

NE-1333 Technical Committee Meeting
Biological Improvement of Chestnut through Technologies
that Address Management of the Species, its Pathogens and Pests

Genesee Grand Hotel, Syracuse, NY

September 30-October 1, 2016

Attendance:

- Connecticut: Sandra Anagnostakis (Connecticut Agricultural Experiment Station)
Kentucky: Lynne Rieske-Kinney, Anna Conrad, Albert Abbott (University of Kentucky),
Tyler Dreaden (USFS-Lexington)
- Maryland: Donald Nuss (University of Maryland Institute of Bioscience and
Biotechnology Research, Shady Grove)
- Michigan: Evan Fannosi (Michigan State University)
- Mississippi: Angus Dawe, Di Ren, Soum Kundu, Gisele Andrade (Mississippi State
University)
- New Jersey: Bradley Hillman, Administrative Advisor (Rutgers University)
- New York: Bill Powell (Chair), Charles Maynard, Kristen Stewart-Russell, Linda
McGuigan, Andy Newhouse, Vernon Coffey, Yokshitha Reddy Bathula, Alex
Levine, Allison Oakes, Erik Carlson, Tyler Desmarais, Dakota Matthews
(SUNY-ESF)
- North Carolina: Paul Sisco (TACF®, Asheville)
- Pennsylvania: Sara Fitzsimmons, John Carlson (Pennsylvania State University), Gary
Micsky (Penn State Extension, Mercer), Mike Marshall (Shippensburg
University)
- Tennessee: Hill Craddock (Chair-elect), (UT Chattanooga)
- Vermont: Kendra Collins (TACF®, South Burlington)
- Virginia: Fred Hebard (TACF®, Meadowview), Laurel Rodgers, Fawzia Bhatti, Dillon
Richardson (Shenandoah University)
- West Virginia: William MacDonald, Mark Double, Cameron Stauder (West Virginia
University)

The meeting was called to order by Linda McGuigan at 8:30 am on 30 Sept 2016 at the Genesee Grand Hotel in Syracuse, NY. Dr. Quentin Wheeler, President, The State University of New York provided a welcome address. Dr. Neil Ringler, Vice Provost of SUNY research, provided information on history and facts about SUNY-ESF. Bill Powell, Professor and Director, Council on Biotechnology in Forestry at SUNY-ESF indicated that Charles Maynard, now retired, received the exemplary research award two years ago.

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OBJECTIVE 1. To develop and evaluate blight resistant chestnut trees for food and fiber through traditional and molecular techniques that incorporate knowledge of the chestnut genome

William MacDonald, West Virginia University

B₃F₃ Planting at the University Forest, Morgantown, WV. Two hundred advanced backcross seedlings were planted in April/Sept 2015 at the University Forest near Coopers Rock in Preston County. WVU forestry students, members of the Urban Forestry Club, helped with the planting. An additional 100 backcross seedlings will be planted in October 2016.

Backcross orchard for assessment of host resistance combined with hypovirulence (in cooperation with Fred Hebard and Sara Fitzsimmons, The American Chestnut Foundation®). Six replicate plots each containing 150 trees have been established at the Plant and Soil Sciences Farm in Morgantown, WV to assess the interaction of host resistance and virulent/hypovirulent strains of *Cryphonectria parasitica*. In three plots, naturally occurring cankers were treated with hypovirulent isolates; three plots were not inoculated. Seeds were planted annually from 2006-2011. As of July 2016, overall survival was 70%. Average diameter, height and survival data for 2016 are listed in the following table.

Species	Total	Percent Dead Since 2013 Inoculations	Average		
			Diam. (cm)	Ht. (m)	Tallest (m)
American	181	25%	4.2	3.3	8.7
B2F2	22	5%	8.6	4.8	6.3
B2F3	220	12%	4.9	3.8	11.5
B3F2	134	20%	4.1	3.6	10.3
Chinese	189	3%	7.2	5.5	10.9
European	154	41%	3.5	2.5	7.4

On 31 July 2013, eighty-seven trees >3 cm (17 American; 42 BF2; 11 BF3; 25 Chinese; and 13 European) were inoculated with Weekly-2, a moderately virulent *C. parasitica* strain. Growth, sporulation and canker morphology have been assessed annually to determine host response to the inoculation with the virulent strain. Canker size $[(L+W)/2]$ was measured in Aug 2016 three years after inoculation. The percentage of trees that have died from either artificial inoculation with WK-2 or from natural infections also was assessed in 2016, and is listed above.

