Field measurements of biological ice nuclei

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A) Measurement of ice nuclei in the field in real time: 1960s and 70s.

In the 1960s Gabor Vali developed a small, 15 lb. highly portable drop freezer using dry ice as the coolant that could measure ice nucleation activity of samples 10 minutes after set-up in the field (see item 1). Russ Schnell used this apparatus in the field, and where electricity was available, adapted this technique to use a small electric cooling plate. By heating samples, the biological component of the ice nucleus activity was measurable in the field within minutes of sample collection.

These systems were used by Schnell (1969-78) to establish the ubiquity of biological ice nuclei in plant litter and marine plankton spanning the globe including the Arctic, Siberia, Asia, Africa, Central America and the Atlantic Ocean, to name a few (see items 2-7) .

To determine the ice nucleus activity of aerosols, Schnell collected samples on 45 mm Sartorius hydrophobic filters, mounted these filters on the cold plates, added an array of clean water drops and measured the ice nucleus activity of aerosols. Heating the filters removed the biological component of the ice nucleus activity.

Possible item for section 1.c, History of bioaerosol measurements.

As an undergraduate student In the 1960s, Schnell worked summers on a hail research project in Alberta, Canada. Gabor Vali, who had recently noted that some soil samples had active ice nucleation activity, asked him what he thought were the source of hail ice nuclei. Schnell had observed that the hailstorms in Alberta generally started over forested and grassland areas devoid of any open soil. He collected living tree leaf and grass samples, washed them and tested for ice nucleation activity. There was none of note.

Accidently leaving a wet grass sample in a plastic bag, 10 days later he noticed the the water was cloudy. Testing it showed ice nuclei I active at -1.3^0 C. He showed the data to an eminent cloud physicist (not Vali) who stated that the data was probably not good and dismissed the observation. Schnell discarded the sample and packed up the freezer as it was time to go back to school. Two years later when Schnell was now a graduate student at the University of Wyoming, he tested decaying tree leaves and again found the presence of ice nuclei active at -1.3^0 C which turned out to be produced by *P. syringae* bacteria which he named Bacteria Derived Nuclei (BDN).

In associated research, Schnell tested decayed leaf litter from under trees and grasses and found high concentrations of stable ice nuclei active at -3.5^0 C that he named Leaf

Derived Nuclei (LDN). Since ice phase precipitation falls over oceans, he tested plankton collected in the Atlantic Ocean off Nova Scotia and found high concentrations of ice nuclei active at -3.5^0 C which he labeled Ocean Derived Nuclei (ODN). Later research found that cultures of the marine bacteria *Cachonina niei* produced ice nuclei active at -3^0 C.

Possible items for section 3.a. Lessons learned

The identification of *P. syringae* as an active ice nucleus source could have been accomplished 2-3 years earlier had the original sample showing ice nucleus activity at -1.3^0 C being frozen and taken to a microbiologist for analysis.

Similarly, the active ice nuclei in LND were for years thought to be a by-product of BDN. More recent research has suggests that one stable ice nucleus in LDN is associated with a fungal isolate *Mortierella alpina*. We missed this by 40 years by not following up on the disconnect between BDN and LDN stability and initial temperature of the ice nucleus activity.

Supercooling of Water and Nucleation of Ice (Drop Freezer)

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The principle and operation of a simple drop-freezing device is described. Such a unit can be effectively used in a teaching laboratory or as a demonstration apparatus to illustrate the general features of nucleation phenomena through the example of the freezing nucleation of water.

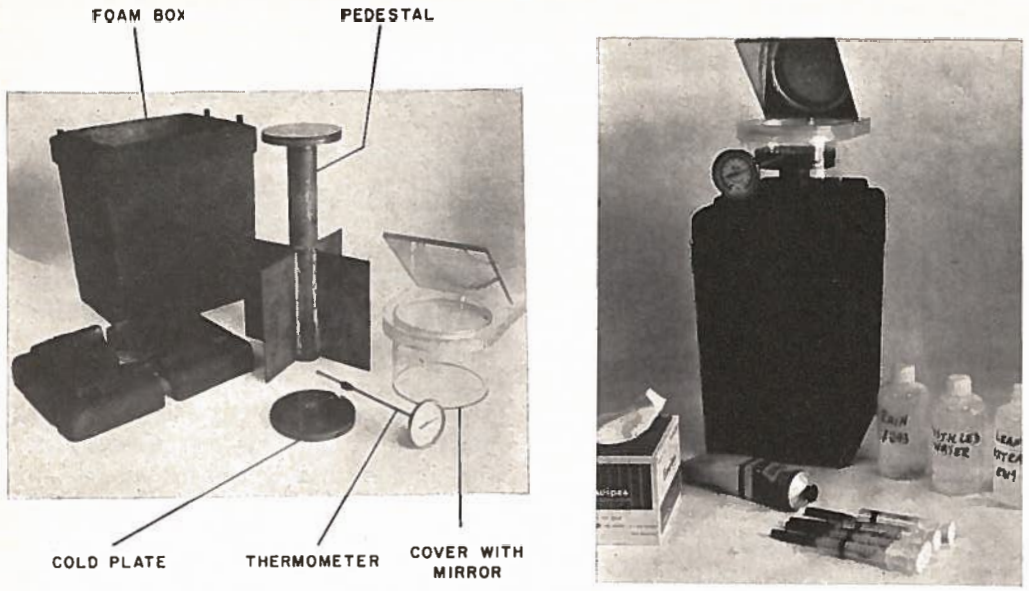
[*The apparatus described in this article was first prize winner in the 1971 AAPT Apparatus Competition. This contribution was in the area of undergraduate laboratory—Editor*]

SUPERCOOLING OF WATER

The "freezing temperatures" of substances are not equal to their "melting temperatures." Fahrenheit in 1724 reported that water can be supercooled; supercooling and the related phenomenon of supersaturation have since that time received widespread attention.

The new, more ordered solid phase can only form from a liquid on germs or embryos of that solid and these embryonic aggregates have to be created in the face of an energy barrier, which results from the large surface to volume ratios of small bodies.[1] The liquid can thus exist in its metastable, supercooled form until a sufficiently large, stable embryo forms within it. The formation of such a stable embryo and the consequent transformation into solid of the entire body of liquid is called a nucleation event.

If the liquid is pure, chances are that super-



(a) (b)

FIG. 1. (a) Photograph of the disassembled unit. (b) Photograph of the complete unit with water drops on the cold stage.

coolings of many tens of degrees will be required before a viable embryo appears and nucleates the sample. Water has been found[2,3] to nucleate "homogeneously" at appreciable rates only at temperatures near −40°C. Yet, ice is observed to form quite commonly at temperatures much warmer than −40°C; ice formation at small supercoolings is due to "heterogeneous" nucleation, the aiding of embryo building by impurity particles in the liquid. Each particle of impurity is capable of nucleating ice at a different degree of supercooling. Broadly speaking, the effectiveness of a particle depends on its composition and on its surface structure. Nucleation at very small supercoolings of only a few degrees is known to be initiated, for example, by silver iodide and by some organic substances.

NUCLEATION EXPERIMENTS

Both homogeneous and heterogeneous nucleation can best be studied by breaking up the liquid into numerous small drops. If nucleation is homogeneous, all of the drops will freeze at very closely the same temperature; if on the other hand the nucleation is heterogeneous there may be differences of up to tens of degrees between the freezing temperatures of the different drops. The reason for this is that each drop freezes at the nucleation temperature of the most active impurity it happens to contain. The allocation of impurities into the various drops is a random process and therefore, when an experiment is performed with many drops from the same sample, the drops can be observed to all nucleate at different temperatures. From such observations one can determine quantitatively the concentrations of impurity nuclei of different activities in the sample.[4–7]

DROP-FREEZING APPARATUS

The most convenient way to study the heterogeneous nucleation of water drops is to place the drops on an inert supporting surface and cool them at a relatively slow rate that allows observation of the freezing temperature of each of the drops. A simple and inexpensive drop-freezing device is shown in Fig. 1. The supporting surface for the drops is a heavy copper plate, which is thoroughly cleaned and coated with a solid silicone film each time a new set of drops is tested. The drops are placed on this surface using a hypodermic syringe and needle. The copper plate

with the drops is placed on a pedestal, which is cooled at its lower end by dry ice. A cover is placed over the assembly to keep off undue air circulation and to minimize the amount of condensation from the surroundings onto the cold surfaces. A probe (a bimetallic dial thermometer) is inserted in the copper plate to provide temperature readings. A slanted mirror over the transparent cover allows the drops and the temperature dial to be conveniently observed from one position. For the unit shown in Fig. 1, the diameter of the copper plate is 10 cm, but smaller as well as larger dimensions could also be chosen. In the construction of the pedestal, the masses and the heat conductivities of the various pieces were chosen to provide a rate of cooling which is approximately 3°–5°/min at 0°C tapering off to approximately 1°/min at −25°C. These cooling rates were found to allow observations to be made with relative ease without an unduly long total time for an experiment.

The freezing of the drops can readily be detected visually as the nucleated drops change abruptly from clear to opaque. A fraction of the drop's total volume solidifies instantaneously, following which solidification is completed as heat is conducted away from the drop. The opaqueness of the drops is due to the formation of air bubbles within the drops as the dissolved air is excluded from the ice, and since the slower freezing drops, at warmer temperatures, trap less of the excluded air, they tend to be a little more transparent than the drops freezing at colder temperatures. The formation of ice can, in all cases, be unambiguously detected. An example of what an array of drops may look like when some are frozen and some are still liquid in shown in Fig. 2.

During the course of an experiment, the freezing temperatures of the drops are recorded as they occur. It is helpful to record these temperatures in the same geometric pattern as the drops are placed on the cooling stage so that at any time a quick reference is available on which of the freezing events have already been noted.

A most convincing method for comparisons of different samples of water is to cool simultaneously two groups of drops on the plate, one group from each of the samples to be tested. Differences

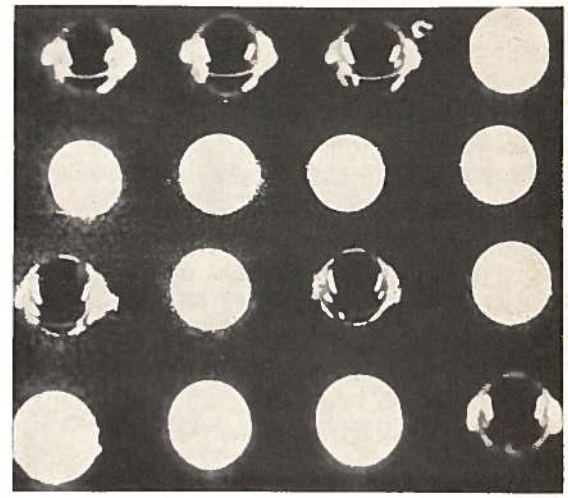


FIG. 2. Typical appearance of the cold stage during an experiment. Some of the drops are still unfrozen (clear) while others are already frozen (white).

in freezing temperatures become quite apparent in such an experiment.

In addition to the basic types of experiments described above, several other observations can be made. In order to show that the apparently random sequence of nucleation events among the drops is in fact determined by the invisible impurities contained in the drops, sets of drops may be melted and refrozen several times in succession. The freezing temperatures for each drop will be found to repeat themselves with very little variation. At the end of a freezing experiment the drops on the plate may be allowed to warm up slowly and it can be then confirmed that all drops melt at the same temperature, 0°C, independently of their nucleation temperatures.

ANALYSIS

The most readily deduced measure of the "freezibility" of a sample of water is the average freezing temperature for a set of drops taken from that sample. The average freezing temperature is a relative measure of the nucleus content; the average freezing temperature depends on the volumes of the water drops as well as on the amounts of impurities in the water. Smaller drops have colder average freezing temperatures than larger drops taken from the same sample.

More complete representations of the experi-

mental results can be given by the drawing of histograms showing the frequencies of freezing events in different temperature intervals. In connection with these histograms, other measures such as the mode, quartiles, or the temperatures at which 10% and 90% of the drops are frozen could also be used.

The most complete descriptions of nucleus content can be given in terms of the so-called differential and cumulative nucleus spectra. These are representations of the concentrations of nuclei per unit volume as functions of temperature and can be readily calculated from the observed freezing temperatures.[7]

APPLICATIONS

Using the simple device and the techniques described above it is possible to perform large varieties of experiments. For example, the samples to be analyzed could come from diverse sources: distilled water, rain water, snow, melted polar ice, and many other sources. A large group of experiments can be performed by adding different substances to distilled water. Tiny amounts, only a few parts per million, of substances such as silver iodide or of humus, for example, can raise the freezing temperatures of drops from −20°C to as warm as −4° to −8°C. Experiments can be performed to show that the addition of a solute to a sample depresses the nucleation temperature by approximately the same amount as the melting point is depressed. The lack of a mechanical effect on nucleation can be shown by tapping the stage with the supercooled drops on it or by the addition of acoustic waves generated in the vicinity of the drops.

The experimental apparatus can readily be embellished with more sophisticated temperature sensing and data handling systems. The whole gamut of temperature probes and displays can be called into use and recording systems from simple homemade paper tape devices to chart recorders and to photographic time lapse systems can be utilized. Automatic detection of the freezing of the drops is also possible but is probably not warranted for a teaching laboratory or demonstration experiment. The simplicity of the device as shown in Fig. 1 is probably one of its merits and should perhaps not be given up easily in favor of complexities.

IMPLICATIONS

Water in its many forms constitutes a major factor in our environment and in our lives. Some very spectacular phenomena are related to the phase transformations of water: the formation of clouds in the atmosphere, the initiation of precipitation within these clouds, the steam engine, cloud and bubble chambers used in atomic physics research, and so many more. Nucleation events initiating these phase transformations are therefore important as well as intriguing problems. The drop freezing experiments provide a readily available and easily comprehended example of such nucleation phenomena. The main features of all nucleation phenomena are present in these experiments: The metastable supercooled state of water can be observed; the probablistic nature of the nucleation process can be demonstrated; and the influence of foreign materials on the nucleation temperatures can be made evident. The knowledge gained from these experiments can be used beneficially in discussing such diverse topics as cloud and bubble chambers, atmospheric clouds and precipitation processes, weather modification theories, and the survival of insects and plants at cold temperatures.

ACKNOWLEDGMENTS

I want to thank R. S. Wywiorski and Dr. D. L. Veal for their help in constructing the drop-freezing device. I also want to thank Professor R. G. Layton of Northern Arizona University for his advice regarding the use of drop-freezing experiments in the undergraduate laboratory.

[1] J. W. Gibbs, Amer. J. Sci. **16**, 441 (1878), reprinted in *The Scientific Papers of J. Willard Gibbs* (Dover, New York, 1961), pp. 354–371.

[2] G. R. Wood and A. G. Walton, J. Appl. Phys. **41**, 3027 (1970).

[3] I. E. Kuhns and B. J. Mason, Proc. Roy. Soc. (London) **A302**, 437 (1968).

[4] N. E. Dorsey, Trans. Amer. Phil. Soc. **38**, 247 (1948).

[5] R. G. Dorsch and P. T. Hacker, U. S. Nat. Adv. Comm. Aero. Tech. Note **2142** (1950).

[6] G. Vali and E. J. Stansbury, Can. J. Phys. **44**, 477 (1966).

[7] G. Vali, J. Atmos. Sci. **28**, 402 (1971).

SHORTER CONTRIBUTION

Freezing nuclei in marine waters

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(Manuscript received May 20, 1974)

1. Introduction

Inorganic soil materials (Isono et al., 1959; Mason, 1960) and extraterrestrial meteoritic particles (Bowen, 1956; Bigg, 1963) have been suggested as possible sources of atmospheric ice nuclei which play important roles in the production of precipitation. Recently, decomposition products of terrestrial vegetation (Schnell & Vali, 1972) have been shown to be good ice nucleators and these sources were found to be world-wide (Schnell & Vali, 1973) with the abundance of the nuclei dependent upon local climate.

As an extension of the research on the land sources of nuclei, it seemed desirable to examine whether sources of nuclei might also exist in the oceans. Samples of surface seawater were collected in the Pacific Ocean off Vancouver, B.C., Canada (January) and Huntington Beach, California, USA (June); in the Caribbean off Nassau, Bahamas (April) and in the Atlantic at Bedford, Nova Scotia, Canada (May). The freezing nucleus contents (drop freezing technique) of the samples were determined within two hours to three days of sample collection following the technique given by Vali (1971).

2. Results

From the results shown in Fig. 1 it may be seen that the Bedford samples (curve 4) exhibited considerably greater freezing nucleus concentrations than seawater from the other areas (curves 1, 2, 3). Beford Basin had experienced a "bloom period" of phytoplankton growth just prior to sample collection so the possibility suggested itself that the high concentration of nuclei might be related to this fact.

Masses of plankton and associated detritus were sieved from the top two feet of the ocean surface at Bedford. Sieving was done with ship-towed 20 μm plankton nets which were allowed to saturate so that even the smallest particles would collect on them. Analysis showed that 95% (by mass) of the captured material was phytoplankton and 5% zooplankton. No accurate measurements were made of the amount of organic debris in the samples though it appeared from microscopic examination that this mass was greater than the living component. A portion of the moist captured material was prepared for freezing nucleus measurements (within two hours of collection) by reinserting one gram of material into 100 g of the original seawater (i.e., seawater shown in curve 4). The mixture was agitated, allowed to settle for five minutes and then filtered through coarse paper. The turbid filtrate was tested for freezing nucleus content and yielded curve 5 in Fig. 1. The difference between curves 4 and 5 (ratio of 10^3 in concentration at -10°C) shows that there indeed are nuclei associated with the plankton matter and suggests that the original activity in this seawater sample was also due to the presence of plankton matter. These nuclei are referred to in the following as ocean-derived nuclei (ODN).

Seawater has a melting point depression of 2°C due to dissolved salts. The inherent nucleating ability of the plankton material can be better evaluated by suspending the sample in distilled water—one gram of plankton matter in 100 g of distilled water yielded the spectrum given by curve 6 in Fig. 1 (the concentration

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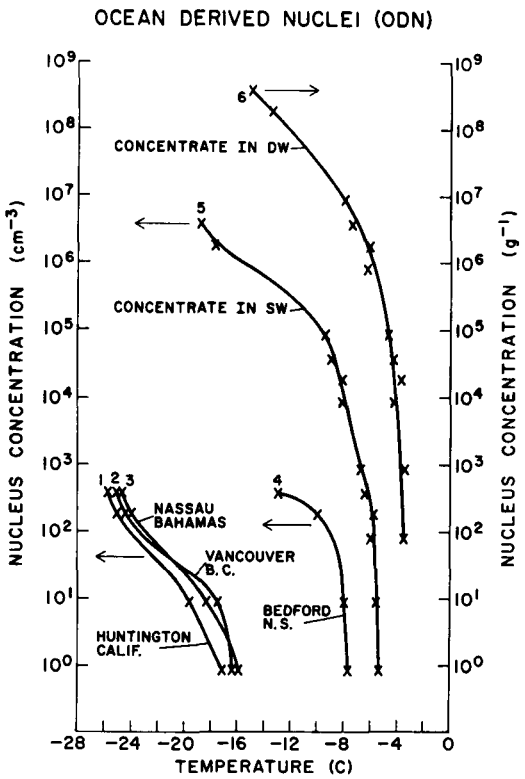

Fig. 1. Freezing nucleus spectra of seawater of low activity (curves 1, 2 and 3) and high activity (curve 4). Activity of plankton concentrate from Bedford Basin is given by curves 5 and 6.

here is expressed per unit mass of solid matter). It may be seen that one gram of Bedford Basin sievings contained nuclei active even at −3.5°C, and 10^7 nuclei per gram were active at −10°C.

The seawater samples were kept at room temperature for seven months and their freezing nucleus contents were measured periodically. Within three weeks the concentration in the Bedford sample (curve 4) had deteriorated to 1% of its former value, and after seven months, this sample had a nucleus spectrum similar to the low-ODN samples (curves 1, 2, and 3) which showed no change at all over the same period. A portion of the Bedford sample was stored frozen over the same period; there was no loss of freezing nucleus activity in this sample. Tests have shown the ODN to be less than 1 μm in diameter (membrane filtration), susceptible to heat (destroyed at 95°C) and easily aerosolized and recovered with nucleation activity intact. These characteristics of ODN are similar to those for leaf-derived nuclei (LDN) (Schnell & Vali, 1972 and 1973).

3. Discussion and conclusions

The transfer of material from the oceans to the atmosphere is well documented. Specifically for organic matter, Blanchard (1964) found that bubbles bursting at a water surface could eject high concentrations of organic material into the atmosphere. Goetz (1965) captured submicron-sized organic particles emanating from oceanic areas. ZoBell & Matthews (1936) and Stevenson & Collier (1962) showed that air above oceans contained numerous microorganisms indigenous to marine waters. Research into the source of organic compounds found in snow and rain falling in New Zealand (Wilson, 1959) and in Sweden (Newman et al., 1959) suggested a marine source for the materials.

Atmospheric ice nuclei measurements over the southern seas were reported by Bigg (1973) for a three-year period. His data showed the presence of bands of high concentrations of airborne ice nuclei along latitudes 40° and 55° S; these bands coincide with zones of the highest primary marine production in the world (Fairbridge, 1966). This correlation supports (although does not uniquely prove) our suggestion.

In summary, the evidence indicates that oceans harbor, in association with phytoplankton, copious numbers of active freezing nuclei. These nuclei may contribute strongly to the local concentrations of atmospheric ice nuclei in regions of high primary oceanic productivity and may also add to the general global background of ice nuclei.

Acknowledgements

The support of Dr T. Platt and B. Irwin of the Marine Ecology Laboratory, Beford Institute of Oceanography, in collecting plankton samples substantially aided this research project. Research was supported in part by Grant GI-32555X from the National Science Foundation.

REFERENCES

Bigg, E. K. 1963. Ice nuclei in the atmosphere. *Proc. Int. Assn. Meteor. Atmos. Phys.*, IAMAP Publ. No. 13, pp. 139–140.

Bigg, E. K. 1973. Ice nucleus measurements in remote areas. *J. Atmos. Sci. 30*, 1153–1157.

Blanchard, D. C. 1964. Sea-to-air transport of surface active material. *Science 146*, 396–397.

Bowen, E. G. 1956. The relation between rainfall and meteor showers. *J. Meteor. 13*, 142–151.

Fairbridge, R. W. 1966. *Encyclopedia of oceanography*. Reinhold, New York, 1021 pp.

Goetz, A. 1965. The constitution of aerocolloidal particulates above the ocean surface. *Proc. Int. Conf. on Cloud Phys.*, pp. 46–47. Tokyo-Sapporo.

Isono, K., Komabayasi, M. & Ono, A. 1959. The nature and the origin of ice nuclei in the atmosphere. *J. Meteor. Soc., Japan, 27*, 211–233.

Mason, B. J. 1960. Ice nucleating properties of clay minerals and stony meteorites. *Quart. J. Roy. Meteor. Soc. 86*, 553–556.

Neumann, G. H., Fonselius, S. & Wahlman, L. 1959. Measurements on the contents of non-volatile organic material in atmospheric precipitation. *Int. J. Air Poll. 2*, 132–141.

Schnell, R. C. & Vali, G. 1972. Atmospheric ice nuclei from decomposing vegetation. *Nature 236*, 163–165.

Schnell, R. C. & Vali, G. 1973. World-wide source of leaf-derived freezing nuclei. *Nature 246*, 212–213.

Stevenson, R. E. & Collier, A. 1962. Preliminary observations on the occurrence of airborne marine phytoplankton. *Lloydia 25*, 89–93.

Vali, G. 1971. Quantitative evaluation of experimental results on the heterogeneous freezing nucleation of supercooled liquids. *J. Atmos. Sci. 28*, 402–409.

Wilson, A. T. 1959. Surface of the ocean as a source of airborne nitrogenous material and other plant nutrients. *Nature 184*, 99–101.

ZoBell, C. E. & Matthews, H. 1936. A qualitative study of bacterial flora of sea and land breezes. *Proc. Natl. Acad Sci. 22*, 567–572.

Ice Nuclei in Seawater, Fog Water and Marine Air off the Coast of Nova Scotia: Summer 1975

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(Manuscript received 9 November 1976, in revised form 15 April 1977)

ABSTRACT

Ice nuclei were measured in seawater, fog water and the free atmosphere from 28 July to 11 August during the 1975 *Hayes* Fog Cruise off the east coast of Nova Scotia, Canada. Some seawater samples were found to contain ice nuclei active at −4 to −5°C, although the majority of seawater samples contained no nuclei active at temperatures warmer than −14°C. Half of the fog water samples contained ice nuclei active at temperatures warmer than −10°C; some nuclei were active at −2°C. Atmospheric ice nucleus concentrations varied from 1.1 to 580 nuclei m^{-3} active at −15°C. Some bacteria isolated from fog water were observed to initiate ice at −1.5°C. High concentrations of active ice nuclei in seawaters and fog waters were associated with high concentrations of biological materials in the same samples.

1. Introduction

Atmospheric ice nuclei (AIN) play an important role in the initiation of much of the precipitation that falls on the earth's surface. The sources for these rare yet pivotal particles have most often been thought to be soil particles or extraterrestrial meteoritic materials (Isono *et al.*, 1959; Kumai, 1961; Bowen, 1956). More recently, Schnell and Vali (1972, 1973, 1976) have presented evidence that decayed litters from terrestrial plants release copious amounts of ice nuclei (IN) in concentrations of up to 10^9 nuclei per gram of leaf litter active at −10°C, and that some litters exhibit IN activity at −4°C. These nuclei are called leaf-derived nuclei (LDN).

