How Safe Are Engineered Organisms?

Ecologists and molecular biologists disagree on how to decide when genetically engineered organisms should be used in agriculture, and how

Any thoughts that molecular biologists and ecologists more or less knew what each other were doing were dispelled by a recent conference on "Engineered Organisms in the Environment: Scientific Issues."* The idea of the meeting was to foster communication between the two disciplines and to consider what precautions should be observed before new organisms created by genetic engineering are set loose.

To some extent, the meeting succeeded. Ecologist Robert Colwell of the University of California at Berkeley remarked, in summing up the meeting, "My own sense of the conference is that most of us have managed at least one essential step—moving from a battle of absolutes to a disagreement about adjectives. Will some, many, or most genetically engineered organisms prove safe in the environment? Should some, many, or most experiments be exempted from review? I don't hear as many 'alls' or 'nones' as there once seemed to be and I myself have changed *some* of my views as a result of what I have learned here."

But despite Colwell's conclusions, the meeting brought into sharp relief the differences between molecular biologists and ecologists and made clear to everyone that it is still impossible to agree on a general set of procedures to be followed in using engineered organisms, at least in agriculture.

Basically, a number of the ecologists think that most molecular biologists know precious little about the ecosystems they want to invade with their organisms and do not appreciate the possible adverse consequences. Says ecologist David Pimentel of Cornell University, "I think many molecular biologists are too cavalier."

Molecular biologists, on the other hand, tended to view some of the ecologists as alarmists. Nina Fedoroff of the Carnegie Institute of Embryology in Baltimore, for example, remarked, "The hazards of recombinant DNA and recombinant DNA–engineered organisms remain hypothetical." Yet, she continued, "the terrible spectre of uncontrolled reproduction is evoked over and over."

*The meeting, Engineered Organisms in the Environment:Scientific Issues, was held in Philadelphia from 10 to 13 June and was organized by the American Society for Microbiology in collaboration with other biological societies.

At issue were four specific proposed experiments, three of which are in agriculture. The most benign of these agricultural experiments, the conference participants agreed, was a proposal to use engineered bacteria to make plants frost-resistant. This experiment, which has gotten a good deal of attention because it was blocked by a lawsuit brought by activist Jeremy Rifkin, was devised by Steven Lindow and his colleagues at the University of California at Berkeley.

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A bacterial species, *Pseudomonas syringae*, that colonizes plant leaves makes a protein that initiates the formation of ice crystals. Lindow's group has produced in the laboratory a mutant strain of bacteria that does not make this ice-crystallizing protein and these investigators want to apply the strain to potato plants to make them frost-resistant. The idea is that the mutant bacteria would colonize the plants to such an extent that the nonmutant strains would be excluded.

Lindow and his colleagues have done a large number of greenhouse studies to show that the experiment works in principle and, Lindow notes, they tested the mutant bacteria on more than 50 species of plants to show it does not seem to be a pathogen. But the reason his experiment received only mild criticism from some of the ecologists is that the mutant bacterial strain is, in a sense, a natural one. Such "ice minus" mutants occur naturally—a small proportion of the *Pseudomonas syringae* found on plants is incapable of producing the ice nucleation protein. So, at least in the numbers that they occur in nature, the mutants do not seem to be causing any harm.

An experiment that was the focus of a bit more concern was described by Lidia Watrud of Monsanto in St. Louis. The idea of this one is to make a genetically engineered microbial pesticide. To do this, the Monsanto researchers took the *delta*-endotoxin gene from *Bacillus thuringiensis* of the variety Kurstake and put this toxin gene in another kind of bacteria, *Pseudomonas fluorescens*, which can colonize the roots of plants such as corn. When root-eating pests ingest the genetically engineered bacteria on the plant roots, the toxin in the bacteria will get into the guts of the pests where it will be activated and will kill them. The agricultural pests that are vulnerable to this microbial pesticide are members of the genus *Lepidoptera* and include tobacco hornworms, black cutworms, cabbage and soybean loopers, and corn earworms.

Watrud remarks that the toxin-producing bacteria, *Bacillus thuringiensis*, is well known in agriculture. The bacteria are sold commercially to be applied to the leaves of plants and have been used for 20 years worldwide. From this experience, agricultural scientists have concluded that it is not toxic to beneficial insects such as honey bees. But molecular biologists and ecologists at the meeting would like to see the Monsanto group test their toxin-producing bacteria to ensure that the engineered organisms do not kill beneficial insects and to be certain that the toxin does not persist in the environment. In late December, the Monsanto group submitted an 800-page package to the Environmental Protection Agency asking for permission to test their microbial pesticide in the field. The proposal, says Watrud, is still under review.

The third agricultural experiment discussed at the meeting was a proposal by Robert Goodman and his associates at Calgene in Davis, California, to employ plants that were genetically engineered to be resistant to a new class of herbicides. Goodman explains that new herbicides have been produced that have some real advantages over the herbicides that are most often used. Some, including sulfonylureas and imidazolinones, are effective in low doses—grams per acre rather than the kilograms per acre required for traditional herbicides. Others, such as glyphosates, are not very toxic to species other than plants and kill most varieties of weeds. But all have a serious drawback: they are nonselective. They kill some major crop plants. So the Calgene researchers propose to use genetic engineering to make crop plants tolerant of the herbicides.

Luca Comai at Calgene isolated from enteric bacteria a mutant gene that confers resistance to the glyphosate herbicides. Then Comai and his colleagues used plant plasmids to transfer the herbicide resistance gene to plants.

