NEWS

Finding New Ways to Fight Plant Diseases

With plant pathogens and the insects that spread them exploding worldwide, researchers must continually search for better ways to protect plants against infectious diseases

In March of this year, the city of New York dedicated a memorial park to a scourge that starved hundreds of thousands of people and spurred a huge wave of immigration to the United States: the Irish potato famine of 1845 to 1847. A fitting reminder of the kind of devastation unlikely to occur in today's world of high-tech agriculture? Not exactly. "Worldwide, plant diseases with the potential to wipe out crops are exploding," says Cornell University agricultural scientist David Pimentel.

There are several reasons for this explosion. Many plant pathogens and the insects that often spread them have overcome the pesticides, agricultural practices, and biocontrols that once held them in check. At the same time, some effective chemicals, such as the fungicide methyl bromide, are being banned because of environmental concerns. And efficient global travel is spreading viral, bacterial, and fungal plant pathogens into new areas, while global warming is allowing insect vectors to expand their ranges.

For example, *Phytophthora infestans*, the fungal-like alga responsible for the potato famine, caused devastating losses when it was transported on fruits and vegetables from northwest Mexico to Canada and the northwestern United States in the early 1990s. And geminiviruses, once a major threat mainly in Africa and the Middle East, have wiped out vegetable crops in the Caribbean and Florida (*Science*, 3 December 1999, p. 1835). "Few people realize that our 'scientific advantage' in agriculture is dwindling rapidly, and pest problems are as serious today as they were 100 years ago," says University of California, Berkeley, plant pathologist Milton Schroth. And like mid–19th century Ireland, developing countries with no food surpluses are especially vulnerable, Schroth points out.

Plant researchers are struggling to maintain their scientific advantage, often in the face of public opinion wary of genetically modified foods. On the scientific level, they are achieving success on several fronts. They are developing an armory of new weapons, from high-tech genetic engineering, to techniques that induce a plant to turn up its own defense mechanisms, to relatively low-tech cultivation strategies aimed at disrupting plant-pest interactions. "Disease resistance is turning out to be a lot more complicated than we thought," says Cornell plant pathologist William Fry. Still, he says, there are "hundreds of opportunities for reducing disease" waiting to be explored.

Protected. The papaya trees at right, which carry a gene from papaya ringspot virus, resist infection, while the unmodified trees at left are susceptible. The inset shows fruit from the modified papaya strain.

CREDITS: (LEFT TO RIGHT) R. MANSHARDT/UNIV. OF HAWAII; S. FERREIRA/UNIV. OF HAWAII AND D. GONSALVES

The best defense

The first line of defense against plant diseases is natural resistance, which can often be transferred between species by crossbreeding. But plant breeding is time-consuming, and microbial pathogens evolve quickly to overcome breeders' painstaking work. "Durable resistance is elusive," says University of Florida, Gainesville, plant pathologist Ernest Hiebert. By allowing researchers to introduce genes directly into plants, genetic engineering has offered a new tool for staying a step ahead of rapidly evolving pests. But it has also sparked a huge international backlash and introduced a new term into the lexicon of public protest: GM, for genetically modified, foods.

Until this backlash gathered force in the late 1990s, GM crops made rapid progress. The most prominent development involved the transfer of a gene encoding an insect-killing protein from the bacterium *Bacillus thuringiensis* (Bt) into crop plants, including corn, cotton, and potato. Bt-transformed crops are now widely planted, particularly in the United States, resulting in reduced application of chemical insecticides for some crops, such as cotton.

Among the specific concerns raised by Bt-modified crops is that insects that feed on the plants will become resistant to the toxin. Researchers are already trying to head off that possibility. Different strains of Bt produce different toxins, and entomologist Leigh English of Monsanto Corp in St. Louis, which has pioneered the technology, says the company is transforming crop plants with several different Bt toxin genes at once. Resistance to such multiple toxins is unlikely to develop rapidly.

Other companies, such as Ecogen in Langhorne, Pennsylvania, and Syngenta (formerly Novartis) in Greensboro, North Carolina, are also developing products that combine genes from different Bt strains to increase activity against key pests, including the armyworm and cotton bollworm. Farmers are also encouraged to plant unmodified plants as insect refuges. These steps will "allow us to stay ahead of the possibility of insects developing resistance to Bt," English says.

Modification with Bt genes makes plants toxic to insects that eat them. Another strategy, which has already saved one crop from a devastating infection, aims to boost a plant's resistance to specific disease-causing pathogens. It involves a process somewhat akin to vaccination: revving up the plant's own defenses by exposing it to the pathogen's proteins. In this case, researchers stitch bacterial or viral genes, or their fragments, into the plant's genome.

