Research on Biological Pest Control Moves Ahead

Companies are giving greater emphasis to developing biological agents to control the insects that prey on farmers' crops

THIS SPRING MARKS A NEW ERA FOR PIONEER Hi-Bred International, Inc., of Johnston, Iowa. The company, one of the United States' major producers of seed corn, is putting aside its long-time reliance on chemical insecticides and turning to a biological pest control agent, the bacterium *Bacillus thuringiensis* (Bt), to protect most of its acreage against the European corn borer.

Don't credit Pioneer International with a first, though. During the past few planting seasons, thousands of other growers across the country—many of whom are conventional, mainstream farmers and not members of the environmental avant garde—have been making the same decision, relinquishing the synthetic chemicals that have dominated the pesticide markets for the past four decades in favor of biocontrols (also see box on p. 212). "I am astounded at the recent change in attitude," says entomologist Marjorie Hoy of the University of California, Berkeley. "Growers are clamoring for new techniques to raise crops."

And pesticide industry scientists are responding. During the past 2 to 4 years, the development of new chemical pesticides has taken a back seat to increasing investment in research aimed at producing more effective biocontrol agents. To take just one example, Sandoz Crop Protection Corp. of Palo Alto, California, has recently doubled its research and development effort on biopesticides, and has also greatly expanded its biopesticide production capacities. Although biopesticides now capture only a small percent of the $1.2 billion spent annually on insecticides in the United States, their market share will grow dramatically within the next 10 years, UC's Hoy predicts.

The biocontrol products now moving through the R&D pipeline range from pheromones that act to keep insect populations under control by disrupting mating behavior to insect pathogens, including bacteria such as Bt, viruses, and fungi.

In the past, these agents left a great deal to be desired, so much of the current research effort is aimed at correcting the problems that made farmers reluctant to use biocontrols. Take, for example, the old workhorse, *Bacillus thuringiensis*, which has been marketed in a small way since the early 1950s. Bt is lethal to insects because it produces spores that release toxic proteins when eaten by insect larvae.

Although spraying a mixture of the spores and the protein crystals on tree foliage has proved effective in controlling the gypsy moth, among other insects, Bt has had difficulty gaining a major market foothold. The bacterium has many subspecies, and each is active only against a narrow range of insects belonging to the same order. While this specificity is an environmental asset, it is also a commercial liability: The market niche for any single Bt variant is too narrow for profitability. But that situation is changing.

Bt is produced in large volume by fermentation techniques, and Sandoz and Abbott Laboratories, both pharmaceutical giants, have applied the knowledge of fermentation techniques, gained during years of antibiotic manufacturing, to perfect the Bt production process. That's already helped to make Bt more competitive with chemical insecticides, especially those used on high-value crops such as strawberries and lettuce.


Monsanto Corp.

Unfriendly habitat. *The cotton boll on the right is from a plant engineered with a bacterial gene that produces a toxin for the pest larvae.*

But the big improvements may come at the hands of the genetic engineers who have set about expanding Bt's range. Their efforts are based on work done independently by David Ellar's group at Cambridge University, England, and by that of the late Helen Whiteley at the University of Washington in Seattle. These researchers found that most Bt toxic proteins have two distinct functional domains: One is a binding domain that determines what insect species the toxin attacks and the other has the larvicidal activity. Whereas the amino acid sequence of the binding domain varies dramatically from one Bt strain to another, the larvicidal domain is about the same in all toxins.

Those findings suggested that the species range for a given Bt toxin could be extended by making chimeric proteins that couple several different binding domains to the toxic segment. And that's what Brian Frederici and his colleagues at the University of California, Riverside, found when they used recombinant DNA techniques to fuse two different Bt proteins together. The result: The chimeric protein proved toxic to a wider range of insects than the original proteins.

Genetic engineering methods can also be used to introduce the gene coding for the Bt toxin into new bacterial species, thereby extending the practical range of the toxin. Bt itself is normally a soil bacterium, but researchers in several labs have put the Bt toxin gene into *Pseudomonas fluorescens*, which grows in roots, and into *Clavibacter xyli*, which grows harmlessly in the vascular tissue of corn and other plants. As plants infected with the genetically engineered bacteria mature, they receive a continuous supply of insecticidal proteins to protect then from insect attackers.

Andy Barnes and Frank Gaertner at the Mycogen Corp. in San Diego have developed a different way of using bacteria genetically engineered to contain the Bt toxin—and they think their method might have an edge when it comes to negotiating the regulatory maze. They kill the engineered bacteria, producing what is effectively Bt toxin encapsulated in dead host cells. The researchers hope that it will be easier to get regulatory approval of a dead recombinant microbe than a live one. They also anticipate that their preparation will be among the most popular biocontrols because it will have a long shelf-life and be more persistent in the field than other forms of Bt.

But perhaps the most dramatic genetic engineering achievement in the past few years has been the introduction of Bt toxin genes directly into the genomes of plants, including cotton, tomato, and potato. Such transformed plants show increased resistance to the pests that commonly feed on them—the cotton bollworm, the tomato pinworm, and the Colorado potato beetle, respectively. Moreover, David Fischhoff and colleagues at Monsanto Chemical Corp. in St. Louis, have shown that they can greatly increase the synthesis of the Bt toxin in plant cells—and therefore the plants' insect resistance—by tinkering with the structure of the transferred gene. In field trials conducted last year, cotton plants carrying such a modified Bt gene fared as well as plants treated with conventional insecticides. Both groups had about 4% damaged bolls, compared to 30% in untreated controls.

