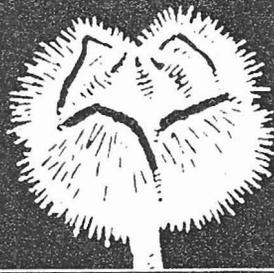


THE BUR



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

Volume 15, No. 2

Fall/Winter 2009

PRESIDENT'S MESSAGE

July 2, 2009

As we anxiously await the fall TACF-NY meeting and the much anticipated reports on blight resistance from Drs. Maynard & Powell of ESF Syracuse, we all need to keep our fingers crossed that the results will be very positive. These results will be the very first constructs that have been produced and work will continue to strengthen resistance levels if it becomes necessary on future constructs.

In the mean time TACF, national, has appointed a committee to produce a bio-tech strategy chaired by Dr. Kim Steiner and charged with having a first draft of the plan ready for the Fall TACF meeting in Pittsburgh, PA. This will be a major step forward towards getting the gene transfer program for trees accepted by the public.

The New York State Chapter and its partners ESF Syracuse, University of Georgia, IFB, Arborgen, NYS DEC, and Monsanto Fund have been working on this for eighteen years and are proud of our progress but the time has come to fully recognize bio-technology as a good route to insure the future of many other species of trees, as well as the American Chestnut tree. I hold great hopes it will happen quickly so other well-qualified organizations can get involved in the team for the future of the nations trees.

Long Live the American Chestnut,

Herbert F. Darling
President, TACF-NY

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NOMINATIONS

Any member may submit suggestions for nominations to the Board of Directors to be voted on at the next Annual Meeting. If you know of someone who might be interested in our mission, send his/her name by October 10 to the Nominating Committee Chairman, Jack Mansfield, 349 Roycroft Blvd. Buffalo NY 14226 or by e-mail to sonjack@Roadrunner .com

DIRECTORS, CLASS OF 2009 1 Year

Jim Donowick; John Dougherty; Adrien Gaudreau; Craig Hibben; Ted Kozowski; Roy Hopke; Enrico Nardone; Robert Nowack; Dr. John Potente

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DIRECTORS, CLASS OF 2010 3 Year

Douglas Campbell; Herbert Darling, Jr. John Gordon; Jack Mansfield Richard Radel; John Spagnoli Arlene Wirsig; Stanley Wirsig

NEW CLASS OF DIRECTOR, 2010

Dale Travis

Don't forget to register for the 19th Annual Meeting of the New York State Chapter of the American Chestnut Foundation

We will be meeting at the Adam's Mark Hotel, Buffalo 120 Church Street Buffalo, NY 14202

716-875-5100 American Chestnut Block *If you have already submitted your registration, mailed under separate cover no need to fill out this form.*

Vince's Chestnut Tree!

Let me introduce myself. My name is Vince Bedient. I live on the north fork of Keuka Lake. On the western slope of the lake lies our 220 acre farm mostly involved in the production of grapes.

I have always been interested in and involved in forestry. I am a 3rd generation woodsman who spent 25 years cutting timber and supplying my father's saw mill with logs.

After a severe accident, I decided to make a major career change. I bought this farm and started raising grapes.

I soon discovered that I now owned a fairly good sized American Chestnut Tree. It was a sucker that came up from a stump which was once a large, healthy American Chestnut which had contracted and eventually succumbed to the dreaded blight. The root system apparently lives to a degree enough to generate new growth.

This tree was a few inches in diameter in 1966. Now, forty + years later, it is almost 20" in diameter breast high. I have cleared an area around it. and fertilize it every spring. I am tempted to prune the lower limbs. but I have not done so because I fear that it may leave places for infection. I have placed bee hives near the tree to help with pollination, with limited success. I have harvested a few nuts each year over the last 10 years, so I am convinced that there is another tree within 'bee range.'

While logging a neighbor's woods around 25 years ago, I discovered another American Chestnut a half a mile away at this neighboring farm. Since that time, I would monitor the tree's health each year. I was disheartened to find that it became sick around the year 2000, and watched helplessly as it slowly died within 2 or 3 years thereafter.

