium sp., P. brevicaespactum) are in red (open symbols with vertical line). Also shown are $n_s$ values for Asian mineral dust [Niemand et al., 2012] (half-filled diamonds).

After accounting for surface area, Figure 5.4 shows that the $n_s$ spectra of the Agaricomycetes, the Eurotiomycetes, and two of the Ustilagomycetes are similar. The remaining Ustilagomycetes spore, U. nigra, is a slightly better IN on a per surface area basis than the other spores studied here. Figure 5.4 also shows that a difference in surface area cannot explain the difference in freezing temperatures within the same genus observed in Figure 5.3 for species of Penicillium. Finally, Figure 5.4 shows that, at temperatures below -20 °C, all of the fungal spores
studied here are less efficient ice nuclei compared to Asian mineral dust on a per surface area basis.

5.3.5 Modelling Results

We used the global chemistry-climate transport model EMAC to study the effects of ice nucleation on transport and distribution of fungal spores in the atmosphere. To do this, we compare two scavenging scenarios, one in which there is no scavenging of particles from heterogeneous ice nucleation (IN-Inactive), and one in which particles are removed by ice nucleation on particles at temperatures below -25 °C (IN-Active), a temperature representative of the immersion freezing temperatures for the fungal spores in the current study. Results are presented in terms of percentage difference in the mixing ratio between the two scavenging scenarios (i.e. (IN-Active – IN-Inactive)/IN-Inactive × 100 %)). Positive non-zero percentages indicate increased concentrations due to scavenging from heterogeneous ice nucleation (-25 °C > T > -35 °C), whereas negative non-zero percentages indicate decreased concentrations due to scavenging from heterogeneous ice nucleation (-25 °C > T > -35 °C).

The results from these simulations are given in Figure 5.5, showing the percent change in surface annual mean mixing ratio, and in Figure 5.6, showing the percent change in the zonally averaged vertical profiles of the annual mean mixing ratio. In both figures, the top and bottom panels correspond to 3 μm and 8 μm particles, respectively. Figure 5.5 shows that the inclusion of scavenging of fungal spores by the ice phase at temperatures between -25 °C and -35 °C mainly modifies surface concentrations over the oceans and polar regions, and that ice nucleation under these conditions is expected to limit the long-distance transport of fungal spores from land surfaces to these remote regions. Interestingly, scavenging by the ice phase increases surface concentrations in some areas (40-70 °S); this may be due to increased sedimentation of the
particles in the clouds to the surface from ice scavenging and subsequent precipitation. Additionally, these conclusions are consistent for different particle sizes (3 μm and 8 μm).

Figure 5.6 shows that the inclusion of scavenging by the ice phase at temperatures between -25 °C and -35 °C modifies concentrations of fungal spores in the upper troposphere. These results provide further support that scavenging by the ice phase at temperatures between -25 °C and -35 °C, can limit the long-distance transport of fungal spores to remote regions (Arctic and oceans). As with Figure 5.5, these conclusions are consistent for different particle sizes (3 μm and 8 μm).
Figure 5.5 EMAC results showing percent change between IN-Active and IN-Inactive simulations in surface annual mean mixing ratio, with percent change being calculated as (IN-Active – IN-Inactive)/IN-Inactive × 100 %. Top: 3 µm particles; bottom: 8 µm particles. In all simulations, spores are assumed to be CCN-active. IN-Active assumes that particles are removed by ice-phase nucleation at temperatures between -25 °C and -35 °C. Emissions were simulated from land surfaces except for land covered with ice.
Figure 5.6 EMAC results showing percent change between IN-Active and IN-Inactive simulations in the zonally averaged vertical profiles of the annual mean mixing ratio, with percent change being calculated as \((\text{IN-Active} - \text{IN-Inactive})/\text{IN-Inactive} \times 100\%\). Top: 3 μm particles; bottom: 8 μm particles. In all simulations, spores are assumed to be CCN-active. IN-Active assumes that particles are removed by ice-phase nucleation at temperatures between -25 °C and -35 °C. Emissions were simulated from land surfaces except for land covered with ice.
5.3.6 *Ascomycota* vs. *Basidiomycota*

The phyla *Basidiomycota* and *Ascomycota* together make up 98 % of known fungal species [James et al., 2006]. Recent field measurements found increased concentrations of *Ascomycota* spores relative to *Basidiomycota* spores in remote marine regions and it was suggested (based on preliminary results from the current study) that these findings might be explained by *Basidiomycota* spores being better IN compared to *Ascomycota* spores [Fröhlich-Nowoisky et al., 2012]. To investigate whether there might be a systematic difference in the ice nucleating ability of the *Ascomycota* and the *Basidiomycota*, we have compiled all previous measurements of ice nucleation on these types of fungal spores, including this study, Haga et al. [2013], Iannone et al. [2011], Jayaweera and Flanagan [1982], and Morris et al. [2013]. These results are shown in Figure 5.7 as the cumulative number of ice nuclei per spore (\textit{INperSpore}), and in Figure 5.8 as the ice nucleation active surface site density (\textit{n_s}).
Figure 5.7 Comparison between INperSpore spectra for Ascomycota and Basidiomycota fungal spores from this study, as well as previous studies, including Haga et al. [2013] (Chapter 4), Morris et al. [2013], Jayaweera and Flanagan [1982], and Jannone et al. [2011] (Chapter 3). Also shown in the figure are INperParticle values for submicron Asian mineral dust [Niemand et al., 2012].
To calculate \( \text{INperSpore} \) for \textit{Cladosporium} sp. studied by \textit{Iannone et al.} [2011] (Chapter 3), we used the number of spores per drop (determined using the same method described in Sections 4.2.2 and 5.2.2), along with the freezing temperatures of the drops using the method described in Section 5.3.3 (data not shown in \textit{Iannone et al.} [2011]). All of the other \( \text{INperSpore} \) values shown in Figure 5.7 were provided in the original studies [\textit{Haga et al.}, 2013; \textit{Jayaweera and Flanagan}, 1982; \textit{Morris et al.}, 2013]. Comparing freezing temperatures for which the cumulative number of ice nuclei per spore was equal to 0.01 from Figure 5.7, some of the \textit{Ascomycota} spores (\textit{P. chrysogenum} and \textit{P. brevicompectum}) are the poorest IN but, on the other hand, some of the spores from this phylum are also the best IN (\textit{P. digitatum}, \textit{P. notatum}, \textit{P. frequentens}, \textit{C. herbarum}). Within both phyla (\textit{Ascomycota} and \textit{Basidiomycota}) there is a wide range of freezing properties. Overall, we conclude that the freezing properties of \textit{Basidiomycota} spores are not inherently better IN than \textit{Ascomycota} spores. Finally, from Figure 5.7 and again comparing the freezing temperatures for which the cumulative number of ice nuclei per spore or particle is equal to 0.01, the ice nucleating ability of mineral dust particles lie approximately in the middle of the range of temperatures found for each phylum (\textit{Basidiomycota} and \textit{Ascomycota}).

Figure 5.8 shows \( n_s \) values for all previous measurements of fungal IN from the classes \textit{Basidiomycota} and \textit{Ascomycota}. To calculate \( n_s \) for the spores studied by \textit{Morris et al.} [2013], we approximate the spores as prolate spheroids based on literature values for spore morphology and size ranges [\textit{Anikster et al.}, 2005; \textit{Anikster et al.}, 2004; \textit{Bruckart et al.}, 2007; \textit{Cummins and Husain}, 1966; \textit{Laundon and Waterston}, 1964]. Specifically, we used dimensions of length and width as follows: 29.0 \( \times \) 25.0 \( \mu \text{m} \) for \textit{P. aristidae}, 14.6 \( \times \) 12.5 \( \mu \text{m} \) for \textit{P. lagenophorae}, 25.0 \( \times \) 20.6 \( \mu \text{m} \) for \textit{Puccinia} sp., 25.0 \( \times \) 21.7 \( \mu \text{m} \) for \textit{P. allii}, 24.4 \( \times \) 19.7 \( \mu \text{m} \) for \textit{P. striiformis}, 28.3 \( \times \)
17.5 μm for *P. graminis*, 32.0 × 23.0 μm for *H. vastratrix*, and 23.3 × 20.4 μm for *P. triticina*. The calculation of $n_s$ for the *Penicillium* spores studied by Jayaweera and Flanagan [1982] was discussed in Section 5.3.4. The other *Ascomycota* spore studied by Jayaweera and Flanagan [1982] was *Cladosporium herbarum*, and to calculate $n_s$ this spore was approximated to be a prolate spheroid with dimensions 19.5 × 6.0 [Schubert et al., 2007]. To calculate $n_s$ for *Cladosporium* sp. from Iannone et al. [2011] (Chapter 3), we used microscope images of the spores to determine their average length and width (data not shown in Iannone et al. [2011]); these measurements were done using the same microscope and method for determining the length and width of the spores for the current study (described in Section 5.2.2).

