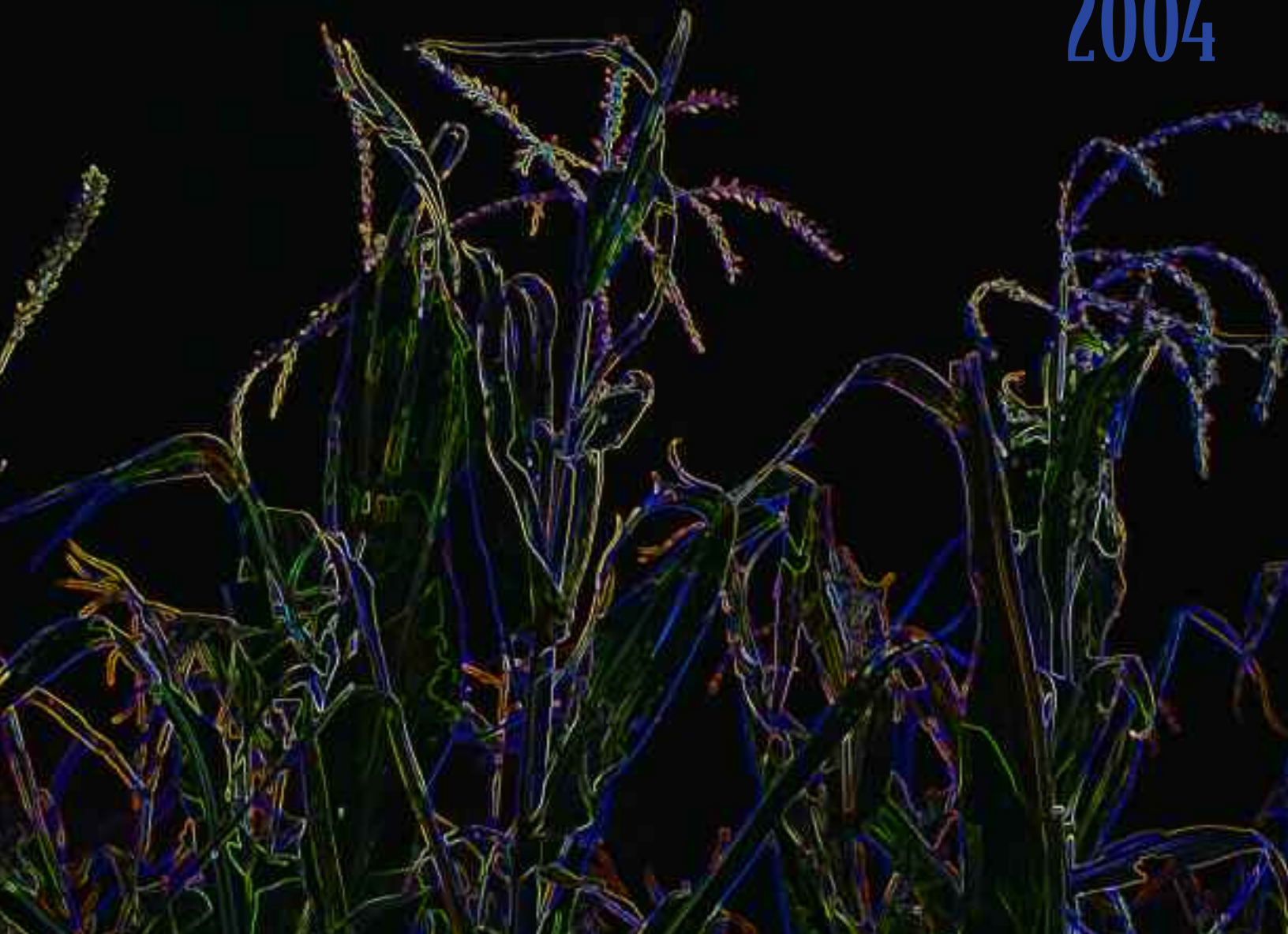


BTI



Boyce Thompson Institute for Plant Research

2004





What is BTI?

Since 1924, the non-profit Boyce Thompson Institute for Plant Research has explored the principles underlying our food supply and environment, with the ultimate goal of improving human lives. Located on the campus of Cornell University, BTI also promotes plant science education and strives toward environmental responsibility.



David Stern with undergraduate intern Mabel Thomas

BTI history began in Yonkers, New York, in 1924, when doors opened on Colonel William Boyce Thompson's dream: a research institute dedicated to exploring the secrets of plant life. The 80 years since are remembered and represented by hundreds of photographs, scientific articles, commercial products, and both living and written narrative. Were he among us today, the Colonel would have much to be proud of.

The institute was originally located near Thompson's estate, Alder, because he wanted to observe scientists going about their business. Five decades later, however, BTI's leadership realized that close association with a major research institution would best allow its science to flourish. The institute moved to Ithaca in 1978. Today BTI finds itself in a world-class nexus of plant biology in partnership with a great university, Cornell, as well as the USDA Plant, Soil and Nutrition Lab, which lies across the street.

While BTI retains its identity as envisioned by Colonel Thompson, our faculty integrate seamlessly into the greater campus community. We embark on common initiatives to build facilities, hire new scientists, recruit and train graduate students, and create new research directions.

BTI and Cornell also work together on educational outreach efforts. We have expanded these efforts by housing a full-time education and outreach coordinator, Nicole Markelz, whose varied activities are highlighted on pg. 2. Most area residents have heard BTI-sponsored radio spots on public radio's "MicrobeWorld" feature. And in 2004, the estate of George and Helen Kohut made a generous gift to the institute, which will provide permanent income to fund new education initiatives such as a teaching lab or internships.

BTI is fortunate to have a dedicated staff and active group of emeritus faculty. We owe much to them, including a flower-filled atrium that warms us through the winter months; a newly renovated plant growth facility; state-of-the-art apparatus for the analysis of proteins, metabolites, and gene structure, and effective administrative support for researchers. Our emeriti serve on committees, help preserve BTI's history, and participate in a variety of research or public service projects.