I have planted chestnut seedlings for the past 10 years in the hopes of pollinating my mature tree, and I am just recently having some success. I have 2 smaller trees that are about 15 feet tall that have bloomed for the past 3 or 3 years. Because they both bloom later than the mature tree, I am of the opinion that there may be another mature tree within pollination range. Otherwise, it is possible that they alone are managing to set some blossoms on the bigger tree. Last fall, I collected as much as a cigar box full of mature, sound nuts from the original tree. which I sent to Herb Darling. He has stored them for planting this spring. Hopefully, we can gather more this coming year.

I would truly like to see a new beginning for this wonderful wood. Maybe with genetic engineering that is possible now and we can renew these magnificent trees.

I am 80 years old and can remember as a kid when my father would cut the dead but very sound trees that dotted the hillsides. He would truck them to PA to the coal mines and trade them for coal. They were used in underground coal mines to prop up the roofs of the mines. They were strong and very rot resistant, so they lasted a long time.

Anyone who would like to see my Chestnuts are welcome. Just give me a call or write.

Vincent Bedient
118 West Lake Road
Branchport, NY 14418

315-595-2593

Name(s):

Address(es):

Phone and e-mail address(es):

Friday Reception and Harvest Exchange in Olmstead Room at the Hotel. 6-9 p.m.

Saturday in Wright Room, coffee and tea. There will be a brief meeting and then leave for the White Seed Orchard and lunch at Herb's Camp.

Back to the hotel at 3 p.m. for research reports in the Wright Room till 5 p.m. then dinner with a Speaker and a Harmony Group for entertainment.

Prior to October 2, 2009 Fees

Registration
\$40 each. # ____ X \$40 = \$ ____ total

Zoar Valley Tour and Lunch
\$40 each. # ____ X \$40 = \$ ____ total

Dinner Reservations
\$35 each. # ____ X \$35 = \$ ____ total

Dinner Choices: Vegetarian, Chicken Wellington, Prime Rib of Beef, Baked Halibut w. Crabmeat

Student rate:
Registration, \$20, Zoar Valley Tour & Lunch \$30. Dinner Reservations \$20
Late fees: Add \$5 to each item.

Send to: Richard Radel, 23 Carriage Circle, Williamsville, NY 14221

It Takes a Team

By

Chuck Maynard and Bill Powell

It has been a busy summer! And a busy spring, and a busy winter before that. We currently have 16 people (Figure 1) working on some portion of the New York State American Chestnut Research and Restoration Project. This is by far the largest and most diverse team we have ever assembled. We are extremely proud of these folks and what they have accomplished.

Figure 1. The Chestnut Team, 2009. From left to right: Chuck Maynard, Amelia Zhang, Linda McGuigan, Tim Cumberbatch, Kathleen Baier, Brian Harvey, Katie D'Amico, Lilibeth Northern, Meagan Collins, Carrie Miller, Allison Oakes, Andy Newhouse, Bill Powell. Not pictured: Susana Serrazina, Tim Kuss, David Meyering



It's impossible to describe in a few words what everyone on this complicated team is doing, so we decided to give brief descriptions of what the different groups are doing and then let the individual team members speak for themselves about it.

Figure 2. Susana Serrazina and Chuck Maynard discussing the finer points of treeplanting.



The Gene Hunters

Good genes are hard to find! We might have a blight-resistant chestnut tree already planted in the field, or in the greenhouse, or still in the Petri dish, or traveling down the development pipeline from some other point. But we might not! For that reason we have a group of people dedicating their efforts to finding new potential blight resistance genes and feeding them into the pipeline.

A Visiting Scientist from Portugal

There are several laboratories around the world doing research similar to ours. We recently set up a cooperative arrangement with a laboratory in Portugal. They were interested in learning how we transform American chestnut so that they could try the technique on European chestnut and cork oak. We were interested in trying some of the genes that they had developed. The best way to accomplish our mutual goals was to send a representative from their lab to ours. Susana Serrazina (Figure 2) was with us for 12 weeks. Below is her brief description of her research.

