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Ice Ice Maybe. Bacteria that trigger frost in grapes could also be responsible for forming raindrops in the atmosphere.

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The freezing of a single young shoot in a vineyard would go unnoticed come harvest time. However, the subsequent frosting of hundreds of thousands more shoots later that night could wipe out the entire vintage. Likewise, the freezing of a single cloud droplet would have no impact on the weather. But the subsequent freezing of millions more in the same updraft could kick-start a thunderstorm. A causal agent in both scenarios may be, oddly enough, bacteria. That is, ice nucleation active (INA) bacteria, which, by freezing water at high temperatures, could be both triggering frost and making rain.

So who are these strange bugs, and how do they do it? So far, we know of around 10 species, all fairly closely related, the most notorious (or admirable, depending on your viewpoint) being *Pseudomonas syringae*. They're very common. Gaze out the window and you'll be looking at them on the plants and in the sky. If you walked over soil, plant debris or grass today they'll be on your shoes.

They freeze water by making a special protein, the most effective, natural ice-triggering particle known. The protein is anchored on the outside of the bacterium, and contains amino acids arranged into an ice-like lattice structure, which, like snowflakes, is hexagonal. Because the amino acids are also hydrophilic, water molecules bind to them and in the process are made to mimic a tiny, one-layer-thick ice crystal. Once big enough this "embryo" grows rapidly and the solution freezes. Furthermore, each bacterium fabricates many copies of the protein which stick together to make giant rafts. These aggregates can hold many more water molecules in the right alignment, and so trigger freezing at up to -1°C, although -3°C is more common for a colony.

But -1°C, let alone -3°C, doesn't sound impressive at all. It seems like they're suppressing freezing. Quite the opposite. It is little appreciated that while pure water melts at 0°C it won't freeze until -38.5°C! This is because, while ice is stable below 0°C, its initial formation is strongly inhibited by the chaotic motion of the water molecules. Imagine trying to get a crowd of commuters to stop and form lines. Freezing only occurs because particles suspended in the water have surfaces that hold water molecules still and help to align them. All the water we see in our normal lives is full of impurities, a few with ice nucleating abilities. And since we never actually measure the temperature that ice cubes or the dew on the grass freezes we just mistakenly assume it happened at zero.

But why do they do it? We think primarily to cause frost damage and so gain nutrients from the damaged plant material, but there are other benefits. Most plant tissues will not freeze until below -5℃. So on a moderate subzero evening, they and the pure water dew drops on their leaves could remain unfrozen all night long. But when INA bacteria are present they trigger freezing. An ice front will spread rapidly over the leaf and then plunge into it. There, growing ice crystals crush and rupture the delicate cell membranes. In a few minutes it's all over. In the morning when the dead leaf thaws the bacteria start to feast and multiply. While they can survive freezing other bugs can't, reducing the competition.

INA bacteria are everywhere. They colonise leaves and decaying plant tissues. In a US study they were found on around 80% of plants. And the numbers are impressive. On leaves of fruit and nut trees, and in leaf mulch, there can be 10 million per gram, and on cereals 1000 or more per gram. In Australia, a handful of studies have found them on cereals, legumes and stone fruits, but we know nothing of their occurrence on natives. Nor did we know anything about them on grapevines, which is rather remiss considering that in late October 2006 a series of frosts caused widespread damage across southeast Australia, contributing to a 0.5 million tonne drop in the grape harvest compared to the previous year. It was also a drought year, and when it's dry that means clear, dry-air nights: a recipe for frosts. Vineyards use various methods to tackle frost including overhead sprinklers, large fans, even helicopters to suck down warm air. But sprinklers use large amounts of valuable and now expensive water, and fans and helicopters are just plain expensive.