Another source of very active IN has been observed to be a function of the intact bodies of a very few species of terrestrial bacteria. Specifically, *Pseudomonas syringae*, *Pseudomonas fluorescens* and *Erwinia herbicola* have been observed to initiate ice in supercooled water at temperatures warmer than −3°C; some nucleation events have been observed at −1.3°C (Maki *et al.*, 1974; Lindow *et al.*, 1975; Vali *et al.*, 1976). These nuclei are called bacteria-derived nuclei (BDN).

Within the marine environment, a prolific source of IN has been observed in close association with marine waters of high primary productivity (Schnell and Vali, 1974). For instance, waters off Bedford, Nova Scotia, produced IN active at −8°C in concentrations of up to 10^2 nuclei active at −10°C per gram of seawater. Marine waters of low primary productivity were observed to contain few IN. In keeping with earlier nomenclature, these nuclei are called ocean-derived nuclei (ODN). ODN have been successfully grown *in vitro* in concentrations of up to 10^6 nuclei active at −10°C per gram of plankton with some first ice embryos detectable at −3°C (Schnell, 1975). More recently, a marine bacterium living in close association with phytoplankton has been observed to be an active ice nucleant at temperatures approaching −1°C (Schnell, unpublished to date). The bursting bubble phenomenon (Blanchard, 1964, 1970) could be a method for ODN transfer from the oceans to the atmosphere.

On the basis of this information, shipboard measurements were conducted during the USNS *Hayes* 1975 Fog Cruise (28 July–11 August, 1975) to monitor the presence of IN in seawater, in air above the water and in marine fog water encountered during a trip that crisscrossed an area approximately bounded by 43°N, 66°W; 45°N, 59°W; 43°N, 62°W; 41°N, 66°W which is off the east coast of Nova Scotia, Canada. The research procedures utilized to conduct the IN measurements, the data gathered, and an analysis of the results form the body of this paper. All times referenced are Atlantic Daylight Saving Time (ADST).

On board the *Hayes* were 24 scientists from across the United States, each measuring some parameter of the ocean and/or the lower atmosphere that might bear upon fog formation. For instance, measurements of sea temperature, air temperature, cloud condensation nuclei, radon gas, air conductivity, sea surface chemistry, aerosol concentration and air microbiology were conducted throughout the cruise. During fog episodes measurements of fog droplet spectra, visibility, fog water chemistry, fog microbiology, etc., were conducted in addition to the regular measurements. Taken together, these measurements form a body of knowledge

on factors involved in marine fog formation, the extent of which has probably never been attained previously.

2. Sample collections

a. Seawater samples

Seawater was collected off the starboard side and stern of the ship by dipping a rope-lowered plastic bucket into the top half meter of the sea surface or by bottle collections at 1–3 m depths.

b. Fog water samples

Fog water samples were obtained from a centrifugal collecting device at a point high above the deck near the bow of the *Hayes* or from a nylon mesh bow kite. Control washes and IN spectra comparisons of samples collected by the two techniques indicated that there was no contamination in samples collected by the centrifugal system and only slight residual contamination from the bow kite, the latter being considered insignificant. Because of the one-man operation of IN measurements, fogs occurring between 2400 and 0800 were generally not tested for IN content.

c. Atmospheric particulates

Membrane filters (Millipore 0.45 μm, 4.7 cm diameter) were exposed in pairs from a forward position on the uppermost deck of the *Hayes*. Air was drawn through a coiled plastic hose (2 m length, 2 cm diameter) into a bifurcated chamber where the membrane filters (in their plastic holders) were mounted. This sampling arrangement allowed spray, rain and fog droplets to fall out prior to passage of air through the filters. A constant airflow of 350 $\ell\ h^{-1}$ was maintained through each filter for periods ranging from 2 to 12 hr. Undoubtedly, some very small particles were lost by diffusion while passing through this system.

Measurements of possible contamination by exhaust plumes from the ship's galley and engines were conducted by purposely exposing filters to the effluent streams. No IN from either source were observed. In order to produce field-exposed control filters, no air was drawn through every tenth pair of filters mounted on the sampling apparatus. These filters were later processed to obtain a mean IN background count, which subsequently was subtracted from each observed count to obtain a true count. For data presented in this report, a background correction of 10 IN per filter active at -15°C was used.

3. Ice nucleus measurements

a. Drop freezing technique

It has been shown by Vali (1971a) that the concentrations of suspended ice nuclei in samples of water can be determined quantitatively by using the drop freezing technique. This technique involves placing equal-sized drops on a thermally controlled surface (cold stage) and monitoring the freezing of the drops as the temperature of the sample is gradually lowered. Nucleus spectra can be constructed from the observed freezing temperatures of the drops. The particular cold stage for these experiments consisted of a copper fin and plate arrangement immersed in crushed dry ice (Vali, 1971b). During cooling of the cold stage, freezing events were detected visually as the drops changed from clear to opaque upon freezing. To characterize the nucleating ability of a sample, the freezing point temperatures (T_1, T_{10}, T_{90} and T_{100}) were noted for the first drop and for 10%, 90% and 100% of 30 drops in a test. For the drop sizes used, T_1, T_{10}, T_{90} and T_{100} correspond to nucleus concentrations of 0.82, 8.7, 192 and 383 cm^{-3}, respectively. For purposes of this paper, the ice forming nuclei measured by the drop freezing technique (variously called freezing nuclei or immersion freezing nuclei) and the ice nuclei measured by the filter membrane technique (variously called deposition nuclei or contact-followed-by-freezing nuclei) are all referred to as ice nuclei.

b. Membrane filter technique

The membrane filter technique is used to measure IN in the deposition mode utilizing a thermal diffusion chamber. Deposition ice nucleation occurs when ice forms on a nucleus by direct transfer of water vapor onto that nucleus. This process can occur below, at, or above water saturation, but only at or above ice saturation at any particular temperature.

The static thermal diffusion chamber is a widespread laboratory device used to measure deposition nucleation (Bigg *et al.*, 1963; Gagin and Aroyo, 1969). The dynamic thermal diffusion chamber utilized in this study was as described by Langer and Rodgers (1975) except for one modification: three thermally controlled ice-covered plates were substituted for the ice cubes to supply water vapor to the nuclei.

To calibrate the system at water saturation, or possibly slight supersaturation, the filter substrate and ice temperatures were adjusted so that a fog, and eventually droplets, appeared on the aluminum foil placed in the processing chamber. Further evidence of proper vapor control was seen when water depleted "halos" formed around growing ice crystals on the filters being processed. Observed ice crystal counts on the exposed filters varied from 11 to 432. In this study, all data presented are for the filters processed at -15°C with each filter corrected for hygroscopic nucleus competition (i.e., volume effects) by use of a correction curve similar to that derived by Mossop and Thorndike (1966). This correction curve may have limited validity for data presented in this study due to differences in air masses encountered during the cruise. It should be noted at this point that there exist unanswered questions in general regarding the validity of the membrane filter technique

for detecting atmospheric ice nuclei within marine environments rich in hygroscopic nuclei, especially for the larger than normal volumes of air sampled in this study. Thus to test the effect of hygroscopic nuclei on the activation of associated ice nuclei, the second filter in each duplicate set was processed in the University of Wyoming static thermal diffusion chamber (Huffman and Vali, 1973) or a commercial version of the static diffusion chamber described by Gagin and Aroyo (1969). Both of these chambers were calibrated to operate at −15°C and nominal water saturation.

The results of these comparison tests showed that the dynamic diffusion chamber activated 1.2–1.8 orders of magnitude more ice nuclei on the duplicate filters than did either static diffusion chamber. This indicates that the moisture supply under static conditions (all other factors assumed to be equal) may be somewhat inadequate at a filter surface both to overcome the effect of hygroscopic particles and activate all of the potential ice nuclei. Further evidence supporting the argument that the dynamic diffusion chamber overcomes the moisture supply problem comes by way of Fig. 6, where it may be seen that ice nucleus concentrations varied independently of air volume sampled.

It is not within the scope of this paper to suggest that the atmospheric IN concentrations depicted in Fig. 6 are absolute, rather that the measurements should be viewed as being relative until questions surrounding the membrane filter technique of measuring IN in a marine environment can be fully answered.

4. Results

a. Ice nuclei in seawaters

Representative spectra for all the seawater samples tested are shown in Fig. 1. From Fig. 1 it may be seen that a large majority of the seawater samples contained IN inactive at temperatures warmer than −14°C. Five seawater samples contained IN active at temperatures warmer than −10°C, including the phenomenally active spectrum designated 1 (collected at 0900 ADST 8 August) which contained freezing nuclei active at −4°C. This spectrum represents the most active sample I have ever collected from ambient seawater. Spectrum 5, collected at 2300 ADST 4 August, is anomalous in that its very shallow slope indicates that the sample contained many IN active at warmer temperatures and relatively few IN active at colder temperatures. Repeat tests of this sample produced similar results.

b. Ice nuclei in fog waters

Representative spectra for all of the fog waters tested are shown in Fig. 2. This figure shows that fogs generally contained more IN per unit volume of water than did seawaters and that the most active fog water tested was collected at 0845 ADST 8 August just 15 min prior to collection of the most active seawater sample (Fig. 1). The fog waters collected between 0600 and 0930 ADST

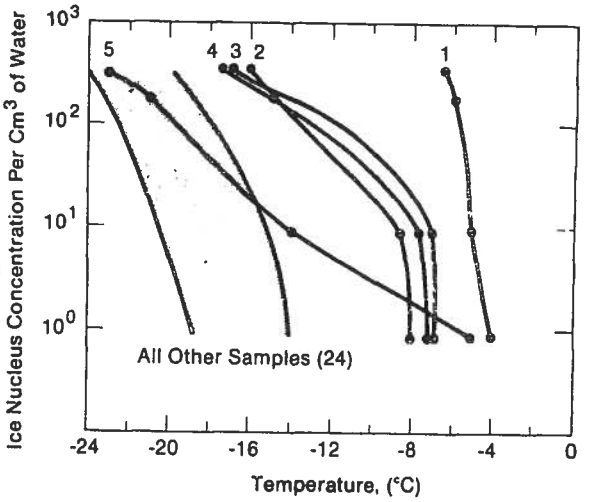

FIG. 1. Freezing nucleus spectra of seawater samples tested onboard the USNS *Hayes*. Collection times for the numbered samples are 1) 0900/8/8, 2) 1900/28/7, 3) 2000/1/8, 4) 2400/2/8, 5) 2300/4/8. All other samples exhibited ice nucleation activity beginning at temperatures lower than −14°C and fell within the shaded portion of the figure.

6 August also exhibited consistently high IN concentrations. These spectra represent what I believe to be the first published data on IN concentrations in marine fog waters at sea. In keeping with the earlier nomenclature applied to biogenic ice nuclei, the name given nuclei present in fog water is fog-derived nuclei (FDN).

c. Relationships between freezing nuclei in seawaters and fog waters

During periods of fog water collection, attempts were made simultaneously to collect seawaters in order that

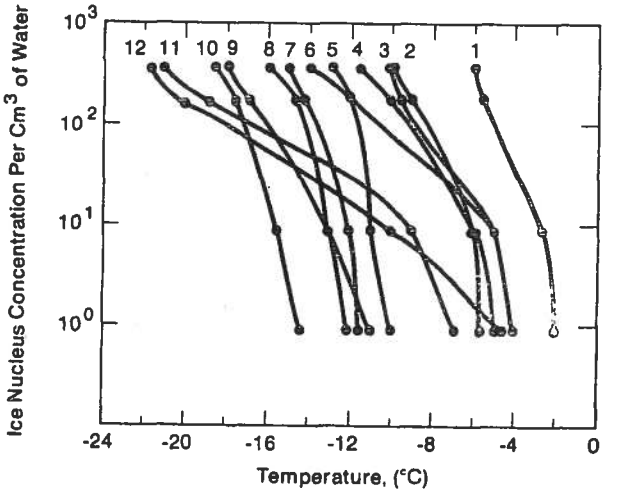

FIG. 2. Freezing nucleus spectra of fog water samples tested onboard the USNS *Hayes*. Collection times for the samples are 1) 0845/8/8, 2) 0600/6/8, 3) 0930/6/8, 4) 0700/6/8, 5) 1800/9/8, 6) 1000/6/8, 7) 2400/2/8, 8) 1000/31/7, 9) 1730/3/8, 10) 1030/31/7, 11) 1900/4/8, 12) 1830/7/8.

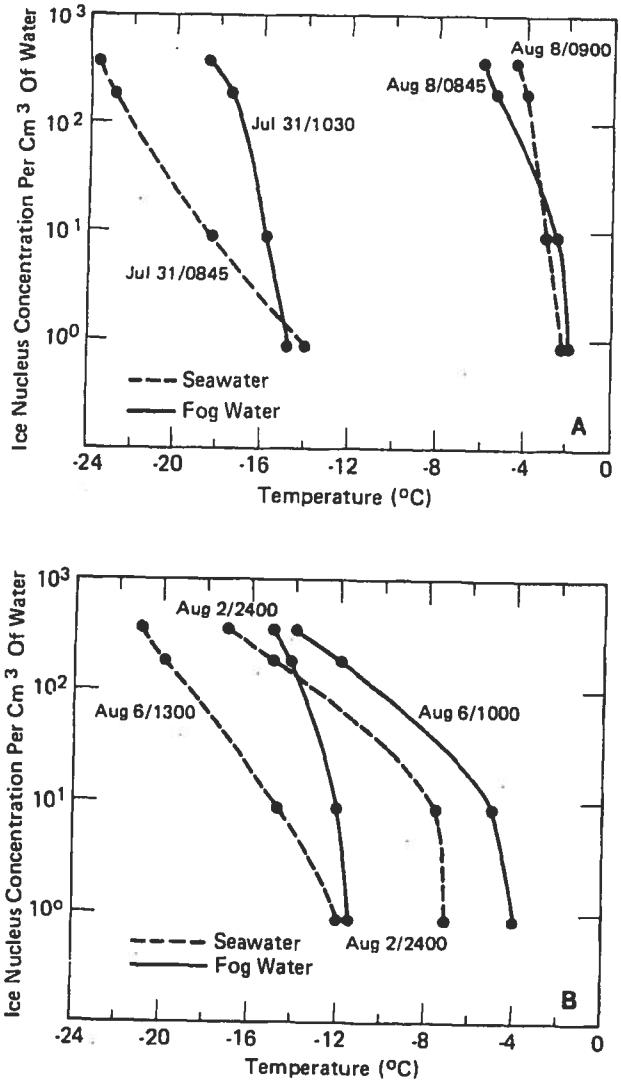

FIG. 3. Relationships between freezing nuclei in seawater and associated fog waters. Graph A shows cases where seawater and fog water spectra are similar and graph B shows cases where spectra are opposites. The seawater spectra shown in this figure have been corrected for a 2°C melting point depression.

comparisons could be made between fog water and seawater IN spectra from similar locations. It was observed that on some occasions IN concentrations in fog water mirrored IN concentrations in seawaters immediately beneath the fogs. As Fig. 3a indicates, this coincidence was observed for both high concentration (8 August) and low concentration (31 July) cases. On the other hand, Fig. 3b shows that there were cases where fog water contained considerably more IN than the associated seawater (6 August) and where fog water contained fewer IN than the associated seawater (2 August). The remaining fog water-seawater spectra pairs exhibited relationships that covered the full range from mirror images to opposites. It should be noted that all spectra for seawaters shown in Fig. 3 have been corrected for a 2°C melting point depression induced by salts inherent in seawater. This correction moves the relevant spectra 2°C to the right on the abscissas of Fig. 3.

d. Ice nucleus activity losses

It is known from previous work that ODN in seawater are subject to activity losses during short periods of storage. The results of tests designed to monitor changes in IN activity of ODN and FDN collected on this cruise are shown in Fig. 4. From this, it can be seen that both ODN and FDN lost activity rapidly when stored at ambient ship temperature (in this particular case, storage was in 10 cm³ syringes). Storage of samples in 100 cm³ and 1000 cm³ volumes under anaerobic and aerobic conditions produced similar results.

e. Ice nucleus activity of fog-derived bacteria

Bacteria were isolated from some fog waters and cultured on board the ship by other members of the *Hayes* scientific crew. These cultures were then removed from the nutrient media upon which they grew by gently washing the agar plates with clean distilled water. The IN activity imparted to the distilled water is shown in Fig. 5, from which it can be seen that IN activity was observed at −1.5°C in water suspensions of both salt-requiring and non-salt-requiring bacteria species. It is interesting to note that low IN activity was also exhibited by other cultures of bacteria with the same salt requirements. Studies on the bacteria are continuing.

f. Atmospheric ice nucleus concentrations

AIN concentrations measured during the cruise are shown in Fig. 6. From Fig. 6 it can be seen that these concentrations varied between a high of 580 m^{-3} (filter 12) to lows of 1.1 m^{-3} (filters 21 and 36).

Fig. 6 also indicates that there is no direct relationship between fogs and the presence or absence of AIN,

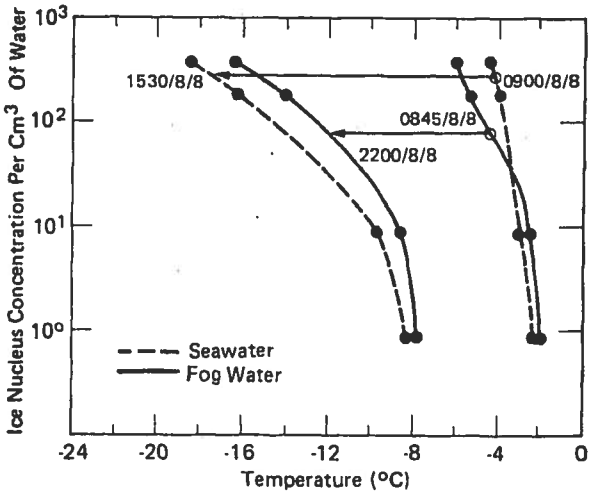

FIG. 4. Freezing nucleus activity losses observed in ODN and FDN samples stored at ambient ship temperature. The seawater samples have been corrected for a 2°C melting point depression.

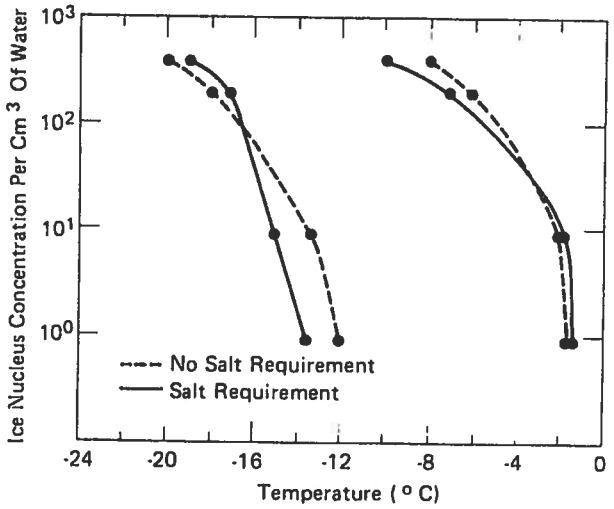

FIG. 5. Freezing nucleus activity produced by bacteria isolated from marine fog water and grown on nutrient agar.

but that most low AIN concentrations (with two notable exceptions, filters 18 and 21) are associated with periods of fog. There also does not appear to be any strong relationship between the presence of IN in seawaters and/or fog waters and atmospheric IN concentrations (cf. data shown in Figs. 1 and 2 with data shown in Fig. 6).

g. Infrared reflectance measurements

Infrared reflectance analyses on seawater and fog water samples were conducted throughout the trip (Baier, 1976). Many of these samples were duplicates of samples tested for IN content. It was interesting to note that samples containing the highest concentrations of IN also contained the highest biological (glycoprotein) content found on the cruise.

Similarly, cloud water collected from the Denver Research Institute cloud chamber, in which the cloud droplets had formed on natural aerosols, exhibited a one to one correlation between high IN concentration and high biological content on one hand (Fig. 7, Curve 1) and between low IN concentrations and low biological content (Fig. 7, Curve 2) on the other (*ibid*). This suggests that some fraction of the IN observed on this cruise may either act as condensation nuclei to form cloud droplets, or be closely associated with the particles that do act as cloud condensation nuclei, and that some of these aerosol particles contain appreciable amounts of biologically derived organic material.

5. Discussion and summary

At the outset of this research project, it was hoped that a clear picture of the source of the AIN observed during the *Hayes* cruise would emerge. This did not come about. Instead, a pattern of IN concentrations in seawater, fog water and air, appearing to vary quite independently of each other, was observed. To confuse the issue, it is known from radon gas measurements conducted during the cruise that the ship was in three continental air masses over the two-week cruise period; AIN, ODN and FDN concentrations did not follow any obvious trend related to radon concentrations.

Since the ship was in constant motion relative to the atmosphere and sea, which in turn were in constant motion relative to each other, the possibility of unambiguously pinpointing a single IN source is remote. Attempts were made to relate AIN and FDN concentrations to ODN source regions measured in the sea taking into account air mass trajectories and ship positions.

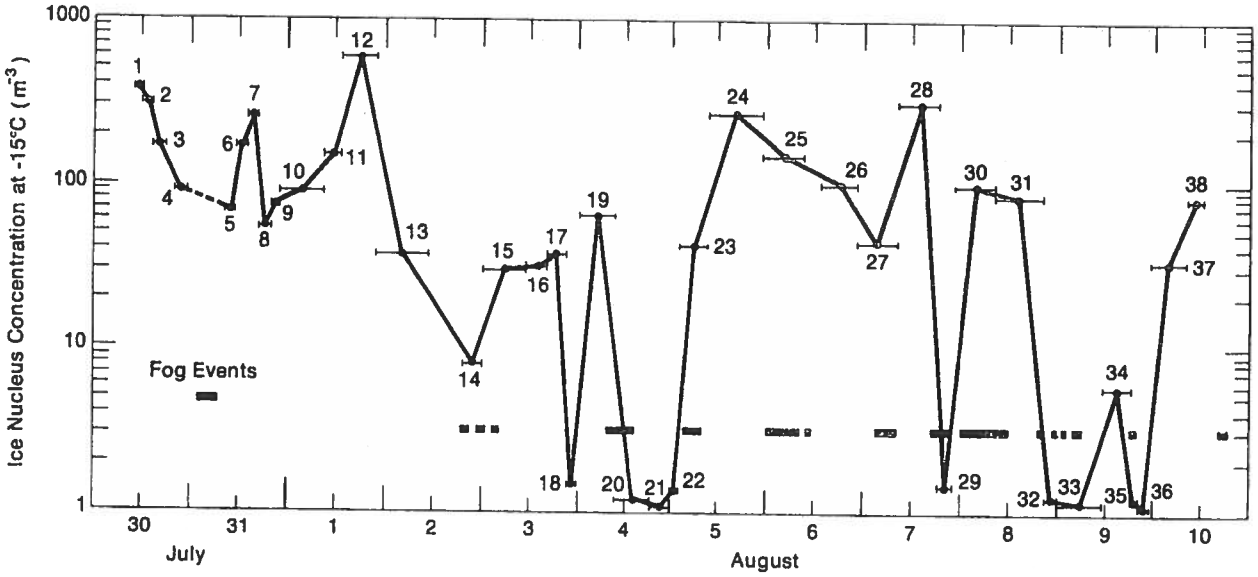

FIG. 6. Atmospheric ice nucleus concentrations over the period 30 July–10 August as measured by the membrane filter technique. The IN concentrations are plotted at the middle of the time period over which the filters were exposed. All filters were processed at −15°C and water saturation.

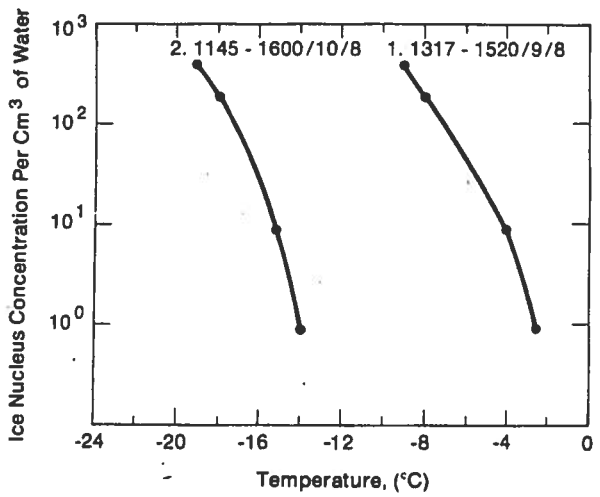

FIG. 7. Freezing nucleus spectra of cloud water collected from the Denver Research Institute cloud chamber operated onboard the USNS *Hayes*. Sample 1 contained high concentrations of biological material, whereas sample 2 contained low concentrations as tested by the Calspan infrared reflectance system (Baier, 1976).

The results were discouraging other than for the relationships shown in Fig. 3a.

Thus until more data on the other atmospheric and oceanic parameters measured on the cruise become available for comparison and study, it is possible only to state in general terms that the ice nucleus measurements taken onboard the *Hayes* appear to be sound and reproducible, that both land and sea could have contributed to the IN concentrations observed, and that many if not most of the IN observed may have been of biological origin or contain biological material, living and dead.

Specific results are as follows:

1) About 17% of the seawater samples tested (5 out of 29) contained freezing nuclei active at temperatures warmer than −10°C, including some nuclei active at −4°C.

2) About 58% of the fog water samples tested (7 out of 12) contained freezing nuclei active at temperatures warmer than −10°C, including some nuclei active at −2°C.