All naturally-occurring cankers in the three hypovirus-introduction plots were treated during the 2013-2016 growing seasons with a hypovirulent slurry (Euro 7, COLI, GH2 and Weekly/Ep155-pXHE7). In August 2014, naturally-occurring cankers that had been treated were sampled (4 plugs/canker). Sixty-five percent (15/23) of the cankers yielded at least one hypovirulent isolate. The treated cankers will be sampled and subjectively rated annually to assess growth, sporulation and host response.

A 0-4 subjective scale was used to assess tree health (4=main stem healthy; 3=main stem alive with some dieback; 2=main stem alive but badly blighted with dieback; 1=main stem dead with epicormics shoots; 0-main stem dead and no living epicormics shoots). The hv-treated and non-hv-treated plots were averaged based on species/hybrids. Ratings from an August 2016

assessment are listed in the following table summarizing trees that were living in 2013 when hypoviruses were first introduced.

Species/Hybrids	Tree Rating (0-4)	
	HV Plots	Non-Hv Plots
American	2.56	2.51
B2F2	2.57	1.2
B2F3	3.18	3.24
B3F2	2.5	2.86
Chinese	3.75	3.82
European	2.57	1.36

Cameron Stauder, West Virginia University

Observations of chestnut blight resistance, susceptibility testing, and hypovirulence.

The objectives of this study were: 1) to conduct comparisons of host resistance among American (*C. dentata*), European (*C. sativa*), Chinese (*C. mollissima*), and three American x Chinese hybrid generations (B2F2, B2F3, B3F2) generated by The American Chestnut Foundation (TACF®) to isogenic virulent and hypovirulent (CHV1) strains of *C. parasitica*; 2) to validate the high-throughput use and reproducibility of the chestnut leaf susceptibility assay with the same fungal strains on representatives across the various host backgrounds; and 3) to conduct comparisons among hypovirulent strains of *C. parasitica* using living branch inoculation, excised leaf, and apple assays. The comparisons of host resistance were conducted on a population of trees grown at the West Virginia University agronomy farm. Living stem infections were initiated with a virulent strain designated 'Weekly' and an isogenic, hypovirulent 'Weekly-CHV1' strain (CHV1-Euro7).

Subsequent canker measurements and stomata counts were performed every two months for a year to assess host resistance. For virulent Weekly inoculations, Chinese chestnuts had significantly smaller canker areas, but no significant differences were observed among the other hosts. Weekly-CHV1 cankers grew during the first two months of the study, but no subsequent growth was observed on any host despite the recovery of these isolates nine months post-inoculation. The excised leaf assay was conducted using leaves from a subset of trees included in living stem assay. Weekly and Weekly-CHV1 were used to inoculate the midvein of leaves from all previously mentioned host backgrounds. No significant differences were found for the Weekly isolate inoculations but the average leaf lesion area for American chestnut (78.5 mm²) was largest and Chinese chestnut (33.1 mm²) was smallest. For Weekly-CHV1 inoculations, Chinese chestnut (42.7 mm²) had significantly smaller lesions while all other hosts had similar leaf lesion areas with the exception of B2F2 (63.72 mm²). Weekly (58.1 mm²) produced a significantly smaller average lesion area across all hosts than Weekly-CHV1 (86.4 mm²). The virulence of selected hypovirulent isolates was studied through a living branch assay using a clonal clump of wild American chestnut sprouts, an excised leaf assay using leaves from the same wild clump, and an apple assay. Weekly-CHV1 once again produced significantly smaller cankers in the living branch assay than Weekly. Interestingly, Weekly-CHV1 produced larger lesions than Weekly in the leaf and apple assays while

all other virulent strains produced larger lesions than their hypovirulent counterparts. Here, a selection of American, European, TACF hybrid chestnuts were shown to be equally susceptible to stem infections of *C. parasitica*. The excised leaf assay produced similar results with regards to host response, but hypovirulent Weekly-CHV1 was found to produce larger lesions than virulent Weekly. This same observation was made for an apple assay and a second excised leaf assay. These findings provide evidence for unique interactions between a *C. parasitica* strain and the Euro7 hypovirus not previously observed.

