

THE BUR

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

Volume 18, No.1

Summer 2013

PRESIDENT'S MESSAGE

It will have been four years this October since TACF accepted TACFNY's gene transfer program as part of the National effort to restore the timber type American Chestnut. Under the capable leadership of Chairman of the TACF Science Cabinet Dr. Kim Steiner and John Dougherty, a member of the Cabinet, the gene transfer program is now part of the National program. For the first time all methods of introducing resistance to the blight are being examined by qualified scientists from all over the United States, giving TACF a complete attack on the disease.

Long Live the American Chestnut

Herbert F. Darling, Jr.
President TACFNY



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H. F. Darling, Jr.,-President; Frank Munzer-VP; Allen Nichols VP; Richard Radel-Treasurer/ Secretary

**Regenerating Transformation Events into Whole Plants and Expansion of Field Trials
 Progress Report Prepared for The New York State Chapter of The American Chestnut Foundation
 By: Drs. Charles Maynard & William Powell SUNY-ESF
 Departments of Forest and Natural Resources Management and Environmental and Forest Biology
 July 31, 2013 Executive Summary**

- ~40 genes or combinations of genes assembled into **constructs**.
- 37 of these **constructs** have been successfully transformed into transgenic chestnut **events**.
- The mean number of **events** per **construct** is approximately 9.
- There are a total of 344 **events** in the pipeline.
- 8 locations have been chosen, fenced, approved by USDA, and planted.
- 1,324 transgenic American chestnut trees are in the ground.
- 1,311 additional trees are in the greenhouse to be planted this fall or held over for next spring.
- ~2,500 shoots are being propagated for planting next season.

**Really Big Deals (RBD) RBD #1:
 The Chestnut Project received national attention.**

TEDx DeExtinction Event

Dr. Powell was an invited speaker at the **TEDx DeExtinction, National Geographic**, Washington DC. 3/14/13 –3/15/13, “*Reviving the American Forest with the American Chestnut.*” Bill spoke before an audience of over 300 and a live feed to over 3,000 viewers on the web. Bill’s talk is linked to ESF’s Chestnut Project’s web page (<http://www.esf.edu/chestnut/>).

NY Times Article

Our research was highlighted in the July 14, 2013 New York Times article, “*Like-Minded Rivals Race to Bring Back an American Icon.*”

RBD #2:

Identifying Blight Resistant Events

To date, the Darling events containing the oxalate oxidase (OxO) gene driven by a constitutive promoter (CaMV 35S), have demonstrated the highest level of blight resistance in leaf assays (Fig. 1). These events have not only smaller necrotic lesions than the American chestnut control, the lesions are smaller than the ones on the Chinese chestnut control. Other observations are discussed in the Figure 1 caption. On the basis of these leaf assays, all of the events showing Chinese-chestnut levels of resistance, as well as a sample from each of the other categories of events have been planted in, or are being multiplied up for field trials. We are also terribly curious about the events that appear to be worse-than-wild-type, so we included all of these in the next round of field tests.