As president, I am immensely proud of both the accomplishments and potential of the institute and its staff, students, postdoctoral researchers, and faculty. I invite you to explore the remainder of this report, as well as our Web site, to learn how BTI scientists are carrying on the mission borne of Colonel William Boyce Thompson.

— David Stern



A typical 1950s biochemistry lab at BTI.

*Inset:
William Boyce Thompson
at his home, Alder.*





High school student Dardoh Sowe

BTI OUTREACH

Eight research labs at the institute hosted ten undergraduate and eight high school students in summer 2004—the largest group of students in the three years of the summer internship program. The interns worked with graduate student and postdoctoral mentors on projects directly related to their host lab's goals. They also participated in lab meetings and other activities, including seminars especially for interns. At the end of the summer, 28 interns from BTI and Cornell presented their research



Visiting high school students transfer bacterial colonies

accomplishments in either a talk or poster at the annual Summer Student Symposium. Much of the BTI community came to show their support, and a committee of several faculty and emeritus scientists selected the best talk and poster. Karen Laubengayer, a student from Heidelberg College working in Georg Jander's lab, won the **Colonel's Cup** for best presentation. Bryan Ellerbrock, a student at Ithaca High School who also worked in the Jander lab, won the Plant Genome Research Project (PGRP) Award for best poster.

For a full week in July, eight high school teachers came to the institute to learn about current plant research through presentations by scientists; tours of the microarray, sequencing, and tissue culture facilities, and hands-on activities. Teachers also had the opportunity to incorporate what they learned about plant science into lesson plans for their classes.

In 2004, BTI began running long-term afterschool programs in local elementary schools. In spring the institute ran a six-week program on plant biology topics for third-grade students at the Greater Ithaca Activities Center. In collaboration with another outreach program on campus, BTI also developed an ongoing "Nature Explorers Club" afterschool program at Northeast Elementary, which highlights topics such as seed dispersal, leaves, germination, insects and spiders, and water transport in plants.

For more on BTI's outreach activities, see <http://outreach-pgrp.cornell.edu>.

HONORS AND AWARDS

Gregory Martin was named the new holder of the **Boyce Schulze Downey Chair** at BTI's 80th Anniversary celebration on December 17, 2004. The chair was created in 1981 with donations earmarked for molecular biology research. President David Stern recommended Martin for the position based on his many accomplishments in the field, including his co-discovery of the first plant disease resistance gene.

The **American Association for the Advancement of Science (AAAS)** named **Martin** and **David Stern** fellows in October. They were among 308 scientists named new fellows this year by the AAAS, which publishes *Science* magazine. The organization recognized Martin for molecular and biochemical characterization of recognition and signal transduction events involved in plant disease resistance and susceptibility. Stern was recognized for molecular, genetic and biochemical studies exploring the modes of post-transcriptional gene regulation in plant organelles, including nuclear regulation of chloroplast RNA processing and stability.

Barbara Warland received the third **Brooks/Colavito Award for Distinguished Service**, the institute's highest honor, just before her retirement in July. This award recognizes an individual from the service support staff who demonstrates diligence and perseverance, commitment to high standards, professional and gracious demeanor, flexibility, and integrity. Warland joined BTI in 1991 as Program Secretary for Environmental Biology and consistently provided superb levels of service during her 13 years in administrative support at the institute.



David Stern and Gary Blissard

NEW ADMINISTRATION

BTI's search for a new president ended August 1 when former Vice President for Research **David Stern** assumed the position. Educated at the University of California, Berkeley; Cambridge, and Stanford, Stern came to BTI as an assistant scientist in 1989.

Gary Blissard, the new vice president for research, earned his Bachelor of Science at Auburn University in Alabama and his Ph.D at Texas A&M. He did postdoctoral stints at Texas A&M and at Oregon State University, joining the BTI faculty in 1990.

BTI AND THE ENVIRONMENT

The institute is in the midst of an environmental overhaul. "We're making the building run like it's supposed to," explained Director of Operations **Larry Russell**. With multiple subtle changes, like adjusting air flow valves throughout the building and putting lights on timers, BTI reduced its energy bill from \$1.4 million in 2002 to \$878,000 in 2004. The more-efficient air system alone is expected to cut the institute's costs by \$22,000 per year. BTI also switched to using recycled paper in all printers and copy machines.

IN MEMORIAM

We are very saddened by the death of **Dorothy Reddington** on August 9, 2004 from breast cancer. Dorothy joined BTI as director of development in 2001, after more than 20 years in development at Cornell University. A skilled and established fundraiser, she was well known and highly respected for her insight and abilities. In the short time that Dorothy was at BTI, she was instrumental in obtaining several substantial gifts and donations to support our research and further our mission.

Dorothy's unique, dynamic personality, her wit, and her energy and sparkle left a lasting impression on all of us. She will truly be missed.



Dorothy Reddington

Other losses in the BTI family

Cheryl Dentes, the wife of Vice President for Finance and Operations John Dentes, passed away on January 8, 2004. A native Ithacan, Cheryl taught pre-K, kindergarten, and Head Start for many years.

Former BTI scientist **Alvaro Goenaga** passed away on February 13, 2004. Alvaro was a plant pathologist at the institute from 1950 to 1978.

Richard Mandl, a scientist at BTI from 1952 to 1990, passed away on March 17, 2004. Dick specialized in the effects of air pollution on plants, and invented the open-top chamber now used widely in the field.



Research assistant Jocelyn Handley

NEW WEB SITE

Look for the latest BTI news on our revamped Web site, <http://bti.cornell.edu>



NUTRITIONAL UPGRADE: BTI scientists are working to make fruits and vegetables even better for you

Vitamin A deficiency kills up to 2.5 million children each year worldwide, according to a study from Johns Hopkins University, and blinds many more. Deficiency is especially rampant among people with rice-based diets who have little access to foods—such as tomatoes and carrots—that contain ample beta-carotene. (Our bodies can't concoct Vitamin A from scratch, but can make it from beta-carotene.)

