BAMS Article

Looking Back: An Account of How Ice Nucleation by Bacteria Was Discovered (1963 to about Mid-1980s). Part I: The Basics

Gabor Vali[a] and Russell C. Schnell[b]

KEYWORDS:
Atmosphere;
Cloud microphysics;
Biosphere–atmosphere interaction;
History

ABSTRACT: An overview is given of the path of research that led from asking how hailstones originate to the discovery that ice nucleation can be initiated by bacteria and other microorganisms at temperatures as high as –2°C. The major steps along that path were finding exceptionally effective ice nucleators in soils with a high content of decayed vegetative matter, then in decaying tree leaves, and then in plankton-laden ocean water. Eventually, it was shown that *Pseudomonas syringae* bacteria were responsible for most of the observed activity. That identification coincided with the demonstration that the same bacteria cause frost damage on plants. Ice nucleation by bacteria meant an unexpected turn in the understanding of ice nucleation and of ice formation in the atmosphere. Subsequent research confirmed the unique effectiveness of ice nucleating particles (INP) of biological origin, referred to as bio-INPs, so that bio-INPs are now considered to be important elements of lower-tropospheric cloud processes. Nonetheless, some of the questions which originally motivated the research are still unresolved, so that revisiting the early work may be helpful to current endeavors. Part I of this manuscript summarizes how the discovery progressed. Part II (Schnell and Vali) shows the relationship between bio-INPs in soils and in precipitation with climate and other findings. The online supplemental material contains a bibliography of recent work about bio-INPs.

SIGNIFICANCE STATEMENT: The story here recounts how the authors found, in the 1970s, that bacteria from soils and vegetation when lifted into clouds as small aerosol particles are very efficient in initiating ice crystals for snow, hail, and rain. Curiously, the same bacteria are also involved in causing frost damage to plants. The importance of this finding for weather and climate is underscored by the knowledge accumulated over the years about this connection between the biosphere and the atmosphere.

DOI: 10.1175/BAMS-D-23-0114.1
Corresponding author: Gabor Vali, vali@uwyo.edu
Supplemental information related to this paper is available at the Journals Online website: https://doi.org/10.1175/BAMS-D-23-0114.s1.

Manuscript received 2 June 2023, in final form 25 January 2024, accepted 13 February 2024

AFFILIATIONS: [a] Department of Atmospheric Science, University of Wyoming, Laramie, Wyoming; [b] NOAA/Earth System Research Laboratories, Global Monitoring Laboratory, Boulder, Colorado

1. Introduction

Ice formation in the atmosphere is crucial for precipitation formation and is a large factor in Earth's radiation budget. At Earth's surface, it influences diverse processes and has a large economic and social impact. This article recounts the discovery of ice nucleation by bacteria.

The initial discovery, in the early 1970s, consisted of finding efficient ice nucleation in soils of high organic content, in decayed tree litter, in plankton, and finally identifying bacteria as being able to nucleate ice with minimal cooling below 0°C. Two papers in the *Journal of the Atmospheric Sciences* summed up these results: Schnell and Vali (1976) and Vali et al. (1976). Research spread rapidly to a variety of disciplines. Results from the first two decades filled a 350-page book citing hundreds of publications (Lee et al. 1995, hereafter LWG95). By now, about 100 publications appear per year dealing with ice nucleation by biological entities [bio–ice nucleating particles (bio-INPs)].[1]

[1] This and other related terms are defined in Vali et al. (2015).

A large variety of organisms are now known as potential bio-INPs. The widespread presence of bio-INPs in atmospheric aerosol has been confirmed. Potential sources of bio-INPs at Earth's surface and in oceans and their role in atmospheric clouds are better known. The roles of bio-INPs in the initiation of frost damage in plants, in the winter survival of insects, in snow-making, in cryogenic tissue preservation, and more have become areas of research and application.

It is striking how this huge range of knowledge had relatively simple origins. We attempt here to document the threads of the initial work, questions asked, serendipity, and collaboration across many fields that led to the rather amazing recognition that bacteria can be highly effective catalysts of ice nucleation. While this story is likely to be similar to how scientific knowledge generally develops, its specific features are perhaps of interest both as science history and as prompters of thinking about the issues involved. This overview covers the period from 1963 to the mid-1970s. Part II will extend that by another decade. Some background about ice nucleation is given in the appendix. Additional references to recent work on bio-INPs are included in the online supplemental material.

2. Hailstorms, ice nucleation, and soils

Soon after World War II, the Stormy Weather Group at McGill University, Montreal, Quebec, Canada, under the leadership of Professor Stewart Marshall, began using microwave radars to study clouds and precipitation. By the 1960s, the research included hailstorms and that inevitably led to the question of how freezing was initiated in convective storms. Laboratory experiments were called for. Following the work of Bigg (1953) and others, water drops from melted hailstones were placed on a cold stage chilled by dry ice to see at what temperatures they froze. As this work progressed, indications emerged that the rate of cooling of the samples has an influence on the freezing temperatures. A newly arrived master of science (M.Sc.) student, Gabor Vali (GV), was given the task of building a new instrument

capable of good control of that rate. His work led, in 1962, to a thermoelectrically cooled device, with an analog control system assembled using sundry parts from the radar laboratory. Constant rates of cooling were achieved from 0° to about −25°C. Freezing events were recorded, like for radar scopes, with a 16-mm time-lapse camera (Vali and Stansbury 1966).

