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Volume 19, No. 1 Newsletter of the New York State Chapter of the American Chestnut Foundation Spring 2014

Dear Members:

After 25 years since the forming of TACF-NY with Stan and Arlene Wirsig, I have chosen to resign as president at this October's annual meeting.

Planning for a smooth transition to the new President has been underway for the past year. After much conversation with many active members, it was decided to propose Mr. Allen Nichols, currently TACF-NY's District 4 Director for the President's job. Since Allen has been a member and passionately involved for some years. Allen and I have been working together since the October 2013 annual meeting and he has been involved in all of this year's major activities. He has been copied on correspondence and kept right up to date. He has also attended important meetings involving TACF-NY.

I am very comfortable turning the leadership of TACF-NY over to Allen. The future requires the production of transgenic seedlings in tremendous quantities. He will need a robust plan as soon as he takes over to accomplish this next step. It will be extensive work, seeking permits and deregulation of transgenic seedlings, with the government agencies. This is no small task! Allen and John Dougherty, TACF-NY Science Director, will need to work closely to accomplish this. Allen has already been involved in these first steps. Allen along with John, Chuck, Bill and I just met in Syracuse with three US government regulation agencies and the process has already been started.

Thank you to all the members new and old who have donated working hours, financial support, planted seedlings, attended meetings and manned the booths and tables to allow us to get this job done.

I am proud of all we have accomplished over the past 25 years and will remain a very interested member and do anything I can to help keep things moving for Allen and his crew.

Fran and Allen, I wish you well in your new adventure!!

Long Live the American Chestnut,

Herb and Jane Darling

Mr. Darling received a carving and Letter of Authenticity from Drs. Maynard and Powell from the two original Darling4 transgenic American Chestnut trees produced in 2008.

In other TACF-NY news, an opening for Secretary Treasurer also needs to be filled.

The complete slate of officers, directors, and leadership will be presented at the meeting for election. Nominations can be sent to Richard Radel, Chairman
TACFNY
23 Carriage Circle
Williamsville, NY 14221

For another option for free time, check:
http://www.hanfordmills.org/visit-a-historic-workingmill/hours-admissions/

Craig Hibben, a long time friend and member to TACF-NY passed away this past year. His dedication and work in the field will be missed.

New York State Chapter of the American Chestnut Foundation, Inc.
24th Annual Meeting

Where:

Oneonta Country Club and
9 Country Club Drive
Oneonta, NY 13820
(607) 432-9074

Hampton Inn Oneonta
225 River Street
Oneonta, NY 13820
(800) 426-7866

When:
Friday, October 10, 2014
Saturday, October 11, 2014

To make your room reservations, call the Hampton Inn Oneonta (http://www.hoteloneonta.com) at (800) 426-7866 or (607) 433-9000 and ask for "The NY Chapter of the American Chestnut Foundation Room Block". Reservations must be made prior to **September 10, 2014** to receive the rate of $99 per room per night. (King or two Queens and includes breakfast) Nut exchange will be in conference room at Hampton Inn and Saturday's meeting will be at the Oneonta Country Club. Check out the website www.oneontacountryclub.org for more details.

Registration Form

Name (s): _____ Address: _____

_____ _____

Phone: _____ City: _____

E-mail: _____ State: _____ Zip: _____

	Cost	# Attending	Total Cost
Saturday			
Registration Fee (**before October 1, 2014**) Includes coffee, danish, and buffet lunch, including ham and turkey, Caesar salad, rolls, soup, beverage, chips and cookie platter.	$20 each	_____	$_____
Dinner Reservation Buffet style, with salad, sautéed chicken, roasted beef brisket, penne pasta w/tomato sauce, seasonal veg., dessert and beverage.	$30 each	_____	$_____
		Total Registration:	$_____