Figure 5.8 shows that, once normalized to surface area, there remains no inherent difference in the ice-nucleating ability of *Basidiomycota* spores compared to *Ascomycota* spores, as suggested above from *INperSpore* spectra (Figure 5.7). Figure 5.8 also shows that the variability in freezing properties within both phyla (*Basidiomycota* and *Ascomycota*) cannot be accounted for by a difference in surface area between different spore species. Finally, Figure 5.8 shows that, at temperatures below -20 °C, all fungal spores are poorer IN than Asian mineral dust.
Figure 5.8 Comparison between $n_s$ spectra for *Ascomycota* and *Basidiomycota* fungal spores calculated using the data in Figure 5.7 and using approximations for spore size and shape detailed in the text. Included are results from the current study as well as all known previous studies, including: Haga et al. [2013] (Chapter 4), Morris et al. [2013], Jayaweera and Flanagan [1982], and Iannone et al. [2011] (Chapter 3). Also shown in the figure are previous results for submicron Asian mineral dust [Niemand et al., 2012].
5.4 Conclusions

5.4.1 Ice Nucleation on Fungal Spores

Spores from three classes of fungi that are believed to be abundant in the atmosphere (Agaricomycetes, Ustilagomycetes, and Eurotiomycetes) were found to act as heterogeneous ice nuclei in the immersion freezing mode. For the fungal spores studied, the cumulative number of ice nuclei per spore is 0.001 at temperatures between -20°C and -30 °C, 0.01 between -25 °C and -34 °C, and 0.1 between -29 °C and -38 °C. (Figure 5.3). Comparing freezing temperatures needed for the cumulative number of ice nuclei per spore to equal 0.01, Ustilagomycetes had the warmest average freezing temperatures (-27 °C), followed by Agaricomycetes (-29 °C) and the Eurotiomycetes had the lowest freezing temperatures (-31 °C). On a per surface area basis, all of the fungal spores studied had similar freezing properties with the exception of one of the Ustilagomycetes (U. nigra), which was a slightly better ice nuclei (Figure 5.4).

It has recently been suggested that spores from the phylum Basidiomycota might be better IN than spores from the phylum Ascomycota [Fröhlich-Nowoisky et al., 2012]. To investigate this further, the freezing results from the current study were combined with all previous measurements on fungal spores from the Basidiomycota and the Ascomycota. These results, shown in Figure 5.7 as the cumulative number of ice nuclei per spore and in Figure 5.8 as the ice nucleation active surface site density, indicate that Basidiomycota spores are not inherently better IN than Ascomycota spores and that both phyla show significant variability in the ice nucleation properties of their spores. On a per particle basis, submicron Asian mineral dust had freezing temperatures approximately in the middle of the range of temperatures found for each phylum. Once normalized to surface area, all previously studied fungal spores are poorer IN than Asian mineral dust at temperatures below -20 °C.
5.4.2 Global Transport of Fungal Spores

Using a global chemistry-climate transport model, we show that ice nucleation on these spores can modify the atmospheric transport and global distributions by providing an additional removal mechanism of the spores from the atmosphere. Specifically, we show that ice nucleation on spores emitted from land surfaces (excluding surfaces covered by ice) with sizes between 3 and 8 μm in diameter affects their long-distance transport to remote polar and marine regions (Figure 5.5). Also, ice nucleation on these spores limits their transport to the upper troposphere (Figure 5.6).

This study is among the first attempts to model the effect of IN activity on long-distance fungal spore transport. While the treatment of ice nucleation scavenging is simplified, it gives an initial indication of the regions that are most sensitive to the rate of removal of particles due to scavenging by ice nucleation, as well as the potential magnitude of these effects.
Chapter 6. Ice Nucleation in Droplets Containing Bacteria Which Have Been Identified in the Atmosphere: Immersion Freezing Results and Atmospheric Implications

6.1 Introduction

Bacteria are ubiquitous in the atmosphere and a range of surface concentrations have been reported for different ecosystems. Surface concentrations over oceans and tundra have been estimated to be approximately 10 \( \text{L}^{-1} \); coastal and forest ecosystems have slightly higher estimated surface concentrations of approximately 60 to 80 \( \text{L}^{-1} \); and crops, grasslands, shrubs, and urban ecosystems are all expected to have the highest surface concentrations of bacteria, in the 100’s \( \text{L}^{-1} \) [Burrows et al., 2009a]. Bacteria have been measured at high altitudes in the free troposphere at concentrations ranging from 1 to 10^4 \( \text{L}^{-1} \) [Bowers et al., 2012; Bowers et al., 2009; DeLeon-Rodriguez et al., 2013; Xia et al., 2013], and have even been measured in the stratosphere at altitudes up to 77 km [Imshenetsky et al., 1978; Smith et al., 2010]. Modelling results have suggested that bacteria can be transported large distances in the atmosphere [Burrows et al., 2009b; Womack et al., 2010].

IN from bacterial sources may influence the radiative and precipitation properties of mixed-phase clouds. From numerous field studies [Conen et al., 2011; Creamean et al., 2013; Garcia et al., 2012; Pöschl et al., 2010; Pratt et al., 2009; Prenni et al., 2009b], it has been suggested that biological particles (including bacteria) may be an important source of IN. However, the current level of understanding of the ice nucleating properties of biological particles is incomplete and there remain significant uncertainties in understanding their effects on mixed-phase clouds. Here, we study the ice nucleation properties of bacteria that have been identified to the species level in the atmosphere. These results should be useful for modelling
studies, which currently rely on limited knowledge of the ice nucleation properties of biological particles.

From previous studies, there can be significant variability in the ice nucleation properties of bacteria species belonging to the same genus. Using the genus *Pseudomonas* as an example, Obata et al. [1999] reported that the cumulative number of IN per *P. antarctica* cell was approximately $10^{-4}$ at -6 °C, where as Attard et al. [2012] reported that the cumulative number of IN per *P. syringae* cell was approximately 0.05 at -6 °C. As a result, here we focus on bacteria that have been identified at the species level in the atmosphere.

Although there have been numerous studies identifying taxonomic groups of bacteria in the atmosphere, to our knowledge only 15 of them have included species level identification. These studies are: Ahern et al. [2007]; Amato et al. [2005]; Amato et al. [2007]; DeLeon-Rodriguez et al. [2013]; Fang et al. [2007]; González-Toril et al. [2009]; Griffin [2004]; Harding et al. [2011]; Imshenetsky et al. [1978]; Maki et al. [2010]; Maki et al. [2011]; Ravva et al. [2012]; Smith et al. [2010]; Wainwright et al. [2003]; and Zweifel et al. [2012]. Combined, these previous studies have identified 49 different bacteria species and here we study 5 of these species, which are listed in Table 6.1, and the details of their taxonomy are provided in the Appendix A, Figure A.3.
Table 6.1 Description of bacteria species studied.