The new apparatus was put to use with hail and rain samples from Alberta. The cooling-rate dependence was confirmed, and a new theory of ice nucleation was developed (Vali and Stansbury 1966; Vali 1971). Another finding was a systematic trend for drops from melted hailstones to freeze at higher temperatures than drops from rain. In a simple view of storms, this seemed to indicate that hailstones started growth earlier in the updraft than the graupel which eventually melted into rain.

In these experiments, some of the drops from hailstones were observed to freeze already at −5°C. Such activity was difficult to reconcile with what was then known about atmospheric INPs. Minerals such as kaolinite were thought to be the most prevalent sources of INPs, and many laboratories studied these materials. Purified mineral particles generally showed ice nucleation starting in cloud chamber experiments at −10°C or lower. Clearly, the activity found in melted hailstones was not explained by mineral INPs and other substances known to be present in the atmosphere. This made for a real puzzle.

Ice nucleation by soils. An unexpected avenue of investigation opened through a playful impulse to test a "dirty snow" sample (as opposed to the sterile collections of rain and hail). As detailed elsewhere (page 30 in LWG95), a snow sample that had garden soil blown over it contained a surprisingly high number of INPs active near −5°C. Evidently, the garden soil had some other component than the minerals in it producing the high activity.

Fortunately, the Soil Science Department of MacDonald College had a great collection of mineral and soil samples. Using a selection of samples from that collection, the pure minerals, as expected, initiated freezing below −10°C. But soil samples had higher nucleation temperatures and also showed a trend toward more activity with more fertile soils. These results are shown in Figs. 1a and 1b. Further exploration was greatly facilitated by the suggestion made by the Head of the Soil Science Department, Professor Angus MacKenzie, to test pairs of samples from the same location, one from near the surface and one from the subsoil further down. This turned out to be key to all what follows in this paper; the comparisons revealed that samples of surface soils (like the garden soil in the dirty snow sample) consistently contained more active INPs than the subsoil samples (see Fig. 1c). For given temperatures, the concentrations of INPs are about an order of magnitude higher in the surface samples. The conclusion drawn in Vali (1968) was

> the most active nuclei in the surface soils samples appear to be associated with some minor (perhaps organic) component of these soils.

The tentative mention of organics in the above quote turned out to be a good insight. One proof is shown in Fig. 1d from Schnell and Vali (1972).

An important and unexpected boost to the evolution of ideas about INPs came from the work of entomologist Dr. Reginald W. Salt[2] working in Lethbridge, Alberta, Canada. GV visited him during the 1968 field campaign of hailstone collections. Salt experimented with insect larvae in a cold room in search of factors governing the winter survival of wheat plants. His experiments with hundreds of larvae (Salt 1966) and GV's with water drops had many similarities. Results too were in agreement

[2] http://esc-sec.ca/wp/wp-content/uploads/2017/02/Obit_Salt_Reginald.pdf.

