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## American Chestnut Restoration Through Biotechnology

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Our overall goal is to establish a system for transformation and regeneration of American chestnut trees engineered with anti-fungal candidate genes for chestnut blight resistance. We have been working on establishing somatic embryogenesis technology for American chestnut mass propagation and gene transfer purposes for over 17 years, and have found it to be a very challenging system. However, during the past few years, with support from ArborGen LLC, the Institute of Forest Biotechnology and the Consortium for Plant Biotechnology Research, we have made substantial progress towards our goal. We initiate new embryogenic American chestnut cultures each summer, using immature nuts supplied by cooperators at The American Chestnut Foundation (TACF) and The American Chestnut Cooperators Foundation, which has allowed us to build a substantial collection of American chestnut germplasm. Copies of these embryogenic cultures are cryostored so that we can draw from this collection for years to test them for their potential for transformation and somatic embryo/somatic seedling production.

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### New Website Launched

We are pleased to announce the the Institute's updated website at [forestbiotech.org](http://forestbiotech.org). The site was completely redesigned with usability in mind. Following the IFB's new focus on science, dialogue and stewardship, the site has new tools and updated information including an online calendar, news, regulatory resources, and other tools.

We will be making additions to the site over time so please take a minute to check out the website and let us know what you think.

## Pine Genome Initiative

The PGI is a scientific plan, supported by a broad coalition of scientists, universities, foresters, land owners, and industry, to use the revolutionary tools of genomics to increase our knowledge of the molecular processes that control economic and ecological traits in pine and other coniferous trees. The PGI will be implemented through a competitive grants program administered by federal agencies with responsibilities and expertise in tree genomics research.

The PGI Implementation Committee co-chaired by Adam Costanza, IFB and Randy Johnson, US Forest Service has had several successes this year. Meetings with House members raising awareness of the PGI resulted in report language specific to PGI in the House Farm Bill. Similar efforts were undertaken in the Senate and a letter supporting PGI was submitted to the Chairs of the Senate Agriculture Committee from Senator Gordon Smith of Oregon. Thank you to Dr. Hal Salwasser, PGI Implementation Committee member from Oregon State University, for spearheading the request for the letter. At time of press, negotiations between the House and Senate on a final Farm Bill were still ongoing.

The American Forest & Paper Association is contributing time and resources to this initiative. On December 6 AF&PA sponsored a meeting for agency personnel in Washington, DC. This meeting focused on strategy for the next two years. The Implementation Committee updated members of industry and U.S. agencies on progress and anticipated outcomes, and received input from the agencies. Thank you to Nadine Block and Al Goetzl at AF&PA who lead this endeavor.

Drs. Ron Sederoff, NC State University and Jeff Dean, University of Georgia gave presentations that were recorded and available on the IFB's website at: [www.publications.forestbiotech.org](http://www.publications.forestbiotech.org).

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## American Chestnut Restoration

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However, until recently, all of the chestnut germplasm we have propagated has been derived from open-pollinated seeds. During 2006, for the first time, we initiated cultures from several controlled pollinations made by TACF cooperators between long-term surviving American chestnut parents, including some Georgia trees, so that we now have embryogenic cultures of known parentage to use for our propagation and transformation research.

Several years of hard work by students and technical personnel in the lab have led to enhancements in our ability to regenerate somatic seedlings from our

embryogenic cultures. Recent breakthroughs employing such factors as manipulation of suspension cultures, cold stratification, medium supplements and light quality during germination have allowed us to raise somatic seedling production efficiency as high as 60% for some

genotypes. Our first American chestnut somatic seedlings to be grown in the nursery last season made impressive growth, with some seedlings reaching almost 2 m in one season. Embryogenic suspension cultures have proven to be excellent target material for Agrobacterium-mediated transformation. Using these cultures, we have defined a repeatable transformation protocol that appears to work well with a range of

embryogenic American chestnut lines. Dozens of somatic seedlings transformed with marker genes, representing over 20 putative transgenic lines, have been produced and transferred to the greenhouse.