Exactly how this produces resistance has been somewhat mysterious, but within the past 2 or 3 years, David Baulcombe of the Sainsbury Laboratory in Norwich, United Kingdom, Herve Vaucheret of the Laboratory of Cellular Biology in Versailles, France, and others have identified a likely mechanism. They have shown that, in ways that are not yet completely understood, the viral genes trigger a plant defense mechanism that degrades viral RNAs, thus disabling the infectious agent (see Review by Vance and Vaucheret on p. 2277).

Researchers, such as Roger Beachy—who pioneered pathogen-derived resistance, first at Washington University and now at the Donald Danforth Plant Science Center, both in St. Louis—have transformed various plants with genes from many of the 40 or more families of plant viruses. This work has produced melon, squash, tomato, tobacco, and papaya strains that are protected from a variety of viral diseases. Indeed, a modified strain of papaya produced by Dennis Gonsalves and Carol Gonsalves of the New York State Agricultural Experiment Station in Geneva, New York, and colleagues from the University of Hawaii and Upjohn company has essentially saved the Hawaiian papaya industry.

In the mid-1980s, the researchers began attempting to modify papaya with a gene from ringspot virus, a pathogen for which there is no known natural resistance. Their initial field trial showed success in 1992, the year the ringspot virus started causing widespread damage to Hawaiian papaya trees. Now, about 60% of papaya trees in the state are protected by this means.

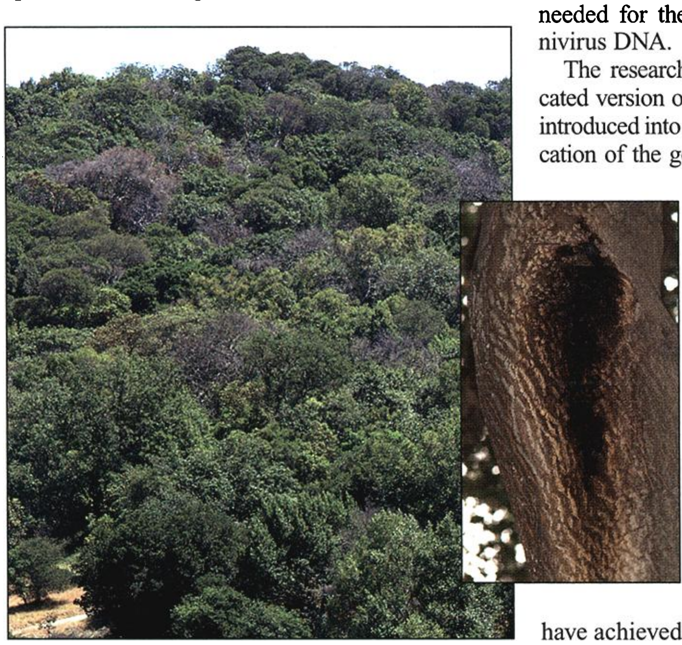
Last year, the Gonsalves team took another step forward. They introduced a chimeric transgene, containing sequences from the turnip mosaic and tomato spotted wilt viruses—two of the top 10 viral pathogens of vegetables—into tobacco. As reported in the August 2000 issue of the *Journal of General Virology*, the resulting plants became resistant to both viruses—the first time researchers achieved multiple virus resistance by transferring viral genetic material. Others are hot on their heels.

In a paper published electronically on 7 May by the *Journal of Biological Chemistry*, Claude Fauquet of the Danforth Center and his colleagues describe results that may lead to a way to make plants resistant to multiple geminiviruses. About 150 sorts of these viruses are plant pathogens, causing, for example, tomato yellow leaf curl and tomato mottle diseases. Hoping to find a viral component that might protect against many, if not all, of these viruses, the Fauquet team turned to the gene encoding a protein needed for the replication of geminivirus DNA.

The researchers found that a truncated version of the gene could, when introduced into plants, inhibit the replication of the geminivirus from which they obtained the gene. But it also inhibited replication of three other geminiviruses, although to a lesser degree. This suggests that a few transgenes could disrupt replication of a wide range of geminiviruses. "We're on a search for a universal strategy for controlling plant viruses," says Fauquet.