Improving American and European Chestnut Tolerance to Fungal Pathogens

By Susana Serrazina^a, Maria Salomé Pais

The European chestnut, *Castanea sativa* Mill, covers a total area of 2.53 million hectares of which two million hectares are chestnut forests, where chestnut is the dominant tree species. The remaining 0.53 million hectares are chestnut orchards devoted to nut production (20.9% of the total chestnut-growing area). Chestnut nut production in Europe declined considerably during the twentieth century to the current 200,000 t (worth almost 300 million Euros – approximately 423 million U.S. dollars). This decline was due to both serious fungal diseases and changes in the structure of society. Towards the end of the twentieth century, there was a sharp

increase in chestnut demand, which triggered new plantings and the restoration of old orchards throughout Europe. Despite the plantings, ink disease (*Phytophthora* spp.) and canker blight (*Cryphonectria parasitica* (Murrill) M.E. Barr.) are still major threats. *Phytophthora cinnamomi* is a serious threat to chestnut across Europe and North America. Both diseases are thought to have been introduced, likely coming from Asia in the late 1800's. In the Iberian Peninsula, *P. cinnamomi* is perhaps the most pathogenic agent to *C. sativa*. In Portugal, it dramatically affects the production of chestnuts, which are of considerable economic importance to the country.

In order to better understand the plant defense mechanisms of *Castanea* spp. to *P. cinnamomi*, Serrazina¹ identified, using reverse transcription-PCR, putative defense genes (allene oxide cyclase (aoc), cystatin, β -1,3-glucanase and thaumatin-like protein) to enhance resistance to *P. cinnamomi*. The expression of these genes in tobacco plants dramatically decreased ink disease^{1,2}. The aoc gene will be tested first³. In collaboration between UBMBP-Lisbon Faculty of Sciences and Drs. William Powell and Charles Maynard at SUNY-ESF, transformation experiments with the aoc gene were initiated in June of 2009 in American chestnut. If successful, this gene will give us another path to improve both European and American chestnut's blight tolerance as well as resistance to other fungal pathogens.

"Plant Molecular Biology and Biotechnology Unit (UBMBP), Center for Biodiversity, Functional and Integrative Genomics (BioFIG), Edifício ICAT, Campus da Faculdade de Ciências de Lisboa, Campo Grande 1749-016 Lisboa, Portugal

References

¹Serrazina, SMT, 2004. Isolation and characterization of resistance genes to ink disease in *Castanea sativa* Mill. PhD dissertation in Molecular biology, Lisbon Faculty of Sciences. 301 Pp

²Serrazina, SMT, Fonseca, SCM, Balde, A, Pais, MSS. 2004. *Castanea sativa* Mill. gene codifying for allene oxide cyclase, cystatin, B-1.3-Glucanase and thaumatin-like protein and their use. Applicant CASTANIA SOCIEDADE AGROFLORESTAL, S.A., international patent application no. PCT/PT2004/000015

³Wasternack, C., Hause, B. 2002. Jasmonates and octadecanoids: signals in plant stress responses and development. *Prog. Nucl. Acid Res. Mol. Biol.*, 72, 165-221

In addition to our visiting scientist, a couple of our graduate students are also involved in searching for genes, putting them into vector constructs, and transforming American chestnut. Below are brief summaries from Kathleen Baier and Amelia Zhang.

A New Candidate Gene For Blight Resistance

By Kathleen Baier, Master's student

I have been studying genetic differences between blight-susceptible American chestnut and blight-resistant Chinese chestnut. In particular, I have been looking at differences in gene expression in canker margin tissue. I have been able to identify several genes that appear to be more highly expressed in the Chinese chestnut because these might be associated with resistance. For example, a gene for the enzyme, laccase, was very highly expressed in Chinese chestnut. Therefore, I decided to study it in more detail. Laccase is suspected to play a part in the process of lignification, the process that helps put up a barrier to canker growth, and has been implicated in pathogen defense in other plant species. We found that American chestnut does have the same gene and although it is not expressed in canker margins or stems, it is expressed in other tissues such as in the leaves or the buds. Recently, I have constructed new vectors (containing genes) for genetic modification of American chestnut embryos. In the first vector, the gene of interest is this laccase while a second vector has a pyramid construction, including genes for both oxalate oxidase (OxO) and laccase. I have begun working on another pyramid vector that will have three genes: laccase, OxO and ESF39.