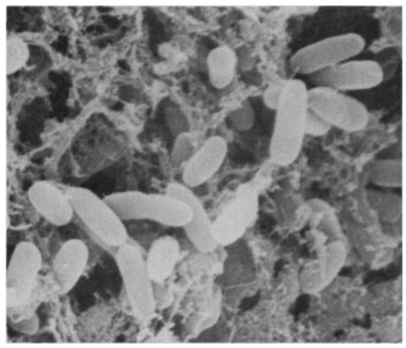
"Fundamentally, the strategy works," says Goodman. "We tried it in tobacco first and we're at various stages of transfer and showing it is expressed in tomatoes, cotton, rapeseed, soybeans, corn, and poplar. The idea is to get away from dangerous herbicides." Other companies are pursuing the same strategies, according to Goodman. Dupont, for example, has isolated a gene that confers resistance to sulfonylureas and is transferring it to crop plants. On 1 June, Calgene submitted an application to the Department of Agriculture to test the genetically engineered tobacco plants in the field.

Although, as Goodman notes, "there is no question that field and lab work need to be done," including studies of whether the herbicide resistance genes will be transferred through the plant plasmids to weeds, the ecologists at the conference were fairly comfortable with the proposed experiments.

The only experiment that did not involve agriculture was one to make genetically engineered vaccines. This experiment, which was described by Bernard Moss of the National Institute of Allergy and Infectious Diseases, involves altering the vaccinia virus, which is the virus used to give smallpox vaccinations. Moss and his colleagues have inserted genes for a number of vaccines into this virus, including herpes simplex, influenza, vesicular stomatitis virus (which infects cattle), and hepatitis B. The advantage of administering vaccines with vaccinia viruses is that they would be very inexpensive, costing only a few pennies per dose; the vaccines would not need refrigeration; and the immune response with vaccinia vaccines, which are administered by scratching the virus on the skin, tends to be better than that with injected vaccines.

Moss gave a number of reasons for expecting that vaccinia virus vaccines would be environmentally safe. One is that although vaccinia virus has been used for smallpox vaccinations for more than 200 years, it has never established itself in the environment. And the recombinant vaccinia viruses Moss and his colleagues are making are even less virulent than the original vaccinia because the researchers insert the foreign genes into the viruses' thymidine kinase genes. The result is a weakened virus.

But one reason that few seemed worried about vaccinia viruses may be that there are regulatory mechanisms in place to deal with vaccines. The regulations for genetically engineered organisms in agriculture are still under study and the conference was populated with scientists from the Environmental Protection Agency and the Department of Agriculture who were looking for clues on how to proceed. There is a huge body of regulations on toxic chemicals and ecologists have a great deal of experience with biological pest control, but regulations for genetically engineered organisms really will break new ground.


Monsanto Co.

Biological pesticide

Genetically engineered bacteria on the roots of corn will kill any agricultural pests that feed on the roots.

The arguments for relaxed regulations are that genetic engineering is actually nothing new. Genes are exchanged among organisms all the time. "We can see in bacteria and in higher organisms that a lot of genetic engineering is going on," says Goodman. "I'm not certain that I see much difference between what happens in the lab and what happens in nature except that bacteria are better at it than we are." Molecular biologists at the meeting urged that this natural genetic engineering be taken into account and that regulatory agencies also consider the experience of research guidelines set by the National Institutes of Health for recombinant DNA studies. Those guidelines now are much less stringent than the guidelines originally adopted because most investigators are convinced that laboratory research with genetically engineered organisms is fairly safe. Susan Gottesman, a member of the NIH Recombinant DNA Advisory Committee (RAC) remarks, "Let me make the plea that we not ask every possible question before we do the first test." Fedoroff points out that the ecologists can learn from the molecular biologists' experience. "Much of the RAC's time was spent undoing what was done quickly and without adequate analysis," she says.

But, say the ecologists, it is their duty to caution. James Tiedje of the University of Michigan says, "I've gone through a number of scenarios. I can end up with hazardous situations or other scenarios [that are not at all alarming]. I feel that I have a responsibility as an ecologist to point out the sobering scenarios." Craig Nelson of Indiana University notes that although recombinant DNA does occur in nature, it can be misleading to compare sporadic genetic transfers to the types of experiments being contemplated in which huge numbers of novel organisms are purposely introduced in such a way as to make it most likely that they survive and thrive. "We only try to introduce organisms that will grow in the environment, which means that they have already passed hurdles that exclude most new organisms. Then we help these organisms preempt resources by introducing them in the proper way and at the proper time so that they have a leg up on their competitors," Nelson remarks.

And although ecologists can sometimes predict what will happen when new species are introduced into an environment, they also have learned to expect surprises. "I personally am struck not by what we know but by what we don't know. I am impressed by how sure we are when we have very little information," says Martin Alexander of Cornell University.

Pimentel argues that the only way to proceed is on a case-by-case basis. He suggests knowing before a new organism is released what other organisms it will affect, how likely it is that the new genes will be transferred to other species, how the organisms carrying the new genes will move and spread in the environment and what other organisms will spread them. For example, if a recombinant organism is sprayed on blossoms, it may be transferred long distances by honey bees. "There is no such thing as a 100 percent safe protocol. However, I think we can design these protocols to minimize hazards," Pimentel says.

Ecologists stress that they have something positive to offer. They have learned from experience to ask questions that may not occur to molecular biologists. As Colwell remarks, "Ecologists have more to contribute to genetic engineering than worrisome carping. The critical balance between effectiveness and safety that we worry about can only be a challenge if there *is* effectiveness, and I think ecologists have much to offer in helping to design effective organisms. If genetic engineering is the cutting edge, then maybe ecology is the whetstone."

—Gina Kolata