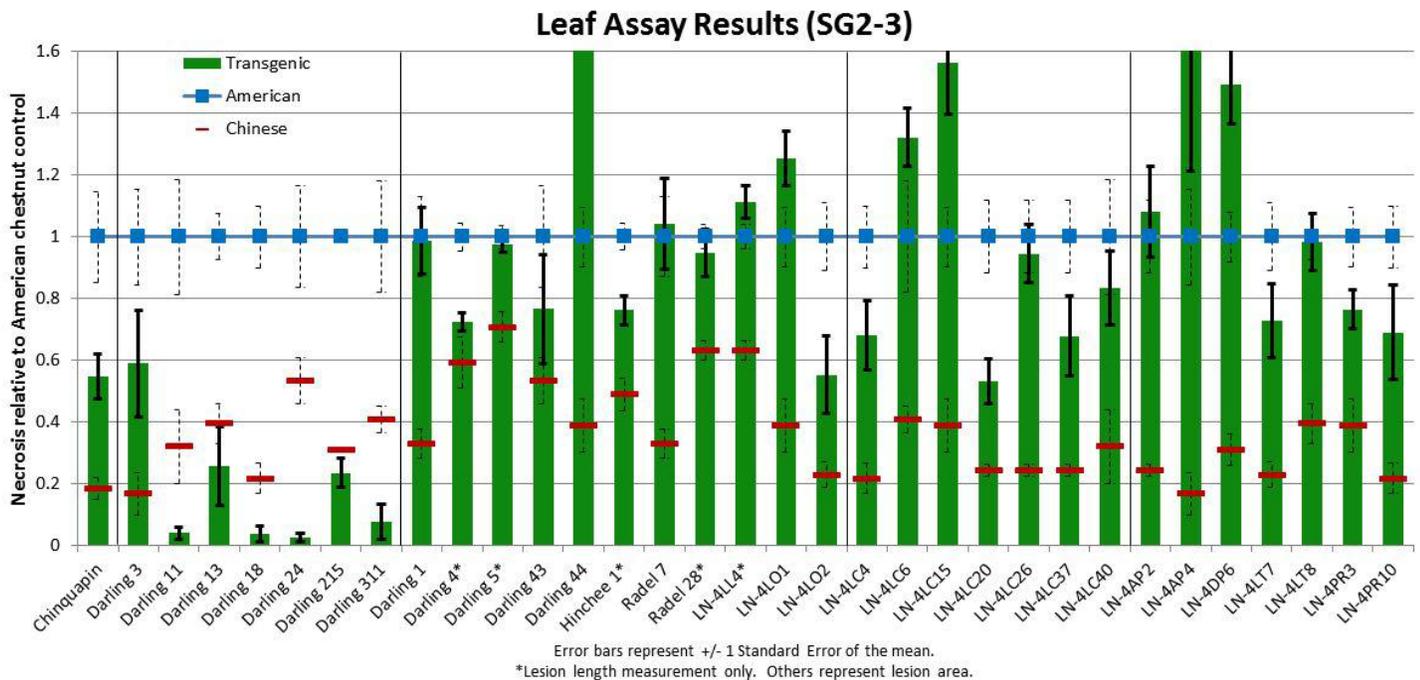


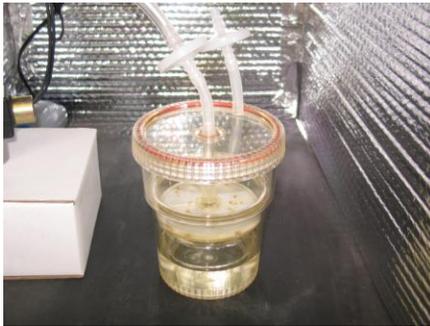
Figure 1. Leaf assays (Newhouse et al. 2013) of transgenic events. The horizontal blue line is the normalized (set to 1.0) necrotic lesion size of susceptible American chestnut controls. The heavy red horizontal lines accompanying each green bar are relative necrotic lesion size for the blight-resistant Chinese chestnut control. Green bars represent the necrotic lesion size of the transgenic events. How to interpret the graph: Green vertical bars that extend above the blue line are events (7) that are actually more susceptible than American chestnut. Green vertical bars that extend above the red lines but below the blue line indicate events (23) intermediate in blight resistant. The tiny green bars just above the baseline indicate events (5) that are more resistant than the Chinese chestnut controls.

RBD #3

Shortening the Transformation Process

In our standard system, we plated out the *Agrobacterium*-exposed somatic embryos onto a semisolid medium and waited patiently for them to either die or grow into a new batch of transgenic cell lines. A limitation of semisolid media is that only the very bottom of each tissue sample is exposed to full-strength medium, including the selection agent. The rest of the cells are exposed to a progressively more dilute concentration. Because of these concentration gradients, it would take as long as 4 to 6 months before we could be positive that all of the surviving cell lines were transgenic and not just escapes. Linda McGuigan has been working to adapt a dramatically different selection process to chestnut somatic embryos. Rather than doing selection in Petri plates on semisolid media, she is testing liquid media in intermittent immersion vessels

The vessels have two chambers separated by a perforated plate. They work by periodically (every four hours) pumping sterile air into the bottom chamber, forcing the sterile liquid medium up through the holes in the plate into the top chamber, bathing the somatic embryos in the medium. After two minutes, the air pump shuts off and the liquid drains back into the lower chamber. This ebb and flow between the chambers thoroughly mixes the medium so that all the cells in each clump are exposed to the medium components including the selection agent at full-strength once every four hours.