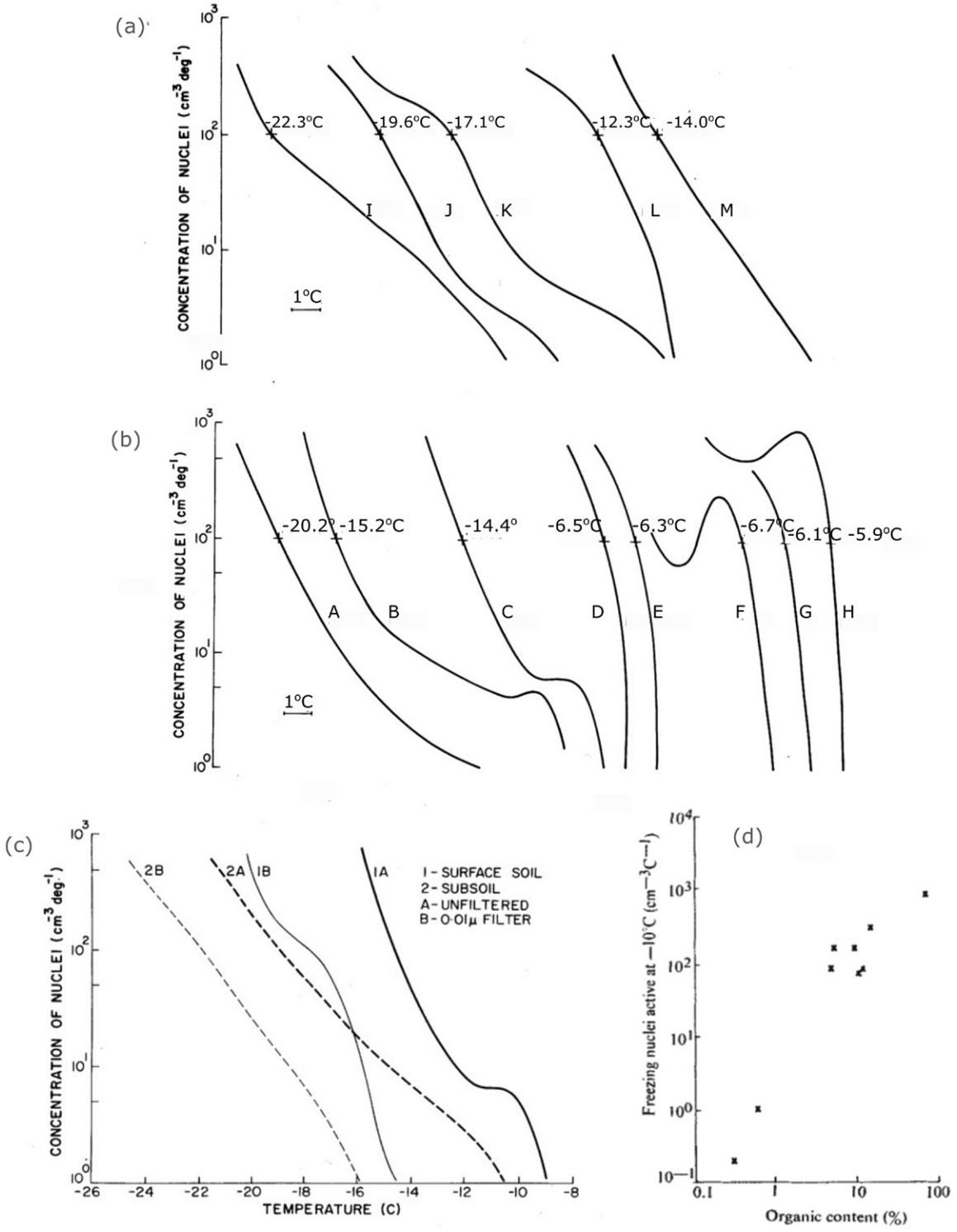

Fig. 1. (a) Nucleation spectra for 0.05 wt.% suspensions of samples of clay minerals: I—kaolinite, Georgia; J—montmorillonite, Mississippi; K—illite, Illinois; L—bentonite, Wyoming; M—bentonite, Alberta. (b) INP spectra for 0.05 weight percent (wt.%) suspensions of samples of surface soils: A—Volcanic ash, St. Lucia; B—clay soil, Quebec; C—clay soil, Quebec; D—humus soil, Alberta; E—humus soil, Quebec; F—loam soil, Quebec; G—loam soil, Saskatchewan; H—peat soil, Quebec. (c) Comparison of surface and subsurface samples. Panels (a)–(c) are from Vali (1968). (d) The correlation of nucleation temperature with organic content, from Schnell and Vali (1972). In (a) and (b), curves are referenced with temperature at an abscissa value of 10^2 and with scale for 1°C indicated.

on many points. Many years later, it became clear why that had to be so. **Garden soil in GV's laboratory and insect larvae in Salt's connected freezing nucleation to biology and organic matter.**

3. Leaf-derived ice nuclei (LDN)

A small group of researchers from the University of Wyoming (UW) conducted aircraft measurements of cloud properties within the Alberta Hail Studies. The leader of the group, Dr. Donald Veal, and GV saw promise in relating the in-cloud observations with the measurement of INPs in rain and hail. This led to GV taking up a faculty position at UW in 1969 and for Russell C. Schnell (RCS) to start graduate research there. RCS's biology background was a good starting point for taking on the question of what the major source of INP activity in organic soils could be. Very shortly, already during the 1970 field project in Alberta, he made a discovery. As RCS recounts,

> I collected fresh tree and grass leaves, washed them and tested the water for ice nucleating activity on the portable drop freezer GV had at the hail studies facilities in Penhold. To my great surprise there were no active ice nuclei in the washes. Luckily, I neglected to throw out one of the sodden leaf batches. So, three weeks later I tested that sample; all drops froze near −1.5°C!!!

In the fall of 1970, the experiment was repeated with deciduous tree leaves from Alberta and Wyoming (Schnell 1971). Samples were collected of fresh leaves from aspen trees (*Populus tremuloides*), of leaf litter beneath the trees, of humus–loam mix in a shallow hole beneath the trees, and of loam in a deeper (2 ft deep) hole and fine clay at a 4-ft depth. Samples were kept at room temperature with the leaves half immersed in water. Duplicate samples were either open to room air or sealed. Analyses were done on the computer-controlled "Nucleus Spectrometer" with electro-optical detection of drop freezing (Vali and Knowlton 1970).

For fresh leaves, the formation of active freezing nuclei was found to peak roughly 3 weeks after collection. For fallen leaves, activity near −2°C developed within a week. Such activity developed only in samples exposed to air indicating a crucial role for aerobic microorganisms in INP formation. In the well-decayed leaf litter, activity started near −4°C and reached a concentration of 10^9 INPs active at −10°C per gram of material. The loam and clay samples contained considerably less active INPs.

In both spruce and poplar litter, the freezing nucleus content was shown to be steady over at least 7 months. Large fractions of the active freezing nuclei were found to pass filters of 0.02-μm pore size. Nucleating ability persisted under repeated freeze and melt cycles, after heating till no more smoke emerged, evaporation and prolonged drying, and after the addition of organic solvents (Schnell 1971).

One of the leaf litter samples collected in 1970 has a most noteworthy story: INPs in sample 70-S-14 were found to be exceptionally stable over more than 40 years. The sample was stored in a plastic bag at room temperature and tested regularly over the following 20 years (1970–90) and more recently by Vasebi et al. (2019). In this latter work, it was determined that the stable INP activity in sample 70-S-14 was most probably produced by a fungus, *Mortierella alpina*.

