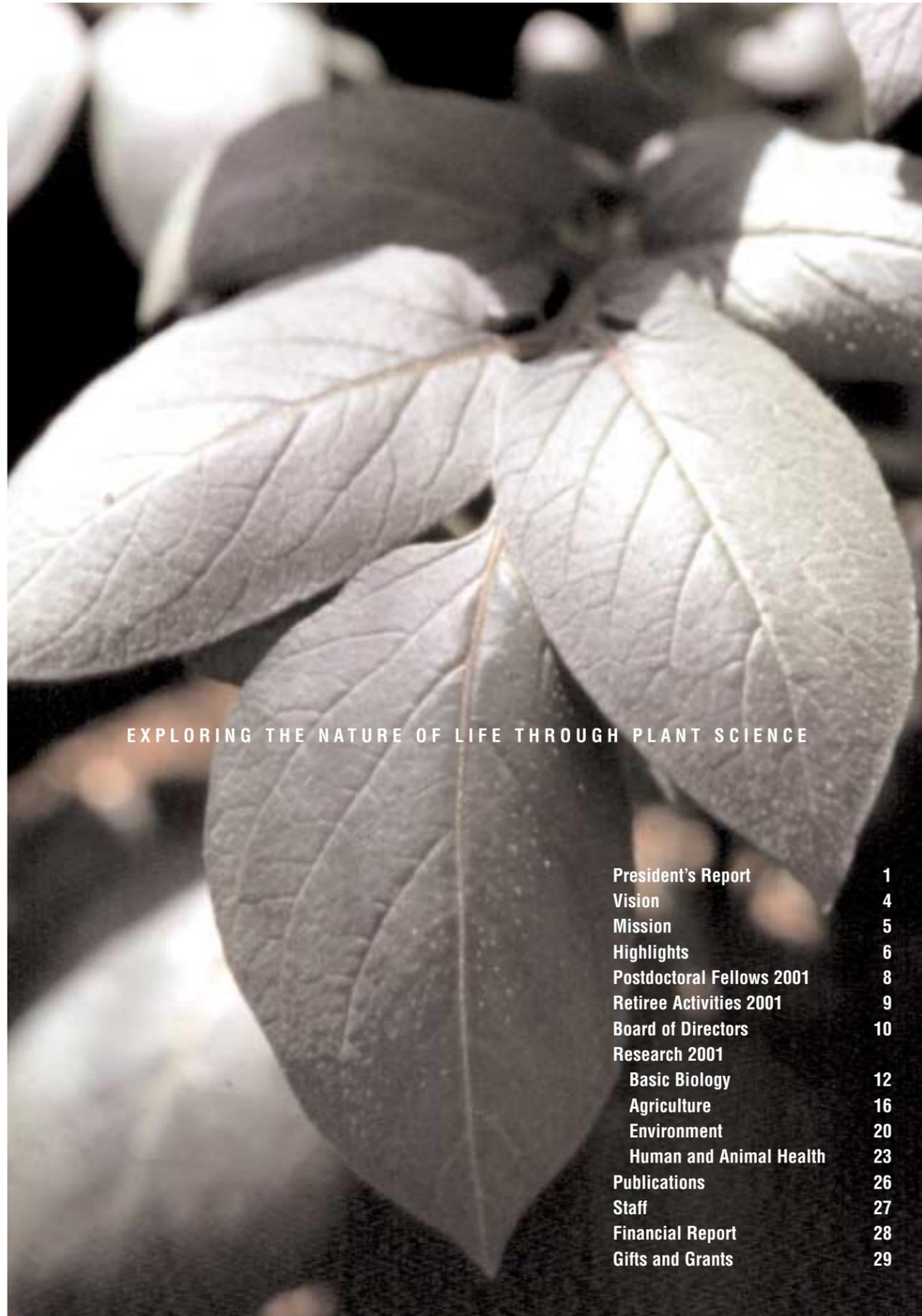


BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH
The 78th Annual Report

EXPLORATIONS

2001





EXPLORING THE NATURE OF LIFE THROUGH PLANT SCIENCE

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BTI / President's Report 2001 /

The "Explorations" theme of this annual report has symbolic meaning on a number of levels. Over time, our Institute has gained an international reputation for its leading explorations in such scientific disciplines as ecology and environmental biology, plant pathology, plant growth and development, and insect biology. And day-to-day, our scientists explore how plants grow, develop and respond to their environment, thereby obtaining knowledge that can enhance our world.



In 2001, we also devoted significant time and thought to an exploration of our research mission in an effort to sharpen and define our focus for the years to come. Interestingly, this exploration has brought us back to our earliest roots as a plant science institute, with renewed emphasis on understanding the basic biology of plants. Established in 1924, BTI is a result of William Boyce Thompson's vision to create "a scientific institution to study why and how plants grow, why they languish or thrive, how their diseases may be conquered, how their development may be stimulated by the regulation of the elements which contribute to their life."

Moreover, the knowledge we have gained in the intervening years has led to an even greater appreciation of Thompson's foresight. He said:

"I want to do something to get at the bottom of the phenomena of life processes and I think a good place to study them would be in the realm of plants. Any principles concerning the nature of life that you can establish for plants will help you to understand man, in health and disease. So, by helping men to study plants, I may perhaps be able to contribute something to the future of mankind."

The more we explore life at its most basic level through plants, the more we recognize and appreciate not only the similarities between plants and people, but the opportunities plant science presents for improving human life.

The majority of BTI's laboratories are focused today on understanding the exchange of information between

plants and their environment, and between cells or organs within a plant. This transmission of information, often called *signal transduction*, enables plants to respond appropriately to changes in their environment – responses that enable them to survive, grow and reproduce. This is the area of plant biology in which 21st century activity and breakthroughs will predominate, and where BTI, as a result of our internal explorations, is concentrating.

2001 was also replete with change – change that affected our staff, our facilities and our policies. In February, David B. Stern was appointed vice president for research following the departure of Stephen H. Howell, who formerly held that position. In April, Dorothy Reddington joined the Institute as our director of development after more than 15 years of service to Cornell University, where she served as director of external relations for The Plantations, among other positions.

Staff changes in 2001 also included the promotion of two BTI scientists to prestigious positions at other research institutions. In April, after 33 years of service to BTI, virologist H. Alan Wood accepted a new challenge as director of the Life Sciences and Biotechnology Institute at Mississippi State University.



In December, after 25 years as a BTI plant pathologist, John Laurence accepted a position with the U.S. Forest Service as manager of the Ecosystems Processes Program at the Pacific Northwest Forest Experiment Station. BTI takes pride in Alan and John's career advancement, thanks them for their numerous contributions while they were at BTI, and wishes them success in their new positions.



In May, chemical ecologist J. Alan Renwick retired after 30 years at the Institute. Alan ended his career with the publication of a research paper in the prestigious scientific journal, *Nature*. His May 2001 article described the strong preference of the insect *Manduca sexta* to feed on only certain plants – an addiction that results from the food source it encounters as a youngster. He and his colleagues described the plant compound/chemical and the molecular mechanism responsible for this addiction. Alan will continue his association with BTI as chemical ecologist, emeritus.

Two new facilities were completed at BTI in 2001. In March, we opened the Plant Tissue Processing Facility, which enables researchers to more efficiently handle large quantities of plant material. In July, the Central Dishwashing and Media Preparation Facility was completed, enabling support staff to service all four research floors from a central location.

Several critical policy changes were also made in 2001 that affect BTI's approach to new faculty appointments and performance reviews. The new policy was drafted by a Faculty Appointment Committee and was considered at the May meeting of the Board of Directors. A revised policy, which calls for appointment of all new faculty in a single track, a rigorous program of performance and promotion reviews for tenure, and an aggressive post-tenure review process, was accepted by the Board in October.

