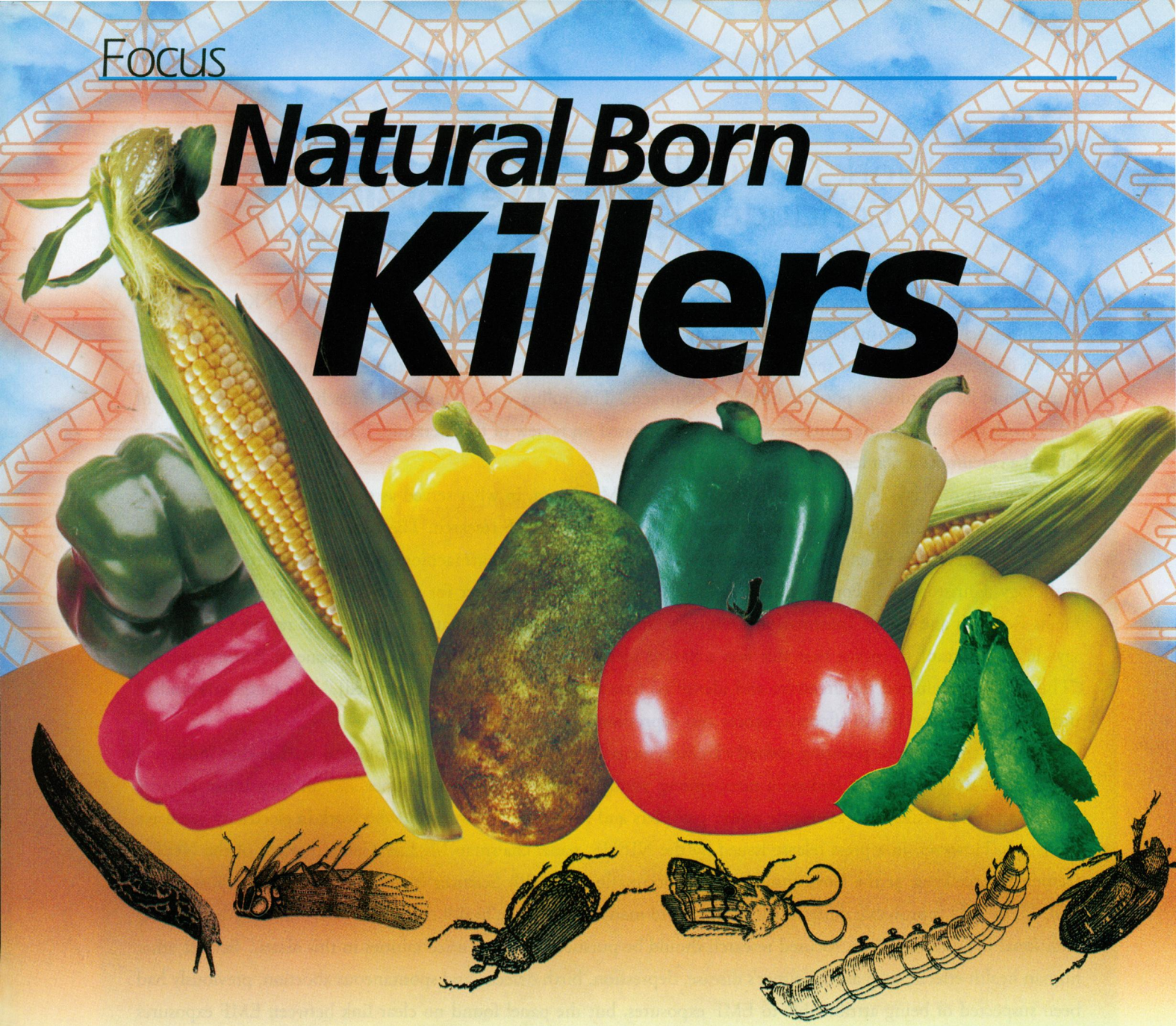
Natural Born Killers



The next time you're about to bite into an ear of corn or spread some butter on a baked potato, consider this: there is a good chance that the food you're about to eat has come from a plant that has been genetically engineered to produce a pesticide. But don't worry—if the side dish in question is indeed transgenic, the pesticide is a familiar endotoxin produced by the microbe *Bacillus thuringiensis* (Bt) that is toxic only to target insects. About 30 million acres of transgenic corn, cotton, and potatoes—the only such crops currently approved for commercial use—will be planted this year. And genetic engineering for pest resistance is an expanding technology, with a number of new crops nearing commercialization and dozens more in the pipeline. These crops are attractive on a number of levels. They provide a cost-effective option for farmers looking for chemical-free alternatives to pesticides. This is a worthy goal, especially since the passage of the 1996 Food Quality Protection Act, which is helping to turn public attention towards reducing pesticide exposures, particularly among infants and children. Furthermore, many plant breeders enthusiastically advocate the continued development of genetically engineered plants as a way to help feed the world's population with minimal adverse environmental impact.

Even so, the public is often wary of transgenic foods, and the EPA has been watching the development of these pest-resistant strains with a cautious eye. Recombinant DNA (rDNA) techniques allow breeders to obtain genes for pest resistance from totally unrelated species of plants, microorganisms, and even animals. The genetic variants produced by these efforts could, some fear, introduce foreign biological substances into the food supply, with unforeseen effects on public health and the environment. The problem is compounded by the difficulties associated with predicting the toxicity of transgenic proteins, as well as identifying susceptible populations that may be at greater risk of exposure. Ecological issues also warrant attention. Some scientists warn of dire ecological consequences in the event that pest-resistant genes are inadvertently transferred to wild plant populations. And environmental groups such as the Union of Concerned Scientists in Washington, DC, are voicing concern that the overuse of Bt crops will help speed the rate at which insect pests become resistant to this popu-

lar, effective, and environmentally friendly pesticide.