_____ 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 the following Member Sharing Topics.
_____.

Any questions contact Allen Nichols at (607)263-5105
Or fajknichols.75@gmail.com

Make checks payable to:
TACFNY

*Mail before **October 1st** to:*
TACFNY
23 Carriage Circle
Williamsville, NY 14221

Regenerating Transformation Events into Whole Plants and Expansion of Field Trials
Progress Report covering the period from January 1 – June 30, 2014
Prepared for
The New York 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

The Chestnut Project as of July 2014

- 43 genes or combinations of genes have been assembled into constructs.
- 37 of these constructs have been successfully transformed into 416 American chestnut events and have either been regenerated into whole plants or are proceeding down the pipeline towards whole plants.
- 6 events containing the Oxalate Oxidase gene (OxO) show **very high** levels of resistance[1] (similar to or even better than Chines chestnut).
- 12 events (some with OxO and others with 3 different Chinese chestnut genes) show intermediate levels of resistance (better than American chestnut but not as resistant as Chinese chestnut[1]).
- 8 field test sites have approximately 1,500 transgenic American chestnut trees.
- We are starting up a new high-production laboratory in the Central New York Biotechnology Accelerator (BAC) building.
- We published two groundbreaking papers about blight resistance in our transgenic American chestnut trees: Newhouse, *et al*. 2014a and 2014b.

The 2013-2014 Chestnut Project Team

Managers & Technicians: Linda McGuigan (supported by TACFNY), Kathleen Baier, and Andy Newhouse
Graduate students: Allison Oakes (PhD) (supported by TACFNY), Kristen Russell (MPS received Spring 2014), Dale Warner (MS)
Undergraduate Lab Assistant & Field Assistant: Shaler Garrett, Alex Coven, Tasha Doulos
Undergraduate research projects: Kevin Johnson, *Embryo encapsulation effects on American chestnut;* Josh Weber-Townsend, *Leaf assays on new chestnut events*; Stephanie Wong, *Testing a soil bacterium for potential blight treatment or preventative properties*; Dakota Matthews, *qRT-PCR on new chestnut events and OxO assays on older trees*; John McGivney, *PCR on chestnut embryos and new elm events;* Michelle McMullen, *Screening new chestnut events with PCR.*
Summer High School Students: Bethany Regan, Jennifer DeRosa, Meg Lovier, Clara Miller, Elizabeth Lane, and Colby Buell (Skaneateles High School)

Updated Website

We have updated the ESF chestnut website (http://www.esf.edu/chestnut) with new information and new videos. One of particular interest is a time-lapse video of a small stem assay that shows the transgenic American chestnut trees, 'Darling 311' and 'Darling 215,' to be as resistant as the Chinese chestnut control. You can view the video at http://www.esf.edu/chestnut/resistance.htm. Enjoy the website and check back as new information is uploaded.

Reports from the Tissue Culture Lab

Linda McGuigan continues to do an outstanding job managing the Tissue Culture Laboratory. She also maintains the American chestnut somatic embryo cell lines, both transgenic and wild type. These embryo lines are sub-cultured (divided and put onto fresh medium) every 3 to 4 weeks. The newly transformed cell lines are regenerated into shoot cultures as soon as possible, but until we have a stable shoot culture, Linda continues to subculture the embryo cell line. In other words, she spends a lot of time taking care of chestnut tissue at the very earliest stages of our process. Once a shoot culture

1. According to leaf assays

has been established, she places the corresponding embryo cell line into suspended animation in a Cryofreezer cooled with liquid nitrogen to a chilly -130 C. This significantly reduces the amount of time she spends maintaining cultures.

Additionally, Linda has been working with Shaler Garret to improve transformation efficiency by using bioreactors during the selection step of the procedure. She was able to increase the number of transformation events per gram of embryogenic tissue by at least 10-fold over the control embryos on standard medium.

