The price of broad patents. Despite the challenges to the Agracetus patents and the antisense altercation, many experts predict that broad patents are here to stay—and that they could have major implications. "I don't read a rejection of broad patents necessarily in [the PTO's office action on the cotton patent]," says John Barton, an expert on biotech patents at Stanford University, who points out that PTO based its decision on the invention's "obviousness," rather than its breadth. According to Barton, the PTO is prone to issuing broad patents—take, for instance, the patent on gene therapy awarded in March to the National Institutes of Health, which covers all procedures in which a therapeutic gene is inserted into cells that have been temporarily removed from the patient's body.

The issuance of broad patents could reshape how agricultural biotech companies do business with one another. "It will undoubtedly increase the use of cross-licensing and various intercompany arrangements for use and development of technologies and genetics," says Drake's Hamilton. One result might be the emergence of a cross-licensing network among the companies that hold the broad patents. And if that were to occur, it would likely drive smaller companies out of business, says Barton, because "you would have to have something to bring to the table," and small, research-based companies might not have anything to put down.

But at least one small company is aiming to run with the big dogs. Last January, PTO awarded Mycogen a patent covering any method of modifying *Bacillus thuringiensis* (*Bt*) gene sequences to make them resemble plant genes. This enables a target plant to turn on the *Bt* gene, which produces an insect-killing protein. The patent leaves a big question mark hanging over efforts at several other companies to develop *Bt* plants, including those at Monsanto, which is conducting large-scale field testing of cotton plants containing a *Bt* gene.

Mycogen and its rivals are going through a shakedown period to define their intellectual property rights and talk about licensing agreements. "We've had discussions and continue to have discussions with Monsanto and other companies," says Mycogen patent lawyer John Sanders, who says Mycogen would like to avoid a costly legal fracas. Judging by the skirmishes embroiling other agricultural biotechnology companies, however, that will be no easy task.

–Richard Stone

Additional Reading

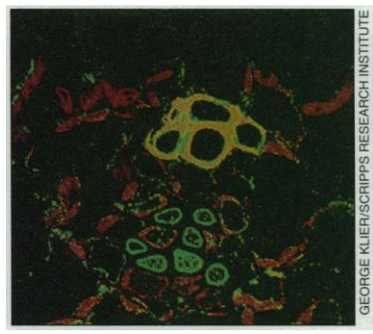
N. Hamilton, "Who owns dinner: Evolving legal mechanisms for ownership of plant genetic resources," *Tulsa Law Journal* **28**, 587 (1993).

P. Umbeck *et al.*, "Genetically transformed cotton plants," *Bio/Technology* **5**, 263 (1987).

MEDICAL APPLICATIONS

Exploring Transgenic Plants As a New Vaccine Source

There was a time not too long ago when most medicinal compounds came from plants: the potent heart stimulant digitalis from foxglove, for example, and opium from the poppy plant. But beginning about 50 years ago, chemistry took over from botany, with most new drugs being synthesized in pharmaceutical labs.



GEORGE KLIER/SCRIPPS RESEARCH INSTITUTE

Glowing success. In this vascular bundle from a transgenic tobacco leaf, the green stain shows the location of the mucosal antibody. (The red signal is from chlorophyll, and the yellow from xylem elements.)

New developments in plant biotechnology may soon reverse this trend, however. Several research groups, including two with papers in this issue, have recent results suggesting that it may be possible to use genetically engineered plants and plant viruses to produce vaccines against human diseases, ranging from tooth decay to life-threatening infections such as bacterial diarrhea, cholera, and AIDS. In perhaps the most far-reaching scenario, it might even be possible to build some vaccines into plants eaten as part of the normal diet.

Jan Holmgren, an immunologist at the University of Göteborg, Sweden, describes the research as having "enormous potential," although he cautions that as it's still in an early stage of development, "there are a lot of unknowns." For example, researchers are only just beginning the animal studies needed to prove that the proteins can evoke protective immune responses. There are also concerns that the plant-produced vaccines, especially those that will be injected, will need to be purified carefully to rid them of alkaloids and other toxic plant materials.

But if these issues can be resolved, production of vaccines in plants could have significant advantages over current methods. Such vaccines might be cheaper than those now available, because plants are easier to grow in large quantities than are the cultured animal or yeast cells now used to make most vaccines. As David Russell, director of plant molecular biology at Agracetus Inc. in Middleton, Wisconsin, puts it, scaling up production of a plant-made protein to the amounts needed for a commercial vaccine would be "as easy as adding acreage." Cheaper vaccines would be a boon in the impoverished countries of the developing world, which often can't afford to buy current vaccines. What's more, plant production eliminates fears about contamination with animal viruses, which always threatens vaccines manufactured in cultured mammalian cells. Plant viruses don't infect humans.