In November 1994, the EPA proposed to strengthen its oversight of the pest-resistant crop industry by regulating the substances produced by plants for pest defense and the genes needed to produce these substances, dubbing them "plant–pesticides" under both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). An important motivating factor for the EPA dates back to the emergence of Bt crops in the late 1980s and early 1990s. Microbial formulations of Bt had been used commercially since 1961 with an unblemished record of safe use. But now, through genetic engineering, the same pesticidal protein was being expressed within the tissues of the plant itself, putting the EPA in a quandary; because the agency had always regulated externally applied Bt under FIFRA, the question became how to approach the agency's regulatory mandate when the only difference between the externally applied powder and the Bt expressed by the plant was the route of delivery.

The rule that the EPA proposed to patch this nascent hole in the regulation sparked a fierce debate among industry stakeholders that continues as the EPA prepares to finalize the rule later this year. Opponents claim it is scientifically indefensible to regulate inherited pest resistance traits under statutes developed for externally applied pesticides. In particular, they find the use of the term "plant–pesticide" by the EPA to be highly pejorative, one that could potentially lead to an erosion of the public's confidence in the safety of the food supply. "All plants [contain] genes for pest resistance," says Calvin Qualset, director of the Genetic Resources Conservation Program at the University of California at Davis. "These products aren't always well understood, and we often don't know how to assay for them. [The EPA] had to produce the name 'plant–pesticide' for their regulation. This essentially says that all plants are pesticides."

Qualset was a principle editor of the 1996 report *Appropriate Oversight for Plants with Inherited Traits for Resistance to Pests*, which was highly critical of the proposed rule. This report was published by the Institute of Food Technologists, a Chicago-based trade group, and endorsed by 11 scientific societies including the American Society of Agronomy and the Crop Science Society of America.

The dispute hinges as much on economics as it does science. Of particular concern to producers is a potentially dramatic rise in the costs of regulatory compliance. Rough estimates indicate that the regulatory price tag attached to each transgenic plant–pesticide for the first time it is registered could range from $60,000 to $1,000,000 (however, the regulation would only apply to plants covered under FIFRA and the FFDCA; other kinds of transgenic plants, e.g., herbicide-resistant plants and those engineered for improved quality factors, would not fall under the new rule). It is no wonder, then, that the loudest opposition is coming from those who stand to lose the most once the rule goes into effect: small biotechnology companies, academic scientists, and agricultural research stations often funded with public dollars. These sectors are concerned that the costs associated with the new regulation could effectively price them out of the pest-resistant crop market altogether.