Allison Oakes handles the majority of shoot multiplication and rooting in the production pipeline, which is the second major step in our process. Although she was on maternity leave for eight weeks in January and February, she still managed to produce ~2,270 rooted shoots between March and May, in addition to the ~10,000 shoots she rooted between August and December. The rooted shoots are passed along the pipeline to Shaler Garrett, who pots them and maintains them in the growth chambers until they are large enough to go to the greenhouse.

Allison actively multiplies 30 key lines of transgenic American chestnuts shoots on a 3-4 week cycle. At the end of each cycle, she divides the clumps of shoots into two groups. One group goes into rooting medium and the other group goes into fresh multiplication medium for another cycle. This season she produced a minimum of 400-550 shoots of each line for the rooting process, except for seven Darling lines that we consider the most promising for blight resistance. She grew out 900 shoots of each of these. Overall, she produced more than 15,000 rooted shoots. Allison also multiplies three lines of Chinese chestnut as controls and maintains 42 older transgenic American chestnut lines as backups. These are transferred on an extended 6-week cycle.

Allison worked with Tom Deacon, a member of the TACFNY from the Buffalo area, to establish two new clonal lines of American chestnut in tissue culture. They were started from sprouts growing from stumps of blight-killed trees that were more than 100 years old. These additional wild-type control lines will be useful for small stem assays and will be used for future leaf transformation experiments.

Shaler Garrett works as a lab technician in the Tissue Culture Laboratory. His responsibilities include washing glassware, preparing media, subculturing, and maintaining many of the new and old cell lines, rooting, potting, and acclimatizing the resulting plantlets. Furthermore, he waters and maintains the plantlets in the growth chambers, does greenhouse care, and also plants, weeds, and waters at the Lafayette Road Experiment Station. Shaler has also carried out a number of pilot studies and projects that have improved several steps in the regeneration and acclimatization process. His most recent study has been transforming American chestnut embryos with a plasmid vector that contains only the OxO transgene as both the selectable-marker and the gene of interest. If his If the research works out, we will be able to identify transformed cells and embryos using oxalic acid as the selection agent. This will dramatically simplify the entire process from constructing simpler vectors to identifying the most promising events for field trials. It will also streamline field trial permit applications and eventually our petition for full release. We are all eagerly awaiting Shaler's results.

Reports from the Molecular Biology Lab

Another essential team member is **Kathleen Baier**, the Lab Manager in the Molecular Biology Lab. Kathleen has several key jobs on the Chestnut Project, one of them is to score all of the events that come out of either lab for gene expression and copy number in order to determine the most promising events to multiply and regenerate into whole plants. Over the reporting period, Kathleen analyzed 36 new events for gene expression at the shoot stage. Most of these events were transformed with either a Chinese chestnut gene or the VST1 gene from grape. Four of the new events were transformed with a pyramid vector that contains the OxO gene from wheat and a second gene, a laccase-like gene, from Chinese chestnut (Figure 1). The events were also analyzed for transgene copy number.

Kathleen is also in charge of hunting for new genes and combining genes that might provide even stronger resistance than the genes we have already tested. She and an undergraduate, Michelle McMullen, tested 42 events transformed with a pyramid vector containing both the OxO gene from wheat and the VST1 gene from grape (pOV). Of the 42 events, 9 contained both genes. Kathleen also produced two new events that have the OxO gene combined with a second gene, RPH, that is expected to improve resistance to chestnut ink disease (caused by *Phytophthora cinnamomi* and *P. cambivora*). These events are being analyzed for gene expression.

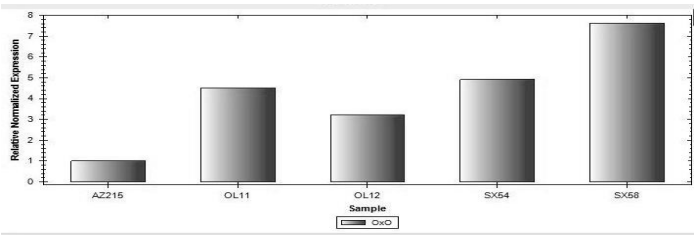

Figure 1. Oxalate oxidase expression in Transgenic American chestnut shoots compared to that in Darling 216.