Researchers are taking several tacks to making plant-based vaccines, but the "edible vaccines" now under development by a team led by plant scientist Charles Arntzen of Texas A&M University in Houston would likely be the cheapest and easiest to administer. The idea behind the edible vaccines is to have people take their dose by eating, as part of their diet, the plant that produces the vaccine. And on page 714, Arntzen, with Texas A&M colleagues Tariq Haq and Hugh Mason, and John Clements of Tulane Medical Center in New Orleans, report the first results indicating that edible vaccines may be feasible, although even the researchers concede that much more work is needed before this approach can be tried on humans.

Arntzen said the idea was prompted by a desire "to do a better job of combining the best of agricultural and medical biotechnology." In particular, he notes, "the dramatic impact of modern vaccines is not reaching the developing world, where it is most needed." That's because such nations often lack the refrigeration and other equipment needed for making and delivering vaccines. But edible vaccines would not require such resources, and in the early 1990s, Arntzen's team started work aimed at developing oral vaccines to prevent enteric diseases, including cholera and diarrhea caused by bacteria such as *Escherichia coli*, *Shigella*, and *Salmonella*. Bacterial diarrheas are a leading cause of infant deaths in the developing world.

The first step was to show that proteins made in plants could elicit immune responses in animals; the Arntzen team achieved that goal by introducing the gene encoding a surface protein from the hepatitis B virus (HBV) into tobacco plants. Not only did the plants make the viral protein, but when injected into mice, it triggered production of antibodies that recognize the hepatitis B protein.

In the next phase, Arntzen and his col-

Plants as Chemical Factories

Synthetic fabrics have been a great boon to busy people. But they're not perfect. "Wrinkle-free polyester," for example, just doesn't have the feel of cotton. But a new specialty fiber that couples the touch and look of natural cotton with the easy care of synthetics may now be on the way—thanks to recombinant DNA.

Scientists at Agracetus Inc., a biotechnology company in Middleton, Wisconsin, are genetically engineering cotton plants to produce fibers with a polyester-like compound in the normally hollow fiber cores (*Science*, 16 December 1994, p. 1811). And Ganesh Kishore, who heads a plant biotech group at Monsanto Corp. in St. Louis, says Agracetus's new cotton plant is "just the tip of the iceberg. Biotechnology is completely changing the concept of plant-based raw materials." Whereas plant geneticists once focused exclusively on improving the quality of food plants, they are now increasingly turning their attention to creating plants that can provide a wide array of nonfood, nonfeed materials.

In addition to Agracetus's natural cotton-polyester blend fibers, the materials on the genetic engineering drawing board include biodegradable plastics, industrial lubricants, and feedstocks for soaps and detergents, as well as drugs and pharmaceuticals, including potential new vaccines (see main text). "Plants make a huge variety of chemical structures with industrial potential," says plant biochemist John Ohlrogge of Michigan State University in East Lansing. Indeed, management consultants Roger Shamel and Michele Keough of Consulting Resources Corp. in Lexington, Massachusetts, predict that sales of nonfood products from genetically transformed plants will grow from about $15 million per year today to $320 million or more by 2005.

AGRACETUS INC.

Blooming business. Modified rapeseed plants may produce a variety of industrial chemicals.

The first nonfood products of plant bioengineering likely to achieve large-scale, commercial success are custom-designed, industrial oils and specialty polymers, such as plastics. (See article by Töpfer *et al.* on p. 681) Calgene, for example, already has in commercial production a genetically engineered rapeseed plant that was modified to produce lauric acid, a 12-carbon fatty acid used to make soaps and detergents.

Inducing rapeseed to make lauric acid was relatively simple, says Andrew Baum, president of Calgene's oils division. The researchers needed to introduce only one gene, which came from the California bay tree. That gene shut off fatty acid synthesis after 12 carbons rather than allowing the acids to grow to the 18-carbon length normal for the plant, while having little effect on productivity. In field tests, the modified rapeseed produced 35 to 40 bushels of seed per acre, compared with 35 to 50 bushels yielded by normal rapeseed. Eric Rey, Calgene oil vice president for operations, says that steering the new rapeseed through the regulatory process was also straightforward. He attributes this to the fact that laurate oils, which are currently obtained from imported coconut and palm kernel oils, "are not a new product. ... They are an existing plant product in a novel packet."

