THE BUR

Newsletter of the New York State Chapter of the American Chestnut Foundation, Inc.

Special Edition Summer 2006

PRESIDENT'S MESSAGE

Herbert F. Darling, Jr., President

WE DO NOT HAVE A RESISTANT SEEDLING AMERICAN CHESTNUT YET!! BUT…WE DO HAVE the first two transgenic American Chestnut seedlings planted outdoors in a secure environment with regulatory permits in place. We have not been able to determine what level of resistance has been produced as they are too small to test yet, 8" +/- tall, and we need many more seedlings to prove results. Also, there are other gene constructs to try to improve the resistance if it turns out we are not satisfied with the first results. More good news is there are 100 more; about 1" tall developing roots. Some of these can hopefully be planted out doors this fall.

As president, I want to congratulate everyone involved in reaching this progress milestone. It took 16 years to get to this point and hopefully will take two years or less to prove resistance. Our goal is in 5 years to have enough seedlings resistant enough to possibly have each TACFNY member receive three seedlings if they want them. The possible hold up on this could be getting release permits for transgenic seedlings. We are working with all the regulatory agencies to make this happen in a safe and timely fashion.

Yes, there are still some hurdles to jump, some of which are bigger than others. I am confident we can have the restoration process all planned and ready to impliment ahead of the mass production of American Chestnut seedling phase.

Nice going! We can ALL be proud of our progress!

Long live the American Chestnut.

SCIENCE REPORT

On June 7th, 2006, representatives of the New York State Chapter, American Chestnut Foundation met at the College of Environmental Science and Forestry in Syracuse, NY to plant the first two transgenic American chestnut trees in the field. Before the planting, Ms. Linda McGuigan, Plant Tissue Culture Lab Manager, gave a tour of the lab where the transgenic plantlets were nurtured from single cells into whole plants. The lab is currently home to more than 2,000 somatic embryos and shoots in various stages of development. Dr. William Powell explained how some of the trees would be planted in the field while others would be tested for blight resistance.

The tour then moved to a controlled-environment room in the basement of the College greenhouse. More than 100 rooted chestnut plantlets were in various stages of the acclimatization process. Upstairs in the greenhouse, everyone got a look at the first fully acclimatized transgenic American chestnut. Linda popped the tree out of its pot to show the developing root system.

At the field-testing site, Mr. Richard Schwab, Director of Forest Properties, described the geological history of the site and how it came to be one of the College properties. Drs. Charles Maynard and William Powell, Co-Directors of the American Chestnut Research and Restoration Project, thanked everyone involved with the project. Dr. Powell spoke about the next steps on the road to a restored American chestnut.

Mr. Herbert Darling read an eloquent letter from Stan and Arlene Wirsig. Herb then unveiled a dedication plaque which read: "American chestnut (*Castanea dentata*) Variety: 'Wirsig'. Named in honor of Stan and Arlene Wirsig for their unfailing dedication to the restoration of the American chestnut." After much applause, the group got around to planting the trees.

Figure 1. The Planting Crew (L to R) Front row: Jim Donowick, Josie and John Ellis, Linda McGuigan, Jack Mansfield, Joyce Fry, John Gordon; Back row: Bill Snyder, Roy Hopke Chuck Maynard, Dick Schwab, Bill Powell, Herb Darling.

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Linda McGuigan discusses the acclimatization process in the growth chamber.

2006 Annual Meeting
October 28-29, 2006
Grand Hotel
Poughkeepsie, NY

***Reservations: made up to 30 days prior to October 28 will be from the rooms reserved for our annual meeting at the rate of $119 per room per night. This includes a full American breakfast.**
Telephone:
800-216-1034
Website:
www.pokgrand.com

*Local Activity: Tours of two of Poughkeepsie National Historic Sites have been arranged for the afternoon of Saturday the 28th as follows:

1. FDR Home and Library $8.00 per person
2. Vanderbilt Mansion $5.00 per person

Please notify us by September 28 which tour you would like to attend or if you would like to attend both tours. This will ensure a group rate and a more personalized tour for all of us. Include your fee for the tours with your registration fee.

*** Look for your registration form to be sent in a separate mailing.**

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TWO FIELD-READY TRANSGENIC AMERICAN CHESTNUT TREES PRODUCED AT ESF.