Recent transformation experiments have resulted in the production of multiple cultures transformed with anti-fungal candidate gene constructs supplied by collaborators at ArborGen and SUNY-ESF. Somatic embryo and somatic seedling production from these cultures is underway. With continued support and help from cooperators, we hope to establish field tests of American chestnut trees with candidate anti-fungal genes within the next few years. We hope our efforts will contribute to the restoration of this valuable heritage forest tree species.

## New Initiative: Forest Fuels

Biofuels are being touted as renewable, environmentally responsible, and overall “green,” but are they sustainable? This question is being asked by a number of environmental organizations. However, understanding the maximum, sustainable potential of forest fuels (those derived from tree cellulose) has not been investigated in relation to advances in forest biotechnology. Information about maximizing forest fuels through biotechnology must be made available to decision makers. The Institute of Forest Biotechnology plans to organize a Forest Fuels® Initiative to address this critical need of information. The IFB is uniquely able to achieve both of these goals as proven by its strong record of forest biotechnology stewardship based on science and driven by dialogue.

### Somatic Embryogenesis

Somatic Embryogenesis is the process of inducing plant reproduction in a controlled environment that allows scientists to select genes that support desirable characteristics and eliminate non-desirable ones. This process allows researchers to introduce genetic material to a plant. One beneficial use of this technique is in helping a species protect itself from invasive pests or diseases.

As discussed, Somatic Embryogenesis is part of a research program that Dr. Scott Merkle is using to help restore the American chestnut. Dr. Merkle has pioneered the use of somatic embryogenesis and gene transfer to lay the groundwork for engineering the tree with anti-fungal genes.

### Dr. Merkle Receives Barrington Moore Award

Dr. Scott Merkle, professor of forest biotechnology at the University of Georgia Warnell School of Forestry and Natural Resources, received the prestigious Barrington Moore Award by the Society of American Foresters. “This award recognizes outstanding achievement in biological research leading to the advancement of forestry.”

As reported in the University of Georgia news article ([www.uga.edu/news/artman/publish/071007Merkle\\_SAF\\_award.shtml](http://www.uga.edu/news/artman/publish/071007Merkle_SAF_award.shtml)) Merkle's research has concentrated on adapting somatic embryogenesis for propagating clones and genetically manipulating southern forest species.



## BIOWORK IX – Forest Biotechnology

The Biowork series is an interdisciplinary forum for discussion of biotechnology. This year's focus was forest biotechnology. The international event was held August 30 – 31, 2007 at the Universidade Federal de Viçosa, Brazil. Dr. Aluizio Borem, IFB Board Member and Professor at the Universidade Federal de Viçosa, organized and hosted the meeting. IFB was one of the meeting sponsors and held a reception for attendees on the first evening of the forum. Fellow IFB board members Drs. Al Lucier and Bob Kellison traveled to the meeting and Dr. Lucier was tasked with giving the summary remarks.

The forum was introduced with opening remarks from Professor Carlos S. Sedyama, Reitor da Universidade de Viçosa, Dr. Waler Colli, Presidente da CTNBio, Dr. Jairon Alcir do Nascimento, Secretário Executivo da CTNBio, and Adam Costanza, President, Institute of Forest Biotechnology.

The two-day forum included talks from 15 international speakers on topics covering plantation forestry, wood quality, propagation, cloning, disease resistance, forest genomics, proteomics and transgenics, legal aspects and competitiveness and was attended by 200 faculty, students and industry representatives. The meeting was presented in Portuguese and the talks were videotaped. Both the powerpoint and video recording are available on the IFB website at [www.publications.forestbiotech.org](http://www.publications.forestbiotech.org).

## Genetically Engineered Forest Trees

Following on the recommendations from the "Growing Trees and Stemming Risks" conference held March 2006 in Vancouver, Canada, the IFB organized a second workshop focused on ecological risk questions.

A workshop identifying priorities for ecological risk assessment was held May 3 – 4, 2007 in Raleigh, NC. The workshop was convened by the IFB and sponsored by The Pew Initiative on Food and Biotechnology, Weyerhaeuser Company Foundation and the Animal and Plant Health Inspection Service of the US Department of Agriculture.