Last year Kristen Russell and Kathleen constructed a new vector that has the OxO gene, but not the NPTII gene usually used for selecting the transformed embryos. This winter Kathleen inserted an additional promoter and terminator (Win3.12 promoter and 35S terminator) so that in the future we can make new pyramid vectors that can be selected using oxalic acid rather than the antibiotic kanamycin. As with Shaler's project described above, this would simplify our transgenic events and potentially facilitate the regulatory process, which means transgenic trees could be distributed sooner. She just finished construction of another new vector, Win3.12-OxO. This vector has a wound inducible promoter controlling the OxO gene and was also engineered for insertion of different genes in the future.

Undergraduate **Dakota Matthews** performed Oxalate Oxidase leaf disc assays on the seedling offspring of the 2013 crosses made with pollen from transgenic trees. If the plant is positive for oxalate oxidase activity, a black pigment appears around the leaf margin or on the midvein. Kathleen then analyzed each OxO positive leaf for presence of the GFP gene because we wanted to determine if any transgenic offspring had inherited the OxO gene but had lost the GFP gene. We found that for these particular crosses the genes for OxO and GFP were always inherited together.

Kathleen also analyzed laccase expression in both leaves and stems of greenhouse–grown Travis events, 4, 37, and 40. These events show intermediate levels of resistance to *C. parasitica* infection when compared to Chinese and American chestnut in the Early Screening Leaf Assay developed by Andy Newhouse. We were interested to see if laccase gene expression correlates with the results of the early screening leaf assay (Figure 2). Ellis 1, Qing, and Hong Kong were used as the controls. American chestnut, Ellis 1, leaves are very susceptible to *C. parasitica* infection and have a low level of laccase expression. Chinese chestnut leaves develop significantly less necrosis when infected with the fungus and have high levels of laccase expression. The Travis events that have intermediate levels of necrosis in the leaf assay have intermediate levels of laccase expression. Since *C. parasitica* infects the bark of larger trees we also looked at laccase expression in the stems of the same Travis events (Figure 3). Ellis 1 stems have very low laccase expression. There was higher laccase expression in the stems of the Chinese chestnut controls and the Travis events, but Travis 4 had higher expression than both of the Chinese chestnut. This suggests that Travis 4 might show higher levels of resistance in stem assays than it did in the leaf assays. It will be very interesting to see what happens when the Travis 4 trees in the field are large enough for stem inoculations.

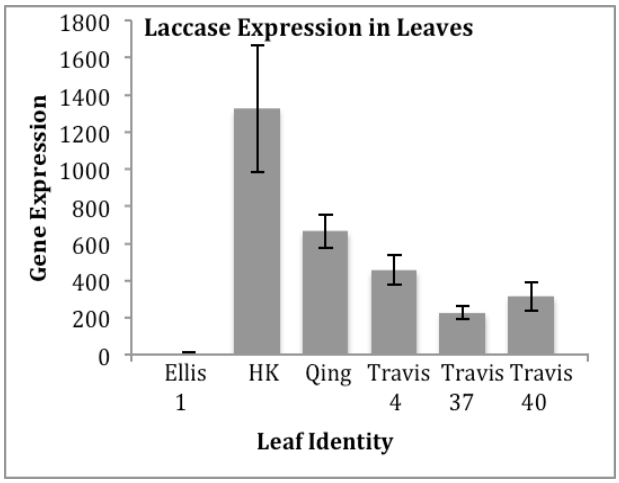

Figure 2. Laccase expression in leaves of three Travis events relative to controls.

