Please Pass the Genes

Experts weigh worries of engineered ills in new food crops

By INGRID WICKELGREN

Plain wrappers masked unusual attributes in a crop of ordinary-looking tomatoes dotting an Illinois field two summers ago. Some of the plants possessed the power to resist certain insects or viral invasions; others could withstand a normally deadly herbicide. The source of these extraordinary qualities lay hidden within the nucleus of each cell. A carefully inserted message, in the form of DNA, directed the production of specific proteins that protected the plants against environmental enemies.

Even more striking than the power of these genetic messages is the way they wound up in the plants. Instead of riding along with many other plant genes inside a reproductive cell during natural or human-aided pollination, the protective genes were inserted separately into leaf tissue. These June tomatoes of 1987 represented the world's first fruits to form in the field from a genetically engineered plant.

Scientists have been tinkering with plant genes since the early 1900s, when breeders started using genetic crosses to create crops with desired traits. But only since the early 1980s have biologists introduced precise changes at the plant's DNA level. Using genes from evolutionarily distant organisms, they are mixing and matching genetic information more rapidly than ever before.

These researchers not only arm plants with genetic defenses but also hope to engineer new foods that offer farmers higher-yield crops and consumers more nutritious produce and grains.

But as the old genetic game takes a new twist, many consumers express concern. Some worry about potential health effects or environmental damage; others fear genetic engineers will irrevocably and unscrupulously disrupt the natural order.

Plant engineers need to anticipate consumer concerns if they wish their products survival in the marketplace. More immediately, they must prepare to satisfy government regulations. No genetically engineered product will reach consumers without first clearing a regulatory obstacle course now being set up by a trio of federal agencies.

The Department of Agriculture approves the field trials of genetically engineered, or "transgenic," plants. The Environmental Protection Agency sets tolerance levels for all chemical pesticides used on food crops and has proposed a new rule that would extend the chemical pesticide regulations to plants engineered to carry genes for virus or insect resistance (SN: 5/13/89, p.300). And the Food and Drug Administration evaluates all genetically engineered plants destined for human consumption to determine whether they should be "generally recognized as safe" or whether their new genetic attribute should be considered a food additive or an otherwise important plant alteration.

Many gene-altered plants are now poised for FDA and EPA evaluation. The USDA has already cleared the way for 45 genetically engineered plants to enter small-scale field trials, and seven applications are pending, says Terry L. Medley, USDA's director of biotechnology regulations.

To try to preempt and evaluate the worries of consumers and federal officials, scientists from a variety of disciplines gathered in May at the Boyce Thompson Institute for Plant Research in Ithaca, N.Y. They did not address the possible environmental impacts of gene-altered plants — the topic of an earlier conference at the institute. Rather, they focused on potential concerns regarding the safety and nutritional quality of genetically altered fruits, vegetables and grains to be sold as food or animal feed. Splitting off into several working groups, they also identified future research needs and attempted to predict which genetically engineered foods will reach the market in the next five years.

To date, all transgenic food plants remain on laboratory benchtops or in small experimental plots, so scientists have few data from which to gauge the precise health consequences of eating such plants. For now, they can only make educated guesses based on what they know about the nature of each inserted gene and its protein product and about similar biological materials or organisms.

Toying with toxins: A new gene might alter a plant's production of toxins either by increasing levels of the plant's natural toxin or by coding for a new toxic product. Addressing the first concern, several workshop scientists argued that genetic engineering is no more likely than traditional plant breeding to alter toxin levels. In both cases, genetic material inserts itself randomly into the plant's genome with unpredictable effects on the expression of other plant cell DNA.

Most proteins are not toxic, notes toxicologist Albert P. Li of Monsanto Co. in St. Louis. Moreover, he says, any simple genetic change seems unlikely to transform a nontoxic protein into a toxic one, because all known poisonous proteins have chemical structures that differ dramatically from those of nontoxic proteins.

Furthermore, since none of the contemplated genetic changes would create proteins containing amino acids other than those normally present in the human body, it seems likely that the digestive system would break down the altered proteins to their standard molecular building blocks. Thus, "the final metabolites [should be] no different from those in the proteins we normally eat," contends Ralph W.F. Hardy, president of the Boyce Thompson Institute.