Many people in industrialized countries don't eat enough fruits and vegetables, either. With multiple studies linking the antioxidants in these foods to a reduced risk of various cancers and heart disease, our preference for junk food carries real consequences.

But what if the fruits and vegetables we do eat packed more punch? What if people in the developing world could get the nutrients they most needed from their staple crops? These ideals drive the effort to nutritionally enhance crops, a problem Jim Giovannoni, Joyce Van Eck, and Tom Brutnell are tackling in different ways.

Giovannoni's lab uses tomato as a model system to learn how plants regulate their levels of beta-carotene and lycopene, an antioxidant associated with a decreased risk of digestive system and prostate cancers. Previous research has revealed the steps plants use to manufacture these and other so-called carotenoids. However, the way plants regulate the carotenoid assembly line remains a mystery.

To find out, Giovannoni uses a library of tomato varieties made from cross-breeding domestic tomatoes with a wild cousin. His lab members measure the

levels of lycopene, beta-carotene, and other nutrients in the lines and compare this with information about which bits of wild DNA they contain.

"We're adding more information about nutritional compounds in this material so plant breeders could go through the collection and see which have higher lycopene, or beta-carotene, or anthocyanins, or Vitamin C, and use them in their breeding programs," Giovannoni explained. "But what we're really interested in is characterizing these lines so we know which are different in terms of the levels of compounds and the genes that control them." Learning more about those lines and their genes will help researchers develop more nutritious varieties not only of tomato, but of other fruits as well.

Giovannoni's lab also uses other methods to expand their knowledge of regulation of lycopene, such as studying mutants. They recently manipulated genes for light signaling in a tomato plant so that it "saw" more light than there actually was. The excess energy from high light can generate destructive free radicals, which carotenoids neutralize. So when a plant gets a high light signal, it makes more carotenoids to deal with the threat. Giovannoni's lab showed that altering the light signaling pathway can throw a plant permanently into high-light mode, so that it produces more lycopene.

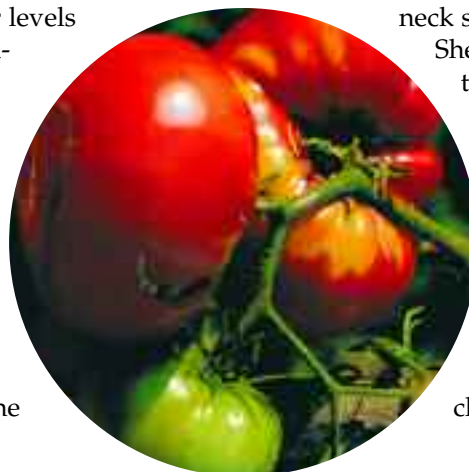
Since breeders already use a similar, naturally occurring mutant to create lines of high-color tomatoes, this discovery could be most useful for other species, Giovannoni said. "Where breeding is so difficult that if you could just introduce a transgenic you'd save years and years of time, that's where the bigger application is," he explained.

One potential target is potato. Joyce Van Eck chose to study the plant because its rapid increase in popularity around the world means varieties with beta-carotene could benefit millions.

Potatoes contain trace amounts of beta-carotene at best, since the nutrient is a transient step in their carotenoid pathway. Van Eck's lab found a way to shut down the protein that converts beta-carotene to the next carotenoid in the line, effectively creating a bottleneck so that beta-carotene could accumulate.

She has tried this experiment in three distinct potato varieties so far, with encouraging results.

Van Eck is now working to increase the amount of beta-carotene produced in the altered varieties (currently the maximum per altered potato is 14% of the recommended daily level). Since the carotenoid assembly line ultimately produces hormones the plant needs, the main challenge is to increase beta-carotene





Joyce Van Eck's lab uses micro-tubers in their potato research.

accumulation while ensuring that enough "downstream" molecules are still produced.

"It's hit and miss now," she explained.

"We need to identify which genes to target."

To learn which genes play the most important roles in the carotenoid pathway,

Giovannoni and Van Eck compare notes about the pathway in tomato and potato. They'll soon add corn to the mix, since Tom Brutnell's newest project focuses on engineering corn to store lycopene.

Corn plants normally convert all their lycopene into other carotenoids, leaving none in the ears that people consume. To change that, Brutnell and graduate student Ling Bai drew on previous research about beta-carotene accumulation in corn. "The idea is once we understand how that pathway is regulated, then we may be able to apply what we learned to other pathways," Brutnell said.

Bai found a way to "knock out" the gene for modifying the lycopene. The resulting corn is pink and rich in anti-oxidants, but lacks a hormone for which lycopene is a precursor. As a result, it germinates on the ear. Bai is trying to fix the problem by making the gene for lycopene conversion less effective, but not removing its function altogether. In theory, the corn could then accumulate some lycopene while also making compounds it needs to thrive.

Managing this feat would confirm Brutnell's understanding of the carotenoid pathway. "If you think you understand the system, you should be able to manipulate the system. If you can't manipulate it, you really don't understand how it works," he said.

Such an understanding could help Brutnell and others interested in changing different aspects of maize makeup, such as protein and starch content.

Ultimately, though, people have to eat fruits and vegetables to benefit from them, which is where other aspects of Jim Giovannoni's research come in. With a better understanding of fruit ripening, he believes, researchers will one day be able to increase shelf life, driving down the cost of fresh fruit and making it available for longer stretches of the year. This could help expand the variety of nutritious foods available in parts of the developing world where access is limited.

Research on ripening could also lead to store-bought fruits that look, smell, and taste more like they grew in your backyard. Giovannoni explains, "If we can make fruit that people find more attractive, tasty, and desirable, they're more likely to consume that than something else that might not be as nutritious."



Ayer Lake, Boyce Thompson Arboretum

Photo: Susan Strom

Boyce Thompson Arboretum

The **Boyce Thompson Arboretum**, a world-class botanical garden founded by William Boyce Thompson in 1929, invites visitors to learn about plants while exploring more than three miles of paths that meander through 320 acres. Located in Superior, Arizona, just one hour's drive east of Phoenix, the arboretum is a research affiliate of the University of Arizona College of Agriculture and Life Sciences.