<table>
<thead>
<tr>
<th>Species</th>
<th>Estimated Cell Shape</th>
<th>Estimated Cell Size (μm)</th>
<th>Bacteria Cells/Drop</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Q1°</td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td>cylinder</td>
<td>0.8 × 2</td>
<td>133</td>
</tr>
<tr>
<td><strong>Pseudomonas putida</strong></td>
<td>cylinder</td>
<td>0.9 × 3</td>
<td>20</td>
</tr>
<tr>
<td><strong>Comamonas testosteroni</strong></td>
<td>cylinder</td>
<td>0.75 × 2.5</td>
<td>30</td>
</tr>
<tr>
<td><strong>Micrococcus luteus</strong></td>
<td>sphere</td>
<td>1.5</td>
<td>11</td>
</tr>
<tr>
<td><strong>Pseudomonas syringae</strong> (strain 31R1)</td>
<td>cylinder</td>
<td>0.95 × 2.25</td>
<td>38</td>
</tr>
</tbody>
</table>

In addition to having been identified to the species level in the atmosphere, *B. subtilis*, *P. putida*, *C. testosteroni*, and *M. luteus* were chosen because (1) they were readily available from existing culture collections, (2) they are classified at a biosafety level appropriate for study in our laboratories, and (3) their ice nucleation properties have either not been studied previously (*C. testosteroni*, *M. luteus*, *P. putida*) or, in the case of *B. subtilis*, in only a few cases ([Edwards et al., 1994; Maki et al., 1974]). *P. syringae* (strain 31R1) was chosen for these studies since there have been numerous studies on the immersion ice nucleation properties of *P. syringae* (see for e.g. Lindow et al. [1989]; Möhler et al. [2008c]; Morris et al. [2008]; Vali et al. [1976]), and here we compare our results with some of these previous studies to confirm the validity of our experimental methods. A summary of previous ice nucleation studies on the species of bacteria investigated here is given in Appendix C (Table C.1).

Of the 15 previous studies that have identified bacteria to the species level, *B. subtilis* was identified in three studies [Fang et al., 2007; Maki et al., 2010; Smith et al., 2010]; *C. testosteroni* was identified in one study [Ahern et al., 2007]; *M. luteus* was identified in two studies [Amato et al., 2007; Fang et al., 2007]; *P. putida* was identified in one study [Ahern et

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Note: Based on cell shapes and sizes from the literature [Buchanan and Gibbons, 1974], cell size given as length × width for cylinders or as the diameter of a sphere.

° Q1: first quartile, 25% of the data were ≤ this value; Q3: third quartile, 75% of the data were ≤ this value.
and P. syringae was identified in two studies [Ahern et al., 2007; Amato et al., 2007]. Also, the specific isolate of B. subtilis used here was sampled from the upper troposphere by DeLeon-Rodriguez et al. [2013] and identified to the species level using DNA analysis (species level identification not shown in DeLeon-Rodriguez et al. [2013]).

One of the main objectives of this study was to determine the cumulative number of ice nuclei per bacteria cell ($IN_{perCell}(T)$) for the full temperature range for mixed-phase clouds (down to -37 °C), as this information is needed to determine whether ice nucleation on these particles (i.e. bacteria cells) might influence the frequency and properties of mixed-phase clouds. Although there have been many previous studies on the ice nucleation of bacteria (see, for example, Table 6 from Desprès et al. [2012]), very few of these studies have investigated the full temperature range for mixed-phase clouds and, also, only a fraction of these studies have reported the cumulative number of ice nuclei per bacteria cell. Of the bacteria studied here, the cumulative number of ice nuclei per bacteria cell has only been reported for P. syringae [Attard et al., 2012; Edwards et al., 1994; Gross et al., 1983; Kozloff et al., 1983; Lindow et al., 1982; Lindow et al., 1989; Maki and Willoughby, 1978; Maki et al., 1974; Möhler et al., 2008c; Morris et al., 2008; Nemecek-Marshall et al., 1993; Vali et al., 1976].

To understand the implications of ice nucleation on bacteria for mixed-phase cloud processes, both the immersion freezing properties and the atmospheric concentrations of bacteria must be considered. We applied the method of Murray et al. [2012] to our immersion freezing results to estimate the atmospheric concentration of IN for the species of bacteria investigated here, using global annual mean concentrations from Hoose et al. [2010b] and assuming that each droplet contains only one particle (i.e. bacteria cell).
6.2 Experimental

6.2.1 Freezing Experiments

To study the immersion freezing properties of droplets containing bacteria, we use a temperature and humidity controlled flow cell that is coupled to an optical microscope (Zeiss AxioLab A with a 10× objective), as has been described in previous studies (Chapter 2-Chapter 5). For most of the freezing experiments a cooling rate of 5 °C min⁻¹ was used; however, for some of the experiments on *P. syringae* a cooling rate of 10 °C min⁻¹ was used (see following paragraph for discussion).

Preliminary experiments on droplets containing *P. syringae* indicated freezing temperatures as warm as -4 °C, in agreement with previous studies (for example, see *Lindow et al.* [1989] and Table C.1). Growth of the ice crystals that froze at these warm temperatures occurred by the Wegener-Bergeron-Findeisen process and as a result, many droplets either froze by contact with the growing ice crystals or evaporated by vapor transfer from liquid droplets to ice crystals. For these experiments, 49 immersion freezing events were observed, 51 droplets froze by contact freezing, and 64 droplets evaporated. To minimize the loss of droplets to evaporation and contact freezing, the ramp rate was increased to -10 °C min⁻¹ for the remainder (and majority) of the *P. syringae* experiments. When a ramp rate of -10 °C min⁻¹ was used, approximately half of the total droplets either evaporated or froze by contact.

6.2.2 Bacteria Samples, Bacteria Surface Features and Slide Preparation

*B. subtilis* isolates came from flights at high altitudes in the area of the Gulf of Mexico and the Caribbean, the details of which are given in *DeLeon-Rodriguez et al.*, 2013. The samples were collected on 0.22 μm pore size filters and bacteria were detached from the filters by incubating in 1× phosphate buffered saline (PBS) and shaking at 200 revolutions per minute.
at 4 °C for 2 hours. The PBS solution containing bacteria cells was then plated separately in Luria Bertani (LB) broth and Reasoner’s 2A agar (R2A) at 25 °C for one week. The isolates were transferred to fresh plates until pure cultures were obtained, at which point the DNA was extracted from the isolates using a Qiagen kit and 16S rRNA PCR analysis was performed using universal primers. The PCR fragment (1,500 bp) was sent to sequence using Sanger sequencing technology and analyzed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the non-redundant NCBI database. The sequence matched *B. subtilis* at 100 % nucleotide identity, confirming that the isolate is *B. subtilis*.

*P. putida* and *C. testosteroni* were acquired from the Bioservices Laboratory bacterial collection (Department of Chemistry, University of British Columbia, Vancouver (UBC), Canada), collection numbers 1062 and 830, respectively. *P. syringae* (strain 31R1) was provided by S. Lindow (Department of Plant and Microbial Biology, University of California, Berkley, U.S.A.). *M. luteus* was ordered from the American Type Culture Collection (ATCC catalogue number 10240).

The bacterial cultures were prepared for ice nucleation experiments according to the method outlined by [Möhler et al., 2008c]. The bacteria were grown on King’s B agar for 3 days at 25 °C, at which time the petri dishes were transferred to a 4 °C cold room and stored there for one week. To isolate the bacteria from the culture substrate, cells were washed off the culture plates using sterile distilled water, centrifuged, and re-suspended in sterile distilled water. A cell number concentration of approximately $10^9$ ml$^{-1}$ was achieved by adjusting the suspension to an optical density of 0.6 at a wavelength of 580 nm. These suspensions were used to prepare slides for freezing experiments and also for imaging of the bacteria using field emission scanning electron microscopy (Hitachi S4700 FESEM, UBC Bioimaging Facility). The bacteria were
fixed directly in suspension using 2.5 % glutaraldehyde and 4 % formaldehyde and held overnight at 4 °C. Then, a 1 mL aliquot was passed through a 0.2 µm filter, rinsed with doubly distilled H₂O, fixed using osmium vapor (1 % OsO₄ (aq)), rinsed again with doubly distilled H₂O, and then dehydrated using a graded ethanol series. Finally, the sample was critically point dried, mounted on an aluminum SEM stub using double-sided tape, and sputter coated with 8 nm gold/palladium. For these experiments, the working distance varied between 11.9 to 14.6 mm, the accelerating voltage was 1.5 kV for P. syringae and 5 kV for the remaining bacteria (B. subtilis, C. testosteroni, P. putida, and M. luteus), and the images were captured with secondary electrons.

To deposit the bacteria on the glass slide, the suspension was passed through a nebulizer (Meinhard Glass Products, Model # TR-30-A1) using high-purity nitrogen as a carrier gas. The flow from the nebulizer was directed at a hydrophobic glass slide, and droplets containing the bacteria were deposited on the surface of the slide upon impaction. Water then evaporated, leaving behind bacteria cells.