On the financial side, a necessary budget reallocation plan was initiated in April to reduce the cost of operations and generate start-up packages to attract new faculty to BTI. While this plan reduced support staff by nearly 14 percent, its impact on research, while less severe, was nonetheless significant.

Meanwhile, BTI development activities, initiated in 2001, began to "bear fruit." With the help and generosity of Dr. Philip Goelet, a BTI Board member, we established the Francis Goelet Distinguished Postdoctoral Fellowship in December.

With the tenure issue resolved, funding in place for faculty start-up packages, and our development efforts beginning to yield results, BTI began a broad-based search for new faculty in fall 2001. By year's end, the search provided us with 120 applicants from whom we chose ten candidates for interviews. We expect to fill three faculty positions from this group of exceptional young and established scientists.

As I write this report during the 2002 Winter Olympics, I'm reminded of the years of sacrifice most of these athletes made in order to compete at the very highest level. BTI has also made sacrifices in the past few years in order to "go for the gold." Our goal is to realize BTI's full potential as one of the world's premier plant research institutions. As a result of the explorations and resulting changes at BTI during 2001, we are now poised to achieve our goal and help fulfill William Boyce Thompson's vision.

I look forward to working closely in the year ahead with Paul Hatfield, Board chair; Roy Park, Jr., vice chair; and the BTI Board of Directors. I also look forward to continuing to work with the Institute's bright, energetic scientists and BTI's dedicated and talented management team and their staff as we continue along our path to excellence.

A handwritten signature in black ink that reads "Daniel M. Kleinig".



BTI / Vision



As the source of oxygen and the foundation of the food chain, plants are a complex and critical component of the earth's ecosystem. Research that leads to a better understanding of the life processes of plants can provide us with valuable insights for improved human health and a more productive, environmentally balanced world.

For more than 75 years, scientists at the Boyce Thompson Institute for Plant Research have worked to reveal the natural processes that underlie plant life. BTI investigates how plants grow and develop, how they ward-off insects and disease, how they produce nutrients in food and how they interact with the environment.

The vision of the Boyce Thompson Institute for Plant Research (BTI) is of a world in which nutritious food is more abundant, the environment is preserved and protected, and people are healthier – a vision that can be realized through research into the basic biology of plants. William Boyce Thompson, the Institute's

founder, first articulated this vision in 1924. Thompson created BTI as a result of his conviction that “any principles concerning the nature of life that you can establish for plants will help you to understand man, in health and in disease.”

Since then, the scientists of BTI have kept their founder's vision in sight. They have made discoveries in biology, environmental science, ecology, agriculture and human health that have directly led to improved crops, a healthier environment and new ways to prevent or treat human disease. As they move forward, they will apply innovative science, such as functional genomics, molecular modeling, computational biology, molecular genetics, bioinformatics and biotechnology, to enhance the understanding of plants for the benefit of people and the environment.

To achieve its 21st century goals, the Boyce Thompson Institute for Plant Research works collaboratively with a variety of non-profit research institutions and universities. Strengthening these relationships is an important initiative in BTI's continuing effort to broaden its multi-disciplinary approach to plant science, provide meaningful, innovative educational opportunities for new scientists, and make significant contributions to the world's body of scientific knowledge.



BTI / Mission



As stated by its Board of Directors, the mission of the Boyce Thompson Institute is “to use basic research to expand the frontiers of plant biology and related areas, and contribute, through science and technology, to the improvement of the environment and the quality of human life.” Broadly, it is a mission that sets worldwide leadership in plant biology as its goal. Specifically, it is a mission to understand the interactions between plants and their environment for the enhancement of life.

Using an interdisciplinary approach, BTI scientists are learning how plants perceive environmental signals, including stress from bacteria, drought or the presence of toxins in the air or soil; how they transmit “knowledge” of these signals within and between cells, and how they respond to these external signals for self-preservation. This interaction between the plant and its environment is called “signal transduction” and it is the primary research emphasis at BTI. Discoveries made in this area will enable BTI scientists and others to enhance a plant's productivity by modifying its natural response to environmental stress and other signals.

Understanding signal transduction in plants will provide important information for the protection of the environment. It will lead to plants that better use their own natural mechanisms to utilize water and nutrients, or fight disease or insects. These are discoveries that, in turn, will reduce the overall use of pesticides and fungicides, preserve natural resources and lead to healthier, more productive crops.

The Institute's research also will lead to improved human health. In fact, BTI scientists discovered that plants and humans share an ancient immune system, handed down from a common ancestor a billion years ago. As a result, scientists at BTI are using biotechnology to develop plants that deliver human vaccines through

food – an advance that could significantly enhance the health of people, particularly in the developing world.

Understanding the genetics of nutrient composition in plants is another major focus of BTI research that can benefit human health. Such discoveries will enable scientists to modify food plants to provide people with better nutrition or a more convenient way to prevent disease – such as high blood pressure – using natural compounds produced in plants.

During the past 75 years, BTI scientists have made important contributions to agriculture; they have been leaders in environmental research, and they have demonstrated the importance of plant biology to medical science. Now and in the future, they will continue to use leading edge technology to reveal the inner workings of plants for the benefit of life.



BTI / Highlights 2001 /

Gary W. Blissard was coordinator for the Cornell Virology Journal Club and served on the executive committee of the Cornell NIH Training Grant in Virology. He was a member of the Baculovirus Study Group of the Invertebrate Virus Subcommittee of the International Committee on Taxonomy of Viruses, and a member of the American Society for Virology Advisory Committee to the American Type Culture Collection. He was on the editorial boards of the *Journal of Virology* and *Virology*.

Tom Brutnell was a member of the Cornell Plant Breeding Faculty Search Committee and gave guest lectures in advanced plant genetics.

Alice Churchill was an *ad hoc* reviewer for the U.S. Department of Agriculture and for the *Journal of Invertebrate Pathology*, and a member of Sustainable Agriculturally-based Bio-Industries Cluster (SABBIC) of Cornell University. She was also a participant in the National Cooperative Drug Discovery Group of the National Institutes of Health, and a member of the Sigatoka Working Group of PROMUSA, an international program for banana and plantain improvement. Churchill gave an invited talk, entitled "Fungi used as a resource for therapeutic agents," at the American Medical Association Media Briefing on Food Biotechnology held in New York City.

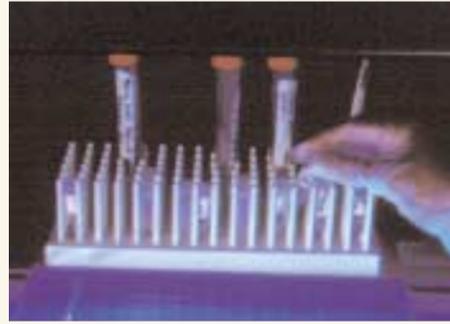
Jonathan Comstock served as director of the Cornell and Boyce Thompson Stable Isotope Laboratory (CoBSIL).



Jim Giovannoni served as monitoring editor for the journal *Plant Physiology* and chaired the U.S. panel for Cell and Molecular Biology of the Binational Agriculture Research and Development program (BARD). He was a coordinator of an international grass-roots effort to sequence the tomato genome. Giovannoni served on several faculty search committees for Cornell and the U.S. Department of Agriculture. He was a member of the Cornell Genomics Task Force.

Robert Granados continued as an adjunct professor in the Department of Entomology at Cornell University, an affiliated faculty member in the Bioengineering Program, and a member of the Cornell Biotechnology-Biocontrol Faculty, the Cornell Center for the Environment, and the Cornell Exploration Program in Biological Sciences.