Paul Sisco, The American Chestnut Foundation® (Asheville)

QTL analysis of resistance to *Phytophthora cinnamomi* derived from Chinese chestnut cultivars ‘Mahogany’ and ‘Nanking’ in BC₁ hybrid families. *Phytophthora cinnamomi* is a lethal, soil-borne pathogen of many plant species, including American chestnut. Asian *Castanea* species are resistant. Because *P. cinnamomi* is found in many locations in the southeastern US as far north as Pennsylvania, chestnut restoration efforts of The American Chestnut Foundation® include breeding resistance to this pathogen as well as to chestnut blight. Knowledge of the genetics of resistance to *P. cinnamomi* in Asian species will aid in development of an efficient and successful breeding program.

To determine the number and chromosomal location of Quantitative Trait Loci (QTLs) associated with resistance to the pathogen, Genotype-by-Sequencing (GBS) was used to analyze hybrid BC₁ families [(*Castanea dentata* x *C. mollissima*) x *C. dentata*] segregating for resistance to *Phytophthora cinnamomi*. The BC₁ families were derived from Chinese chestnut cultivars ‘Mahogany’ and ‘Nanking’, sources of resistance to chestnut blight being utilized by The American Chestnut Foundation®.

The BC₁ families were generated by controlled pollination at the Meadowview Research Farms (‘Mahogany’ family HB2) and at the Cliffs of Glassy, Landrum, SC (‘Nanking’ family NK4). In April of each year of the experiment, seed were planted in a replicated, randomized design at the Chestnut Return Farms (Seneca, SC). In July, seedlings were labeled and leaf tissue was harvested and stored in a -80°C freezer at Clemson University. Seedlings were then inoculated with two isolates of *P. cinnamomi* and left exposed to the pathogen for the remainder of the growing season. In December or January, when the plants were dormant, resistance to *P. cinnamomi* was scored using a 0 (no lesions) to 3 (dead) scale developed by S.N. Jeffers and J.B. James, based on visual examination of the seedling roots, as seen in the following table.

Hybrid families analyzed with Genotype-by-Sequencing

Hybrid Family Code - Year	Total Plants	Root rot symptom severity				Type of family	Source of resistance
		0	1	2	3		
HB2 - 2014	237	0	3	106	128	BC ₁	<i>C. mollissima</i> cv. ‘Mahogany’
NK4 - 2014	318	2	17	135	164	BC ₁	<i>C. mollissima</i> cv. ‘Nanking’

At the Clemson University Genomics Institute, DNA was isolated from the leaf tissue, two restriction enzymes were used to generate fragments of appropriate length for sequencing (~200-700 bp), and linkers were added so that each sequence could be referenced to its seedling source. The DNA fragments were then sequenced at the Medical University of South Carolina (Charleston, SC). A large number of Single Nucleotide Polymorphisms (SNPs) were found to distinguish the parental genotypes of the BC₁ families, as many as 84,000 SNPs for the 'Nanking' NK4 family. A subset of SNPs was chosen, based on the amount of missing data in the seedlings composing each family. The final group of SNPs had less than 10% missing data in any one seedling. Genetic maps were generated using JoinMap4.1 (van Ooijen, 2006) and QTLs were identified using MapQTL6.0 (van Ooijen, 2004).

Four linkage groups corresponding to four of the 12 chromosome pairs of chestnut were found to have significant QTLs for resistance to *P. cinnamomi*. The non-parametric Kruskal–Wallis (KW) test was employed to detect association between markers and traits individually. In a second step, interval mapping (IM) analysis was performed to select markers significantly associated with the trait to find an initial set of cofactors. A backward elimination procedure was applied to the initial set of cofactors. Using a function of MapQTL6.0, the most significant markers were selected and used as cofactors in a multiple QTL method (MQM) analysis for QTL detection. A mapping step size of 1 cM was used for both the IM and MQM analyses. The LOD (Log of odds) thresholds for genome-wide QTL detection were empirically determined based on the Permutation Test with 1,000 iterations. A threshold LOD value of 2.8 was used to declare the presence of a QTL. Regions with a LOD score above 2.0 were also inspected for potential QTLs if in one of the two crosses significant signal was detected nearby.