Linda has run four batches of chestnut somatic embryos through the periodic immersion vessel transformation system. She has also put ~10 batches of embryos received from the Molecular Biology Lab through the system. On her last experimental run, she obtained **nine** times as many putative transformation events in approximately half the time required by the old system which was included as a control. This is especially important because it brings our timeline (aka "pipeline") to slightly over one year from inoculating embryos to having a handful of plants ready to do leaf assays. If you include the other improvements made by our team, our timeline is now under a year.

Personnel and Their Accomplishments

Linda McGuigan serves as lab manager for the Plant Tissue Culture (PTC) Laboratory (217 Marshall Hall). She is also in charge of the cryopreservation of

our base collection of wild type embryogenic cell lines, as well as all of the transformed cell lines that are developed in either the PTC Lab or Dr. Powell's Molecular Biology Lab. This year, in addition to our own cell lines, we agreed to receive and cryostore the cell lines that Dr. Scott Merkel from the University of Georgia established from burs provided to him by TACFNY. As of August 1, 2013, Linda has received 48 cell lines and has put 16 of these into cryostorage. Scott has nearly 300 cell lines from New York trees in his collection. We can only handle a batch of about 24 at a time, so it will probably take us the rest of the year to cryostore them all. In addition, Linda took over the putative transformation events that Lilibeth Northern, a former research assistant in Dr. Powell's lab, had initiated. Out of approximately 127 transformation events that Linda received, she along with the help of a high school student, Sophie Hearn, put half into cryostorage, and has spent the last six months trying to regenerate them into whole plants. To date, she has regenerated 17 events with an additional 66 events in the last stage of the regeneration pipeline. She is still attempting to regenerate the remaining 44 events.

Allison Oakes is both a full-time PhD graduate student and a full-time technician on the Chestnut Project. She has spent countless hours in the lab this year maintaining and multiplying our ever-expanding collection of chestnut shoot cultures. She is currently multiplying and rooting over seventy shoot cultures and maintaining an additional thirty shoot cultures. With the help of two undergraduate assistants, she was able to root and acclimatize over 2,500 American chestnut plantlets for the spring of 2013 field test plantings. On the research front, Allison has conducted numerous studies to improve our rooting and acclimatization procedures. She has tested the effects of different times in the various media, changing the pH of the multiplication medium, varying individual media components, and examining the effects of darkness and light on the rooting process. In total, these studies have led to a dramatic improvement in the survival rate and a ~50% reduction in the time required (Figure 2). Periodic immersion vessels as used by Linda McGuigan to transform American chestnut somatic embryos for rooting and acclimatization. She published these results this summer in her paper "Doubling Acclimatization Survival of Micropropagated American Chestnuts with Darkness and Shortened Rooting Induction Times" (*J. Environ. Hort.* 31(2):77–83. June 2013). She also made two trips to Meadowview Research Farms to collect winter shoots which she used to establish and multiply a number of B3F3 hybrids in tissue culture and is developing a rooting protocol for these hybrid lines.

Andrew (Drew) Teller was hired as a part-time glassware washer in May of 2012. Drew quickly graduated from glassware to transfers, media preparation, and transplanting plantlets from sterile medium into potting mix. He is now responsible for transplanting and babying the chestnut plantlets through the critical growth chamber stage of the acclimatization process. Drew is also programming a field recorder/barcode scanner to automate the collection of data from the inoculation plots at the Lafayette Road Experiment Station and eventually all data collection from all eight of the field-test sites.

Andy Newhouse is a full-time technician on the Chestnut Project. Currently, Andy's salary is coming out of a different grant, but his work is an integral part of the field-testing process. He is responsible for essentially all of the post-growth chamber stages in the blight screening process, beginning with preparing the USDA permit applications for all test sites, growing and hardening-off the plants in the greenhouse, laying out test sites, supervising plantings and plot maintenance, mapping all

plots, and inoculating and scoring for blight resistance. Andy is also responsible for most of our control-pollination work and has done most of the research to develop the leaf assay. A full description of this leaf assay technique was recently accepted for publication in the journal *Plant Disease*.