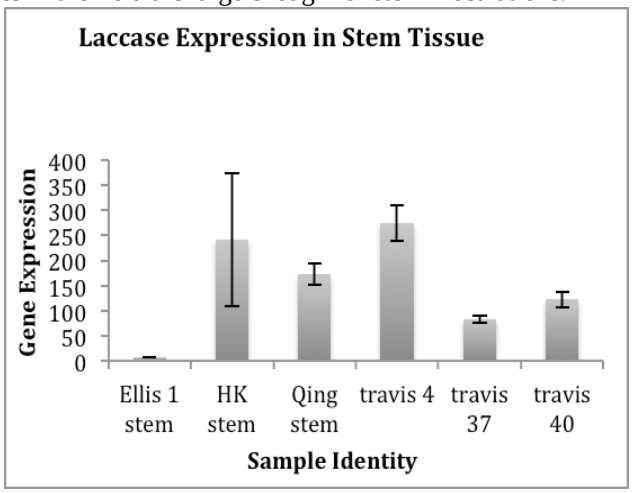

Figure 3. Laccase expression in stem tissue of three Travis events relative to controls.

Andy Newhouse is one of the most versatile researchers we have on the Chestnut Team. This year Andy was first author on two groundbreaking papers. The first paper showed that the leaf-inoculation assay was a good predictor of blight resistance (Newhouse, *et al.* 2014a) (Figure 4). The second publication demonstrated that some of our transgenic American chestnut lines are definitely blight resistant, and that the genes that make them so, can be transmitted through pollen to seedling progeny (Newhouse, *et al.* 2014b).

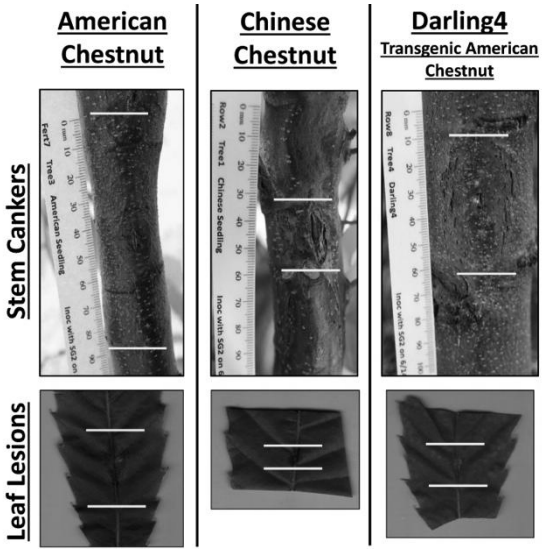

Figure 4. Top row, stem cankers on American, Chinese, and transgenic American chestnut. Second row, necrotic areas on leaves from the same three sources. (From Newhouse et al. 2014a.)

Andy is responsible for maintaining all of the records and USDA permits for field trials. This reporting period Andy submitted and received a renewal of our largest USDA permit. Completing one of these applications for multiple events and locations is a monumental task. This comprehensive 73-page renewal application describes every part of every gene used to transform each of the events. It also includes a detailed description of each planting site. He also completed several other smaller transportation permits and amendments.

Andy supervises field projects (planting, mowing, replacing mortality, doing controlled pollinations, and stem inoculations) and manages the greenhouse. Andy also supervised two undergraduate student research projects. Josh Weber-Town completed leaf inoculation assays to screen transgenic events, and Stephanie Wong

tested a soil bacterium called *Bacillus amyloliquifaciens* as a potential natural blight protectant.

Plans for the Near Future

Much More Blight Resistance Screening

With 416 events in the pipeline, and more genes still to test, we are looking at a lot of additional trees to screen for blight resistance. Field inoculation trials are the ultimate proof of blight resistance. We will never completely eliminate the need for field tests. However, field trials take time, effort, and money. A field trial has to be in the ground for at least three years before the trees are large enough to be inoculated, and it is the end of that third growing season before results are definitive. In contrast, leaf assays can be carried out on a handful of leaves from just a 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 using the leaf assay to testing all new events as soon as they are big enough to spare some leaves and only field-testing the most promising ones.