Building New Vectors and Putting Them in Chestnut

By Amelia Bo Zhang, PhD. Student

During the past year, I have constructed a vector called p35S_OxO that has a 35S promoter (the genetic switch that turns on and off a gene) together with the oxalate oxidase (OxO) gene. This is a constitutive expressing promoter, which means we expect to see OxO activity throughout the transgenic American chestnut. This will be used as a control to compare with our VspB promoter. The VspB promoter only expresses in vascular tissue (mainly stems) and is a good candidate to put in transgenic American chestnut intended for release into the forests.

With these vectors made, together with the vector pGFP (which contains a visual marker gene), I have done many co-transformations to put these genes into American chestnut. So far, I have 10 confirmed events containing both pTACF3 and pGFP, seven confirmed events with pGFP only, seven confirmed events containing both pWVK147 (an empty vector control) and pGFP, and there are more than 30 putative events containing both p35S_OxO and pGFP which still need to be confirmed. When these events are regenerated into whole plants, we will have many more transgenic American chestnut lines to be tested both at the tissue culture stage and in the field.

It is my great pleasure to be involved with the American chestnut restoration program. I have met many wonderful people and made a lot of friends. I will continue working with American chestnut and I believe that with all our effort together, we will definitely defeat the chestnut blight and will give back American chestnut its pride this great tree deserves.

The Tissue Culturists

It's not enough to produce a single transgenic blight resistant tree. We have to have the ability to produce dozens, then hundreds, and then thousands of trees. That, in a nutshell, is the job of the tissue culture group. Thanks to the generosity of the New York State chapter of the American Chestnut Foundation, we have two wonderful new growth chambers. In addition to simply being wonderful for producing large numbers of plants, the fact that we have two chambers allows us to study different variables that might affect the

growth and survival of Chestnut plantlets undergoing acclimatization. Below is a brief report from our most senior lab personnel, Linda McGuigan.

Temperature Preferences of American Chestnut Plantlets

By Linda McGuigan, Lab Manager and Tissue Culture Specialist

I have been spending a great deal of time with the new growth chambers this year. Since we received them in December 2007, we've learned a lot about how to keep the tissue culture chestnuts alive and happy. Because of these new growth chambers, we have gone from approximately 10% survival to approximately 80% survival. This spring, some of you had the opportunity to plant some of the first trees that had been acclimatized in the chambers.

While the chambers work great, I wanted to know if there was anything that could be done to increase the growth rate of the tissue culture chestnut trees. I read a paper in which the authors acclimatized chestnut in a growth chamber with a daytime temperature of 77°F and a nighttime temperature of 68°F. Up until now, we had been using a 68°F daytime temperature and a 61°F nighttime temperature. I decided that since we had two chambers, I would compare our settings with that of the paper. The first batch of chestnuts went in on April 14, 2009 and I took measurements 12 weeks later. I measured the height of the trees, the number of leaves on each tree and the length of the longest leaf. The results: the chestnuts grown in the warmer chamber significantly outgrew those grown in the cooler chamber (Figure 3).

Figure 3. Determining the optimum temperature for acclimatizing chestnut plantlets. Chestnuts on the left grew at the warmer temperature and on the right at cooler temperatures.

The average height, number of leaves and longest leaf in the warmer chamber was 10.2 inches, 13 and 13.7 inches respectively compared to 3.8 inches, 9 and 9.8 inches for the cooler chamber. There are 6 more batches of chestnuts that still have to be measured but I am confident they will show similar results. By making additional adjustments to the system over the next year, I hope to further increase the growth and survival rate of American chestnut plantlets for the 2010-planting season.



Quality Control

To ensure that the trees we produce are as expected, there are several quality control experiments that need to be completed. This keeps us on track and prevents wasting research time on unusable trees. In addition to other duties, these tests are distributed among different researchers. Below are two brief summaries on these types of experiments from Andy Newhouse and Lilibeth Northern.

Checking whether the genes went in, and if so, how many copies?