The pragmatic approach just described, reproducing natural processes in the laboratory, lead to evidence that INPs active at temperatures above −5°C can develop in decaying tree leaves. Not knowing what the active substance actually was, these INPs were labeled LDN. About 6 years after the "dirty snow" sample was analyzed, **it became possible to argue that highly active INPs found in surface soils may have their origin in decayed leaf litter.** However, this connection between soil INPs and LDN is still being explored 50 years later.

4. Ice nuclei in marine waters—Ocean-derived ice nuclei

After the findings with soils and LDN, it seemed logical to examine whether biological ice nuclei might exist in the oceans as well. To that end, RCS collected samples of surface seawater from various ocean basins. The most active INPs were found in a sample from

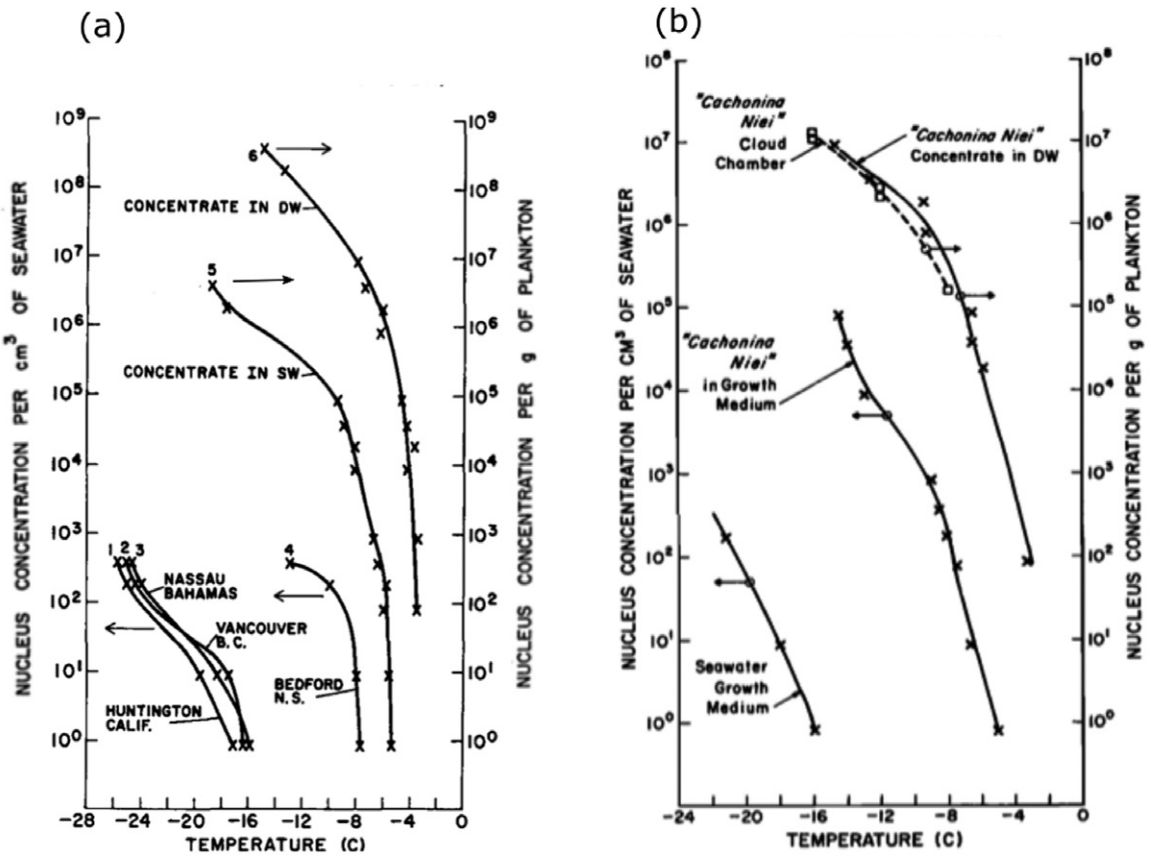

Fig. 2. (a) Measured concentration of INPs in seawater from different locations. Curves 1–4 refer to the left ordinate, and curves 5 and 6 refer to the ordinate on the right (Schnell and Vali 1975). (b) INP spectra for the seawater growth medium (left axis), for a culture of *Cachonina niei* of medium activity, and for a concentrated sample of *Cachonina niei* tested both by drop-freezing and in an isothermal cloud chamber. From Schnell (1975).

Bedford, Nova Scotia, Canada, taken from the top 2 ft of the ocean surface with a plankton net towed with a small ship. Plankton sieved from this sample within 1–2 h after collection produced activity shown in Fig. 2. Activity was destroyed after the sample was heated to 95°C and was also lost within 3 weeks when stored at room temperature. These ice nuclei were named ocean-derived nuclei (ODN) in Schnell and Vali (1975).

Another set of seawater samples was collected and tested by RCS during 2 weeks on board the U.S. Naval Ship (USNS) *Hayes* off Nova Scotia in an area of relatively elevated plankton production and persistent fog. One of the 29 seawater collections had activity (after correcting for melting point depression by sea salt) rising sharply at –2°C (Schnell 1977). Only three other samples had appreciable activity at temperatures above –8°C. These data illustrated that ODN may be as active as LDN but with considerable variation with sample origin and timing.