Please visit the arboretum Web site at <http://arboretum.ag.arizona.edu/> for more information.



White-throated Sparrow, Two-tailed Swallowtail, Northern Cardinal.

Photos: Marceline Vandewater, Phil Lowe, John Ellis



THE TRIAD FOUNDATION

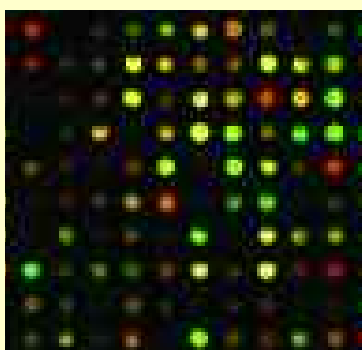
Jim Giovannoni and Joyce Van Eck's work on carotenoid synthesis is partly supported by a grant from the Triad Foundation.

A philanthropic organization headed by the family of Roy H. Park, Jr., the foundation currently donates \$250,000 to BTI each year for research with potential applications to human health. Projects led by **Haiyang Wang, Georg Jander, and Greg Martin** also received Triad Foundation funds in 2004.



IN THE LABS

- Gary Blissard
- Robert Kohut
- Thomas P. Brutnell
- Gregory B. Martin
- Alice C.L. Churchill
- Peter Moffett
- James J. Giovannoni
- David B. Stern
- Maria J. Harrison
- Joyce M. Van Eck
- Georg Jander
- Haiyang Wang
- Daniel F. Klessig
- David A. Weinstein



What are microarrays?

Microarrays took molecular biology by storm in the early 1990s. They're small slides spotted with fixed samples of DNA, each for a different gene. When a researcher prepares a labeled cell extract and incubates it with the slide, messengers in the sample stick to the fixed DNA. This shows which genes in the sample are being actively "expressed," or transcribed into RNA. Microarrays are especially useful for comparing gene expression across different samples. For example, they can show the changes in expression that take place during stages of development, or when a plant encounters a pathogen.

When bananas succumb to fungi, it's not just monkeys and smoothie drinkers who have cause for concern. Bananas are the fourth most economically important crop in the world and are sprayed up to 60 times per year in export plantations to protect them from black Sigatoka disease. This is bad news for the environment, and even worse for the millions of subsistence farmers worldwide who can't afford fungicides to safeguard their livelihoods.

A black Sigatoka-resistant variety of banana might have become available years ago if the plants could be subjected to conventional breeding. But most edible bananas are seedless and sterile: Each new banana plant comes from a cutting of an existing one. To shield the banana, researchers need to find a weakness in the black Sigatoka-causing fungus. In 2004 Alice Churchill's lab took a big step in that direction.

Previous research had demonstrated that applying toxins from the fungus to banana leaves produced symptoms of black Sigatoka. Those toxins were thought to be the key to fungal virulence. Churchill's lab tested this hypothesis more rigorously by shutting down a fungal gene critical for making the suspected toxins. Using one gene-specific method, they found that the altered fungus infected bananas as well as the original, indicating that the suspected toxins do not help the fungus cause disease. However, when they used a second, less specific method for silencing the gene, the fungus became less virulent.



What this means is that the suspected toxins were likely a red herring in the quest to stop black Sigatoka. But the second experiment suggests that the culprit may be a close relative of the targeted gene—so close that it was inadvertently shut off by the method used to target the suspected toxins. Future experiments to identify that gene and others may point the way toward defeating black Sigatoka.

James J. Giovannoni: Toward a Better Tomato 

Jim Giovannoni wants you to eat more fruit. But he's taken a different tack from most crusaders for dietary responsibility: If healthy foods taste better, he reasons, people will naturally choose them more often.

Most shoppers in America have access to a wide selection of fresh fruits and vegetables year-round, but these rarely measure up to home grown varieties. "In general, things are harvested very prematurely so that they have the shelf life and the firmness to survive shipping," Giovannoni explained. "What's always lost in that tradeoff is taste, appearance, aroma—things that are associated with quality."

To make store-bought fruit more palatable, agricultural scientists need a better understanding of how fruits "decide" when and how much to ripen. Giovannoni's lab studies tomatoes and other plants to find the genes responsible for that decision. In 2004 they discovered several such genes, including two that may play a role in a broad range of species. The lab is now working to find similar genes that cooperate with these genes to regulate ripening and associated quality characteristics.

Giovannoni's group also participates in a broader effort, with others at BTI and in nine foreign countries, to map and sequence the tomato genome. The completed genome will represent a giant step toward unlocking tomato's inner workings, just as the Human Genome Project did for people's. In a related project, the lab uses microarrays (see facing page)



and computer analyses to learn how groups of genes switch on and off at the right time at each stage of ripening. This eagle's eye view of the dynamics of development enables them to compare the process across different species, and to pick out genes that may control the timing of ripening.

(For more on Giovannoni's work, see pg. 4.)

How do fruits "decide" when to ripen?

Gary Blissard: The Good Virus



Viruses make great villains. They infect cells with ruthless efficiency, commandeering and sometimes destroying their hosts to reproduce themselves before moving on to the next victim.

But can these highly-evolved killing machines be made to work to our advantage? That's the question Gary Blissard is helping to answer. He works with baculoviruses, which can liquefy their insect hosts in a matter of days, but don't induce so much as a sneeze in mammals. "They're like the Ebola virus of the insect world," Blissard explained. Once a baculovirus gains

entry to an insect cell, it hijacks its host's genetic machinery and produces massive quantities of proteins to build more viruses. Baculoviruses can easily enter mammal cells, too, but once inside they're stymied by the differences in the gene expression system. Researchers hope that with some tweaking, baculoviruses could be used in gene therapy to induce the production of needed proteins in patients with a defective gene. Specialized baculoviruses could also replace chemicals in controlling some crop pests and disease-carrying mosquitoes, thus reducing the impact on farm workers and the environment.