By Andy Newhouse, Molecular Biologist and Chestnut Technician

One important consideration when producing any transgenic organism is determining how many copies of the transgene have been inserted. Multiple copies of a gene may increase expression levels: but it can also lead to confounding effects known as "gene silencing". Also, single copy transgenic events are preferable if the organism will be bred at some point, as the inheritance patterns will be much simpler and more predictable. The best way to test for copy number is through a procedure called a Southern blot, which has been my primary responsibility this year. In a Southern blot, all the DNA from a given organism is basically spread out on a nylon membrane, where it is securely bound. Then a faintly glowing probe is prepared, which has a DNA sequence complementary to our transgene. The probe sticks to the membrane wherever there is a complementary DNA sequence - in this case, wherever the transgene is found. If the probe sticks in one place, it means there's only one copy of the transgene, two places indicates two copies, and so on. I'm still in the process of testing all of our transgenic chestnut lines, but so far I have results from the two lines for which we have the most plants. The "Darling" (aka AN-2XG4) line has two copies of the transgene, and the AN-2XG5 line has a single copy. These results are encouraging, and will enable us to efficiently continue our quest for a blight-resistant chestnut.

Checking The Expression of the OxO Gene in Some American Chestnut Clones

By: Lilibeth Northern, Research Specialist

Restoration of the American chestnut via resistance to the blight is our team goal. Recently we have been using a technique called "co-transformation" with two simultaneous plasmids, one has GFP (a reporter gene) plus a second plasmid that contains the gene of interest (resistance against the chestnut blight). This technique has given us promising results. We have gotten a number of transgenic shoots that grow and multiply nicely. As with any transformation system, it is necessary to check the expression of the gene of interest at several steps before we get the plants rooted and ready to plant in the field. To monitor the expression of the OxO gene, we use an oxalate oxidase assay. We test the transformed embryos as soon as they grow shoots, as well as later, when they are big enough for being planted in pots. This assay is fast (it gives results in 1 week), easy to perform, and it helps us to confirm that we really have a transgenic chestnut with the putative resistance-enhancing gene being expressed as expected.

I have been involved in all of these steps, from growing untransformed embryos to planting the transgenic chestnuts in the field. One of my main contributions to the chestnut team is to validate that the transgenic chestnuts (with the OxO gene) are all checked with the oxalate oxidase assay. Most recently, I have also been involved with the regeneration of new transgenic plants that contain both the OxO gene and the ESF39 antimicrobial peptide gene. This "pyramid" gene construct is predicted to give higher and more durable resistance.

Summary

Above are just a sampling of the work by our team members and we will have many more exciting results to report at the annual meeting. Last year we reported low levels of resistance enhancement in the Wirsig American chestnuts. This summer we are testing two new events, the Darling American chestnut and AN-2XG5, which we will report on then. With the help of TACFNY members, we planted over 300 trees for environmental tests and for field resistance tests. We are now producing the next generation of trees for this fall's and next year's planting. This is an exciting time and the research is advancing quickly due to your support.

We don't usually BRAG, but it has been a great year!
By: Chuck Maynard, Bill Powell, and Linda McGuigan

We had been preparing for the 2009 Spring Planting for nearly 2 years. In January, 2008 we wrote a proposal: *Evaluating environmental impacts of transgenic American chestnut trees to chestnut trees produced by conventional breeding*, to fund a dramatically larger field trial than we had previously attempted with American chestnut. The USDA awarded us a ~\$380,000 Biotechnology Risk Assessment Grant (affectionately called a BRAG). This grant is shared with three other faculty researchers at SUNY-ESF, Dr. Don Leopold (Plant ecologist), Dr. Tom Horton (Mychorrizal specialist), and Dr. Dylan Parry (Entomologist). With adequate funding available, we were off and running to study the environmental impact of our transgenic trees compared to trees produced by conventional breeding. This is a key step in getting governmental approval to distribute these trees.

Last summer, we fenced one of the new areas to be planted (Figure 1). **Figure 1. The ESF Forest Properties crew putting the finishing touches on the gate for the new open-field planting area.**

Last winter, we applied to the USDA for field trial permits. We also worked with Dr. Chris Nowak, a silviculture professor at the College, to prepare two shelterwood sites so that we could see how the chestnut trees perform when planted in a partially shaded setting (Figure 2).