To further examine INP activity in plankton, 23 phytoplankton species maintained in sterile seawater media by the Marine Biology Research Division of Scripps Institution of Oceanography were screened for ice nucleating ability (Schnell 1975). Results for the most active sample are shown in Fig. 2b. Dinoflagellate *Cachonina niei* dispersed in distilled water yielded up to 10^6 nuclei per gram of plankton active at –10°C. As a complementary test, aerosolized *Cachonina niei* was tested in the Colorado State University isothermal cloud chamber (Garvey 1975). Ice nucleating activity was found to be nearly identical to that observed in drop freezing tests. Two other plankton samples from the Scripps depository, *Ochromonas danica* and *Porphyridium aerugineum*, also showed appreciable ice nucleating ability, whereas the remaining 20 species exhibited no ice nucleating activity above –15°C.

The search for ODN was continued in 1983 with the collaboration of Professor Ray Fall of the Department of Chemistry, University of Colorado. At this time, 21 pure, living marine algae cultures from depositories from around the United States were tested. Of these, only the same species, *Heterocapsa niei, PY-5*, contained activity at −4.8°C in seawater. Seven bacterial colonies from *Heterocapsa niei, PY-5*, out of 159 tested, were active above −7°C. Pursuing that lead further, Fall and Schnell (1985) reported that the bacteria isolate "FB 1032 is halotolerant and phenotypically similar to *Pseudomonas fluorescens* biotype G." Similarity of the freezing temperature of this sample to that shown in Fig. 2 for plankton indicated that **INP activity in plankton may be linked to bacteria**.

5. Bacterial ice nuclei (BDN)

***a. Ice nucleation by* Pseudomonas syringae.** The high activity in decayed leaves, LDN, clearly called for investigating what biological agent was responsible for the process leading to INPs. An expert in fungi at UW, Professor Martha Christensen, was approached for help and M.Sc. student Richard W. Fresh working with her undertook the task of isolating the active agent.

Samples of needles and leaves from various tree species were collected at monthly intervals from April to September at National Forest sites in southeast Wyoming. Samples were placed into pairs of sterile jars, and small amounts of water were added. One of each pair of jars was sterilized for control. At regular intervals, small amounts were withdrawn from the jars, macerated, and added to the vials of distilled water. The automated drop freezer was used to test the supernatant for INP content (Fresh 1972; Vali et al. 1976). For simplicity, INP activity was quantitated using T_{90}, the temperature at which 90% of the drops were frozen. For many months, things did not look promising as T_{90} remained in the range from −10° to −16°C for the samples. Finally, the *Alnus tenuifolia* (alder) sample collected in late summer had T_{90} = −2.5°C after just a few days of incubation. This activity slightly exceeded that of LDN.

Sterilized samples did not show any change in activity with time. However, inoculating sterilized *Alnus tenuifolia* leaves with a small amount of the highly active decaying leaves made the sample active in a few days. This proved that the leaves form active INPs only if a critical ingredient is present, and this ingredient is likely to be a microorganism (Fresh 1972).

This finding led to the laborious process of trying to isolate the critical ingredient. Fungal and bacterial species were cultured from the active *Alnus tenuifolia* slurry and then grown as isolates and tested. These findings are described in Vali et al. (1976), and the most important results are summed up in Fig. 3. None of the fungal composites led to increased INP activity. For bacterial isolates, out of 25 pairs tested, only one pair, labeled C-8 + C-9, showed a clear increase in activity with T_{90} = −8.6°C and T_{90} = −3.5°C after 7 and 14 days, respectively. Separation of the two parts of this pair showed that C-9 was responsible for the action of the pair, while C-8 was like the 24 other pairs. Thus, a single bacterial isolate emerged as the agent leading to high nucleation temperatures on the *A. tenuifolia* leaves. Identification of the bacterium C-9 was started right away, with help from Professor Leroy Maki of the Department of Microbiology and his graduate students. The bacterium C-9 was identified as *Pseudomonas syringae* (Maki et al. 1974). The conclusion drawn was that **bacteria are responsible for developing high INP activity in LDN and in soils.**

Were all cells of *P. syringae* equally effective INPs? During the selection tests, the drops contained very high numbers of bacteria. Dilutions with distilled water were used to determine how activity depended on the number of cells in a drop. Nucleation temperatures clearly dropped off after dilution to 10^6 cells cm^{-3} or about 10^4 cells per drop. This revealed that high nucleating temperatures were associated with a small fraction of bacterial cells,