The purpose of the workshop was to establish research priorities with a clear scope and broad-based buy-in to address the most urgent ecological risk questions for genetically engineered trees. Parameters to accomplish this included: what baseline information exists for assessing the environmental impact of genetically engineered trees; what comparators are appropriate; what constitutes a negative or positive effect; and what is an appropriate research timeline. This meeting helped to:

- ❖ Define the terms and concepts for determining ecological risk.
- ❖ Identify the baseline context and experimental comparators appropriate for genetically engineered forest trees.
- ❖ Identify ecological risk issues unique to genetically engineered forest trees.
- ❖ Identify the most important scientific knowledge gaps for ecological research that are relevant and informative to the U.S. policy framework.
- ❖ Identify research priorities with a clear scope and broad based support for the approach.
- ❖ Outline a funding strategy to support cross-disciplinary research on priority issues of ecological risk assessment of genetically engineered forest trees.

A comprehensive summary of the workshop is being finalized and will be available on the IFB website. The papers from the previous conference, "Growing Trees and Stemming Risks", were published in the journal *Tree Genetics and Genomes*, Volume 3, Number 2.

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## Biorefineries Seminar

Sponsored by Resources for the Future (RFF) and the IFB, this symposium was held April 4, 2007 at the RFF offices in Washington, DC. IFB Board Member and RFF Fellow Dr. Roger Sedjo moderated the panel and discussion. The panel addressed the potential of cellulosic biomass for producing energy and transport fuels. Pulp mills, which currently use large volumes of wood energy, could be modified to include biorefineries. Utilizing new gasification systems, these facilities could convert wood and other cellulosic material into energy and biofuels, including ethanol and biocrude. These possibilities were examined, as well as the comparative potential of various feedstocks, including rapidly growing trees and agricultural cellulose as feedstock.

Presentation topics included:

- ❖ **Biorefineries Integrated with Pulp Mills**  
Eric D. Larson, Research Engineer, Princeton Environmental Institute, Princeton University
- ❖ **Improved Trees for Biomass Energy**  
Barbara Wells, President and CEO, ArborGen, LLC
- ❖ **Biomass Feedstock for Energy**  
Stephen S. Kelley, Professor and Department Head Wood and Paper Science, NC State University

Discussants:

- ❖ Theodore H. Wegner, Assistant Director, Forest Products Laboratory, USDA Forest Service
- ❖ John C. Houghton, Office of Biological and Environmental Research, US Department of Energy

The symposium is available on the RFF website at [www.rff.org/rff/events/biomassenergy.cfm](http://www.rff.org/rff/events/biomassenergy.cfm).



# STEWARDSHIP

## Responsible Use Initiative

The Institute of Forest Biotechnology (IFB) has launched an initiative to develop guidelines for the Responsible Use™ of biotechnology in trees. Biotechnology is a powerful tool being used to grow trees with special characteristics. When used responsibly, society and the environment can benefit from advanced tree breeding technologies, such as genetic engineering, to protect threatened species, remove contaminants from soil, and grow more on less land.

Genetically engineered trees will be grown for fiber production in two to five years. However, there are currently no long-term guidelines for the stewardship of these trees. There needs to be a mechanism to determine which uses of this technology will bring the most benefit, and which might cause harm. Without Responsible Use Guidelines™, long-term management of these trees may never be addressed. Through science, dialogue, and stewardship, we can enhance the benefits of these trees while minimizing any risks.

To learn more about the Responsible Use initiative that the IFB just launched in January, go to our website dedicated to providing transparent information about the entire guideline development process: [www.responsibleuse.org](http://www.responsibleuse.org) Contact Adam or Susan for ways to sponsor this initiative, or for opportunities to get involved and help develop the guidelines.

## Protecting the Future of our Forests

This initiative will determine how forest biotechnology can be used responsibly to benefit society and the environment.