By Linda McGuigan, Tissue Culture Specialist

The two small trees we planted on June 7, 2006 started out as part of an experiment performed on April 12, 2004. During the experiment, tiny green dots were seen on clumps of chestnut somatic embryos growing in a Petri dish. The reason some cells fluoresced green while the surrounding cells were creamy white was because the green ones had picked up a small set of new genes which included a green fluorescent protein (*gfp*) gene and an oxalate oxidase (*OxO*) gene. The *OxO* gene is designed to increase the resistance of chestnut tree to the blight and is described by Bill Powell elsewhere in this special issue of *The Bur*. The *gfp* gene has nothing to do with blight resistance, however it marks the cells that have incorporated the new genes and makes them easier to identify.

For months we watched the tiny green dots grow, first into individual embryos and then into many Petri dishes full of transgenic embryos. When it was clear that a particular little green dot (and all of the little green embryos derived from it) were going to continue to both fluoresce and produce new embryos, it was assigned a unique transformation event number. The trees planted in the field were first designated as transformation event WB275-27-LP28 on November 4, 2004.

After transforming the somatic embryos, they need to be grown into whole plants. The three major stages to do this are regeneration, rooting and acclimatization.

The first step, regeneration of embryos into shoots, involves culturing the embryos on a series of three different media. These media contain different concentrations of sugars, hormones and minerals. While progressing through the series of media, the embryos begin to develop cotyledons and start to actually look like the tiny embryos that are found in naturally formed seeds. These developing embryos are transferred to a second medium where they continue to grow and mature. Finally they are transferred to a third medium where some of them develop shoots. This shoot regeneration phase is a bottleneck because for every 25 embryos that develop cotyledons, usually only one or two continue to develop into shoots. Fortunately we know how to multiply the shoots.

The next step is to grow roots on the shoots. To do this, the tips of the shoots are removed to help encourage axillary shoot growth. When these new shoots are approximately 1/16 to 1/8 of an inch long, they are transferred to a pre-rooting medium that contains a low concentration of cytokinin (a hormone that helps shoots multiply but can inhibit root formation). When the shoots are approximately one inch to two inches tall, a small slit (1/16 of an inch long) is cut up the bottom end and the shoots are dipped in an auxin (a hormone that promotes rooting) for 60 seconds. The shoots are transferred to a medium that contains minerals, sugars and finely ground charcoal but no plant hormones. The charcoal is used to remove any excess auxin. After 10 to 12 days in the charcoal medium, the shoots are transferred to a post-rooting medium until roots develop. On average, around 20 to 50 percent of the shoots that start the process will eventually form roots.

The final step is acclimatization. Because the plants have been growing for many months or even years in an environment where their leaves are surrounded by air at almost 100 percent relative humidity and also where they receive all their nutrients (including sugar) all the time, they need to adjust very gradually before they can be planted outdoors. To do this, the plants are put in 4-inch pots that contain a 2:1:1 peat moss, vermiculite and perlite potting mix. A clear plastic lid is placed over them to keep in moisture and they are fertilized at least once a month. They are put in a growth room that is temperature and humidity controlled. The daytime temperature is 68˚F and the nighttime temperature is 61˚F. The relative humidity is set at 65% for both day and night. After a week of adjusting to these new conditions, the lid is lifted slightly (approximately 1/4 inch) for one to two weeks. The plant must be constantly monitored for wilting. If any wilting occurs, the lid is lowered for a few days at which time the process is tried again. Once the plant has adjusted, the lid is completely removed and once again the plant is monitored for wilting. After one to two weeks without the lid, the plant is placed in a shaded greenhouse with higher light levels and lower humidity. Once the plants have adjusted to the greenhouse, they are ready to be planted outside.

The whole process, from first identification of tiny transgenic cells to fully acclimatized small chestnut trees requires an average of 14 to 20 months. The two chestnuts that were planted on June 7, 2004 required 26 months because of the learning curve involved in developing the process.

Figure 2. Linda McGuigan shows Herb Darling the root system of a transgenic American chestnut tree.

THANK YOU TO ALL CONTRIBUTORS

By **Chuck Maynard, Professor**

June 7th, 2006 meant a lot to me personally. Ever since I was a graduate student and plant genetic engineering was the latest and greatest thing on the horizon, I have had the dream of applying it to a forest tree species. This planting represents a large step along the path to fulfilling that dream.

Elsewhere in this special issue of *The Bur*, Linda McGuigan describes how she produced the transgenic trees, and Bill Powell describes the genes they contain and the milestones ahead, so I will devote my "column inches" to thanking the people that helped make this event possible.

