In contrast, other studies suggest that soil microbes themselves might use any extra nitrogen produced; in that case, plant growth will eventually be limited, says Kurt Pregitzer of Michigan Technical University, who works on the problem with Teeri. And in the long term, high CO_2 levels could slow the nitrogen cycle: If there is less nitrogen per leaf, leaf litter may decay more slowly and limit the amount of nitrogen available to plants, says Pregitzer. For now, there's evidence for both cycles. "It may be that both positive and negative cycles can occur," says Bazzaz. "The trick for us is to find out which scenario operates under which conditions and ecosystems."

Despite all these uncertainties, most ecologists expect terrestrial ecosystems to absorb some—but not all—of the extra carbon that will be pumped into the air in the next century. Bazzaz estimates the global response to doubled CO_2 will be no more than a 10% to 20% growth increase. In that case, plants will almost certainly not be able to balance the carbon budget.

As for ecosystems, although researchers can't predict the effects of high CO_2 in detail, they do forecast change. Even if global warming never comes, if you take a walk in a northern Michigan forest 50 years from now, you'll probably find a changed world, with a different mix of trees and other species, says Teeri. Denser forest canopies may favor shade-tolerant species, and the identity of key pathogens may shift as various insects decline, prosper, or switch from one plant to another under high-CO_2 conditions. In some species, high CO_2 can also trigger earlier flowering, which could disrupt insect pollinators. Researchers are just beginning to explore such changes in Duke's ring of 21st-century air, as well as in other facilities.

For now, there's consensus that air rich in CO_2 will be a boon for many farmers, at least in developed nations. But many ecologists believe it's too soon to say whether humans will celebrate or mourn the biodiversity shifts triggered by our changing atmosphere. One thing seems certain: Whether air enriched in CO_2 warms the globe or not, the gas will alter the growth of green plants and so act as a potent force for global change.

–Elizabeth Culotta

Additional Reading

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INTELLECTUAL PROPERTY

Sweeping Patents Put Biotech Companies on the Warpath

In October 1992, the U.S. Patent and Trademark Office (PTO) stunned the agricultural biotechnology community by awarding a patent to a single company, Agracetus Inc. of Middleton, Wisconsin, for rights to all forms of genetically engineered cotton—no matter what techniques or genes are used to create them. "It was as if the inventor of the assembly line had won property rights to all mass-produced goods, from automobiles to washing machines," says Jerry Caulder, chief executive officer of San Diego–based Mycogen Corp.

While the patent's breadth took the plant biotech community by surprise, what happened next was less surprising: a round of legal challenges that hasn't ended yet. And it would not be the last such battle. At least three major legal tussles over the awarding of broad patents for genetically altered plants are now tying up agricultural biotechnology companies, and more such conflicts are on the horizon. "It's like the Superfund program," says Neil Hamilton, director of Drake University's Agricultural Law Center, referring to the U.S. Environmental Protection Agency's embattled program to clean up toxic waste sites. "Instead of money getting spent to benefit society, it's getting diverted to fights over ownership and liability."

These test cases—along with some similarly broad patents issued in Europe—are being closely followed in the biotech industry. For Agracetus and other companies, the outcomes of these patent decisions could significantly affect their bottom lines. Some smaller companies could even be forced out of business if they have to pay licensing fees for use of the patented technologies. According to Caulder, for example, Agracetus has asked $1 million for a license to exploit its cotton technology—a significant sum for a small start-up company. (Agracetus refused to confirm the figure.)

A budding dispute. Since the early 1980s, the PTO has awarded 112 patents for genetically engineered plants and recombinant DNA approaches to manipulating plants. But the legal skirmishes have been touched off by a handful of patents that stand to be real moneymakers: high-value crops or technologies experts expect to be widely used.

