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Urediospores of *Puccinia* spp. and other rusts are warm-temperature ice nucleators and harbor ice nucleation active bacteria

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In light of various features of the biology of the rust fungi and of the epidemiology of the plant diseases they cause that illustrate the important role of rainfall in their life history, we have characterized the ice nucleation activity (INA) of the aerially disseminated spores (urediospores) of this group of fungi. Urediospores of this obligate plant parasite were collected from natural infections from 7 species of weeds in France, from coffee in Brazil and from field and greenhouse-grown wheat in France, the USA, Turkey and Syria. Immersion freezing was used to determine freezing onset temperatures and the abundance of ice nuclei in suspensions of washed spores. Microbiological analyses of spores and subsequent tests of the ice nucleation activity of the bacteria associated with spores were deployed to quantify the contribution of bacteria to the ice nucleation activity of the spores. All samples of spores were ice nucleation active having freezing onset temperatures as warm as -4°C . Spores in most of the samples carried cells of ice nucleation-active strains of the bacterium *Pseudomonas syringae* (at rates of less than 1 bacterial cell per 100 urediospores), but bacterial INA accounted for only a small fraction of the INA observed in spore suspensions. Changes in the INA of spore suspensions after treatment with lysozyme suggest that the INA of urediospores involves a polysaccharide. Based on data from the literature, we have estimated the concentrations of urediospores in air at cloud height and in rainfall. These quantities are very similar to those reported for other biological ice nucleators in these same substrates. We suggest that air sampling techniques have ignored the spatial and temporal variability of atmospheric concentrations that occur under conditions propitious for precipitation that could increase their local abundance intermittently. Nevertheless, we propose that the relative low abundance of warm-temperature biological ice nucleators in the atmosphere corresponds to optimal conditions for the processes of evolution to positively select for INA.

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1 Introduction

Over the past few years there has been a renewed interest in biological particles that could act as warm temperature ice nucleators in the atmosphere. By catalyzing ice formation at temperatures warmer than -10°C , ice nucleation active spores, pollen and bacterial cells transported into clouds could initiate the process of raindrop formation at temperatures where mineral particles are incapable of having an effect. Particles of biological origin are among the only particles naturally present in the atmosphere known to be effective ice nucleators at temperatures warmer than -10°C and hence they have the potential to incite processes needed for rainfall that would otherwise not occur in mid and high latitude clouds at such temperatures. The potential importance of this environmental function has stimulated renewed research to discover, characterize and quantify biological ice nucleators in the atmosphere for subsequent parameterization of cloud physics models that will contribute to estimating the impact of biological ice nucleators on rainfall (Morris et al., 2011).

The most well-described biological ice nucleator in the atmosphere is the bacterium *Pseudomonas syringae*. This bacterium, also known for its pathogenicity to plants, is disseminated via aerosols to clouds, in snow and rainfall, and via snowmelt, rain runoff and flowing streams between terrestrial and freshwater habitats mostly in temperate regions (Monteil et al., 2011; Morris et al., 2008). In spite of the biological and ecological data available for this bacterium, it is unclear if there are sufficient numbers of ice nucleation active cells in the atmosphere to have an effect on the quantity or frequency of rainfall (Hoose et al., 2010; Phillips et al., 2009). Hence, in addition to the effort to accumulate supplementary data on atmospheric concentrations of *P. syringae*, there has been a search for other biological ice nucleators potentially more abundant in the atmosphere. One approach in this direction has been to assess the ice nucleation activity (INA) of one of the most abundant and ubiquitous micro-organisms in the atmosphere, *Cladosporium* spp. However, this fungus has not proven to be ice nucleation active at temperatures warmer than -15°C (Iannone et al., 2011). Likewise, data for INA of air

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and snow samples suggest that warm-temperature biological ice nucleators constitute only minor components of the microbial populations in these samples (Bowers et al., 2009; Mortazavi et al., 2008).

It has been suggested that one of the major roles of INA in the adaptation of *P. syringae* to its habitats is in fostering fallout from clouds with raindrops or aggregates of ice formed from the catalysis of glaciation (Morris et al., 2008). Without a force to precipitate the ultra-light cells of bacteria from the atmosphere, they could remain suspended in the atmosphere indefinitely. Another potential role for INA for this bacterium is to enhance access to host tissue by creating wounds due to frost damage. This led to one of the key field observations linked to the discovery of the INA of *P. syringae*: the accrued frost damage to crops in its presence compared to when it was absent (Army et al., 1976). This same observation has also been made for the various other plant pathogenic bacteria that are ice nucleation active (Azad and Schaad, 1988; Lindow et al., 1978; Zeng et al., 1999). If we use these properties – fallout with rain and enhanced frost damage to plants – to search for other micro-organisms that might benefit from INA, there is evidence from the epidemiology of rusts that suggests that this trait would be very useful for this group of plant pathogens.

Since the early 1900's it has been demonstrated repeatedly that rusts are transported to altitudes of clouds and can travel for 100's to nearly 1000 km before returning to the Earth's surface (Stakman et al., 1923; Wolfenbarger, 1946; Nagarajan and Singh, 1990). The initiation of rust epidemics depends critically on rainfall as a source of primary inoculum that effectively deposits spores traveling these long distances (Nagarajan and Singh, 1990); dry deposition accounts for only a very small fraction of spores deposited from the atmosphere (Li et al., 2009; Rowell and Romig, 1966). Furthermore, the viability of urediospores of rusts is greatly reduced by temperatures below about -5°C (Eversmeyer and Kramer, 1995; Gladders et al., 2007; Jurick et al., 2008; Pfender and Vollmer, 1999). This strongly influences their overwinter survival rate thereby re-enforcing the importance of new incoming primary inoculum in the spring to initiate epidemics. It also suggests that survival during aerial transport would

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be severely limited if the fungus remained airborne at very cold temperatures. Furthermore, rusts are among the very few diseases for which there are several reports of enhanced frost sensitivity of plants when they are present (McAinsh et al., 1990; Kessler, 1970; Paul and Ayres, 1991).

5 Apart from our report of the preliminary characterization of coffee rust (Gonçalves and Massambani, 2011), the INA of rusts has not been investigated. Rusts are obligate parasites and this entrains two obstacles in particular to the measurement of their INA. Firstly, as they cannot be grown on microbiological culture media, they must be collected from plant material. This can be accomplished with green house-grown plants,
10 or by direct collection in the environment from wild or cultivated plants. Secondly, by culturing micro-organisms in the laboratory, it is possible to obtain pure cultures that contain only a single strain of the target micro-organism. In absence of such purification, it is likely that rusts harbor “contaminants” such as bacteria. Bacteria are well-known to be naturally associated with rust spores. Bacteria can be attached to spore surfaces and some of them can parasitize rust spores (Doherty and Preece, 1978; French et al.,
15 1964; Perelló et al., 2002). Furthermore, contamination of rust fungal DNA with bacterial DNA can be a significant problem for sequencing the genome of obligate parasites such as rust fungi (Barnes and Szabo, 2008). Therefore, the presence of bacteria on rust spores could influence their INA, and in particular if the bacteria themselves were
20 ice nucleation active. In this work we demonstrate that urediospores of all species of rusts collected here have marked ice nucleation activities at temperatures warmer than -10°C . We have quantified the rate of ice nuclei per spore at a range of temperatures and have attempted to determine if the microflora associated with the spores is responsible for this activity. In light of the available data on abundance of rust spores in the
25 atmosphere at cloud height and in rain, and knowledge about physical processes in clouds leading to ice multiplication, our results suggest that it might be critically important in the life history of warm temperature ice nucleators to be relatively rare in the atmosphere.