3) In some cases high freezing nulceus concentrations in seawater were mirrored by high freezing nucleus concentrations in associated fog waters, and in some cases they were not. Also, there were cases where fog waters contained high concentrations of ice nuclei, and associated seawaters were devoid of any active freezing nuclei.

4) Storage of active ODN and FDN at ambient room temperature, under both aerobic and anaerobic conditions and in volumes from 10 to 1000 cm^3, induced rapid losses of freezing nucleus activity in the samples.

5) Measurements of AIN exhibited a wide range of concentrations from 580 down to 1.1 nuclei m^{-3} with considerable variation over the space of a few hours. With one exception, the lowest atmospheric ice nucleus concentrations were observed for measurements within or predominantly within fogs. No firm correlation was noted between the presence of freezing nuclei in seawaters and fog waters on one hand, and atmospheric ice nucleus concentrations on the other.

6) Some bacteria isolated from fog waters were observed to initiate ice at temperatures warmer than −2°C. Other bacteria, similarly isolated, were observed to be very poor ice nucleators.

7) All the seawater, fog water and cloud chamber water samples exhibiting high freezing nucleus concentrations also exhibited high concentrations of biological material.

Acknowledgments. This research was supported by funds from the Advanced Study Program of the National Center for Atmospheric Research (supported by the National Science Foundation), and from the National Academy of Sciences and the National Research Council under the Research Associate Program. Ship time was provided by the Naval Research Laboratory through the courtesy of Dr. Lothar Runke. A special thanks to the scientific staff and crew of the USNS *Hayes* who made this research possible, and to Dr. Jayne F. Carney and Chris E. Carty for providing bacteria isolates.

REFERENCES

Baier, R. E., 1976: Surface-active and infrared-detectable matter in North Atlantic aerosols, sea fogs, and sea-surface films. Tech. Rep. VA5788-M, Calspan Corporation, Buffalo, N. Y. 56 pp.

Bigg, E. K., S. C. Mossop, R. T. Mead and N. S. C. Thorndike, 1963: The measurement of ice nucleus concentration by means of Millipore filters. *J. Appl. Meteor.*, **2**, 266–269.

Blanchard, D. C., 1964: Sea-to-air transport of surface active material. *Science*, **146**, 396–397.

——, 1970: Mechanisms for the water-to-air transfer and concentrations of bacteria. *Science*, **170**, 626–628.

Bowen, E. G., 1956: The relation between rainfall and meteor showers. *J. Meteor.*, **13**, 142–151.

Gagin, A., and M. Aroyo, 1969: A thermal diffusion chamber for the measurement of ice nuclei concentrations. *J. Rech. Atmos.*, **4**, 115–122.

Huffman, P. J., and G. Vali, 1973: The effects of vapor depletion on ice nucleus measurements with membrane filters. *J. Appl. Meteor.*, **12**, 1018–1024.

Isono, K., M. Komobayasi and A. Ono, 1959: The nature and the origin of ice nuclei in the atmosphere. *J. Meteor. Soc. Japan*, **27**, 211–233.

Kumai, M., 1961: Snow crystals and the identification of the nuclei in the northern United States of America. *J. Meteor.*, **18**, 139–150.

Langer, G., and J. Rodgers, 1975: An experimental study of the detection of ice nuclei on membrane filters and other substrata. *J. Appl. Meteor.*, **14**, 560–570.

Lindow, S., R. W. Barchet and C. D. Upper, 1975: The relationship between populations of bacteria active in ice nucleation and frost sensitivity in herbaceous plants. *Trans. Amer. Geophys. Union*, **56**, 994.

Maki, L. R., E. L. Galyan, M. C. Chien and D. C. Caldwell, 1974:

Ice nucleation induced by *Pseudomonas syringae*. *Appl. Microbiol.*, **28**, 456–459.

Mossop, S. C., and N. S. C. Thorndike, 1966: The use of membrane filters in measurements of ice nucleus concentration. 1. Effect of sampled air volume. *J. Appl. Meteor.*, **5**, 474–480.

Schnell, R. C., 1975: Ice nuclei produced by laboratory cultured marine phytoplankton. *Geophys. Res. Lett.*, **2**, 500–502.

——, and G. Vali, 1972: Atmospheric ice nuclei from decomposing vegetation. *Nature*, **236**, 163–165.

——, and ——, 1975: Freezing nuclei in marine waters. *Tellus*, **27**, 321–323.

——, and ——, 1976: Biogenic ice nuclei: Part 1, Terrestrial and marine sources. *J. Atmos. Sci.*, **33**, 1554–1564.

Vali, G., 1971a: Quantitative evaluation of experimental results on the heterogeneous freezing nucleation of supercooled liquids. *J. Atmos. Sci.*, **28**, 402–409.

——, 1971b: Supercooling of water and nucleation of ice (drop freezer). *Amer. J. Phys.*, **89**, 1125–1128.

——, M. Christensen, R. W. Fresh, E. L. Galyan, L. R. Maki and R. C. Schnell, 1976: Biogenic ice nuclei. Part II: Bacterial sources. *J. Atmos. Sci.*, **33**, 1565–1570.

Biogenic and Inorganic Sources for Ice Nuclei in the Drought-Stricken Areas of the Sahel - 1974

Interim Report to the Directors,
Rockefeller Foundation
New York City, New York

December, 1974

Field Study Period
August 8 September 20, 1974

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Advanced Study Program
National Center for Atmospheric Research*
Boulder, Colorado 80303

* The National Center for Atmospheric Research is sponsored by the National Science Foundation.

ABSTRACT

Recent research has shown that organic decay products of tree and grass litters (i.e., biogenic nuclei) may act as atmospheric ice nuclei at relatively warm temperatures (-2 to -10C). Theory has suggested a sensitive relationship between the availability of such ice-forming nuclei and the amount of precipitation.

A hypothesis is advanced that massive overgrazing in the sub-Saharan (Sahel) region of Africa which preceded the recent drought, resulted in depletion of the sources of these biogenic nuclei and a subsequent reduction in total precipitation. The hypothesis was tested by sampling the availability of surface-derived ice nuclei in the Sahel.

The results show that where organic plant decay products were available, ice forming nuclei active at -7C were present in concentrations to 10^3 per gram of litter and nuclei active at -10C were present to 10^4 per gram. Soils relatively free of such organic decay products were found to contain no ice forming nuclei active at temperatures warmer than -14C and only 10^3 per gram of material active at -18C.

It is suggested that poor agricultural management contributed to the persistence and severity of the drought, and a feedback mechanism between the vegetation and local precipitation is proposed.

INTRODUCTION

Mankind has been affected by drought from time immemorial. The most recent drought in sub-Saharan Africa, in the area known as the Sahel, has been the subject of countless articles in both the popular (see Sterling, 1974) and the scientific (Bryson, 1973; Macleod, 1974; Wade, 1974; among others) press, and has inspired a flurry of hypotheses and research designed to explain both its occurrance and its persistence. Some investigators have concluded that droughts are normal in the earth's climatological history (Winstanley,1973; Lamb, 1974; Landsburg, 1974) and controlled by immeasurable and unpredictable forces. Others (AID, 1972; Bryson, 1973; Macleod, 1974; Otterman, 1974) suggest that man, by his increasing use of natural resources, induces or aggravates the conditions leading to drought. A new theory which explains drought in terms of the availability of ice nuclei from which precipitation is formed is presented below. Evidence to support this theory was obtained in the Sahel during the summer of 1974.

The basis for this theory is discussed in detail in the report (ARIII) which accompanies this paper. Briefly, it states that evidence has recently been presented to show that submicron organic particles (i.e., biogenic nuclei) produced during the decay of vegetable matter may be important in providing nuclei in clouds upon which ice may form and from which precipitation may develop. This evidence is supported by recent work showing that biogenic nuclei at the earth's surface may be released into the atmosphere in appreciable amounts (Allee, 1974; Braham and Speyers-Duran, 1974). Evidence is also given in the report to show

that the availability of ice nuclei from vegetation is closely correlated with concentrations of airborne ice nuclei and ice nuclei measured in precipitation.

Until recently it had been assumed that clouds in tropical regions were too warm to form precipitation other than by the coalescence process in which small cloud droplets merge and grow to form raindrops. More recent investigations by Trewartha (1966), Portig (1963), Griffiths (1972), Booker (1973), and Bollay (1974) suggest the possibility that much of the precipitation in sub-Saharan Africa may, in fact, involve the ice process.

If precipitation is indeed formed through the ice process in tropical continental cumulus clouds, it appears that there is a possible series of physical reasons which may explain, at least in part, the long-term reduction in rainfall in the region and the ensuing drought. The Sahel was overgrazed (AID, 1972; Sterling, 1974) and much of the vegetation which may have been the source of local ice nuclei was removed, thus potentially affecting the probability and efficiency of local precipitation. This reduced rainfall leads to less vegetation production and, therefore, an accelerated reduction of ice nuclei available from such vegetation. When the numbers of grazing animals have been sufficiently reduced by death or removal, that vegetation which remains will then continue to decay and to produce increasing numbers of ice nuclei and, eventually, increased rainfall as well as more vegetation. This cycle may repeat itself if vegetation again becomes sufficient to support massive grazing by steadily increasing numbers of cattle.

The area affected by the drought of 1963-1974 is shown in Fig. 1 as a grey band spreading from east to west across Africa. The area is roughly coincident with the geographical entity known as the "Sahel," from the Arabic word for border. This is a 200- to 400-mile-wide sweep of grassland and shrubs which separates the Sahara desert to the north from the semitropical forests lying to the south.

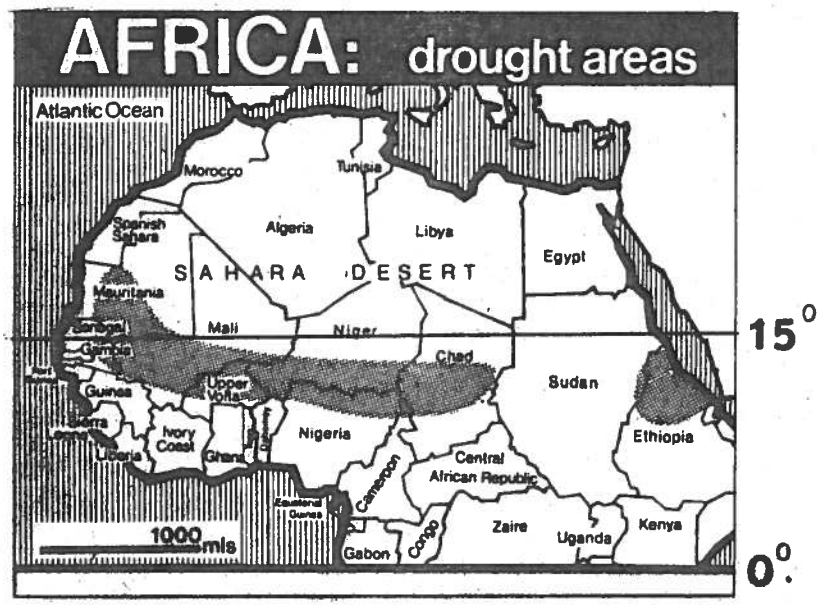

Figure 1. Approximate Sahel drought areas as of February, 1974 (shaded areas). The country of Sudan is generally believed to have also suffered a drought but due to sparse population in the hinterland of Sudan, information confirming the severity of the drought is not readily available (Canadian Press News Service, April, 1974).

Most of the data cited below are based on materials collected in Niger, chosen for its central location, accessibility, and because most of its usually omnipresent cattle herds had either starved or been moved to better grazing during the past five years. The absence of both cattle and their attendant human population in an area usually teeming with both, permitted the vegetation to grow undisturbed during 1972 and 1973. Nearly normal precipitation in the rainy season of 1974 had also produced fresh vegetation, "the most lush in living memory."

Figure 2 gives the rainfall averages for Niger over the pre-drought period, 1931-1960. There is an evident diminishing of the rainfall from south to north. The area between the 900 and 600 mm per year isohyets (lines of equal rainfall) is characterized by semitropical forest in the south which changes to savanna as one goes north. The savanna gives way to vast areas of grasses and shrubs in the region between the 650 and 300 mm per year isohyets and to shorter grass and fewer shrubs as one continues north from the 300 to the 100 mm per year isohyet. The most severe aspects of the drought occurred north of the region whose southern boundary approximates the 800 mm per year isohyet.

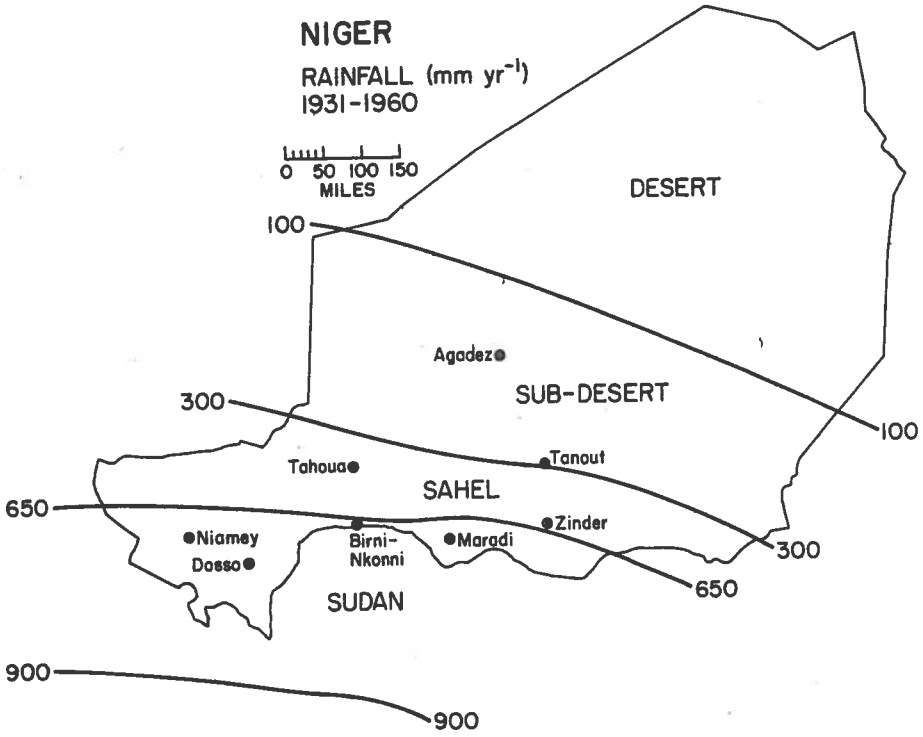

Figure 2. Pre-drought average isohyets and vegetation zones for Niger, Africa (WMO Records).

Rainfall averages for representative locations in Niger are given in Fig. 3 for 1953-1973. This figure shows that the rainfall did not cease abruptly but generally declined during these twenty years. In 1974, the rainfall totals (not shown in Fig. 3) generally returned to the 1956 levels.

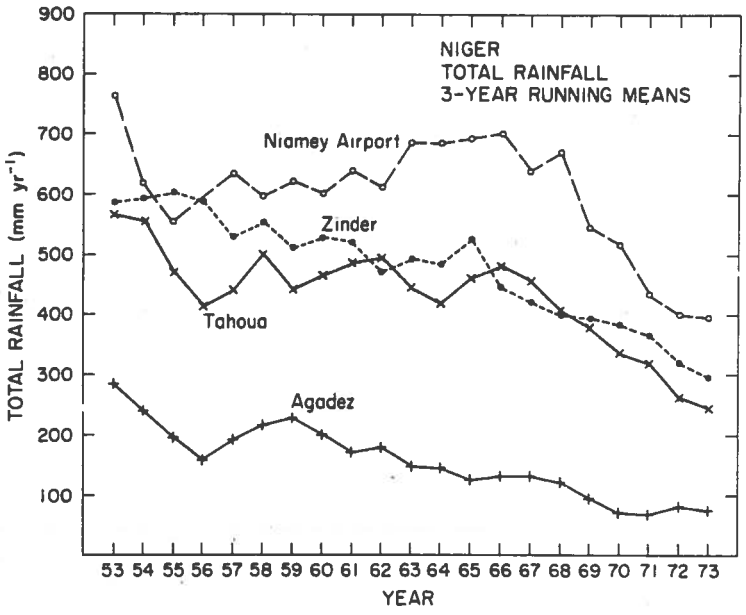

Figure 3. Three-year running means of total yearly rainfall for selected stations in Niger, Africa (WMO Records).

TRAVEL AND OBSERVATIONS

Tri-weekly air service connects the capital of Niger, Niamey, with Europe. Five of Niger's largest cities are served on a weekly basis, weather and equipment permitting. Ground transportation varies from large, four-wheel-drive trucks and high-wheel-based buses to Land Rovers and light European cars. During the rainy season all of these vehicles are restricted to a few main roads. Shorter journeys of 10 to 15 miles are usually conducted on foot.

Accommodations and food in Niger varies from the best available in local French hotels to sleeping in the open and eating local food which is risky on occasion.

As knowledge of the drought and its effect upon the people of Niger reached the rest of the world, people and governments responded with massive aid. Evidence of this aid is everywhere apparent. The nomad population is tented and fed in central camps. People from permanent settlements collect aid allotments from depots scattered throughout the country. One often saw pack camels carrying grain from these depots, miles from any distribution center.

SAMPLING AND TESTING

Sampling consisted of collecting materials from living plants, trees, and grasses, and litter from the bases of the same vegetation. Samples were also taken from surrounding soils within a three-foot radius. All of the samples were placed in sterile plastic bags and stored either open, which duplicated the natural decay process, or tightly sealed, in an anaerobic environment which retarded decomposition.

One hundred and six samples were collected on zig-zag traverses which passed through all of the towns shown on Fig. 2 as well as many places in the area bounded by a line joining Birni N'Konni, Zinder, and Agadez. These samples will be compared with others collected in the non-drought areas of Dahomey, Nigeria, and Kenya.

Freezing nuclei in these samples were determined at the University of Wyoming using a Vali-Knowlton nucleus spectrometer and the standard procedure outlined in ARIII. Seventy-six samples have been examined and the remainder will be processed in early 1975.

RESULTS

In general, it was observed that fresh litters contained more active freezing nuclei than old litters, which are the decayed matter from previous seasons. The old litters in turn contained more active nuclei than did the surface soils. A typical set of freezing nucleus spectra from one site (site #1, Niamey) is given in Fig. 4. As is shown, the recently decayed leaf matter from the 1974 growing season contained some nuclei active at -8C and 10^3 - 10^4 nuclei per gram of litter active from -10 to -11C. Bare surface soil at this site, less than two feet away from the growing vegetation, contained no nuclei active at temperatures warmer than -14C and 10^3 nuclei per gram of soil active at -16C.

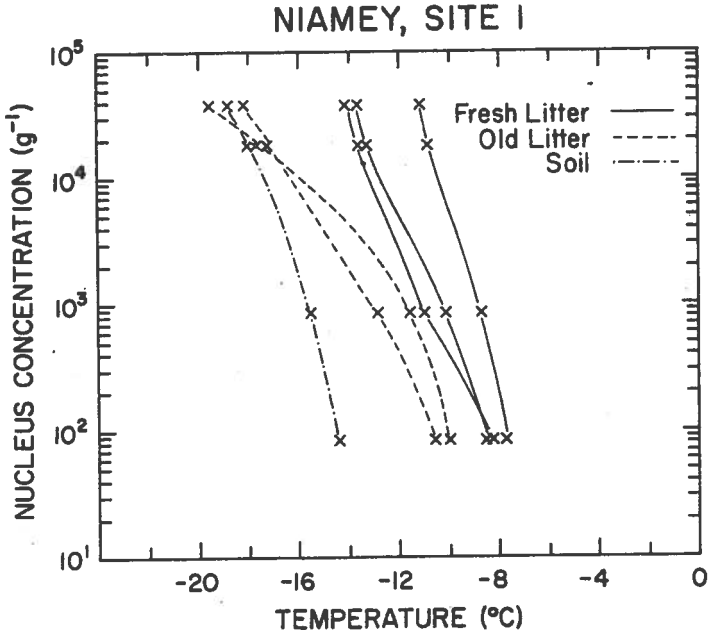

Figure 4. Freezing nucleus spectra for samples collected near Niamey, Niger, during the summer of 1974.

Site #4 at Birni N'Konni is a moist, oasis-like area and the spectra from this site are shown in Fig. 5. Here the recently decayed vegetation contained many more active nuclei than were present in the surface soils. No old litter was available at this site.

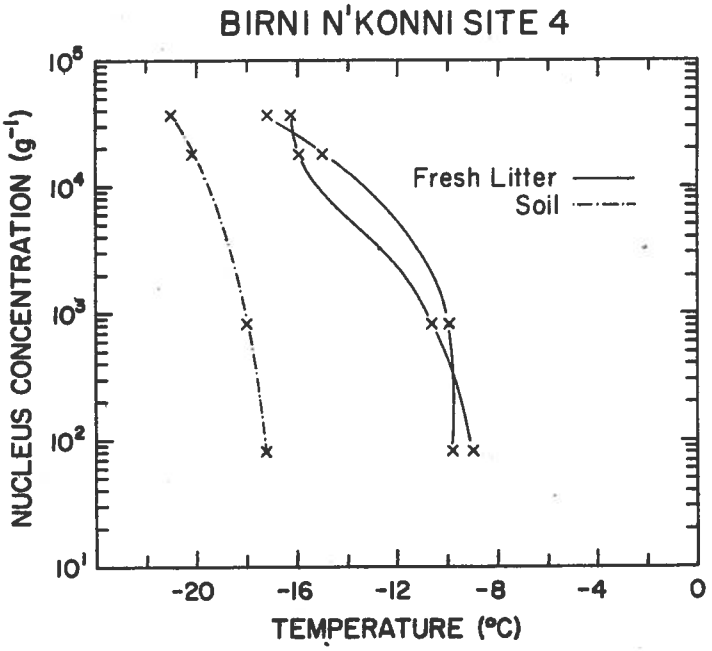

Figure 5. Freezing nucleus spectra for samples collected near Birni N'Konni, Niger, during the summer of 1974.

Agadez South, site #3, was the sample location closest to the barren Sahara and may be somewhat representative of the nucleating properties of inorganic Sahara dusts which have been suggested as prime contributors to atmospheric ice nucleus budgets (Mason, 1971). The spectra from this site are given in Fig. 6 and recently decayed vegetation and old litter again contained many more active nuclei than did the adjacent soils.

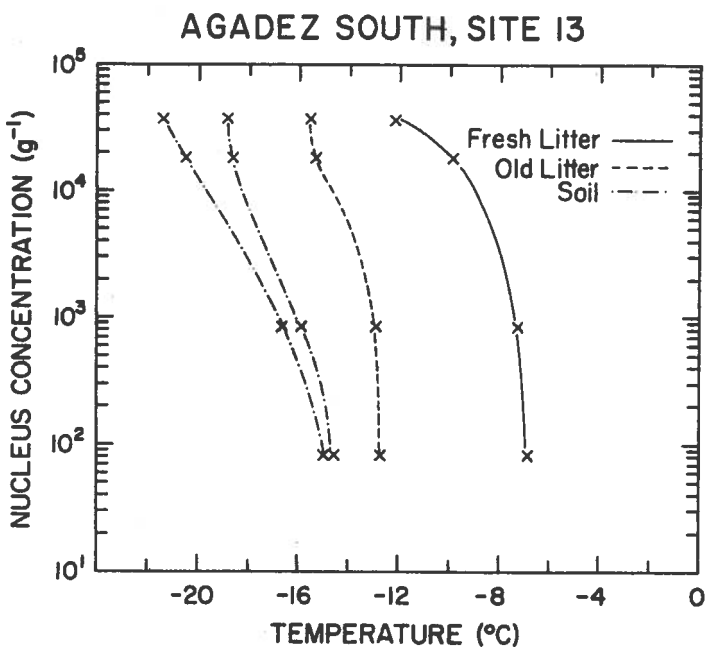

Figure 6. Freezing nucleus spectra for samples collected near Agadez, Niger, during the summer of 1974.

Figure 7 gives the spectra obtained from samples collected at successively greater distances from a clump of grass growing near Zinder, site #10. The most active nuclei, shown in the spectrum furthest to the right, are for decaying leaves from the 1974 growing season. The next spectrum is from blades which had withered on the stalk and begun to decay; the next from a mixture of old litters immediately adjacent to the clump; and the last from a mixture of soil and litter which was collected from an eroded and sparsely vegetated slope above the clump. Again, those samples containing the largest amounts of organic materials also contained the most active freezing nuclei.

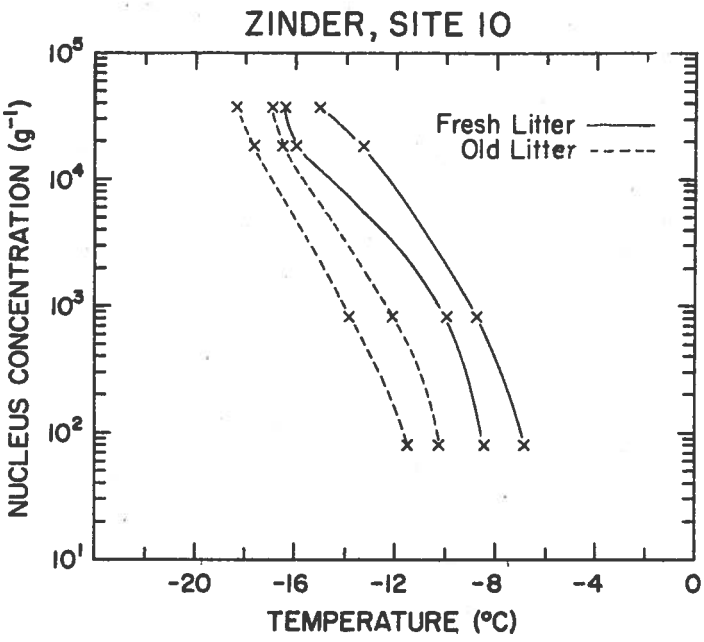

Figure 7. Freezing nucleus spectra for samples collected near Niger, during the summer of 1974. The curve furthest to the left was a mixture of old litter and surface soil; because of its high organic content it has been classed as old litter for purposes of illustration.