First, though, scientists must learn more about what makes baculoviruses tick. To that end, Blissard studies what enables them to get into and out of cells. His lab is now characterizing a crucial protein, GP64, which the virus uses both to fuse with and enter the host cell, and to assemble new viruses at the end of its infection cycle. Using microarrays (see pg. 6), Blissard is also learning what proteins are needed at each stage of the infection cycle, and how the virus makes them at the right time. Ultimately, what he learns may be used to recruit baculoviruses—at least in modified form—to our side.

"Baculoviruses are like the Ebola virus of the insect world."

Georg Jander: Plants Versus Aphids



Picture this: You can't move, and small insects are eating you alive. That's the nightmare scenario many plants cope with every day. One way they do so is with chemical weapons.

Glucosinolates, for example, sit benignly inside a cell until it's punctured, usually by predator feeding. They then mix with certain enzymes from other cells, reacting to yield sharp-tasting, sometimes toxic compounds such as those that give mustard and horseradish their distinctive tanginess.

Because of this phenomenon, "a radish doesn't taste like a radish until you bite into it," explained Georg Jander. In

one project, he studies interactions of green peach aphids with *Arabidopsis* to learn more about how plants deploy glucosinolates—and how insect pests get around them.

In 2004, Jander's lab let aphids snack on a certain *Arabidopsis* variety and analyzed plant extracts to find which glucosinolates the plant responded with. Although this variety can make 20 different glucosinolates, they found that aphid feeding caused the plant to produce only some of these. The lab also fed aphids sugar solutions containing different glucosinolates to find which affected their reproduction. Of all the glucosinolates in the *Arabidopsis* strain's arsenal, only those induced by aphid feeding had a significant negative effect on the insects.

These results seem to indicate that the plant can somehow tell aphids apart from other pests and react accordingly. Its other glucosinolates may be intended for other insects or hungry animals. Jander plans to test both these hypotheses and find out more about how plants differentiate between predators and communicate the need for defensive responses. In the future, such knowledge could help agricultural scientists make crops better equipped to ward off insect herbivores.

"A radish doesn't taste like a radish until you bite into it."



When Tom Brutnell drives past a field of hearty green corn plants, he doesn't see a slice of bucolic Americana. The plants' towering height is, he knows, the result of a struggle that pits each individual against its neighbors in a bid for precious sunlight—and draws resources away from its edible ears. This problem has increased in recent decades as farmers have found ways to pack more and more plants into each acre. They've managed to breed maize lines less inclined to pour energy into growth when surrounded by other plants, but per-plant yield has still declined as per-acre yield increased.

In 2004 Brutnell and colleagues identified the basis of corn's competitive tendencies, a gene for a photoreceptor in maize that "sees" red and far-red light. The green chlorophyll pigments in leaves selectively filter red wavelengths of light while allowing far-red light to pass through. This change in the light signals the plant that it's in the shade. Targeting the photoreceptor could "blind" the plants to shade and lead to fields of shorter plants with bigger ears. Such plants would benefit not only farmers, but also the environment, since they would require less water and fertilizer.

One of Brutnell's graduate students is now working to make a more nutritious variety of corn (see pg. 5). In yet another project, Brutnell's lab has just begun to compile a seed bank representing thousands of naturally occurring gene mutations



in maize plants. They make the mutants with jumping genes, short stretches of DNA that insert themselves into other genes, disrupting their function. Scientists from BTI and elsewhere can then grow the mutants and compare them to normal plants to root out the roles of the interrupted genes.

Researchers could "blind" corn to shade, yielding fields of shorter plants with bigger ears.

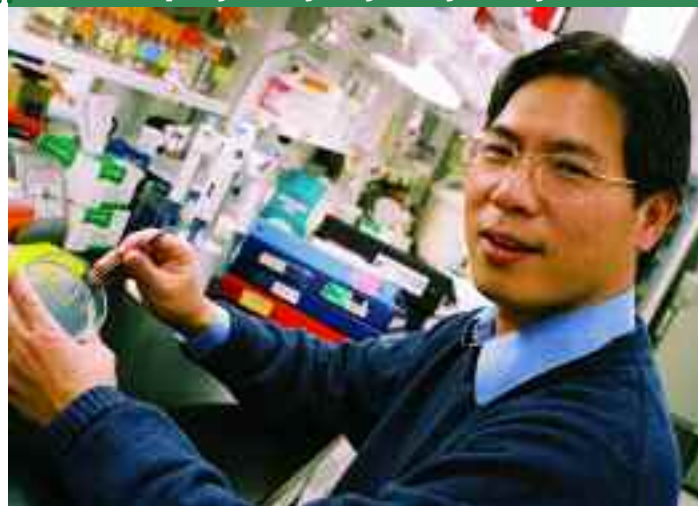
Plants must constantly gauge and adjust to their environments in order to survive. One way they "learn" about their surroundings is from the color, intensity, direction, and duration of light around them. Different kinds of light determine when seeds will sprout; how fast, how tall, and in which direction they grow, and when they flower—all traits with palpable impacts on agriculture.

Yet scientists are only just beginning to understand how plants perceive and respond to light. When light photons hit a specialized plant protein called a photoreceptor, the receptor passes a message to another molecule, which relays it to another, and so on. Understanding this relay system could help in designing new means of crop improvement.

Haiyang Wang's lab uses several techniques to piece together details of the so-called light signaling cascades. In one project, they look for randomly mutated *Arabidopsis* plants that respond differently to light than do normal plants. They then use molecular techniques to learn more about the altered genes and their role in passing along the light signal.

In another project in 2004 Wang studied a family of signaling molecules, finding that some helped steer *Arabidopsis*' response to red light at the early seedling stage, while others influenced flowering time. The lab is working to find what the other molecules in the family do.