### 6.2.3 Surface Area Density of Bacteria, Surface Area and Number of Bacteria Per Drop

Once bacteria were deposited on a glass slide, images of the slide were obtained using an Olympus IX70 microscope (20× objective). These images were then used to calculate the projected 2-dimensional (2-D) surface area density of bacteria on the glass slide (µm² bacteria/µm² glass slide) using the National Institute of Health’s image processing software, ImageJ. The surface area of bacteria per droplet was then estimated by first measuring the projected 2-D surface area of each droplet, and then by multiplying the 2-D droplet area by the surface area density of bacteria. Next, we used cell sizes and shapes from the literature (see
Table 6.1 and Buchanan and Gibbons [1974]) to estimate the projected 2-D surface area of a single bacteria cell, and from this we calculated the number of bacteria per droplet.

Using this method to estimate the number of bacteria per droplet assumes that (1) there is a uniform distribution of bacteria over the slide and (2) that there exists only a single layer of cells on the slide, that is, that there are no bacteria stacked on top of each other. Careful examination of images of the prepared slides (data not shown) support the first assumption that a uniform distribution of bacteria was observed in most cases. However, it was difficult to control the amount of bacteria deposited on the slides, and in some images it is apparent that bacteria are stacked on top of each other (images not shown). Therefore, we present the freezing results for the species of bacteria studied here with the caveat that the calculated number of bacteria per droplet should be considered as lower limits, providing upper limits for both the cumulative number of ice nuclei per particle (Section 6.3.3 and Figure 6.3) and the ice nucleation active surface site density (Section 6.3.4 and Figure 6.4).

6.3 Results and Discussion

6.3.1 Bacterial Cell Properties

Shown in Figure 6.1 are SEM images for the bacteria investigated. The sizes and shapes of the bacteria agree with the literature [Buchanan and Gibbons, 1974]. B. subtilis, C. testosteroni, P. putida, and P. syringae have rod-shaped cells and have approximate dimensions of 0.7-0.8 × 2-3 μm, 0.7-0.8 × 2.1-2.9 μm, 0.7-1.1 × 2.0-4.0 μm, and 0.7-1.2 × 1.5-3 μm, respectively; M. luteus has spherical cells approximately 1.0-2.0 μm in diameter. In addition to the bacteria cells, there are smaller fibrous features present in the SEM images of the bacteria shown in Figure 6.1. These additional features may be either: (1) the flagella, the motile structure associated with some of these bacteria (B. subtilis, C. testosteroni, P. putida, P. syringae)
[Buchanan and Gibbons, 1974], or (2) components of a biofilm, an extracellular matrix that can be produced by many types of bacteria [Hall-Stoodley et al., 2004]. Of the bacteria studied here, *B. subtilis* [Vlamakis et al., 2013], *M. luteus* [Matsuura et al., 2013], *P. putida* [Martínez-Gil et al., 2012], and *P. syringae* [Penaloza-Vazquez et al., 2010] have all been shown to produce biofilms.

Figure 6.1 SEM images of the bacteria studied.

6.3.2 Fraction of Droplets Frozen as a Function of Temperature

The fraction of droplets frozen as a function of temperature for the bacteria species studied here are given in Figure 6.2, as well as previous results for homogeneous freezing from Iannone et al. [2011] using the same experimental method as the current study. The number of bacteria per droplet for each species, given as a median, first quartile (Q1), and third quartile (Q3) are given in Table 6.1. The median number of bacteria per droplet was between 11-133 for the species studied here. Figure 6.2 shows that *P. syringae* is active as a heterogeneous IN and that most of the droplets containing *P. syringae* froze at temperatures above -10 °C. As discussed in Section 6.2.1, for *P. syringae* at temperatures below -10 °C, only a small number of
immersion freezing events were observed due to a high number of droplets either evaporating or freezing by contact. Further studies are needed to investigate immersion freezing by *P. syringae* at temperatures below -10 °C. The remaining bacteria (*B. subtilis, P. putida, C. testosteroni, M. luteus*) are either not active as IN, since droplets containing these bacteria froze in the temperature regime for homogeneous freezing; or these bacteria are active as heterogeneous IN, but the same temperatures as homogeneous freezing (~ -37 °C).

Figure 6.2 Fraction of droplets frozen as a function of temperature for the bacteria studied here and also for homogeneous freezing of pure water droplets from *Iannone et al.* [2011] (Chapter 3) using the same experimental method as the current study.
6.3.3 *Cumulative Number of Ice Nuclei Per Bacteria Cell as a Function of Temperature, INperCell*

Using the fraction of droplets frozen as a function of temperature (Figure 6.2), we calculated the cumulative number of ice nuclei per bacteria cell as a function of temperature, \( \text{INperCell}(T) \), using the method of Vali [1971] and adapted for the current study as described previously for rust and bunt fungal spores [Haga et al., 2013](Chapter 4, Section 4.3.3). In Figure 6.3, \( \text{INperCell} \) values are given for the bacteria studied here. Included for comparison in Figure 6.3 are previous results from Lindow et al. [1989] and Möhler et al. [2008c] for the same strain of *P. syringae* as was used in the current study (*P. syringae* 31R1), as well as the cumulative number of ice nuclei per particle (\( \text{INperParticle}(T) \)) for submicron Asian mineral dust [Niemand et al., 2012].
Figure 6.3 The cumulative number of ice nuclei per bacteria cell, $IN_{per\text{Cell}}(T)$. Results from the literature are included for the same strain of P. syringae as studied here (strain 31R1) [Lindow et al., 1989; Möhler et al., 2008c]. The $IN_{per\text{Cell}}$ value at -5.6 °C from Möhler et al. [2008c] is an upper limit. Also included are the cumulative number of IN per particle, $IN_{per\text{Particle}}(T)$, values for submicron Asian mineral dust [Niemand et al., 2012].

$IN_{per\text{Particle}}$ was calculated for the Asian mineral dust (experiments ACI04_16 and ACI04_19) from Niemand et al. [2012] using the ice nucleation active surface site density ($n_s$) values according to the method described in Haga et al. [2013](Chapter 4, Section 4.3.3), and by assuming the dust particles are spherical with diameters of 0.70 μm (ACI04_16) and 0.76 μm (ACI04_19) [Niemand et al., 2012]. The ice nucleation active surface site density corresponds to the number of ice nuclei per unit of surface area for a certain type of IN [Connolly et al., 2009]. Of the bacteria studied here, B. subtilis, P. putida, C. testosteroni, and M. luteus are poor IN on a per particle basis: the cumulative number of ice nuclei per bacteria cell was $3 \times 10^{-4}$ at temperatures between approximately -27.5 °C and -34 °C, and 0.01 between approximately -35.5
°C and -37 °C. As expected from previous studies *P. syringae* is an excellent IN, with the cumulative number of ice nuclei per bacteria cell equal to $3 \times 10^{-4}$ at approximately -4 °C and equal to 0.01 at approximately -9 °C. Niemand *et al.* [2012] reported that, for submicron Asian mineral dust, the cumulative number of ice nuclei per particle was approximately $3 \times 10^{-4}$ at -20.5 °C and 0.01 at approximately -27 °C. Compared to the bacteria studied here, on a per particle basis Asian mineral dust is a better IN than *B. subtilis*, *P. putida*, *C. testosteroni*, and *M. luteus*, and a poorer IN than *P. syringae*. From Figure 6.3, the freezing temperatures are significantly different for *P. putida* and *P. syringae*, two species within the same genus. For example, the difference between the temperatures at which the cumulative number of ice nuclei per bacteria cell equals 0.01 is roughly 27 °C for these two bacteria species. Similar inter-species variability in the cumulative number of ice nuclei per spore has been observed for fungal spores from the genera *Penicillium* (Chapter 5, Section 5.3.3).