But the EPA stands firmly behind the proposal, and is adamant about its mandate to regulate these substances to protect public health and the environment. Janet Anderson, director of the Biopesticides and Pollution Prevention Division at the EPA, says, "If you make a claim that you have a substance that can control a pest, then under FIFRA that substance is a pesticide. We regulate a lot of substances under FIFRA, even baking soda and certain pheromones. As far as we're concerned, if a pesticidal claim is made, it's a pesticide."

> If you make a claim that you have a substance that can control a pest, then under FIFRA that substance is a pesticide. . . . As far as we're concerned, if a pesticidal claim is made, it's a pesticide.
>
> — JANET ANDERSON

A Historical Perspective

Breeding plants for traits such as increased yield, fitness, and pest resistance has a long history. For decades, beginning with Gregor Mendel in the 1880s, breeders have strived to improve crops by introducing genetic variation with controlled breeding, or hybridization. The biggest challenge has always been to select for the right combination of genes. Often these crude attempts were shots in the dark, and breeders spent a lot of time experimenting with different variations to produce hybrids that not only expressed the desired trait but could also be successfully cultivated. In the early days, and to a large extent even today, breeders had virtually no knowledge of the biological mechanisms of pest resistance. Furthermore, they were constrained by the fact that hybridization could only occur among related and sexually compatible plant species.

Over time, breeders began using more sophisticated cytological techniques, such as chromosome substitution, in their efforts. Among the greatest achievements of this technology was the development of a variety of wheat that was bred to resist stem and leaf rust using resistance genes obtained from rye. The emergence of this new variety in the 1950s helped control a disease that had often devastated wheat fields in much of North America.

But with the advent of molecular rDNA technology, plant breeders were afforded a seemingly endless array of possibilities that allowed them to produce genetic variants in a way only previously imagined. Most important, molecular techniques eliminated the previously insurmountable barrier of sexual compatibility. Because the structure of DNA is universally conserved, it can—with the help of sophisticated laboratory procedures—be moved easily between plants of any species. Even DNA from microorganisms and animal cells can be inserted into a plant's genetic sequence with unprecedented levels of precision. For example, cold-tolerance genes from North Atlantic fish have been spliced into plant genomes to boost their tolerance to freezing. According to Alison Snow, a professor in the department of plant pathology at Ohio State University in Columbus, in an article published in the February 1997 issue of *BioScience*, the absence of key traits in sexually compatible plants has stimulated the search for genes in unrelated organisms. Breeders have used this technology to increase crop yield, fitness, and shelf life, as well as boost resistance to drought and other environmental stressors.

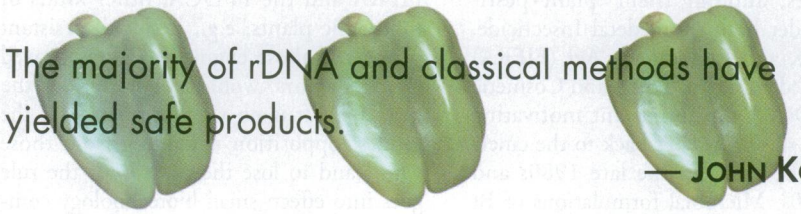
Health Effects Evaluation

Plant breeders contend that varieties bred using rDNA techniques are no more dan-

gerous than those developed using more conventional techniques, and that the level of specificity afforded by direct gene transfer allows plant breeders to maximize the safety of their products overall. Even so, the expression of pest-resistant proteins in plants with no natural precedent remains, for some, an unsettling prospect.