Plant engineers have achieved their earliest successes against viruses, but they are now turning their attention to bacteria and fungi, with some promising results. As reported in the November 2000 issue of *Nature Biotechnology*, William Kay and Santosh Misra of the University of Victoria, British Columbia, and their colleagues engineered potatoes with a chimeric gene encoding segments of two insect proteins: a cecropin, an antimicrobial peptide made by moths and other organisms, and melittin, a component of bee venom. The plants proved resistant to the potato blight fungus, and the harvested tubers were also protected against a bacterium that causes "soft rot" in stored potatoes (*Science*, 16 March, p. 2070). Feeding the modified raw potatoes to mice produced no obvious toxic effects.

Wilt preventer. Spraying the tomato plant at right with the protein harpin protected it from the bacterial wilt pathogen, while the untreated plant at left shows characteristic wilt symptoms.



Sudden death—for oaks. A newly discovered species of *Phytophthora* has killed many of the oaks on this hillside in Marin County, California. The inset shows the characteristic lesion in the bark of an affected tree.

CREDITS: (TOP TO BOTTOM) K. LOEFFLER/CORNELL UNIVERSITY; D. SCHMIDT/UC BERKELEY

Genetic options

The anti-GM movement has focused its opposition largely on the transplanting of genes from entirely different organisms into crop plants. Some researchers are trying what may be a less contentious strategy: tinkering with a plants' own disease-resistance genes. For example, plant pathologist Brian Williamson and his colleagues at the Scottish Crop Research Institute in Dundee have found that polygalacturonase-inhibiting protein (PGIP) can control the gray mold *Botrytis cinerea*, which is the most serious cause of disease in ripe raspberries. The problem is that PGIP is normally expressed only in immature green fruit.

The researchers would like to alter the PGIP gene's regulatory sequences in raspberry so that the protein would be made all the time, but so far they haven't been able to produce such modified raspberry plants. They have, however, introduced the raspberry PGIP gene, with a regulatory element from cauliflower mosaic virus that keeps the gene continuously active, into chickpea—a valuable crop in India, the Middle East, the southern Mediterranean, and Australia that is vulnerable to fungal diseases. "The germ plasm has been exported to our partners at ICRISAT [the International Crops Research Institute for the Semi-Arid Tropics in Patancheru, Andhra Pradesh, India] for testing," says Williamson. If successful, it would be a significant breakthrough, he says, because plant breeders have worked without success for many years to make this nutritious legume resistant to gray mold.

Researchers have numerous other candidates for such genetic manipulations. In the last decade or so, they have isolated several dozen disease-resistance genes, defending against, for example, tobacco mosaic virus and *Pseudomonas syringae*, from the experimental plant *Arabidopsis* and also from about a dozen crop plants, such as potato, tobacco, flax, rice, and tomato. Work by plant molecular biologist Barbara Baker at the U.S. Department of Agriculture (USDA) lab in Albany, California, and others has shown that, in many cases, disease-resistance genes from different species are similar. This suggests that the resistance mechanisms they encode have been evolutionarily conserved and may be general.

At this point, however, it's too soon to tell whether it will be possible to beef up disease defenses in crop plants by giving them additional copies of resistance genes or by altering the genes' regulatory sequences to make them more active. Some practical problems remain to be solved. For example, one source of unwanted variability in transgenic plants is the unpredictable placement of transgenes. Also, unknown point mutations generated during tissue culture, which is needed to grow transformed cells into whole plants, can induce unexpected traits or loss of desirable traits.

Meanwhile, an alternate approach to crop protection is showing promise: the use of externally applied chemical stimulators that elicit plants' natural defensive mechanisms. These include the so-called hypersensitive response (HR), which causes cells to die in the immediate vicinity of infection sites, thereby preventing further pathogen spread, and systemic acquired resistance (SAR), which first results in necrotic lesions but also activates a signal system that results in a marked reduction in disease symptoms after subsequent infection.

Researchers have begun to identify compounds that elicit these defenses. Steven Beer and his colleagues at Cornell found that *Erwinia amylovora*, the bacterium that causes fire blight in apple and pear trees and related plants, encodes harpin, a glycine-rich protein that elicits HR and SAR to pathogens and insects. As an added benefit, it also enhances growth. The harpin technology was licensed to Eden BioScience in Bothell, Washington, and last summer its harpin product, known as "Messenger," went on sale for use on a broad array of plants, including strawberry, cotton, and tomato.