Figure 2 below. Preparing Shelterwood plots



Shelterwood plantings is a key step in returning the chestnut to the forest. The whole trial includes 4 sites (two wooded, two open fields) and 14 test entries (including American, Chinese, backcross and various hybrid chestnut seedlings, as well as 4 different transgenic events.)

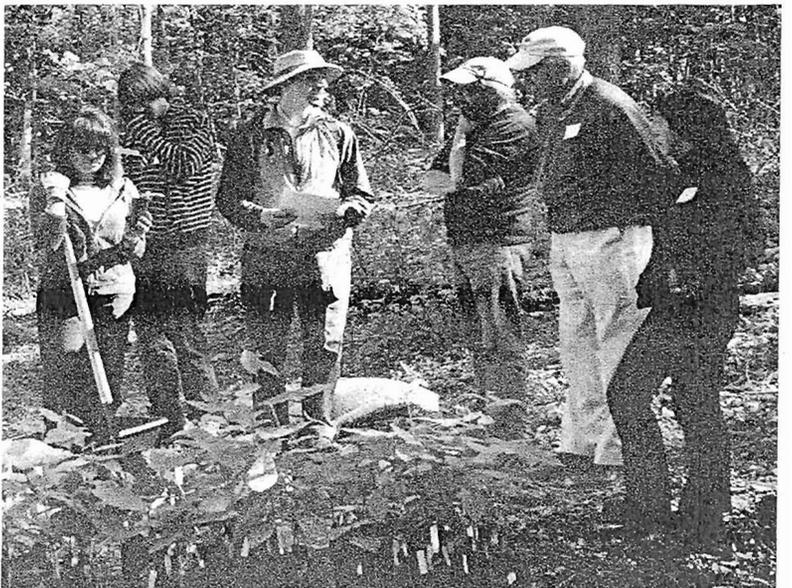
The biggest single task, however, was growing sufficient planting stock. This was made possible through the culmination of many years of research from regeneration of whole plants from embryos to the best size pots to use in the greenhouse. The high number of plants produced was made possible by the two new growth chambers generously donated by TACFNY. To do this task, we hired two people, Allison Oakes and Andy Newhouse, whose primary jobs were to propagate tissue culture plant material for the test. In addition, Allison was responsible for producing the American and Chinese chestnut seedling controls (Figure 3)



Figure 3. Allison Oakes thinning and inspecting chestnut seedlings and Andy was responsible for DNA fingerprinting of the transgenic plants. Andy also was a crew leader on planting day (Figure 4).

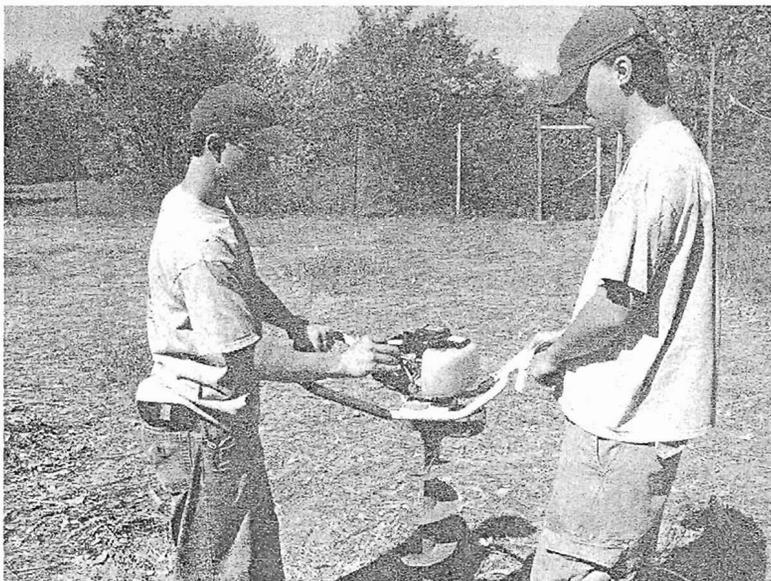
Figure 4. Andy Newhouse Explaining the planting directions to his eager crew. (L to R) Lilibeth Northern, David Meyering, Andy, Michael Satchwell, Herb Darling, Susana Sen-azinn.

Lilibeth Northern helped propagate as well as check many of the plants to see if the added genes were being properly expressed. Linda McGuigan helped propagate and coordinated the overall stock production activities.