However, shuffling genes has elevated natural plant toxins in the past, says Jack Doyle, director of the agriculture and biotechnology project at the Environmental Policy Institute in Washington, D.C. In the 1960s, he notes, scientists bred a new kind of potato that caused human illness. It turned out that the new breed's creators had inadvertently mixed genes in such a way that the hybrid produced dangerous levels of a toxin most potatoes harbor in innocuous amounts. The USDA later pulled the poisonous "Lenape" potato off the market, Doyle says.

To help prevent such unpleasant surprises, workshop participants recommend testing any technologically modified food both for unidentified toxins and for toxins known to exist in the host species. But such tests may have limited value, because a plant or fruit as a whole often has biological properties far different from those of its individual molecular constituents, warns nutritionist Joan D. Gussow at Columbia Teacher's College in New York City. Citrus fruits, for example, contain certain chemicals that appear carcinogenic in the Ames assay, but an assessment of epidemiologic studies led a National Research Council committee to conclude last March that these fruits may actually help protect people from stomach cancer.

Arguing that any ill effects will most likely show up within two weeks, workshop scientists conclude that standard animal feeding studies lasting about that long should adequately test for toxins or altered nutrient levels. However, researchers may need to follow up with human studies monitoring longer-term nutritional effects of the marketed product to address concerns about chronic adverse effects, says Peter L. Pellett, a nutritionist at the University of Massachusetts at Amherst.

The fact that scientists and regulators can make morbid mistakes like the Lenape potato underscores the need for careful monitoring, especially as transgenic plants enter the picture. "Classical genetics takes longer [than genetic engineering] to do, so a mistake would be more catchable," Doyle says. In evaluating engineered foods, he adds, "the FDA needs to pay attention to [safety] much more than they have in the past."

Allergy unknowns: Like all foods, transgenic plants pose the risk of allergic reactions. Scientists have difficulty assessing allergenic potential because animals don't mimic human allergies and because of the large number of humans needed to estimate the "allergic subset," should one exist.

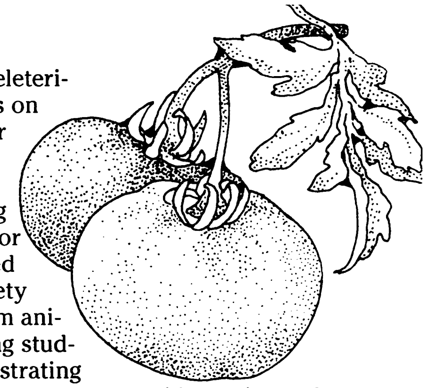
In evaluating the significance of these data, scientists would need to compare the allergenic potential of an engineered food to that of currently marketed food allergens such as peanuts, soybeans, milk and eggs, says Stephen L. Taylor, a food chemist at the University of Nebraska at Lincoln.

Working the bugs out: Of all the transgenic food plants, those endowed with a bacterial gene coding for an insect-killing protein may prove the simplest to evaluate for safety. So far, *Bacillus thuringiensis* (Bt) is the only bacterium found to have major biopesticidal potential. It carries a gene that codes for a protein called Bt toxin. The protein breaks down in the gut of some insects to form poisonous polypeptides, disrupting the gut membrane and eventually killing the insect, explains molecular biologist David A. Fischhoff of Monsanto.

Plants genetically engineered to express the gene produce the toxin, which quickly deters insect feeding and so minimizes damage to the plant. One version of the toxin protects against a range of caterpillars, including the European corn borer, the cotton bollworm and the beet armyworm. Other versions from different Bt strains work against beetle, fly and mosquito pests, Fischhoff says.

The gene and its protein product are well characterized, and for 28 years farmers have used the crystalline protein itself as a pesticidal spray on dozens of food crops, including peaches, bananas, lettuce, tomatoes and cabbage. In fact, Bt-toxin-producing plants may represent a unique case for the FDA because of the toxin's long history of use and the wealth of data regarding its toxicology, chemistry and environmental effects, says Ronald Meeusen, plant biotechnology research director at Sandoz Crop Protection Corp. in Palo Alto, Calif., which markets several forms of the pesticidal spray.