First, we would like to thank the organizations that, over an 18-year period, have collectively invested more than 1.2 million dollars in the Chestnut Project:

The NY State Chapter, American Chestnut Foundation
ArborGen LLC
Monsanto Fund
USDA National Research Initiative
USDA McIntire-Stennis Research Program
The Wild Turkey Federation
The National Arborist Association
NY State Legislative Appropriation
NY State Science & Technology Foundation
NY Center for Forestry Research and Development
SUNY-ESF

Next our thanks to the many people that have worked so tirelessly in the laboratory (in approximately chronological order):

Mike Satchwell – Worked on a chestnut rooting procedure.

Janna Beckerman - Screened natural gene products to determine if any could be used to control chestnut blight.

Heather Engelman – Developed a prototype chestnut rooting procedure.

Cathy Catranis – Tested *in vitro* the first synthetic antimicrobial peptides for use against chestnut blight and constructed one vector to test these in transgenic poplar.

Zizhuo Xing – Suggested that we use somatic embryos, established several cell lines in culture, and produced our first small field-test of tissue culture plantlets.

Hongyu Gao – Made a self-processing, two-gene construct for testing in chestnut.

Sharon Bickel LaPierre – Developed repeatable rooting & acclimatization procedures and produced enough acclimatized plants to start significant field tests.

Ryan MacFee – Examined the genetic diversity in surviving American chestnut trees in New York State.

Rosy Mukherjee – Produced the first sizable batch of transformation events (exciting, but they turned to callus).

Haiying Liang – Transformed model species, poplar and Arabidopsis, and tested several gene constructs *in vivo*. Constructed the two new vectors, including OxO gene construct that these chestnut plantlets carry.

Bernadette Connors – Isolated three gene promoters from American chestnut, one that we used in producing transgenic elms.

Gisella Stallock – Tested a seed transformation procedure.

Jason Corwin – Compared gene expression among American, hybrid, and Chinese chestnut.

Linda Polin McGuigan – Put all the pieces together and produced the first fully acclimatized transgenic American chestnut plantlets.

Ron Rothrock – Devised an alternative transformation system called plate flooding.

Andy Newhouse – Performed DNA hybridizations to prove that we really have produced transgenic chestnut trees.

Special thanks to the undergraduate lab assistants who have taken on the invisible jobs like washing mountains of glassware, making bathtub quantities of media, doing endless transfers of tiny chestnut shoots into fresh media, and many other tedious but vital tasks.

We have had help from other scientists (in alphabetical order):

Dr. Danny Fernando, an expert in pine pollen physiology, and his graduate student **Javonna Richards** – Developed techniques for transforming individual chestnut pollen grains.

Dr. Joyce Fry, consulting tissue culture specialist – Provided the key to transforming chestnut: gentle desiccation.

Dr. Maud Hinchee, Dr. **Dayton Wild, Dawn Parks** and other ArborGen scientists – For providing a dozen somatic cell lines, sharing their expertise in woody plant transformation, & whole-plant regeneration, and research planning skills.

Dr. Fred Hebbard, a plant pathologist and plant breeder from the National ACF in Virginia – Collected the burs that provided some of the embryo lines that Linda transformed.

Dr. Tom Horton, an expert on mycorrhizal root systems, and his graduate student **Kris Dolmer** – Determined that a wealth of suitable mycorrhizal fungi are still out there waiting to colonize the roots of our tiny chestnut trees.

Dr. Scott Merkle, a tissue culture scientist from the University of Georgia and his research coordinator **Gisele Andrade** – Generously shared their expertise on chestnut tissue culture and somatic embryogenesis.

Dr. Joe Nairn, a molecular biologist from the University of Georgia – Supplied two promoters and a new binary vector for our chestnut trees.

Jun Wang, a visiting scholar from China – Developed a chestnut callus assay to test resistance enhancing potential of transgenes.

Others who made many significant contributions (in alphabetical order):

Herb Darling, President, TACFNY – For his endless enthusiasm and amazing organizational skills.

John Dougherty, Consulting project manager – Keeps us striving for our next milestone.

John Ellis, James Donawick, and all the other "bur collectors" – For supplying us with the raw materials we needed, often on short notice.

Craig Hibben, Roy Hopke, and all the other seed orchard managers – For maintaining and expanding the American chestnut gene pool.

Lucy Popkess, former ESF Assistant to the President – Guided us in seeking State funds.

Senator Mary Lou Rath – Convinced the State Legislature to form the American Chestnut Research and Restoration Center and provided four years of State Funding.

Dr. William Tully, former Provost, and **Dr. Edwin White**, former Dean of Research – Provided a year of funding after our State appropriation ended.