Included for comparison in Figure 6.3 are INperCell values from two previous studies on the same strain (31R1) of *P. syringae* that was studied here. Lindow *et al.* [1989] reported that the cumulative number of ice nuclei per *P. syringae* cell was approximately $3 \times 10^{-4}$ at -2 °C and approximately 0.01 at -3 °C. Möhler *et al.* [2008c] reported that the cumulative number of ice nuclei per *P. syringae* cell was approximately $3 \times 10^{-4}$ (as an upper limit) at -5.6 °C and approximately 0.01 at -8 °C. Our results for *P. syringae* (INperCell equal to $3 \times 10^{-4}$ at -4 °C; INperCell equal to 0.01 at -9 °C) agree well with the results from Möhler *et al.* [2008c], and are roughly consistent with the data from Lindow *et al.* [1989], although shifted to colder temperatures. The ice nucleation site in *P. syringae* is a protein located in the outer cell membrane of the cells [Lee *et al.*, 1995], and previous work has shown that the expression of the ice nucleation protein can vary significantly depending on the culture conditions used, including
culture medium, temperature and time [Lindow et al., 1982; Nemecek-Marshall et al., 1993; Pooley and Brown, 1991; Rogers et al., 1987]. We used the same culture conditions as Möhler et al. [2008c], but different culture conditions (medium, time, temperature) as Lindow et al. [1989]. Considering the variability of INperCell values expected for different culture conditions, our results for P. syringae are in good agreement with those from Lindow et al. [1989].

There have been two previous studies on the ice nucleation properties of B. subtilis [Edwards et al., 1994; Maki et al., 1974], both using the droplet-freezing assay developed by Vali [1971]. Edwards et al. [1994] studied the ice nucleation activity of B. subtilis at temperatures between -5 °C and -9 °C, and Maki et al. [1974] did a preliminary test for the ice nucleation activity of B. subtilis at temperatures down to -25 °C. Both studies found that this bacterium was inactive as an IN at these temperatures, in agreement with the results presented here for B. subtilis (Figure 6.3).

The INperCell values in Figure 6.3 should be considered as upper limits because the number of bacteria per drop was likely underestimated for some droplets (see discussion in Section 6.2.3). This is especially true for B. subtilis, which had very high amounts of bacteria per droplet (median 133 bacteria per drop, see Table 6.1). Despite the high surface area of B. subtilis per droplet, Figure 6.2 indicates that B. subtilis is a very poor IN with freezing temperatures overlapping with the homogeneous freezing of pure water droplets. Experiments studying the immersion freezing of droplets containing small amounts of B. subtilis (or P. putida, C. testosteroni, M. luteus) would, therefore, not be of great interest since these bacteria are not likely important atmospheric IN. Further discussion on the atmospheric implications of ice nucleation on the bacteria studied here is provided in Section 6.3.5 and Section 6.4.2.
6.3.4 Ice Nucleation Active Surface Site Density, $n_s$

To normalize for the surface area dependence of ice nucleation, we calculated the ice nucleusation active surface site density, $n_s$, using the INperCell results in Figure 6.3 and the method described by Haga et al. [2013](Chapter 4, Section 4.3.4). Figure 6.4 shows the $n_s$ values for the bacteria in the current study, for the results by Lindow et al. [1989] and Möhler et al. [2008c] on the same strain of *P. syringae* as studied here (strain 31R1), and for submicron Asian mineral dust [Niemand et al., 2012]. For each bacteria studied here and for the *P. syringae* results from Lindow et al. [1989] and Möhler et al. [2008c], the cell sizes and shapes from Table 6.1 were used to estimate surface area for each bacterial cell. Comparing $n_s$ values at temperatures below roughly -25 °C, once normalized to surface area, *B. subtilis*, *P. putida*, *C. testosteroni*, and *M. luteus* are poor IN compared to the Asian mineral dust studied by Niemand et al. [2012]. It is difficult to compare the $n_s$ values of *P. syringae* and Asian mineral dust due to the lack of overlap between these two data sets. Compared to all of the bacteria studied here, the Asian mineral dust studied by Niemand et al. [2012] has the highest ice nucleation active surface site density.
Figure 6.4 Ice nucleation active surface site density, $n_s$, calculated from the cumulative number of IN per bacteria cell or particle results (Figure 6.3). Included are $n_s$ values from Lindow et al. [1989] and Möhler et al. [2008c] for the same strain of *P. syringae* (strain 31R1) as was studied here, and also $n_s$ values for Asian mineral dust [Niemand et al., 2012].

### 6.3.5 Atmospheric Implications

To understand the implications of ice nucleation on the bacteria studied here for mixed-phase cloud processes, both the immersion freezing properties and the atmospheric concentrations of bacteria must be considered. To do this, we used the method of Murray et al. [2012] to estimate the global annual mean concentration of IN for the species of bacteria studies here by assuming that each droplet contains only one particle that may be active as an IN at a
given temperature and using the following equation [Hoose and Möhler, 2012; Murray et al., 2012; Vali, 1971]:

\[ \text{IN}(T) = [\text{Bacteria}] \times \left\{ 1 - \exp(-\text{INperBacteria}) \right\}, \]  

(6.1)

where \([\text{IN}(T)] \text{ (L}^{-1}\text{)}\) is the global annual mean concentration of IN, \([\text{Bacteria}] \text{ (L}^{-1}\text{)}\) is the global annual mean concentrations of bacteria cells, and the \text{INperCell} values were calculated in Section 6.3.3 and are shown in Figure 6.3. This calculation was done for the inefficient bacterial IN studied here considered together (\textit{B. subtilis}, \textit{P. putida}, \textit{C. testosteroni}, \textit{M. luteus}) and for \textit{P. syringae}. A similar calculation was done for the Asian mineral dust studied by Niemand et al. [2012] using the following equation:

\[ \text{IN}(T) = [\text{particles}] \times \left\{ 1 - \exp(-\text{INperParticle}(T)) \right\}, \]  

(6.2)

where \([\text{particles}]\) is the global annual mean concentration of dust and the \text{INperParticle}(T) values are taken from Figure 6.3.

From the global modelling study by Hoose et al. [2010b], bacteria concentrations were estimated to be between \(10^{-2} - 10 \text{ L}^{-1}\) and dust concentrations were estimated to be between \(10^2 - 5 \times 10^4 \text{ L}^{-1}\) between 200-800 hPa. For the inefficient bacterial IN studied here (\textit{B. subtilis}, \textit{P. putida}, \textit{C. testosteroni}, \textit{M. luteus}), \text{INperCell} values for \textit{B. subtilis} were used to estimate lower limits for \([\text{IN}(T)]\) and \text{INperCell} values for \textit{P. putida} were used to estimate upper limits for \([\text{IN}(T)]\). Shown in Figure 6.5 are the global annual mean IN concentrations estimated using this method. IN concentrations for the bacteria should be considered as upper limits since not all bacteria in the atmosphere are expected to belong to either the inefficient bacterial IN (\textit{B. subtilis}, \textit{P. putida}, \textit{C. testosteroni}, \textit{M. luteus}) or to \textit{P. syringae}. From Figure 6.5, all of the bacteria studied here have lower estimated IN concentrations compared to Asian mineral dust. However, \textit{P. syringae} may be an important IN for mixed-phase cloud processes on a global
scale, in particular because this bacterium is active as an IN at warm temperatures (≥ -10°C) where ice multiplication is thought to be important. This supports previous findings by Murray et al. [2012].

Figure 6.5 Global annual mean IN concentration calculated using Equations (6.1) and (6.2), particle concentrations from Hoose et al. [2010b], and INperCell and INperParticle values from Figure 6.3. IN concentrations for the bacteria should be considered as upper limits since not all bacteria in the atmosphere are expected to belong to either the inefficient bacterial IN (B. subtilis, P. putida, C. testosteroni, M. luteus) or to P. syringae.