In addition to Agracetus's cotton patent, these include a patent awarded on 2 March 1993 to biochemist Masayori Inouye of the State University of New York, Albany, who now works at the University of Medicine and Dentistry of New Jersey. The patent, licensed to New York City's Enzo Biochem Inc., gives the firm broad rights to use novel RNAs, called antisense RNAs, to block the activity of specific genes in any crop. On the same day PTO issued the patent, Enzo sued Calgene Inc., a Davis, California–based company that uses an antisense gene it patented to produce the Flavr Savr tomato, a vine-ripening tomato that resists spoiling. And in March 1994, the European Patent Office (EPO) granted broad rights to Agracetus for all forms of genetically engineered soybeans. This patent, too, is under legal challenge.

While none of these cases may have the longevity of Jarndyce and Jarndyce, all of them are likely to be long-running. The story of the cotton patent, in fact, is already a decade old. About 10 years ago, a team of Agracetus scientists led by Paul Umbeck launched a program to develop a system for inserting foreign genes into cotton, using as a carrier the bacterial pathogen *Agrobacterium tumefaciens*. This bacterium easily infects cotton, and in the course of the infection, transfers a plasmid, a small circular piece of DNA, into the plant cells, where the plasmid DNA splices itself into the cellular genome. A foreign gene inserted into the plasmid will also be incorporated in the host plant's DNA, and after plant scientists infect cotton cells in culture with *A. tumefaciens* carrying such a modified plasmid, they can regenerate whole plants carrying the new gene from the single cells transformed by the bacterium.

But when Umbeck filed an application for a patent on the technique in August 1990, he claimed to have invented more than just a modified *Agrobacterium* technique for use in cotton: He claimed "cottonseed capable of germination into a cotton plant comprising in its genome a chimeric recombinant

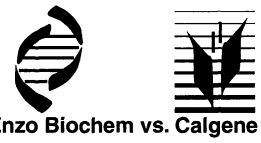
A Tale of Four Broad Patents

1992

October
U.S. Patent and Trademark Office (PTO) awards patent to Agracetus granting rights to all forms of genetically engineered cotton.

1993

March
PTO awards patent, licensed to Enzo Biochem, granting rights to antisense technology used in crops; Enzo sues Calgene for infringing its antisense patent.



Enzo Biochem vs. Calgene

gene construction" as well as "cotton plants germinated from [such] seeds." In other words, Umbeck's patent claimed rights to all genetically engineered cotton plants and seeds, regardless of the method used to engineer the plants. PTO examiner David Fox accepted the claims, and the office issued a patent to Umbeck 2 years later.

The patent provoked an outcry from many in the agricultural biotech community. "I was violently opposed to the idea of such broad patent claims," says Peter Day, director of the Rutgers Agricultural Biotechnology Center. Researchers at the U.S. Department of Agriculture (USDA) also began grumbling that the patent might crimp their research. These concerns drew national attention in December 1993, when USDA cotton research Jerry Quisenberry lambasted the Agracetus patent in an interview with National Public Radio. The patent, Quisenberry said, "is not good for the country, and it's not good for American agriculture."

Agracetus complained to USDA, and in subsequent comments to the press Quisenberry and other USDA scientists made it clear they were speaking on their own behalf. Official USDA spokespersons appeared more comfortable with the Agracetus patent, saying it would not affect USDA research. And in a public statement, Agracetus said it would "make research licenses available, free of charge, to all academic or USDA researchers upon request."

But that didn't mean all the waters had been smoothed. The patent forced companies developing their own transgenic cotton to strike a licensing agreement with Agracetus or abandon research on transgenic cotton before it proceeded to the development stage. Agricultural biotech giants Calgene and St. Louis–based Monsanto, with transgenic cotton products in the pipeline, could afford to ante up for the license. But Mycogen, a much smaller company, decided to save the $1 million licensing fee and hope that the patent would be challenged in court.

Indeed it was. In January 1994, the Alexandria-based law firm Breneman & Georges filed a request on behalf of an unidentified party for PTO to re-examine the cotton patent. A few months later, USDA filed its own request.

A key reason cited in both challenges was that the Agracetus patent had neglected to refer to a discussion of cotton transformation and regeneration in another company's earlier patent application. Therefore, the challengers argued, genetically engineered cotton had already been "obvious" to scientists in the field, and Agracetus's broad claim to having invented genetically engineered cotton was invalid.

