2011-2012 Progress Report

Prepared for

The American Chestnut Foundation-New York State Chapter

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The 2011-2012 Chestnut Team

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Undergraduate students: Aaron Barrigar, Mike Cook, Ashoor Howell, Jessica Miller, Melody Papapietro, Caitlyn Snyder, Logan Will, Rachel Burris, Kristen Scroger, and Jesse Spitzer
Non-student: Andrew Teller

Past and present members of ESF's chestnut team (L to R) Bill Powell, Mike Satchwell, Sharon LaPierre, Kathleen Baier, Katie D'Amico, Andy Newhouse, Kristen Russell, Bernadette Connors, Amelia Zhang, Linda McGuigan, Haiying Liang, Lilibeth Northern, and Chuck Maynard

INTRODUCTION

We have been working with The American Chestnut Foundation, NY State Chapter since 1989. Until now, we have given our annual reports in the form of narrated PowerPoint presentations at the yearly meeting in October. We believe that this has worked pretty well for everyone. The drawback, however, is that many details and events that happen during the year either get left out or glossed over in the time we have to make our presentations.

Therefore, we are breaking with tradition and presenting a supplemental mid-year written report. There are three reasons for this. First, we are very excited about some of the things we have accomplished. Second, we hope this report will fill in the above-mentioned gaps. Third, because, candidly, we need additional funding by mid-summer or we are going to lose some key people who have been integral to our success. (A new grant proposal will follow shortly.)

The first section we titled "Really Big Deals" (RBD). We decided on this somewhat un-scientific sounding section heading because of Dale Travis, a regional coordinator for TACF-NY, and an ESF alumnus. Dale was the driving force behind the New York Botanical Garden Chestnut Event. He approached the administration of the Garden over a year ago, telling them about the Chestnut Project being a "really big deal." He then approached the ESF Alumni office once again calling the event, you guessed it, "a really big deal." In the spirit of Dale, an RBD to us signifies a major accomplishment that will have an impact on the whole project.

The second section "Ongoing Research and Production" describes some of the ongoing research the Chestnut Team is doing and a glimpse of how much material we still have traveling through the pipeline toward field trials. Even though we don't list these items as RBDs, they are still very important because they provide the foundation research that leads to RBDs.

The third section is titled "The Near Future." This is the preamble for a new grant proposal.

REALLY BIG DEALS (RBD)

NY Botanical Garden Chestnut Planting Event, RBD #1

On April 18, 2012, at the New York Botanical Garden in NYC, Bill and Chuck presented a lecture on the history of the American chestnut and highlights of their chestnut research to an audience of over 120 people.

This was followed by a tree planting ceremony, as shown in the photo below. We planted two

transgenic trees, the other eight having been professionally planted the day before. Based on the leaf assay test developed by Andy Newhouse (see RBD #5 for more detail), we are cautiously confident that one or more of these events will be blight-resistant.

The NY Botanical Garden was a very fitting place to plant our transgenic chestnut trees. It is literally across the street from where the blight was discovered in 1904.

At one point in the planting ceremony, Bill mentioned that not only was this the first time we had planted our trees in an arboretum, that it was to the best of his knowledge the first time ***anybody*** had planted transgenic plants of ***any*** kind in an arboretum. The ESF Chestnut team, TACF-NY, and the Botanical Garden can proudly share this distinction.

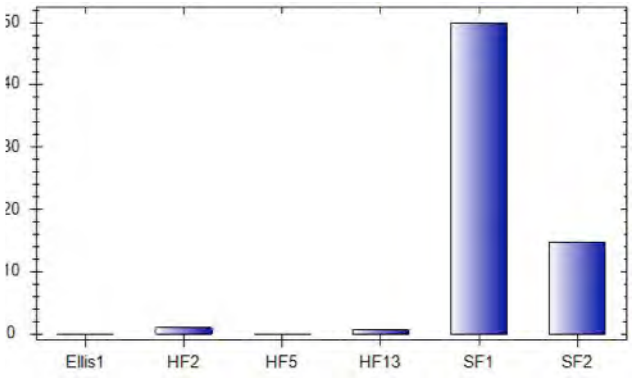
The evening was capped off by a formal dinner. Speakers included (L to R): Neil Murphy (on the right), President of the College of Environmental Science and Forestry; Bryan Burhans, President of The American Chestnut Foundation; Maude Hinchee, Chief Science Officer for ArborGen; and Dale Travis, a regional coordinator for the NY chapter, and the driving force behind the event.