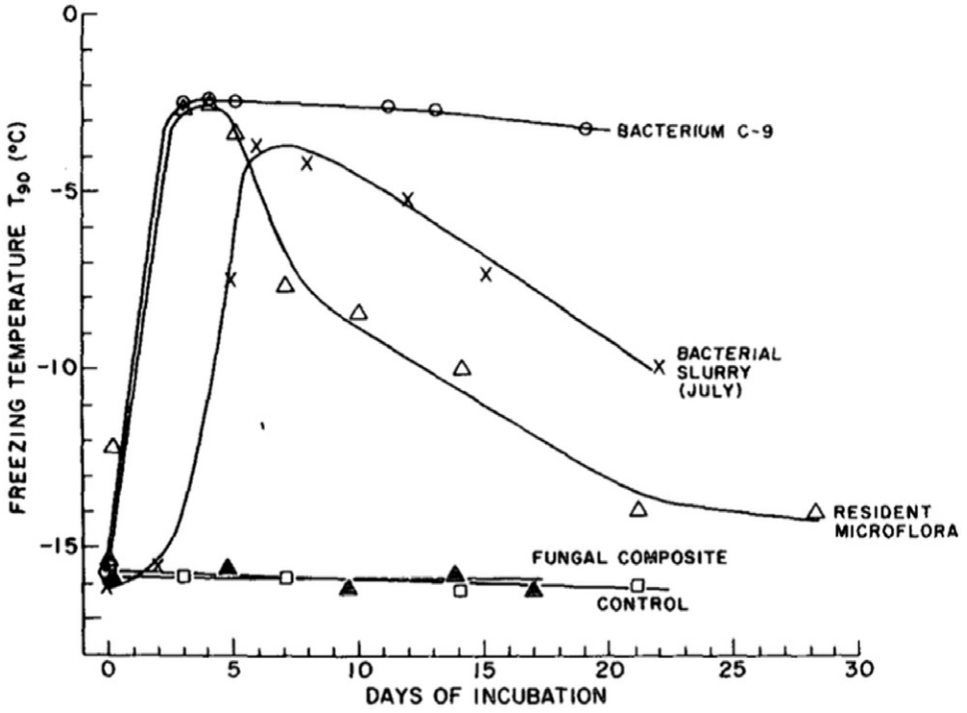

Fig. 3. Evolution of nucleation activity in different *Alnus tenuifolia* samples. Resident microflora refers to the sample allowed to decay as collected. From Vali et al. (1976).

about 1:10 000. It became clear that **exceptional features on a small fraction of bacterial cells form the most active nucleation sites.** That rare structural feature was later identified to be built around a specialized protein molecule (Wolber and Warren 1989), but the search for full understanding of these features is continuing to this day.

b. Frost injury on corn. Soon after the identification of *P. syringae* as the ice nucleating agent in decayed tree leaves, an unexpected and revealing connection emerged between two totally different and independent lines of research. At one end of the bridge was the University of Wyoming group and at the other the University of Wisconsin's Department of Plant Pathology. The link that emerged was described in Upper and Vali (1995) and more recently by Lindow (2023).

The search for corn strains more resistant to frost damage went on over many years. In particular, it was noted that powdered dry leaves from previously damaged plants increased the likelihood of frost and this was thought to be due to some fungus affecting the plants. Steven Lindow was given the assignment to find out how that worked. When one sample developed unexpected turbidity when left unattended over a weekend, it was tested in spite of that. Early freezing was noted, and *P. syringae* was isolated from the sample as being responsible for causing plants to freeze. The mechanisms of the effect were unknown. Then, Richard Barchet, an agrometeorologist at Wisconsin, mentioned that he heard during a visit to Wyoming that *P. syringae* nucleated ice effectively. Indeed, a quick test of adding a drop of the isolate from the corn freezing tests to distilled water proved its nucleating effect.

Thus, quite remarkably, a search for the causes of frost damage in plants and the search for INPs in hailstones, soils, and in the atmosphere led to finding the same bacterium, *P. syringae*, as the answer for what the researchers were looking for. Key papers providing the scientific evidence on ice nucleation by *P. syringae* are those by Maki et al. (1974), Vali et al. (1976), Arny et al. (1976), and the recent article by Lindow (2023).

6. From today's perspective

Only a few general and subjective observations are given here. The supplemental material contains an annotated bibliography of recent literature which shows in detail how widely the work on bio-INPs has extended, and how deeply knowledge about them infuses work in cloud physics, atmospheric chemistry, climatology, and many other areas.

Perhaps the most general point that can be made is that essentially all of the early results described in the foregoing have been found to be robust. No significant contradictions emerged with time. Rather, subsequent work confirmed and amplified the findings here described.

The role of LDN, ODN, and BDN as atmospheric INPs active at temperatures above about –10°C continues to be a viable assumption. Classifications through modern analytical tools identify more specific links to bio-INPs in the current literature, but thinking of LDN, ODN, and BDN as sources is still broadly valid. Thermal deactivation and other analyses show that INPs both in aerosols and in precipitation are linked to the presence of biological components. Strong support for the importance of LDN sources is given by the correlation of INPs with climate zone and hence with soil types (Part II; Schnell and Vali 2024). There is much to unravel still about the sources of nucleating ability in leaf litters, in fertile soils, and in plankton. Indications are that bacteria and fungi play roles. More needs to be known about the diversity of these, their abundance, their time evolution and resistance, and their potential for dispersion into the atmosphere.

The first bio-INP microorganism identified, *P. syringae*, is still recognized as one of the most abundant and most active species of ice nucleating bacteria. In reverse, perhaps that was the reason for it to be the first to be found and identified.

Many other species of bacteria, fungi, pollen, and a growing list of other microorganisms and macromolecules are now known to be effective bio-INPs. The bio-INPs have significant impacts in the atmosphere, in the biosphere, and also in cryopreservation of tissue and organs and artificial snow for ski resorts. Ice nucleation by bacteria was found to be responsible not only for frost on corn but also for the freezing of insects, as in Salt's experiments described earlier, in frogs, and other small creatures. More is being learned about bio-INPs daily. Publication rate on the topic is accelerating from work all around the globe. A most interesting question, pursued by numerous modeling and experimental research groups, is to learn what is common and what is different on the molecular scale in the nucleating sites responsible for the activity of the many different kinds of bio-INPs.