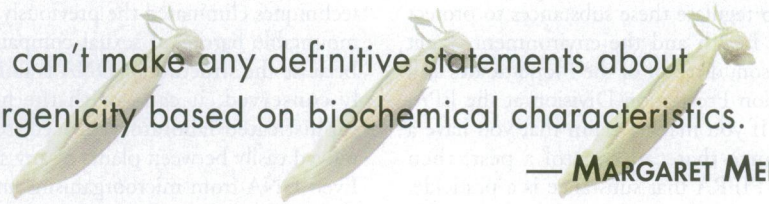
Genetically engineered foods may carry a stigma of being unknown and unnatural, and many consumers are suspicious of them. Margaret Mellon, director of the Agriculture and Biotechnology Program with the Union of Concerned Scientists, says that perhaps the most alarming health issue posed by transgenic crops is the transfer of allergens throughout the food supply. "We're talking about the prospect of moving proteins from nonfood sources like bacteria and microorganisms, and then presenting them in foods to people who might not know that they're allergic to them," she says. Mellon is also particularly concerned that researchers have limited tools available to assess allergenicity in the first place, noting that "we can't even predict whether a protein candidate is an allergen."

John Kough, a biologist with the Biopesticides and Pollution Prevention Division at the EPA, agrees that the lack of predictive models for allergenicity testing is a problem. "Food allergies don't lend themselves to classical models of toxicity testing," he says. "There is no animal



The majority of rDNA and classical methods have yielded safe products.

— JOHN KOUGH



You can't make any definitive statements about allergenicity based on biochemical characteristics.

— MARGARET MELLON

Furthermore, allergies are highly variable in their appearance in the human population. Once a person is sensitized to a food allergen, that person can be adversely affected by low levels of the allergen in food. However, the mechanisms behind the original sensitization event aren't clear."

Nonetheless, Kough is confident that existing procedures for evaluating allergenicity and other potentially toxic properties have done much to ensure the safety of genetically engineered foods before these crops ever reach market. "The majority of rDNA and classical methods have yielded safe products," he says. "Obviously we have much less experience with rDNA, but we're doing our job."

Fortunately, there is no evidence that transgenic plants (or traditionally bred plants) have inadvertently introduced any new allergens into the marketplace thus far. Of the two known instances in which this might have occurred, the problem was recognized early in the products' development stages. One case involved a soybean that researchers were trying to nutritionally enhance with recombinant genetic material obtained from Brazil nuts, to which some people are allergic. But the transgenic product in this case tested positive for reactivity with IgE in human serum obtained from people with Brazil nut allergies, and the product was promptly pulled from further development. The other case involved the attempted transfer of pesticidal traits through traditional, nonrecombinant breeding techniques from the Solanaceae plant family, which includes certain varieties of peppers, tomatoes, and tobacco. These plants may contain varying levels of glycoalkaloids, which can be toxic if eaten in sufficient quantities. these substances could pose a problem for some consumers, and routinely screen for levels of glycoalkaloids when working with these particular varieties.

According to Kough, a number of effective, albeit imperfect, tests are available to scientists looking to screen transgenic proteins for allergenicity. For example, one can compare them with any of the roughly 300 currently known allergens for structural similarities in the amino acid sequence. According to Kough, researchers can conduct sequence homology comparisons to break down the three-dimensional structure of the proteins in question into their primary amino acid sequence, and compare this sequence to that of known allergenic or toxic proteins. As this process can compare for similarity between proteins as small as 8 amino acids long, it affords the analysis a considerable degree of specificity. Another approach takes advantage of the fact that food allergens tend to withstand heat and a low pH environment, and pass through the lumen of the GI tract intact. Using *in vitro* digestibility assays, scientists can assess whether a transgenic protein is likely to break down in the gut. If it doesn't, this sends up a red flag that the protein should be evaluated with further tests for reactivity with human IgE in serum obtained from allergic individuals.

If the specific pesticidal protein can be isolated, researchers can conduct toxicity testing on it using a standard animal bioassay. As an example, one can look to Monsanto's experience in developing a transgenic version of the Russet Burbank potato to be resistant to the tuber's number one pest: the Colorado potato beetle. The Monsanto Russet Burbank, released in 1995, was the first pest-resistant potato variety to be commercially distributed in the United States. Researchers successfully induced resistance in the variety by introducing the *cryIIIA* gene from Bt into the plant's genome. The resultant transgenic gene coded for the pesticidal Bt protein, which Monsanto isolated in pure form, and tested in an acute gavage study conducted in mice. The results of this study, published in *Genetically Modified Foods: Safety Issues*, helped confirm its safety for human consumption prior to marketing.