Although products based on Bt are the clear current leaders in the biocontrol sweepstakes, there are other serious contenders, including insecticides based on viruses and fungi that infect insects. "In the '90s, we are going to see the development of more and more novel control strategies," predicts Robert Granados, an insect pathologist at the Boyce Thompson Institute for Plant Research in Ithaca, New York.

Several groups have been trying to develop the baculovirus group of insect viruses as insecticides, although with limited success so far. The main problem is that the viruses kill too slowly to be of much value in the field, even when they have been genetically engineered to contain genes encoding toxins or other proteins harmful to the target insects. However, Lois Miller and her colleagues at the University of Georgia in Athens have recently identified a protein, isolated from female mites, that paralyzes insects fast. Introducing the gene encoding the protein into the baculoviruses genome "offers significant improvement in the speed of kill," Miller says. Infected insects die in 1 or 2 days, instead of 3 to 5.

Granados is working on another way of accelerating insect kills by viruses. He has identified a baculovirus protein that destroys the midgut of insects. Adding tiny—even nanogram—amounts of the protein to the virus that infects alfalfa loopers decreased the time of kill of the insects by 12 hours, Granados says. He suggests that by breaking down the insect gut, the "enhancing factor protein" allows the virus to penetrate insects more rapidly. Granados is now testing mixtures of the protein with viruses, Bt, and chemicals to determine the best brew for insect kills.

Fungal insecticides are also beginning to rack up some successes. At the Boyce Thompson Institute, for example, researcher Anne Hajek is developing a Japanese fungus, *Entomophaga maimaiga*, for use in gypsy moth control. The fungus produces microscopic spores that invade the gypsy moth caterpillar, where they quickly multiply, with fatal results for the host. In a field trial conducted last summer, the Boyce Thompson workers found that soil infected with the fungus, when placed around the base of a small stand of oaks, protected the trees against infestation.

And urban householders can look forward to a new weapon in the war against their nemesis, the German cockroach. A fungal insecticide active against the cockroach is in an advanced stage of development at EcoScience Corporation in Amherst, Massachusetts. The fungus is dispensed in a chemical bait station, which looks somewhat like a "Roach Motel." When the roach checks in, it picks up the deadly fungus and then carries it to other roaches back in the nest.

Lastly, there is hope for wider application of pheromones, even though they are now costly. These sex attractants are very occasionally used for the mass trapping of insects. But they have been most successful when used to monitor the density of insect populations, to detect the arrival of exotic pests, or to disorient mating males, thereby keeping populations from building up to damaging levels. This in turn allows growers to time, and limit, applications of pesticides more efficiently.

New pheromone products, albeit for niche markets, continue to be introduced. Just last week Wendell Roelofs and his colleagues at Cornell's New York State Agricultural Experiment Station in Geneva announced a new pheromone device that disrupts the mating of the grape berry moth, the most serious pest of grapes grown east of the Rockies. The researchers claim that it should greatly reduce the need for application of toxic parathion.

Although researchers are confident that biocontrols are at last poised to make significant inroads in the pesticide market, they note that the agents differ in a significant respect from the chemicals. Biocontrols strive to manage, rather than eradicate, harmful pests. And that means that the users must become more skillful observers and managers of the manmade ecosystem known as the farm. But as the Pioneer Corporation has proved, it can be done.

■ ANNE SIMON MOFFAT

Why Farmers Are Switching

In recent years, even mainstream farmers have begun shifting from the use of chemical insecticides to biological control agents to protect their crops (also see story on p. 211). Underlying the shift is a mixed bag of social, scientific, and, perhaps most important, economic concerns, says Frank Zalom, director of integrated pest management at the University of California, Davis. Farmers themselves are becoming convinced that there are environmental and health risks associated with repeated exposures to some chemical pesticides, and are increasingly eager to protect their lands and groundwater, as well as their workers. They are also aware that more and more insects—including the mosquito, blackfly, leaf miner, German cockroach, and Colorado potato beetle, to name a few—are now resistant to traditional chemical insecticides.

Still, mainstream farmers might not have become so amenable to change if they hadn't learned they can cut out, or at least reduce, their reliance on potentially hazardous chemicals—and still make money. Says Marlin Bergman of Pioneer Hi-Bred International, Inc., who helped make that company's decision to go with the biological pest control agent Bt on its corn fields: "Bt does the job as well at equal or less cost than chemical insecticides."

And farmers know that consumers are demanding food free of unnecessary chemicals. Justin Micheli of Yuba City, California, has come to depend on another type of biocontrol, namely, pheromone "confusers." These are pipe cleaner-like devices that he hangs in his peach orchards where they emit sex attractants that disrupt the mating of the oriental fruit moth, a serious peach orchard pest. Micheli credits the confusers with reducing his use of organic phosphate insecticides by 75% over the past 4 years. But he turned to biocontrols at least in part because he sells his peaches to a baby food manufacturer, which insists on minimal chemical residues.

The administrative costs of using chemical pesticides have also persuaded growers to seek alternative controls methods. Farmers, especially those in states with strict environmental legislation, such as California, must deal with massive amounts of paperwork when working with potentially harmful chemicals. In addition, they are faced with rising insurance premiums associated with the use of such chemicals. With all these factors pushing farmers toward biocontrols, it's not surprising that more and more are deciding that they would rather switch than fight. "Progressive farmers are enthusiastic about the switch to biocontrols," says Zalom. "Others are just fatalistic about the change."

■ A.S.M.