The range of spectra and the number of samples in each nucleant group are shown in Fig. 8. All three groups overlap at the colder temperatures but at warmer temperatures the most active freezing nuclei are associated with recently decayed leaves followed by old litters. Samples classified as containing predominantly inorganic materials consistently exhibited less effective freezing nuclei than those samples containing appreciable amounts of organic material. It should be noted here that while the amount of visible organic material varied with the distance from decaying vegetation, no accurate measurement of the amount of organic material in the samples has been made.

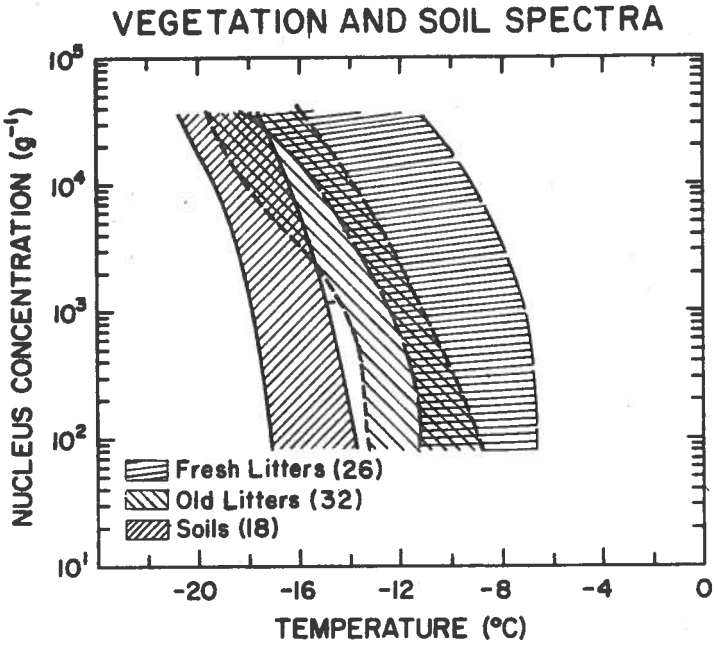

Figure 8. Range of nucleus spectra for samples collected in Niger, Africa, during the summer of 1974. The bracketed numbers indicate the number of samples in the respective categories.

Although these data show that there are more active freezing nuclei in decayed vegetation than adjacent soils, no measurements were made to determine whether such nuclei were in fact being lifted into the Sahelian atmosphere. Surface material was observed being drawn into the bases of cumulus clouds on several occasions and these observations are supported by Macleod who reported in 1974 that in many Sahelian cumulus clouds, "the undersides of the clouds were a brick-red color... (This) color is just that of the fine dusts of the Sahel collected on white cloth."

The organic content of the dusts has not been measured in this case either, but Delaney et al. (1967) measured the organic content of

Sahelian dusts as far downwind of Africa as the Barbados in the Western Atlantic. Many of their measurements showed the organic content of the dusts to be in excess of the inorganic content and the arrival time and amount of organic content of the dusts correlated with both the growth-decay cycle and the position of the Inter-Tropical Convergence Zone in the Sahel.

Delaney and Zenchelsky (1974) reported flux measurements of wind-ablated dust rising from fields in northwestern Texas. They found that samples of aerosols taken 3.5 meters above the surface contained a far greater percentage of organic material than did samples taken from the surface immediately below. These observations confirm earlier work by Junge (1963) in which it was suggested that organic plant debris might be lifted from the surface in preference to denser, inorganic soil particles and that the denser, inorganic particles might fall out faster because of their greater fall velocities. It is, therefore, conceivable that some dust clouds could consist primarily of organic materials.

DISCUSSION

The research results reported here suggest the possibility that poor agricultural practices in the Sahel, which led to the removal of vegetation in that area, might also have removed the local sources of warmer range ice nuclei which, in turn, may have adversely affected the formation of precipitation in the cumulus clouds that form above these denuded areas. The reduced rainfall would support scant vegetation and thus accelerate animal starvation. In the absence of large numbers of

grazing animals, the vegetation would recover and again produce litter with its attendant organic nuclei, allowing rains to increase in subsequent seasons.

At this point in the study, the above chain of events should be taken as speculation, not fact. Over the next six months a study of rainfall frequency distributions and daily rainfall occurrence prior to and during the drought will be undertaken. From these data coupled with open literature data on condensation and ice nucleus concentrations related to physical processes within Sahelian cumulus clouds, a clear picture of the role that biogenic ice nuclei may have played in the current drought should emerge.

Any conclusions will have to be related to investigations being undertaken by other workers who are studying the relationship between the drought and the changes in surface albedo produced by overgrazing, by the abnormal (and as yet unexplained) shifts in the intertropical convergence zone, and by the effect of dust in the Sahelian atmosphere.

FURTHER RESEARCH AND ACTIVITIES

The work reported above is to be extended as follows:

A. Completion of testing samples for their freezing nucleus content.

B. Sizing of the active freezing nuclei.

C. Testing soils and litters in the Colorado State University Cloud Simulation Chamber for their ice nucleating activity in supercooled clouds.

D. Conducting chemical tests on Sahel litters to determine some of the chemical characteristics of their ice nucleants.

E. Publication of results of the above research.

F. Preparation of a final report by July 1975.

7.6

Reprinted from Preprint Volume: Seventh Conference on Inadvertent and Planned Weather Modification, October 8-12, 1979, Banff, Alberta, Canada. Published by the American Meteorological Society, Boston, Mass.

A NEW TECHNIQUE FOR MEASURING ATMOSPHERIC ICE NUCLEI ACTIVE AT TEMPERATURES FROM -20°C TO APPROACHING 0°C, WITH RESULTS

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I. INTRODUCTION

Atmospheric ice nuclei (IN) are thought to be scarce and are rarely observed (using present measurement methods) to be active at temperatures warmer than -10°C. Yet, in the atmosphere, ice crystals may be observed in clouds whose temperatures are not colder than -5°C to -8°C. At colder temperatures (say, -16°C) observed ice crystal concentrations are, on many occasions, an order of magnitude (or more) greater than measured IN concentrations (Pruppacher and Klett, 1978). In this paper, I describe a simple technique for measuring natural atmospheric IN which appears to be capable of detecting IN active at temperatures as warm as -2°C in concentrations of up to 1 ℓ^{-1}, active at -8°C.

II. METHODS

Air is drawn through Sartorius hydrophobic membrane filters (0.45 or 0.2 μm pore diameter) which capture IN along with other aerosols (sample volumes of 100 ℓ to 300 ℓ). These filters are quartered and mounted on IN-free aluminum foil which has been bonded onto a copper disc having high thermal conductivity. The foil is made IN-free by covering its surface with silicone varnish. Equal-volume IN-deficient distilled water drops are placed on the filters with a medical pipette. The hydrophobic nature of the filters causes the drops to form hemispheres covering equal areas of the filter; for a 35-mm-diameter filter surface, I find 30 drops of 2.5-mm diameter to be a good amount. The disk supporting the filter is cooled at a steady rate of from 1°C to 0.5°C per minute, using a thermo-electric cooler. The plate temperature is monitored with thermometers. Each water drop on the filter freezes at the threshold temperature of the most active nuclei that the drop covers or is in contact with. It is assumed that immersion freezing nuclei are detected with this technique and that the statistics on the freezing of the supercooled water drops are the same as those developed by Vali (1971). In this study, the maximun difference in temperature at which the first drops froze on sets of six filters exposed to the same volume of air varied from 0 to 1.5°C, with a standard deviation of 0.7°C. Control blanks were processed with each exposed filter set. On occasion, ice nucleants with known threshold activity were aerosolized and deposited on the filters to check the technique and to monitor the repeatability of tests.

The concentrations of IN in the atmosphere are calculated by relating the temperature at which the drops freeze to the ratio of the area of the filter covered by the drops to the area of the whole exposed filter, and relating these values to the volume of air sampled. Filters (generally duplicates or triplicates) have been exposed at 20 m AGL using standard Millipore filter holders, and in isokinetic probes mounted on a light aircraft flown at altitudes from 100 m to 4500 m AGL. It has been noted that when there are high concentrations of IN on the filters some nuclei are "masked" by their more active cousins. This problem may be overcome by reducing the sample volume.

In an adaptation of this method, filters were pre-cooled in a Bigg-Warner isothermal cloud chamber, and water droplets were allowed to drop through 1/3 meter of air (at the temperature of the filters) onto the filter surfaces. It is assumed that this method, which produced higher IN concentration than reported in this paper, measured contact or a combination of contact and immersion freezing nucleation. Results from these experiments will appear in a later paper.

III. RESULTS

IN spectra from filters collected near Erie, Colorado, are shown in Fig. 1 where it may be

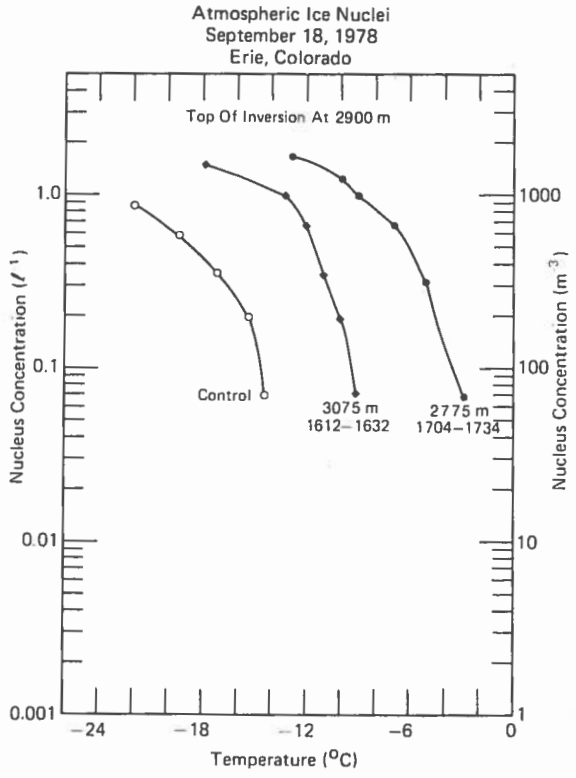

Figure 1. Atmospheric IN concentrations measured above and below a temperature inversion on September 18, 1978, over northeastern Colorado.

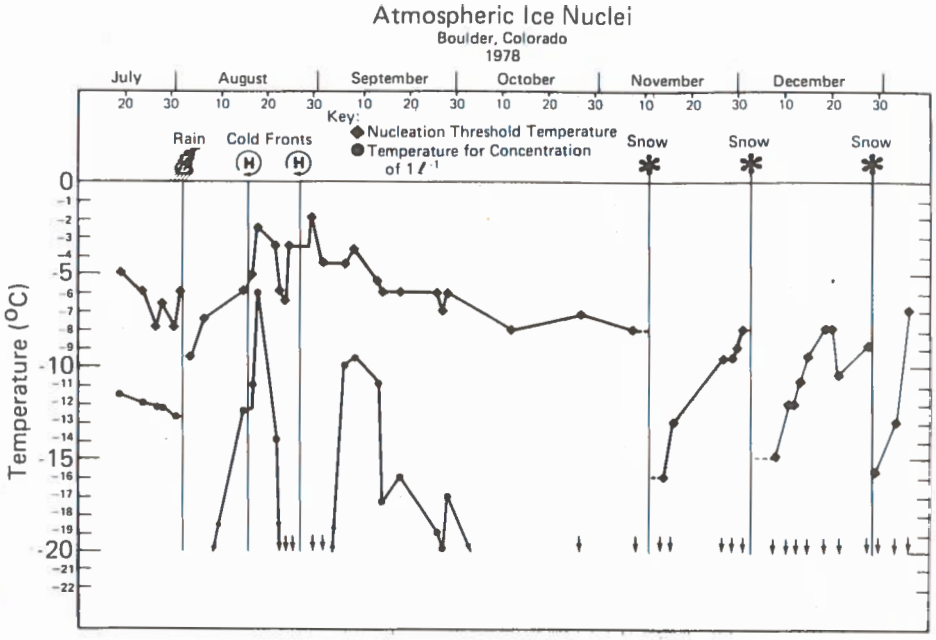

Figure 2. Seasonal variations in the threshold activity of IN measured at 20 m in Boulder, Colorado (1978), and the temperature at which IN concentrations were 1 ℓ^{-1}. The increase in IN activity in August, related to successive days of stable air, is of interest as is the decrease in IN concentration associated with large-scale changes in air masses in winter.

observed that aerosols trapped below a temperature inversion contained considerably more IN than air 300 m higher, above the inversion. It is interesting to note that on this day threshold IN activity was observed at -3°C with concentrations of 1 nucleus per liter, active at -8°C. More recent measurements under similar situations suggest IN concentrations of 10 per liter, active at -14°C to -16°C, may not be unrealistic.

The results from a seasonal inventory of IN measured at 20 m AGL in Boulder are shown in Fig. 2 where it may be observed that IN with the warmest threshold temperatures and highest total concentration were associated with stable synoptic situations best described as "Indian Summer" days which were typified by light easterly winds and reduced visibility due to aerosols trapped in the shallow boundary layer. A marked decrease in IN activity was observed in the air masses behind snow-producing cold fronts; often this change occurred over the space of 3 or 4 hours. This same pattern has been observed to occur in air mass changes in June in Boulder.

Measurements of IN in the "Denver Brown Cloud" on December 7 and 8, days of record breaking smog and complete snow cover, indicated there were few IN on the filters. (Exposed filters were visibly grey.) Filters exposed in cleaner air upwind of the city exhibited the same activity as those collected in Denver on the same day. The IN spectra for November 27 and 30 and December 1, days with smog but no snow cover, exhibited considerably more IN. Again, upwind and city-center filters exhibited the same spectra.

In other field tests, IN measurements in the environments of large coal-fired power plants have been conducted in 3 western and 1 eastern state on 8 occasions covering all seasons of the year. From the data collected it was observed that no power plant plume had any real effect on upwind IN concentrations as measured by this technique. Measurements in the Los Angeles Basin suggest that natural production of atmospheric IN far outweighs production of IN produced by petroleum refineries and the Los Angeles smog. Similar results were obtained in central Utah and southern Montana.

IV. DISCUSSION

The technique described appears effective for measuring atmospheric IN having relatively low threshold activity and therefore raising the sensitivity of atmospheric IN measurements. If the results are representative of processes in the atmosphere (nuclei on/in filters and large supercooled drops may not be), this would suggest that the atmosphere contains more IN potentially active at lower threshold activity than previously observed. Also, on the basis of the above data, there is a strong suggestion that these nuclei are of natural, local surface origin, and that particular air masses may have distinctive IN populations which can be measured and described in synoptic-scale terms.

Acknowledgments

The impetus for this research came from Dr. H. K. Weickmann, Director, NOAA/APCL with support from Dr. R. F. Pueschel, NOAA/APCL. Funds were provided by the National Research Council Research Associate Program and, in part, by EPA through Interagency Agreement 78 REG/EHE-625/D5-E693.

References

Pruppacher, H. R. and J. D. Klett, 1978. Microphysics of Clouds and Precipitation. Reidel, 714 pp.

Vali, G. 1971, Quantitative evaluation of experimental results on the heterogeneous freezing nucleation of supercooled liquids. J. Atmos. Sci., 28, 402-409.

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Reprinted from Preprint Volume: Fourteenth Conference on Agriculture & Forest Meteorology and Fourth Conference on Biometeorology, April 2-6, 1979, Minneapolis, Minn. Published by the American Meteorological Society, Boston, Mass.

SOME SIZE AND COMPOSITION CHARACTERISTICS OF AEROSOLS AT MOUNT KENYA, EAST AFRICA

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1. INTRODUCTION AND METHODS

As a component of a larger project, studies of the concentration, size, and composition of aerosols captured at the 4000 m MSL level on Mount Kenya were undertaken during January of 1978. The aerosols were captured on Nuclepore membrane filters (0.1 μm pore diameter) by drawing air through the filters with a battery powered air pump. Each filter was exposed for 1.5 hours for a total sample volume of 800 liters of air. Five filters were exposed between the hours of 1300 and 1600 LST (maximum convective turbulence) and five between the hours of 0100 and 0400 LST (stable, cooling, descending air). The particles captured on the filters were analyzed within six weeks of collection for chemical composition, and sized and counted using the facilities of the Atmospheric Physics and Chemistry Laboratory of the National Oceanic and Atmospheric Administration, Boulder, Colorado (NOAA/APCL).

The particles were first coated with a thin layer of gold (Au) to stabilize them for photography, then studied with a scanning electron microscope (SEM) having a lower size detection limit of 0.02 μm. Particles of particular interest were then analyzed for elemental composition using an energy dispersive X-ray analyzer (XEDA) built into the SEM. When the electron beam of the SEM scans over a particle, every elemental atom with an atomic number greater than 10 (i.e. Neon) in the particle will emit a characteristic X-ray energy pattern. One can also measure, semi-quantitatively, the contribution of an element to the particle based upon the intensity of its X-ray emission (Parungo et al., 1975). Purely organic particles containing the elements carbon (C), oxygen (O), and hydrogen (H), all with atomic numbers less than 10, do not emit any X-ray pattern.

Other portions of the same filters were studied with a transmission electron microscope (TEM) with a resolution of 20 angstroms. Here the particles were sized and counted over representative areas of the filter to produce particle size and number distributions for each exposure period. On each filter, between 801 and 1652 separate particle diameters were determined.

2. RESULTS

Representative photographs and XEDA analyses of a number of larger particles are shown in Figs. 1 through 4. In Fig. 1 may be seen two large organic particles, one obviously a plant pollen grain or related structure and the other probably also of plant origin, although its structure is undeterminable. Attached to the pollen grain are particles of soil origin which contain aluminum, silicone, and potassium. An enlarged view of the analyzed soil particle appears behind the XEDA spectra of the particle.

In Fig. 2 may be observed another natural organic particle supporting much smaller soil particles of different composition. Recall that the gold (Au) was used to shadow the particles and, therefore, is not found naturally in the particles.

Two rather interesting fibrous organic structures may be observed in Fig. 3 along with a clump of 4 soil particles. The fiber resembling a hair pin is probably of plant origin whereas the wispy brush-like fiber may be of insect origin.

Of particular interest is the particle shown in Fig. 4, which is a dual salt composed of sodium chloride (NaCl) in the center and calcium sulfate ($CaSo_4$) in the wings (McCrone and Delly, 1968). Its probable origin is in the Indian Ocean some 350 km east of Mount Kenya. Taken together, these photos show that the air passing Timau Hill on Mount Kenya contains a variety of large and small particles of both organic and inorganic composition, and that large organic particles (probably of local origin) are present even during nighttime when one might expect their absence.

The above study in itself does not provide definitive answers on the overall nature of the aerosol since the larger and more dramatic particles were selected for illustration. A more rigorous study of the captured aerosol was undertaken by counting and sizing all particles on random areas of the filters to obtain particle number, diameter and volume information as shown in the $\Delta V/\Delta$ log Dp versus Dp plot in Fig. 5. From Fig. 5 it may be seen that there are two particle volume modes which stand out — one with a peak in the 0.2 to 0.3 μm particle diameter range and the other in the greater than 1 μm range. The spectra, of course, return to the particle diameter axis, but not until the 50-100 μm particle diameter range is reached. Using the XEDA probe it was observed that the identifiable particles in the smaller diameter mode were predominantly of soil origin (Al, Si) and that 90% of the particles in the larger diameter mode were organic in composition (no X-ray return).

This same volume-diameter depiction of aerosol characteristics was applied to unpublished

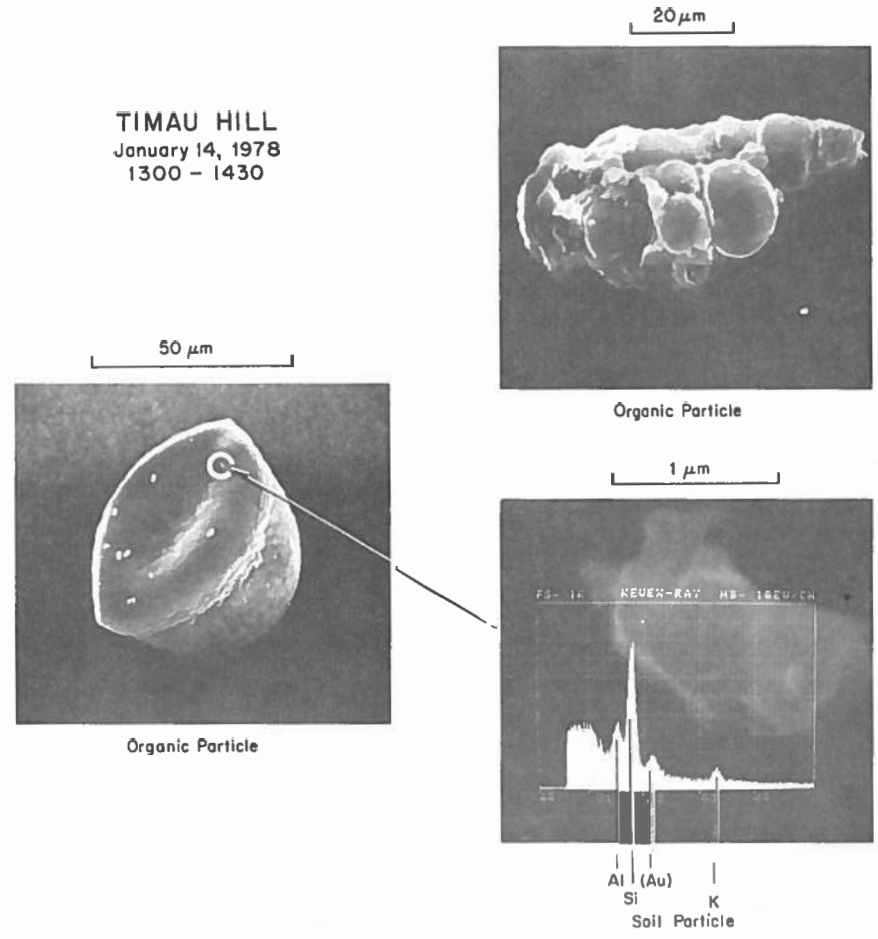

Figure 1. Organic aerosol particles captured on Timau Hill, Mount Kenya. Observe that the pollen-like particle is supporting smaller soil particles.

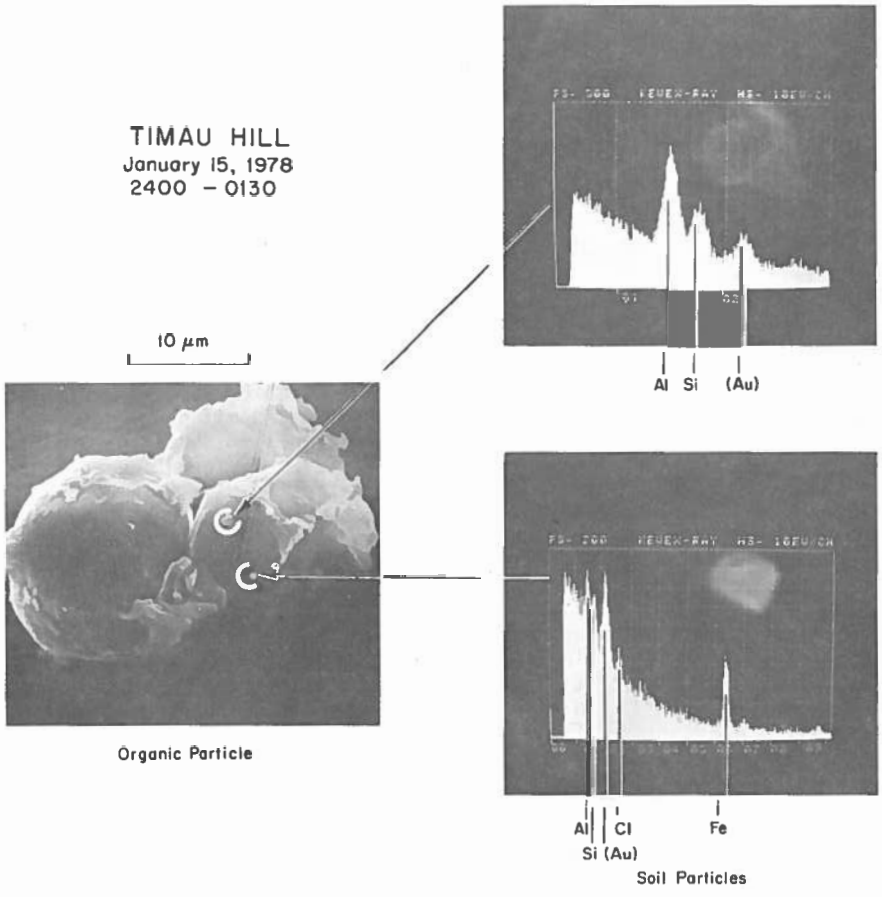

Figure 2. An organic aerosol particle with smaller soil particles of differing composition upon its surface.

measurements of aerosols previously collected at the Mauna Loa Baseline Station (a volcanic peak in Hawaii) and provided to the project courtesy of Dr. R. F. Pueschel, NOAA/APCL. The results are shown in Fig. 6 where it may be seen that during "background air" situations, the aerosol distribution at Mauna Loa was uni-modal with a maximum in the 0.1 to 0.4 μm range. During upslope conditions, the aerosol was completely different being characterized by a bi-modal distribution and high concentrations of large particles which probably originated in the lowland urban and agricultural areas surrounding the base of the mountain.