So far, the commercial insecticide has produced no detectable adverse effects on human health. But the amount of toxin a person consumes in a sprayed plant may differ from that in a plant engineered to manufacture its own. Because the sprayed product's half-life on leaves and fruit is only about three days, researchers think much of it may dissipate by the time the food reaches consumers. On the other hand, notes Fischhoff, Bt toxin sprayed on stored soybeans and grain remains stable for months, apparently without deleterious effects on humans or animals.



More compelling evidence for the sprayed toxin's safety comes from animal feeding studies demonstrating its harmlessness to a wide variety of species, says Yousef H. Atallah, director of environmental sciences at Sandoz Crop Protection's Des Plaines, Ill., office. The feeding studies use doses much larger than those a human would encounter in sprayed produce or in genetically engineered plants, Fischhoff says. The toxin spares animals, he explains, because it does its job only in the alkaline gut of certain insects, where it reacts with particular proteins they harbor.

Nonetheless, the workshop group focusing on Bt gene transfer concludes that "limited information is available on the specific form of the [Bt] protein produced in transgenic plants." It also cautions that "short-term metabolism studies comparing the sprayable product with the engineered product are needed to evaluate the applicability of the existing toxicology database to plants."

Such studies, says Fischhoff, should include animal tests using the form of the protein produced by gene-altered plants as well as chemical experiments to determine whether this form also exists in the spray. And although biochemical studies suggest that heat-processing procedures render the Bt protein inactive and benign to all organisms, the group says researchers need further experiments to determine at what temperatures and how quickly the protein is denatured.

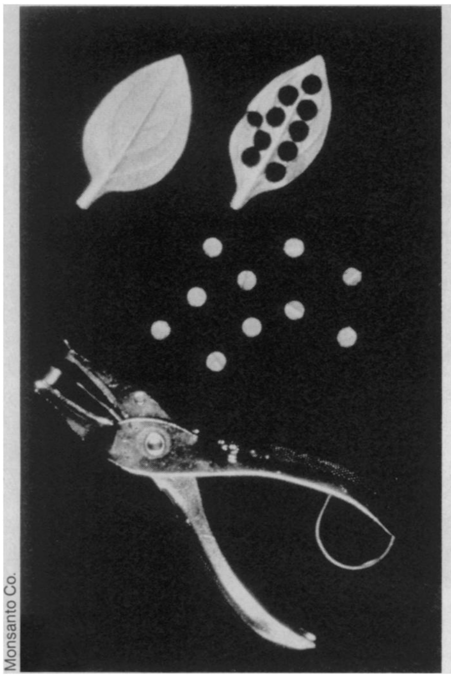
These studies seem likely to begin soon. Scientists already have inserted the Bt gene into tomatoes, tobacco and cotton and have started field tests with all three crops. Corn represents a major candidate for the gene but is difficult to manipulate genetically, mainly because it tends to resist bacteria-mediated DNA transfer, Fischhoff says. By 1995, the working group predicts, scientists will overcome these problems, and Bt-endowed corn, potatoes, rice, lettuce and cole crops (such as broccoli and cabbage) will emerge from laboratories for field-testing – just before the field-tested tomato crops reach the market.

Monsanto Co.

Researchers sprayed all of these petunias with the herbicide glyphosate, killing or nearly killing those in the foreground. The healthy plants were engineered to contain a gene that boosts production of the enzyme inhibited by glyphosate, enabling them to overcome the herbicide's toxic effects.

Withstanding weed killers: Biologists also are engineering plants to tolerate specific herbicides. By planting crops designed to stand up to weed killers that are more environmentally benign than those on today's market, farmers might someday be able to abandon the more damaging herbicides. Endowing plants with herbicide-tolerance genes might also help farmers control weeds in crops that would otherwise wither under weed-killing treatment. At present, farmers can apply an herbicide only to crops that naturally tolerate the chemical or, in rare cases, to crops specially bred for such tolerance, notes molecular biologist S. Carl Falco at Du Pont Co. in Wilmington, Del.