Daniel Klessig participated in the Pathogenesis-Related Protein Workshop in Spa, Belgium; the 7th International Conference of Plant Growth Substances in Brno, Czech Republic; the 27th FEBS and America Association of Biochemical and Molecular Biology Meeting in Lisbon, Spain; the 10th International Congress on Molecular Plant-Microbe Interactions in Madison, WI; the joint annual meeting of the American Society of Plant Biologists and the Canadian Society of Plant Physiologists in Providence, RI; the annual meeting of the Phytopathology Society in Salt Lake City, UT; and the 1st U.S.-Korea Joint Seminar on Plant Molecular Genetics and Biology, held in Ithaca, NY.



Robert Kohut gave a presentation to the New Visions Program at Cornell entitled, "The effects of changes in air quality on genetic diversity," and hosted a student conducting an independent research project in his laboratory. He was also an invited speaker at the Ozone Education Day held by the Forest Health Monitoring Program of the U.S. Forest Service at the University of Massachusetts, Amherst, and is serving as program co-chairman for the April 2002 Air Pollution Workshop at Pennsylvania State University.

Gregory Martin is co-principal investigator on an NSF-funded project that is developing a large, publicly available tomato express sequence tags (EST) database, administered by The Institute for Genomic Research (TIGR). Martin also co-directed BTI's collaboration with Cornell Plantations to develop the Tomato Biodiversity Garden. He was a member of the Cornell Genomics Task Force, the Cornell Genomics Technology and Infrastructure Planning Subcommittee, and a participant in the Biomedical Research Alliance of Upstate New York.

Hugh Mason gave a presentation, entitled "Structure and function of vaccine antigens expressed in edible plants," at the 13th Annual International Scientific Meeting on Scanning Microscopies in New York City in May. He also presented "Plant-based vaccines: Expression and Immunogenicity" at the 2001 Congress on *In Vitro* Biology held in St. Louis, MO, in June, and "Clinical trials with edible plant vaccines: present status and future challenges" at the EuroConference on Vaccines of the Future held at the Pasteur Institute in Paris, France, in October.



Alan Renwick and colleagues presented a poster at the February Gordon Conference, held in Ventura, CA, on Plant Herbivore Interactions, entitled "Chemical basis for host recognition by solanaceous-feeding insects." He also presented a paper, entitled "The chemical world of crucivores: lures, treats and traps," at the 11th International Symposium on Insect-Plant Relationships held in Helsingør, Denmark in August. Renwick served as a member of the editorial board of the *Journal of Chemical Ecology*.

Joyce Van Eck was coordinator of the BTI-Cornell Plantations Tomato Biodiversity Garden, workshop leader for Cornell's "Expanding Your Horizons" program, and a participant in the New Visions program.

David Weinstein chaired the Cornell Plantations Advisory Board, was a member of the Dryden Town Planning Board, and the Land Committee of the Finger Lakes Land Trust, and an associate member of the Tompkins County Environmental Management Council.



BTI / Attracting the Best and the Brightest /

BTI's continuing tradition of innovative plant science research attracts graduate students, post-doctoral and research assistants from countries throughout the world. In 2001 alone, 34 young scientists from 13 countries augmented BTI's staff. Though each has a different reason for joining BTI, four of their stories are representative.

Seoul, Korea, is the home of **Bong Ghi Hong** who is pursuing his doctorate at Cornell. Hong, who received his undergraduate degree from Seoul National



University, was attracted to BTI by David Weinstein's work in plant ecology. "It's the best in ecological modeling," he says. In fact, Hong was so intent on studying with Weinstein that he changed his major from forestry to environmental toxicology in order to realize his goal.

Meena Haribal joined Alan Renwick's lab as a post-doctoral fellow and now works as a research associate. She holds a Ph.D. from the Indian Institute of Technology in Bombay, her hometown. Haribal has always been interested in natural history, particularly



butterflies. When Cornell chemist Jerrold Meinwald was lecturing in India, he invited Haribal to Ithaca where she was attracted by Renwick's work on the interaction between insects and their host plants.

A talk by Greg Martin in Copenhagen attracted **Sophia Ekengren**, a native of Stockholm, to BTI. A graduate of Stockholm University with a Ph.D. in developmental biology, Ekengren sought and received a two-year grant from the Swedish Royal Academy, which made her post-doctoral position at BTI possible. After completing her postdoctoral fellowship, Ekengren plans to return to Sweden where she will continue her research on plant parasites.



International recognition of Dan Klessig's research was the factor that brought **Frank Menke**, a native of the Netherlands, to BTI. Says Menke, "The Klessig lab is well known for a good mixture of techniques in molecular biology, biochemistry and genetics." Menke, who holds a Ph.D. in plant molecular biology from Leiden University, received funding from the Dutch government and, subsequently, from the prestigious Human Frontiers of Science Program – a multi-national organization – for his BTI work. Menke plans to return to the Netherlands and continue his research on plant defense signaling.



BTI / Keeping up with BTI's Retirees /

In the course of its 78 year history, BTI has been blessed with a number of individuals who have devoted their scientific careers to the Institute. Three maintain active offices in our facility.

In the six years following his "retirement" in 1992, **Dick Staples**, Ph.D, plant pathology, worked with Harvey Hoch in the Department of Plant Pathology in Geneva, N.Y., where he studied the development of appressoria in rust fungi, which is an aggressive parasite that attacks a wide variety of crop plants. Staples is also a receiving editor for the Federation of European Microbiological Societies journal, entitled *FEMS Microbiology Letters*, and he writes commentaries for the publication, *Trends in Plant Science*. Staples also writes occasional reviews. He, Carl Leopold and Mark Jaffe recently co-authored a paper, "Thigmo responses in plants and fungi," which reviews their findings about the plant/fungi interaction that is based on touch. Their paper will be published in the *American Journal of Botany*.

Dubai, Brazil and Spain are just a few of the countries **Leonard Weinstein**, Ph.D, plant physiology and William Boyce Thompson Scientist Emeritus, visited in the past year in his role as an environmental expert on fluorides and their effects on plant life. When he's not traveling, Weinstein works on a book, tentatively titled *Fluoride and the Environment*, with co-author Alan Davison, a 1982 BTI visiting scholar. The manuscript will be submitted to CABI, a leading international publisher in the applied life sciences, in September. Weinstein's last graduate student, Mari Reeves, completed her studies in 2001. She will publish the results of their collaborative research on the phytoremediation of fluoride and complex metal cyanides.

Carl Leopold, Ph.D., plant physiology, is currently involved with four ecological organizations. As vice president of the Wisconsin-based Aldo Leopold Foundation, he is concerned with environmental education based on the ethical treatment of the land. In his role

as chair of the Tropical Forestry Initiative, he is helping to restore rain-forests in Costa Rica. In fact, his recent article, "Attempting restoration of wet tropical forests in Costa Rica," was recently published by Elsevier. As founding president of the Finger Lakes Land Trust, Leopold is active in several land trust committees that focus on the protection of quality lands in the region. A board member of the Black Locust Initiative, Leopold also promotes the use of native hardwoods for the construction of buildings and outdoor structures, such as playgrounds. The replacement of pressurized wood with hardwood for construction will ultimately reduce the amount of arsenic, chromium and other toxic compounds that leach into the environment.

In May, the New York Academy of Sciences honored **Karl Maramorosch**, Ph.D., an entomologist and former BTI program director, for his "distinguished and caring service to science, technology and society as an Academy member over the past 50 years." Maramorosch chaired the Academy's Microbiology Section and served as vice president and recording secretary during the course of his scientific career. In June, Maramorosch was also honored by the *In Vitro* Biology Society at their congress, held in St. Louis, MO. He received the Society's Distinguished Lifetime Achievement Award for his exemplary research in and pioneering contributions to the field of cell culture. Maramorosch is well-known for his studies of insect/plant/virus interactions.