The book by LWG95 demonstrates that very substantial results were obtained already in the first two decades after the discovery of bacterial ice nucleation. A guide to more recent results, going much beyond LWG95, is given in the online bibliography of the supplemental material.

The story described here is an example of the importance of seeing problems from many different perspectives and demonstrates how, in the pursuit of an elusive problem, curiosity aided by serendipity helped arrive at unexpected answers. Institutional support and collaboration across disciplines had a major role. It is notable that a very large fraction of the discoveries was made by graduate students pursuing M.Sc. and doctorate (Ph.D.) degrees. That includes both authors of this paper.

Acknowledgments. At McGill University, Professors J. Stewart Marshall, Edward J. Stansbury, Kenneth Gunn, and Walter Hitchfeld were GV's mentors. Their support and encouragement of a young immigrant cannot be thanked enough. The help of laboratory assistants John Stevenson and others is gratefully acknowledged. The work at McGill was supported by the National Research Council, Canada, and the Alberta Hail Project. RSC was the Research Technician of the Hail Project. GV collected rain and

hail samples as part of that project and his Ph.D. work at McGill. In 1969, GV assumed a faculty position at the University of Wyoming and RCS completed his M.Sc. and Ph.D. research there, with GV as thesis director. Ice nucleation research at the University of Wyoming (UW) during 1969–73 was part of a project led by Donald L. Veal and was supported by Project Skywater of the U.S. Bureau of Reclamation headed by Dr. Archie Kahan. Dennis Knowlton designed the control and data systems of the automated drop-freezing apparatus. Bureau Reclamation funding was obtained via Contract 14-06-d-6801 with the University of Wyoming. From 1973 on, work on bio-INPs was supported by the National Science Foundation with Grant GI-32558 to G. Vali as P.I. Professors Martha Christensen (Botany) and Leroy R. Maki (Microbiology) at UW directed the work of graduate students Richard Fresh, Elizabeth Galyan, Karen Willoughby, and Mai-Mon Chang-Chien. Professor Daniel Caldwell helped with questions of cell physiology. Special thanks are also due to several laboratory assistants, most notably Victoria Sutherland and Robert D. Kelly. Research at the University of Wisconsin was under the direction of Professors Christen D. Upper and Deanne Arny, with major contributions by Susan Hirano and Steven Lindow. Major landmarks in the work here described were the meeting (later dubbed the Zeroth Conference on Biological Ice Nucleation) held in Laramie with Drs. Christen Upper and Dean Arny from the University of Wisconsin and the more officially designated First Conference on Biological Ice Nucleation in San Francisco hosted by Professor Lloyd Kozloff. The authors want to thank those mentioned above and the many more people who helped professionally and personally. The contributions of colleagues and families are immeasurably greater than can be expressed in a few words. We thank the Editor, Dr. Manfred Wendisch, for guiding the paper through the review process. Two anonymous reviewers and Dr. Alexei Kiselev provided insightful comments, excellent questions, and useful recommendations. The authors are greatly appreciative of their contributions. The statements, findings, conclusions and recommendations are those of the author(s) and do not necessarily reflect the views of NOAA or the U.S. Department of Commerce.

Data availability statement. No new data or new analyses were used in this article. Data depositories were not in use at the time the work here described was published. Some of the data used in the original papers may be available by request to the authors.

APPENDIX

Ice Nucleation Topics

For simplicity, and to allow focus on the history being described, ice nucleation is treated cursorily in the main text. Some background is added here.

First, only freezing nucleation is discussed. Ice nucleation by deposition is not considered. The main justification for this is the predominant role of freezing nucleation in clouds of the lower troposphere. Little is known at this time about deposition nucleation by bio-INPs.

The major measurement of nucleation activity in this paper is by the drop-freezing method. Even today, that is the major research approach, though the specifics of the devices used vary considerably. Data presented in this paper all derive from measurements with drops of near 2-mm diameter on a cold stage, as described in Vali and Stansbury (1966). The data are interpreted in terms of differential nucleus spectra defined in Vali (1971, 2019). Relationships to fraction frozen and other measures of activity are presented in Vali et al. (2015). Arguments justifying these time-independent descriptions are given in Vali (2014).

The exploratory nature and the limited availability of analytic tools at the time are the reasons why, in this paper, the freezing nucleation measurements are accompanied by very little data on the physical and chemical characteristics of the bio-INPs. Current research presents a much more diverse and complete view.

References

Arny, D. C., S. E. Lindow, and C. D. Upper, 1976: Frost sensitivity of Zea mays increased by application of *Pseudomonas syringae*. *Nature*, **262**, 282–284, https://doi.org/10.1038/262282a0.

Bigg, E. K., 1953: The supercooling of water. *Proc. Phys. Soc.*, **66B**, 688–694, https://doi.org/10.1088/0370-1301/66/8/309.

Fall, R., and R. C. Schnell, 1985: Association of an ice-nucleating pseudomonad with cultures of the marine dinoflagellate *Heterocapsa niei*. *J. Mar. Res.*, **43**, 257–265, https://doi.org/10.1357/002224085788437370.