But the situation is not always so simple. According to James Cook, a professor in the department of agriculture at Washington State University in Pullman, the exact nature of the pesticidal substance may not be readily apparent. Sometimes the resistance is a function of a cascading series of events, and without knowing all of the biochemical steps involved, it may be difficult to determine exactly which substance to test. Cook points to the unblemished safety record of transgenic foods as demonstrating the efficacy of the existing regulatory framework for evaluating health effects. "It has become standard to screen early generation material and reject it before it gets to field trials if there's a suspicion that a plant is potentially harmful," he says.

But Mellon remains unconvinced, and warns that some potentially allergenic proteins could slip through these standard testing protocols. Her concerns center on the observation that food allergies are, for the most part, rare in the population, and that it is impossible to know if someone is allergic to a substance until they've been exposed to it. "You can't make any definitive statements about allergenicity based on biochemical characteristics," she says. "The only reliable test is to find someone who is allergic to that protein and then test it using serum from the person you know to be allergic."

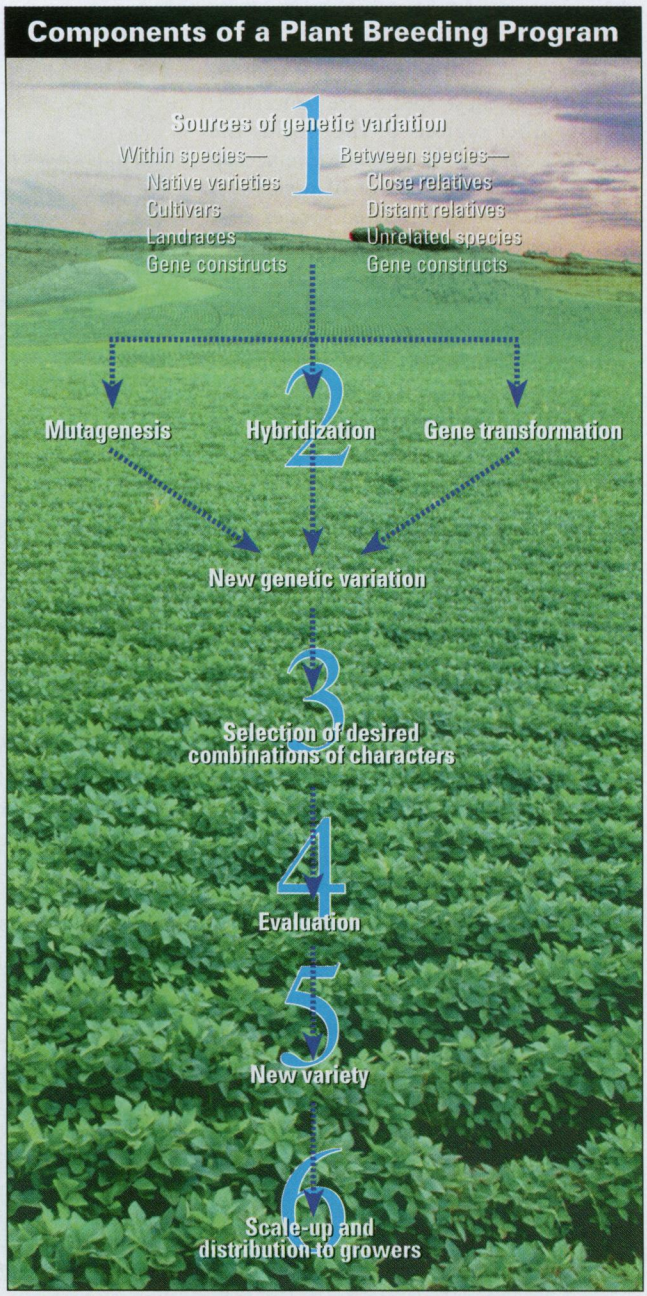
Wild and Wily Weeds

In addition to health effects, some scientists also worry that pest-resistant crops may be ecological disasters waiting to happen. Many of the United States' most important crops are often surrounded by their sexually compatible weed relatives. In a phenomenon known as outcrossing, resistance transgenes are transferred into weeds and confer upon them a competitive advantage over other plants in the ecosystem. From there, one can envision a scenario in which resultant hybrids could grow basically unchecked to gradually choke out their neighbors. The prospect is even more alarming if one assumes that certain endangered plant species could be threatened by such transgenic weeds.