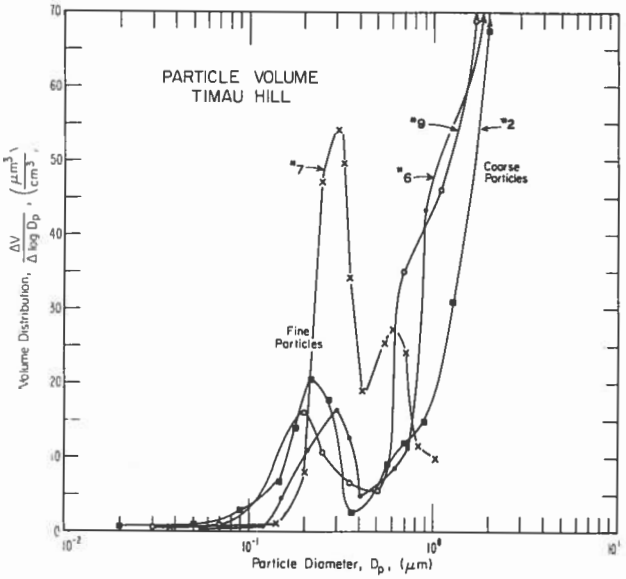

Figure 5. Particle volume versus particle diameter relationships for aerosols captured at 4000 m MSL at Timau Hill 1978. Spectra 2 and 7 are for samples collected between the hours of 1300 and 1430 LST and 6 and 9 are for samples collected between 0200 and 0330 LST.

This method of displaying aerosol characteristics is beginning to be widely applied to the study of aerosols, so some explanation of its meaning is in order. It has been suggested (Whitby, 1973) that aerosols are universally distributed in trimodal volume versus diameter distributions with maxima at the 0.02 μm, 0.1 to 0.8 μm and greater than 1 to 2 μm diameter ranges. The first peak is produced by fresh "combustion" aerosols which coagulate and condense into the second or "fine particle accumulation" within 6 - 12 hours of their formation. The third distribution of "coarse particles" is produced by aerosolization of soil and vegetation by winds. These coarse particles sediment out of the atmosphere within a few hours to a few days of their production due to their large mass. The coarse particle mode does not interact appreciably with the fine particle mode although it may contribute to the fine particle accumulation mode. Thus, it is now becoming the feeling, among some scientists within the field of aerosol science, that the central particle mode represents aged, stable, background aerosol probably relatively constant throughout large portions of the earth's atmosphere remote from large combustion sources. If the above is reality, we may now have a new, powerful, inexpensive, and easily implemented method of characterizing background aerosol.

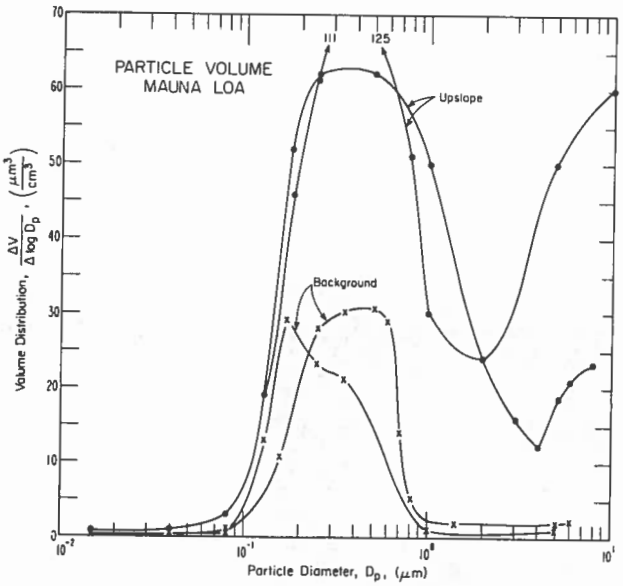

Figure 6. Particle volume versus particle diameter relationships for aerosols captured at 3400 m MSL at Mauna Loa Observatory in 1973. Note the absence of the coarse particle mode in the "background air" samples.

3. DISCUSSION

From the above results it may be observed that there are appreciable numbers of large aerosol particles (mostly organic) present in the air sampled at 4000 m on the side of Mount Kenya. Since the sample location was some 1000 m vertically above and 10 km horizontally distant from the tree line and on an isolated and exposed rock hill, it is suggested that the large particles originated in the forest and/or out in the plains at the foot of the mountain some 30 - 50 km horizontal to and 3000 m vertically below the sample location. The origin of the salt particles is most likely in the Indian Ocean, 350 km to the east (prevailing winds are from the east). The smaller soil particles probably originated in the drier lowlands of central or northeast Kenya at least 50 to 200 km from the sampling location.

The fact that the aerosol volume distribution measured at Mount Kenya did not change appreciably at night suggests that the aerosol particles represent sources in a general regional area, not strong local sources on the mountain. Since there are no appreciable industrial sources for particulates in the Mount Kenya area, we may assume then that the composition and size distribution of the aerosols measured on Mount Kenya are probably representative of a major portion of East Africa and possibly are even representative of a large area of tropical Africa.

ACKNOWLEDGEMENT

This research was supported by the World Meteorological Organization/United Nations Environment Program as part of the Mount Kenya Baseline Station Feasibility Study, 1976-1978.

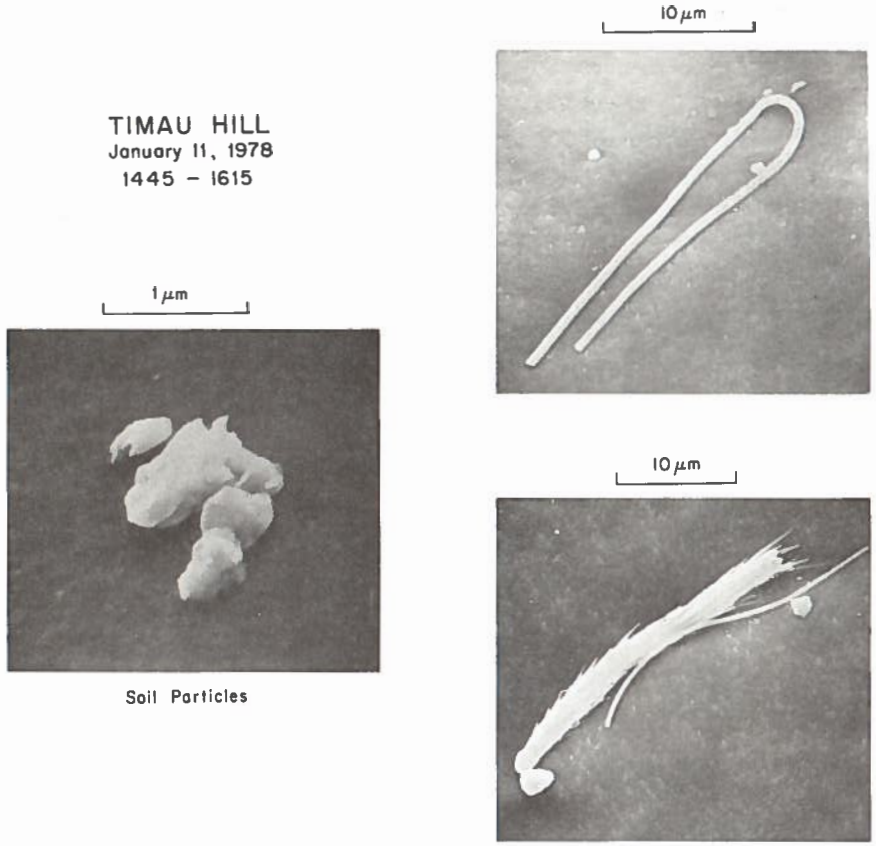

Figure 3. A clump of soil particles and two organic particles of interesting shapes collected at the same time and location.

REFERENCES

McCrone, W. C. and J. G. Delly, The Particle Atlas. ed. Two, Ann Arbor Science Publishers Inc., Ann Arbor, Mich. pp. 575-794, 1968.

Parungo, F. P., R. F. Pueschel, B. T. Patten, and D. Eyre. Physical and chemical properties of AgI aerosols formed at simulated airborne conditions. NOAA Tech. Rpt. ERL 337-APCL 36, pp. 43, 1975

Whitby, K. T. On the Multimodal Nature of Atmospheric Aerosol Size Distribution. Particle Technology Laboratory Publication No. 218, Univ. of Minnesota, Minneapolis, pp 13 1973.

Figure 4. An aerosol particle composed of two different salts of marine origin. The nearest ocean is 350 km east of Mount Kenya.

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1981

Reprinted from Preprint Volume of Extended Abstracts: 15th Conference on Agriculture and Forest Meteorology and Fifth Conference on Biometeorology. April 1-3, 1981, Anaheim, Calif. Published by the American Meteorological Society, Boston, Mass.

ICE NUCLEATION STUDIES ON BACTERIA AEROSOLS

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1. INTRODUCTION

One species of epiphytic bacteria, Pseudomonas syringae has been identified as a principal natural ice nucleant (IN) initiating frost damage in leaves of frost-prone North American plants (at temperatures warmer than -5°C (Lindow et al. 1978)). In the absence of these specific bacteria, living vegetation has been observed to supercool to -6°C and to recover without loss of vigor. This IN active bacteria has been collected in the atmosphere in a viable state above and downwind of field crops (Lindemann, et al. 1979) and in clouds over the Arctic Ocean (Flanagen and Jayaweera, 1980). In both studies, the bacterium was found to have maintained its ability to initiate ice at supercoolings of -5°C or warmer.

In cloud chamber tests, P. syringae, has been observed to be a good IN at temperatures colder than -8°C (Schnell, 1976, Maki and Willoughby, 1978) which is somewhat colder than the -1.5°C to -2.5°C threshold nucleation temperatures observed in bulk suspensions of the bacteria (Vali, et al. 1976). In this report we present data on a series of experiments in which the IN properties of P. syringae aerosols were determined under controlled laboratory conditions.

2. METHODS

Water suspensions of actively growing P. syringae cultures were nebulized into a 2.75 m^3 aerosol tent constructed of Mylar. A typical aerosolization consisted of approximately 10^8 bacteria suspended in 10 cm^3 of water sprayed into the tent over a 1 minute period. The relative humidity in the tent never exceeded 50% during the tests reported in this paper.

Aerosol size and number distributions in the tent were monitored with two overlapping laser aerosol spectrometers (active scattering aerosol spectrometer (ASASP-X) and a forward scattering aerosol spectrometer (FSSP)) measuring particle radii from 0.045 μm to 23.5 μm in eight partially overlapping ranges of 15 bins each (Knollenberg, 1976). Total suspended aerosol mass per unit volume of air sampled in the tent (averaged over a 5-min period) was determined by integrating under the DV/D log R curves in a manner outlined by Barrett et al. (1979), assuming a density of 1 for the suspended bacteria.

At intervals while the aerosols were aging in the tent, 25-ℓ samples of the tent contents were filter sampled using 0.45-μm pore diameter Sartorious hydrophobic membrane filters, and the IN content of the captured particulates determined within 10 minutes of collection using a combination membrane filter-drop freezing technique (Schnell, 1979). In this IN measurement technique, a filter is bonded to a clean aluminum-foil-covered copper plate which can be thermoelectrically cooled at a slow and steady rate such that the freezing temperatures of an array of distilled water drops placed on the filter can be monitored. The freezing temperatures of the drops are then used to produce a freezing-nucleus spectrum for the aerosol as illustrated in Figures 2 and 3 of this report.

3. RESULTS

A DV/D log R plot of a P. syringae aerosol at 1 hour after aerosolization is shown in Figure 1. From this Figure, it may be observed that the aerosol distribution peaked in the range of 1.0 μm to 1.5 μm radius (2.0 to 3.0 μm diameter) and had a mass of 225.6 μgm^{-3}. Since individual P. syringae cells are about 1.0 to 1.5 μm in length, these data suggest that some clumping of cells occurred in the aerosol. This suggestion is supported by observations that when exceptionally high concentrations of bacteria were aerosolized into the tent, (thereby increasing the probability for clumping), the peak of the distribution shifted to larger diameters, in one case, up to a diameter of 8 μm.

The IN spectra from a typical P. syringae aerosol aging experiment are shown in Figure 2. From this figure it may be observed that immediately following aerosolization (time, 0) the aerosol contained IN active at -1.6°C in concentrations of 10 ℓ^{-1} active at -2.0°C. One hour later, the concentration of active IN had

decreased appreciably (time, 1 hr) and by 3 hours after aerosolization (time, 3 hr) threshold nucleation had decreased to -7.2°C with a concentration of 10 ℓ^{-1} active at -12.5°C. The aerosol distribution shown in Figure 1 corresponds to the time of the nucleus spectra 1 hour after aerosolization in Figure 2.

As mentioned earlier, the IN activity of the bacteria on the filters was determined within 10 minutes of filter exposure. This was done as earlier research (Schnell et al. 1980) had shown that natural IN, once deposited upon filters, tended to lose activity over relatively short periods of time. To monitor the potential change of IN activity on the filters exposed in this study, triplicate filters were exposed simultaneously, then developed later. The results of one such experiment are shown in Figure 3 where it may be observed that appreciable IN activity was lost within the first 2 hours of storage with a proportionately smaller loss over the following 24 hours. In other tests on these aerosols, it was observed that beyond 24 to 36 hours further loss of ice nucleus activity was not appreciable (up to one month).

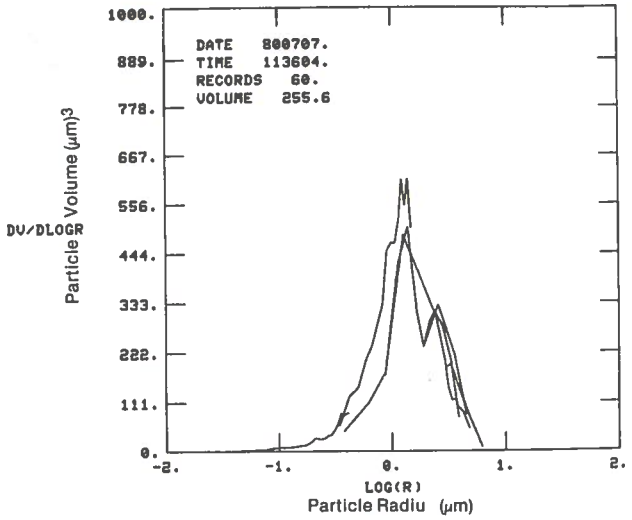

Figure 1. Volume-size plots of a P. syringae aerosol at 1 hour after aerosolization. The aerosol at this time possessed the ice nucleation characteristics depicted in Figure 2, time 1 hr.

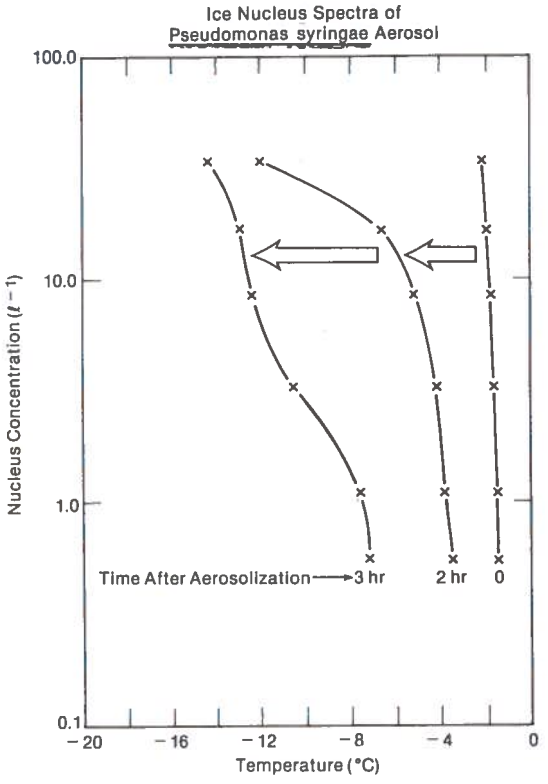

Figure 2. Ice nucleus spectra of P. syringae aerosol in a 2.75 m^3 tent as a function of time after aerosolization.

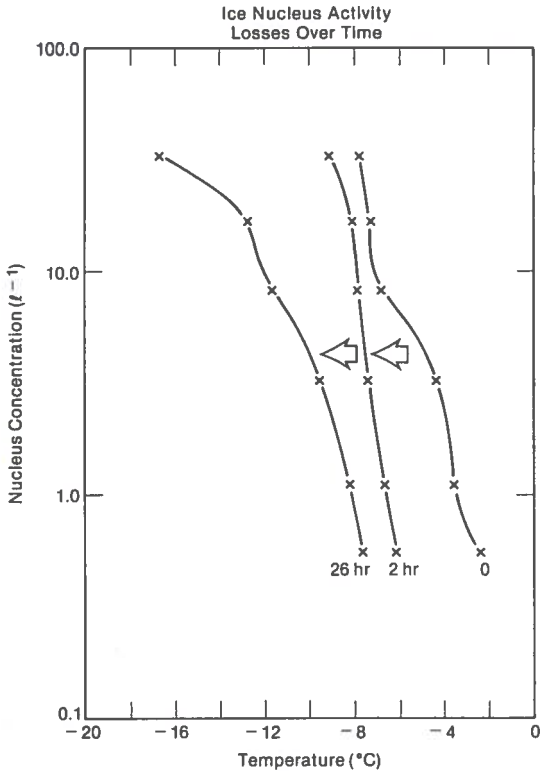

Figure 3. Ice nucleus activity losses of P. syringae aerosols stored at room temperature on membrane filters.

4. DISCUSSION

The above data show that it is possible to aerosolize P. syringae into an enclosed volume and recover associated IN activity in appreciable concentrations over periods up to 3 hours. In other tests conducted on the same bacteria, IN were recovered up to 8 hours after aerosolization although the IN were present only in small concentrations and possessed activity only marginally better than background IN.

The average concentration of ice forming bacteria (as measured by active IN on the filters) in the tent aerosol was in the region of 1 ℓ^{-1} active at -10°C which is less than the average concentration of 1.7 ℓ^{-1} viable bacteria (maximum 6.5 ℓ^{-1}) observed in natural air over midwest agricultural crops (Lindemann et al., 1979). But, the concentrations of bacteria in the tent were considerably greater than the average of 0.04 ℓ^{-1} living bacteria in the atmosphere capable of producing IN reported in this same study. In the only other study of atmospheric bacterial IN known to the authors, Flanagen and Jayaweera (1980) report concentrations of up to 150 viable bacteria per liter of air sampled in stratus clouds over the Arctic Ocean in June 1980, with the total count of bacteria, living plus dead, occasionally observed to be in excess of 2000 ℓ^{-1}. A proportion of the bacteria they collected were of the genus Pseudomonas and were active IN at temperatures as warm as -5°C. It is possible that some of the dead (dormant) bacteria could have been IN as Maki et al. (1974) have shown that dead P. syringae may retain IN active at -5°C.

In summary, we suggest that at some point in the tent studies, the concentration of IN active bacteria may have been similar to those observed in the natural atmosphere. If this was so, our data show that aerosolized P. syringae may remain airborne for appreciable periods of time and maintain threshold IN activity at temperatures in the region of -5°C and warmer. Thus, our observations support the results of other scientists which suggest that IN active bacteria in the atmosphere may be playing important roles in transmitting frost sensitivity between agricultural crops, and that the bacteria have a potential to act as atmospheric IN.

Acknowledgments

This research was supported by grant 8G130532 from the Weather Modification Program Office of the National Oceanic and Atmospheric Administration, and by use of laboratory facilities provided by Dr. R. F. Pueschel, NOAA/ARL, Boulder.

REFERENCES

Barrett, E. W., F. P. Parungo and R. F. Pueschel 1979. Cloud modification by urban pollution: a physical demonstration. Meteoral. Rdsch., 32, 136-149.

Flanagen, P. W. and K. Jayaweera 1980. Evidence for microbial ice nucleation over the arctic ocean. EOS, 61, 968.

Knollenberg, R. G. 1976. Three new instruments for cloud physics measurements: the 2-D spectrometer, the forward scattering spectrometer probe, and the active scattering aerosol spectrometer. Proceedings, International Conference on Cloud Physics, Boulder, Colorado. AMS, Boston, Mass., 554-561.

Lindemann, J., H. A. Constantinidou, W. R. Barchet and C. D. Upper 1979. Epiphytic bacteria, including those that are ice nucleation active, in the atmosphere above plant canopies. Preprints, 14th Conf. on Ag. and Forest Met., Minneapolis, Minn. AMS, Boston, 221-223.

Lindow, S. E., D. C. Arny, C. D. Upper and W. R. Barchet 1978. The role of bacterial ice nuclei in frost injury to sensitive plants. pp 244-263. P. Li and A. Sakai Editors, Plant cold-hardiness and freezing stress mechanisms and crop implications. Acad. Press, Inc. N.Y.

Maki, L. R., E. L. Galyan, M. M. Chang-Chien and D. R. Caldwell 1974. Ice nucleation induced by "Pseudomonas Syringae". Appl. Microbiology, 28, 456-459.

Maki, L. R. and K. J. Willoughby, 1978. Bacteria as biogenic sources of freezing nuclei. J. Appl. Meteor. 17, 1049-1053.

Schnell, R. C. 1976. Bacteria acting as natural ice nucleants at temperatures approaching -1°C. Bull Amer. Met. Soc., 57, 1356-57.

Schnell, R. C. 1979. A new technique for measuring atmospheric ice nuclei active at temperatures from -20°C to approaching 0°C, with results. Preprints, 7th Conf. on Inadvertant and Planned Weather Modification. Banff, Canada. American Meteorological Society, 110-111.

Schnell, R. C., B. Wrobel, and S. W. Miller 1980. Seasonal changes and terrestrial sources of atmospheric ice nuclei at Boulder, Colorado. Preprints VIII Int. Conf. Cld. Phys., Clermont-Ferrand, France, 53-56.

Vali, G., M. Christensen, R. W. Fresh, E. L. Galyan, L. R. Maki and R. C. Schnell, 1976. Biogenic ice nuclei, Part II: Bacterial sources. J. Atmos. Sci. 33, 1554-1564.

SEASONAL CHANGES AND TERRESTRIAL SOURCES OF ATMOSPHERIC ICE NUCLEI AT BOULDER, COLORADO

IAMAP-ICCP VII Conference
Clermont-Ferrand, France
pp 53-65,
July 1980

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I. INTRODUCTION

Atmospheric ice nucleus (IN) concentrations have been measured at 20 m AGL at Boulder, Colorado on a regular basis since July of 1978 and at irregular intervals at ground level over various terrestrial surfaces, using a combination membrane filter-drop freezing technique in which the freezing temperatures of clean distilled water drops placed on exposed membrane filters are taken to be a measure of the IN in the air (Schnell, 1979). The data in that report (ibid.) suggested that atmospheric IN concentrations decreased immediately following the passage of snow producing cold fronts with recovery to pre-snow levels over the space of a few weeks, as the snow cover melted. In this present report, the results from 3 months of IN measurements in a summertime period are presented along with data from tests on aging and exposure of IN to vacuum treatments.

The IN measurement technique used in these studies has not undergone the rigors of extensive intercomparison with more established methods of IN detection, thus the data presented herein are best regarded as being internally comparable and consistent but not absolute values until the results of intercomparison studies are published. It may be observed though, that in the data presented below, the relative concentrations and changes in observed IN activity can be related to meteorological and physical factors with precision and, on some occasions, with a degree of predictability.

II. ATMOSPHERIC ICE NUCLEUS CONCENTRATIONS

The temperature at which threshold IN activity and concentrations of 500 nuclei m^{-3} were observed over the period of June 18 through August 15, 1979 are shown in Figure 1. From this Figure it may be seen that the passage of cold fronts which introduced new air masses of northern origins to the Boulder area were coincident with decreases in IN threshold temperatures and in IN concentrations. The IN concentrations are seen to recover within a few days following frontal passage as is well illustrated by the measurements associated with the frontal passage of July 24, 1979. Another interesting feature of the summer's IN record is the persistent period of low IN concentrations from August 2 through 6, 1979. This period was coincident with a heat wave and dry period over the Western USA resulting in part from a large zone of persistent subsiding air of upper level origins. Relative hu-

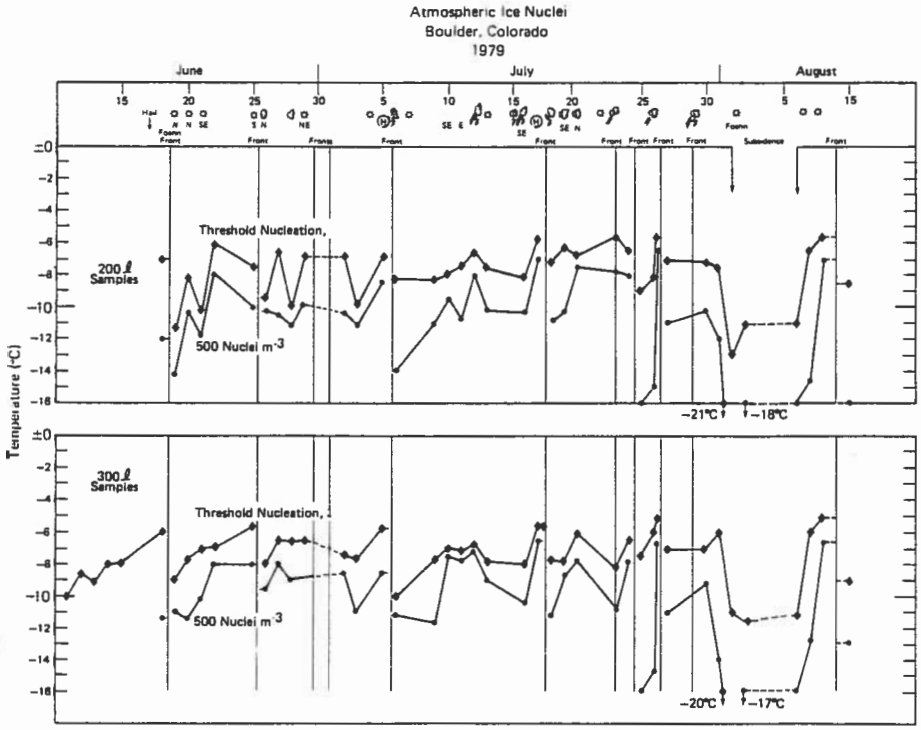

Figure 1. Atmospheric ice nuclei measured at 60 m AGL at Boulder, Colorado in the summer of 1979 showing threshold activity and the temperatures at which 500 nuclei m^{-3} were observed for 200 ℓ and 300 ℓ air samples. The decrease in atmospheric ice nucleus concentrations following frontal passages and the persistent low concentrations measured during a period of large scale subsidence (August 1-6) are worthy of note.

midities in Boulder during this period were occasionally as low as 17%. Along with a change in the air mass on August 6 and 7, IN concentrations returned to previous levels. This pattern has been observed on two subsequent occasions.