6.4 Conclusions

We studied the immersion ice nucleation properties of five bacteria that have been identified to the species level in the atmosphere. This information is then combined with their atmospheric abundance to infer their potential role on cloud formation.
6.4.1 Immersion Freezing of Bacteria

On a per particle basis, we find that four of the species studied (B. subtilis, P. putida, C. testosteroni, and M. luteus) are poor IN (Figure 6.3): the cumulative number of ice nuclei per bacteria cell for these bacteria was $3 \times 10^{-4}$ between approximately -27.5 °C and -34 °C, and 0.01 between approximately -35.5 °C and -37 °C. The fifth species studied, P. syringae, is an excellent IN with the number of cumulative ice nuclei per cell equal to $3 \times 10^{-4}$ at -4 °C and 0.01 at -9 °C, in agreement with previous studies (see for eg. Lindow et al. [1989]; Möhler et al. [2008c]; Morris et al. [2008]; Vali et al. [1976]). We found a large difference in the ice nucleating ability of P. putida and P. syringae, two species within the same genus, on a per particle basis: for similar $IN_{perCell}$ values (i.e. 0.01) the freezing temperatures of P. syringae cells were approximately 27 °C warmer than for P. putida cells (Figure 6.3). On a per surface area basis and at temperatures below roughly -25 °C, four of the bacteria (B. subtilis, P. putida, C. testosteroni, and M. luteus) are poor IN compared to Asian mineral dust (Figure 6.4). A per surface area comparison between P. syringae and Asian mineral dust is difficult due to the lack of overlap between these two data sets (Figure 6.4). However, compared to all of the bacteria studied here, the Asian mineral dust studied by Niemand et al. [2012] has the highest ice nucleation active surface site density values (Figure 6.4). Figure 6.4 also shows that the difference in ice nucleation ability between P. putida and P. syringae cannot be explained by a difference in surface area of the bacteria cells, supporting the conclusion from Figure 6.3 that there can be significant variability in the ice nucleation properties of species from a single genus.

6.4.2 Atmospheric Implications

The global average IN concentration for the bacteria studied here, as well as for the Asian mineral dust studied by Niemand et al. [2012] was calculated according to the method of Murray
et al. [2012] (Figure 6.5). Four of the bacteria (B. subtilis, P. putida, C. testosteroni, M. luteus) are not expected to be important IN for mixed-phase cloud processes on a global scale because their estimated IN concentrations are much lower compared to Asian mineral dust, and also, they are active at colder temperatures. P. syringae is active at warm temperatures (≥ -10°C) where ice nucleation is thought to be important and this bacterium may therefore be an important atmospheric IN for mixed-phase cloud processes on a global scale (as previously suggested by Murray et al. [2012]). To confirm the importance of P. syringae for mixed-phase cloud processes, further studies are needed that determine the fraction of bacteria in the atmosphere belonging to P. syringae.

Although there have been numerous field studies identifying bacteria in the atmosphere, very few of them have provided species level identification. This study has shown that there can be significant variability in the ice nucleation properties of bacteria species belonging to the same genus, indicating that generalizations about the ice nucleation properties of genera or higher taxonomic classifications may not be appropriate. Future studies that not only identify bacteria to the species level from atmospheric samples, but that also quantify their number concentrations, would be extremely useful in assessing the potential implications of bacteria acting as IN on ice and mixed-phase cloud processes. The results presented here are applicable to mixed-phase cloud conditions and additional studies on bacterial ice nucleation targeting cirrus conditions are also needed.
Chapter 7. Conclusions

7.1 Ice Nucleation Properties of Mineral Dusts, Fungal Spores, and Bacteria

The heterogeneous ice nucleation properties of four mineral dusts, eighteen fungal spores and six bacteria have been studied using a temperature and humidity controlled flow cell coupled to an optical microscope.

In Chapter 2, the deposition mode ice nucleation properties of uncoated and acid coated mineral dusts were studied. It was found that the presence of acidic coatings greatly reduces the ability of mineral dusts to nucleate ice. For kaolinite and illite, sulfuric acid coatings increased the onset RH\textsubscript{i} by approximately 30 %; for montmorillonine and quartz, sulfuric acid coatings increased the onset RH\textsubscript{i} by about 20 %. Our studies also show that ammonium bisulfate coatings influence the heterogeneous ice nucleation properties of kaolinite particles: the coated particles are less effective at nucleating ice than uncoated particles, with the onset RH\textsubscript{i} increasing by approximately 18 to 26 %, depending on temperature. These results support the idea that anthropogenic emissions of SO\textsubscript{2} and NH\textsubscript{3} may influence the heterogeneous ice nucleating properties of mineral dust particles by increasing the relative humidity required for ice nucleation.

In addition to mineral dusts, the effect of sulfuric acid coatings on the deposition mode ice nucleation properties of SNOMAX, a biological ice nucleus made from the bacterium Pseudomonas syringae, was also investigated in Chapter 2. Interestingly, there was no significant difference in the ice nucleating ability of uncoated and sulfuric acid coated SNOMAX particles.

The immersion ice nucleation properties of biological particles was studied in Chapter 3-Chapter 5 (fungal spores) and Chapter 6 (bacteria). Shown in Figure 7.1 are the cumulative
number of ice nuclei per spore or bacteria cell for all of the species of fungal spores and bacteria studied in this dissertation.

![Diagram showing cumulative number of ice nuclei per spore or bacteria cell](image)

**Figure 7.1** The cumulative number of ice nuclei per spore or bacteria cell for the fungal spores (Chapter 3-Chapter 5) and bacteria (Chapter 6) studied in this dissertation.

In Chapter 3-Chapter 5, the immersion ice nucleation properties of 18 different species of fungal spores were investigated. Prior to this research, there had only been a few studies on the ice nucleation properties of fungal spores [Jayaweera and Flanagan, 1982; Morris et al., 2013; Pouleur et al., 1992]. We present a new method for aerosolizing fungal spores that are grown under culture conditions in Chapter 3, and use this method to determine the ice nucleation
characteristics of Cladosporium sp., one of the most abundant types of fungal spores in the atmosphere [Al-Subai, 2002; Herrero et al., 2006; Li and Kendrick, 1995; Lim et al., 1998; Mallo et al., 2011; Mitakakis and Guest, 2001; Pyrri and Kapsanaki-Gotsi, 2007]. It was found that these spores are poor IN, with the cumulative number of ice nuclei per spore being equal to 0.01 at approximately -29.2 °C (this calculation was done in Chapter 4, see Table 4.2). Chapter 4 was a study on the ice nucleation properties of rust (Puccinia graminis, Puccinia triticina, Puccinia allii, Endocronartium harknessii) and bunt (Tilletia laevis, Tilletia tritici) spores. We found that rust and bunt spores are active as heterogeneous ice nuclei and that, at approximately -24 °C (rust spores) and -28 °C (bunt spores), the cumulative number of ice nuclei per spore equals 0.01. In Chapter 5, the ice nucleation properties of spores from the taxonomic classes Agaricomycetes (Agaricus bisporus, Amanita muscaria, Boletus zelleri, Lepista nuda, Trichaptum abietinum), Ustilagomycetes (Ustilago nuda, Ustilago avenae, Ustilago nigra), and Eurotiomycetes (Penicillium brevicompactum, Penicillium chrysogenum, and Penicillium sp.) were studied. There was a wide range of ice nucleating abilities for the spores studied, with the cumulative number of ice nuclei per spore being equal to 0.01 at approximately -27 °C for the Ustilagomycetes spores, -29 °C for the Agaricomycetes spores, and -31 °C for the Eurotiomycetes spores. The studies of ice nucleation on fungal spores in Chapter 3-Chapter 5, combined with previous studies on fungal spores [Jayaweera and Flanagan, 1982; Morris et al., 2013; Pouleur et al., 1992] demonstrate that there can be a wide range of freezing properties of fungal species belonging to the same genus. For example, there is a difference of approximately 24 °C between the temperatures at which the cumulative number of ice nuclei per spore is equal to 0.01 for Penicillium chrysogenum (studied in Chapter 5) and for Penicillium digitatum (studied by Jayaweera and Flanagan [1982]) (see Figure 5.3). This variability highlights the
importance for identifying atmospherically abundant fungi to the species level; this information would be useful for motivating future laboratory studies on the ice nucleation properties of fungal spores.

In Chapter 6, the ice nucleation properties of five species of bacteria were studied: *Pseudomonas syringae, Pseudomonas putida, Bacillus subtilis, Comamonas testosteroni,* and *Micrococcus luteus.* With the exception of *Pseudomonas syringae,* all of the bacteria studied were poor IN. Specifically, for these four bacteria (*Pseudomonas putida, Bacillus subtilis, Comamonas testosteroni,* and *Micrococcus luteus*) a temperature of approximately -36 °C was required to achieve a cumulative number of ice nuclei per bacteria cell equal to 0.01. *Pseudomonas syringae* was an excellent IN, with the cumulative number of ice nuclei per bacteria cell being equal to 0.01 at -9 °C, in agreement with previous studies on this bacterium (see for eg. *Lindow et al.* [1989]; *Möhler et al.* [2008c]; *Morris et al.* [2008]; *Vali et al.* [1976]).