Scientists use two methods to engineer plants to tolerate an herbicide. One is to insert an altered gene for the specific plant enzyme inactivated by the herbicide. The change either makes the enzyme insensitive to the weed killer or causes the plant to produce enough of the enzyme to overcome the chemical's effects. The other approach is to insert a gene for a new enzyme that detoxifies the herbicide.



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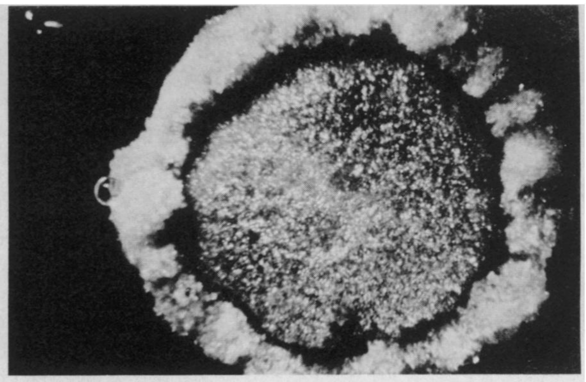
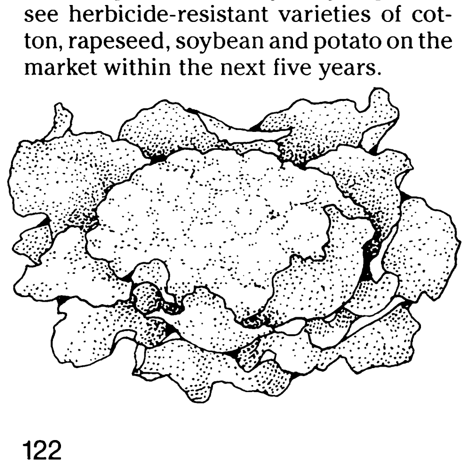


Gene insertion: Using a sterilized paper punch, scientists remove disks from plant leaves (left), generating wounded tissue that Agrobacterium tumefaciens *bacteria can penetrate. Having endowed the bacteria with the gene of interest, the researchers mix them with the disks, where the bacteria insert the new DNA into the nucleus of one or more plant cells. The disks are then placed on a selective medium that allows them to form an outer ring of cells (above), each capable of growing into a whole plant.*

In transgenic plants containing an altered enzyme that differs from its natural counterpart by a single amino acid, the workshop scientists deem human and animal health risks to be low. They reason that researchers have never shown such a small, well-defined change to alter an enzyme's function except to protect it from an herbicide, and so the change would seem unlikely to cause any adverse health effects, says Klaus M. Herrmann of Purdue University in West Lafayette, Ind. He adds that the risks from plants endowed with herbicide-detoxifying enzymes appear unlikely to exceed those from natural herbicide-resistant varieties, all of which use the detoxification method.

But because no one can or will assume the risks to be zero, the working group focusing on this issue recommends testing transgenic herbicide-resistant plants for their nutrient and natural toxin levels and comparing these with the levels in control plants. In addition, the group urges measuring herbicide residues within the plants and testing the plants in animals through feeding and injection. Workshop scientists say they expect to see herbicide-resistant varieties of cotton, rapeseed, soybean and potato on the market within the next five years.



Invulnerability to viruses: A wide variety of viruses infect important crops, including wheat, corn, potato, tobacco, tomato and citrus. They often invade the whole plant, including edible parts. Sometimes the infection results in reduced crop yield, or blotched or misshapen fruit that cannot be sold. But often the viral infection remains invisible.

Over the past three years, by endowing vulnerable species with a gene for a coat protein of their viral enemies, researchers have produced plants that tolerate tobacco, alfalfa and cucumber mosaic viruses, potato viruses X and Y, and tobacco etch and rattle viruses, says biologist Roger N. Beachy of Washington University in St. Louis. So far, scientists have field-tested virus-resistant strains of tomato and potato plants.

The new genes reduce the chance that the virus will invade the plant – and if it does successfully invade, they reduce its spread and multiplication within the plant. Scientists do not fully understand, though, how a gene coding for a viral-coat protein manages to protect a plant cell.