Opening a New Lab

Because of the widespread publicity the Chestnut Project has received in the last few years, we receive numerous requests for trees as well as many offers to help test our resistant trees. We asked our College administration for additional lab space to gear up production of our best lines. We made a case that within three to five years we expect to have a Notice of Non-Regulated Status for at least one of these lines and we would need additional space to grow enough trees to meet the demand. Apparently we made a good case because a few weeks later Provost Bruce Bongarten told us that we could keep our existing labs and could expand the Chestnut Project in a new lab in a brand new building called the Central New York Biotech Accelerator (BAC) (Figures 5 & 6). The target date for opening the new lab is late August.

Figure 5. The Central New York Biotechnology Accelerator: A new home for the Chestnut Project is located about a mile from campus. The facility is a joint venture between the College of Environmental Science and Forestry and the Upstate Medical University.

Figure 6. The new scale-up laboratory. A blank slate, but what potential!

Ramping up Production of Plantlets and Pollen from Our Elite Lines

The BAC lab is designed for two purposes. First is to produce large batches of plantlets of the selected blight resistant lines that we plan to move forward toward deregulation. The second purpose is to produce pollen from the same lines, as well as a few others, for outcrossing and other breeding research. The new lab will have two growth chambers: one will be a high humidity chamber similar to the two TACFNY helped us buy in 2006. The second is close to a duplicate of the high-light growth chamber Kathleen Baier used in her research on early flowering (Baier, *et al*. 2012). Both chambers are going to be equipped with automatic watering systems.

We are looking forward to the convenience of having the chambers in the same building as the laminar-flow hoods and the illuminated growing benches. We expect to improve survival, plant quality, and pollen production simply because we can take better care of the plants.

Figure 7. Linda McGuigan and Andy Newhouse bagging trees in the Zoar Valley Seed Orchard, near Buffalo, NY.

Outcrossing

A key goal of our reintroduction plan is to conserve as much of the remaining genetic diversity in the original American chestnut population as possible. We don't want to replace the remnants of the original American chestnut with our resistant trees. Instead, we hope to eventually introduce the resistance genes they carry into the original population by outcrossing the blight-resistant

trees onto flowering trees in the seed orchards established by TACFNY members (Figure 7). We will recommend planting the resulting seedlings, not in large blocks where they will cross with each other, but as widely spaced individual trees in natural stands containing isolated American chestnut trees to encourage another round of outcrossing. The resulting introduction foci will each contain a different sample of the genetic diversity of the original American chestnut, and with the assistance of members of The American Chestnut Foundation as well as squirrels, jays, chipmunks, turkeys and the many other wildlife species that dispersed the original chestnuts, we expect to see blight-resistant American chestnut trees once again recapturing a dominant position throughout the eastern forests.

Academic Recognition of the Chestnut Project

- Dr. Bill Powell received the 2013 Forest Biotechnologist of the Year award from the Institute of Forest Biotechnology. (Note: Dr. Powell received the award on December 12, 2013, just before the start of this reporting period, but this award is enough of a Big Deal to warrant repeating. – Chuck Maynard.)
- Drs. Bill Powell and Chuck Maynard received ESF's Exemplary Researchers award, April 2014.

National and international recognition in the popular press

- "The American Chestnut's Genetic Rebirth" by William Powell, *Scientific American*, March 2014.
- "How Genetic Engineering Can Save the Iconic American Chestnut Tree", *The Motley Fool*, March 16, 2014.
- "Chestnuts Are Making a Comeback", *The Daily Star*, March 28, 2014.
- "American Chestnut: A Test Case for Genetic Engineering?" *Wisdom* (Forest Guild Publication), April 2014.
- "NIFA Program Helps Re-establish the American Chestnut Tree in the United States", NIFA Newsroom blog, May 5, 2014. (National Institute of Food and Agriculture)
- "American Chestnut Set for Genetically Modified Revival", *New Scientist*, May 30, 2014.