As with the ice nucleation studies on fungal spores (Chapter 3-Chapter 5), again we found a large range in freezing properties for bacteria species belonging to the same genus. For example, the temperatures required for the cumulative number of bacteria cell to be equal to 0.01 was approximately 27 °C warmer for *Pseudomonas syringae* than for *Pseudomonas putida.* This emphasizes the need for field studies that identify abundant bacteria to the species level in the atmosphere.

7.2 Implications for the Formation Conditions and Properties of Ice and Mixed-Phase Clouds

Mineral dusts make up a significant component of the atmospheric aerosol population (for example, see Figure 1.1 and Table 1.1), are known to be effective ice nuclei [*Hoose and Möhler,* 2012; *Murray et al.,* 2012], and field studies have indicated that mineral dusts can
influence cloud formation and properties [DeMott et al., 2003; Sassen, 2002; Sassen et al., 2003]. Although it is well understood that mineral dusts can become coated with inorganic or organic material in the atmosphere [Hinz et al., 2005; McNaughton et al., 2009; Sullivan et al., 2007; Usher et al., 2003; Wiacek and Peter, 2009], little is known about how these coatings might affect the ice nucleation properties of mineral dusts. This information is needed to improve existing parameterizations of ice nucleation in climate models, and will help reduce the uncertainty associated with the indirect climate effect from atmospheric IN (for an explanation of the effect of atmospheric ice nuclei on climate, see Chapter 1, Section 1.3). For example, a previous study by our group on the ice nucleation properties of uncoated and sulfuric acid coated kaolinite particles [Eastwood et al., 2009] was recently used to parameterize ice nucleation in a modelling study on Arctic cloud properties [Girard et al., 2013]. When acid coated dust particles were included in the model, Girard et al. [2013] found significant changes in the microstructure and radiative properties of ice and mixed-phase clouds compared to simulations that included only uncoated dust. Here, we expanded on the study by Eastwood et al. [2009] and show that sulfuric acidic coatings can significantly decrease the ice nucleating ability of other abundant mineral dusts (i.e. illite, montmorillonite, quartz) in addition to kaolinite. In addition, we show that ammonium bisulfate also decreases the IN ability of kaolinite particles. The results from Chapter 2 could be used to include multiple types of dusts and coatings in existing parameterizations for ice nucleation in modelling studies that investigate the indirect effect of atmospheric ice nuclei.

Some recent modeling studies have suggested that biological ice nuclei, including fungal spores (Chapter 3-Chapter 5) and bacteria (Chapter 6), do not influence ice and mixed-phase cloud processes on a global scale [Hoose et al., 2010a; Sesartic et al., 2012, 2013]. To
investigate this further, the results from our ice nucleation experiments and particle concentrations from *Hoose et al.* [2010b] were used to calculate the global annual mean IN concentrations for the fungal spores and bacteria studied here following the method of *Murray et al.* [2012], and these results are summarized in Figure 7.2. The bacteria have been divided into two groups given the large difference in ice nucleation ability between *Pseudomonas syringae* and the remaining four species studied (*Bacillus subtilis*, *Micrococcus luteus*, *Comamonas testosteroni*, *Pseudomonas putida*). Also included for comparison in Figure 7.2 are global annual IN concentrations for the Asian mineral dust studied by *Niemand et al.* [2012] (see Section 4.4.2 for details on how this calculation was done). From Figure 7.2, the fungal spores and four of the bacteria (*B. subtilis*, *P. putida*, *C. testosteroni*, *M. luteus*) studied here are expected to have lower IN concentrations that Asian mineral dust on a global and annual scale and are not expected to be important atmospheric IN for mixed-phase cloud processes, supporting the modeling studies by *Hoose et al.* [2010a]; *Sesartic et al.* [2012, 2013]. *P. syringae* is active at warm temperatures (≥-10 °C) where ice nucleation is believed to be important and, therefore, this bacterium may be an important atmospheric IN (this has been previously suggested by *Murray et al.* [2012]). However, the atmospheric number concentration of this species is uncertain and to investigate this further, field studies are required that quantify the amount of *P. syringae* in the atmosphere at freezing heights.
Figure 7.2 Summary of global annual IN concentrations for the fungal spores and bacteria (P. syringae, B. subtilis, P. putida, C. testosteroni, M. luteus) studied here. IN concentrations were calculated using the freezing results from Chapter 3-Chapter 6, using particle concentrations from Hoose et al. [2010b] and according to the method of Murray et al. [2012]. Also included for comparison are global annual IN concentrations for Asian mineral dust studied by Niemand et al. [2012].

Ice nucleation on fungal spores and bacteria may be important for ice and mixed-phase cloud processes on a seasonal and regional scale i.e. during periods of high expected number concentrations of these aerosols [Phillips et al., 2009]. This topic is challenging to study because of the significant spatial and temporal variability in the concentrations of fungal spores and in the atmosphere [Desprès et al., 2012], the scarcity of studies quantifying biological particles to the species level, and the low level of understanding on the ice nucleation properties of biological particles. The ice nucleation results presented in Chapter 3-Chapter 6 can be used to improve existing parameterizations for ice nucleation on fungal spores and bacteria in future modelling.
studies investigating the effects of biological IN on mixed-phase cloud processes on a regional and seasonal scale.

7.3 Implications for Predicting Long-Distance Transport

Mineral dusts have been shown to travel large distances in the atmosphere [Chiapello et al., 2005; Duce et al., 1980; Glaccum and Prospero, 1980; Prospero, 1999; Prospero et al., 1981], and ice nucleation on these aerosols may effect their long-distance transport. The results from Chapter 2 indicate that acid coated mineral dusts are much less efficient ice nuclei compared to uncoated mineral dusts, and these results may have important implications for predicting their long-distance transport. These results will help to improve the description of ice nucleation on mineral dusts as a removal mechanism of these aerosols from the atmosphere in models used to predict their long-distance transport.

Ice nucleation on biological particles, followed by precipitation, might be an important removal mechanism for these aerosols and could effect their long-distance transport. This is particularly important for biological allergens and disease-causing microorganisms that have adverse health impacts, and also for biological pathogens that can cause economic losses on agricultural crops.

In Chapter 4 and Chapter 5, the results from the ice nucleation experiments on fungal spores were used to describe ice nucleation in two modelling studies that simulated atmospheric transport. The HYSPLIT particle dispersion model was used in Chapter 4 to determine the fraction of rust and bunt spores (large spores having diameters between approximately 15-19 µm) that reach high altitudes where they could cause heterogeneous freezing. It was found that a significant fraction of the spores emitted from the surface can reach freezing altitudes, between 3-6 % for 19 µm spores and between 6-9 % for 15 µm spores, suggesting that ice nucleation on
these types of spores may be an important removal mechanism and should be considered in future models used to predict their long-distance transport. In Chapter 5, the EMAC global chemistry-climate transport model was used to simulate the transport of fungal spores with diameters of 3 μm and 8 μm. When ice nucleation on the spores was included in the model, their long-distance transport to remote polar and marine regions was effected, indicating that ice nucleation on these fungal spores may be an important removal mechanism and should be considered in future modelling studies on their long-distance transport. These two modelling studies are the first that we know of to examine the effect of ice nucleation on fungal spores on their long-distance transport. The ice nucleation results on bacteria (Chapter 6) could be used similarly in future studies investigating the long-distance transport of bacteria.