Evaluating the safety of food from these plants remains impossible without results from chemical and animal tests, but scientists say such inserted genes are probably harmless. Because plant viruses are ubiquitous in nature, ordinary plants grown in the field often become infected. Thus, today's produce probably contains varying amounts of different plant viral-coat proteins, says Boyce Thompson plant virologist Candace W. Collmer.

A few studies indicate a person would consume considerably less viral-coat protein in a transgenic plant than in its currently marketed counterpart, Beachy says, adding that a protein from a foreign gene constitutes only 0.01 to 0.1 percent of a transgenic plant's total protein content. In the case of one ordinary, virus-infected tomato variety, Beachy determined that a person would have to eat 2,000 to 5,000 transgenic tomatoes to ingest the same amount of viral protein contained in one infected tomato.

In the future, adds Collmer, scientists will compare virus levels in transgenic plants with those in ordinary plants as one approach to assessing safety. But plant viruses do not harm and cannot infect humans and animals, she says.

Beachy notes that endowing plants with viral-coat-protein genes resembles a long-standing agricultural practice known as classical cross protection, in which scientists inoculate plants with a mild viral strain to prevent a more pathogenic strain from overwhelming a crop. For 50 years, he says, farmers worldwide have used cross protection in crops headed for the market, apparently without causing adverse health effects.

According to the viral-resistance working group, transgenic virus-tolerant tomatoes, potatoes, alfalfa, melons, soybeans, strawberries, sugar beets and various cole crops will reach the commercial market within five years.

Superfoods: On the nutritional front, genetic engineers seek to improve the protein composition of plant foods and feeds. They have conducted only a few laboratory tests on protein-enhanced plants – among them a tobacco variety engineered to produce abnormally high levels of the amino acid methionine, in the hope of eventually producing more nutritious soybeans (SN: 5/13/89, p.300). But progress has been steady, leading the USDA last month to issue a permit for the first field tests of tobacco genetically

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altered for an increase in the amino acid lysine.

Indeed, protein-improved produce may be just around the corner. Five years from now, predicts the workshop's nutrition group, people will be eating protein-enhanced beans, corn, soybeans and wheat, and livestock will chow down on altered corn, soybeans, wheat, alfalfa, rapeseed and sunflower.

Any risks posed by these crops would vary depending both on the plant host and on the source of each new protein. And although scientists have few actual examples on which to base safety predictions, they say they suspect that plants receiving a gene for a well-characterized protein from a known source will pose little if any health risk.

Scientists find themselves handicapped not only by the lack of experimental data but also by their incomplete understanding of genetic expression in plants. This makes it difficult to predict how a given manipulation will affect the plant's other genes, says food scientist John E. Kinsella of Cornell University. In addition, gene transfer technology has yet to reach the point where scientists can control where in a plant's genome a new piece of DNA will insert itself, notes Stephen H. Howell, Boyce Thompson's director of plant molecular biology.

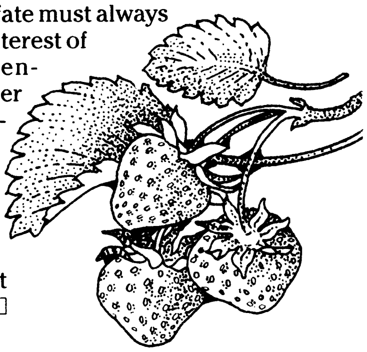
However, because genetic engineering results in a well-defined genetic change — as opposed to an unknown alteration later selected as an observable trait, as occurs in plant breeding — it theoretically introduces fewer unknowns into the product. Thus, genetic engineering should ultimately enable scientists to predict more accurately whether a new gene will affect a plant's overall health and its usefulness as food.

As the technology advances, genetic engineering should provide a much more efficient way to endow plants with agriculturally and nutritionally important traits than is possible with conventional breeding. With genetic engineering, scientists would need to isolate a gene only once in order to use it on subsequent occasions without much additional work. Moreover, by linking beneficial genes to marker genes, they could more easily detect the presence of the desired gene in the transgenic plant and follow it through subsequent generations. And because genetically engineered plants would have more predictable properties, researchers could more easily identify plants with the desired traits, Falco says.