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2 Materials and methods

2.1 Collection of urediospores and associated bacteria

Urediospores of rusts were collected from naturally infected plants in the field and from greenhouse-grown host plants inoculated with spores of fungal cultures from laboratory collections (Table 1). Spores were collected with a suction device that allowed them to be taken up through sterile pipette tips and impacted onto polycarbonate filters (pore diameter, 8 μm). To remove bacteria that were not firmly bound to the spores, the spores were washed twice by passing 10 ml of sterile distilled water across the filter for each wash. Spores were then scraped from the filter surface and suspended in sterile distilled water to a concentration of about 10^5 spores ml^{-1} depending on the total number of spores that were obtained (indicated in Table 1). The concentration of spores was determined by direct microscopic counts. It was difficult to harvest enough spores to obtain a sufficient volume of spore suspensions at a concentration of greater than 10^5 spores ml^{-1} . Furthermore, the total volume of spore suspension that could be prepared varied among samples and had consequences on the detection levels and the range of tests that could be performed for each sample.

To determine the quantity of bacteria on the urediospores after washing, aliquots of the suspensions used for microscopic counts were dilution plated on two culture media, on King's medium B (for detection of fluorescent pseudomonads) (King et al., 1954) and 10% tryptic soy agar (for total culturable mesophilic bacteria). Plates were incubated at 22–25 °C and colonies were counted at 2 and 5 days after incubation. Fluorescent colonies on KB were counted under UV light (312 nm). Population sizes of putative *P. syringae* were determined as previously described (Morris et al., 2008) by verifying the absence of cytochrome C oxidase on pure cultures made from 10 to 20 randomly selected fluorescent colonies from the same dilution of each sample. About 90 strains of putative *P. syringae* were put into storage at –80 °C in 40% glycerol for later characterization of their INA. When fluorescent pseudomonads were not detected

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in samples, colonies of the dominant morphological types were purified and characterized for their INA as described below.

2.2 Quantification of ice nucleation activity

The capacity of the spores to induce freezing of water at temperatures from -2°C to -10°C was determined for droplets of the spore suspension placed on a metal surface floated on a cooling bath as described previously (Morris et al., 2008). The objective of these tests was to estimate the cumulative number of ice nuclei per spore according to the calculation of Vali (Vali, 1971)

$$K_T = [\ln(N_{\text{total}}) - \ln(N_{\text{unfrozen}})]/d$$

where N_{total} is the total number of drops, N_{unfrozen} is the number of drops not frozen (liquid) at the each temperature, and d is the quantity of spores per drop. Depending on the total volume of spore suspension that was obtained for each sample, from 20 to 50 drops of $20\ \mu\text{l}$ of the original suspension were tested. To determine if the INA of spore suspensions was due to bacteria, an aliquot of the suspension was incubated with lysozyme (final concentration of $3\ \text{mg ml}^{-1}$) for 72 h at 4°C as previously described (Christner et al., 2008a). To verify that INA was sensitive to boiling and therefore likely to be proteinaceous, an aliquot of the suspension was boiled for 10 min. For the lysozyme-treated suspension, 20 droplets were tested and 10 droplets were tested for the boiled suspension. For all tests, strain CC0094 of *P. syringae* (Morris et al., 2008) was used as a positive control and sterile distilled water was used as the negative control.

The INA of *P. syringae*-like and non-pseudomonad strains was quantified in a similar manner as for spores. Suspensions of 48-h cultures on KB medium were prepared in sterile distilled water, adjusted to 10^7 bacteria ml^{-1} and stored at 4°C for about 1 h to foster optimal conditions for formation of the ice nucleation protein (Nemecek-Marshall et al., 1993). For each of 5 ten-fold dilutions of the bacterial suspension (10^3 to 10^7 bacteria ml^{-1}) 32 drops at $20\ \mu\text{l drop}^{-1}$ were tested at -2° to -9°C . Data obtained under identical conditions for strains representing the range of INA observed in a large

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collection of *P. syringae* established during several studies (Morris et al., 2000, 2008, 2010) were used for comparison.

3 Results

3.1 Ice nucleation activity of rust spore suspensions

5 Warm temperature INA was observed for all suspensions of rust spores characterized in this study. The temperature of onset of ice nucleation was between -4°C and -9°C for all samples (Table 1). For the sample with the coldest temperature of onset of freezing (-9°C for wheat rust on cv. Michigan Amber plants cultivated in the greenhouse), the onset temperature increased to -6°C after 2 additional weeks of incubation of the plants in the greenhouse. The frequency of ice nuclei per spore was in the range of the frequency of ice nuclei produced per bacterial cell of reference strains of *P. syringae* (Fig. 1). The rates of ice nuclei presented in Fig. 1 represent examples of the range of activities for the rust spores characterized in this study and for strains of *P. syringae* from plants among the many strains we have characterized in our collection and those reported in the literature (Orser et al., 1985). These frequencies of INA activity are also in the same range observed for strains of *P. syringae* and related species isolated from clouds (Attard et al., 2012).

3.2 Abundance of bacteria on washed rust spores

20 The size of the bacterial population carried on rust spores, after washing, was evaluated for 15 of the samples (those samples with sufficient spores to obtain working suspensions of at least 10^5 spores ml^{-1} , see Table 1). All spores carried detectable densities of total mesophilic bacteria at rates of 8×10^{-4} to 2.1×10^3 bacterial cells spore $^{-1}$ with 10 of the 15 samples having less than 1 bacterial cell spore $^{-1}$. *P. syringae* was detected on 11 of the 15 samples at rates of 8.4×10^{-5} to 0.18 bacterial cells spore $^{-1}$. In general, there was not a correlation between the abundance of bacteria on spores

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and the onset temperature of freezing. However, the spores with the warmest onset temperature carried the greatest amounts of total bacteria and among the greatest amounts of *P. syringae* (Fig. 2).

3.3 Evaluation of the hypothesis that ice nucleation activity of rust spores is attributable to bacteria on the spores

Given that bacteria are present on rust spores, we calculated the INA of spore suspensions at the freezing onset temperature in terms of the number of bacteria contained in the spore suspension. This calculation addresses the hypothesis that the bacteria, rather than the spores that carry them, are responsible for the INA of the spore suspensions. For the hypothesis that total bacteria are responsible for INA, rates of ice nuclei per bacterial cell varied widely relative to the rates per spore, ranging from 100-fold greater to 1000-fold lower frequencies than for those calculated for the hypothesis that the spores themselves were responsible for the INA (Fig. 3). For the hypothesis that *P. syringae* is responsible for INA, rates of ice nuclei per bacterial cell were calculated to be 10-fold to 10 000-fold greater than the rates for spores. However, for four samples, INA of spores could not be attributed to *P. syringae* because the bacterium was not detected in these samples. For two other samples, if *P. syringae* was responsible for the INA then it would be due to the presence of exceptionally active strains (10^{-2} nuclei cell $^{-1}$ at -4°C and 10^{-1} nuclei cell $^{-1}$ at -7°C) having an ice nucleation rate equal to that of strain Cit7 under the conditions reported by Orser and colleagues (Orser et al., 1985) (Fig. 1).