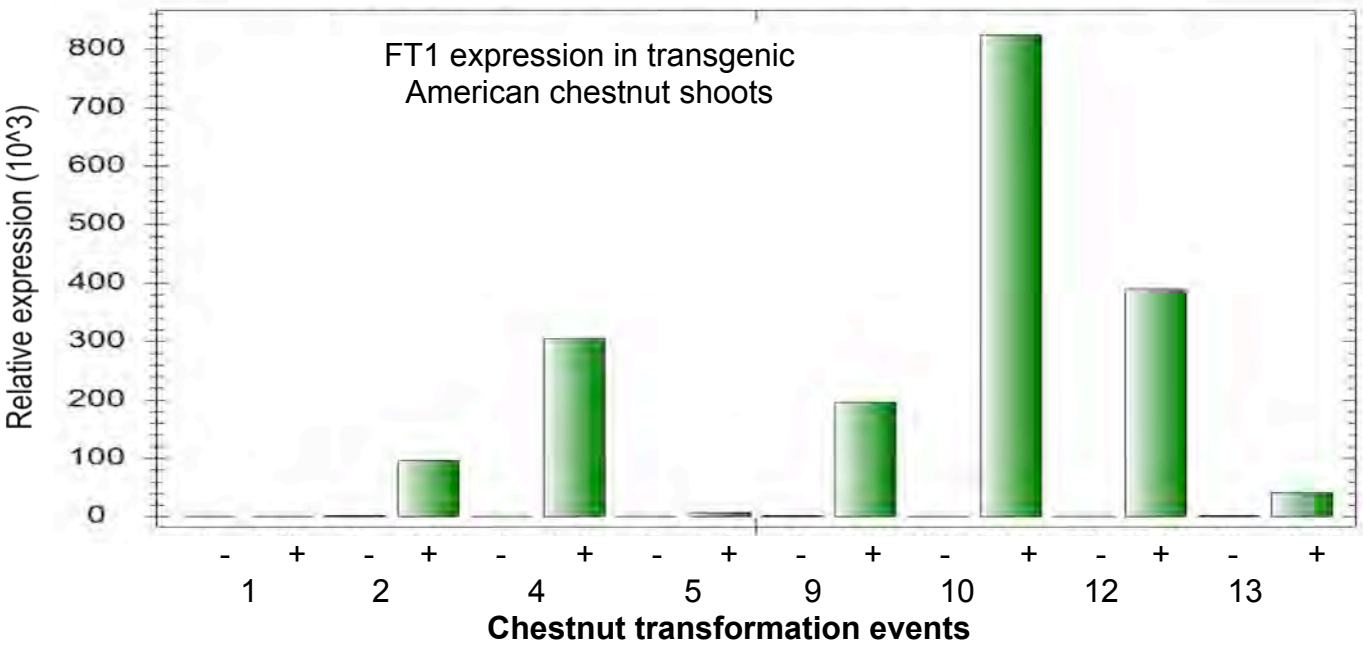
Early Flowering Gene Tests, RBD #2

We tested a gene from poplar (called PtFT1) that induces very early flowering in other tree species. The gene worked amazingly well in chestnut. Too well, in some cases! When coupled with a very strong promoter, the gene was so powerful that the transformed chestnut tissues began to flower in the Petri dish. Only two of these strongly promoted events ever reached the shoot stage, and they immediately formed male flowers (below left). These events will never be useful for breeding, but they did tell us that the gene clearly influences flowering in chestnut. We also tried the gene with a heat shock promoter. This is a promoter that keeps genes "off" as long

as the plants are at or below room temperature, and turns genes "on" when the plant is exposed briefly to high temperatures. Nine transformation events with this promoter were regenerated. Some of these events formed catkins spontaneously and will be discarded, but a few events have been regenerated into whole plants and appear to be growing normally.

FT1 expression, non-induced, in transgenic American chestnut embryos. In HF events, the FT1 gene is under control of a heat shock promoter. In SF events, the FT1 gene is under control of a 35S constitutive promoter.





RNA was extracted from chestnut shoots at ambient temperature (~22C) and from shoots induced at 40C for 4 hours. Uninduced shoots (-) had essentially 0.0 expression. Induced shoots (+) showed a wide range of expression.