Kathleen Baier is a full time technician on the chestnut project. Currently, Kathleen's salary is coming from the Forest Health Initiative phase 2 grant that ends Dec. 31, 2013. Kathleen is responsible for all the molecular assays, which include all the gene expression studies and insert copy number. Without these, we would not know which plants to produce. She also helps with early identification of events using PCR. She is also key to our vector constructions where we are staking resistance genes and checking them by DNA sequencing. In addition to the molecular work, Kathleen is responsible for the rapid transgenic pollen production (which made last year's RBD list). With her pollen-forcing procedure, we can cross the resistance-enhanced events with wild-type mother trees in the TACFNY orchards, increasing the genetic diversity in the trees we produce. Lastly, she is the molecular lab manager, ordering supplies and training students.



Kristen Stewart and **Dale Warner** are two MS graduate students on the chestnut project. We are seeking funding for these two students. Kristen has been a significant help with screening our new transgenic events using multiplex PCR. Most of this work has been done as an unpaid volunteer. She is also working on her MS thesis studying a flowering gene in transgenic chestnut. Dale Warner is our newest graduate student working on the mycorrhizal association with American chestnut. He is taking over where our past graduate student, Katie D'Amico, left off. Dale has also been helping Andy with the leaf assays over this summer.

Undergraduate Student Assistants:

Lab Assistants:

This year we hired two undergraduate assistants to work part-time in the lab. Both of them are an important part of the project. Rachel Arnold is the glassware washer. Without her, we would not have the necessary equipment to do experiments, make media, or even perform routine transfers of our cultures. Shaler Garrett has been making media as well as transferring, regenerating, and rooting cultures. He will also be performing an experiment to test the toxicity of oxalic acid on transformed and non-transformed American chestnut embryos. We also have a visiting undergraduate student, Allison Chartrand, from Northwestern University who is working to find a better antibiotic than we currently use (cefotaxime) at the right concentration to kill off *Agrobacterium* after a transformation.

Field Assistants:

In addition to undergraduate staff in the labs, we have two part-time summer field assistants: Collin Bartholomew (re-hired from last summer) and Drew Teller (introduced above). These people help with planting, plot maintenance, mowing, weeding, watering, and experiments as needed.

Plans for the Near Future

More Pollinations

A key part of our restoration plan is to increase genetic diversity by out crossing our transgenic trees with the mother trees collected by the New York State Chapter of The American Chestnut foundation, as well as many additional selections from the remnant populations throughout the American chestnut's original range. Since only half of the offspring are expected to inherit the transgene, it is important that the seedlings go through our battery of molecular tests to identify the trees with enhanced blight resistance.

Lots More Blight Resistance Screening

With 344 events in the pipeline, and many more genes to test, we are looking at a lot of additional field trials. Field inoculation trials are the ultimate proof of blight resistance. We will never completely eliminate the need for them. There are, however, several significant problems with field trials. It takes time, effort, and money to grow the 20 or more plants from each event that are required for a statistically accurate field trial. A field trial has to be in the ground for at least two, but probably three, years before the trees are large enough to be inoculated, and it is the end of the growing season before results are definitive. In contrast, leaf assays can be carried out on a handful of leaves from just the few plants as soon as they have enough leaves to spare, and the results are "in" at the end of the week. We are now testing new events with the leaf assay before putting that event into field trials. We are very excited about the inoculation trials we planted this spring, because, in the leaf assays, some of the events have shown stronger blight resistance than the Chinese chestnut controls.

Publications

Baier, K.M., C.A. Maynard, and W.A. Powell. 2012. Early flowering in chestnut species induced under high intensity, high dose light in growth chambers. *Journal of The American Chestnut Foundation* 26:8-10

Nelson, C.D., W.A. Powell, C.A. Maynard, K.M. Baier, A.E. Newhouse, S.A. Merkle, C.J. Nairn, L. Kong, J.E. Carlson, C. Addo-Quaye, M.E. Staton, F.V. Hebard, L.L. Georgi, A.G. Abbott, and B.A. Olukolu. 2013. The Forest Health Initiative,

American chestnut (*Castanea dentata*) as a model for forest tree restoration: biological research program. *Acta Hort* (in press).