Eighty-eight strains of *P. syringae* associated with rust spores were isolated and tested for INA. For each strain, 32 drops of 20 μl of each 10-fold dilution from 10^8 to 10^4 cells ml $^{-1}$ were tested from -2° to -9°C . Hence at any given temperature, the maximum frequency of ice nuclei that could be detected was 1.7×10^{-2} nuclei cell $^{-1}$ (31 drops frozen for the suspension at 10^4 cells ml $^{-1}$) and the minimum frequency detectable was 1.6×10^{-8} ice nuclei cell $^{-1}$ (1 drop frozen for the suspension at 10^8 cells ml $^{-1}$). The maximum rate of INA at the corresponding onset freez-

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ing temperature of the sample for the strains from each sample ranged from the minimum to the maximum detectable (Table 2). We then used these rates to estimate the maximum number of ice nuclei in each sample that could have been contributed by *P. syringae* at the onset freezing temperature. This was accomplished by multiplying the abundance of *P. syringae* in the spore suspension by the maximum rate of INA of this bacterium observed among the strains of each sample. These values of potential abundance of *P. syringae* ice nuclei were compared to the total number of ice nuclei in the sample (Table 2). Among the 10 samples in which *P. syringae* was detected and for which strains were isolated and checked for INA, in five of these there were about 100 × more ice nuclei in the spore suspension than those that could have been contributed by *P. syringae*. In three of the samples there was less than 10 × more ice nuclei in the suspension that could have been contributed by *P. syringae* and in two samples *P. syringae* ice nuclei could have accounted for all of the ice nuclei detected. Likewise, the rates of ice nucleation activity that *P. syringae* would have needed to account for the ice nuclei in the spore suspension were very high and in some cases much greater than what we have observed among the numerous strains we have characterized as depicted in Fig. 1. The INA of strains other than *P. syringae* was not systematically characterized in this study. However, the dominant colony types were collected and characterized from a few samples and in particular from samples on which *P. syringae* was not detected. None of these strains were ice nucleation-active. Furthermore, we calculated the rates of ice nuclei production that would have been necessary among the total bacteria under the hypothesis that the ice nuclei in the spore suspensions came from the total bacterial population on spores. Under this hypothesis, the total bacterial population would have needed to produce ice nuclei at similar or higher rates than observed among the *P. syringae* strains collected here.

3.4 Effect of lysozyme and heat treatments on ice nucleation activity of rust spore suspensions

Boiling eliminated all detectable ice nuclei at the freezing onset temperature and at one degree below this temperature for all samples. Lysozyme, on the other hand, had
5 a more erratic effect on ice nuclei. Lysozyme treatment eliminated all detectable ice nuclei at the freezing onset temperature for 8 samples (Fig. 4) and at one degree below the freezing onset temperature for 5 of these samples. For 2 samples, lysozyme treatment had little or no perceptible effect on the abundance of ice nuclei in the sample. Surprisingly, lysozyme treatment caused a 150 % to a 700 % increase in the abundance
10 of ice nuclei in four samples. But this increase was not always observed at all the temperatures tested for the sample.

4 Discussion

All rust spores collected in the field in this work had onset freezing temperatures in immersion freezing tests at -8°C or warmer. This suggests that warm-temperature
15 INA is a wide-spread trait among urediospores of rusts. Urediospores harbor bacteria including strains that are ice nucleation active. However, quantitative analyses of bacterial abundance and the rates of INA of the associated bacteria suggest that the rust spores themselves have inherent INA under the conditions of the tests here. Many of the bacteria associated with spores at the time of their harvest from leaves were removed by the washing step used in this study. Here, spores were washed in an attempt to reduce as much as possible the influence of bacteria on the INA of the spores
20 to test the hypothesis that the urediospores themselves were INA. But in their native state on leaves and during their dry release into the atmosphere, rust spores would most probably carry the bacteria that we removed during washing. We quantified the bacteria removed during washing (data not shown) and this allowed us to estimate that
25 spores carried several orders of magnitude more bacteria (both total and *P. syringae*

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populations) before than after washing, with several samples having up to equal numbers of urediospores as bacteria. In this light, it is likely that on spores liberated into the atmosphere directly from leaves under dry conditions, bacteria could account for much more of the INA of spores than observed here.

Although the results of the microbiological analyses suggest that bacteria do not account for the INA of washed rust spores, it should be noted that the analyses conducted here were based on the capacity of bacteria to grow on culture media. Dead or non culturable bacteria would not have been quantified via these methods. Dead cells of *P. syringae*, for example, can maintain INA if the cells remain intact after death as illustrated by the INA of Snomax[®]. To account for the INA of rust spores, dead and non culturable cells would have needed to be at least 10 times more frequent on spores than culturable cells based on the rates of INA described for these bacteria. We observed rust spores under scanning electron microscopy (data not shown). Spores that had surface contaminants that could have been bacterial cells were rare (less than 1 in about 50 spores). Alternatively, bacteria might have made a greater contribution to urediospore INA if the INA of bacteria adhering to the spores were in fact one or more orders of magnitude greater than what was measured in cell suspensions grown in the laboratory. For many samples, lysozyme markedly reduced INA. This result is compatible with the hypothesis that the INA is due to bacteria. However, in some cases lysozyme treatment enhanced INA, a phenomenon that we have never observed for the INA of pure culture suspensions of bacteria.

Lysozyme is a glycoside hydrolase. It hydrolyses the 1,4- β -linkages between the glycoside residues in the peptidoglycan of bacterial cell walls and those between the N-acetyl-D-glucosamine residues in chitodextrins (Strynadka and James, 1991). In other words, it can react with the oligosaccharides from the hydrolysis of chitin, and the chain length of these oligosaccharides determines the rate of their hydrolysis by lysozyme (Rupley, 1964). Chitin is the principal cell wall constituent of the Eumycota. Along with hemicellulose and glucomannan it is the major component of the outer layer of urediospores (Ehrlich and Ehrlich, 1969; Staples and Wynn, 1965). The chemistry

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of the urediospore surface is highly dynamic as the spore matures, as its spines form and elongate, and as it prepares for germination. Urediospores are highly hydrophilic. When in contact with free water or under high relative humidity, the under layers of the spore wall absorb water in preparation for germination. This sets off chitin synthesis, degradation of the surface mucilage of urediospores and other enzymatic processes that lead to increased surface quantities of soluble carbohydrates (Staples and Wynn, 1965). It is plausible that variations in amounts and configurations of chitin oligosaccharides occur during these processes, and that these oligosaccharides were substrates for the lysozyme used in our treatments. INA of chitin building blocks or derivatives have not been demonstrated. However, there is evidence for the INA of certain plant polysaccharides (Pummer et al., 2012; and citations within). The chemical nature of the INA of rust spores will need to be further explored.

Rust fungi sporulate prolifically. A hectare of slightly rusted wheat, for example, can harbor over 10^{15} urediospores, and a heavily rusted field would produce several times as many (Stakman and Christensen, 1946). These spores can be liberated into the atmosphere via wind and other mechanical agitation of the plants. This is highly accentuated during harvest: 1 h of combining (about 5 ha of wheat) can liberate about 10^{13} urediospores (Friesen et al., 2001). Likewise, for stem rust of perennial ryegrass it has been estimated that 10^{14} spores can be liberated from a 50 ha field in 24 h (Pfender et al., 2006). Spore release can result in near-ground (2 m) urediospore concentrations of 10^3 to 10^6 m^{-3} (Gregory, 1978). Since the early 1920's it has been known that these rust spores can be transported up to several km into the troposphere (Stakman et al., 1923). Data collected during extensive sampling with manned aircraft indicate an overall logarithmic decrease in concentration with height, and at 1 to 2 km altitude the urediospore concentrations detected are on the order of 10 or fewer spores m^{-3} (Gregory, 1978; Hirst et al., 1967). If these concentrations of urediospores were associated with typical clouds containing about 3 ml m^{-3} distributed into about 10^8 droplets m^{-3} (Pruppacher and Klett, 1997) then this would likely be insufficient to initiate rainfall via

primary glaciation where about 1 ice nucleus per 10^5 or 10^6 (Pruppacher and Klett, 1997) or in some cases per 10^3 (Young, 1993) drops is required.