Detailed genetics maps also were generated with both the HB2 and NK4 families, allowing the ordering of >400 scaffolds (HB2 map) and 4,196 scaffolds (NK4 map) in the *C. mollissima* reference map. The report by John Carlson for the PA Chapter – TACF® in these minutes references only the HB2 map results, because the much-improved NK4 map had been generated just before this meeting.

The results of this study clearly showed: 1) that more than one locus from *C. mollissima* was correlated with resistance to *P. cinnamomi* in these hybrid families; and, 2) different subsets of loci were correlated with resistance in each cultivar. In the HB2 family derived from 'Mahogany', loci on LGs A, E, and K were significant in the MQM mapping, whereas in the NK4 family derived from 'Nanking', loci on LGs C and E were significant, with a locus on LG K identified as just below the significance level.

LG_E appeared to have more than one significant locus, confirming previous work by Tom Kubisiak and Bode Olukolu (Kubisiak, 2010; Olukolu et al. 2012). The most significant locus in the HB2 family was near the central part of LG_E, whereas the most significant locus in the NK4 family was near the distal end of one arm of LG_E. The NK4 family also had a less significant locus near the central part of LG_E, perhaps the same locus as the significant one in the HB2 family.

Future work will focus on narrowing down the significant loci identified in this study with the goal of finding useful molecular markers for screening for resistance to *P. cinnamomi* in seedlings. A 'Nanking' F2 family of 325 seeds has also been phenotypically screened for resistance to this pathogen, which will help to identify any recessive factors in disease resistance.

John Carlson, Schatz Center for Tree Molecular Genetics, Pennsylvania State University

The Chestnut Genome Sequencing Project. In addition to John Carlson, Schatz Center for Tree Molecular Genetics, the project team includes Charles Addo-Quaye, Nathaniel Cannon, Lynn Tomsho, Daniela Drautz, Lindsay Kasson, Tyler Wagner, Nicole Zembower, Abdelali Barakat, Richard Burhans, Webb Miller, and Stephan Schuster at Penn State University; Steven Ficklin, Tatyana Zhebentyayeva and Chis Saski at Clemson University; Margaret Staton and Nathan Henry at the University of Tennessee; Bert Abbott and Dana Nelson at the University of Kentucky at Lexington; Jason Holliday and Mihir Mandal at Virginia Tech University; Nurul Islam-Faridi at Texas A&M University; and Fred Hebard, Tom Kubisiak, Jared Westbrook, Sara Fitzsimmons and Laura Georgi of The American Chestnut Foundation®.

Update. Version 1 of the Chinese chestnut genome has been available to the public since January 2014 at the website <http://www.hardwoodgenomics.org/content/tools>, curated by Margaret Staton at the University of Tennessee-Knoxville. The version 1 genome assembly (for TACF cv. Vanuxem) consisted of 724.4 Mb in 41,270 scaffolds, averaging app. 40,000 bp in length. A total of 36,146 gene models and 38,146 peptide sequences were machine-predicted, with gene expression support. In addition, BAC contigs spanning the 3 blight resistance QTL (identified in the early F2 QTL mapping population) were sequenced and assembled into a total of 395 scaffolds. A total of 1,952 genes were predicted and annotated in the QTLs, including 194 known stress-response genes, from which 15 candidate genes for blight resistance were selected for further study. The website has had thousands of visits from across the globe for use of the genome browser and the QTL browser, and for searches and downloads of data from the scaffolds, gene models, predicted transcripts and predicted proteomes databases there (bigger pieces averaging ~40K bp).

They will soon release an improved and validated version 2 of the Chinese chestnut genome, for which the assembly consists of only 14,358 scaffolds representing 784Mb of genome sequence, or app. 98% of the estimated genome size. The 5,745 largest scaffolds were anchored to the integrated genetic-physical map to produce a set of 12 pseudo-chromosome sequences, representing the 12 linkage groups and providing 798 Mbp (98%) of genome coverage. The predicted gene positions have been transferred over to the pseudo-chromosomes, as well as the previously assembled QTL sequences. The arrangement of scaffolds in the pseudo-chromosome sequence assemblies has been validated by comparison to the order of thousands of DNA markers on new high density genetic linkage maps produced by Tatyana Zhebentyayeva at Clemson. We also await the production of very long genome sequences by the PACBio technology for further validation and gap closing. PACBio data generation is supported by a new USDA AFRI program grant that was awarded to TACF during the past reporting period. Vanuxem genomic DNA was prepared several times at PSU, but did not meet Washington State University PACBio service lab standards. Vanuxem leaves were collected from a tarp-shaded branch and sent in June from TACF to Arizona Genomics Institute for DNA extraction by their PACBio sequencing support staff, which proved successful. Presently we are in a queue for PACBio sequencing at Arizona Genomics Institute in February.