III. RELEASE OF ICE NUCLEI FROM TERRESTRIAL SURFACES

It has been shown by Schnell and Vali (1976), that naturally decayed plant litter contains numerous freezing nuclei active within the temperature range from -4°C and -8°C. In experiments designed to measure the release rates of these IN from terrestrial surfaces, filters were exposed at 5 cm above a variety of vegetated and cultivated soils in and near Boulder. The results from one set of tests are shown in Fig. 2 where it may be observed that on July 13, 1979, under calm wind conditions, (left-hand graph) the ambient air above the grass contained IN active at -6.5°C and -7.8°C in 50 ℓ and 25 ℓ samples in concentrations in the region of 1 nucleus ℓ^{-1} active at -8°C. At 20 m vertical and 400 m horizontal from the grass plot, an air sample of 250 ℓ exhibited threshold nucleation at -8.1°C in concentrations of 1 nucleus ℓ^{-1} active at -11.5°C. Disturbing the surface by agitation of the grass with a 20 cm long piece of coiled rope released greater numbers of nuclei than were in the ambient air. The freezing nuclei in a bulk sample of the surface leaf litter tested with the drop freezing technique (Schnell and Vali, ibid.) is also shown. It is interesting to note that the threshold activity of the IN from the larger (50 ℓ) sample of air above the agitated surface is equal to that in the bulk sample of the leaf litter suggesting that the IN on the filter were the same as the IN in the leaf litter.

A somewhat different picture was observed over the same grass plot on September 14, 1979 following a 3-day period of rain (right-hand portion of Fig. 2). There it may be seen that there were few IN in the ambient air above the wet grass. By disturbing the surface, appreciably greater numbers of IN were released to the air although their numbers and activity were lower than when the same grass was dry. The IN spectra for the bulk leaf litter and for ambient air at 20 m AGL for this day are also presented.

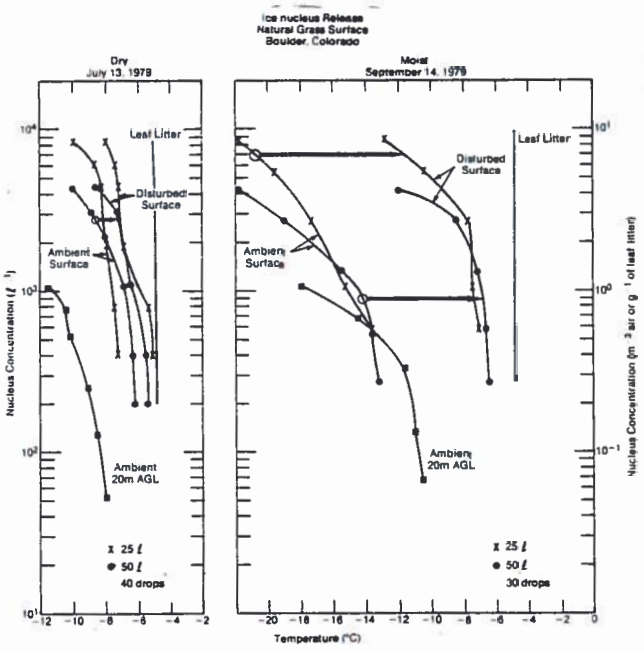

Figure 2. Ice nucleus concentrations measured in air at 5 cm above a dry grass surface (left-hand graph) and above the same grass surface (right-hand graph) following a 3-day period of rain. The open circles indicate the number of freezing nuclei measured in samples of decayed leaf litter from the surface of the soil.

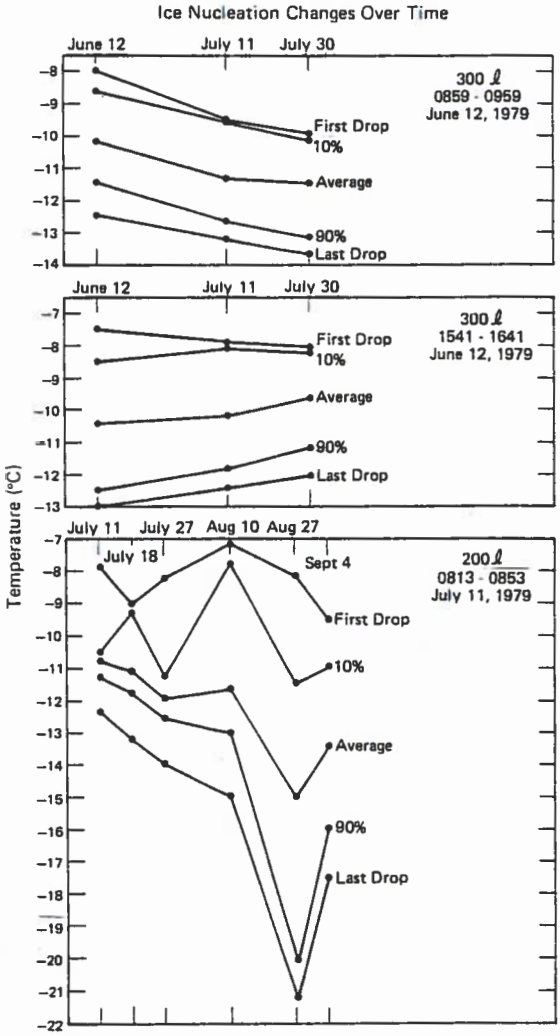

Figure 3. The freezing ranges of 20 drops of distilled water placed on membrane filters which were exposed in sets and stored for various periods of time prior to testing. The general decrease in the nucleation characteristics of the aerosols on the filters collected July 11, 1979 are of interest.

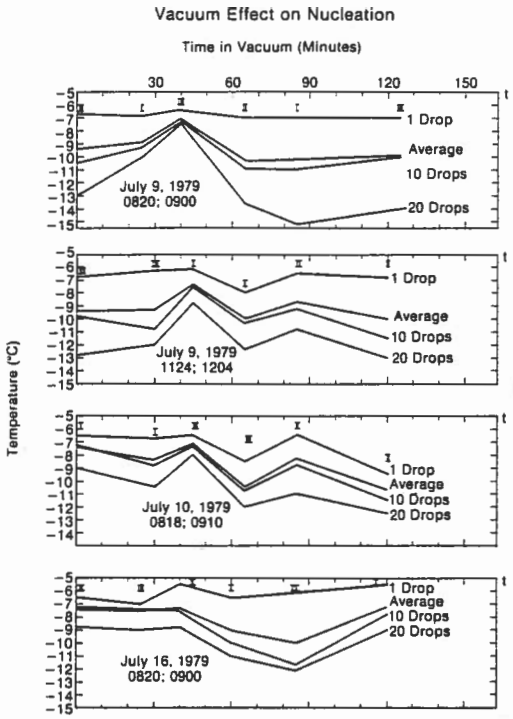

Figure 4. The freezing range of 20 drops of distilled water placed on membrane filters which were exposed in triplicate, halved, then placed in a 10^{-6} Torr vacuum for various periods of time prior to testing. Halves from the same filters are indicated by Roman numerals. A general increase in ice nucleation activity after a vacuum treatment of about 40 minutes is predominate on filters exposed on July 9 and 10, but not on July 16.

IV. ICE NUCLEUS ACTIVITY CHANGES WITH TIME

During the early stages of this research project, it was noted that separate halves of a filter occasionally produced different IN spectra when processed at times separated by a few days. To quantify these observations, a series of triplicate and sextuplicate filters were exposed to natural air, stored at room temperature in sealed petri dishes, and then processed singly over time intervals varying from a few hours to a month. The range of changes observed in the IN activity are shown in Fig. 3 where the freezing temperatures of 20 drop samples are plotted. From Fig. 3 it may be seen that there was a slight but steady decrease in IN activity on the filters collected on the afternoon of the same day. The filters exposed on July 11, 1979 exhibited a notable spreading of the freezing range after 1 month of storage, followed by a slight recovery of activity. In other tests, little change in IN activity was observed to occur on filters stored less than 2 days. These results suggest that the IN on the filters are either changing in themselves over time, or are reacting with the filters in some as yet unexplained and apparently random manner. If the nuclei are indeed changing, this suggests that they may contain unstable organic materials or adsorbed gases which can change or be removed over time. These changes also raise questions about the validity of data obtained from filters stored more than a few days.

V. EFFECT OF VACUUM ON NATURAL ICE NUCLEI

In an experiment to test the volatility of natural IN, triplicate filters were exposed to natural air, halved, and then all but one portion subjected to a vacuum of 10^{-6} Torr for time periods ranging from 20 minutes to 125 minutes prior to testing for IN activity. The results from 4 such tests are shown in Fig. 4 when it may be seen that in 3 out of 4 cases the IN activity was significantly increased at around 40 minutes of treatment, to be followed by deactivation at from 60 minutes to 80 minute of treatment. At 80 minutes to 120 minutes, 2 filter sets exhibited further deactivation and 2 sets exhibited reactivation. In other tests, 36 out of 48 filters collected between June 2 and July 16, 1979 exhibited an activation of IN at and around 40 minutes of treatment, to be generally followed by some deactivation between 80 and 120 minutes. These results suggest that the vacuum may have removed some gaseous or liquid component of/on the IN which was suppressing nucleation or that removal of some component of the nuclei produced a new structure which was more active. Continued exposure to the vacuum further changes the nature of the nuclei such that either deactivation and/or further reactivation occurs over time. Again, the possibility that the filter material and the deposited aerosols may somehow be reacting to produce the observed results must not be discounted at this point.

VI. DISCUSSION

The changes in atmospheric IN concentrations which were observed during this study (using a combination membrane filter-drop freezing technique) can be related to large scale meteorological effects, thereby suggesting a cause and effect relationship controlled by the meteorological characteristics /life history/ source region of a particular air mass. These results are similar to those embodied in the data collected in an ice nucleus benchmark as reported by Allee (1974). Also, Rogers and Vali (1978) and Langer et al.,(1979)have presented data which suggests that atmospheric IN concentrations fluctuate on the mesoscale in response to gust fronts and local wind patterns, and that these effects are measurable from ground level up to cloud base. The present results differ from these earlier results mainly in that the threshold nucleation temperatures we detect are generally 6 to 10°C warmer than reported in the other studies, and in that the concentrations of IN we measured were appreciably greater at temperatures warmer than -10°C. The initial increase in IN activity resulting from the outgassing treatment on the filters is similar to that noted by Parungo et al., (1978) for filter samples collected in powerplant plumes, although in the present study the samples were collected in relatively clean

air. In the powerplant studies, removal of adsorbed IN suppressing pollutant gasses from the surface of the IN was suggested as being a factor in the enhanced nucleation affects observed.

Based on the results from the surface measurements of IN, and the results from the ambient temperature and pressure tests, we suggest that the IN observed on filters both at the surface and at 60 m AGL were surface derived, and that the nuclei probably contained an appreciable component of biogenic material.

ACKNOWLEDGMENTS

This research was supported by Dr. H. K. Weickmann, then Director, NOAA/ERL/APCL with support from Dr. R. F. Pueschel, NOAA/ERL/ARL. Funds were provided by the National Research Council Research Associate Program, the Cooperative Institute for Research in Environmental Sciences, and, in part, by the Environmental Protection Agency through Interagency Agreement 78 REGEHE-625/D5-E693.

REFERENCES

Allee, P.A., 1974: Ice nuclei concentration measured by a benchmark network. Preprints, 4th Conf. on Weather Modification, Ft. Lauderdale, Florida. American Meteorological Society, pp. 242-245.

Langer, G., G. Morgan, C. T. Nagamoto, M. Solak and J. Rosinski, 1979: Generation of ice nuclei in the surface outflow of thunderstorms in northeast Colorado. Jr. Atmos. Sci., 36, pp. 2484-2494.

Parungo, F., E. A. Ackerman, H. Proulx, and R. Pueschel, 1978: Nucleation properties of fly ash in a coal-fired powerplant plume. Atmos. Environ., 12, pp. 929-935.

Rogers, D. C. and G. Vali, 1978: Summer climatology of aerosols and nuclei in the central high plains of the U. S. Preprints, Conf. on Cloud Physics and Atmospheric Electricity, Issaquah, Washington. American Meteorological Socity, pp. 25-30.

Schnell, R. C., 1979: A new technique for measuring atmospheric ice nuclei active at temperatures from -20°C to approaching 0°C, with results. Preprints, 7th Conf. on Inadvertant and Planned Weather Modification. Banff, Canada. American Meteorological Society, pp. 110-111.

Schnell, R. C. and G. Vali, 1976: Biogenic ice nuclei; part I. terrestrial and marine sources. Jr. Atmos. Sci., 33, pp 1554-1564.

Tellus (1982) *34*, 92–95

Kenyan tea litter: A source of ice nuclei

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(Manuscript received March 5; in final form May 18, 1981)

ABSTRACT

A strong source of relatively active ice nuclei has been identified within the surface litter layer of tea plantations around Kericho, Kenya. This area also has the earth's largest known hailfall frequency. Aerosols produced from the tea litter retain their ice nucleation activity.

1. Introduction

Hail falls an average of 132 days a year on to the tea estates in the Kericho area of Western Kenya–a possible world record (Alusa, 1976). These localized storms damage tea crops and, in many cases, are one of the largest single natural variables affecting tea production. Approximately 70% to 85% of all storms in this area (07 lat., 35° E. long.) produce hail reaching ground level (1800 to 2000 m ASL) (Alusa, 1976). The hail is, for the most part, less than 10 mm in diameter. At some early stage in the production of hail in these clouds embryonic ice crystals form and grow by the collection of super-cooled water vapor or water drops. Inorganic soil materials (Isono et al., 1959) have been suggested as possible sources of atmospheric ice nuclei (IN) from which the embryonic ice crystals would initiate. More recently, decomposition products of terrestrial vegetation have been found to be good IN with IN concentrations in the atmosphere, and in precipitation (rain and hail) observed to mirror the activity of the IN found in the local plant litter (Schnell and Vali, 1976). Rosinski et al. (1980) have observed that up to 50% of the ice nucleating particles collected in the outflow regions of Colorado thunderstorms were natural organic particles in the size range from 1 to 10 μm radius.

This note reports on a study of the availability and ice-forming power of IN in and around the Kericho tea-growing (and hail) areas. On three occasions between 1974 and 1978 samples of soils, plant litter, and plant leaves were collected in the tea estates near Kericho and at lower elevations (to 1000 m ASL) on a line west of Kericho to Lake Victoria (50 km) and east of Kericho to Nakuru (90 km). These samples were tested for IN (freezing nucleus) content within 1 to 4 weeks of collection in the manner described by Schnell and Vali (1976). Later, composite samples of the tea litter were dispersed into a large containment tent and the size distribution and IN activity of the tea aerosol monitored over time.

2. Results

In September 1974 tea litter was found to contain more active IN (some active at −5 °C) than plant litters collected in the adjacent indigenous Mau Forest or in litter from eucalyptus groves (planted to replace portions of the Mau Forest). Plant litters collected away from the Kericho area also contained fewer IN than the tea litters. In February 1977 and June 1978 the IN activity of the litters from the eucalyptus groves and Mau Forest was similar to that observed in

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1974, whereas the tea litters were slightly less active than in the previous period. In Fig. 1 may be seen the range of the tea, eucalyptus and Mau Forest litter IN activity measured in 1977 and 1978; the bracketed numerals indicate the number of separate sample areas represented.

In 1977 and 1978, samples of tea litter from the surface, and from 5 cm below the surface from five widely separated tea estates were tested for IN activity. Results showed that the surface of the litter layer contained far more active IN than did samples collected 5 cm lower in the litter layer; threshold IN temperatures were uniformly 3 °C to 5 °C warmer in the surface samples at the litter–air interface (where they would most easily be dispersed into the atmosphere). Vali et al. (1976) have documented the production of highly active IN under such favourable aerobic conditions of natural leaf decay such as those at the top of tea litter layers.

In tea litter aerosolization tests, 1.5 g samples of tea litter (produced by mixing 11 litters from 1977 and 1978) were single puff aerosolized into a 2.75 m^3 Mylar-coated aerosol tent and the aerosol distribution monitored with two PMS overlapping laser aerosol spectrometers measuring particle radii from 0.045 μm to 23.5 μm. Total suspended aerosol mass per unit volume of air sampled in the tent (averaged over a 5 min period) was determined by integrating under the $dV/d\log R$ curves assuming a density of 1 for the suspended tea litter. At intervals while the aerosols were settling in the tent, 200 l samples of the tent contents were filter sampled using 0.45 μm pore diameter Sartorious hydrophobic membrane filters, and the IN content of the captured particulates determined using a combination membrane filter-drop freezing technique (Schnell, 1979).

The IN spectra from one such set of tests are presented in Fig. 2 for a tea litter aerosol produced at 09.26 local time. At 09.51, 25 minutes after aerosolization of the tea leaf litter, the tent contained a nominal 801.7 $\mu g\ m^{-3}$ of suspended litter concentrated in the 7 to 10 μm radius range

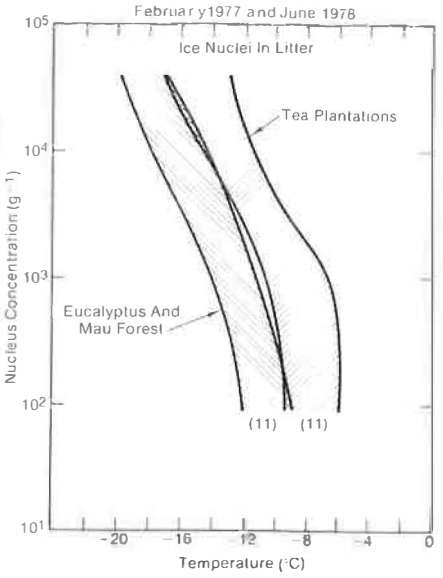

Fig. 1. Ranges of IN activity of litters collected in the Kericho, Kenya, area, February 1977 and June 1978. The IN spectra from the eucalyptus groves and Mau Forest are essentially the same and both have lesser IN activity relative to tea litters. Bracketed numerals indicate the number of separate samples contributing to each range. Spectra to the right of the graph represent the most active IN.

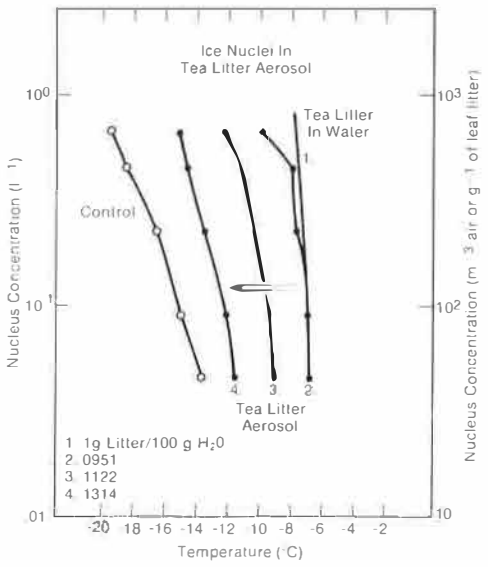

Fig. 2. Ice nucleus activity of bulk tea litter in water (1) and tea litter aerosols collected on filters (2–4). The control is obtained by measuring the IN on an unexposed filter. Spectra 2, 3 and 4 are from successive samples of a single charge of aerosol aging in a containment tent. Aerosol concentrations in the tent at the time of the respective IN spectra were: (2) 801.7 $\mu g\ m^{-3}$, (3) 71.8 $\mu g\ m^{-3}$, (4) 9.8 $\mu g\ m^{-3}$.

with IN activity as shown by IN spectrum 2, Fig. 2. It is interesting to note that the IN activity of the aerosol is nearly the same as that measured by adding 1 g of tea leaf litter directly into 100 g of distilled water (spectrum 1, Fig. 2). At 2 hours after aerosolization (11.27), particle concentrations in the tent had fallen to a nominal 71.8 μg m^{-3} with IN activity as shown by spectrum 3, Fig. 2. At 3.5 hours after aerosolization (13.14), particle concentrations had fallen to 9.8 μg m^{-3} with IN activity shown by spectrum 4, Fig. 2. Aerosol concentrations in the range of 50 to 100 μg m^{-3} are normal in unpolluted rural atmospheres.

3. Discussion

Data presented in this report indicate that tea litter on the ground surface in the Kericho area contains relatively greater concentrations of active IN than other plant litters in the region, with the most active IN observed at the litter–air interface of the tea litter layer. Aerosols produced from the tea litter had a size distribution similar to that found in natural aerosols associated with thunderstorms (albeit on different continents) and maintained appreciable IN activity at aerosol mass loadings observed in natural atmospheres. Similar aerosolizations of plant litters into the Colorado State University isothermal cloud chamber have produced reliably repeatable IN concentrations over a wide range of cloud temperatures in direct proportion to the mass of leaf litter aerosolized (Schnell and Vali, 1976).

In a study of the cumulus clouds over the Kericho tea estates, Dye and Breed (1979) have shown that the clouds may be classified as continental, suggesting that the ice phase may be the predominant precipitation formation mechanism in these clouds. Alusa (1976) has shown that Kericho cumulus clouds and resultant hailstorms are characteristically triggered when surface level westerly winds from Lake Victoria flow up the Mau escarpment and converge with easterly winds over the higher ground near Kericho. The bases of these hail-producing clouds are typically between 500 to 1500 m AGL, somewhat lower than cloud bases of hail-producing clouds in Colorado, which are typically from 1500 to 2500 m AGL (Summers et al., 1979). Data presented by Rogers and Vali (1978) for Colorado cumulus clouds suggest that surface aerosol and nucleus measurements are representative of the inflow air at cloud base.

Thus, as an early step leading to the formation of hail in the Kericho cumulus clouds, we suggest that IN from the tea litter could be released to the atmosphere either naturally or assisted by the feet of hundreds of tea pickers going about their daily jobs. Once airborne, these small, light particles could be drawn aloft into the growing cumulus clouds to be involved, subsequently, in hail formation processes.

This scenario has a number of weaknesses, including the lack of data on IN activity and concentrations at cloud base. Also, it is not known what role atmospheric IN concentrations and aerosol size and number distributions play in hail formation (National Hail Research Experiment, Final Report, 1981). Probably, the major factors in producing the high hail incidence in the area are a combination of unique topographical influences and dynamic air flows conducive to hail formation. The prolific source of IN in the tea litter may be coincidental.

Some useful additional data on the role of tea litter aerosols in the formation of hail in the area should be forthcoming when planned measurements of the atmospheric IN concentrations and aerosol distributions above the Kericho tea litters, and at the bases of the hail-producing cumulus clouds, are undertaken. At that time it should also be possible to make some inferences as to why, if there are potentially as many active IN in the area as suggested by the above data, the local clouds are not fully glaciated, and thereby reduce the potential for hail formation of a size damaging to the tea.

4. Acknowledgements

This research was made possible and supported directly or indirectly by Professor Gabor Vali, Department of Atmospheric Science, University of Wyoming, Laramie; the Rockefeller Foundation, New York; the World Meteorological Organization, Geneva; the National Research Council, Washington, D.C.; the Environmental Research Laboratories of the National Oceanic and Atmospheric Administration; the Cooperative Institute for Research in Environmental Sciences, Boulder, Co; and the Tea Research Institute of East Africa, Kericho, Kenya. The assistance of the Kenya Meterological Department and Alex Alusa, Director, East African Institute for Meteorological Training and Research, is gratefully acknowledged.

REFERENCES

Alusa, A. L. 1976. The occurrence and nature of hailstorms in Kericho, Kenya. Preprints, Second WMO Scientific Conference on Weather Modification, Boulder, Colorado, August, WMO Geneva, 249–256.

Dye, J. E. and Breed, D. W. 1979. The microstructure of clouds in the high-frequency hail area of Kenya. *J. Appl. Meteor. 18*, 95–99.

Isono, K., Komobayasi, M. and Ono, A. 1959. The nature and origin of ice nuclei in the atmosphere. *J. Meteor. Soc. Japan 27*, 211–233.

National Hail Research Experiment, Final Report, Chapter 3, Aerosols, cloud nuclei and ice nuclei. CSD, NCAR, Boulder, Colorado 80303. (In press.)