Nevertheless, the evidence for the importance of rainfall in dissemination of rusts is compelling. Initiating processes that lead to the formation of rain drops would be a useful adaptation for rusts, which are obligate biotrophs, to assure their deposition back to the Earth's surface as has been suggested for *P. syringae* (Morris et al., 2008). Rain fallout of long distance disseminated spores has been the source of primary inoculum in initiating numerous rust epidemics such as in the first appearance of coffee rust in Brazil in 1966 – most likely resulting from the transport of spores with trade winds from regions of coffee production in Angola that coincided with the seasonal heavy rain in Brazil (Bowden et al., 1971; Nagarajan and Singh, 1990). Soybean rust, an emerging disease in North America, is spreading across the Eastern US with rain (Barnes et al., 2009). Long distance dissemination of wheat rust across India is another example of the role of rainfall deposition of urediospores in the spread of this pathogen (Nagarajan et al., 1976). The presence of urediospores in rainfall has been demonstrated by collection and direct observation of rainwater (Rowell and Romig, 1966). Observations were made during periods prior to the start of wheat rust epidemics at experimental farms when local sources of urediospores were absent. Therefore the spores collected in the rain were not accumulated by scrubbing of the air as the drops fell. Based on these data we calculated that there were between 0.01 and 1.2 urediospores of *Puccinia recondita* and *P. graminis* per ml of rainfall when these fungi were detected in rain. Interestingly, these values are similar to the concentrations of *P. syringae* in rain and snow ($1\text{--}10$ bacteria ml^{-1} , Morris et al., 2008) and those for total biological ice nucleators in snow active at -7°C ($0.001\text{--}0.23$ ice nuclei ml^{-1} (Christner et al., 2008a,b)). The coherence of these observations incites us to wonder if the available data on spore concentrations at cloud altitudes is representative of conditions conducive to precipitation.

We suggest that there are several factors that could explain why the currently available data on atmospheric concentrations of biological ice nucleators at cloud height

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are misleading relative to the processes considered to be involved in rainfall formation. Firstly, sampling from manned aircraft is generally conducted under stable atmospheric conditions with flight times that are very short relative to the volume of air for which estimates of particle concentrations will be made. Under these conditions, the data might not represent conditions most favorable for particle uplift and the turbulence favorable for precipitation. Micro-organisms released from plant surfaces can be uplifted in thermal plumes. These plumes are not uniformly spaced across a landscape and can lift “packages” of air that are not markedly diluted as they rise thereby preserving the near-surface particle concentrations. These packages of convected air will butt up against inversion layers where they can accumulate until the inversion layer degrades. Hirst and colleagues noted a trace of this phenomena in the uplift of urediospores in a case where concentrations increased with altitude up to 1 km (Hirst et al., 1967). Hence, it is likely that current data on microbial concentrations in the air at cloud height do not reflect the spatial and temporal variability of these concentrations. Secondly, the values for ice nucleation rates that we report here are for immersion freezing. But contact freezing is the process most likely to occur in clouds and it is generally more efficient than immersion freezing (Möhler et al., 2007). Cumulus clouds entrain dry air from their edges (Pruppacher and Klett, 1997) thereby ensuring that ice nuclei in dry aerosols can act as contact nuclei. Thirdly, in light of the temperature range of INA of urediospores, the freezing that they initiate could lead to ice multiplication via the Hallett-Mossop process which is restricted to the -3°C to -8°C temperature range and can result in the production of several orders of magnitude more ice crystals than ice nuclei (Hallett and Mossop, 1974). Equipment to permit sampling that is more representative of the conditions under which clouds form and to describe the spatial and temporal variability in air-borne concentrations of INA micro-organisms would make an important contribution to elucidating the conditions under which these micro-organisms can influence rainfall.

Whatever the real concentrations of rusts and other INA micro-organisms in the atmosphere, it seems that they are nevertheless minor components of air-borne microbial populations. In atmospheric samples, warm-temperature ice nucleators were no more

frequent than 91 in 6.6×10^6 total microbial particles (Bowers et al., 2009). In fresh snowfall, biological ice nucleators were much less than 1 % of the total DNA-containing particles in the size range of micro-organisms (Christner et al., 2008a). Likewise, ure-diospores detected at 1–2 km altitude were in background fungal populations that were generally 10 to 100 times more dense (Gregory, 1961; Hirst et al., 1967). This leads us to wonder if the relative low density of rust spores, *P. syringae* and other INA micro-organisms is not also a clue to how INA is advantageous in their life history. Excessive ice nuclei in clouds can inhibit the formation of large precipitable ice particles because the competition among crystals for water reduces the ultimate size of the ice crystals (Young, 1993). If ice that is induced by warm-temperature biological ice nucleators multiplies via the Hallet-Mossop process, then could this inhibit growth of cloud drops due to the competition among crystals? Our observations also raise questions about the significance of the association of *P. syringae* and rust spores for their dissemination and survival. Cells of *P. syringae* could be protected in the mucilaginous matrix that covers rust spores, and this bacterium might also use rust spores as a food base. Ure-diospores are likely to be liberated more readily from plant surfaces than are bacterial cells and hence they could serve as dissemination vectors for bacteria. The spores in turn could benefit from the INA of the bacteria when spores had low levels of activity. *P. syringae* and many of the rusts are favored by similar environmental conditions (cool, moist climates) and hence it is not surprising to find them together on plants and it could be expected that they have been subjected to similar selective pressures of the physical environment.

Human activities clearly have had an impact on the abundance of rust spores in Earth's atmosphere. In North America, for example, cultivation of winter wheat spread widely across the Great Plains in the 1900's after American plant breeders successfully obtained cold-tolerant wheat varieties. This created a vast belt of wheat production from Mexico to Canada throughout the months of March to July that led to the emergence of severe and continuous epidemics of rust caused by *Puccinia graminis* f. sp. *tritici* as the spores of this highly specific fungus flew northward throughout the artificially

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prolonged period of availability of the host plant. Did this have any impact on cloud processes involving warm-temperature glaciation? It would be fascinating to explore the regional patterns of rainfall in light of wheat rust epidemics and in particular with regard to the Dust Bowl that devastated the Great Plains in the late 1920's. Wheat and the accompanying rusts spread across several continents in the early 1900's and were accompanied by intense disease monitoring. Meteorological records could be mined in this light.