Better late than never, the University of Melbourne's Viticulture Research Group (VitUM), made a start last spring and we have made rapid progress. Sonja Needs and I (TH) took a few samples from two vineyards. In one, hit badly by a -3℃ frost, we isolated INA *Pseudomonas syringae* from frozen Chardonnay shoots but not from unfrozen shoots. In the other vineyard, which had escaped the frost, the bug was there in abundance on the Chardonnay shoots, ready and waiting. In pure culture they triggered freezing at around -3℃. A month later, after warm weather, we took some young leaves from vines that were growing so fast that they were essentially microbe-free. When sprayed with pure water they survived -5℃ without any damage.

But when sprayed with our INA bacteria, freezing would almost always be triggered at that temperature. So now we're developing a DNA-based method to directly count the number of copies of the ice protein gene in samples. There is one per bacterium so we are, in effect, counting the number of bugs. It's exquisitely sensitive, using the same technology used to count HIV virus in a patient's blood. With this tool we can test existing chemical treatments that claim to protect grapevines, and try out some new ideas to either remove them or break up their protein rafts. Any insights may hopefully also apply to other susceptible crops.

We're by no means the first to try and beat them. An elegant but ill-fated approach for controlling ice bacteria was developed by Steve Lindow, at The University of California at Berkeley, in the 1980s. He used the then new recombinant DNA technologies to knock out the ice nucleating protein from *Pseudomonas syringae*. He happened to also be making the world's first GMO, and it sparked a storm. The idea was to spray this ineffective ICE– mutant onto crops to overwhelm and displace the naturally occurring frost-inducing strains. Because it was living it would persist and provide ongoing protection. The problem was that people worried that ICE– might spread and replace ICE+ strains on many plants worldwide. This could reduce the number of ice nucleating particles in the atmosphere which could lower the amount of snowfall and rainfall. As a frost protectant ICE– showed promise, but the seeds of doubt had been sown and it failed commercially.

The possible global role of INA bacteria as atmospheric ice nuclei, essential for rain drop formation in temperate climates, was first mooted by Gabor Vali and Russ Schnell, both leading atmospheric scientists (Schnell now also oversees the famous long-term CO2 monitoring lab at Mauna Loa). Gabor Vali is also the person who discovered them, with a little serendipity. In the late 1960s he tested some dirty snow from beneath his daughter's swing and found it caused ice nucleation at exceptionally high temperatures compared with pure snow. He and Schnell went on to show that decaying grasses and leaves in the snow were carrying the nuclei, and later, with Leroy Maki, that the bacterium *Pseudomonas syringae* living in the organic matter was the true source.

Later, in 1982, the importance of crops as an atmospheric source, and the tightness of the link between earth and sky, was proposed by plant pathologist David Sands. After seeing his entire 360 ha test field of wheat, hitherto completely free of disease, suddenly and simultaneously be afflicted by a disease-causing strain of

Pseudomonas syringae that was also INA he knew that they could only have come down in the rain. Sampling flights confirmed their presence in precipitation falling on the field. Intriguingly, the first report of

Pseudomonas syringae on grapevines in Australia also noted symptoms first appearing after heavy rains. Falling is always easy, but taking off and flying is even simpler. On warm days, dried aggregates of bacteria on leaves will just flake off like tiny dandruff and be carried away in the wind. This year, Cindy Morris, Dave Sands and co-authors showed that INA *Pseudomonas syringae* is also widespread in non-agricultural habitats, finding it in 100% of snow samples, >80% of alpine plant, lake and stream samples, and even in rock biofilms. The same team, led by Brent Christner, then confirmed that biological INA particles that cause freezing at high temperatures are abundant in fresh snow and ubiquitous in precipitation worldwide.

Back to the vineyard, let's say in southwestern Australia. On the frost-killed shoots, the bacteria multiply, the shoots dry out, and they are shed in the breeze and carried eastward. They join updrafts and get swept up into cloud droplets. Clouds loft, droplets supercool, and the ice nucleation protein may trigger them to freeze. Frozen droplets suck up moisture from the air at the expense of liquid ones. So they grow, eventually becoming large enough to start falling, becoming "collectors", and growing ever faster as they collide with and freeze to droplets on the way down. The little hail stones fall out of the cloud, melt, and the bacteria, safe in their Earth re-entry vehicles splash down in some distant place to start the cycle all over again.