Recent Publications & Reference Cited

Refereed 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, CD, WA Powell, CA Maynard, KM Baier, AE Newhouse, SA Merkle, CJ Nairn, L Kong, JE Carlson, C Addo-Quaye, ME Staton, FV Hebard, LL Georgi, AG Abbott, BA Olukolu. 2014. The Forest Health Initiative, American chestnut (*Castanea dentata*) as a Model for Forest Tree Restoration: Biological Research Program. Acta Hort 1019:179-189 http://www.actahort.org/books/1019/1019_27.htm

Newhouse, A. E., J. E. Spitzer, C.A. Maynard, and W.A. Powell. 2014. Chestnut leaf inoculation assay as a rapid predictor of blight susceptibility. *Plant Dis.* 98:4-9. http://dx.doi.org/10.1094 / PHIS-01-13-0047-RE.

Newhouse, A.E., L.D. Polin-McGuigan, K.A. Baier, K.E.R. Valletta, W.H. Rottmann, T.J. Tschaplinski, C.A. Maynard, W.A. Powell. (2014) Transgenic American chestnuts show enhanced blight resistance and transmit the trait to T1 progeny, *Plant Science* (in Press) Available online as of 4/13/2014 at: http://dx.doi.org/10.1016/j.plantsci.2014.04.004.

Book chapter (Accepted for publication)

Maynard, C.A., L.D. McGuigan, A.D. Oakes, B. Zhang, A.E. Newhouse, L.C. Northern, A.M. Chartrand, L.R. Will, K.M. Baier, and W.A. Powell. (2014). Chestnut, American (*Castanea dentata* (Marsh.) Borkh.) Chapter 13 In: Wang, K. *Methods in Molecular Biology. Third Edition: Agrobacterium Protocols.*

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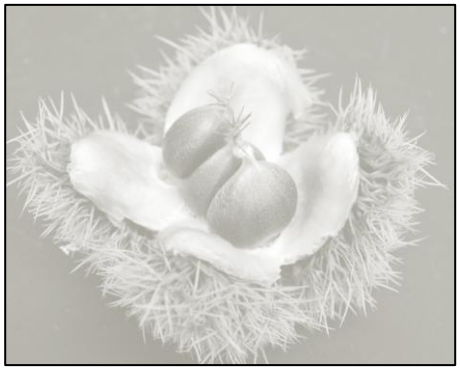


New York State Chapter of the American Chestnut Foundation, Inc.

24th Annual Meeting Agenda

Where: The Oneonta Country Club
9 Country Club Drive, Oneonta, NY 13820
www.oneontacountryclub.org

When: Friday, October 10, 2014 - Saturday, October 11, 2014

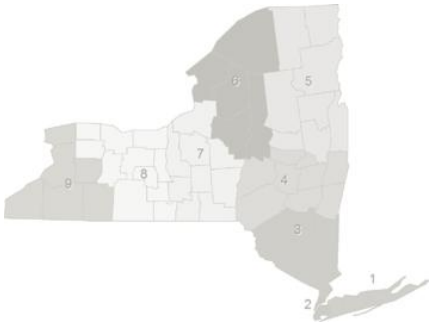
Friday (10/10) – Hampton Inn, 225 River Street, Oneonta NY 13820

6:00 pm till done Dinner at Pizza Land, 24 Oneida Street, Oneonta

7:30 pm – 10 pm Harvest Exchange and informal get together in conference room.

Meeting Agenda Continued**Saturday (10/11) – Oneonta Country Club**

7:30 am Coffee, tea, danish *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 **Herb Darling**
(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
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
1 – 3 pm Crumhorn Mountain **Afternoon Field Trip**

Workshops

3 – 3:45 pm - Session 1 **" Two tree orchards, why we need people to plant a "mother" tree now."**
4 – 4:45 pm - Session 2 *Announce winners to silent auction/50:50*
5:00 pm Afternoon session closes
6:00 pm Dinner
After Dinner Closing Remarks

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