Fresh, R. W., 1972: Microbial production of freezing nuclei from decomposing tree leaves. M.S. thesis, Dept. of Botany, University of Wyoming, 85 pp.

Garvey, D. M., 1975: Testing of cloud seeding materials at the Cloud Simulation and Aerosol Laboratory, 1971–1973. *J. Appl. Meteor.*, **14**, 883–890, https://doi.org/10.1175/1520-0450(1975)014<0883:TOCSMA>2.0.CO;2.

Lee, R. E., G. J. Warren, and L. V. Gusta, 1995: *Biological Ice Nucleation and Its Applications.* APS Press, 370 pp.

Lindow, S., 2023: History of discovery and environmental role of ice nucleating bacteria. *Phytopathology*, **113**, 605–615, https://doi.org/10.1094/PHYTO-07-22-0256-IA.

Maki, L. R., E. L. Galyan, M. M. Chang-Chien, and D. R. Caldwell, 1974: Ice nucleation induced by *Pseudomonas syringae*. *Appl. Microbiol.*, **28**, 456–459, https://doi.org/10.1128/am.28.3.456-459.1974.

Salt, R. W., 1966: Factors influencing nucleation in supercooled insects. *Can. J. Zool.*, **44**, 117–133, https://doi.org/10.1139/z66-009.

Schnell, R. C., 1971: Freezing nuclei from decomposing tree leaves. M.S. thesis, Dept. of Atmospheric Resources, University of Wyoming, 71 pp.

——, 1975: Ice nuclei produced by laboratory cultured marine phytoplankton. *Geophys. Res. Lett.*, **2**, 500–502, https://doi.org/10.1029/GL002i011p00500.

——, 1977: Ice nuclei in seawater, fog water and marine air off the coast of Nova Scotia: Summer 1975. *J. Atmos. Sci.*, **34**, 1299–1305, https://doi.org/10.1175/1520-0469(1977)034<1299:INISFW>2.0.CO;2.

——, and G. Vali, 1972: Atmospheric ice nuclei from decomposing vegetation. *Nature*, **236**, 163–165, https://doi.org/10.1038/236163a0.

——, and ——, 1975: Freezing nuclei in marine waters. *Tellus*, **27**, 321–323, https://doi.org/10.3402/tellusa.v27i3.9911.

——, and ——, 1976: Biogenic ice nuclei: Part I. Terrestrial and marine sources. *J. Atmos. Sci.*, **33**, 1554–1564, https://doi.org/10.1175/1520-0469(1976)033<1554:BINPIT>2.0.CO;2.

——, and ——, 2024: Looking back: An account of how ice nucleation by bacteria was discovered (1963 to about mid-1980s). Part II: Broadening the scope. *Bull. Amer. Meteor. Soc.*, **105**, https://doi.org/10.1175/BAMS-D-23-0115.1, in press.

Upper, C. D., and G. Vali, 1995: The discovery of bacterial ice nucleation and its role in injury of plants by frost. *Biological Ice Nucleation and Its Applications*, R. E. Lee, G. J. Warren, and L. V. Gusta, Eds., APS Press, 370 pp.

Vali, G., 1968: Ice nucleation relevant to hail formation. McGill University, Stormy Weather Group Scientific Rep. MW-58, 62 pp.

——, 1971: Quantitative evaluation of experimental results on the heterogeneous freezing nucleation of supercooled liquids. *J. Atmos. Sci.*, **28**, 402–409, https://doi.org/10.1175/1520-0469(1971)028<0402:QEOERA>2.0.CO;2.

——, 2014: Interpretation of freezing nucleation experiments: Singular and stochastic; sites and surfaces. *Atmos. Chem. Phys.*, **14**, 5271–5294, https://doi.org/10.5194/acp-14-5271-2014.

——, 2019: Revisiting the differential freezing nucleus spectra derived from drop-freezing experiments: Methods of calculation, applications, and confidence limits. *Atmos. Meas. Tech.*, **12**, 1219–1231, https://doi.org/10.5194/amt-12-1219-2019.

——, and E. J. Stansbury, 1966: Time-dependent characteristics of heterogeneous nucleation of ice. *Can. J. Phys.*, **44**, 477–502, https://doi.org/10.1139/p66-044.

——, and D. Knowlton, 1970: An automated drop freezer system for determining the freezing nucleus content of water. *Proc. Second Int. Workshop on Condensation and Ice Nuclei*, Fort Collins, CO, NSF, 75–78.

——, 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, https://doi.org/10.1175/1520-0469(1976)033<1565:BINPIB>2.0.CO;2.

——, P. J. DeMott, O. Möhler, and T. F. Whale, 2015: Technical note: A proposal for ice nucleation terminology. *Atmos. Chem. Phys.*, **15**, 10263–10270, https://doi.org/10.5194/acp-15-10263-2015.

Vasebi, Y., M. Mechan, R. Hanlon, D. Schmale, R. Schnell, and B. Vinatzer, 2019: Comprehensive characterization of an aspen (*Populus tremuloides*) leaf litter sample that maintained ice nucleation activity for 48 years. *Biogeosciences*, **16**, 1675–1683, https://doi.org/10.5194/bg-16-1675-2019.

Wolber, P., and G. Warren, 1989: Bacterial ice-nucleation proteins. *Trends Biochem. Sci.*, **14**, 179–182, https://doi.org/10.1016/0968-0004(89)90270-3.