But other scientists downplay this threat, proposing that in most cases crop–weed hybrids are either sterile or so reduced in fitness that their chances of survival are fatally diminished. Furthermore, they argue, there is no guarantee that a transgenic gene will be expressed in the hybrid at levels high enough to boost the plant's fitness. "Outcrossing has been demonstrated over the last 30–50 years," says Qualset, "but the real issue is this: will the gene that is carried in the cross be established in the wild population? Hybrids are often sterile, or potentially sterile. Some of them could be fertile, produce seeds, and become weeds. But it's important to keep in mind that half the genes are coming from the domesticated plant. Domesticated plants aren't fit for wild conditions, and the hybrids may not be as fit as the weeds themselves."

However, Norman Ellstrand, a geneticist at the University of California at Riverside, points to growing evidence suggesting that some crop–weed hybrids could actually thrive in the field. Ellstrand is one of a group of scientists that has been warning of the potential dangers of transgene escape. "Hybrids are not necessarily wimpy plants," he says. "Wild radishes are a good model. We created hybrids [between radishes and weeds] by cross-pollination. Then we grew the hybrids with pure weeds and observed almost a 20% fitness boost. They produced more seeds and fruits than wild pure weeds under the same field conditions." Ellstrand published the results of this study in the February 1994 issue of *Ecological Applications*. It's also important to remember, says Cook, that current crop plants came from weeds, and that scientists are currently inserting genes for pest resistance from weeds into crop plants using more conventional breeding methods.

For a specific transgenic feature such as pest resistance to enhance weed growth, a number of conditions need to be in place. First and foremost, the gene has to be expressed in the wild plant itself. Second, the wild plant needs to be controlled by the pest in question. If the wild plant is already resistant, then the transgene won't confer any fitness boost whatsoever. Given these limitations, the ecological impact of pest-resistant strains is still uncertain. Also,



Source: Adapted from: IFT. Appropriate Oversight for Plants with Inherited Traits for Resistance to Pests. Chicago:Institute of Food Technologists, 1996;28.

The real issue is this: will the gene that is carried in the cross be established in the wild population?

— **Calvin Qualset**

Hybrids are not necessarily wimpy plants.

— **Norman Ellstrand**

there are no weed relatives for corn, soybeans, and potatoes in the United States, so crop–weed transfer wouldn't be a problem as far as those species are concerned. But in other regions of the world, the impacts could be far greater. Every crop has its origin in a "center of diversity," where a multitude of related species exist in a delicate ecological balance. Such centers are where plant breeders look to find genes for pest resistance. For corn, the center of diversity is Mexico, for potatoes it is Peru, for soybeans it is China, and for sunflowers it is the United States. These regions could be hit particularly hard by the emergence of a resistant strain that would outcompete its other wild relatives.

Evolution of Pest Resistance

One of the more worrisome outcomes of resistant crops is tolerance development among insect pests, particularly with respect to Bt, a favored defense among the environmental community. Tolerance is a familiar problem with chemical pesticides, in which the hardy insects that survive exposure multiply and transfer resistance genes to their offspring. Eventually, the entire species becomes tolerant to the pesticide, thereby nullifying its efficacy. With externally applied Bt, this isn't usually a problem because the pesticide breaks down rapidly in sunlight. However, the diamondback moth, which ravages cole crops including cabbage, broccoli, and cauliflower, has developed resistance to externally applied Bt after repeated spraying in Hawaii, the Philippines, and East Asia. And with Bt being expressed continually in plants, it is likely that the rate at which insects develop tolerance will accelerate. According to Snow, some resistant biotypes could emerge within 3–5 years of commercial cultivation of a Bt crop. Resistance to Bt is a serious issue to environmental groups, who are clamoring for expanded resistance management programs. "Bt is in serious danger of being lost," says Mellon. "There are forces acting to put it into every crop. The only counterpressures are coming from the government."