5 Conclusions

Urediospores of several species of rust fungi are warm-temperature ice nucleators capable of initiating ice formation in immersion freezing tests at temperatures as warm as -4°C for suspensions at 10^5 spores ml^{-1} . Spores in most of the samples carried cells of ice nucleation-active strains of *P. syringae* (at rates of less than 1 bacterial cell per 100 urediospores), but the INA of the bacteria accounted for only a fraction of the ice nuclei in the spore suspensions. INA of urediospores was heat-labile. Changes in the INA of urediospores after treatment with lysozyme, which is a glycoside hydrolase attacking the 1,4- β -linkages between the glycoside residues in the peptidoglycan of bacterial cell walls and those between the N-acetyl-D-glucosamine residues in chitodextrins, suggest that the basis for INA of urediospores involves a polysaccharide. The concentrations of urediospores in the atmosphere at cloud height reported in the literature do not represent the spatial and temporal variability of spore dissemination and they are the result of measurements taken under conditions unlike those associated with precipitation. The compelling evidence for the importance of rainfall in the life history of rust suggests that further investigations are needed to elucidate the conditions under which they could be involved in fostering rainfall. The ensemble of data for the abundance of INA micro-organisms in the atmosphere nevertheless suggests that these organisms are minor components of atmospheric microbial communities. This relative low abundance might, in fact, be advantageous for rainfall deposition to

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be a positive natural selection for ice nucleation activity if ice multiplication is involved in their role in cloud glaciation.

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References

Arny, D. C., Lindow, S. E., and Upper, C. D.: Frost sensitivity of *Zea mays* increased by application of *Pseudomonas syringae*, *Nature*, 262, 282–284, 1976.

Attard, E., Yang, H., Delort, A.-M., Amato, P., Pöschl, U., Glaux, C., Koop, T., and Morris, C. E.: Effects of atmospheric conditions on ice nucleation activity of *Pseudomonas*, *Atmos. Chem. Phys. Discuss.*, 12, 9491–9516, doi:10.5194/acpd-12-9491-2012, 2012.

Azad, H. and Schaad, N. W.: The relationship of *Xanthomonas campestris* pv. *translucens* to frost and the effect of frost on black chaff development in wheat, *Phytopathology*, 78, 95–100, 1988.

Barnes, C. W. and Szabo, L. J.: A rapid method for detecting and quantifying bacterial DNA in rust fungal DNA samples, *Phytopathology*, 98, 115–119, 2008.

Barnes, C. W., Szabo, L., and Bowersox, V.: Identifying and quantifying *Phakopsora pachyrhizi* spores in rain, *Phytopathology*, 99, 328–338, 2009.

Bowden, J., Gregory, P. H., and Johnson, C. G.: Possible wind transport of coffee leaf rust across the Atlantic Ocean, *Nature*, 229, 500–501, 1971.

Bowers, R. M., Lauber, C. L., Wiedinmyer, C., Hamady, M., Hallar, A. G., Fall, R., Knight, R., and Fierer, N.: Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei, *Appl. Environ. Microbiol.*, 75, 5121–5130, 2009.

Christner, B. C., Morris, C. E., Foreman, C. M., Cai, R., and Sands, D. C.: Ubiquity of biological ice nucleators in snowfall, *Science*, 319, 1214, 2008a.

Christner, B. C., Rongman, C., Morris, C. E., McCarter, K. S., Foreman, C. M., Skidmore, M. L., Montross, S. N., and Sands, D. C.: Geographic location, season, and precipitation chemistry

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influence on the abundance and activity of biological ice nucleators in rain and snow, P. Natl. Acad. Sci. USA, 105, 18854–18859, 2008b.

Doherty, M. A. and Preece, T. F.: *Bacillus cereus* prevents germination of uredospores of *Puccinia allii* and the development of rust disease of leek, *Allium porrum*, in controlled environments, Physiol. Plant Pathol., 12, 123–132, 1978.

Ehrlich, M. A. and Ehrlich, H. G.: Uredospore development in *Puccinia graminis*, Can. J. Bot., 47, 2061–2064, 1969.

Eversmeyer, M. G. and Kramer, C. L.: Survival of *Puccinia recondita* and *P. graminis* urediniospores exposed to temperatures from subfreezing to 35 °C, Phytopathology, 85, 161–164, 1995.

French, R. C., Novotny, J. D., and Searles, R. B.: Properties of bacteria isolated from wheat stem rust spores, Phytopathology, 54, 970–973, 1964.

Friesen, T. L., de Wolf, E. D., and Frankl, L. J.: Source strength of wheat pathogens during combine harvest, Aerobiologia, 17, 293–299, 2001.

Gladders, P., Langton, S. D., Barrie, I. A., Hardwick, N. V., Taylor, M. C., and Paveley, N. D.: The importance of weather and agronomic factors for the overwinter survival of yellow rust (*Puccinia striiformis*) and subsequent disease risk in commercial wheat crops in England, Ann. Appl. Biol., 150, 371–382, doi:10.1111/j.1744-7348.2007.00131.x, 2007.

Gonçalves, F. L. T. and Massambani, O.: Bacteria and fungi spores as ice nuclei in *Coffea arabica* L., Revista Ciencia Natura, 33, 73–93, 2011.

Gregory, P. H.: The Microbiology of the Atmosphere, Interscience Publishers, Inc., New York, 251 pp., 1961.

Gregory, P. H.: Distribution of airborne pollen and spores and their long distance transport, Pure Appl. Geophys., 116, 309–315, doi:10.1007/bf01636888, 1978.

Hallett, J. and Mossop, S. C.: Production of secondary ice particles during the riming process, Nature, 249, 26–28, 1974.

Hirst, J. M., Stedman, O. J., and Hogg, W. H.: Long-distance spore transport: methods of measurement, vertical spore profiles and the detection of immigrant spores., J. Gen. Microbiol., 48, 329–355, 1967.

Hoose, C., Kristjánsson, J. E., and Burrows, S. M.: How important is biological ice nucleation in clouds on a global scale?, Environ. Res. Lett., 5, 024009, doi:10.1088/1748-9326/5/2/024009, 2010.

- Iannone, R., Chernoff, D. I., Pringle, A., Martin, S. T., and Bertram, A. K.: The ice nucleation ability of one of the most abundant types of fungal spores found in the atmosphere, *Atmos. Chem. Phys.*, 11, 1191–1201, doi:10.5194/acp-11-1191-2011, 2011.
- Jurick, W. M., II, Narvaez, D. F., Brennan, M. M., Harmon, C. L., Marois, J. J., Wright, D. L., and Harmon, P. F.: Winter survival of the soybean rust pathogen, *Phakopsora pachyrhizi*, in Florida, *Plant Dis.*, 92, 1551–1558, 2008.
- Kessler, W.: Leaf-rust infection and early-frost damage in the Osier Willow variety “Apia”, *Sozialistische Forstwirtschaft*, 20, 346–347, 1970.
- King, E. O., Ward, M. K., and Raney, D. E.: Two simple media for the demonstration of pyocyanin and fluorescein, *J. Lab. Clin. Med.*, 44, 301–307, 1954.
- Li, X., Yang, X., Mo, J., and Guo, T.: Estimation of soybean rust uredospore terminal velocity, dry deposition, and the wet deposition associated with rainfall, *Eur. J. Plant Pathol.*, 123, 377–386, 2009.
- Lindow, S. E., Arny, D. C., and Upper, C. D.: *Erwinia herbicola*: a bacterial ice nucleus active in increasing frost injury to corn, *Phytopathology*, 68, 523–527, 1978.
- McAinsh, M. R., Ayres, P. G., and Hetherington, A. M.: The effects of brown rust and phosphate on cold acclimation in winter barley, *Ann. Bot.-London*, 66, 101–110, 1990.
- Möhler, O., DeMott, P. J., Vali, G., and Levin, Z.: Microbiology and atmospheric processes: the role of biological particles in cloud physics, *Biogeosciences*, 4, 1059–1071, doi:10.5194/bg-4-1059-2007, 2007.
- Monteil, C. L., Guilbaud, C., Glaux, C., Lafolie, F., Soubeyrand, S., and Morris, C. E.: Emigration of the plant pathogen *Pseudomonas syringae* from leaf litter contributes to its population dynamics in alpine snowpack, *Environ. Microbiol.*, doi:10.1111/j.1462-2920.2011.02680.x, 22 December, Epub ahead of print, 2011.
- Morris, C. E., Glaux, C., Latour, X., Gardan, L., Samson, R., and Pitrat, M.: The relationship of host range, physiology, and genotype to virulence on cantaloupe in *Pseudomonas syringae* from cantaloupe blight epidemics in France, *Phytopathology*, 90, 636–646, 2000.
- Morris, C. E., Sands, D. C., Vinatzer, B. A., Glaux, C., Guilbaud, C., Buffière, A., Yan, S., Dominguez, H., and Thompson, B. M.: The life history of the plant pathogen *Pseudomonas syringae* is linked to the water cycle, *ISME J.*, 2, 321–334, 2008.
- Morris, C. E., Sands, D. C., Vanneste, J. L., Montarry, J., Oakley, B., Guilbaud, C., and Glaux, C.: Inferring the evolutionary history of the plant pathogen *Pseudomonas syringae* from its bio-