7.4 Future Research Directions

Our understanding of atmospheric ice nucleation is still in its infancy and many outstanding research questions remain. For example, several studies have shown increased levels of biological organisms during dust storms [Grishkan et al., 2012; Hara and Zhang, 2012; Hua et al., 2007; Jeon et al., 2011; Maki et al., 2010]. A combined field and laboratory approach examining the ice nucleation properties of field samples of mineral dust aerosols harboring biological organisms would be very interesting. From Chapter 2, we found no effect from acid coatings on the ice nucleation ability of a commercially available biological ice nucleus, SNOWMAX; it would be useful to do similar experiments on fungal spores and bacteria that haven’t been modified to optimize IN activity, such as is the case for SNOMAX. Although it is well understood that the IN site on Pseudomonas syringae is a protein located on the outer cell membrane [Lee et al., 1995], little is known about the IN site on other types of biological IN. A detailed investigation to identify the IN site on fungal spores and bacteria (other than P.
Syringae) would be very interesting. Finally, although the results presented here on the ice nucleation properties of fungal spores and bacteria will significantly contribute to the understanding of biological ice nucleation, there remain significant gaps in knowledge on this topic. What are the most abundant biological species in the atmosphere and what are their number concentrations at high altitudes? How are the surfaces of these abundant species modified from ageing and exposure to harsh conditions (temperature, sunlight, etc.) and does this change their ice nucleation properties? Field studies aimed at quantifying the number concentrations of biological aerosols at various heights in the atmosphere would be an excellent starting point to address these questions, and would provide motivation and direction for future laboratory studies on the ice nucleation properties of biological particles.
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Appendices

Appendix A Taxonomy for the Species of Fungi and Bacteria Studied

A.1 Taxonomy for the Species of Fungi Studied

The species of fungi studied in this dissertation are from the phyla *Ascomycota* (Chapter 3, Chapter 5) and *Basidiomycota* (Chapter 4, Chapter 5). Details for the taxonomy of *Ascomycota* species studied are given in Figure A.1 and for the taxonomy of *Basidiomycota* species studied are given in Figure A.2.

![Taxonomy Diagram](image)

**Figure A.1** Taxonomy for the *Ascomycota* species of fungi studied in Chapter 3 (*Cladosporium* sp.) and Chapter 5 (*P. chrysogenum*, *Penicillium* sp., *P. brevicompactum*) [Benson et al., 2009; Kirk et al., 2008; Sayers et al., 2009].
Figure A.2 Taxonomy for the Basidiomycota species of fungi studied in Chapter 4 (*P. graminis*, *P. triticina*, *P. allii*, *E. harknessii*, *T. tritici*, *T. laevis*) and Chapter 5 (*U. nuda*, *U. nigra*, *U. avenae*, *L. nuda*, *A. bisporus*, *A. muscaria*, *B. zelleri*, *T. abietinum*) [Benson et al., 2009; Kirk et al., 2008; Sayers et al., 2009].
A.2 Taxonomy for the Species of Bacteria Studied

Details for the taxonomy for the species of bacteria studied in Chapter 6 are provided in Figure A.3.

![Taxonomy Diagram]

Figure A.3 Taxonomy for the bacteria species studied in Chapter 6 [Garrity et al., 2004].
Appendix B Global Chemistry-Climate Transport Model (EMAC) Simulation: Results for CCN Inactive Conditions

EMAC simulations were run assuming the fungal spores studied in Chapter 5 are inactive as CCN (i.e. $R_{\text{nuc,liq}} = 0$). These results are shown in Figure B.1 and Figure B.2.

**Figure B.1** EMAC results showing percent change in surface annual mean mixing ratio, IN-Active vs. IN-Inactive. Top: 3 µm particles. Bottom: 8 µm particles. For both IN cases, spores are assumed to be CCN-inactive. IN-active assumes that particles are removed by the nucleation of the ice-phase at temperatures $< -25$ C. Emissions were simulated for land surfaces except for land covered with ice.
Figure B.2 EMAC results showing the percent change in zonally averaged vertical profiles of annual mean mixing ratio, IN-Active vs. IN-Inactive. Top: 3 μm particles; bottom: 8 μm particles. For both IN cases, spores are assumed to be CCN-inactive. IN-active assumes that particles are removed by the nucleation of the ice-phase at temperatures between -25 °C and -35 °C. Emissions were simulated for land surfaces except for land covered with ice.
Appendix C Summary of Previous Ice Nucleation Studies on the Species of Bacteria Studied in Chapter 6

Table C.1 Summary of previous ice nucleation studies on the bacteria studied here.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Additional Taxonomy</th>
<th>T Range(^q) (°C)</th>
<th>(IN)perParticle(^r)</th>
<th>Freezing Mode(^s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td><em>Bacillus subtilis</em></td>
<td>-9 ≤ T ≤ -5</td>
<td>Y(^t)</td>
<td>IF</td>
<td><em>Edwards et al.</em> [1994]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ -25</td>
<td>Y(^t)</td>
<td>IF</td>
<td><em>Maki et al.</em> [1974]</td>
</tr>
<tr>
<td>γ-P</td>
<td><em>Pseudomonas syringae</em></td>
<td>-10 ≤ T ≤ -2</td>
<td>Y</td>
<td>IF</td>
<td><em>Attard et al.</em> [2012]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-10, -5</td>
<td>N</td>
<td>IF</td>
<td><em>Constantinidou et al.</em> [1990]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-9 ≤ T ≤ -5</td>
<td>Y</td>
<td>IF</td>
<td><em>Edwards et al.</em> [1994]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-5, -9</td>
<td>Y</td>
<td>IF</td>
<td><em>Gross et al.</em> [1983]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ -42</td>
<td>N</td>
<td>IF</td>
<td><em>Junge and Swanson</em> [2008]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-43 ≤ T ≤ -8</td>
<td>N</td>
<td>DF/CDF</td>
<td><em>Kanji et al.</em> [2011]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ -15</td>
<td>Y</td>
<td>IF</td>
<td><em>Kozloff et al.</em> [1983]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-10, -5</td>
<td>N</td>
<td>IF</td>
<td><em>Lindemann et al.</em> [1982]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ -10</td>
<td>Y</td>
<td>IF</td>
<td><em>Lindow et al.</em> [1982]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-12 ≤ T ≤ -1</td>
<td>Y</td>
<td>IF</td>
<td><em>Lindow et al.</em> [1989]</td>
</tr>
</tbody>
</table>

\(^p\) A: Actinobacteria; F: Firmicutes; β-P: β-Proteobacteria.
\(^q\) Temperature range studied in ice nucleation experiments.
\(^r\) Y: \(IN\)perParticle was reported; N: \(IN\)perParticle was not reported.
\(^s\) IF: immersion freezing; CDF: condensation freezing; DF: deposition freezing.
\(^t\) Cumulative number of ice nuclei per bacteria particle was reported, but no ice nucleation activity was observed for *B. subtilis.*
<table>
<thead>
<tr>
<th>Phylum(^p)</th>
<th>Additional Taxonomy</th>
<th>T Range(^q) (°C)</th>
<th>(INperParticle(^i))</th>
<th>Freezing Mode(^s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ -25</td>
<td>Y</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Maki et al. [1974])</td>
</tr>
<tr>
<td>≥ -10</td>
<td>Y</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Maki and Willoughby [1978])</td>
</tr>
<tr>
<td>-15 ≤ T ≤ -5</td>
<td>Y</td>
<td>IF/CDF</td>
<td></td>
<td></td>
<td>(Möhler et al. [2008c])</td>
</tr>
<tr>
<td>-6 ≤ T ≤ -2</td>
<td>Y</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Morris et al. [2008])</td>
</tr>
<tr>
<td>≥ -8</td>
<td>Y</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Nejad et al. [2006])</td>
</tr>
<tr>
<td>≥ -10</td>
<td>Y</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Nemecek-Marshall et al. [1993])</td>
</tr>
<tr>
<td>≥ -10</td>
<td>N</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Ponder et al. [2005])</td>
</tr>
<tr>
<td>-4</td>
<td>N</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Sands et al. [1982])</td>
</tr>
<tr>
<td>≥ -10</td>
<td>N</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Turner et al. [1990])</td>
</tr>
<tr>
<td>T ≥ -25</td>
<td>Y</td>
<td>IF</td>
<td></td>
<td></td>
<td>(Vali et al. [1976])</td>
</tr>
<tr>
<td>γ-P</td>
<td>(Pseudomonas syringae/P. virflava)</td>
<td>-15 ≤ T ≤ -5</td>
<td>Y</td>
<td>IF/CDF</td>
<td>(Möhler et al. [2008c])</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ -10</td>
<td>Y</td>
<td>IF</td>
<td>(Pooley and Brown [1991])</td>
</tr>
</tbody>
</table>