When they are a bit bigger, we will "cook" them and see if they flower. Preliminary tests with tissue culture plants indicate that exposure to higher temperatures does indeed activate the promoter and start expression of this gene.

The reason this item made the RBD list is because it will significantly speed up the restoration part of the Chestnut Project. If it works as planned, this gene will be added to one of the constructs we transform into chestnut, causing the resulting transgenic tree to flower "on command." We then intend to collect the pollen and start making crosses to the TACF-NY "mother" trees. The seedlings from these crosses will be collected and grown in the greenhouse. When they are a foot or so tall, we will briefly "cook" them and wait. Some of them will contain resistance genes and the early flowering gene. These will flower and they will be used to pollinate more trees. Some will contain only the resistance gene. These won't flower and can be distributed for commercial use. Each generation will be similar, producing field-ready transgenic seedlings as well as breeding plants. Only the offspring *without* the flowering gene will be moved to the field, but using this gene in the greenhouse will greatly speed up generation times and allow faster integration of broader genetic diversity into the restoration program.

Physiologically Induced Early Flowering, RBD #3

Kathleen Baier discovered that 16 hours per day of high intensity light (approximately 1/3 full-sun), produced by high pressure sodium and metal halide lamps in our large walk-in growth chamber, will induce catkin formation in both Chinese and American chestnut, and female flowers in Chinese chestnut, in as little as six months. This treatment could shorten the breeding cycle to a single year, even without additional transgenes. Theoretically, and we emphasize the word theoretically, a backcross B3F3 tree could be produced in about 6 years starting from new F1 hybrid American x Chinese seedlings.

(A manuscript has been submitted to the Journal of The American Chestnut Foundation.)

This one made the RBD list for the same reason the flowering gene item did; it could significantly speed up the breeding cycle. In addition, this one involves no genetic engineering, and, therefore, no USDA permits. The backcrossing team at Meadowview could start this TOMORROW. It would cost a small fortune to buy enough growth chambers to bring the breeding program entirely indoors, but for elite lines, it could be a quicker way to produce pollen in young trees.

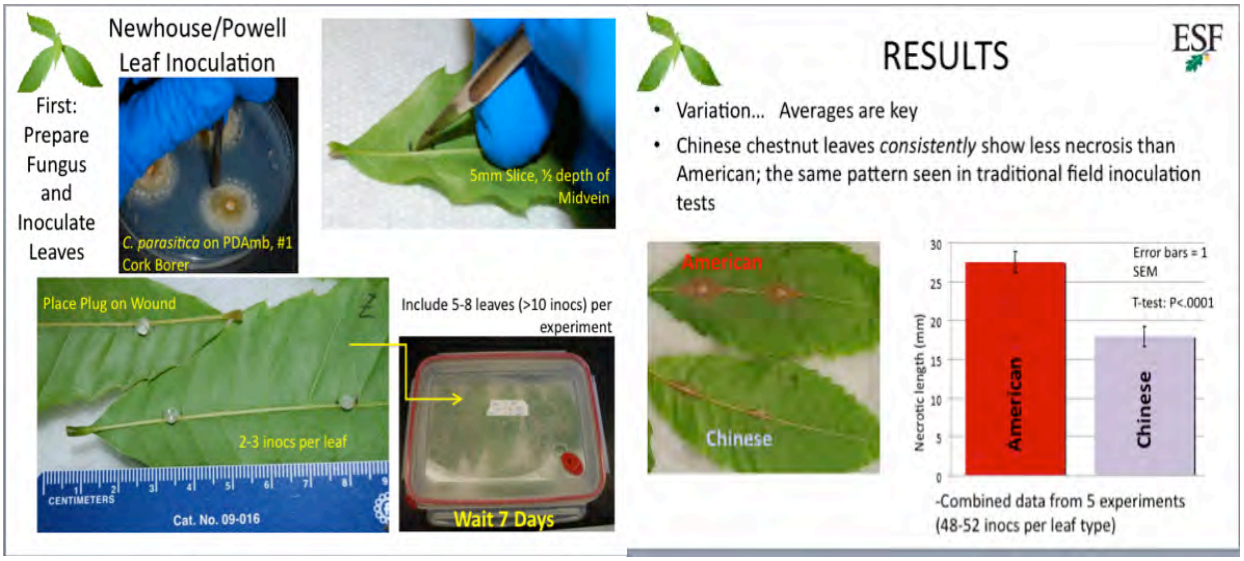
The first cross between transgenic and wild-type American chestnut trees produced viable (and definitely transgenic) progeny, RBD #4