In a recent experiment Ottmar Möhler, Dimitri Georgakopoulos and others sprayed a suspension of highly INA *Pseudomonas syringae* (a commercial product, called SnomaxTM, used here for making artificial snow) into a cloud chamber. Immediately this mist of single cells wetted up and became droplets: a little cloud. So it seems they can make their own cloud droplets too! Little surprise then that when our colleague, Helen Ahern, Bruce and I sampled pristine clouds streaming in from the North Atlantic over the mountains of the Outer Hebrides she found that 33% of all bacteria in the cloud water were *Pseudomonas* species. By contrast their relative abundance in the rain was only 2%. Helen was intrigued. She investigated and found that all her *Pseudomonas* cultures produced biosurfactants (natural detergents that help them attach to and colonise leaves), whereas all other isolates did not. Surfactants can help particles become droplets by lowering the surface tension of water (improve their wettability) or by altering their hygroscopicity (their tendency to absorb water). And when I screened natural peat bog vegetation in the Hebrides and

Finland, I found that all isolates that were INA were also the most copious biosurfactant producers. Cloud models suggest that reducing surface tension has numerous impacts affecting cloud droplet number, size and lifespan. Returning to Ottmar Möhler's great cloud chamber, when the INA bacteria were sprayed into it it was already cold at -5.5℃. Having wet up, 1% of them then froze. And when the chamber was cooled to around -8℃, 20% froze, at which point all remaining droplets rapidly evaporated as the growing ice crystals sucked the water vapour out of the air. So the *Pseudomonas syringae* mist transformed itself into a cloud and then froze up to one fifth of the droplets.

This again may not sound very impressive. Until you know that, although crucial, ice nuclei are rare in the atmosphere. While 10 litres of air may contain 100 million particles, 1 million of which will be potential cloud droplet nuclei, only 1 will be an ice nucleus at -10℃. Over the Australian deserts Keith Bigg found only 1 particle active at an even colder -15℃ in every 40 litres of air. Natural sources are mineral dusts, decayed organic matter, bacteria, fungi, fungal spores, viruses, algae and pollen. Ice nucleation by biological particles such as the INA bacteria would have greatest impact in the lower levels of the atmosphere, above plant sources, and be strongly influenced by the weather and the seasons.

There is also limited evidence the INA could be used to seed clouds to make rain. But it's not simple, even if the cloud is right. Zev Levin, another atmospheric scientist with vast experience, from Tel Aviv University cautioned this year that "there is a long chain of processes and feedbacks leading to precipitation, which are not well understood".

Tantalisingly, cloud seeding experiments using silver iodide in Tasmania in the 1960s hinted at the role of bioaerosols released by vegetation. Keith Bigg and Edith Turton noted that, as expected, seeding increased rainfall on the day of flying, but that a second larger wave peaked nine days later and lasted for three weeks. They hypothesised that the silver iodide particles fell on plants and stimulated the growth and release of ice nucleating bacteria. Tantalising, because a recent review of the 46 years of operations over the island found that the apparent impact of seeding depends on the analysis method used. That's not to say the effect wasn't real; just that proof is elusive in this field. Some clarity may emerge from new experiments in the Snowy Mountains, using silver iodide with an indium tracer, and in southeast Queensland, using hygroscopic particles.