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geography in headwaters of rivers in North America, Europe and New Zealand, *mBio*, 1, e00107-10-e00107-20, 2010.

Morris, C. E., Sands, D. C., Bardin, M., Jaenicke, R., Vogel, B., Leyronas, C., Ariya, P. A., and Psenner, R.: Microbiology and atmospheric processes: research challenges concerning the impact of airborne micro-organisms on the atmosphere and climate, *Biogeosciences*, 8, 17–25, doi:10.5194/bg-8-17-2011, 2011.

Mortazavi, R., Hayes, C. T., and Ariya, P. A.: Ice nucleation activity of bacteria isolated from snow compared with organic and inorganic substrates, *Environ. Chem.*, 5, 373–381, 2008.

Nagarajan, S., Singh, H., Joshi, L. M., and Saari, E. E.: Meteorological conditions associated with long distance dissemination and deposition of *Puccinia graminis tritici* uredospores in India, *Phytopathology*, 66, 198–203, 1976.

Nagarajan, S. and Singh, D. V.: Long-distance dispersion of rust pathogens, *Ann. Rev. Phytopathol.*, 28, 139–153, doi:10.1146/annurev.py.28.090190.001035, 1990.

Nemecek-Marshall, M., Laduca, R., and Fall, R.: High-level expression of ice nuclei in a *Pseudomonas syringae* strain is induced by nutrient limitation and low temperature, *J. Bacteriol.*, 175, 4062–4070., 1993.

Orser, C., Staskawicz, B. J., Panopoulos, N. J., Dahlbeck, D., and Lindow, S. E.: Cloning and expression of bacterial ice nucleation genes in *Escherichia coli*, *J. Bacteriol.*, 184, 359–366., 1985.

Paul, N. D. and Ayres, P. G.: Changes in tissue freezing in *Senecio vulgaris* infected by rust (*Puccinia lagenophorae*), *Ann. Bot.-London-*, 68, 129–133, 1991.

Paulsen, I. T., Press, C. M., Ravel, J., Kobayashi, D. Y., Myers, G. S., Mavrodi, D. V., DeBoy, R. T., Seshadri, R., Ren, Q., Madupu, R., Dodson, R. J., Durkin, A. S., Brinkac, L. M., Daugherty, S. C., Sullivan, S. A., Rosovitz, M. J., Gwinn, M. L., Zhou, L., Schneider, D. J., Cartinhour, S. W., Nelson, W. C., Weidman, J., Watkins, K., Tran, K., Khouri, H., Pierson, E. A., Pierson, L. S. R., Thomashow, L. S., and Loper, J. E.: Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5, *Nature*, 23, 873–878, 2005.

Perelló, A., Simón, M. R., and Arambarri, A. M.: Interactions between foliar pathogens and the saprophytic microflora of the wheat (*Triticum aestivum* L.) phylloplane, *J. Phytopathol.*, 150, 232–243, 2002.

Pfender, W., Graw, R., Bradley, W., Carney, M., and Maxwell, L.: Use of a complex air pollution model to estimate dispersal and deposition of grass stem rust urediniospores at landscape scale., *Agr. Forest. Meteorol.*, 139, 138–153, doi:10.1016/j.agrformet.2006.06.007, 2006.

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- Pfender, W. F. and Vollmer, S. S.: Freezing temperature effect on survival of *Puccinia graminis* subsp. *graminicola* in *Festuca arundinacea* and *Lolium perenne*, *Plant Dis.*, 83, 1058–1062, 1999.
- Phillips, V. T. J., Andronache, C., Christner, B., Morris, C. E., Sands, D. C., Bansemer, A., Lauer, A., McNaughton, C., and Seman, C.: Potential impacts from biological aerosols on ensembles of continental clouds simulated numerically, *Biogeosciences*, 6, 987–1014, doi:10.5194/bg-6-987-2009, 2009.
- Pruppacher, H. R. and Klett, J. D.: *Microphysics of Clouds and Precipitation*, Kluwer Academic Publishers, Dordrecht, 954 pp., 1997.
- Pummer, B. G., Bauer, H., Bernardi, J., Bleicher, S., and Grothe, H.: Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen, *Atmos. Chem. Phys.*, 12, 2541–2550, doi:10.5194/acp-12-2541-2012, 2012.
- Rowell, J. B. and Romig, R. W.: Detection of urediospores of wheat rust in spring rains, *Phytopathology*, 56, 807–811, 1966.
- Rupley, J. A.: The hydrolysis of chitin by concentrated hydrochloric acid, and the preparation of low-molecular-weight substrate for lysozyme, B.B.A.-Mucoprotein. *Mucopolysaccharid.*, 83, 245–255, 1964.
- Stakman, E. and Christensen, C. M.: Aerobiology in relation to plant disease, *Bot. Rev.*, 12, 205–253, 1946.
- Stakman, E. C., Henry, A. W., Curran, G. C., and Christopher, W. N.: Spores in the upper air, *J. Agric. Res.*, 24, 599–606, 1923.
- Staples, R. C. and Wynn, W. K.: The physiology of uredospores of the rust fungi, *Bot. Rev.*, 31, 537–564, 1965.
- Strynadka, N. C. J. and James, M. N. G.: Lysozyme revisited: crystallographic evidence for distortion of an N-acetylmuramic acid residue bound in site D, *J. Mol. Biol.*, 220, 401–424, 1991.
- Vali, G.: Quantitative evaluation of experimental results on the heterogeneous freezing nucleation of supercooled liquids, *J. Atmos. Sci.*, 28, 402–409., 1971.
- Wolfenbarger, D. O.: Dispersion of small organisms, *Am. Midl. Nat.*, 35, 1–152, 1946.
- Young, K. C.: *Microphysical Processes in Clouds*, Oxford University Press, New York, 427 pp., 1993.

Zeng, D., Chao, L., Sun, F., and Zhao, T.: The ice nucleation active bacteria on poplar trees and their effects on the causes of freezing injury and induction of fungal canker, *Sci. Sil. Sin.*, 35, 53–57, 1999.

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Table 1. Sources of rust urediospores characterized for ice nucleation activity (INA) and bacterial contamination.