During the 2011 summer, our first transgenic event (Wirsig) produced pollen, and some of our non-transgenic control trees produced female flowers. This was just too exciting an opportunity for an old-time tree breeder like myself (Chuck) to pass up. We collected pollen from a transgenic Wirsig tree, and Andy Newhouse and Aaron Barrigar used it to pollinate several non-transgenic trees. Both male and female flowers and the resulting burs were contained through the whole season with bags to prevent any escapes. From these crosses, I collected 34 filled nuts. These were stratified over the winter and potted up in February and March. We now have 11 second-generation transgenic chestnuts growing in the greenhouse that will be planted in the field this spring, and four additional trees were shipped to Buffalo that will be planted in the Zoar Valley orchard. The GFP marker gene is very strongly expressed (see above). This is proof of the concept that transgenic American chestnut trees can produce viable nuts that germinate and grow as rapidly as wild-type nuts, and that the transgenes can be expressed in offspring. We are planning to perform a larger cross this summer, hopefully with some of our more recent transgenic events using pollen collected from the growth chamber trees mentioned in the last section.

Early Blight-Resistance Assay Development, RBD #5

"There absolutely *has* to be a better way to test trees for blight resistance than to plant them, wait three or four years, and then stick some fungal hyphae in a hole in the bark."

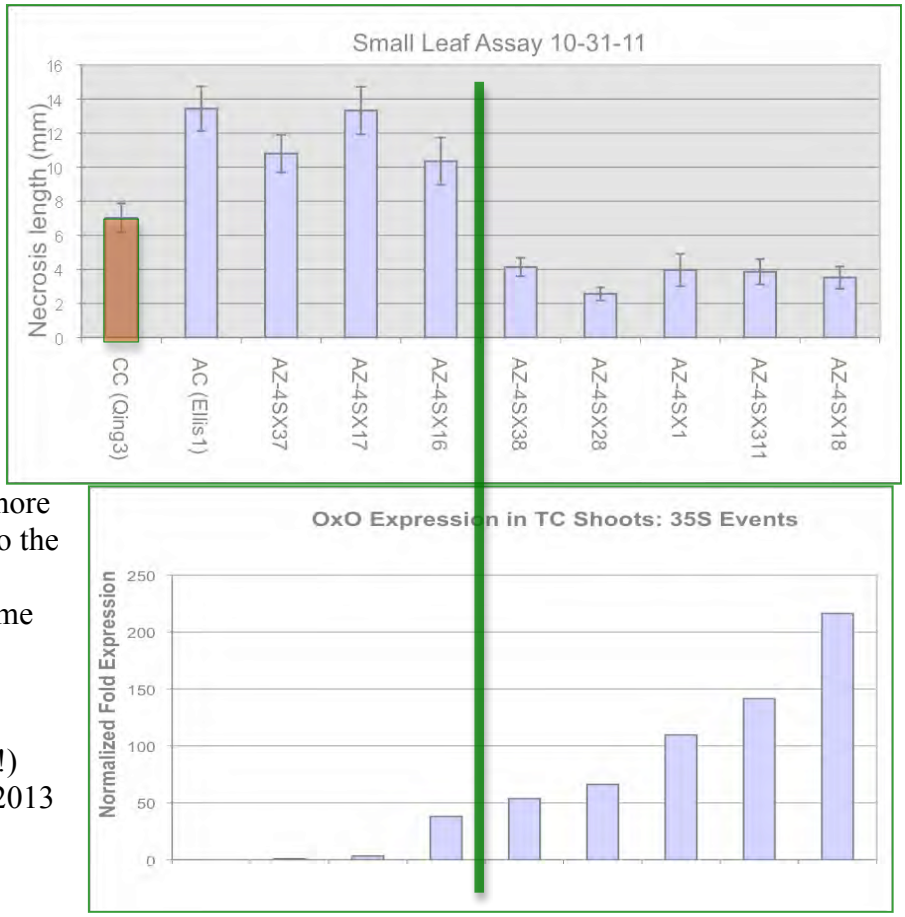
This sums up what Bill Powell and his team have been trying to achieve for more than a decade. The leaf assay, described below, is very close.