Haiyang Wang: Light Signaling in Plants



In a separate study, Wang's lab looked at a key protein that transmits both far-red and blue light signals. This protein binds to a cell's DNA to regulate whether—and how much—a plant will respond to light signals. Wang's lab found that the abundance of this protein is controlled by light via interaction with another light signaling molecule. In this way light controls the quantity of the protein, which in turn determines the plant's behavior by influencing which genes are expressed.

Daniel F. Klessig: Learning from a Plant with “Allergies”



Gardeners have long noted a connection between damp weather and plant disease, which scientists have attributed to a constellation of factors, such as more favorable conditions for germination of fungal spores. In 2004 Dan Klessig’s lab found that high humidity actually suppresses a plant’s immune system, making it more vulnerable to pathogens.

Klessig lab members studied a mutant of *Arabidopsis* whose immune response is always turned on—like having very

bad allergies. Though good at warding off infection, the mutant puts so much energy into immunity that it looks sickly and stunted. When grown in chambers with high humidity, Klessig’s group found, the mutant plant’s immune response shut down, enabling it to grow normally. As a follow-up, the group may test whether non-mutant plants are also unable to defend against pathogens while exposed to high humidity.

Currently the lab studies additional mutations in the “allergic” plant itself that turn off its immune response. Studying such mutants should help Klessig develop a picture of the early steps in the signaling system plants use to counter infection.

In separate studies, the Klessig lab modified an *Arabidopsis* plant to knock out one component of an important signaling pathway. In this pathway, the MAP kinase cascade, a signal such as a hormone from inside or outside of the cell sets off a chain reaction, ultimately kick-starting the cell’s response to the signal. Klessig’s lab found that the elimination of one link in this chain reaction increased the susceptibility of the plant to several different pathogens, suggesting that one of the MAP kinase cascade’s duties is to activate the plant’s immune response. Learning about this pathway could enable scientists to manipulate it to create crop plants that are more resistant to disease.

Peter Moffett: Engineering Defenses



Animals can use their adaptive immune systems to “learn” to ward off infections—which is why vaccines protect us and we can’t get chicken pox twice. But plants only have immunity to viruses, bacteria and other pathogens they’re genetically programmed to recognize. Farmers have limited options for protecting their crops: Where resistant varieties exist, growers can use them for cross-breeding—a time-consuming and sometimes ineffective process.

Genetic engineering has the potential to slash the time needed to develop an immune variety by allowing the transfer of resistance genes between lines or between plant species. In

theory, such genes could even be custom designed for a particular pathogen. First, though, researchers will need a better understanding of how, at the molecular level, resistant plants recognize and respond to invaders.

One key to plant immunity is a family of proteins called NBS-LRR that each recognize a specific pathogen protein. Recognition causes the NBS-LRR protein to change shape, which in turn triggers a cascade of signals that put the plant cell on the defensive.

To learn more about this system, Peter Moffett studies an NBS-LRR protein called Rx that coordinates resistance to a potato virus. To tease out which parts of the protein carry out each stage of recognition, shape change, and signaling, Moffett’s lab first screens plants with mutated versions of the Rx gene. When they find one that doesn’t respond normally to a viral protein, they use molecular techniques to learn more about how the mutation disrupted the protein’s function. Knowing how different parts of the NBS-LRR protein work together is important, since customizing the section that recognizes invaders would affect the rest of the protein as well.

Genetic engineering has the potential to slash the time needed to develop an immune plant.

In the battles between plants and the microbes that prey on them, victory goes to the organisms with the most effective genes. Greg Martin's lab studies tomato plants and disease-causing *Pseudomonas syringae* bacteria to seek the answer to a seemingly simple question: What makes a plant vulnerable to infection? The answer could affect not only agriculture, but also human health.

One type of *Pseudomonas* causes bacterial speck disease in susceptible tomatoes, while different strains of the bacteria can infect other plants, and one even causes antibiotic-resistant infections in people with compromised immune systems. Martin helped sequence the speck-causing bacteria's genome last year, information that sped up his search for genes that enable *Pseudomonas* to skirt tomatoes' resistance.

Martin's lab explored techniques in 2004 for fleshing out the functions of tomato genes involved in the plant's arms race with *Pseudomonas*. Using virus-induced gene silencing, for example (see Joyce Van Eck's work, this page), the Martin lab found signaling molecules in tomato that determine its response to *Pseudomonas* exposure. Resistant plants quickly corral and kill off infected cells, causing brown spots of dead cells to appear on the leaves. But susceptible plants also display specks, as *Pseudomonas* kills infected cells to spread further. Martin recently showed that the same molecular switch sets off both the defensive and disease-induced cell death, though



through different channels. He suggests that this switch could be changed to make crops more resistant.

The lab has identified surprising similarities between immune systems in tomatoes and animals, indicating that in some cases, plants could be used to model human response to disease.

When a plant detects a protein from a pathogen that it's resistant to, it quickly mounts a counter-attack, targeting the invader as well as infected cells. Previous studies of diverse plant species have implicated genes for the lipoxigenase (LOX) family of proteins in this type of response. To learn more about the roles LOX proteins play in tomato immunity, Joyce Van Eck exploits another feature of plants' defensive toolbox.

This phenomenon, called virus-induced gene silencing (VIGS) is triggered when a virus infects a plant cell with its RNA. A versatile molecule, one of RNA's functions in cells is to relay genetic information from DNA to "factories" where it's converted into proteins. This type of RNA exists in single strands. Some viruses, however, use double-stranded RNA instead of DNA to store their genetic information, and to induce host cells to manufacture proteins for new viruses.

To defend against such viruses, plant cells chew up any double-stranded RNA that comes their way. They also go after any of their own RNA molecules that happen to be similar to the viral RNA. Like order forms lost en route from DNA to the protein factories, these single-stranded RNAs never get their message across, and the gene they relay is effectively silenced.

In recent years molecular biologists have turned this defense to their advantage, infecting plants with engineered

Joyce M. Van Eck: Aspects of Immunity



viruses that contain RNA from a gene of interest. Van Eck uses this technique to silence genes for LOX proteins in tomatoes. She then exposes the plants to pathogenic bacteria (*Pseudomonas syringae*) to see how the loss affects their defenses. Though in its early stages, the project has yielded unexpected results: Silencing either of two LOX genes appears to make plants less, not more, vulnerable to *Pseudomonas*.