Plant host	Rust spp.	Location	Date	Log (spores ml ⁻¹) ¹	INA onset (°C) ²	Log (spores per ice nucleus) ³
Field samples						
<i>Chenopodium album</i> Lambsquarters	<i>Puccinia aristidae</i> ⁴	St. Saturnin-les-Avignon, France	Apr 2009	5.68	-8	4.96
<i>Senecio vulgaris</i> Common groundsel	<i>P. lagenophorae</i> ⁴	St. Saturnin-les-Avignon, France	Mar 2009	5.50	-8	4.78
		Montfavet, France	Jan 2010	5.53	-6	4.68
<i>Festuca</i> sp. Perennial tufted grass	<i>Puccinia</i> sp. ⁴	St. Saturnin-les-Avignon, France	Apr 2010	5.45	-8	4.03
			Jun 2010	5.37	-6	5.17
<i>Avena fatua</i> Wild oats	<i>Puccinia</i> sp. ⁴	St. Saturnin-les-Avignon, France	Jun 2010	5.55	-4	3.62
<i>Dactylus glomerata</i> Orchard grass	<i>Puccinia</i> sp. ⁴	St. Saturnin-les-Avignon, France	Jun 2010	5.98	-6	5.28
<i>Allium ampeloprasum</i> Wild leeks	<i>P. allii</i> ⁴	Montfavet, France	Jan 2010	5.36	-6	5.16
		St. Saturnin-les-Avignon, France	Apr 2010	5.40	-8	4.79
<i>Allium schoenoprasum</i> Wild chives	<i>P. allii</i> ⁴	Montfavet, France	Apr 2010	5.45	-7	4.62
<i>Triticum aestivum</i> wheat, local land race	<i>P. striiformis</i>	Gaziantep, Turkey	Apr 2010	4.24	-7	3.88
<i>T. aestivum</i> cv. McNeal, wheat	<i>P. graminis</i> f. sp. <i>tritici</i> ⁵	Bozeman, Montana, USA	Aug 2010	5.78	-5	5.27
			Sep 2011	5.26	-4	3.94
<i>Coffea arabica</i> Coffee	<i>Hemileia vastratrix</i> ⁴	Pinhal City, Brazil	Jul 2008	6.04	-6	5.10
Rusts maintained on wheat in green houses						
<i>T. aestivum</i> cv. Michigan amber, wheat	<i>P. tritici</i> ⁶	Versailles, France	May 2010	5.50	-9	5.30
				5.02	-6	4.51
<i>T. aestivum</i> cv. Morocco, wheat	<i>P. graminis</i> f. sp. <i>tritici</i> ⁶	Aleppo, Syria	Apr 2010	4.38	-7	4.83
	<i>P. striiformis</i> ⁵	Aleppo, Syria	Apr 2010	4.37	-7	4.82
	<i>P. tritici</i> ⁶	Aleppo, Syria	Apr 2010	3.74	-7	3.53

¹ Concentration of washed spores used for determining INA and bacterial contamination rates.

² Warmest temperature at which INA of the spore suspension was detected.

³ Number of spores necessary to detect an ice nucleus active at the threshold temperature.

⁴ Putative identification of rust species based on the known rusts of the plant species sampled here.

⁵ Rust identity was determined based on disease symptoms and spore morphology.

⁶ Rust cultures of known identity maintained in culture on greenhouse-cultivated plants.

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Table 2. Rates of ice nuclei production required for bacteria to account for the ice nucleation activity observed in suspensions of rust spores.

Plant host	INA onset (°C) ¹	Log (ice nuclei ml ⁻¹) ²	Log (total bacteria ml ⁻¹) ³	Log (<i>P. syringae</i> ml ⁻¹) ³	Log (ice nuclei <i>P. syringae</i> cell ⁻¹) ⁴	Log (ice nuclei ml ⁻¹ due to <i>P. syringae</i>) ⁵	Minimum log (ice nuclei cell ⁻¹) to account for freezing onset ⁶	
							<i>P. syringae</i>	Total bacteria
<i>Avena fatua</i>	-4	1.92	7.20	3.95	-4.61	-0.66	-2.03	-5.28
<i>Triticum aestivum</i> cv. McNeal	-4	1.32	8.59	4.18	-4.08	0.09	-2.86	-7.27
<i>T. aestivum</i> cv. McNeal	-5	0.48	3.29	2.61	-5.09	-2.52	-2.13	-2.81
<i>T. aestivum</i> cv. Michigan amber	-6	0.48	5.67	nd ⁷				-5.19
<i>Senecio vulgaris</i>	-6	0.93	4.94	4.00	-3.1	0.90	-3.07	-4.01
<i>Allium ampeloprasum</i>	-6	0.18	5.36	3.48	-3.1	0.38	-3.30	-5.18
<i>Festuca</i> sp.	-6	0.18	5.69	4.64	-5.06	-0.42	-4.46	-5.51
<i>Dactylis glomerata</i>	-6	0.70	5.12	nd		0		-4.42
<i>Allium schoenoprasum</i>	-7	0.81	3.64	1.98	-4.35	-2.40	-1.17	-2.83
<i>Festuca</i> sp.	-8	1.41	5.30	3.46	-3.38	0.08	-2.05	-3.89
<i>Allium ampeloprasum</i>	-8	0.71	3.20	3.85	< -8.0	0	-3.14	-2.49
<i>Senecio vulgaris</i>	-8	0.72	5.51	nd		0		-4.63
<i>Chenopodium album</i>	-8	0.74	2.59	3.32	NC ⁸		-2.58	-1.85
<i>T. aestivum</i> cv. Michigan amber	-9	0.18	4.58	1.43	-1.76	-0.33	-1.25	-4.40

¹ Warmest temperature at which INA of the spore suspension was detected.

² Concentration of ice nuclei, active at the freezing onset temperature, in the spore suspension.

³ Concentration of total bacteria and *P. syringae* in the suspension of washed spores. These are bacterial cells that were most likely adherent to the spores.

⁴ Maximum frequency of ice nuclei per cell of *P. syringae* among the strains isolated from the rust spore suspension and tested for INA under laboratory conditions. The frequency reported here is for the freezing onset temperature of the spore suspension.

⁵ Estimation of the concentration of ice nuclei in the spore suspension based on the amount of *P. syringae* cells in the suspension and the maximum rate of ice nuclei per cell observed among the characterized strains. This concentration is for the freezing onset temperature.

⁶ Frequency of ice nuclei per cell that would be required for the bacteria associated with the rust spores to account for the onset freezing temperature observed for the suspensions of rust spores.

⁷ *P. syringae* was not detected. The detection threshold was 10 bacteria ml⁻¹.

⁸ Bacterial strains were not collected from this sample.