Because we wanted the planting technique to be as uniform as possible among treatments and sites, we hired two undergraduate students, Tim Kuss and David Meyering, to do the final site preparation on all four plots (Figure 5).

Figure 5. Tim Kuss and David Meyering using a power auger to prepare planting holes



They have also been maintaining the plots all summer.

It was a huge effort, but on May 19, 2009 it all came together. With many volunteers from the College of Environmental Science and Forestry and from the New York chapter of The American Chestnut Foundation, planting day was a resounding success (Figure 6).

I am sorry that I will not be able to make our annual meeting this year because of a wedding. But I am still active with my endeavor to produce resistant nuts as soon as Syracuse can release a resistant tree. Some of the 60 trees in my orchard started to produce catkins this year, but no burs yet.

I have also started planting small orchards of about 10 trees in each. I planted three of them this year. This is with the intention of grafting all but one of the trees in each orchard with the same scion material so they will not pollinate each other. The remaining tree I plan to graft with blight resistant material as soon as it is available, thereby eliminating the need to do any bagging or hand pollinating to produce blight resistant nuts.

Allen and Fran Nichols

The BUR
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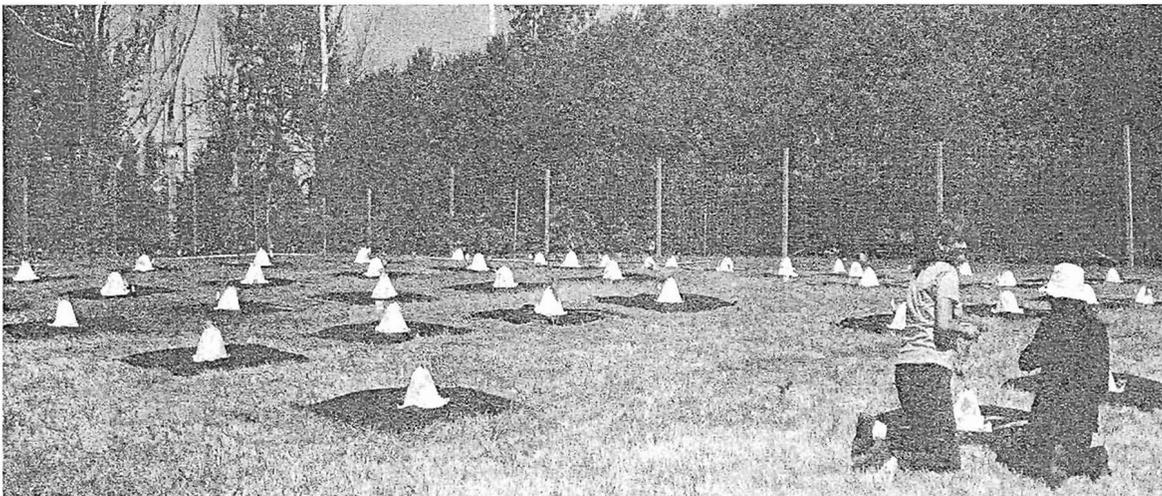
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Figure 6. The 2009 Chestnut Planting crew. (Back row, L to R) Roy Hopke, Herb Darling, Chuck Maynard, David Meyering, Jerry Pinkley, Jim Donowick, Chuck Carpenter, Chris Lyons, Doug Jewell, David Webb, Tim Kuss. (Middle row:), Andy Newhouse, Dale Travis, Lilibeth Northern, Susana Serrazina, Katie D'Amico, Lee Herrington, Bill Powell. (Front row:), Sigrid Freundorfer, Bridget McMaster, Nick Kaczmar, Linda McGuigan, Amelia Zhang, Kathleen Bayer.

The two wooded plots were fenced the following week. Several smaller studies were planted in the following weeks. In total, more than 350 trees were planted—far and away the largest chestnut planting we have ever attempted (Figure 7).

Figure 7. The chestnut open-field planting showing the "HotKaps" used to protect seedlings and plantlets from desiccation and sunburn.



We look forward to many more of these planting days in the future.

For more pictures, visit our web site at <http://www.esf.edu/chestnut/default.htm>