Exactly how the EPA will approach resistance management under the proposed rule is still being negotiated. Says Lynn Goldman, assistant administrator for the Office of Prevention, Pesticides, and Toxic Substances at the EPA, "We're very concerned about resistance management in the context of Bt. If we have resistance, it's a lose–lose situation. So we've put conditions on registration to assure that there is appropriate resistance management. But the process has to be acceptable to the farmer."

One approach being advocated by Monsanto is the creation of non-Bt crop refuges on farms that allow insects to thrive. The idea is that the Bt-susceptible insects will breed with those exposed to Bt plants, thereby diluting potential resistance genes out of the gene pool. Fortunately, pesticidal Bt proteins are designed to be highly specific, targeting only the pest that affects a given crop. Therefore, resistance to Bt may not occur across the board, but may rather develop in one species at a time. But this theory is by no means proven. According to Snow, there is some evidence that insects that develop resistance to one form of Bt also develop resistance to others. She warns that if cross-resistance is common, multiple Bt toxins may not be able to provide adequate protection against evolving pests.

In the end, some scientists take a philosophical view of the problem. Says Qualset, "We've been faced for 100 years with resistance breeding. This is not a new issue, and a lot of extraordinary steps are being taken to minimize resistance to Bt in a new pest. When insects do overcome the Bt gene, we'll have to go back to old methods or find another set of resistance genes to work with. This is part of normal pest breeding, and nothing has really changed with the biotech products."

Changing Regulatory Policies

Regulatory oversight of transgenic crops is currently divided among three federal agencies. The U.S. Department of Agriculture's Animal Plant Health

EPA Review under the Proposed Rule

Under the EPA's proposed rule, before the agency will approve a field test of a genetically modified plant, it must issue an Experimental Use Permit. To obtain such a permit, the applicant must supply the EPA with documentation describing the following in detail:

- Genetic makeup of both the host and donor organisms
- Genetic modification
- Stability of the genetic modification
- Proposed field test design and monitoring procedure
- Available health and environmental information on the host and donor organisms
- Results of tests performed in the laboratory and growth chambers

To sell or distribute a crop plant–pesticide, the EPA's proposed rule requires that the developer register the new plant–pesticide and seek a tolerance, or a tolerance exemption, for the plant–pesticide in each new crop variety. To register a plant–pesticide and obtain tolerance exemption, the applicant must supply the EPA with documentation describing the following in detail:

- Identity and genetic makeup of the host and donor organisms
- Genetic modification that took place on these organisms
- Genetic stability and expression of the plant–pesticide
- Chemical characterization of the plant–pesticide
- Environmental fate (e.g., biological, biochemical) of the plant–pesticide
- Ecological effects on nontarget organisms
- Human health effects

The proposed rule requires that the registrant comply with all conditions of the registration, including standard and supplemental conditions on the sale or distribution, resistance management, and monitoring for adverse effects.

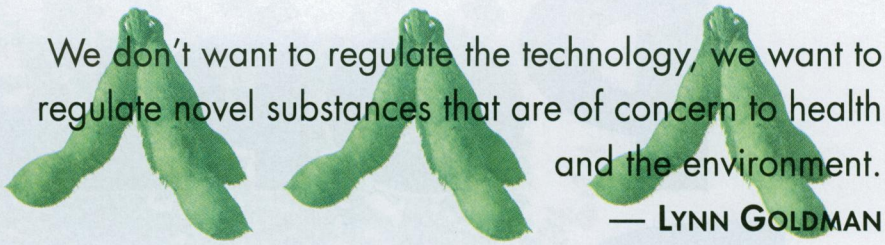
Source: Adapted from: IFT. Appropriate Oversight for Plants with Inherited Traits for Resistance to Pests. Chicago:Institute of Food Technologists, 1996;28.

Inspection Service monitors importation, interstate transfer, and environmental release of genetically modified organisms. The Food and Drug Administration (FDA) regulates the safety of transgenic crops for human consumption under the FFDCA. In this capacity, genetically conferred traits are regulated by the FDA in the same way as food additives. Finally, the EPA regulates pest-resistant crops as a specific subset of transgenic plant varieties under both the FFDCA and FIFRA. In this respect, the EPA shares its regulatory authority with the FDA as that agency is responsible for regulating transgenic plants for nutritional composition and endogenous toxins.