Some food scientists and policymakers worry that plant geneticists might engineer crops with traits that improve processing and yield at the expense of good nutrition. Plant breeders have already contributed to such "nutritional erosion" by endowing tomatoes with genes for uniform ripening, "self pruning" and other commercially beneficial traits that significantly decrease vitamin C content, Doyle says. He wonders: "Where are the priorities, and who's going to set them?"

Many believe that genetic engineering will, on balance, promote better health by enhancing plants' nutritional value and even ridding some foods of natural toxins. Nonetheless, scientists must first ensure that potential risks will not cast a shadow over the technology's budding blessings.

Warns Kinsella, paraphrasing advice from Albert Einstein: "Concern for man himself and his fate must always form the chief interest of all technical endeavors, in order that the creations of our minds — and, I would add, our laboratories — shall be a blessing and not a curse." □



News of the week continued from p. 119

Surveys slash away at forest estimates

Two new studies indicate Earth's forests may hold far less vegetation than commonly believed — and therefore may be much less able to store the carbon dioxide emitted by human activities, primarily fossil-fuel burning. These findings, reported last week at the American Institute of Biological Sciences' annual meeting in Toronto, could complicate scientific and political efforts to balance the planet's carbon budget and to slow a climate-altering buildup of atmospheric carbon dioxide.

Green plants inhale carbon dioxide, exhale oxygen and harness carbon for growth. To gauge how much of the world's carbon dioxide emissions such plants can absorb, scientists need an accurate tally of vegetative mass, or biomass. However, maintains Daniel B. Botkin of the University of California, Santa Barbara, until now "there have been no statistically valid estimates of biomass for any large area of the Earth."

Two years ago, Botkin began surveying North America's boreal forests — the largely coniferous woodlands running from the Arctic tree line down through Canada and dipping into the northern United States. His statistically representative sampling of 760 circular plots, each 10 meters in diameter, indicates they contain a mean biomass of 4.2 kilograms per square meter (kg/m^2). Botkin says that adds up to 1.9 billion metric tons of stored carbon within the roughly 5 million km^2 boreal forest he surveyed — only one-third the total indicated by most previous assays of that forest.

He attributes most overestimates to ecologists' practice of trekking into known mature forests, measuring what's there and then multiplying those values by the presumed area of the forest. He thinks the "same casual [survey] techniques" have probably exaggerated biomass estimates for all other ecosystems. In contrast, Botkin randomly surveyed all regions able to support forests. This enabled him to identify not only existing forest but also areas cleared for agriculture, burned, logged or covered by bedrock or water.

Forest ecologist Sandra Brown of the University of Illinois at Urbana-Champaign reports that accepted biomass figures for tropical woodlands may be similarly exaggerated. As recently as 1980, she says, "we thought we knew what the biomass of the tropical forest was" — generally about 35 kg/m^2. But she says she and other researchers had obtained those estimates by averaging values from about a dozen places worldwide, using a total sampling of less than 30 hectares (0.3 km^2). Having recently tapped into "statistically sound" forestry inventories conducted for economic reasons and stored at the United Nations Food and Agricultural Organization, she says she's now finding that tropical biomass values vary regionally — from about 5 to 55 kg/m^2. And because of "rampant" cutting and degradation of tropical woodlands, she says the average of 35 kg/m^2 is no longer valid in many places.

For years, policymakers in industrial nations have pressured developing nations in the tropics to preserve their trees from overexploitation because forests play such an invaluable role in the global environment, notes Charles A.S. Hall at the State University of New York at Syracuse. Hall thinks Botkin's data hint at some hypocrisy in that stance by intimating that "we in the United States and Canada are also chopping down our forests as fast as we can."

Together, Botkin's and Brown's studies suggest the world's forests "have been degraded more than we had thought," says Sandra Postel, a natural resource analyst with the Worldwatch Institute in Washington, D.C. If so, she says, "there's even more reason to step up reforestation rates — not just to recapture the carbon we've lost, but also to regain the ecosystem services [such as erosion protection and water-holding capacity] that we're obviously losing." — *J. Raloff*