- ✓ Ecosystem, forest sustainability, and public concerns will be addressed
- ✓ The guidelines will be developed in a transparent mechanism, open for public comment
- ✓ Governments around the world can evolve regulations to incorporate guideline elements if they wish
- ✓ Business risks are lowered by following only responsible uses of forest biotechnology
- ✓ Guidelines will be robust and verifiable, yet small in number
- ✗ The guidelines will NOT increase transaction costs along forest products supply chain
- ✗ They are NOT a certification scheme
- ✗ The guidelines can NOT be used in place of sustainable forestry practices

## Responsible Use Guidelines

### Complementing Regulations

These guidelines are not intended to replace government regulations. Instead, they will provide a consistent level of assurance that Responsible Use guidelines have been met, regardless of where genetically engineered trees are used.

If a country's regulations incorporate all of the Responsible Use elements, then the guidelines have been met. If only some elements are covered, then additional steps must be taken to comply with these guidelines.



### For example:

#### Country X has extensive regulations:



3 guideline elements need to be addressed

#### Country Y has less comprehensive regulations:



7 guideline elements need to be addressed

## Species Protection through Disease Resistance:

### Moving from the Lab to the Field

IFB held its first Partners meeting November 28, 2007. This meeting focused on opportunities to protect threatened tree species and strategies to expand field testing; it was only open to IFB's Partners. For more information about the benefits of being a Partner, please visit our new website ([www.forestbiotech.org/partners.htm](http://www.forestbiotech.org/partners.htm))

A number of human and environmental factors are threatening tree species around the world. There is tremendous potential for appropriately used biotechnology to assist in the protection of these species. This meeting will:

- ❖ Update stakeholders on the status of American chestnut and English Elm research efforts.
- ❖ Expand the breadth of the Species Protection initiative to include additional species or research.
- ❖ Explore species protection via genetic engineering for climate adaptation and insect resistance.
- ❖ Meeting sponsors include the North Carolina Biotechnology Center, ArborGen, LLC, and CellFor.

In addition to IFB's day-long meeting, The Partnership for Saving Threatened Forests held a meeting the following day, on November 29th, in Raleigh, NC. Information about this meeting is available online at [www.threatenedforests.com](http://www.threatenedforests.com).

## Partner Information

Many thanks to our Forest Biotechnology Partners. The Partnership has been significantly overhauled in the past year. Today, it is a structured network and management mechanism that spans organizational boundaries. Indeed, we have representation from sectors including government, non-profit, academia, and industry. We are working diligently to bring the benefits our partners want and the international perspective that this field of science needs. The Partnership works directly with the IFB to develop initiatives that accelerate the responsible use of forest biotechnologies to benefit society and the environment. Organizations formally linked through the partnership have direct access to the world's largest forest biotechnology network of experts.

The current Forest Biotechnology Partners are in the list on the right. Those with an asterisk [\*] have contributed sustaining resources to the Institute of Forest Biotechnology in excess of the nominal partnership requirements.

In addition to the networking and collaboration opportunities, Forest Biotechnology Partners operate in a strategic planning role to the IFB. Partner meetings are held annually to highlight initiatives, raise new areas of interest, and address any questions about the Institute in general. For additional information about partnership requirements and benefits, please visit our Partner's webpage at: [www.partners.forestbiotech.org](http://www.partners.forestbiotech.org) and contact Adam Costanza or Susan McCord.

## FOREST BIOTECHNOLOGY PARTNERS

- ❖ U.S. Forest Service\*
- ❖ MeadWestvaco\*
- ❖ Weyerhaeuser Company Foundation\*
- ❖ International Paper Company\*
- ❖ North Carolina State University\*
- ❖ North Carolina Biotechnology Center\* (Meeting Sponsor)
- ❖ ArborGen LLC
- ❖ Clemson University
- ❖ Oregon State University
- ❖ Purdue University (Newest Partner – Welcome!)
- ❖ CellFor Inc
- ❖ Michigan Technological University
- ❖ Penn State University
- ❖ University of Washington
- ❖ State University of New York College of Environmental Science and Forestry
- ❖ Virginia Tech University
- ❖ Universidade Federal de Vicosa (Brazil) (Featured Meeting)
- ❖ Warnell School of Forestry and Natural Resources at the University of Georgia (Featured Article)

The Institute of Forest Biotechnology is a non-profit organization whose programs are directed to the ecological, economic and societal benefits of forest biotechnology. It works with a diverse group of stakeholders to foster science, dialogue, and stewardship in this burgeoning field.