Left to right: Carl Leopold, Leonard Weinstein, and Richard Staples



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Cornell University, Ithaca, NY

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New York, NY

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Maria Moors Cabot Professor of
Biology, Harvard University,
Cambridge, MA

Joanne Chory
Investigator, Howard Hughes
Medical Institute
Director and Professor, Plant Biology
Laboratory, Salk Institute,
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University, Ithaca, NY

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Red Abbey LLC, Baltimore, MD

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/ Explorations / BASIC BIOLOGY

The basic biological mechanisms that enable plants to grow, defend themselves from disease and insects, use water, and react to stress are not well understood. At BTI, scientists are working to open a new window of knowledge on the interaction between plants and their environment. Studying these interactions will enable BTI scientists to unravel the mysteries of plants at the molecular level. Knowledge gained in such basic biology explorations will provide important information for enhancing the health and productivity of plants, the sustainability of the environment, and the quality of life for animals and people.

HOW DOES A PLANT'S IMMUNE SYSTEM FUNCTION?

Although it's well known that humans and other mammals have highly evolved immune systems, work by Daniel Klessig, his colleague Greg Martin, and their co-workers, has helped establish that plants also have a type of immunity. It is an ancient system shared by plants, insects, people and other animals and is frequently referred to as "innate immunity."

Klessig and his colleagues discovered that when a plant is attacked by a pathogen, such as a virus, bacterium or fungus, chemical and biochemical signals are exchanged between the two organisms. Recognition of the pathogen through these signals is mediated by disease resistance genes (several of which have been identified and isolated by both the Klessig and Martin groups) and triggers a signal transduction cascade that leads to production of defense signaling molecules.

Klessig's group established that nitric oxide, which plays an important role in

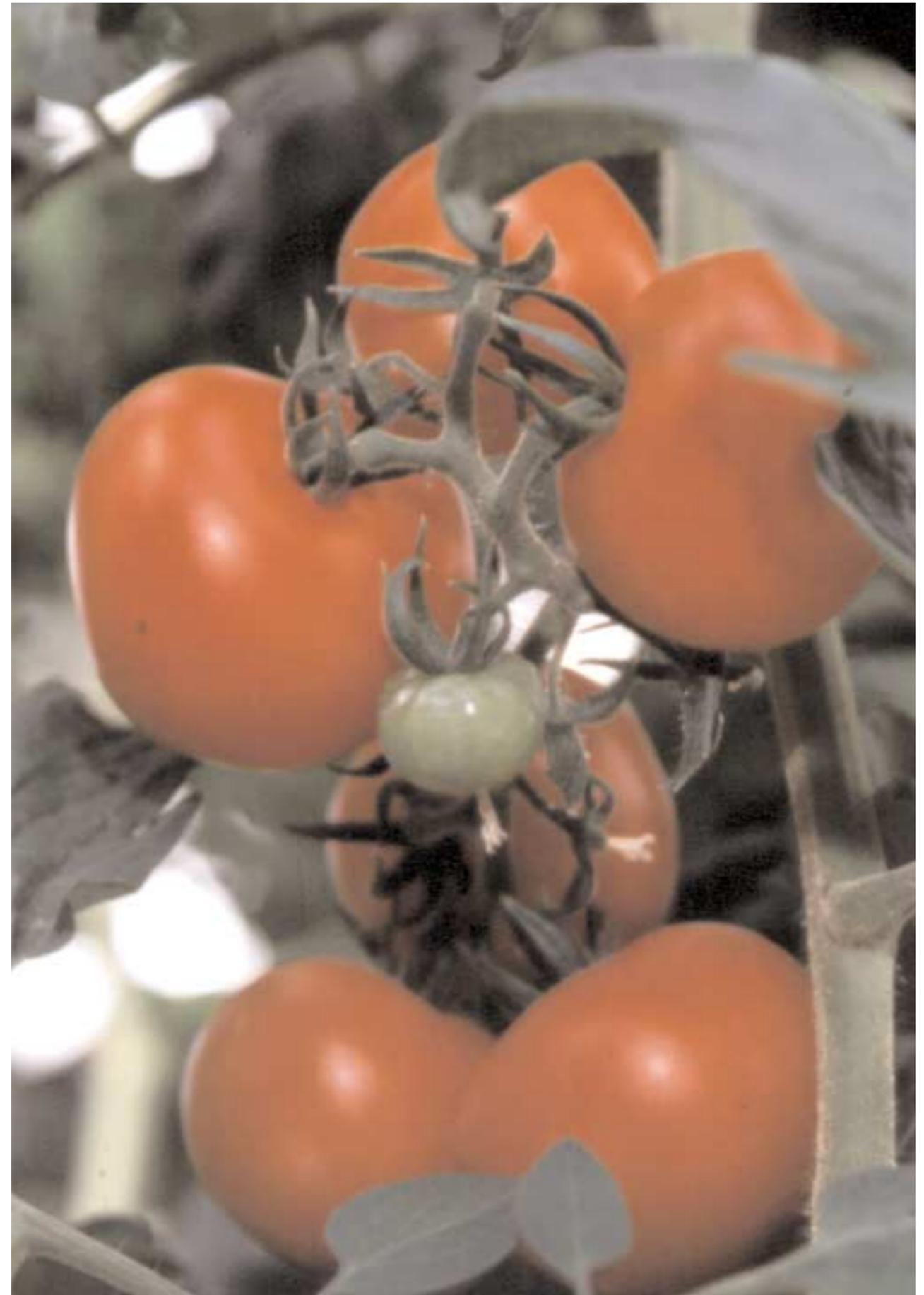


immunity in animals, and salicylic acid, a derivative of which is aspirin, are two critical defense signals in plant innate immunity. Using genetic and molecular approaches, they identified a new defense signal in 2001, which is the fatty acid, oleic acid, or a derivative of it. It appears that oleic acid normally suppresses the salicylic acid-mediated defenses while it activates other defenses controlled by another defense signal, jasmonic acid.

Their work continues to determine the kind of molecules plants produce under attack, the order in which they are produced, and how they work to activate defenses against a variety of pathogens.

Understanding how this happens at the molecular level could lead to improved disease resistance in crops and, therefore, more environmentally sustainable agricultural practices. It may also provide insights into innate immunity in humans (or how humans ward off infection by microbial pathogens).

Daniel F. Klessig, Ph.D.
Scientist / Plant Pathology / President & CEO



WHAT IS THE GENETIC BASIS OF DISEASE RESISTANCE IN PLANTS?

Gregory Martin's lab at BTI focuses on the genetic basis of plant disease resistance through studies of the changes pathogens cause in plants at the molecular level. Currently, Martin is using functional genomics to identify and study the genes in a bacterium (*Pseudomonas syringae*) that enable it to cause speck disease in tomatoes, and to identify the genes in the tomato plant that are involved in defending it against the disease.

Funded by a grant from the National Science Foundation Plant Genomics Project, Martin has identified about 20 genes out of a total of 6,000 that enable the *Pseudomonas* bacterium to infect tomatoes. In 2001, his group also identified over 400 tomato genes (out of a total of 35,000) whose expression is altered when the plant is infected. Work continues to inventory the entire array of genes in *Pseudomonas* that cause disease and to identify the plant's full complement of defense mechanism genes.

The next goal for Martin's research is to understand the interaction between the pathogen and the plants. To do that, he infects tomato leaves with the bacteria and then uses microarray techniques to "watch" the plant's genes turn on and off during the first few hours after infection. Together with computer modeling, this work will enable him to determine the function of each affected plant gene – knowledge that ultimately could lead to crop plants with an improved ability to fend off disease.