Stepping into the fray, PTO examiner Patricia Moody last December issued an "office action" that rejected all claims under the Agracetus patent—essentially throwing out the patent. Quoting patent law, she wrote, "a patent may not be obtained" if the subject matter "would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains."

"That's a pretty potent rejection," says Howard Silverstein, a USDA lawyer involved in the challenge. "I've seen much weaker statements used by the patent office to uphold a rejection," he says.

Agracetus filed a response to the PTO action in March. However, officials at Agracetus and Grace, its parent company, refused to provide *Science* with Agracetus's response, nor would they comment on the PTO's action until it's made final or reversed, an action expected later this month. In the meantime, Agracetus's patent remains in force. If PTO finally rejects the patent, Agracetus can appeal to federal court.

And federal court is not a new venue for plant biotech patent fights—it's where the struggle over rights to antisense technology is playing out. Despite months of negotiations in anticipation of the patent being awarded, Enzo sued Calgene in March 1993 for infringing on a patent that appears to give Enzo rights to all crops engineered to express antisense genes. Calgene, meanwhile, already has begun marketing its Flavr Savr tomato, which uses an antisense gene patented by Calgene. The case was being tried last month in U.S. District Court in Wilmington, Delaware; no decision had been reached as *Science* went to press.

Similar squabbles in Europe. Even as controversy over the breadth of the cotton and antisense patents raged in the United States, another sprang up in Europe over the similarly broad patent the EPO issued to Agracetus covering all genetically engineered soybeans. In this case, Agracetus scientists did not use *Agrobacterium* for inserting foreign genes into plant cells. Instead, they used a "gene gun," which shoots foreign DNA encapsulated in biologically inert gold spheres directly into soybean tissue. Whole soybean plants expressing the foreign DNA can then be regenerated from the treated tissue. In addition to claiming rights to this technique, Agracetus again made a sweeping patent claim covering all soybean plants that express foreign gene products.

As in the case of the cotton and antisense patents, the breadth of the soybean patent invited challenge. Last December the Rural Advancement Foundation International (RAFI), a nonprofit based in Ottawa, Ontario, filed one with EPO. RAFI claims the Agracetus patent should be disallowed under a section of the European Patent Convention called *l'ordre publique*, or "public morality." Under this vague statute, EPO is not supposed to issue plant or animal patents that would threaten public morality. "A patent granting a single corporation monopoly control over genetic research on one of the world's most important food crops is a threat to world food security," says Patrick Mooney, RAFI's executive director. The soybean patent, charges Mooney, "demonstrates the patent system is recklessly out of control."

RAFI is not alone. Monsanto, Sandoz, DeKalb Genetics Corp., Pioneer Hybrid, and Ciba-Geigy all have filed challenges over the last year, although the company briefs argue against the patent on technical grounds. Their main criticism is that the invention was "obvious" because information about "gene gun" transformation and regeneration existed in the literature before the patent was filed. "Based on what was known, Agracetus was asking for far too broad a coverage on its patent," says Monsanto spokesperson Karen Marshall.

Agracetus would not comment on the challenges to the soybean patent. An Agracetus statement says the company "recognizes the potentially substantial benefit to society, and therefore intends to make nonexclusive licenses broadly available at reasonable terms." Research licenses, it says, will be made available free of charge. The EPO is expected to respond to the challenges at the end of this year or in early 1996.

1994

January
Breneman & Georges law firm files challenge to Agracetus's cotton patent.

March
European Patent Office awards patent to Agracetus granting rights to all forms of genetically engineered soybeans.

April
PTO examiner Patricia Moody agrees to re-examine Agracetus's cotton patent.

June
U.S. Department of Agriculture files second challenge to Agracetus's cotton patent.

1995

December
PTO issues "office action" throwing out the Agracetus's cotton patent.

Rural Advancement Foundation International joins several companies in challenge to Agracetus's soybean patent.

January
PTO awards patent to Mycogen granting rights to any method of modifying *Bt* insecticidal protein genes to insert them into plants.

March
Agracetus files response to PTO office action.

April
Enzo Biochem vs. *Calgene* trial held in U.S. District Court in Wilmington, Delaware.

Agracetus

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

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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-