Interest in seeding clouds with hygroscopic (water absorbing) particles to create large cloud droplets, rather than silver iodide to create ice crystals, arose after reports of significant rain enhancement using hygroscopic salt flares in South Africa and Mexico. In 1991, Graeme Mather and colleagues were flying their cloud physics Learjet when they spotted a thunderstorm developing over a large paper mill. Flying into its flanks they found updrafts ascending at 55 km/h and supporting huge raindrops up to 6 mm wide. Mather reasoned that hygroscopic particles emitted by the factory were acting as cloud condensation nuclei (CCNs). When water condenses into cloud it releases enough energy to warm the air by more than a degree. Like a hot air balloon it then lifts, expands and cools, and more water condenses onto the droplets releasing more heat, creating a selfsustaining reaction that fuels the growth of these great air pistons that power a thunderstorm. Around the same
time, modelling by Tamir Reisin and Zev Levin predicted that while well-timed seeding to make ice could increase rainfall by 10-35% the injection of hygroscopic particles at the right time to make fewer bigger droplets could double the rainfall in amenable clouds. More recently, simulations by Daniel Rosenfeld's team, at The Hebrew University, Jerusalem, predicted that seeding with giant hygroscopic CCNs, 1.5 to 3 µm wide, can increase raindrop production when natural nuclei such as sea salt are lacking. Large droplets are also inherently more likely to freeze.

Funnily enough, microbe-bearing particles shed by plants are typically large, >2 µm. If these aggregates are composed of surfactant-producing, ice-nucleating bacteria then they seem perfectly designed for making raindrops. As Ottmar Möhler, Paul DeMott, Gabor Vali and Zev Levin concluded in a review published late last year that "If the biological particles act as both giant cloud condensation nuclei and ice nuclei their effect could be very important."

Back in 1982, David Sands suggested "that the desertification occurring in eastern Africa may be due, in part, to the destruction of sources of ice nucleating bacteria by overgrazing with a subsequent decrease in rainfall, thus initiating a cycle which allows neither the vegetation nor the climate to return to normal." Aggravating this is another and parallel positive feedback cycle. Rosenfeld and colleagues used new satellite and aircraft observations to show, in 2001, that clouds forming from desert dust contain too many small droplets and so produce little rain. This leads to drier soils, which in turn raise more dust, and so on. A couple of years ago, he and colleagues from Monash University also argued strongly that air pollution emitted by cities and industrial areas in Australia, such as the brown coal power stations in the La Trobe Valley in Victoria, also suppress rain downwind by the same small droplet collective starvation mechanism. A grand solution, promoted by Walter Jehne, from the Nature and Society Forum based in Canberra, would be to extend and restore rainforests and savannahs in northern and inland Australia to boost natural rain nucleation processes. Bioaerosols released by the vegetation could seed both cloud and rain in the humid air of the former Australian monsoon that flows diagonally from top-left to bottom-right across the country. Rainfall would increase and carbon would be sequestered. From very little things, very big things can grow.

Time for our bugs to finally show their Yin (gentle and cloudy), as opposed to their Yang (aggressive and clear sky), side. The afternoon downpour, in the Goulburn Valley in Victoria, that they helped trigger is now reviving a parched wheat crop that was close to being cut early, yet again, for hay. They multiply harmlessly on the leaves in the now warmer weather. A couple of weeks later a whirlwind cuts through the field, hoovering up millions of INA bacterial aggregates, spinning them straight up into the mid-troposphere. A couple of days later a vineyard manager in Marlborough, New Zealand, admires the billowing clouds entering the valley.

Fig. 1. Ice nucleating bacteria are everywhere, on tree leaves, vine shoots and grass. Ice nucleating *Pseudomonas syringae* was readily isolated from the vine shoots in this small vineyard in Gippsland, Victoria. The inset shows leaf bacteria and fungi growing on an agar plate after a shrub and clover leaves were pressed onto it.

Fig. 2. Cloud collectors set up on *An Clisean* mountain, Outer Hebrides, Scotland. In the cloud water collected here, more than ¼ of all bacteria were *Pseudomonas* species.

Fig. 3. Bacteria in rain that fell on Dookie, northern Victoria, from 10 am to 2 pm on 18th November, 2007. This rain contained 1.5 million bacteria per litre of rain able to grow on this nutrient agar, equating to perhaps 1% of the true number.