Gregory Martin, Ph.D. / Scientist / Plant Pathology

HOW DO VIRUSES INFECT CELLS?

Understanding how viruses enter cells, duplicate themselves and then exit the cell could have important implications for disease therapy in humans. To that end, Gary Blissard studies large DNA viruses, called baculoviruses, that infect certain insects. Long term, his work may also have important insect control applications in agriculture.

Blissard discovered a protein, called GP64, located on the outside covering, or envelope, of the virus that enables it to attach to the surface and then enter a host cell where it releases its DNA. Blissard also found that GP64 is involved in the final phase of infection when progeny of the virus bud out from the host cell surface. Current research in his lab is focused on understanding how the structure and organization of GP64 facilitates these important infection functions.

Blissard is also working to understand how the 150 genes in baculovirus are regulated and expressed. It is known that about half the virus' genes are turned on when it enters a host cell, and Blissard's studies have examined how the host cell recognizes these genes and reacts to them as if they were its own genes. These "early genes" then turn on "late genes" that enable the virus to take over its host. Blissard's research focuses on this cascade of events, how it is triggered, and what role early genes play in turning on late genes.

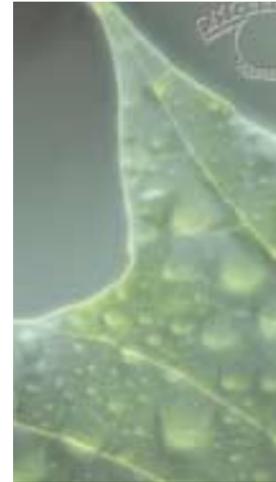
Through his work on baculovirus envelope proteins, Blissard is uncovering important details of the viral infection process. The results of his work could lead to new, environmentally sound ways to control insect pests in crop plants. It could also lead to new gene therapy methods for people.



Gary W. Blissard, Ph.D.
Scientist / Molecular Biology



baculovirus DNA



Thomas P. Brutnell, Ph.D.
Assistant Scientist / Plant Molecular Genetics

HOW DO PLANTS RESPOND TO LIGHT?

It's well known that plants adjust their rate of growth according to the amount of light they receive. In lower than ideal light, plants grow tall – a physical characteristic gardeners call "leggy" – because

they allocate the majority of their energy to leaf and stem growth. When this happens in crop plants, they produce less food. Understanding how plants sense and respond to light at a biochemical level could lead to genetically modified plants that divert their energy into grain production rather than height.

Thomas Brutnell's lab at BTI is working to understand light signaling pathways in corn. As a result of light signaling studies in the model lab plant, *Arabidopsis*, it is known that exposure to direct sunlight causes a plant protein (Phytochrome B) to become active which, in turn, tells the plant to divert its energy into seed production. When the plant is shaded, the protein deactivates – an event that tells the plant to grow taller. This appears to be the case in corn as well, evidenced by the fact that corn plants in the center of a field, which are shaded on all sides by other corn plants, grow taller than the plants at the edge of the field.

Brutnell is working to identify which genes in corn control light recognition. He's using transposons (pieces of DNA that "jump" from one place in the genome to another) to deactivate or "knock-out" specific genes in the pathway. When these genes are knocked-out, there is an observable effect on the plant's physical characteristics, enabling him to link a particular gene to a particular trait. In so doing, he can determine the function of each gene in the light-signaling pathway. In the long term, better understanding of light signaling in crop plants like corn or rice could lead to plants that produce significantly more food.

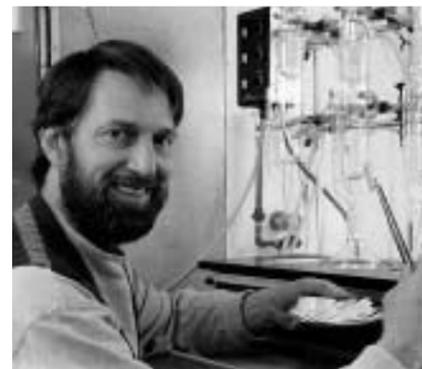
Brutnell's lab is also using a technique called transposon mutagenesis to create multiple lines of corn that are genetically identical except for the position of a single transposable element in the genome of each line. Funded by the National Science Foundation's Plant Genome Project, the work will result in lines of corn other researchers can use to identify the function of any gene in the corn genome. Once developed, the corn lines will be available without charge from the Maize Cooperative Stock Center at the University of Illinois.

HOW DO PLANTS REGULATE WATER USAGE?

Water is critical to a plant's ability to absorb carbon dioxide from the air and carry out photosynthesis – the process that enables it to grow. But photosynthesis uses nearly 95 percent of the water a plant needs in its life. In fact, plants that are highly efficient at using water do not photosynthesize at the highest rates, and plants that show a high rate of photosynthesis do not use water efficiently. It's clear there is a highly complex genetic basis for water use that enables plants to maintain a delicate balance between the amount of carbon dioxide they capture from the air and the amount of water they lose in the process.

In 2001, Jonathan Comstock's lab at BTI received a major collaborative grant from the National Science Foundation to study water use efficiency in plants. A consortium of scientists from BTI, Cornell University, the University of North Carolina and Oklahoma State University is conducting the research. At BTI, Comstock's lab will focus on the identification of the genes in tomato and rice plants that control the plants' water usage.

Knowledge gained will enable scientists to understand how plants coordinate and carry out the complicated control processes necessary for growth and development, such as effective water utilization; what genes are involved in these processes, and the order in which they function. Ultimately, this work could lead to crop plants that are more tolerant of drier conditions, which, in turn, could expand the world's food-producing area.



Jonathan P. Comstock, Ph.D.
Associate Research Scientist / Plant Ecophysiology

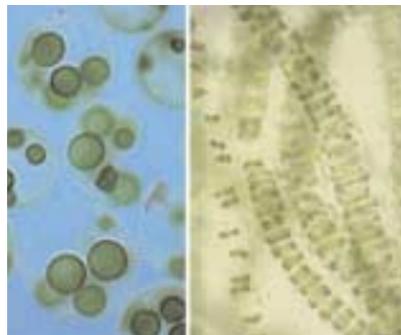
/ Explorations / AGRICULTURE

Finding ways to make farming more sustainable and food more nutritious are critical areas of research at BTI. With world population predicted to reach 9 billion people by 2050, new ways must be found to provide farmers in developed and developing countries with plants that provide nutritionally enhanced food and crops that protect themselves from insects, disease and environmental stress. Work is on going at BTI to speed the development of sustainable agriculture and healthier food through biological research and the application of biotechnology.

HOW DO PLANTS RESPOND TO STRESSES IN THEIR ENVIRONMENT?

There is a form of algae that grows in the snow high in the mountains. There's another that lives inside sponges in a Wyoming lake. Both of these life forms have learned to photosynthesize under conditions that are extremely stressful to most other plants. What is the genetic basis of their ability to survive?

BTI scientist David Stern is trying to address that question by better understanding how a plant perceives its environment, how it signals itself to deal with environmental stress, and what genes are involved in the complex signaling networks the plant employs. To do this, he's studying the chloroplasts of a green alga, called *Chlamydomonas reinhardtii*, in the laboratory. Chloroplasts are the small organs in a plant cell that carry out photosynthesis and produce other substances necessary for the plant's growth and survival. Understanding what happens in the chloroplast when the



Light micrographs of two green algal species, Volvox and Ulothrix.



environment stresses the plant will help Stern determine the signaling networks the plant uses for communication among its cells.

Stern is using specialized computer programs to help him analyze the plant's signaling networks, and he's working to create a computer-based "virtual organism" as a study model. Using the virtual organism, Stern will be able to simulate environmental signals and then predict and test the genetic changes that occur in the organism as a result. Understanding the complex mechanisms plants use to interact with their environment may lead to crops that can survive in currently inhospitable places – like the algae that grows in the snow.