After obtaining key approvals last year, Calgene planted a few thousand acres of the modified rapeseed near Albany, Georgia. The oil from the first crop, which will be harvested this month, has already been sold to a major manufacturer of soaps and detergents for what Baum describes as "a reasonable profit."

Because rapeseed is easy to grow and a good oil producer, it's also been the target of other efforts aimed at creating plants that produce industrial oils. Calgene has induced it to produce erucic acid, which is used as a lubricant and as a feedstock for making nylon 13-13, by introducing two genes, one from the jojoba plant and the other from meadowfoam. And Michigan State's Ohlrogge wants to get rapeseed to produce petroselinic acid, an isomer of oleic acid which can be used to make margarine and shortening.

These plants are several years from commercial production, however. Calgene's erucic acid producer is undergoing greenhouse testing, and the Ohlrogge team hit a snag when the researchers found that they needed not one gene as originally thought, but two or three. "Things are more complicated than they seemed originally," Ohlrogge says. This, he notes, points up one of the problems that can hinder efforts to develop plants as chemical factories. Researchers first have to understand the synthetic pathways with which they are tinkering.

Commercial plastic-producing plants are also several years away, although researchers have been making progress. One such effort comes from plant molecular biologist Chris Somerville of the Carnegie Institution of Washington's plant lab in Stanford, California, and his colleagues, who have introduced bacterial genes that make enzymes for synthesizing the biodegradable plastic polyhydroxybutyrate (PHB) into the tiny plant *Arabidopsis*. The original gene transfer worked, although the resulting plants were sickly and made very little PHB. Somerville's group recently solved the problem by adding a sequence to the transferred genes that causes the enzymes they make to be targeted to storage vesicles called plastids. This both provides the enzymes with high levels of a key starting compound for PHB synthesis and protects the rest of the plant cell from the possibly harmful effects of PHB accumulation. As a result, PHB synthesis showed a 100-fold increase with no significant ill effects on plant growth or seed yield.

Agracetus scientists are using a similar approach in their efforts to make natural permanent-press cotton fibers. They have already gotten production of PHB in the fiber cells of cotton plants transformed with the bacterial genes, but are still working to get the right amount of the plastic into the fiber cores. To do this, they will need to hitch the right regulatory sequences to the bacterial genes, says Agracetus's director of fiber development, Maliyakal John. He predicts that the company can achieve its goal in 3 to 5 years.

If Agracetus and the other companies succeed in their efforts, the availability of plants that produce industrial chemicals might both reduce reliance on the oil-derived chemicals that now provide some of these materials and also help redirect U.S. agriculture away from the production of surplus foods, such as corn. This might help reduce agricultural subsidies, which have ranged between $6 billion and $26 billion in recent years. And then society, as well as the companies, could reap broad benefits from the new wave of bioengineered plants.

–A.S.M.

leagues started with an *E. coli* gene that makes part of the protein, called an enterotoxin, that induces diarrhea in susceptible animals. They then introduced the gene into potato plants in such a way that the enterotoxin proteins were made in the potato tubers themselves. Mice that ate these raw potatoes developed antibodies to the enterotoxin, including mucosal antibodies, which are secreted into the digestive system, where they are most needed to protect against bacterial infections. The researchers are now planning experiments to determine whether this oral vaccine will in fact prevent *E. coli* infections in animals.

The medical community's reaction to this work is one of intense curiosity. "Oral vaccines from transgenic plants ... are worthy of a lot of attention in research labs," says Joseph Melnick, a virologist at Baylor College of Medicine in Houston. Robert Daum, an infectious-disease expert at the University of Chicago, agrees that the idea is worth pursuing. "For countries with a per capita health budget of a couple of dollars, the possibility of producing [domestically grown] vaccines is very exciting," he says.

Still, several issues will have to be resolved before this intriguing idea can become a reality. For one thing, the enterotoxin protein used by the Arntzen group is an extraordinarily potent inducer of immune responses. Other immunizing proteins may not work so well when taken orally. In fact, they can have the opposite effect, because many proteins in the diet induce tolerance, making the immune system less able to mount a response against them. Or compounds in plants may compromise the ability of the vaccine protein to induce immunity. And, of course, the food containing the vaccine protein must be palatable. And that's a problem with potatoes, which must be cooked before they're eaten. The heat used in cooking would cause the vaccine protein to denature, reducing or eliminating its ability to elicit immunity.