In a related strategy, Alison Tally and others at Syngenta took a page from the drug developers' book. In 1989, after screening at least 40,000 compounds for their ability to trigger defense mechanisms in plants, they identified an isonicotinic acid derivative, known as "Actigard," that induces SAR. Once activated by this compound, a plant's defense mechanisms may remain active for many weeks, even when the inducer is degraded, says Tally. Actigard was registered in August 2000 by the U.S. Environmental Protection Agency and is being introduced into the marketplace to prevent bacterial spot and speck infections of tomatoes, downy mildew on spinach, and blue mold on tobacco.

SOME EMERGING PLANT PATHOGENS

Disease	Hosts	Geographic Distribution
FUNGAL		
Late blight	Potato, tomato	Spreading worldwide
Downy mildew	Corn, sorghum	Spreading out of Southeast Asia
Rust	Soybean	Spreading from SE Asia and Russia
Karnal bunt	Wheat	Pakistan, India, Nepal, Mexico, U.S.
Monilia pod rot	Cocoa	South America
Rust	Sugarcane	The Americas
Blast	Rice	Asia
VIRAL		
African mosaic	Cassava	Africa
Streak disease	Maize, wheat, sugarcane	Africa
Hoja blanca (white tip)	Rice	The Americas
Bunchy top	Bananas	Asia, Australia, Egypt, Pacific Islands
Tungro	Rice	Southeast Asia
Golden mosaic	Bean	Caribbean Basin, Florida, Central America
Plum pox virus	Stone fruits (peaches, etc.)	Europe, India, Syria, Egypt, Chile
High Plains virus	Cereals	Great Plains, U.S.
BACTERIAL		
Leaf blight	Rice	Japan, India
Wilt	Banana	The Americas

An ecological approach

After bolstering plants' own defenses, the next best strategy for protecting crops is managing the environment to give plants a competitive edge over pathogens. This may involve using biocontrols, organisms or their products that can kill plant pathogens, or tinkering with physical aspects of crop growth, such as altering light, temperature, or the times of planting and harvesting.

The biocontrols include Bt, both the whole organism and the toxic proteins, which were sprayed on crops to control insects for 50 years before researchers began genetically engineering the toxins directly into plants. Also, growers have had decades of experience with pheromones, chemicals that elicit certain behavioral responses from insects. For example, traps containing pheromones that attract insects searching for a mate are used to determine insect populations and the need for pesticides.

More recently, a new product, called CideTrak, which was developed by scientists at the USDA and Trece Inc. of Salinas, California, couples an insect feeding stimulant with a low-dose pesticide. When sprayed on corn, this preparation kills adult corn rootworms, preventing egg-laying in the soil. This holds down next year's population of ravenous worms, thus reducing by 90% the amount of pesticide needed to control the rootworm.

Whole organisms can be used as insecticides as well. These include baculoviruses, which are safe for nontarget insects, humans, and the environment, and insect-killing fungi, which infect a broader range of insects than do other microbes. The largest use of baculoviruses is in Brazil for protection of soybeans against the velvetbean caterpillar. The fungal insecticide *Beauveria bassiana* is used on a moderate scale in eastern Europe and China to control the silverleaf whitefly, which infects hundreds of plants.

Cultivation practices can also help control plant diseases. For example, vegetable growers have begun adopting a strategy long known to wine enthusiasts. Some of the best grapes are grown by grafting vines that produce superior fruit onto the rootstocks of varieties that produce less desirable grapes but are resistant to soil-borne pathogens. In southern Europe, tomatoes are grafted onto the rootstock of eggplant and peppers, and melons are grafted onto cucumber rootstocks, often with the aid of machines. One thing that helps make this feasible is cultivating the plants to produce over longer periods. "It is now possible to grow productive tomato vines to a length of more than 20 feet [6 meters]," says plant virologist Jane Polston of the University of Florida, Bradenton.

Even plastic, generally thought to be detrimental to the environment, can be the farmer's friend. For decades, some growers have protected vegetables with reflective plastic mulch, which repels many insect pests, such as troublesome whiteflies and aphids. Lately, there have been some new entries to this field.

For example, Yehezkel Antignus and his colleagues at the Volcani Center in Bet Dagan, Israel, are testing ultraviolet (UV)-absorbing, clear plastic films to protect greenhouse crops against virus-carrying insects, such as aphids, white flies, and thrips.

Kibbutz workers first noticed that tomatoes grown under such plastic to protect them from burning by the sun escaped viral diseases. A study of insect behavior beneath the plastic revealed why: It disrupts the spread of microbes carried by insects that need UV light to navigate. "By interfering with vision behavior, contact between the vector and the plant may be prevented, and, therefore, virus spread is decreased," says Antignus.