To test the value of the chestnut reference genome for use in genetic variation studies and in Genome-Wide-Selection in the TACF breeding program, we produced app. 10X depth sequence data in 2015 for the following chestnut genotypes from CAES and TACF orchards: one *C. alnifolia* genotype, one *C. crenata* genotype, five *C. dentata* genotypes (GMBig, Ted Farm A, Alex R, Huan Row1Tree18(MK5), and Ellis 1), one *C. henryii* genotype (Chinese chinkapin), four *C. mollissima* genotypes (Mahogany, Nanking, PA Fat Camp, and PA Stone Valley), one *C. ozarkensis* genotype, one *C. sativa* genotype, one *C. sequinii* genotype, three third backcross hybrids from the TACF breeding program (from parents B3119 x B3176), and the BC3 *C. dentata* x *C. mollissima* parental genotypes - B3119 and B3176. Alignment of the parental and BC3 genotype sequences to the Vanuxem reference genome provided a very clear display of the varying extents of transition of the genomes towards American genome content as a result of the backcrossing process. These results were presented at the TACF annual meeting in October 2015. Jason Holiday and Jared Westbrook are now developing a Genome-Wide-Selection Model for use in the TACF breeding program, with funding from USDA. The Staton group produced a set of potentially diagnostic 714,039 SNPs supported by sequencing from all three American genotypes for use in developing the GWS model(s).

Jason Holiday also produced deep RNA sequence data from 9 tissues from grafted clones of the Vanuxem reference genotype. Margaret Staton's group is mapping the RNAseq data to the new pseudochromosomes to validate and update the computer-predicted gene models.

The 2015 annual meeting of The American Chestnut Foundation was hosted by The Schatz Center for Tree Molecular Genetics and held at the Penn State conference center on October 23 and 24, 2015. The focus of the meeting was to update the TACF® membership on the status of and discoveries from chestnut genomics, and plans for integrating the genome resources into TACF advanced breeding efforts. Excellent keynote talks were presented by Antoine Kremer and Ronald Sederoff on the history of forest tree genomics and biotechnology. Talks on the state of genomics with chestnut and other Fagaceae species were presented by Albert Abbott, Catherine Bodénès, Nathaniel Cannon, John Carlson, Rita Costa, Angus Dawe, Jason Holliday, Nurul Islam-Faridi, Scott Merkle, C. Dana Nelson, William Powell, Jeanne Romero-Severson, Margaret Staton, Jared Westbrook, and Isacco Beritognolo for Fiorella Villani. Over 200 people attended, and the TACF® membership uniformly expressed their sincere appreciation to all of the speakers. The meeting also included hands-on workshops on chestnut DNA extraction and use of the chestnut genome browsers, along with a tour of Sara Fitzsimmons' and Kim Steiner's BC3 trial in the Arboretum at Penn State. Finally, a discussion forum was held that brought together chestnut genomics researchers and TACF® members to discuss next steps in use of genomics tools in the breeding and reforestation efforts.

Plans for the coming year. Work in the coming year will focus on:

- Validate and improve the Chinese chestnut pseudochromosome sequences using very long genome sequences produced from the PacBio single molecule sequencing technology (USDA AFRI grant to TACF®).
- Obtain deep RNA sequence data from several tissues of Chinese chestnut cv. 'Vanuxem' to refine the identification and annotation of genes in the reference genome.
- Submit refereed journal article on Chinese chestnut reference genome (in preparation).

Bill Powell, SUNY-ESF

American chestnut research and restoration project. Powell thanked Chuck Maynard for his many years of significant contribution as co-director of the project. Maynard has retired.