Andy Newhouse developed a quick and simple leaf assay. This test uses individual leaves removed from greenhouse-grown seedlings. Tiny wounds are made on the leaf midveins, an equally tiny volume of *Cryphonecria parasitricia* inoculum is placed on the wound, and the leaves are incubated for 5 to 7 days in mini-humidity chambers (which bear a striking resemblance to Tupperware sandwich boxes). The apparatus can be assembled in just a few hours, and the resulting necrotic areas are observable in 5-7 days. Andy has demonstrated that the leaf assay will consistently distinguish blight-susceptible American chestnut from blight-resistant Chinese chestnut. This means that the necrosis length in the leaf assay correlates to canker size in field inoculations. Furthermore, preliminary results indicate some of our transgenic events expressing oxalate oxidase (OxO) appear to have intermediate levels of susceptibility between American and Chinese chestnut seedlings. This one made the RBD list because it will help screen out the least promising events in the greenhouse stage. We will now be able to efficiently focus our field-testing resources on events with the best potential for blight resistance. This also sets the stage for the last item on our Really Big Deal list.

Better-Than-Chinese Blight Resistance? Maybe, maybe not… (We really need to find out!), RBD #6

Amelia Zhang built a transgene construct with a constitutive (always-on) promoter (CaMV 35S) driving an OxO gene and used it to transform American chestnut. Not surprisingly, there was a good deal of variation in transgene expression level among the events (bottom graph). More interestingly, there was a strong inverse correlation between the OxO expression and leaf assay necrosis (top graph). In other words, when there is MORE OxO enzyme produced by the transgene, there is LESS necrosis visible in a leaf assay, suggesting that these events would have smaller cankers in the field. Amelia's most important finding was that five events had leaf necrosis scores (bottom graph) equivalent to, or even better than, the Chinese chestnut (CC) control (orange bar). Her best five events, Darling 18, 311, 1, 28, and 38 (to the right of the vertical green bar connecting the two graphs), are being multiplied as rapidly as possible. We only have about 10 of these ready to go into the field this spring (2012), but many more are being propagated to go into the Fall 2012 and Spring 2013 inoculation-trial plantings. Some plants from these events have already been moved into the flower-inducing, high-light growth chamber to (hopefully!) produce pollen for crosses in 2013 (See RBDs # 3 & 4). The inoculation-plot trees will be



ready to inject and score for resistance in 2014 or 2015. If the traditional field inoculation test is strongly positive, it will validate the leaf assay. Far more important, we will have one or more blight-resistant American chestnut trees, produced by careful application of the best of modern biotechnology. Now, that will be ***The*** **Really Big Deal!**

ONGOING RESEARCH AND PRODUCTION

The Chestnut Project has become a mixture of research studies and simple bulk production. To start with, we had to learn how to assemble genes from many sources into little packages of genes called constructs. Then we had to produce many constructs. This was followed by learning how to put those constructs into chestnut cells. Next we had to produce many chestnut cells with constructs. This goes on for each stage of the pipeline, culminating in (at least for now) with field trials.

There are two important points to the above narrative. First, the research part is fun and exciting, and probably publishable. The subsequent "production phase," however, is often mind-numbingly tedious, boring, and consumes enormous quantities of supplies. As more and more of the pipeline enters the production phase, we will have to make sure whenever possible to distribute both the research and the drudgery so that no one experiences burnout (or has to undergo carpal tunnel surgery!).

Research

Putting new clones into culture

Allison Oakes obtained a grant from TACF to develop a method to induce bud break in dormant chestnut stems so they can be used to start shoot cultures. This will provide a quick way to clonally propagate elite lines of chestnut from either the backcross breeding program or our transgenics program. For example, if there is a particular B3F3 tree that shows good blight multiplied into many thousands of field-ready little trees, so this same B3F3 individual could be tested far more thoroughly. If it proves to be blight resistant, the clonal "progeny" called ramets, can then be distributed for further tests, be planted into wild-type chestnut orchards, or be sold to the public for commercial plantation use.

Storage of embryo cultures and shoot cultures

The Tissue Culture Lab is in danger of being overwhelmed by maintenance of cultures, both embryos and shoots. We badly need to find ways to reduce the amount of time and money we are spending on routine transfers of both these tissue types.

Long-term cryo-storage

Cryostorage (liquid nitrogen temperature -320F!) is usable for embryo tissue, and it has the advantage of longevity. Ultra-frozen tissue samples will keep for decades, but the recovery time when cultures thaw out can be several months. Cryostorage is like a safety deposit box; it is a great place to store the fancy jewelry, but you wouldn't keep your billfold in there. In our case, we plan to store small backup samples of each embryo cell line we might ever want to use again. Other than regular replacement of liquid nitrogen tanks, no maintenance is required until the cell lines are eventually removed and regenerated.