David B. Stern, Ph.D.
Scientist / Plant Molecular Biology
Vice President for Research



HOW CAN INSECT PESTS BE CONTROLLED WITHOUT CHEMICAL INSECTICIDES?

Interfering with insect development or causing insect pests to be more vulnerable to pathogens in the environment, such as viruses, could reduce the use of chemical insecticides. Robert Granados is pursuing this theory in novel research aimed at identifying insect baculovirus genes that could be used to develop new kinds of insect-resistant crop plants.

Granados discovered that certain genes in baculovirus produce enzymes that attach to insect proteins located on the chitin membrane that lines the insect's digestive tract. When this happens, the protective membrane is damaged, which, in turn, interferes with the insect's ability to develop and makes it more susceptible to microbial infections. Disruption of the membrane eventually kills the insect.

In another study, Granados produced antibodies to the insect membrane protein, which he fed to larvae in the laboratory. The larvae that ate the antibodies became more susceptible to microbial infection and died, indicating that the antibodies may also provide a novel, non-chemical means of controlling insect pests.

Both projects could lead to the development of genetically modified, insect-resistant plants that produce the insect control proteins or antibodies. In fact, Granados has inserted the baculovirus protein gene into tobacco and rice plants, which have shown resistance to certain insect pests, including armyworms and various species of loopers.

Funded by the U.S. Department of Agriculture, Granados is currently working to identify the chitin-binding sites on the insect membrane protein in order to isolate its active portion. This work may lead to the use of the insect protein itself to control insect pests.



Robert R. Granados, Ph.D.
Charles E. Palm Scientist / Virology

WHAT MAKES PLANTS VULNERABLE TO INSECT ATTACK?

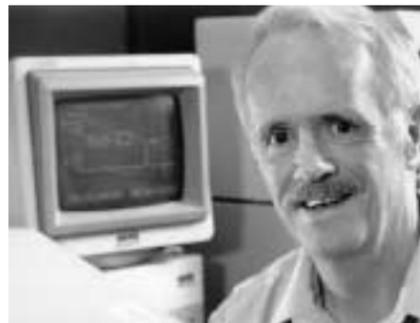
Alan Renwick's lab is approaching the control of insect pests from a different angle. He has identified compounds produced in plant leaves that either attract or discourage insects based on taste. These compounds are recognition factors that signal the insect to accept or reject the plant for egg laying or feeding.

In comparative studies of insects that prefer potatoes, tomatoes and eggplant, Renwick has found that specialized insects actually become addicted to specific compounds produced in the plant's leaves. Colorado potato beetles and tobacco hornworms feed on the same kinds of plants, but the beetles are tuned to non-steroidal glycosides while the hornworms prefer steroidal glycosides.

Renwick has also isolated two active compounds from cabbage leaves that stimulate egg laying in diamond-back moths. If these compounds could be produced in large quantities, they could be used to trick the moth into laying its eggs on a hostile, non-crop plant, such as a weed, where the moth's larvae would be unable to survive.

Long-term, Renwick's research may lead to genetically modified crop plants that no longer produce compounds attractive to particular pests or produce compounds that repel particular insect pests. Both approaches could lead to novel, non-chemical ways to protect crop plants from insect damage.

Currently, Renwick is determining whether insects can provide a good model system for understanding the sense of taste in humans and the basis of people's addiction to certain foods.



J. Alan A. Renwick, Ph.D.
Scientist / Chemical Ecology



James J. Giovannoni, Ph.D.
Scientist / Plant Molecular Biology, USDA

WHAT IS THE GENETIC BASIS OF FRUIT QUALITY AND RIPENING?

Ripening enhances the texture, color, flavor and nutrient content of fruits and vegetables. As a result, understanding the ripening process at the genetic level – and developing the ability to control it – could lead to higher quality food. James Giovannoni's USDA laboratory at BTI focuses on fruit ripening and the production of nutrients in fruit, using tomato as a model system.

One of Giovannoni's projects focuses on the production of lycopene in tomatoes. Lycopene is an important precursor of Vitamin A, and it is the substance that makes tomatoes red: the redder and riper the tomato, the more lycopene it contains. Understanding the genetic basis of lycopene production in tomatoes may enable scientists to produce genetically enhanced tomatoes and other fruits that are more nutritious.

Because fruit ripeness is important for both nutritive and commercial reasons, Giovannoni is also studying the genetic basis of ripening in tomatoes. How does a tomato signal itself to ripen? What gene or genes turn on the production of ethylene – the substance that causes tomatoes to ripen? Can the production of ethylene be regulated so that commercial tomatoes have better taste, texture and nutritive value? And is there a common genetic mechanism that controls ripening in all fruit?



To answer these questions, Giovannoni turned to today's commercially available tomatoes, which, for shipping purposes, have been traditionally crossbred with a mutant tomato variety that never fully ripens. Though tomato breeders have used this mutant tomato for 10 to 15 years, the gene controlling its ripening capabilities was not

known until now. In 2001, Giovannoni's lab isolated and cloned the single gene that is responsible. This gene, which controls ethylene production in the plant, is turned off in the mutant variety and is an important key to the overall understanding of ripening in tomatoes.

Using biotechnology, Giovannoni may be able to develop lines of tomatoes in which the ethylene production gene is active at varying degrees – an advance that would enable breeders to produce tomatoes that can be shipped at a riper stage for increased nutrition, flavor and texture. He is also using the tomato gene to "fish out" and identify similar ripening genes in other kinds of fruit to understand whether the ripening process in tomatoes is common among all plants.

/ Explorations / THE ENVIRONMENT

Understanding the genetic basis of a plant's ability to adapt to environmental stress can have significant positive future impact. At BTI, scientists are discovering how pollutants like carbon dioxide and ozone affect plant diversity. They're studying the interaction between plant roots and the soil environment to determine how optimal growth can be achieved in trees. And they're using computer modeling to predict the future health of U.S. forests under various climatic and environmental conditions.

DO AIR POLLUTANTS AFFECT THE GENETIC DIVERSITY OF PLANTS?

Acknowledging that habitat destruction is the primary reason for the loss of plant diversity on earth, Robert Kohut is studying subtle, but widespread atmospheric pollutants that may also affect diversity. Kohut's lab is investigating whether genetic adaptation to increasing levels of carbon dioxide and ozone pollution negatively impacts the genetic diversity of plant populations and alters their ability to cope with other environmental stresses, such as insects, disease or climatic change.

Kohut is using a fast-growing model plant system (*Brassica rapa*) to study the effects of air pollutants. With a life cycle of only 50 days, *Brassica rapa* enables Kohut to study changes in the physiological, phenotypic and genetic properties of these plants over several generations as levels of carbon dioxide in the air are gradually increased. The theory is that as populations of plants adapt to higher concentrations of pollutants, they may be losing other genetically based survival characteristics.