Genetic engineering approach to blight resistance—what genes are being tested? To date, they have seven:

1. Acid phosphatase (*C. mollissima*)
2. Laccase-like protein (*C. mollissima*)
3. Lipid transfer protein (*C. mollissima*)
4. Cystatin (*C. mollissima*)
5. Glutathione s-transferase (*C. mollissima*)
6. Deoxy-arabino-heptulosonate phosphate synthase (*C. mollissima*)
7. Subtilisin-like protease (*C. seguinii*)

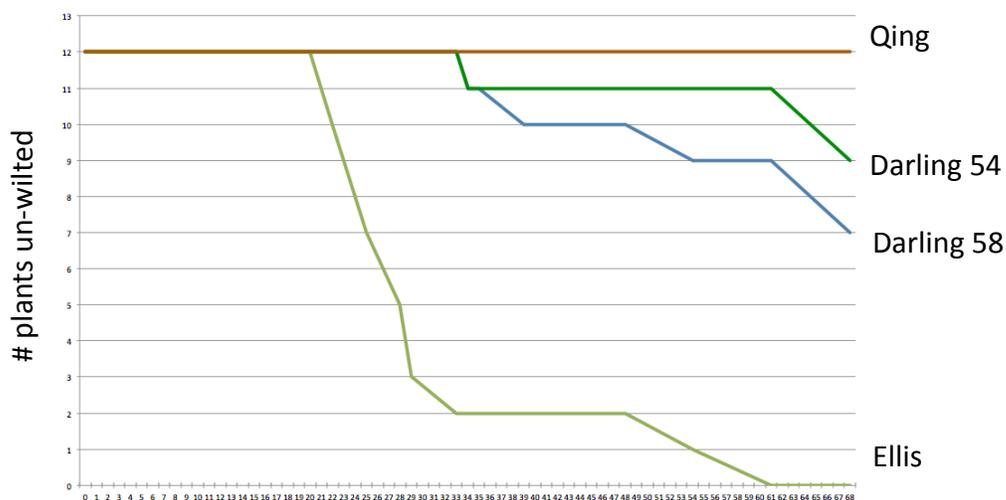
Funding for the above 7 genes has run out, so these projects are on hold. Powell has a few students working on some of these genes but it is not their current focus.

Genes from other plants. Powell reiterated that it is not the source of the gene that is important—it is the function of the gene that is key. Their current focus is on:

- Stilbene synthase (grape) – phytoalexins like resveratrol (Dr. Joe Nairn, UGA)—this gene has been shown to have enhanced resistance to *Phytophthora*
- Oxalate oxidase [OxO] (wheat and many other plants) (Dr. Randy Allen, Texas Tech)

The OxO gene has been the most promising to date. The ‘Darling’ transgenic lines show a great deal of promise, based on a small stem assay with virulent *C. parasitica* strain Ep155. After the Penn State meeting (mentioned by John Carlson, these minutes), there was some discussion on the Chinese chestnut control (‘Qing’) used in stem assays when comparing the transgenic lines. To test ‘Qing’, Powell obtained some Chinese chestnut ‘Nanking’ to also use in comparison trials. ‘Nanking’ has been put into tissue culture by Allison Oakes, but the plants are not large enough to test.

Powell showed the results from a 68-day assay on small stems (12 plants/cultivar) using: 1) Qing; 2) Darling 54; 3) Darling 58; and 4) Ellis. The latter three are all clones except that Darling 54 and Darling 58 have the OxO construct located in different parts of the genome. The number of wilted plants for each cultivar after inoculation with Ep 155 are seen in the figure below.



In order to assure that the virulent isolate they use in these assays maintains virulence, they inoculate a tree with Ep 155 and reisolate from resulting cankers.

In addition to greenhouse testing, field testing also is being conducted with T1 offspring. (T1 = F1—it's an outcross with a transgenic tree). They had offspring from Darling 311 with high blight resistance that had enough size to be field tested this year. Field inoculations are made with a nit-picker (similar to a crochet hook). The small hook allows for a slight scratch in the bark.

Powell discussed the unique feature of the 'Darling' transgenic chestnut. His goal is to use pollen from 'Darling' trees to pollinate TACF-NY mother trees, surviving wild American chestnuts, and backcross trees. Using 'Darling' pollen with regionally adapted trees will allow allelic rescue, provide local adaptation and increase genetic diversity. Because this is a dominant resistance gene, it will allow the ability to rescue the genetic diversity of the currently surviving trees. Half of all offspring will be fully resistant and can be identified by an easy leaf assay and each offspring will have a different complement of the mother's alleles. Outcrossing will increase genetic diversity and allow local adaptation through a "mother tree" program.