Linda McGuigan and Kevin Johnson, an undergraduate student working on an independent study project, are carrying out cryopreservation experiments with the new Liquid Nitrogen Storage system purchased with funds from our last grant from TACF-NY. When the bugs are worked out of this system, we will be able to put many transgenic events, as well as parent cell lines, into essentially suspended animation. This may also be usable for shoot cultures we aren't actively using. Early tests with embryos are promising, and we hope to have a workable system in place by mid-summer.

Short-term cold storage

Cold storage (refrigerator temperature ~36F) is usable for embryo or shoot cultures, but it only slows growth of the tissues; cultures still need to be transferred to fresh media at 6 to 12 month intervals. However, they can be removed from storage, warmed up in a few hours, and be growing happily within a week. We plan to use this type of cold storage to store shoot cultures of events that have gone into field tests but have not yet been scored for resistance. This will allow us to propagate more of those plants if necessary, but will greatly reduce the time and costs associated with regular maintenance transfers.

Allison Oakes is currently testing whether American chestnut embryos can be stored for 3-6 months at 4C to reduce the number of transfers per year from 12 to 4 or even 2. She is multiplying up extra shoot cultures to see if these can also be placed in cold storage.

Increasing Growth and Survival

We still need to improve the survival and initial growth of our transgenic plantlets. The first move is from the tissue culture vessel into a container with potting mix in a growth chamber. The next move is into the greenhouse. The third move is into the field. We lose plants in all three of these moves. The studies described below are our current efforts to ameliorate these losses.

During Acclimatization

We have come a long way in getting American chestnut into tissue culture and back out again. Although our techniques have improved, we feel they can get even better. One major bottleneck of the tissue culture process is during the acclimatization stage in the growth chamber. Our old growth chamber was very inefficient. Our new ones, purchased with matching funds from the New York Chapter of The American Chestnut Foundation, are terrific. However, the chestnuts in the chambers are showing signs of nutrient deficiencies. Linda McGuigan and Logan Will are performing fertilizer studies to determine what is causing these deficiency symptoms in older chestnut leaves, specifically interveinal chlorosis. We have been planting our tiny trees in Fafard's Super-fine Germinating Mix and have been using Miracid and Hoagland's solution to fertilize; however, neither fertilizer is sufficient in keeping away the chlorosis. Miracid lacks both magnesium and calcium. By trying different combinations of Miracid with these two nutrients as well as other micronutrient fertilizers, we feel we can increase growth and survival during this very crucial stage.

During Hardening Off

We have had rather mediocre success growing chestnut plantlets as well as seedlings in the greenhouse. As an alternative, Andy Newhouse built two shade tents with simple drip-irrigation systems at the Lafayette Road Experiment Station. He then carried out several studies using some plantlets that had been hardened off in the greenhouse and some that came directly out of the growth chambers. In total, Andy planted 153 chestnut plantlets in the shade tents. Initial results look promising. Trees in these tents readily acclimatized to outdoor conditions, even without "hardening" time in the greenhouse, and the root systems look very healthy.

Transplanting tests, to move trees from these tents to other plots, and shipping them bare-rooted to more distant sites, are underway.

Production

Constructs and events in the upstream part of the pipeline

The 'Travis' events contain a gene that codes for the enzyme laccase that is just like the laccase found naturally in Chinese chestnut. The 'Radel' events are pyramid constructs, containing three genes (OxO, ESF39, and laccase). Forty more 'Travis' events are still in the pipeline, and many will be ready for late 2012 or 2013 plantings. Eleven 'Mansfield' events, which contain a PRP1 gene from Chinese chestnut, are in the pipeline for a targeted planting in 2013.

Moving on down the pipeline

Since May 2011, Allison Oakes has regenerated 65 new transgenic American chestnut events from embryo cultures and as of May 1, 2012, has more than 50 shoots of each event. In total, she has over 3,250 shoots from these new lines in tissue culture. She is multiplying these rapidly.

All together (including controls) we have 35 gene constructs in the pipeline (see Appendix). We intend to produce around 10 events from each construct, and for each event, we will need approximately 25 trees to test for blight resistance. Taken all together, 35 constructs x ~10 events/ construct x 25 trees per event, this equals 8,750 trees that we will have to plant, prune, water, mow around, inoculate, and score for blight resistance.