(For more on Van Eck's work, see pg. 4.)



Maria J. Harrison: Of Phosphates and Fungi



Each summer, agricultural runoff from the Mississippi River creates a 5,000 square mile Dead Zone off the coast of Louisiana: Excess nutrients feed algal blooms, which leach most of the oxygen from the water. Getting crops to take up phosphorus and other nutrients more efficiently would drastically reduce the fertilizer running off fields, saving money as well as watery ecosystems. One promising strategy is to harness a symbiotic relationship as ancient as land-dwelling life.

If you pull up a plant and look at its roots, chances are that part of what you see is actually fungus. Fossil records show

plants and mycorrhizal fungi teamed up hundreds of millions of years ago, perhaps enabling water dwelling plants to colonize land. Yet modern farming regimes don't take full advantage of the fungi's potential to harvest phosphate for the plant. Learning more about the plant-fungus symbiosis could help farmers come up with ways to benefit from this interaction.

Maria Harrison studies the signaling system that allows plants and mycorrhizal fungi to work in tandem. Her lab is scanning the genes in the model plant *Medicago truncatula* (a relative of alfalfa) to find which coordinate its relationship with mycorrhizal fungi. In the initial phases of a four-year project, they looked for genes whose level of activity changes as fungi move into the root. The lab identified 92 such genes using microarrays (see pg. 6). They next fine-tuned a system for knocking out each candidate gene in turn and looking at the resulting plant to see whether symbiosis was affected. Working with collaborators in Minnesota, Harrison's lab will plough through hundreds of genes in the next few years to discover which are most essential for symbiosis.

Harnessing an ancient symbiosis could revolutionize modern farming.



David B. Stern: The Meaning of Teamwork



A long time ago, in a land of primordial goo, two bacteria lent new meaning to the word "teamwork." It didn't start out that way, most scientists think: One bacterium probably ate the other. But it turned out that the two survived better together than they had independently, and their progeny evolved into a single, more sophisticated organism. This process happened at least twice, yielding the specialized cellular compartments chloroplasts and mitochondria.

David Stern's lab studies chloroplasts, light-harvesting factories unique to plants and algae. While structures called nuclei contain most genes in plant cells, chloroplasts hold a few genes of their own. Most of these genes have to do with harnessing

energy from sunlight to make sugars, a process agricultural scientists hope can be made more efficient through genetic engineering. But since the nucleus controls how the chloroplast's genes operate, understanding their interaction—the theme of Stern's research—is essential.

In one 2004 project, the Stern lab used a mutant single-celled alga to find how chloroplast gene activity decreases when cells are starved of sulfur. The mutation occurred in a gene in the nucleus that scaled down copying of DNA to RNA under low-sulfur conditions. By comparing the mutant with normal algae, lab members found that the mutated protein affected gene copying not only in the nucleus (as previously thought), but also in the chloroplast. Since plants need sulfur-containing proteins to process light energy and avoid its potentially harmful effects, this system may protect a sulfur-starved cell by cutting how much light it absorbs.

This mechanism is just one step in the delicate choreography between nuclei, chloroplasts, and the environment. Future experiments on their relationship will add a novel chapter to our understanding of evolution—one that explains how three organisms became one.

Chloroplasts harness energy from sunlight, a process we hope can be made more efficient.



he found 64 to be at high risk of showing ozone injury to plant leaves. Kohut also wrote a handbook that the Park Service will use to design and conduct assessments of leaf injury. Such assessments could help park visitors make the connection between air quality and the health of their favorite getaways.

Of the 270 national parks, monuments, and historical sites studied, 64 are at high risk of showing ozone injury to plant leaves.

David A. Weinstein: Ecosystems in Silicon



Weinstein's lab recently published the results of another modeling study on the reasons for a change in the behavior of northeastern watershed forests. For decades, nitrates from fertilizer in agricultural runoff flowed unhindered through the forests, but during the early 1980s the forests suddenly began absorbing the nitrates—a boon to cities downstream. Most researchers believed that a confluence of one-time events must have caused the switch, but Weinstein's group showed it could be explained by long-term changes in temperature and soil moisture.

Millions of people visit national parks each year to admire nature at its pristine best. The US National Park Service is charged with protecting park ecosystems, but with limited resources, park managers need information about what puts local environments most at risk in order to decide what to monitor. Bob Kohut's research in 2004 will help the Park Service determine which sites have plants most at risk for ozone injury, and how to monitor that injury.

A highly reactive molecule made up of three oxygen atoms, ozone forms low in the atmosphere when air pollutants react with sunlight and oxygen. Ozone and its fossil fuel antecedents often get caught up in air currents and travel hundreds of miles from the cities that produced them. When plants open their stomata to harvest carbon dioxide for photosynthesis, ozone enters as well and injures leaves.

Several factors play into whether a park is at high risk of ozone injury. Ozone-sensitive plant species have to grow in the park, and the ozone level has to be high enough to injure leaves. Finally, the environment has to favor ozone uptake. While hot sunny weather speeds up the formation of ozone from pollutants, it also tends to dry out the soil. In a drought, plants keep their stomata closed much of the time to conserve water, thus protecting themselves from ozone.

Kohut's risk assessment, therefore, compiled information on the concentrations of ozone at or near individual national parks from 1995 to 1999, soil moisture levels during that time, and ozone-sensitive species growing in each park. Of the 270 national parks, monuments, and historical sites studied,

Decades of research demonstrate ozone's deleterious effects on plants: It reduces photosynthesis rates in their leaves, stunting plants' growth and weakening their immunity. Ozone forms a protective layer against UV radiation high in the atmosphere, but closer to earth it can be toxic.

In fact, ozone may endanger whole forested ecosystems. Normally, the danger would be measured by exposing plants to controlled amounts of ozone in a greenhouse—an impractical technique for ecosystems.