The potato problem, however, may be nearing a solution—through a switch to a carrier that is eaten raw: bananas. Arntzen and his colleagues have introduced a foreign gene into banana plants and shown that the gene is expressed there, the first time this has been accomplished with the banana, the world's fourth most important food crop. They did not use a gene for a potential vaccine protein in these experiments, but plan to put the *E. coli* enterotoxin gene in bananas. If that succeeds, Arntzen wants to add genes for additional vaccine proteins. "My vision," he says, "is to have a baby-food jar containing a transgenic banana" that will protect against several infections.

Arntzen's strategy for oral vaccines is to use plant-made proteins to stimulate the recipient's own immune system. This is known as "active vaccination." Another effective immunization strategy is "passive vaccination," achieved by giving protective antibodies that are made outside the body, usually in cell culture. On page 716, Julian Ma at Guy's Hospital in London, Andrew Hiatt of Plant Biotechnology in San Diego, Mich Hein of the Scripps Research Institute in La Jolla, California, and their colleagues report progress in attempts to use plants to make an antibody that might be added to toothpaste to protect against the bacteria that cause tooth decay.

AGRACETUS INC.

Antibody factory. These soybean plants have been engineered to make a monoclonal antibody.

The team wanted to make mucosal antibodies, which are normally secreted through the linings of the mouth and digestive tract. This proved to be difficult, says Hein, because a complete secretory antibody contains four different protein chains: the heavy and light chains that make up all antibody molecules, plus two additional proteins, one of which is added during secretion.

To achieve their goal of making functional secretory antibodies in plants, the group first made four separate lines of transgenic tobacco plants, each of which had a different gene for a secretory antibody protein inserted into it. When they then crossed the four lines, using conventional plant breeding techniques, the progeny produced the expected secretory antibody, which is directed against the bacterium *Streptococcus mutans*, which causes tooth decay. The researchers hope that their plant antibodies can be formulated into a toothpaste. "We're aiming for an oral prophylactic [for tooth decay]," Hiatt says. The group is planning to start human trials, perhaps using volunteer dental students, within a few months.

In their quest to develop plant-based vaccines, researchers are not limiting their efforts to oral therapies, however. Despite the considerable challenges associated with purifying plant materials to a level adequate for injection, some groups are targeting injectable materials for possible development. For example, Arntzen's group is investigating whether the HBV protein they've made in genetically engineered tobacco plants can be used to protect against hepatitis.

Vaccines aren't the only potential uses for proteins made in plants. Take the monoclonal antibodies now under investigation for cancer therapy. They often can't be made in sufficient quantities for commercialization by conventional methods, but plant production might remedy that, says Agracetus's Russell. His company has genetically engineered soybeans to make a monoclonal antibody, known as BR96, that has shown some promise in preclinical trials as a vehicle for targeting the chemotherapeutic drug doxorubicin to breast, colon, ovarian, and lung tumors. The company is growing the antibody-producing soybeans in Puerto Rico with the goal of isolating enough antibody to start clinical trials.

Even plant viruses may be getting into the act as a possible source of human vaccines. Reasoning that plant viruses can't cause disease in animals, a number of groups have redesigned certain of the viruses to express fragments of antigenic proteins on their surface in the hope that these modified virus particles can coax a protective reaction from mammalian immune systems. For example, in a paper in press in *Vaccine*, John Fitchen, Roger Beachy, and Hein, all of Scripps, report that they've engineered a coat protein from tobacco mosaic virus (TMV) to contain a 13–amino acid fragment from a protein found in the mouse zona pellucida, the jellylike material covering unfertilized eggs. The researchers also found that, when injected, TMV particles carrying the modified coat protein triggered production of antibodies in mice that specifically recognize the zona pellucida protein fragment. Hein says that, in theory, vaccination with the modified viral coat protein might work as a form of birth control, because antibodies to the zona pellucida could prevent fertilization of eggs. But, for now, he says, "we just want evidence that [modified] virus coat protein can trigger the immune system into action."

In addition, Jack Johnson of Purdue University, George Lomonosoff of the John Innes Institute in Norwich, U.K., and Lisa Wisniewski of Axis Genetics in Cambridge, U.K., have put fragments of the gp41 surface protein of the HIV virus onto cowpea mosaic virus. In an upcoming issue of *Seminars in Virology*, they report that these modified plant viruses elicit the production of mouse antibodies that neutralize the virus in test tube studies. Says Wisniewski, "The results indicate the possibility of producing a preventive HIV vaccine by presenting a cocktail of specific HIV epitopes on the surface of a plant virus."

While no one expects these experiments to yield commercial benefits soon, there is growing awareness that basic studies in plant biology may reap impressive and unusual harvests in the future. And that may make plants, once again, a dominant source of preventive and therapeutic drugs.

–Anne Simon Moffat