Moving into the field

In addition to the planting out more of our original 'Wirsig' and newer 'Darling' and 'Hinchee' events, we have one 'Travis' event (36 trees total) and five 'Radel' events (60 trees total) ready for field planting this spring (2012).

Field Tests

Three new test sites have been established in the last year. The first is in Belleville, New York, in cooperation with the Belleville-Henderson School District. The plot is located on site with highly productive agricultural soil but in a considerably colder part of the state. Mean January temperatures are 6 degrees colder than in Syracuse. We will use this site to test for cold hardiness. Second, a new test site was established in Saratoga, New York, in cooperation with the Department of Environmental Conservation. The site is located on the DEC Saratoga Tree Nursery. For our third test site, ten trees were planted at the New York Botanical Garden, as described previously. Taken all together, we now have 436 transgenic American chestnut trees, and about an equal number of non-transgenic controls, distributed among eight locations in NY State.

THE NEAR FUTURE

This has been a watershed year for the Chestnut Project. We have accomplished some game-changing RBDs.

- We now have a reliable (and not too leaky) pipeline producing a steady flow of new events with new constructs, ready to be evaluated for blight-resistance.
- We have a leaf assay that will help us sort quickly through the events.
- We have five events, all showing a lot of potential, almost ready for field-testing.

The first little spurt to come out of the pipeline ('Wirsig') was so encouraging. The next few showed even more promise, and now there is a steady flow of potentially blight-resistant events. We think this is a very exciting time to be part of The American Chestnut Research and Restoration Project.

We have submitted an application for renewal of our USDA BRAG grant and are waiting to hear back. We also are in the process of submitting a renewal proposal to the Forest Health Initiative. Soon, we will be submitting a new proposal to TACF-NY. If two or all three proposals are funded, we will make rapid progress toward our ultimate goal of producing a blight-resistant American chestnut tree. If only one of the proposals is funded, we will have to cut back on personnel and will undoubtedly move at a considerably slower pace. If none of these proposals is funded… Well, I guess we (Bill and Chuck) would be spending a ***LOT*** of time in the lab!

We hope that you will continue to help us reach our mutual goal, the restoration of the American chestnut. We are getting closer each day.

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PUBLICATIONS

Published

Zhang, B., A. Newhouse, L. McGuigan, C. Maynard, and W. Powell. 2011. Agrobacterium-mediated co-transformation of American chestnut (*Castanea dentata*) somatic embryos with a wheat oxalate oxidase gene. (Extended abstract for the IUFRO meeting in 2011) BioMed Central (BMC) Proceedings 2011, 5(Suppl 7):O43.

In Press

Oakes, A.D., N.A. Kazcmar, C.A. Maynard, and W.A. Powell. 2012. "Vegetative Propagation of American Elm Varieties from Softwood Cuttings" Journal of Environmental Horticulture. 30(2):73–76.

Submitted

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 (submitted 5/5/2012).

Zhang, B., A. Oakes, C. Maynard, and W. Powell (in review). Enhancing Agrobacterium-mediated co-transformation of American chestnut (*Castanea dentata*) somatic embryos. In Vitro Cellular and Developmental Biology-Plant.

Zhang, B., A. Oakes, A. Newhouse, K. Baier, C. Maynard, and W. Powell (in review). Transgenic American chestnut (*Castanea dentata*) expressing oxalate oxidase shows reduced *Cryphonectria parasitica* necrosis. Transgenic Research.