But once the plant–pesticide rule goes into effect, the regulatory landscape for these plants is likely to change considerably. Although the details have yet to be finalized, under the new EPA rule, plant breeders working with pest resistance genes will have to first register with the EPA under FIFRA; then, if possible, they may take advantage of the myriad exemptions that the EPA has woven into the regulation. If an exemption applies, then regulatory oversight by the EPA will be minimal. However, if research involves development of a "novel substance" or pesticidal substance with a "novel exposure," then a series of data requirements will ensue. These data will be used to establish either a temporary or permanent tolerance level, depending upon how close the product gets to commercialization. Furthermore, once a novel substance has been assessed under the rule, it does not have to be reassessed every time it is introduced into a new variety of that species.

EPA officials are quick to point out that, in issuing the rule, the intent is not to regulate the plants themselves, but the pesticidal substances produced in the plant. "We don't want to regulate the technology, we want to regulate novel substances that are of concern to health and the environment," says Goldman.

Several categories of exemptions are currently on the table. One category applies to viral coat proteins for pest resistance that are incorporated into crop plant DNA. "We've reviewed these proteins enough to feel confident that they are safe," says Goldman. According to Elizabeth Milewski, special assistant for biotechnology in the EPA's Office of Prevention, Pesticides, and Toxic Substances, virus-infected plants have always been part of the domestic food supply, and there is no evidence indicating that they are infectious to humans or animals. Another class of exemptions applies to genetic modifications that alter the physical characteristics of a plant, such as those that prompt a thickening of the cuticle so that insects can't penetrate the outer skin. Finally, a third set of exemptions applies to transgenic species produced by a union of genes from related plant species. In this case, the EPA has reasoned that, because closely related plants share a common pool of genetic material, transgenic hybrids would be unlikely to result in new or otherwise threatening substances.



Some opponents of the proposed rule find the system of exemptions somewhat baffling, however. "The exemption categories seem to me to be arbitrary and capricious," says Qualset. "For example, [the EPA] is saying that plants produced by traditional breeding will be exempt, but we can do some pretty nasty stuff with conventional breeding. Instead of implementing a rule that exempts 99% of the cases, why don't they just regulate 1%?"

But Anderson counters that the system of exemptions has been put into place in order to streamline the process. "If we don't do this, we have to require that they all be tested as if they were chemical pesticides," she says. "This would cost a lot more money. [The system of exemptions] is a much more rational approach."

The emergence of commercially viable, transgenically developed pest-resistant crops could go a long way towards boosting world food production and reducing the use of chemical pesticides in agriculture. But the development of pest-resistant strains remains a painstaking affair, and it can take years of trial and error to a develop a commercially viable crop. Furthermore, according to Cook, genetically-engineered resistance is biologically a very specific kind of pest control. "[It's similar to vaccines], a different one for each pathogen or strain of pathogens," he said. Wheat alone has several hundred important diseases and another hundred or so important insect pests on a global scale. This adds up to many, many genes if all these pests are to be managed by resistance. Bt is one of the first examples of one gene that can be used in more than one crop and control more than one pest on more than one crop."

One important question that remains is whether the new regulation will create bottlenecks in research in this dynamic and rapidly developing area of crop science. Possibly the biggest losers could be small research outfits working on minor-use crops with a smaller payoff. There is a serious concern among stakeholders on both sides of the issue that the only organizations that would be able to pay for new registration requirements would be large multinational corporations such as Monsanto, and that these companies would focus their efforts on the most valuable crops at the expense of fruits and vegetables that are grown on less acreage. According to Goldman, the EPA is attempting to work with the smaller companies to address these issues. "We are concerned that we have a process that is workable to the smaller companies," she says. "Since the 11 Societies Report [published by the Institute of Food Technologists], we've held a national meeting [and] we've spent a lot of time trying to understand small R&D operations. We've put together information to make our procedures more understandable. It's important that we figure out how to live with each other."

Charles W. Schmidt