Newhouse, A.E., J.E. Spitzer, C.A. Maynard, and W.A. Powell. 2013. Leaf inoculation assay as a rapid predictor of chestnut blight susceptibility. *Plant Disease*. (Accepted for publication)

Oakes, A.D., W.A. Powell, and C.A. Maynard. 2013. Doubling acclimatization survival of micropropagated American chestnuts with darkness and shortened rooting induction time. *J. Environ. Hort.* 31(2):77–83

Zhang B., A.D. Oakes, A.E. Newhouse, K.M. Baier, C.A. Maynard, and W.A. Powell. 2013. A threshold level of oxalate oxidase transgene expression reduces *Cryphonectria parasitica* - induced necrosis in a transgenic American chestnut (*Castanea dentata*) leaf bioassay. *Transgenic Res* (in press)

(open access at <http://link.springer.com/article/10.1007%2Fs11248-013-9708-5#>)

Conclusions

We are well on our way to fulfilling the goals set forth in this grant:

- We have regenerated 17 new events into whole plants, with many more in the final stages.
- We have reached our goal of shortening the pipeline to a year or less.
- We planted out more than 1,300 transgenic trees this spring in field trials.

None of this would have been possible without the continuing support of the New York chapter of The American Chestnut Foundation. We thank you.

An abbreviated Treasurers Report Jan 1st thru June 30th, for the Bur is as follows:

In Bank 1/1/13.....				\$131,345
Income		Expenses		
2013 Getting Events in the Ground & Tested donations		2012-13 Research Grant for Getting Events in the		
Greater than \$500	\$13,581	Ground & Tested	\$64,482	
less than \$500	\$ 7,400	Other expenses	\$ 5,112	
Total received in 2013 to date	\$20,981	Total Expenses	\$69,594	
Other income	\$ 5,430			
Total Income	\$ 26,411			
In bank 6/30/13.....				\$88,162
		Committed for Research Grant 2013-14	\$65,161	
		Balance for other expenses	\$23,001	

Commitment for 2013-2014 is \$73,669 less \$65,161 is \$8,508 needed by June 2014

Commitment for 2014-2015 is \$75,765 and we need to raise all of it by June 2015

TACF-NY has planted a combination of 214 trees at the White Plantation in Zoar Valley, mostly transgenic, some Chinese, some backcross, some pure American Chestnut.

Of those trees some are 160 cm high or 5' 3" with trunk in the 5.0 cm range or 2". Of the 214 trees planted prior to 2013, only 22 are currently dead or about 10%. Of the first 9 rows of trees or 106 plantings only 6 trees have died. These are the old trees some 5 feet tall.



A coincidental public lecture on the development of the TACF/ESF transgenic American Chestnut is being held in the Gateway Building on Thursday evening at 7:00PM. It's open to the public and the lecturer is Bill Powell. TACF inspired this lecture series. Dr's. Powell & Maynard are two of about 18 doctors working under Don Leopold, Distinguished Teaching Professor and Chair of the Department of Environmental and Forest Biology.

These guys are slaving away, just like Dr's. Powell & Maynard on absolutely fascinating projects and doing it incognito.

Last year, Dr's. Powell & Maynard were absolutely thrilled to have the chance to have a public lecture at the New York Botanical Garden, a chance for them to "show their stuff". Dale Travis decided to fund an ongoing lecture series that would be open to the public, publicize the efforts going on at ESF (which is great advertizing for ESF).

The first lecture was last fall in the new Gateway building, by Dr. James Gibbs. He has been working to determine the remaining population of Snow Leopards in Siberia/Mongolia/Nepal, and how to enhance their survival rate.