That's where David Weinstein's computer models come in. Using finely tuned simulations, he can plug in numbers for factors such as soil moisture, temperature, ozone concentration, and forest type to predict how an ecosystem would react over time. In 2004 his lab completed a project commissioned by a consortium of southern Appalachian state governments, industries, and environmental groups to compute the impact of several ozone-reduction scenarios on the growth of various kinds of forests in the region.

For a few sensitive species, they found, dramatic reductions in ozone would increase growth by up to 60 percent. Without such reductions, less ozone-sensitive species would gain ground on their more-sensitive neighbors over time. Weinstein warned, "if current ozone levels remain the same through the year 2040, the familiar species of many of these forests—some of which are vital to the timber industry—will become much more rare."



Emeritus

Holding over 40 patents, **Robert R. Granados** still works as an intellectual property consultant for BTI. Parts of Granados's impressive insect cell culture collection are being used to make human vaccines and other pharmaceuticals. Granados and retired scientist Karl Maramorosch teamed up to convene a symposium on molecular engineering and biology of invertebrate cell cultures in May 2004, part of the Eleventh International Invertebrate Cell and Tissue Culture Conference. Granados received a Society for In Vitro Biology Fellow award and Presidents Award at the conference.



Lynn Leopold and Lise Grace with four-year-old trees planted as part of a forest restoration effort in Costa Rica.

A. Carl Leopold plays an active role in several environmental organizations both locally and abroad. Leopold was the founding president of the Finger Lakes Land Trust and is active in the Aldo Leopold Foundation, through which he promotes his father's vision of ethical treatment of land. He has created a new local organization called Greensprings, which advocates burial without chemical treatment, and initiated a pioneering ecological restoration program in Costa Rica, which strives to return depleted land to its natural state as healthy tropical rainforests. Ten years after initiating the program, a once grassy pasture of approximately 100 acres is now a closed tropical forest with monkeys bounding overhead. He is now working on his fourth scientific article on the reforestation project.

Since retiring in 2001, **Alan Renwick** has continued work supported by the National Science Foundation on plant compounds that taste good or bad to insects. He serves on the Molecular and Chemical Ecology and Museum committees at BTI, and advises a graduate student in entomology. Renwick recently co-authored a book chapter on insect adaptation to changes in plant chemistry. He also regularly reviews papers and serves on the editorial board of the *Journal of Chemical Ecology*. Since retirement his "fieldwork" focuses on investigating golf courses and ski slopes.

Richard Staples frequently publishes reviews on plant pathogens, and occasionally lectures for Cornell's Department of Plant Pathology. He has been on the editorial board of *FEMS Microbiology Letters* for ten years and serves as the reviews editor for the journal. In collaboration with colleague Len Weinstein, he is also writing a history of BTI from 1974 to 2000. His latest research paper, based on a gene from the rust fungus he cloned in the 1980s whose importance has resurfaced, will appear sometime in 2005. Staples enjoys gardening, exploring the area's finer restaurants with his wife, and reading novels to his grandchildren—*The Lord of the Rings* reigns as his all time favorite.

Leonard Weinstein heads BTI's Museum Committee and is co-writing the history of BTI from 1974 to 2000. In 2004 he co-authored the book *Fluorides in the Environment*. He has been a consultant for a number of years to governments and industries. Iceland is Weinstein's current "laboratory": He is co-directing external environmental monitoring for an aluminum smelter there in order to gauge effects of fluoride emissions on plant and animal life.

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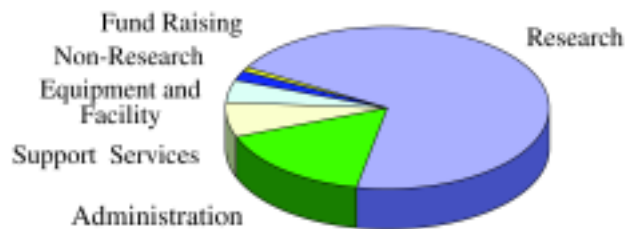
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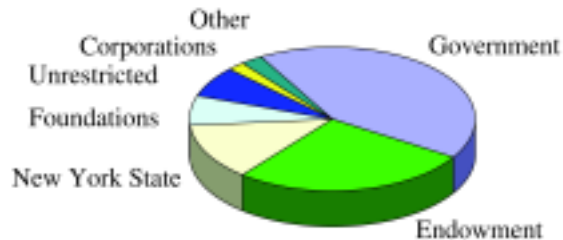
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Research	\$8,506,000	70%
Administration	1,968,000	16%
Research support services	862,000	7%
Equipment & facility	528,000	4%
Non-research	245,000	2%
Fund raising	111,000	1%
Total	\$12,220,000	100%

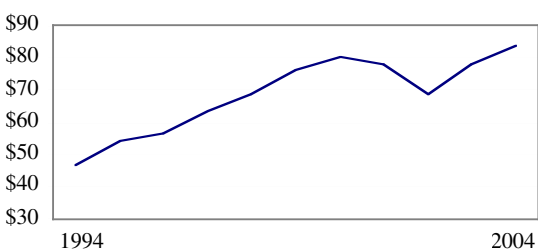


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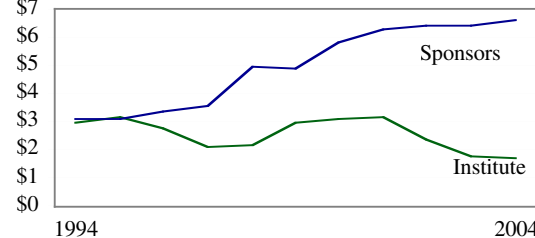
U.S. government	\$5,241,000	42%
Institute endowment	3,341,000	26%
New York State	1,718,000	13%
Foundations	842,000	7%
Unrestricted revenues	850,000	7%
Corporations	236,000	2%
Other private sources	314,000	3%
Total	\$12,542,000	100%



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"2004 was a very good year for BTI."

— John Dentes

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