Clear plastic can also help when used to cover the soil for 3 to 6 weeks before crops are planted. During this period, temperatures can rise to about 50 degrees Celsius, enough to kill some fungal spores, weed seeds, and nematodes. The technique has been used in Florida to protect vegetables and ornamentals, in Egypt and Italy to protect tomatoes and carnations, and in Italy and Turkey to protect peppers. In some cases, this technique replaces synthetic chemicals that are being banned or restricted, says plant pathologist R. J. McGovern of the University of Florida, Bradenton.

But despite the wide range of pest-control strategies under development, researchers know that they, like the Red Queen in *Alice's Adventures in Wonderland*, have to keep running just to stay in place. History has shown that no control, no matter how clever, is immune to pest evolution. "The battle against crop pests is ongoing, with short periods of relief when science temporarily gains the advantage," says Berkeley's Schroth. Adds plant pathologist Carolee Bull of the USDA in Salinas, California, who has just begun studies to learn why some organic farming techniques succeed while others fail: "There is an enormous amount of work to be done."

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NEWS

The Push to Pit Genomics Against Fungal Pathogens

Despite the damage done by these serious plant pests, researchers have barely begun sequencing their genomes

Ask a plant pathologist to draw up a "most wanted" list of dangerous microbes, and, chances are, many fungi would be near the top. Fungi and funguslike organisms called Oomycetes destroy crops, kill trees, ruin lawns and golf courses, and contaminate foods and animal feed with deadly toxins. Their dirty work causes some 10,000 different diseases in plants alone. Notorious examples include the late blight of potatoes, which caused the great Irish famine in the 19th century, and Dutch elm disease, which wiped out many elms in the United States in the mid–20th century. And now, a relative of the late blight potato pathogen, called *Phytophthora*, is taking out century-old live oaks in California.

Given this trail of destruction, most plant pathologists would put fungi near the top of another most wanted list: microbes whose genomes should be sequenced. Determining the complete sequences of pathogenic and nonpathogenic fungi could be a big help in determining what makes some fungi the microbial equivalents of Bonnie and Clyde while others never cause trouble—information that could help in the design of much-needed antifungal agents.

But although academic researchers have sequenced the genomes of dozens of bacteria, including important pathogens such as those causing cholera and Lyme disease, they have so far completed the sequence of only one fungal genome: that of yeast. A few others—such as those of the nonpathogenic bread mold *Neurospora crassa* and white rot fungus, which destroys deadwood—are in draft form. But even the white rot fungus was chosen more to test a new sequencing strategy rather than because of its economic importance. Although several biotech companies are sequencing the genomes of fungal pathogens, what they are learning largely remains behind closed doors. For the most part, "fungi have been left out in the cold," says Ralph Dean, a plant pathologist at North Carolina State University in Raleigh.

Money is the main problem. "The government funding agencies haven't committed the resources to get fungal genomics going," says Olen Yoder, a plant pathologist at the Torrey Mesa Research Institute in La Jolla, California. The National Institutes of Health, the Department of Energy (DOE), and the National Science Foundation (NSF) have poured hundreds of millions of dollars into genome sequencing; the draft of the human genome sequence alone cost more than $300 million. In contrast, plant pathology's fungal camp has had virtually no support. And fungi, with genomes that are several times larger than that of the average bacterium, are relatively expensive to sequence and assemble.

Rice menace. When the spores of the rice blast fungus (*inset*) infect rice plants, they can cause the rice head to droop and die.

PHOTOS BY H. DAVID THURSTON

Still, there are signs that the situation is beginning to improve. As the human genome project winds down, the big sequencing centers are beginning to take an interest in fungal genomes. In September 2000, the Whitehead Institute/Massachusetts Institute of Technology Genome Sequencing Project in Cambridge got NSF support to sequence the genome of *Neurospora crassa.* With that under its belt, the Whitehead would like to start on a new "Fungal Genome Initiative," with the goal of sequencing the genomes of 15 fungi, at least some of which will be plant pathogens. Moreover, this past spring, the U.S. Department of Agriculture (USDA), in conjunction with NSF, initiated a new $9 million program for sequencing the genomes of agricultural and forest pests, which could get the Whitehead's fungal initiative or other fungal sequencing efforts off the ground.

These efforts won't be sufficient by themselves, but they should at least begin to provide plant pathologists with a new arsenal of knowledge for neutralizing the threats that fungi pose to both food crops and ornamental species. And the information could be applicable to much more than plant diseases. Some of the fungi that cause problems in people "are the same pathogens that cause plant diseases," says Ann Vidaver, a plant