New York State Chapter of the American Chestnut Foundation, Inc. 23rd Annual Meeting Agenda

Where: SUNY-ESF (www.esf.edu)
Gateway Building
1 Forestry Drive, Syracuse, NY 13210

When: Friday, October 11, 2013
Saturday, October 12, 2013
page 1 of 2

Pre-Meeting (Optional - for those who may be interested)

Thursday (10/10) - Gateway Building

- 7:00 pm The Dale Travis Lecture Series
- The Return of a King: A New Beginning for the American Chestnut – Bill Powell
- ### Meeting Agenda

Friday (10/11) - Gateway Building

- 6:00 pm Registration (Sign up to become a member or renew membership/Set up posters)
(Free 1 year membership for students with ID registering for the meeting)
- 6:30 pm Dinosaur BBQ dinner (Catered at the Gateway Building)
- 7:30 – 10 pm Harvest Exchange
- Chestnut Beer/Chestnut Ice Cream/Chestnut Liqueur
 - Silent Auction/50:50 (To continue on Saturday)

Saturday (10/12) - Gateway Building

- 7:30 am Continental Breakfast for early birds (catered by ESF Chestnut Committee)
- Silent Auction/50:50 (all day until 4:45 pm)
- 8 am – 9 am Registration (Sign up to become a member or renew membership)
- 9:00 am Welcome & President's report by **Herbert F. Darling Jr.**
(Other reports on the trees at NY Botanical Garden, Zoar Valley, Lasdon Arboretum, Saratoga Springs and Bellville)
- 9:20 am Treasurer's Report by **Richard R. Radel**
- 9:30 am Election of Board - **Richard R. Radel**
- 9:35 am District Director's Reports

District 1	Enrico Nardon
District 2	Dale Travis
District 3	Frank Munzer
District 4	Allen Nichols
District 5	(we need you)
District 6	Urling Walker
District 7	Roy Hopke
District 8	Alec Newlands
District 9	William Snyder



- 10:00 am Coffee Break
- 10:30 am Science Reports – **Drs. Maynard & Powell and Staff**
- 12:00 pm Lunch (catered by American Food and Vending)

Afternoon Field Trips or Workshops

- 1 – 3 pm Field Trip - Lafayette Road Experiment Station, 452 Lafayette Road, Syracuse, NY
OR:
1 – 3 pm Field Trip – Destiny USA Mall, 9090 Destiny USA Dr., Syracuse, NY
(www.destinyusa.com)
OR:

- 1 – 1:45 pm - Session 1 (choose one of the following)
- Room A – The Path to Deregulation - John Dougherty
 - Room B – Grafting - Allen Nichols
 - Room C – Cooking with Chestnuts – Dale Warner
 - Room 240 – Pollination - Sara Fitzsimmons
 - Concourse – Open Space for Free Time

- 2 – 2:45 pm - Session 2 (choose one of the following not chosen in Session 1)
- Room A – The Path to Deregulation - John Dougherty
 - Room B – Grafting - Allen Nichols
 - Room C – Cooking with Chestnuts – Dale Warner
 - Room 240 – Pollination - Sara Fitzsimmons
 - Concourse – Open Space for Free Time

- 3 – 3:45 pm - Session 3 (choose one of the following not chosen in Session 1 or 2)
- Room A – The Path to Deregulation - John Dougherty
 - Room B – Grafting - Allen Nichols
 - Room C – Cooking with Chestnuts – Dale Warner
 - Room 240 – Pollination - Sara Fitzsimmons
 - Concourse – Open Space for Free Time

3:45 pm Coffee Break/Fun Games on the Concourse

4:45 pm Announce winners to silent auction/50:50

5:00 pm Afternoon session closes

6:00 pm The MOST (The Museum of Science and Technology), 500 S Franklin St., Syracuse, NY
(<http://www.most.org/>)

7:00 pm Dinner at the MOST

After Dinner Closing Remarks

New York State Chapter of the American Chestnut Foundation, Inc. 23rd Annual Meeting

Where:

Genesee Grand Hotel and
1060 E. Genesee St.
Syracuse, NY 13210
(315) 476-4212

SUNY-ESF
Gateway Building
1 Forestry Drive,
Syracuse, NY 13210

When:

Friday, October 11, 2013
Saturday, October 12, 2013

To make your room reservations, call the Genesee Grand Hotel (www.geneseegrande.com/) at (315) 476-4212 and ask for "NY Chapter American Chestnut Room Block". Reservations must be made prior to September 20, 2013 to receive the rate of \$94 per (single or double) room per night or king suites at \$139 per night. Meetings and facility touring will be at SUNY College of Environmental Science and Forestry. Check out the website <http://www.esf.edu/outreach/chestnut> for more details.