Appendix

vector	Variety	Host Line	Event Code	Control	CG	Events SE	PCR+	Tissue Events Culture	Events having Shoots	Events in Pots	Trees in Pots	Trees in Field
totals:						**140**		**81**	**2316**	**97**	**958**	**437**
pVspB-OxO	Wirsig	WB275-27	LP-2VX28	V	OxO	1	+	1	32	1	2	39
pGFP only	control	WB275-27	AN-2XG#	none	none	2	+	2	32	1	13	52
pTACF3	Darling	WB275-27	AN-2XG#	V	OxO	7	+	9	96	9	58	171
pESF-KBO	Darling	Ellis 1	ALD-4XX#	V	OxO	1	+	1	112	61		4
p35S-OxO	Darling	Ellis 1	AZ-4SX#	C	OxO	17	+	17	500	11	250	1
pTACF7	Hinchee	WB275-27	KS-2PG#	V-V	OxO + ESF39 AMP	2	+	2	12	2	114	158
pESF-KBLOE	Radel	Ellis 1	LN-4LE#	V-V-V	codon optimized chestnut laccase +OxO + ESF39 AMP	7	+	7	672	6	464	9
pESF-KBLO	-	Ellis 1	LN-4LO#	V-V	codon optimized chestnut laccase +OxO	3	+					
pESF-KBL	Travis	Ellis 1	LN-4LL#	V	codon optimized chestnut laccase	6	+	1	96			1
pGFP only	control	Ellis 1	AZ-4G#	none	none	4	+	4	224	4	42	2
pWVK147	empty vector control	Ellis 1	AZ-4WK#	none	none	2	+	2	?	2	15	
pFHI-CmLac	Travis	Ellis 1	LN-4LC#	C	*Cm* laccase / diphenol oxidase	40	+	20	325			
pFHI-PRP1	Mans-field	Ellis 1	LN-4PR#	C	*Cm* Proline-rich protein (PRP1)	11	+	4	75			
pFHI-DAPH		Ellis 1	LN-4DP#	C	*Cm* DAPH synthase (DHS1)	7	+	4	65			
pFHI-AcPhos		Ellis 1	LN-4AP#	C	*Cm* Acid Phosphatase	4	+	2	45			
pFHI-LTP1		Ellis 1	LN-4LT#	C	*Cm* Lipid transfer protein identified by SSH	8	+	5	30			
pFHI-ETF1		Ellis 1	LN-4ET#	C	*Cm* Ethylene response transcription factor	5	+					
pFHI-AcOx1		Ellis 1	LN-4AO#	C	*Cm* ACC oxidase (1-Aminocyclopropane-1-Carboxylic Acid)	4	+					
pFHI-MIP1		Ellis 1	LN-4MP#	C	*Cm* Myo-inositol-1 phosphate synthase	5	+					
pFHI-VST1		Ellis 1	LN-4VS#	C	stilbene synthase gene (grape?) – phytoalexin resveratrol synthesis	4	+			p		
pFHI-CaAMP (Nov. selection)		Ellis 1		C	Animicrobial peptide (pepper?)							
pFHI-CcAOMT (Dec. - selection)		Ellis 1		C	*Cm* caffeoyl-CoA-O-methyltransferase							
pFHI-TAGL1 ((Dec. - selection)		Ellis 1		C	*Cm* Triacylglycerol lipase							
pSKDH1 (Dec. - selection)		Ellis 1		C	*Cm* Shikimate dehydrogenase							
pFHI-Gluc2 (Dec. - selection)		Ellis 1		C	*Cm* Glucanase (glucan endo-1,3-glucosidase)							
pFHI-GST7 (Dec. - selection)		Ellis 1		C	*Cm* GST U7 (glutathione S transferase)							
pFHI-PrOx (Dec. - selection)		Ellis 1		C	*Cm* Peroxidase							
pFHI-CAD (Dec. - selection)		Ellis 1		C	*Cm* CAD (Cinnamy-alcohol dehydrogenase)							
pFHI-RPH1 (Feb. - selection)		Ellis 1		C	*Cm* RPH1 (*Phytophthora* resistance)							
pFHI-Thau (Feb. - selection)		Ellis 1		C	*Cm* Thaumatin-like protein							
pFHI-SBTL1 (March - selection)		Ellis 1		C	*Cm* Subtilisin-like protease (Cucumisin precursor)							
pFHI-NPR34 (March - selection)		Ellis 1		C	*Cs* NPR3/4 (*Phytophthora* resistance?)							
pFHI-LTP2 (March - selection)		Ellis 1		C	*Cs* Lipid transfer protein (LTP) /proteinase inhibitor							

Key

Vector - a coded description of the genes in the construct

Variety - the name we have given to all transformants that are derived from a single construct

Host line - the name of the embryogenic cell line before it was transformed

Event code - molecular jargon that tells what transgenes went into the event

CG - a key word that describes the gene of interest

Event SE - the number of events in the somatic embryo stage

PCR+ - the new transformation event has been screened via Polymerase Chain Reaction; if the event is marked +, you have successfully inserted the gene of interest

Events in Tissue Culture, shoots, pots- the number of events, growing as embryos, regenerated into shoots, rooted and growing in soil, respectively.