Kohut exposed five generations of *Brassica rapa* to atmospheric carbon



Brassica rapa

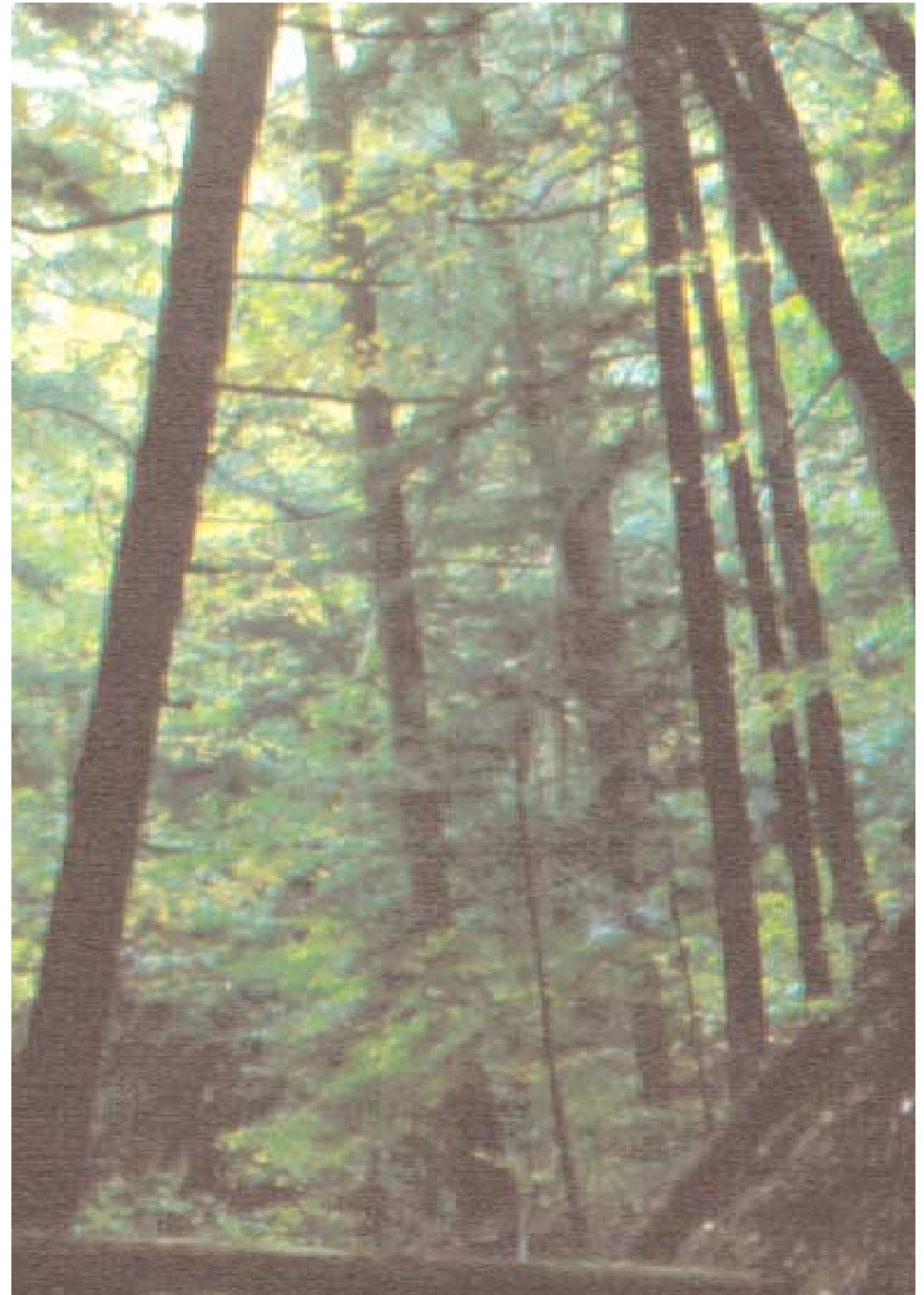


dioxide concentrations that increased with each generation, rising from 360 to 660 PPM. Comparing succeeding generations of control and experimental plants, he observed a modest increase in the experimental plants' rate of photosynthesis and a thickening of their leaves, but he saw no significant change in the size, number or germination rate of the seeds the plants produced. Though these results lead Kohut to believe that a plant's ability to exploit carbon dioxide in the air may provide no reproductive advantage, he is now assessing what changes may have taken place at the genetic level.

Kohut is also interested in determining whether ozone – the most widespread air pollutant in the United States – is altering the genetic diversity of sensitive species of native plants.

The results of Kohut's work promises to provide important information about the long-term effects of atmospheric pollutants on plants. In turn, his work may help set new air pollution standards that will better protect the quality and diversity of plant life for future generations.

Robert J. Kohut, Ph.D.
Scientist / Plant Pathology



HOW DO TREE ROOT SYSTEMS INFLUENCE THE RATE OF TREE GROWTH?

The interactions between a tree's roots and the soil in which it grows are complex and little understood. How do roots respond to stress? Do root-colonizing fungi help trees tolerate stress and alter their growth rates? Why do some trees within a species grow faster than others in the same species? These are currently unanswered questions that BTI scientist Mary Topa is striving to answer.

Forest trees, which exhibit unusually high genetic variation among individuals within a species, are among the most genetically variable organisms on earth. By studying a genetically controlled stand of loblolly pine trees in North Carolina, Topa is working to understand why some trees grow faster than others and whether the rate of growth is a result of an individual tree's efficiency in using the carbon it extracts from the air. Because more than half the annual carbon fixed by a tree is allocated below ground, studying various root systems under various environmental conditions is key to understanding tree growth.

Using a variety of scientific techniques, Topa is analyzing the efficiency of the trees' rooting strategies under optimal and stressed soil environments. She has found that fast growing trees have a different mycorrhizal community on their roots than slow growers. Mycorrhizae are root fungi that are beneficial to the tree, providing nutrients in exchange for tree carbon. She also has found that fast growing trees reduce their fine root growth in the presence of fertilizer, while slow growers do exactly the opposite. These results suggest that fast growers use carbon more efficiently.

In the longer term, Topa's efforts to understand the genetic basis of root-soil interaction and the effect of rooting strategies on the rate of tree growth may enable scientists to develop trees that are ideally suited to their soil environment. Her work could lead to trees that require less fertilizer or water and grow faster – an important goal, particularly for sustainable forestry where improved yield through faster growth would be a significant benefit.



Mary A. Topa, Ph.D.
Associate Scientist / Plant Physiology



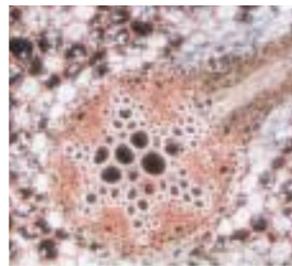
David A. Weinstein, Ph.D.
Associate Research Scientist
Plant Ecology

CAN THE AFFECTS OF POLLUTANTS AND HUMAN ACTIVITY ON FORESTS BE PREDICTED?

The slow growth and longevity of trees make it difficult to determine the long-term effects of air pollutants and human activity on the health of forests. To that end, David Weinstein's lab is using advanced computer modeling techniques to simulate forest growth over long periods of time under varying environmental conditions.

In 2001, Weinstein completed a study of the effect of ozone reduction on forest growth in 13 regions of the Southern Appalachian Mountains. The study's goal was to determine which of three control scenarios proposed for the regulation of ozone in the southern U.S. states would have the most beneficial long-term effect on forests throughout the Southern Appalachian Mountains. All three scenarios were predicted to prevent the loss of the number and diversity of major tree species due to climbing ozone levels. These central strategies were predicted to be critical to the maintenance of key species in several national forests.

Weinstein's computer modeling software revealed that drastic ozone reduction wasn't the best strategy for every forest. Though all 37 forests in the 13 regions were predicted to have a stable abundance of trees over time under all three scenarios, some areas were predicted to lose diversity if ozone were drastically reduced. This is because reduced ozone would increase competition among the individual tree species in the forest, with some species proliferating while others declined.



Electron micrograph of a root.

/ Explorations / HUMAN AND ANIMAL HEALTH

Plants are critical for human and animal health. In addition to providing carbohydrates and essential vitamins and minerals, they are the source of nearly half the pharmaceuticals used to treat human and animal diseases. As a result, understanding the genetic basis of nutrient production in plants, whether plants can be used to manufacture and deliver vaccines to people, and using fungi to bioprospect for novel pharmaceuticals are key areas of research at BTI. This research will ultimately result in healthier, more nutritious food, and novel ways to immunize people against disease.

