Microbial Antifreeze: Gene Splicing Takes to the Field

The nip in the air of early autumn, or for that matter late spring, can cost a farmer his crop. Frost damage in the United States runs more than $1 billion per year, and bacteria—by seeding ice formation—are responsible for almost all of it. Thus investigators are applying tools of both traditional plant pathology and modern genetic engineering to rid crops of ice-triggering bacteria. The first authorized, deliberate release into the environment of genetically engineered bacteria is expected within weeks; it is to be the spraying of a small potato plot with bacteria altered to protect the plants from early frost damage.

"You can look at frost damage as a disease," says Russell C. Schnell of the University of Colorado in Boulder. In the absence of two common types of bacteria on the leaf surfaces, water in plants will not freeze but become supercooled when temperatures drop a few degrees below 0°C. Plants can withstand temperatures of −6°C to −8°C for several hours.

Frost injures plants only if ice is formed. At a few degrees below 0°C, a special material, called a nuclei, center or seed, must be present to trigger crystallization of the supercooled water. Two types of bacteria have been found that can serve as seeds for ice crystals. (These same bacteria are also being considered for use in cloud seeding.)

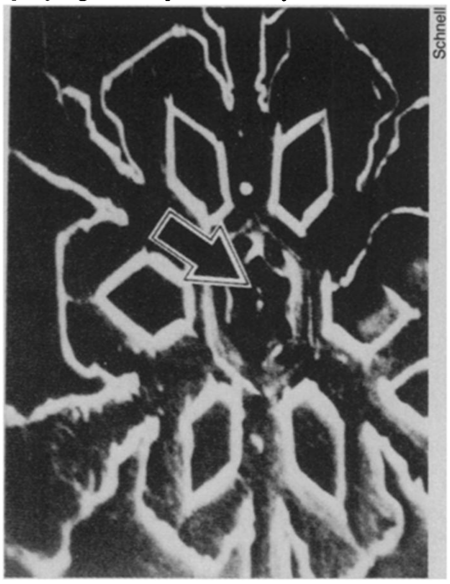
"The fact that these bacteria exist on crop plants such as grasses, vegetables, fruit trees and citrus throughout the U.S., Europe and Asia means that the potential for frost damage could run up to $5 billion and the potential market for frost amelioration products could run up to $.5 billion to $1 billion," says Alan Walton of University Genetics Company, a Norwalk, Conn., firm that has just set up a subsidiary called Frost Technology.

Early experiments by Steven Lindow at the University of Wisconsin reduced crop damage in a frost by spraying a field of corn with the antibiotic streptomycin. But widespread use of drugs is not environmentally sound. Now scientists are attempting to eliminate the ice-seeding bacteria with a more specific, biological agent.

Within two years Frost Technology expects to have on the market a series of viruses that infect only the two types of plant-surface bacteria, *Pseudomonas syringae* and *Erwinia herbicola,* that act as seeds for ice crystals. In laboratory tests with bean plants, within a few hours of application 90 percent of these bacteria are destroyed, the company reports.

The bacteria-infecting viruses, called bacteriophage, were found in extracts of leaves and debris from apple orchard vegetation by Lloyd Kozloff of the University of California at San Francisco and Russell Schnell. The University of Colorado has patented this use of the viruses and has a marketing agreement with University Genetics Company.

The company expects to begin field testing the viruses this winter, and also scale-up production of the virus with fermentation procedures. Gregory Morey of University Genetics says this approach is the most cost-effective potential frost protection for crops. He estimates an expense of $100 per acre, but he says it will vary among the different crops. Spraying an orange tree will take more virus than spraying a corn plant, he says.



An ice crystal formed around three bacteria Pseudomonas syringae. *The arrow points to a single bacterium, and a pair of bacteria lie below it. Each bacterium is about 2 microns long.*

Contrasting this approach to that of genetically engineering bacteria, Morey says, "We think that could be very costly, not as effective, and there may be more public reaction against it and difficulty getting governmental approvals. We're taking something natural and applying it."

Some other investigators, however, strongly disagree. "Bacteria have a much better chance of succeeding than the bacteriophage, which have a history when sprayed on plants of being very unstable," says Cindy Orser, who with Lindow, now at the University of California at Berkeley, and other colleagues, is attacking the problem with genetic engineering techniques.

These scientists have altered one of the ice-nucleation bacterial strains so that it no longer triggers ice crystal formation. They plan to spray such bacteria on field crops early in the growing season, so they become the established strain and crowd out the natural ice-nucleating strains. This approach has proven feasible in laboratory experiments on pinto-bean plants.

So far the scientists do not know what components of the bacterial cell are responsible for the ice-seeding ability. But still they were able to delete from the bacterium *P. syringae* the genes behind those unknown components. First, they made a set, called a library, of pieces of the bacterium's DNA. They next inserted copies of each piece into the standard laboratory bacterium *Escherichia coli,* which normally does not cause ice nucleation. Then the researchers tested to see whether the laboratory bacterium could now seed ice crystals. "It is fortuitous that *E. coli* expresses the ability to nucleate," Orser says.

By this method, the DNA required for ice nucleation was identified. The scientists then constructed DNA pieces lacking this segment. These pieces, which they call "ice minus," were then exchanged for the normal DNA pieces, called "wild ice," in the normal (wildtype) !*P. syringae*. Because the strains of bacteria differ from crop to crop, the "ice minus" bacteria now must be tailored to individual hosts, an expensive laboratory procedure, Orser says. But in the long run she expects such genetic engineering to provide the most feasible approach.

Permission to apply genetically engineered bacteria to plants in the field took more than a year to obtain from the National Institutes of Health (NIH) Recombinant DNA Advisory Committee. Lindow, Orser and colleagues hope to begin a field test in a remote, northern California area within the next few weeks. The NIH committee also approved two other field tests of genetically engineered material, but those involve altering the cells of tomato and tobacco crop plants rather than bacteria.

Because ice nucleating bacteria occur naturally in the upper atmosphere, and may be important in precipitating rain and snow, there has been some speculation that the genetically engineered bacteria, should they reach the upper atmosphere in wind currents, might alter global climate. Schnell says, "I, at this point, wouldn't be overly concerned about it." Although he says he could imagine some strictly local changes, the amount of forest, grassland and other vegetation on earth that would never be treated for frost resistance is so great, "I would rank it [potential global changes] as a very low concern."

—*J.A. Miller*