Rogers, D. C. and Vali, G. 1978. Summer climatology of aerosols and nuclei in the central high plains of the United States. Preprints, Conference on Cloud Physics and Atmospheric Electricity, Issaquah, Wash., AMS Boston, Mass. 25–30.

Rosinski, J., Morgan, G., Nagamoto, C. T., Langer, G., Yamate, G. and Parungo, F. 1980. Size distribution of inorganic and organic ice forming nuclei present in downdrafts of convective storms. Preprints, 8th International Conference on Cloud Physics, Clermont-Ferrand, France, July, 49–51.

Schnell, R. C. 1979. A new technique for measuring atmospheric ice nuclei active at temperatures from −20 °C to approaching 0 °C, with results. Preprints, 7th Conference on Inadvertent and Planned Weather Modification, Banff, Canada, AMS, Boston, Mass. 110–111.

Schnell, R. C. and Vali, G. 1976. Biogenic sources of ice nuclei, part I terrestrial and marine sources. *J. Atmos. Sci. 33*, 1554–1564.

Summers, P. W., Fankhauser, J. C., Morgan, G. M., Foote, G. B. and Modahl, C. A. 1979. Results of a randomized hail suppression experiment in northeast Colorado part VIII: the representative draw analysis. *J. Appl. Meteor. 18*, 1618–1628.

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

Biogeosciences, 16, 1675–1683, 2019
https://doi.org/10.5194/bg-16-1675-2019

Biogeosciences Open Access EGU

Comprehensive characterization of an aspen (*Populus tremuloides*) leaf litter sample that maintained ice nucleation activity for 48 years

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Received: 3 December 2018 – Discussion started: 7 January 2019
Revised: 22 March 2019 – Accepted: 8 April 2019 – Published: 24 April 2019

Abstract. Decaying vegetation was determined to be a potentially important source of atmospheric ice nucleation particles (INPs) in the early 1970s. The bacterium *Pseudomonas syringae* was the first microorganism with ice nucleation activity (INA) isolated from decaying leaf litter in 1974. However, the ice nucleation characteristics of *P. syringae* are not compatible with the characteristics of leaf litter-derived INPs since the latter were found to be sub-micron in size, while INA of *P. syringae* depends on much larger intact bacterial cells. Here we determined the cumulative ice nucleation spectrum and microbial community composition of the historic leaf litter sample 70-S-14 collected in 1970 that conserved INA for 48 years. The majority of the leaf litter-derived INPs were confirmed to be sub-micron in size and to be sensitive to boiling. Culture-independent microbial community analysis only identified *Pseudomonas* as potential INA. Culture-dependent analysis identified one *P. syringae* isolate, two isolates of the bacterial species *Pantoea ananatis*, and one fungal isolate of *Mortierella alpina* as having INA among 1170 bacterial colonies and 277 fungal isolates, respectively. Both *Pa. ananatis* and *M. alpina* are organisms that produce heat-sensitive sub-micron INPs. They are thus both likely sources of the INPs present in sample 70-S-14 and may represent important terrestrial sources of atmospheric INPs, a conclusion that is in line with other recent results obtained in regard to INPs from soil, precipitation, and the atmosphere.

1 Introduction

Ice-nucleating particles (INPs) are necessary to initiate freezing of cloud droplets in mixed-phase clouds in order for precipitation to form. Therefore, the concentration of atmospheric INPs affects frequency and intensity of precipitation (Mülmenstädt et al., 2015). Identifying and characterizing INPs and their sources is thus the subject of intense research with important implications for modeling climate change (Morris et al., 2014; Coluzza et al., 2017).

In their search for sources of atmospheric INPs in the 1970s, Russell Schnell and Gabor Vali at the University of Wyoming found that decaying leaf litter contained unexpectedly high concentrations of INPs active at temperatures as warm as −1.3 °C (Schnell and Vali, 1972). These very active INPs were called leaf-derived nuclei (LDN). Because LDN were absent in green leaves and increased in concentration during leaf decay, it was hypothesized that they were of microbial origin. In fact, *Pseudomonas syringae*, the most active ice-nucleating microbe known to date, was later isolated from leaf litter (Maki et al., 1974). *P. syringae* is a genetically diverse species and includes plant pathogenic as well as non-pathogenic strains (Morris et al., 2013). Parallel to the discovery of *P. syringae* with INA in leaf litter, *P. syringae* with INA were also isolated from dried powdered corn leaves and identified as the culprit of frost damage in crop plants (Arny et al., 1976), and *P. syringae* with INA have been isolated from clouds, precipitation, and surface water (Morris et al., 2013). Because of its ubiquitous presence in compartments of the water cycle and other circumstantial evidence, *P. syringae* might contribute to the formation of precipitation in

clouds (Morris et al., 2013). Other bacteria with INA have been identified in additional *Pseudomonas* species (Failor et al., 2017) and in additional genera within the Gammaproteobacteria: *Erwinia* (Phelps et al., 1986), *Pantoea* (Failor et al., 2017), a genus that includes several species classified in the past as members of the genus *Erwinia*, and *Xanthomonas* (Kim et al., 1987). A strain belonging to the genus *Lysinibacillus* was recently identified and characterized as the first ice-nucleating Gram-positive bacterium (Failor et al., 2017). Some fungal species have been found to have INA as well: *Fusarium acuminatum* and *F. avenaceum* (Pouleur et al., 1992), *F. tricinctum* and *F. oxysporum* (Richard et al., 1996), *F. sporotrichioides* (Huffman et al., 2013), *Mortierella alpina* (Fröhlich-Nowoisky et al., 2015), *Isaria farinosa*, and *Acremonium implicatum* (Huffman et al., 2013). An additional fungus with INA is the lichen fungus *Rhizoplaca chrysoleuca* (Kieft, 1988). Finally, pollen was added to this long list of biological INA (Diehl et al., 2001).

Microrganisms with INA produce different types of INA molecules. The Gammaproteobacteria produce a membrane-anchored INA protein that is inserted into the bacterial cell wall and is thus heat labile and cannot be separated from bacterial cells by filtration through membrane filters with a 0.22 µm pore size (Morris et al., 2004). However, in the case of *Erwinia herbicola* (now known as *Pantoea ananatis*), the INA protein can be released from cells as part of extracellular vesicles (EVs) (Phelps et al., 1986). Since EVs are submicron in size (tens to hundreds of nanometers), these vesicles can pass through a 0.22 µm filter.

All biological INPs outside of the Gammaproteobacteria pass through 0.22 µm filters. The INPs produced by the INA isolate of the genus *Lysinibacillus* are moreover very heat stable (remaining partially active even after an hour of boiling), are proteinase resistant, and do not pass through a 100 kDa protein filter with a pore size of approximately 50 nm. This suggests that they consist of relatively large non-proteinaceous secreted single molecules or stable aggregates of multiple smaller molecules (Failor et al., 2017). Fungal INPs are also mostly retained by 100 kDa filters, suggesting that they also consist of large secreted single molecules or of large aggregates of smaller molecules (Fröhlich-Nowoisky et al., 2015; O'Sullivan et al., 2015). Many fungal INPs are heat stable up to 60 °C but do not resist boiling and are variably susceptible to proteinases, and are thus believed to be mostly proteinaceous (Fröhlich-Nowoisky et al., 2015; O'Sullivan et al., 2015). Pollen INPs are mostly retained by 100 kDa filters, are heat and proteinase resistant (Pummer et al., 2012), and have been proposed to consist of polysaccharides of various chain lengths (Dreischmeier et al., 2017).

Now that all these biological INPs have been identified, and at least partially characterized, we decided to go back to one of the original leaf litter samples, the aspen (*Populus tremuloides*) leaf litter sample 70-S-14, which had been collected by Russell Schnell in 1970 and whose characterization in regard to its INA was published in 1976 (Schnell and Vali, 1976). Intriguingly, sample 70-S-14 maintained a high concentration of INA until today, 48 years after collection, while being stored at room temperature. INPs in this sample had been found to be less than 0.1 µm in size (Schnell and Vali, 1976). Therefore, *Pseudomonas* species can be excluded as the main source of INPs in this sample. While the INPs in the sample could be of plant origin (Pummer et al., 2015), here we tested the specific hypothesis that the INPs in this leaf litter sample were produced by strains of *Lysinibacillus* or by other bacteria or fungi. To test this hypothesis, we performed a comprehensive characterization of sample 70-S-14 in regard to its content in INPs and the composition of its microbial community.

2 Materials and methods

2.1 Description of leaf litter sample 70-S-14 and how it has been stored

Leaf litter sample 70-S-14 was collected in a grove of *Populus tremuloides* (aspen) about 1.5 km west of Penhold Airbase, Alberta, now known as Red Deer Regional Airport (52.1762° N, 113.8870° W) in the summer of 1970. Even though the dominant litter in the grove was from decaying aspen leaves that fell the previous fall, there were decaying leaves from shrubs and grasses that also were collected in the sample. To collect the litter, handfuls of litter were grasped in a 1 m square to fill a garbage bag. The litter was somewhat moist as it was collected in the shade of the trees. The grove is still there and litter samples from the same area have been collected many times over the intervening decades, most recently in June 2018. The litter still produces prodigious numbers of ice nuclei with freezing activity beginning at −4.5 to −5 °C in concentrations of 10^8 g^{-1} of leaf litter active at −10 °C.

Some months after collection, when the litter sample was dry, the twigs and other large debris were removed. Some of the cleaned sample was ground to a fine powder in a fluid energy mill and used for INA tests in the Colorado State University Cloud Chamber, where it proved to produce excellent INPs (Schnell and Vali, 1976, Fig. 3).

Sample 70-S-14 was stored at room temperature inside four different plastic bags and a paper bag from 1970 to 2018 at room temperature open to the air in Schnell's various offices and a portion mailed to Virginia Tech in 2017. At Virginia Tech, it was again stored at room temperature in a paper bag until processing.

2.2 Characterization of the cumulative ice nucleation spectrum of leaf litter sample 70-S-14

One gram of leaf litter was added to 100 mL double-distilled water (DDW) in a 250 mL flask under sterile conditions and stirred for 5 min. The leaf litter suspension was passed through 2.5 µm pore size Whatman cellulose filter paper (GE

Healthcare, USA) to remove large leaf fragments. This primary suspension was then subject to 10 different treatments (see Fig. 1), and INA was tested after each treatment to determine the main characteristics of the INPs present in the leaf litter sample.

In short, 10 mL of the primary suspension was passed through a 0.22 µm filter (Supor® 200 PES membrane Disc Filter, PALL, USA) to select for sub-micron INPs in the filtrate and for intact bacterial and fungal cells in the 0.22 µm filter retentate resuspended from the filter in the same volume (10 mL); 5 mL of the 0.22 µm filtrate was then passed through a 100 kDa protein filter with an approximate pore size of 50 nm (Macrosep Advance Centrifugal Device with 100 K MWCO, PALL, USA) for 10 min at 5000 rpm to separate sub 50 nm sized INPs in the 100 kDa filter filtrate from INPs larger than 50 nm in the 100 kDa filter retentate resuspended in the same volume (5 mL). Portions of the original suspension and of each filtrate and retentate were also boiled for 15 min to determine the heat sensitivity of each fraction.

Dilutions from 10^{-1} to 10^{-6} were made after each of the 10 treatments to obtain cumulative ice nucleation spectra. Thirty drops of 20 µL of each dilution were tested at −8, −10, and −12 °C, maintaining each temperature for 10 min on parafilm boats floating in a cryobath (Lauda Alpha RA24, LAUDA-Brinkmann, Delran, NJ, USA). The entire assay, including the 10 treatments, was repeated three times. The concentration of ice nuclei was calculated using the approach developed by Vali (1971) and described previously (Failor et al., 2017).

2.3 Culture-independent microbial community analysis

To determine the overall composition of the fungal and bacterial communities in the leaf litter sample, a culture-independent approach was used first. The primary leaf litter suspension described above was vacuum-filtered through a 0.22 µm pore-size filter membrane (Supor® 200 PES membrane Disc Filter, PALL, USA). DNA was extracted from the filter with the DNeasy PowerWater kit (Qiagen, USA) according to the manufacturer's protocol. DNA concentration and quality were evaluated by UV spectrophotometry (NanoDrop 1000, Thermo, USA) and visualized on a 1 % agarose gel.

For bacterial community analysis, the V4 hypervariable region of the 16S rRNA gene was amplified and sequenced using barcoded versions of the primers 799F (anti-chloroplast, 5′AACMGGATTAGATACCCKG3′) and 1115R ("universal", 5′AGGGTTGCGCTCGTTG3′). For fungal community analysis, the ITS 2 region was amplified and sequenced using primers ITS9_F (GAACGCAGCRAAIIGYGA) and ITS4_R (TCCTCCGCTTATTGATATGC). All steps from PCR to paired-end (2 × 300 bp) amplicon sequencing on the Illumina MiSeq platform were performed at Molecular Research LP (MR DNA™, Shallowater, TX, USA).

Paired-end sequences were joined together into a single-sequence read. Quality trimming was performed and barcodes and primer sequences were depleted. Then, sequences shorter than 200 bp, sequences with ambiguous base calls, and sequences containing homopolymers longer than 6 bp were removed. High-quality sequences were processed using the Quantitative Insights into Microbial Ecology (QIIME) bioinformatic pipeline (Caporaso et al., 2010). Operational taxonomic units (OTUs) were assigned using an open-reference approach with a threshold of 97 % sequence similarity. OTU picking and taxonomy assignment were performed using UCLUST and the SILVA database (Quast et al., 2013). Fungal ITS paired-end sequences were processed as described above, but UNITE was used as the database instead (Abarenkov et al., 2010).

All OTUs assigned to mitochondria or chloroplasts and OTUs with fewer than five reads were excluded from further analysis. QIIME-generated output files were imported to R for data visualization using the Phyloseq 1.19.1 (McMurdie and Holmes, 2013) and ggplot 2 2.2.1 (Wickham, 2009) packages.

2.4 Culture-dependent bacterial community analysis

In parallel to the culture-independent approach, bacteria were cultured to determine the composition of the bacterial community present in the leaf litter sample using a culture-dependent approach. The undiluted primary leaf litter suspension described above and 10^{-1} and 10^{-2} dilutions were plated on R2A (Reasoner and Geldreich, 1985). Agar plates were incubated at 28 °C for 2–7 days. After incubation, the bacterial population was estimated by counting the bacterial colonies. This assay was performed three times. One hundred colonies were randomly selected for identification using PCR followed by Sanger sequencing of the V4 hypervariable region of the 16S rRNA gene as described previously (Failor et al., 2017).

2.5 INA testing of individual bacterial colonies

The suspensions and 10^{-1} and 10^{-2} dilutions described above were plated on R2A and LEM (Lysinibacillus enrichment medium). LEM is based on a medium originally developed for *Lysinibacillus sphaericus* (Russell et al., 1989) to enrich for *Lysinibacillus* strains similar to the *Lysinibacillus* strain with INA that we recently isolated (Failor et al., 2017). LEM contains, per liter, Na_2HPO_4, 5.57 g; KH_2PO_4, 2.4 g; $(NH_4)_2SO_4$, 2.0 g; $MgSO_4 \cdot 7H_2O$, 50 mg; $MnCl_2 \cdot 4H_2O$, 4.0 mg; $FeSO_4 \cdot 7H_2O$, 810 µg; $CaCl_2 \cdot 2H_2O$, 1.5 mg; H_2SO_4, 0.3 µL; sodium acetate, 321.8 mg; thiamine 200 mg; and biotin, 20 µg. After incubation for up to 7 days, random bacterial colonies were resuspended in 140 µL of DDW. Five drops of 20 µL from each colony suspension were tested for INA at −8, −10, and −12 °C. Colonies for which at least one drop froze at any of the used temperatures were streaked

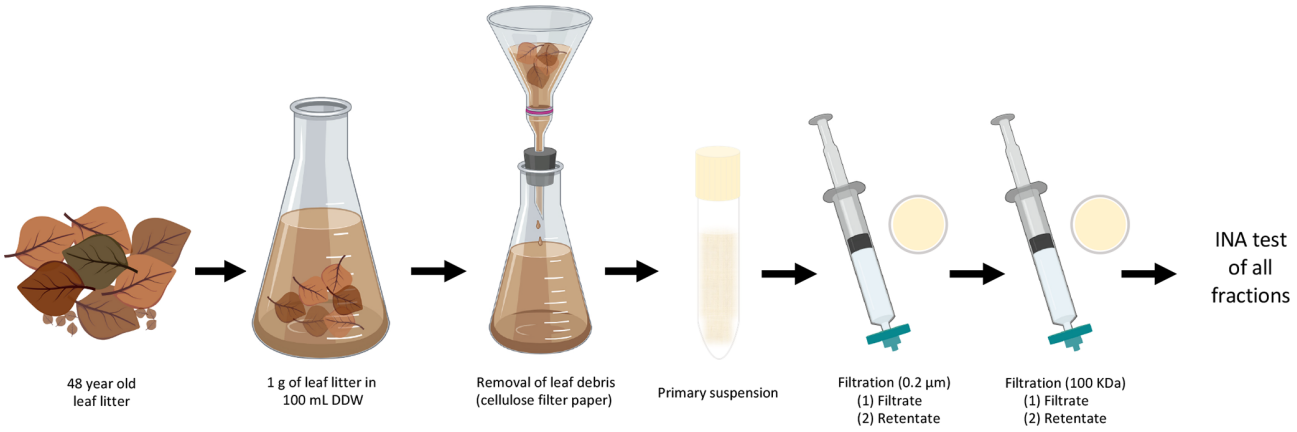

Figure 1. Characterization of INA of aspen leaf litter 70-S-14. For each biological replicate ($n = 4$), 1 g of aspen leaf litter was suspended in 100 mL of sterile double-stilled water (DDW). The primary suspension, the filtrates, and the filter retentates of the 0.22 µm and the 100 kDa filters were tested for INA. In parallel, a portion of the primary suspension and of both filtrates and of both filter retentates were boiled for 15 min and tested for INA as well.

onto new plates. Bacterial suspensions were made from the new plates for these colonies and five drops were tested for INA a second time. If still positive, a third test was performed similarly to the one described to determine the cumulative ice nucleation spectrum of leaf litter 70-S-14: 30 drops of 20 µL each were tested for each dilution of a 10^{-1} to 10^{-6} dilution series starting with a bacterial suspension of approximately 1×10^8 cfu mL^{-1}. The same was done to determine the cumulative ice nucleation spectrum of *Pa. ananatis* BAV 3057. In this case, a bacterial suspension at a concentration of 3.2×10^8 cfu mL^{-1} was used as a starting suspension for the 10^{-1} to 10^{-6} dilution series and the bacterial starting suspension was subject to the same differential filtration and boiling combinations as the leaf litter.

2.6 INA testing of individual fungal colonies

Ten-fold serial dilutions were made from the primary suspension described above. One hundred microliters of each dilution was plated on potato dextrose agar (PDA) medium supplemented with either streptomycin (20 mg L^{-1}) or lactic acid (0.1 %) to restrict bacterial growth. Plates were incubated for 7 days at room temperature. Single fungal colonies were transferred to new PDA plates supplemented with streptomycin (20 mg L^{-1}) and kept at room temperature. For all fungal isolates, mycelium was scraped off the agar and resuspended in 1 mL of DDW, and three drops of 20 µL from each fungal suspension were tested for INA as described above. Fungal isolates for which at least one drop froze at any of the used temperatures were transferred to new PDA plates. To confirm INA in fungal isolates, new PDA plates were used to prepare fungal suspensions, and 30 drops were tested for a second time. For the cumulative ice nucleation spectrum of *M. alpina* strain LL118, 1 mg of mycelium was scraped off the PDA plates and suspended in 10 ml of DDW and processed as described in the protocol used for the cumulative ice nucleation spectrum of leaf litter 70-S-14.

3 Results

3.1 Aspen leaf litter 70-S-14 contains mostly INPs of submicron size that are heat-sensitive

Differential filtration using a 0.22 µm pore size filter and a 100 kDa filter (approximately 50 nm pore size) in combination with boiling each filtrate and each retentate allowed us to determine the concentration of total INPs in the leaf litter sample and their approximate size and heat sensitivity (Fig. 2). While there was no detectable INA at −6 °C and above, INA increased strongly when the temperature was lowered to −8 °C, at which temperature we detected 10^6 INPs per gram of leaf litter. The concentration of active INPs increased further to almost 10^7 g^{-1} at −10 and −12 °C. Based on the earlier tests of ice nucleation on less aged 70-S-14 by Schnell and Vali (1976), it appears that the sample of 70-S-14 lost between 1 and 1.5 °C of threshold ice nucleation activity and 2 orders of magnitude in total INP concentration active at −10 to −12 °C over the 48 years of storage.

Almost all INPs passed through the 0.22 µm filter, confirming the results obtained by Schnell in 1976 that leaf litter mostly contains sub-micron INPs (Schnell and Vali, 1976). The retentate of the 0.22 µm filter further confirmed this result since it had a very low concentration of INPs, approximately only 1/100 to 1/1000 of the 0.22 µm filtrate. Boiling the original unfiltered suspension and the 0.22 µm filtrate also reduced the INP concentration to 1/100 to 1/1000, revealing that the majority of INPs present in the leaf litter is heat sensitive. Further, passing the 0.22 µm filtrate through the 100 kDa filter reduced the concentration of INPs active at −8 °C approximately 100-fold, while the concentration of INPs active

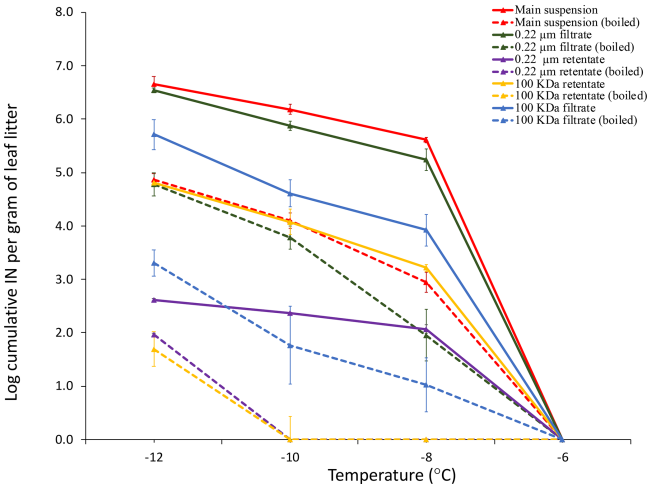

Figure 2. Cumulative ice nucleation spectra of aspen leaf litter sample 70-S-14. Results are shown for all fractions shown in Fig. 1 based on droplet freezing assays at −6, −8, −10, and −12 °C. IN: ice nuclei.

at −12 °C was reduced approximately 10-fold. This suggests that the majority of sub-micron INPs is larger than 50 nm and that a fraction of INPs is even smaller than 50 nm. Intriguingly, resuspensions of the INPs from the 100 kDa filter revealed a concentration of INPs in the 100 kDa filter retentate that was even lower than that in the 100 kDa filtrate. This could be due to the majority of INPs strongly binding to the filter. When the filtrate and the retentate of the 100 kDa filter were boiled, INPs active at −8 °C were almost completely abolished, further confirming the heat sensitivity of the majority of the INPs present in the 70-S-14 leaf litter sample. In summary, the majority of INPs in aspen leaf litter sample 70-S-14 consisted of heat-sensitive sub-micron-sized particles, with some being smaller than 50 nm and some being larger than 50 nm.

3.2 Microbial population analysis of the aspen leaf litter 70-S-14 sample reveals few known microbial taxa with INA

To identify the possible biological origin of the INPs present in the aspen leaf litter sample, the overall composition of the bacterial and fungal communities was determined. This was done using a culture-independent analysis by extracting DNA followed by amplification and sequencing of the bacterial V4 hypervariable region of the 16S rRNA gene and the fungal ITS region (Fig. 3).

The main bacterial phyla found in the leaf litter were Proteobacteria (49 %), Actinobacteria (34 %), and Bacteroidetes (16 %). Within the Proteobacteria, the genera *Pseudomonas* (13 %) and *Sphingomonas* (12 %) were the most common. Within the Actinobacteria, the most common taxon was an undescribed genus in the Microbacteriaceae family (22 %). Within the Bacteroidetes, *Flavobacterium* was the most common genus (14 %). Therefore, of all bacterial taxa known to include strains with INA, only the genus *Pseudomonas* was identified in this culture-independent approach. The fungal leaf litter community contained 42 % Basidiomycota, 38 % unidentified phyla, and 20 % Ascomycota. The most common genus was identified as *Cystofilobasidium* (14 %), followed by *Venturia, Ceratobasidium*, and unidentified genera in the Helotiales and Tremellomycetes. None of these genera is known to include INA strains.

For bacteria, we also performed a culture-dependent analysis by plating the primary suspension on R2A medium. Based on colony counts, the leaf litter contained a total of 3.13×10^5 colony-forming units (CFUs) per gram of leaf litter. One hundred random colonies were then selected for partially sequencing the 16S rRNA gene. This analysis revealed the presence of bacterial genera that are known to include strains with INA. In fact, not only were 22 % of colonies identified as *Pseudomonas*, but 1 % each were identified as *Pantoea* and *Erwinia*. However, the genus with the highest number of colonies (34 %), the Gram-positive bacterium *Clavibacter*, is not known to include any strains with INA.