HOW DO FUNGI PRODUCE BIOACTIVE CHEMICAL COMPOUNDS?

Fungi naturally manufacture chemicals with biological activity. Some protect the fungus from its own predators; others cause disease in plants and animals. Still other fungal products have important medicinal uses in people, such as penicillin, lovastatin and cyclosporin. Of the estimated 1.5 million fungal species on earth, only about five percent have been given names and an even smaller number have been studied for their abilities to produce beneficial chemicals.

The focus of Alice Churchill's work at BTI is understanding the genetic bases for how fungi manufacture bioactive compounds. Specifically, her lab studies fungi as a resource for novel genes and chemicals with medicinal activities, as well as the role of fungal toxins in causing plant disease.

Understanding the molecular basis of natural chemical production in fungi could lead to new sources of medicines for human or animal care. Churchill's lab has screened over 100 fungi and



Penicillium sp. (fungus): The source of penicillin.

identified genes predicted to synthesize novel, biologically active natural chemicals. Churchill's research is conducted in cooperation with Cornell University, the U.S. Department of Agriculture, and a pharmaceutical partner as part of an NIH-funded National Cooperative Drug Discovery Group. Her research could lead to the identification of new fungal chemicals with anticancer or anti-microbial activity.

Fungal chemicals can also be involved in causing plant disease. In 2001, Churchill published the results of her work on toxin production by the sorghum pathogen *Periconia circinata*. She also co-authored a paper describing genetic transformation of the *Mycosphaerella* fungi that cause the most important diseases of bananas and plantains worldwide. She is also studying the production of bioactive pigments by the chestnut blight fungus to understand the role of these pigments in the biology of this tree pathogen.



Alice C. L. Churchill, Ph.D.
Center Scientist / Molecular Mycology

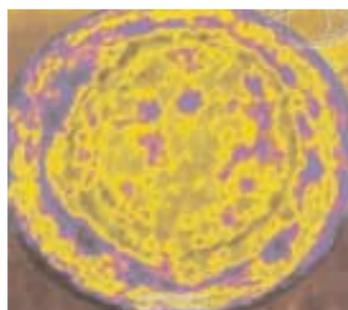
HOW DO PLANTS PRODUCE NUTRIENTS IMPORTANT FOR HUMAN HEALTH?

Folic acid, primarily available in leafy green vegetables, is important to the body's ability to make red blood cells and produce the nervous system chemicals norepinephrine and serotonin. It is also one of the few nutrients known to prevent neural tube birth defects, such as spina bifida. Joyce Van Eck is working in collaboration with Tricia Conklin (formerly of BTI) to understand how plants produce folic acid and how they could be encouraged to produce higher quantities of this important B vitamin.

Van Eck's work, supported by a grant from the Helen Graham Foundation, focuses on the identification of the genes in *Arabidopsis* plants responsible for producing a component of folic acid, called PABA. Her ultimate goal is to elucidate the biochemical pathway in plants that leads to folic acid production. Through this work, she and other scientists may be able to use biotechnology to insert the genes for folic acid production into crop plants, such as rice, potatoes or wheat, that do not naturally produce large quantities of this nutrient.

Plant disease resistance is another important research focus in Van Eck's lab. In 2001 – in cooperation with Cornell University and Syngenta, Inc. – she field-tested 2,000 Russet Burbank potato plants modified to resist late blight (*Phytophthora infestans*). This fungus-borne disease caused the Irish potato famine in the 1840s and continues to devastate potato crops worldwide. Currently, late blight can only be controlled through numerous applications of fungicides – a costly, environmentally unsound practice.

In the 2001 field trials, Van Eck's modified potatoes exhibited genetically based resistance to the fungus. Research in this area is on going, but Van Eck's work promises to provide important ways to enhance agricultural sustainability throughout the world by reducing the use of chemical fungicides.



Hepatitis B virus



Joyce M. Van Eck, Ph.D.
Senior Research Associate /
Plant Biotechnology



Hugh S. Mason, Ph.D.
Associate Research Scientist /
Plant Molecular Biology

CAN VACCINES BE PRODUCED AND DELIVERED IN FOOD?

The ability to produce vaccines in plants could have important implications for human and animal health care, particularly in developing countries. Hugh Mason's lab at BTI is studying "bio-pharming" as a new way to produce pharmaceuticals, including vaccines, in crop plants.

Mason has already produced vaccines for three human pathogens – hepatitis B, *E. coli* enterotoxin (traveler's diarrhea) and Norwalk virus (diarrhea and vomiting) – in genetically modified potatoes. The potatoes, which contain only certain portions of the pathogen in their tissues, stimulated an immune response in humans and animals during clinical trials. Because these are "subunit" vaccines (vaccines that contain only certain proteins from the pathogen), they are safer and less likely to cause disease than live or killed conventional, whole-cell vaccines.

In 2001, Mason produced modified tomato lines that contained vaccines against each of the three diseases. He freeze-dried and powdered the fruit from each line, and then rehydrated the tomato powder and fed it to mice to determine whether the fruit would stimulate immunity after it was freeze-dried and processed. Preliminary results indicate that processing did not interfere with *E. coli* enterotoxin or Norwalk virus immunity.

Currently, Mason is collaborating with Dow Agrosciences to develop animal vaccines that could be delivered through feed. Future work will focus on the development of more potent antigens and the use of other food plants, such as bananas, for the production and delivery of protein vaccines.

For delivery to humans, these innovative vaccines could be extracted from the plants and converted into pills, delivered by injection, or fed to people in food form. Whatever delivery method is ultimately chosen, plant-produced protein vaccines will be administered by health professionals.



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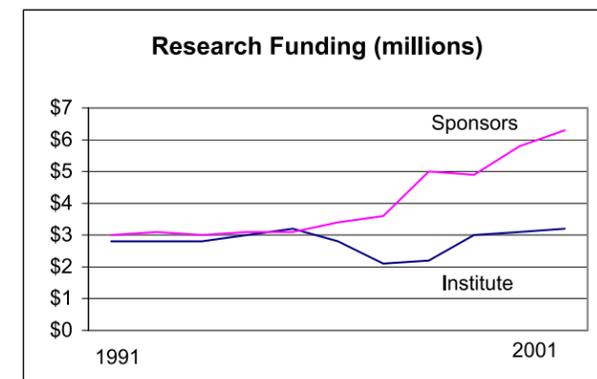
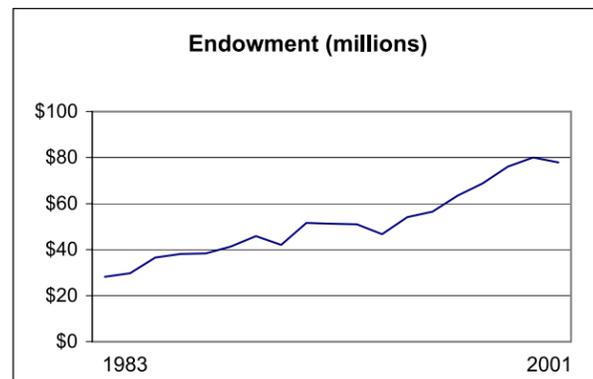
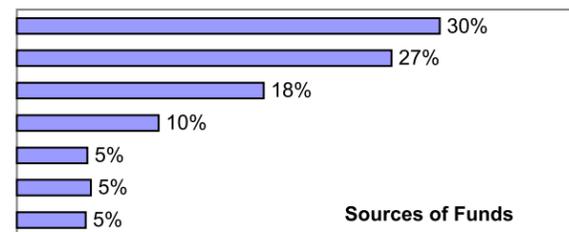
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