Looking forward. 3BUR is an acronym that stands for breeding, biotechnology and biocontrol, united for restoration. There are 13 action points in 3BUR, but Powell focused on just one action point, restoration demonstration forests. This will allow for environmental impacts of restoration. This is important for the general public to know that all impacts will be investigated, both positive and negative.

Powell and colleagues are already gathering foundation environmental data on:

- Terrestrial and aquatic insect feeding
- Leaf litter decomposition and seed germination
- Mycorrhizal colonization
- Metabolomics
- Enzyme activities
- Bumble bee feeding on pollen
- Growth and form

Demonstration forests:

- Part of stewardship plan during and post-regulatory review
- USDA BRAG connection: Comparison of environmental impacts of genetic engineered restoration trees to trees produced by traditional methods
- NSF Long Term Research in Environmental Biology (LTREB)
- Supports model development for forest restoration
- Begin to hand off the research from geneticist to ecologists

Test trees in the proposed demonstration forests include:

Two types of plantings are envisioned:

- **Agroforestry/orchard planting**—using clones or seedlings, planted in rows with some maintenance (more managed plantings)
- **Restoration planting**—25% chestnut and 75% trees and shrubs that typically grow with chestnut vernal pools, habitat shelters, random spacing. Included will be pollen trap trees

(sentinels) on each side of the planting using ‘Colossal’ hybrid trees. Powell envisions several 2.5 acre blocks with a visitor trail.

A key part is public access and surveys--educational trails with smartphone accessible stations will be established. Trees will be identified only as chestnut at each site; this will help reduce bias. Opinion questionnaire will be offered at the end of a visit. The site also would include a recruitment zone where no trees are planted. This would allow natural seeding of chestnut and other tree species. The plantings will include the following:

- **Transgenic** American chestnut (Darling 54 and Darling 58, OxO gene and NTP2 selectable marker)--outcrossed seedling and tissue culture trees
- **Backcross** B3F4 American chestnut--seedling and tissue culture trees
- **Hybrid** chestnut trees, Dunstan (most widely planted commercial) and one other--seedling (maybe TC)
- **Wild-type** American chestnut (with hypovirulence used to control blight--super donor?)--seedling and tissue culture trees
- **Control**--plots with no chestnut

Ideally, Powell would like demonstration plantings in three or four locations within the American chestnut range in conjunction with current and/or potential collaborators. Potential sites include: SUNY-ESF; PA/NJ chapter of TACF; VA TACF or VA Tech; Mississippi fish and wildlife or University of Georgia.

Out from the transgenic blocks in the plantings will be sentinel trees to ascertain distance of pollination. ‘Colossal’ chestnuts will be used as sentinel trees. Why use ‘Colossal’ hybrid chestnut as pollination test traps?

- Regulators are very interested in “effective” pollination distance
 - Information needed for isolation distance from organic crops
 - Information needed for restoration program when you want pollination
 - Current Literature: 1000 ft. for Amer. Chestnut, 400m for Euro. chestnut
- ‘Colossal’ is a Japanese/European hybrid that is male sterile
 - Will not contaminate transgenic American chestnut trees
- We have a clonal line
 - Cannot self-pollinate even if a rare catkin is produced
- Produces large chestnuts
 - Easy to identify burs that have been pollinated from others that are not pollinated
 - Only need to test pollinated burs
 - We have an easy and inexpensive OxO enzyme assay
- It is a popular agricultural variety
 - Model for agricultural orchard distances

Tyler Desmarais, SUNY-ESF

Improving plant health and survival of tissue culture produced blight resistant American chestnuts. Desmarais summarized the progress of their 2016 field production innovations, new orchard installations, and expansions to their various field research plots. These plots included the installation of two open pollination, seed production orchards (one in Tully and one in Zoar Valley),

expansions of their blight inoculation plots at Lafayette, and the expansion of their genetic diversity orchard in Tully. Desmarais covered the recent progress in their transgenic, blight resistant pollen production, which has improved the effectiveness of our controlled pollination efforts.

His presentation also projected ahead toward some of their upcoming field production goals including: 1) the continued expansion of our Tully and Zoar Valley open pollination orchards'; 2) the continued expansion of the Tully genetic diversity orchard; 3) the Lafayette inoculation plots; 4) construction of a shade house for outdoor container production; and 5) the Heiberg Restoration Planting experiment station.