3.3 INA testing of bacterial and fungal colonies reveals presence of INA strains belonging to the bacterial species *P. syringae* and *Pa. ananatis* strains and the fungal species *M. alpina*

To determine whether any culturable bacteria or fungi with INA were still present in the aspen leaf litter 48 years after collection, a total of 1170 bacterial colonies either grown on R2A (881 colonies, including the 100 colonies described above) or LEM (289 colonies) and 277 fungal isolates grown on PDA were tested for INA.

Only three bacterial colonies were found to have stable INA in all tests starting at −8 °C or above. The strains were identified by sequencing the hypervariable V4 region of the 16S rRNA gene. Two strains were identified as members of the genus *Pantoea*: one strain had 99 % DNA identity over 935 nt with *P. ananatis* strain Ta030 (NCBI accession number MH973238), and the other strain had 99 % DNA identity over 922 nt with *P. ananatis* strain 12WE (NCBI accession number MH010898.1). The third strain was identified as a member of the genus *Pseudomonas* since it had 99 % DNA identity over 794 nt with *P. syringae* pv. *syringae* strain CFBP4215 (NCBI accession number LT962480).

Among the fungal isolates, only one was found to have stable INA at −8 °C. This isolate, LL118, was identified as a member of the species *M. alpina* based on ITS sequencing since it had 99 % identity to *M. alpina* isolate F08ID36 with NCBI accession number KJ469836.1 (Fröhlich-Nowoisky et al., 2015).

Unfortunately, the *P. syringae* and the two *Pa. ananatis* strains isolated from the leaf litter were lost and could not be further characterized. However, we characterized the cumulative ice nucleation spectrum of *M. alpina* LL118 and

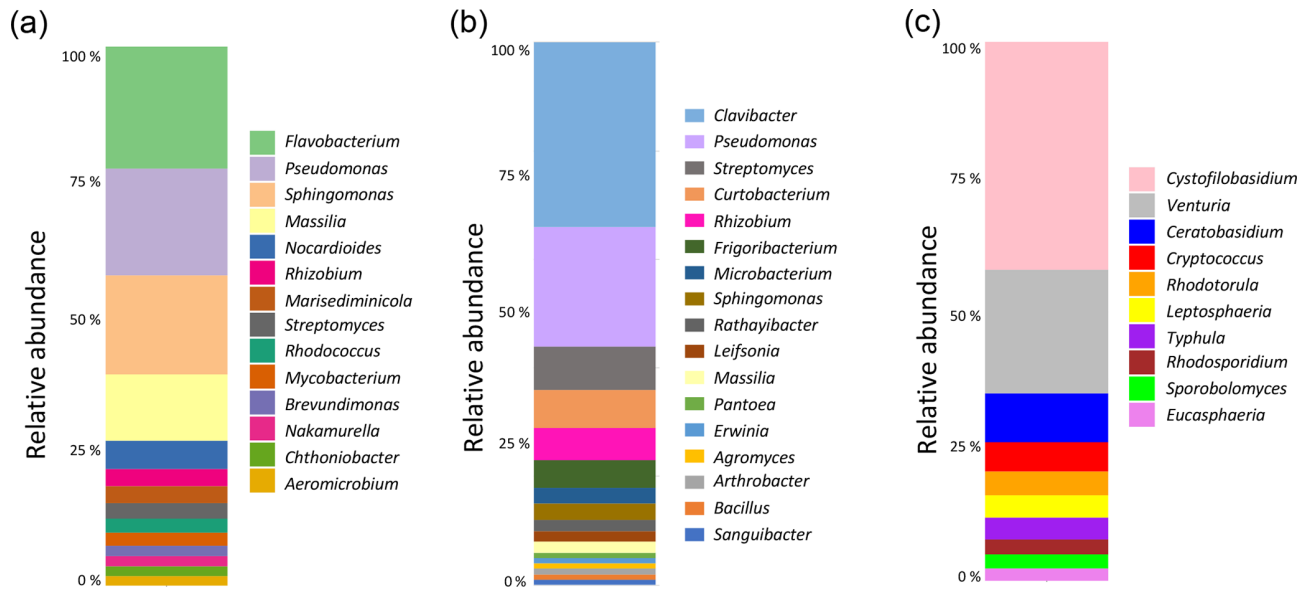

Figure 3. Analysis of the composition of the microbial communities present in aspen leaf litter sample 70-S-14. **(a)** Results from the culture-independent analysis of bacterial diversity based on the V4 hypervariable region of the 16S rRNA gene (considering only classified reads); **(b)** results from sequencing the V4 hypervariable region of the 16S rRNA gene of 100 bacterial isolates cultured from sample 70-S-14; **(c)** results from the culture-independent analysis of fungal diversity based on the ITS region (considering only classified reads).

of *Pa. ananatis* strain BAV 3057, which was previously isolated from rain (Failor et al., 2017) and that had over 99 % identity in its 16S rRNA sequence to one of the two *Pa. ananatis* strains from leaf litter. Because of the genetic similarity of *Pa. ananatis* BAV 3057 to the two *Pa. ananatis* strains isolated from the leaf litter, these strains can be expected to have very similar cumulative ice nucleation spectra. The number of INPs produced by *M. alpina* LL118 per gram of fungal mycelium active at −7 °C (the temperature at which drops of the fungal suspension started freezing) was approximately 1×10^6. The number of INPs produced by *Pa. ananatis* BAV 3057 at −5 °C (the temperature at which drops of the bacterial suspension started freezing) was approximately 1×10^{-5} per CFU. Figure 4 shows how the INPs produced by both *M. alpina* LL118 and *Pa. ananatis* BAV 3057 were mostly heat sensitive. *M. alpina* INPs were all sub-micron in size, and the majority could be resuspended from the 100 kDa filter and were thus larger than 50 nm. Only approximately 1/10 of *M. alpina* INPs passed through the 100 kDa filter and were thus smaller than 50 nm. *Pa. ananatis* INPs active at −5 °C were mostly larger than 0.22 µm, but 1/10th of the *Pa. ananatis* INPs active at −9 °C and below were sub-micron in size. Similarly to *M. alpina*, more of the sub-micron INPs could be resuspended from the 100 kDa filter (and were thus larger than 50 nm) compared to the INPs that passed through the filter (and were thus smaller than 50 nm).

4 Discussion and conclusions

Aspen leaf litter sample 70-S-14 (Schnell and Vali, 1976) collected in 1970 has maintained remarkable INA over 48 years. When its characterization was first published in 1976, *P. syringae* was the only known organism with INA, and microbial community analysis was not possible. Therefore, with the discovery of many bacterial, fungal, and plant INA organisms since then and today's ease in determining the composition of microbial communities, this historic sample represented a great opportunity for re-evaluation of its INA and identification of the INA organisms possibly still present and alive in this sample 48 years later.

The cumulative INP spectra of 70-S-14 obtained after a combination of filtration and boiling (Fig. 2) clearly confirmed the result obtained in 1976 by Russel Schnell; i.e., the INPs in this sample are submicron in size. Moreover, the INPs were found to be mostly heat sensitive. Since INPs produced by *Lysinibacillus* are heat resistant (Failor et al., 2017), this result excludes our initial hypothesis that recently discovered INA strains of the genus *Lysinibacillus* might have contributed to the INA of 70-S-14. Also, heat-resistant INPs produced by pollen can be excluded (Pummer et al., 2012).

The result is more in line with secreted INPs produced by strains of the species *Erwinia herbicola* (Phelps et al., 1986), of which most strains were later assigned to the species *Pantoea ananatis* (Walterson and Stavrinides, 2015), and with INPs secreted by fungi in the genus *Fusarium* (O'Sullivan et al., 2015) and in the species *M. alpina* (Fröhlich-Nowoisky et al., 2015). In fact, *Erwinia*/*Pantoea* cells secrete some of

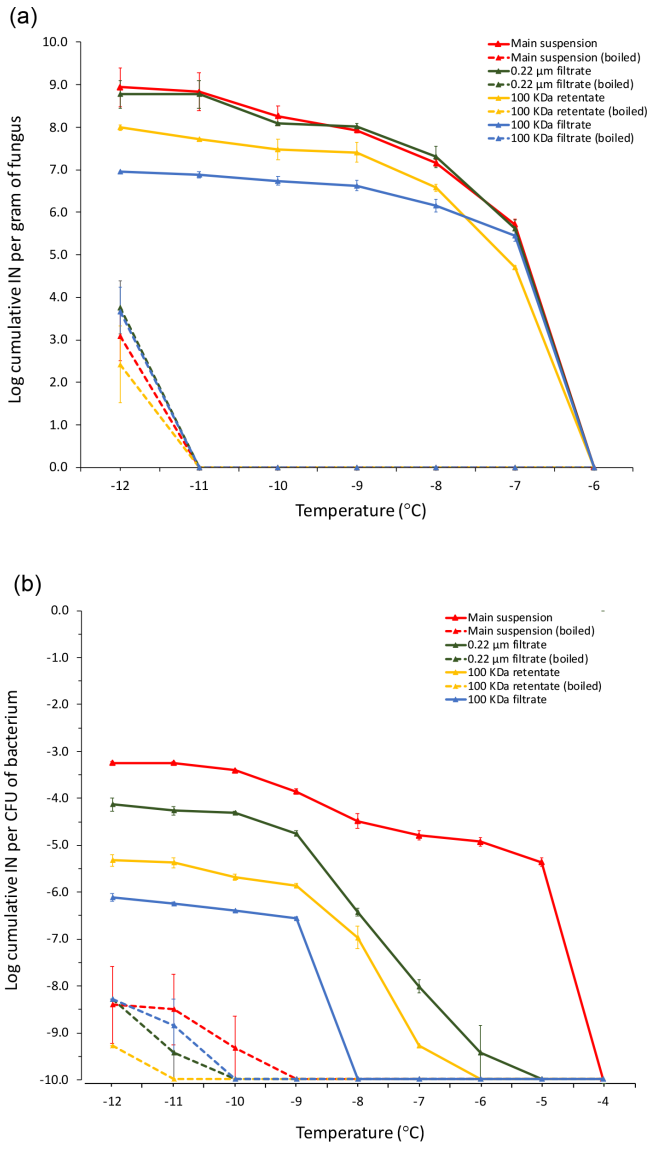

Figure 4. Cumulative ice nucleation spectra of *M. alpina* strain LL118 **(a)** isolated from leaf litter sample 70-S-14 and of *Pa. ananatis* BAV 3057 **(b)** isolated from rain, but over 99 % identical in its 16S rRNA sequence to the two *Pa. ananatis* isolates from sample 70-S-14. The analyzed fractions derived from differential filtration and boiling are the same as shown in Fig. 1; besides that, instead of leaf litter, suspensions of *M. alpina* mycelium and of a *Pa. ananatis* bacterial suspension were used as the starting material. IN: ice nuclei; CFU: colony-forming units.

the heat-sensitive INA protein as part of sub-micron EVs (Phelps et al., 1986), and *F. avenaceum* and *M. alpina* both secrete sub-micron INPs that are heat sensitive and probably proteinaceous in nature (Fröhlich-Nowoisky et al., 2015; O'Sullivan et al., 2015). However, culture-independent analysis of 70-S-14 did not identify any of these organisms, although a high number of bacterial 16S rRNA fragments (131 796 sequencing reads) and a high number of fungal ITS fragments (153 546 sequencing reads) were sequenced. This suggests that these known INA organisms are very minor constituents of the overall microbial communities present in this leaf litter sample.

Interestingly though, sequencing a 16S rRNA fragment of a relatively small number (100) of randomly selected bacterial colonies cultured from 70-S-14 did reveal the presence of *Pantoea/Erwinia* (two colonies). This may be due to enrichment of this genus by the employed culture media. Even more importantly, two *Pa. ananatis* isolates and one *M. alpina* isolate with INA were identified among the 1170 bacterial colonies and the 277 fungal isolates, respectively. The cumulative ice nucleation spectra of *M. alpina* and *Pa. ananatis* fit the overall ice nucleation spectrum of 70-S-14 (with the majority of INPs being heat sensitive and in large part sub-micron in size) and are thus likely sources of the INPs present in 70-S-14.

Only one *Pseudomonas* strain with INA was isolated from 70-S-14. However, the typical ice nucleation spectrum of *Pseudomonas* species does not fit the leaf litter ice nucleation spectrum since most *Pseudomonas* species do not shed the INA protein as part of EVs, but INA of *Pseudomonas* species is generally associated with intact bacterial cells that do not pass through 0.22 µm pores (Failor et al., 2017; Maki et al., 1974; Morris et al., 2004).

Although *Mortierella* was the only fungal genus with known INA identified in 70-S-14, it is possible that other fungal strains are present (and culturable) at very low frequency in this leaf litter sample since the number of tested fungal isolates was relatively low (277 isolates). Therefore, we cannot exclude that testing additional fungal isolates could reveal the presence of additional fungal genera with INA in 70-S-14. However, it is also possible that some INPs still present in the leaf litter sample 48 years after collection were originally produced by bacteria or fungi that are not culturable anymore and whose DNA has been degraded. In fact, it is interesting that the number of INPs produced per gram of pure culture of the isolated *M. alpina* strain is approximately only 100-fold larger than the number of INPs produced per gram of leaf litter. Considering that the total mass of fungi and bacteria with INA in the leaf litter probably constitutes only a very minor fraction of the total leaf mass in the leaf litter sample suggests that organisms that produce a very high number of INPs must have been present in the leaf litter sample at some point. It is also possible that *M. alpina*, *Pa. ananatis*, and other INA organisms produced a much higher number of INPs when they originally grew in the decaying leaves compared to the number of INPs that they produced when grown on nutrient-rich agar plates in our lab before INA testing. In fact, low nutrient availability has been shown to increase production of INPs by various INA bacteria (Failor et al., 2017).

One important question in regard to the present study is whether the presence of viable *Pa. ananatis* and *M. alpina* isolates in a leaf litter sample that has maintained INA for 48 years strengthens the evidence for a role of these organ-

isms as a source of atmospheric INPs. Several lines of evidence support a positive answer to this question. In fact, 106 strains among 593 strains found to have stable INA among 33 134 strains isolated from precipitation and tested for INA in our previous work (Failor et al., 2017) were identified as *Pantoea* or *Erwinia* as well. Also, Du and colleagues recently found submicron INPs in precipitation (Du et al., 2017). Although *Pantoea*/*Erwinia* and *M. alpina* and other known organisms with INA could not be identified in the analyzed precipitation samples using a culture-independent approach (Du et al., 2017), these organisms could still be present since we could not identify them in our culture-independent leaf litter analysis either, but we still found them by culturing. Finally, Conen and Yakutin recently identified a large fraction of heat-sensitive submicron INPs in soils from various continents (Conen and Yakutin, 2018). Since these INPs were only inactivated through boiling but not by incubation at 60 °C, they are more likely of fungal origin than a product of *Erwinia*/*Pantoea*. In conclusion, we think that combining the results from these recent studies with our new finding that 70-S-14 still contains viable *Pa. ananatis* and *M. alpina* with INA supports a role of these organisms as important sources of atmospheric INPs. Importantly, finding that heat-sensitive sub-micron INPs are still active after 48 years in leaf litter suggests that leaf litter might represent an important reservoir of atmospheric INPs. The relative importance of leaf litter compared to live plants and soil as a contributor to the atmospheric pool of INPs is thus an important question that warrants further investigation.

What we could not do in the present study and what we could not do in our previous study of bacterial sources of INPs in precipitation (Failor et al., 2017) was to directly determine the presence of different genes coding for different biological INPs in metagenomic sequences. In fact, while direct culture-independent metagenomic sequencing of environmental samples is possible today (Behzad et al., 2015), the limitation is that the only gene known so far to encode a biological molecule with INA is the INA gene of the Gammaproteobacteria, including *Pseudomonas* species, *Xanthomonas* species, and *Pantoea*/*Erwinia* species (Edwards et al., 1994). Identifying the genetic basis of biological INPs produced by additional bacteria and by fungi would instead allow determination of the presence of all these various INA genes in environmental samples, such as soil, plants, leaf litter, precipitation, and even clouds. Comparison of presence and abundance of various INA genes between samples could in turn help infer the migration of microbes with INA among environments and their relative contribution to atmospheric INPs.

Data availability. All data is is available upon request from the corresponding author.

Author contributions. YV and MEML performed the experiments and analyzed the data with the help and advice of RH for fungal isolation and culturing. DGS contributed expertise during the interpretation of the data. RS provided the leaf litter and suggested the overall research project. BAV, with the help of YV and MEML, developed the experimental plan and wrote the manuscript. All authors read and edited the manuscript. YV and MEML contributed equally to this work.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. This research was supported by the National Science Foundation under grant IOS-1754721. Funding to Boris A. Vinatzer and David G. Schmale III was also provided in part by the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture, US Department of Agriculture.

Review statement. This paper was edited by Kees Jan van Groenigen and reviewed by Cindy Morris and one anonymous referee.

References

Abarenkov, K., Henrik Nilsson, R., Larsson, K.-H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Ursing, B. M., Vrålstad, T., Liimatainen, K., Peintner, U., and Kõljalg, U.: The UNITE database for molecular identification of fungi – recent updates and future perspectives, New Phytol., 186, 281–285, https://doi.org/10.1111/j.1469-8137.2009.03160.x, 2010.

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, https://doi.org/10.1038/262282a0, 1976.

Behzad, H., Gojobori, T., and Mineta, K.: Challenges and Opportunities of Airborne Metagenomics, Genome Biol. Evol., 7, 1216–1226, https://doi.org/10.1093/gbe/evv064, 2015.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R.: QIIME allows analysis of high-throughput community sequencing data, Nat. Method., 7, 335–336, https://doi.org/10.1038/nmeth.f.303, 2010.

Coluzza, I., Creamean, J., Rossi, M., Wex, H., Alpert, P., Bianco, V., Boose, Y., Dellago, C., Felgitsch, L., Fröhlich-Nowoisky, J., Herrmann, H., Jungblut, S., Kanji, Z., Menzl, G., Moffett, B., Moritz, C., Mutzel, A., Pöschl, U., Schauperl, M., Scheel, J., Stopelli, E., Stratmann, F., Grothe, H., and Schmale, D.: Perspectives on the Future of Ice Nucleation Research: Research Needs and Unanswered Questions Identified from Two International Workshops, Atmosphere, 8, 138, https://doi.org/10.3390/ijerph13010019, 2017.

Conen, F. and Yakutin, M. V.: Soils rich in biological ice-nucleating particles abound in ice-nucleating macromolecules likely produced by fungi, Biogeosciences, 15, 4381–4385, https://doi.org/10.5194/bg-15-4381-2018, 2018.

Diehl, K., Quick, C., Matthias-Maser, S., Mitra, S. K., and Jaenicke, R.: The ice nucleating ability of pollen: Part I: Laboratory studies in deposition and condensation freezing modes, Atmos. Res., 58, 75–87, https://doi.org/10.1016/S0169-8095(01)00091-6, 2001.

Dreischmeier, K., Budke, C., Wiehemeier, L., Kottke, T., and Koop, T.: Boreal pollen contain ice-nucleating as well as ice-binding "antifreeze" polysaccharides, Sci. Rep., 7, 41890, https://doi.org/10.1038/srep41890, 2017.

Du, R., Du, P., Lu, Z., Ren, W., Liang, Z., Qin, S., Li, Z., Wang, Y., and Fu, P.: Evidence for a missing source of efficient ice nuclei, Sci. Rep., 7, 39673, https://doi.org/10.1038/srep39673, 2017.

Edwards, A. R., Van den Bussche, R. A., Wichman, H. A., and Orser, C. S.: Unusual pattern of bacterial ice nucleation gene evolution, Mol. Biol. Evol., 11, 911–920, https://doi.org/10.1093/oxfordjournals.molbev.a040172, 1994.

Failor, K. C., Schmale, D. G., 3rd, Vinatzer III, B. A., and Monteil, C. L.: Ice nucleation active bacteria in precipitation are genetically diverse and nucleate ice by employing different mechanisms, ISME J., 11, 2740–2753, https://doi.org/10.1038/ismej.2017.124, 2017.

Fröhlich-Nowoisky, J., Hill, T. C. J., Pummer, B. G., Yordanova, P., Franc, G. D., and Pöschl, U.: Ice nucleation activity in the widespread soil fungus *Mortierella alpina*, Biogeosciences, 12, 1057–1071, https://doi.org/10.5194/bg-12-1057-2015, 2015.

Huffman, J. A., Prenni, A. J., DeMott, P. J., Pöhlker, C., Mason, R. H., Robinson, N. H., Fröhlich-Nowoisky, J., Tobo, Y., Després, V. R., Garcia, E., Gochis, D. J., Harris, E., Müller-Germann, I., Ruzene, C., Schmer, B., Sinha, B., Day, D. A., Andreae, M. O., Jimenez, J. L., Gallagher, M., Kreidenweis, S. M., Bertram, A. K., and Pöschl, U.: High concentrations of biological aerosol particles and ice nuclei during and after rain, Atmos. Chem. Phys., 13, 6151–6164, https://doi.org/10.5194/acp-13-6151-2013, 2013.

Kieft, T. L.: Ice Nucleation Activity in Lichens, Appl. Environ. Microbiol., 54, 1678–1681, 1988.

Kim, H. K., Orser, C., Lindow, S. E., and Sands, D. C.: *Xanthomonas campestris* pv. *translucens* strains active in ice nucleation, Plant Dis., 71, 994–997, 1987.

Maki, L. R., Galyan, E. L., Chang-Chien, M.-M., and Caldwell, D. R.: Ice Nucleation Induced by *Pseudomonas syringae*, Appl. Microbiol., 28, 456–459, 1974.

McMurdie, P. J. and Holmes, S.: phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data, PLOS ONE, 8, e61217, https://doi.org/10.1371/journal.pone.0061217, 2013.

Morris, C. E., Georgakopoulos, D. G., and Sands, D. C.: Ice nucleation active bacteria and their potential role in precipitation, J. Phys. IV France, 121, 87–103, 2004.

Morris, C. E., Monteil, C. L., and Berge, O.: The Life History of *Pseudomonas syringae*: Linking Agriculture to Earth System Processes, Annu. Rev. Phytopathol., 51, 85–104, https://doi.org/10.1146/annurev-phyto-082712-102402, 2013.

Morris, C. E., Conen, F., Alex Huffman, J., Phillips, V., Pöschl, U., and Sands, D. C.: Bioprecipitation: a feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere, Glob. Change Biol., 20, 341–351, https://doi.org/10.1111/gcb.12447, 2014.

Mülmenstädt, J., Sourdeval, O., Delanoë, J., and Quaas, J.: Frequency of occurrence of rain from liquid-, mixed-, and ice-phase clouds derived from A-Train satellite retrievals, Geophys. Res. Lett., 42, 6502–6509, https://doi.org/10.1002/2015GL064604, 2015.

O'Sullivan, D., Murray, B. J., Ross, J. F., Whale, T. F., Price, H. C., Atkinson, J. D., Umo, N. S., and Webb, M. E.: The relevance of nanoscale biological fragments for ice nucleation in clouds, Sci. Rep., 5, 8082, https://doi.org/10.1038/srep08082, 2015.

Phelps, P., Giddings, T. H., Prochoda, M., and Fall, R.: Release of cell-free ice nuclei by *Erwinia herbicola*, J. Bacteriol., 167, 496–502, 1986.

Pouleur, S., Richard, C., Martin, J.-G., and Antoun, H.: Ice Nucleation Activity in *Fusarium acuminatum* and *Fusarium avenaceum*, Appl. Environ. Microbiol., 58, 2960–2964, 1992.

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, https://doi.org/10.5194/acp-12-2541-2012, 2012.

Pummer, B. G., Budke, C., Augustin-Bauditz, S., Niedermeier, D., Felgitsch, L., Kampf, C. J., Huber, R. G., Liedl, K. R., Loerting, T., Moschen, T., Schauperl, M., Tollinger, M., Morris, C. E., Wex, H., Grothe, H., Pöschl, U., Koop, T., and Fröhlich-Nowoisky, J.: Ice nucleation by water-soluble macromolecules, Atmos. Chem. Phys., 15, 4077–4091, https://doi.org/10.5194/acp-15-4077-2015, 2015.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O.: The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucl. Acids Res., 41, D590–D596, https://doi.org/10.1093/nar/gks1219, 2013.

Reasoner, D. J. and Geldreich, E. E.: A new medium for the enumeration and subculture of bacteria from potable water, Appl. Environ. Microbiol., 49, 1–7, 1985.

Richard, C., Martin, J. G., and Pouleur, S.: Ice nucleation activity identified in some phytopathogenic *Fusarium* species, Phytoprotection, 77, 83–92, https://doi.org/10.7202/706104ar, 1996.

Russell, B. L., Jelley, S. A., and Yousten, A. A.: Carbohydrate metabolism in the mosquito pathogen *Bacillus sphaericus* 2362, Appl. Environ. Microbiol., 55, 294–297, 1989.

Schnell, R. C. and Vali, G.: Atmospheric Ice Nuclei from Decomposing Vegetation, Nature, 236, 163–165, https://doi.org/10.1038/236163a0, 1972.

Schnell, R. C. and Vali, G.: Biogenic Ice Nuclei: Part I. Terrestrial and Marine Sources, J. Atmos. Sci., 33, 1554–1564, 1976.

Vali, G.: Quantitative Evaluation of Experimental Results an the Heterogeneous Freezing Nucleation of Supercooled Liquids, J. Atmos. Sci., 28, 402–409, 1971.

Walterson, A. M. and Stavrinides, J.: *Pantoea*: insights into a highly versatile and diverse genus within the Enterobacteriaceae, FEMS Microbiol. Rev., 39, 968–984, https://doi.org/10.1093/femsre/fuv027, 2015.

Wickham, H.: ggplt2, Use R!, Springer, New York, 213 pp., 2009.