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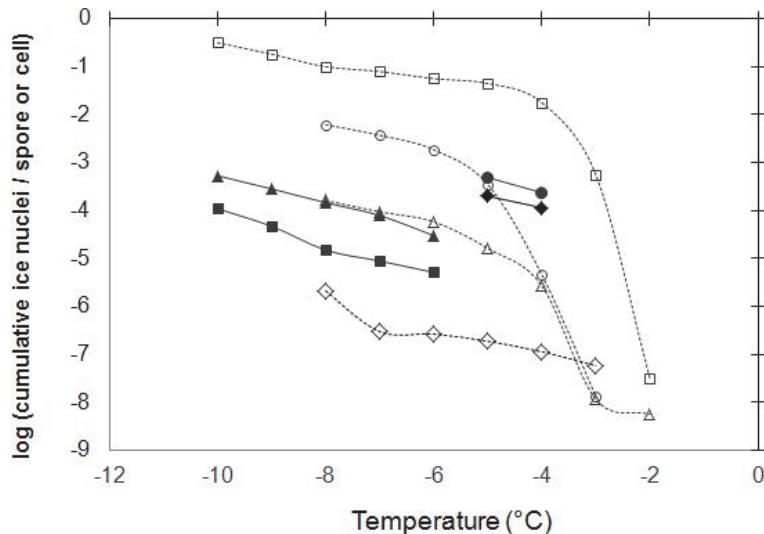


Fig. 1. Cumulative ice nucleation activity of urediospores of rusts (solid lines) compared to that of cells of *Pseudomonas syringae* (dashed lines). Examples are presented to represent the range of activities observed among the rust samples from this study and among strains of *P. syringae* from our collection and reported in the literature (Cit7 as reported in Fig. 4 of Orser and colleagues, Orser et al., 1985). Rusts whose activity is illustrated here were collected from *Dactylus* Orchard grass (■), green house-grown wheat (▲), field wheat (◆) and wild oats (●). Strains of *P. syringae* that illustrate the range of the activity of this bacterium include Cit7 (□), CC0229 (○) CC0094 (△) and from epidemics of cantaloupe blight, and CC0670 from a wild plant of the *Primula* species (◇). Curves for cumulative freezing spectra of rusts are not as complete as those for *P. syringae* because of the lack of sufficient spore quantities to prepare ample volumes for tests across a range of spore concentrations.

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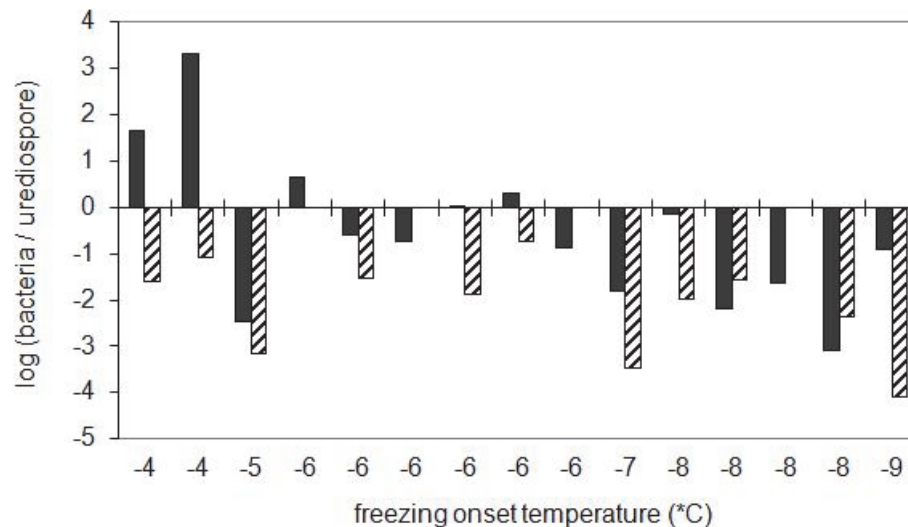


Fig. 2. Rates at which ureidiospores carried total bacteria and *Pseudomonas syringae*. Rates are presented as the log of total bacteria (solid bars) or *P. syringae* (hatched bars) per ureidiospore for samples ranked in the order of decreasing freezing onset temperature.

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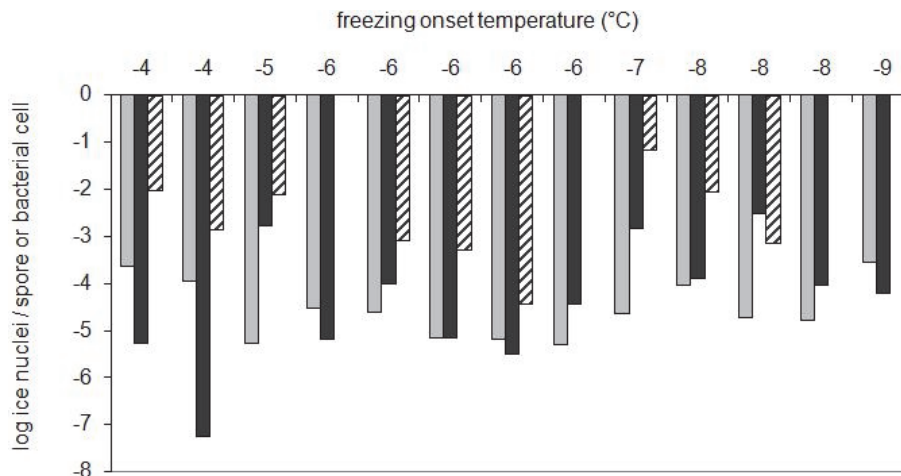


Fig. 3. Ice nuclei per particle in the spore suspensions calculated according to three hypotheses about the origin of the ice nuclei in the suspensions: that the ice nuclei are associated only with (i) the urediospores or the cells (grey bars) of (ii) the total bacteria (black bars) or (iii) *Pseudomonas syringae* (hatched bars) in the spore suspensions. The abundance of ice nuclei per spore or bacterial cells are presented in the order of decreasing freezing onset temperature of the spore suspensions.

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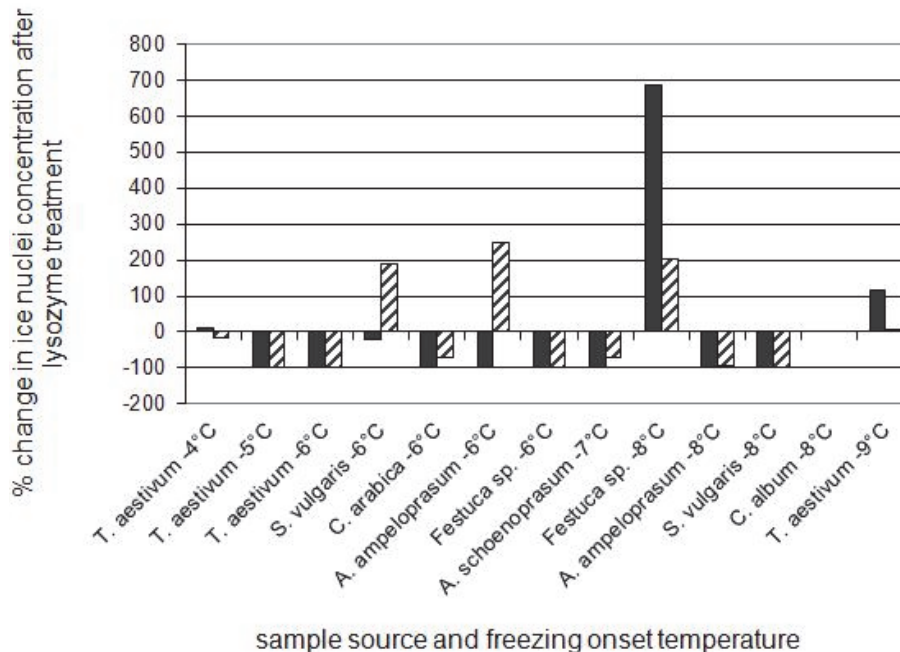


Fig. 4. Effect of lysozyme treatment on the abundance of ice nuclei in spore suspensions. The effect is presented as the percent of change in the number of ice nuclei per ml of spore suspension at the freezing onset temperature (black bars) and at one degree below the freezing onset temperature (hatched bars) for each sample.