Registration Form

Name (s): _____ Address: _____
 Phone: _____ City: _____
 E-mail: _____ State: _____ Zip: _____

Brief Meeting Agenda:

Friday night Dinosaur BBQ dinner followed by the Harvest Exchange at the Gateway Building 6 pm to 10:00 pm with a cash bar (Chestnut Beer & Liqueur) and dessert (homemade Chestnut Ice Cream). Saturday morning Belgium waffles & bacon breakfast at the Gateway Building, with meetings until 11:45 am. Lunch at 12:00 pm with either tours of the facilities, workshops or sightseeing from 1:00 pm to 4:30 pm. Saturday night dinner at the Museum of Science and Technology with time to tour before dinner. Shuttle service will be provided for those who do not want to drive their own car.

	<u>Cost</u>	<u># Attending</u>	<u>Total Cost</u>
Thursday			
Dale Travis Lecture Series	Free	_____	Free
Friday			
Dinosaur BBQ Dinner Reservation	\$22 each	_____	\$ _____
Student Dinosaur BBQ Dinner Reservation	\$15 each	_____	\$ _____
Nut Exchange	Free	_____	Free
Saturday			
Registration Fee (before October 1, 2013)	\$65 each	_____	\$ _____
Dinner Reservation	\$40 each	_____	\$ _____
Student Registration Fee	\$25 each	_____	\$ _____
Student Dinner Reservation	\$25 each	_____	\$ _____
Total Registration:			\$ _____
Please choose: # _____ (Beef) # _____ (Chicken) # _____ (Fish) # _____ (Vegetarian)			

- _____ I desire shuttle service Hotel to/from Gateway Building.
- _____ I desire shuttle service Hotel to/from Museum.
- _____ I desire shuttle service to sightsee at the Destiny USA Mall.
- _____ I expect to bring _____ nuts for Harvest Exchange.
- _____ I would like _____ nuts to plant.
- _____ I would be glad to help at the Annual Meeting.
- _____ I would be interested in _____

Make checks payable to:
TACFNY
Mail before October 1st to:
TACFNY
23 Carriage Circle
Williamsville, NY 14221

New York State Chapter
The American Chestnut Foundation, Inc.

23 Carriage Circle
Williamsville, N. Y. 14221
Tel: (716) 639-0620 e-mail: RichardRRadel@MSN.com

Membership Application

Enclosed is my membership support of

- Gold Leaf, \$1,000
- Silver Leaf \$500
- Bronze Leaf \$250
- Green Leaf \$100
- Regular \$40
- Student \$15
- Other \$ _____
- Special Gift to NY State Chapter
\$ _____

Total Amount \$ _____

NY Chapter membership

for membership in The American Chestnut Foundation which includes subscriptions to **The Bark and Journal of the American Chestnut Foundation.** TACF located at 160 Zillicoa Street Suite D, Ashville, North Carolina, 28801



Should you desire to help the NY Chapter with their three year grant to The Research Fund of SUNY - ESF of \$213,916

My donation is \$ _____

The title of the grant is "Getting events in the ground and tested"

The NY Chapter is presently approximately 45% funded toward our goal.

Enclosed is an additional contribution in the amount of \$ _____ in support of the New York

State Chapters Activities

Name: _____

Address: _____

City/State/Zip: _____

This is a gift membership for the above. from

Name: _____

Address: _____

City/State/Zip: _____

By noting (X) NY Chapter membership you will receive their Newsletter "**The Bur**". The NY Chapter helps guide research at SUNY-ESF and maintains plantings to keep the American Chestnut gene pool. Please make checks payable to The American Chestnut Foundation, NY Chapter (TACF-NY Chapter) and send to 23 Carriage Circle, Williamsville, NY 14221. TACF & TACF-NY Chapter are 501 (c) (3) non-profit organizations. Except for the membership services portion of your contribution (valued at \$15) your gift is tax deductible to the full extent allowed by law.

The Bur

The American Chestnut Foundation Inc.

New York State Chapter

c/o Richard R. Radel

23 Carriage Circle

Williamsville, NY 14221