Arlene Wirsig, former Secretary Treasurer for TACFNY – Always kept us on track and on budget—or really wanted to know why we weren't!

Stan Wirsig, Vice President and Science Director for TACFNY – He is always planning how to produce the first BILLION chestnut trees.

And finally we thank all the members of the New York State Chapter of The American Chestnut Foundation for their continued support and encouragement.

We are on a long and exciting journey, and as you can see from the list above, some very dedicated and gifted people are helping to make it possible. Thank you one and all.

WHERE DO WE GO FROM HERE?

By William A. Powell, Professor

We have passed a very significant milestone by planting the first transgenic American chestnut trees, with a putative blight resistance enhancing transgene, in the field. This is the culmination of many years of hard work and support by many people. Although this is a great achievement, we aren't finished yet. There is still much work ahead. As I stated, these first trees have a putative blight resistance enhancing transgene. This transgene produces a wheat enzyme called oxalate oxidase. This enzyme will detoxify the oxalic acid produced by the blight fungus when it attacks the tree. We still use the word "putative" because it is predicted to enhance resistance in chestnut based on our past experience with this transgene in poplar trees and from other researchers' reports of using this transgene in soybean, peanut, and sunflower. But blight resistance still needs to be confirmed in the actual plants. Therefore the next batch of transgenic plants will be used for greenhouse testing. Once the stems on these small trees reach 3mm in diameter or larger, we will begin the testing using a small-stem resistance assay developed in our lab and demonstrated at the last year's annual meeting.

The small-stem resistance assays will need to be preformed on several different "transgenic events" of the chestnut trees. A "transgenic event" is a clonal line of trees in which the transgene is located at the same position in the chromosomes. Therefore, different transgenic events represent the transgene in different locations in the chromosomes. Why is this important? It is because not all locations are equal. Just like a given house located in different areas of the country can have dramatically different values, our transgene located in different areas of the chromosomes could give different levels of blight resistance. Therefore, we need to test several events and choose the one that gives the highest level of resistance. Currently we have approximately 42 transformation events in various stages of regeneration. If we do not find an event with levels of blight resistance high enough for the restoration program, we will then need to add additional transgenes. But we believe that the oxalate oxidase transgene will likely provide the level needed.

Once we identify a transgenic event that provides strong blight resistance, what is next? The next stage is to increase production of the chestnut trees. We will need hundreds of trees to go through the field trials necessary to gather the information needed by the USDA, EPA, and FDA so that the trees can be released to the public. During this interim period, all of the transgenic trees will be "regulated" and can only be planted with field permits from the USDA. Initially, trees will be planted on SUNY-ESF properties but we hope to quickly include other planting sites in these field trials. Cooperators from TACFNY will need to follow all the USDA-APHIS-BRS regulations when helping with these field trails. Some items that need to be tested are growth under different conditions, the stability of blight resistance across locations and over time, pollination and nut production, and interactions with non-target organisms such as mychorrizal fungi and selected insects.

With the planting of the first transgenic American chestnuts in the field, we are beginning a new phase in the restoration of this extremely valuable tree species. This new phase will not replace the ongoing lab work, but instead will add on an important field component to the research. The researchers at SUNY-ESF thank you for all your support that allowed us to get to this milestone and we look forward to your continued help in the future.

Bill Powell discusses the future of the American Chestnut Research and Restoration Project while John Gordon patiently waits for the planting.

Herb Darling reads a congratulatory letter from Stan and Arlene Wirsig.

The first transgenic and putatively blight-resistant American chestnut trees go in the ground.

The BUR
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Newsletter of the New York State Chapter of the American Chestnut Foundation, Inc.

IF YOU HAVE FRIENDS WHO ARE INTERESTED IN OUR GOAL OF RESTORING THE AMERICAN CHESTNUT, PLEASE GIVE THEM THIS APPLICATION.

Membership Application

Enclosed is my membership support of:

- ☐ Gold leaf, $1000
- ☐ Silver leaf, $500
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- ☐ Student, $15
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- ☐ Special Gift to NY State Chapter $ _____

Total Amount $ _____

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Membership includes subscriptions to The Bark and Journal of the American Chestnut Foundation and enrollment in the New York State Chapter. The Chapter publishes the BUR, helps guide research at CESF, and includes nine Districts for local involvement in maintaining the American chestnut gene pool. Please make check payable to The American Chestnut Foundation, PO Box 4044, Bennington, VT 05201-4044. TACF is a 501(c) (3) non-profit organization. Except for the member services portion of your contribution (valued at $15), your gift is tax deductible to the full extent allowed by law.

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