Andrew Newhouse, SUNY-ESF

Transgenic chestnuts and the regulatory review process. Newhouse gave an update about the current status and upcoming plans for taking transgenic American chestnuts through the federal regulatory review process. Currently, all outdoor plantings are under strict USDA-APHIS permits, which just recently started allowing open pollination under certain circumstances. The FDA reviews new products for food and feed safety; ESF has some preliminary nutrition data indicating transgenic chestnuts are essentially equivalent to non-transgenic nuts, and will probably submit an application to the FDA first. The EPA regulates pesticides, which will be complicated in the case of this tree, but we're making progress with discussions with regulators and the process seems achievable. The USDA regulates environmental safety; again, ESF has a variety of preliminary data contributing to ecological interactions and environmental safety of transgenic trees. The opinions of non-regulatory groups, including federal (e.g. Fish & Wildlife Service) and NGO's (e.g. Nature Conservancy), also matter, even though they're not technically reviewing or approving the applications. Overall, ESF has received very positive feedback from the general public (everyone wants blight resistant trees ASAP!) and from regulators.

Dakota Matthews, SUNY-ESF

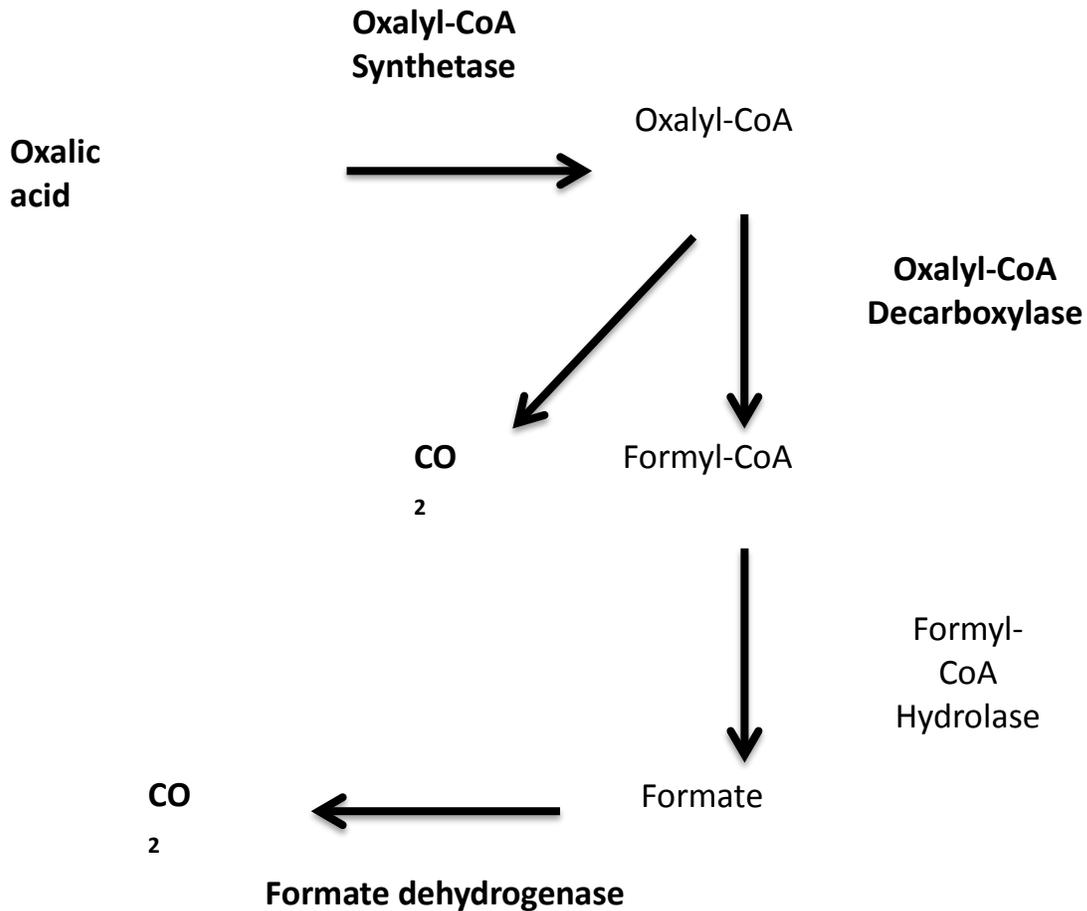
Methods for detecting presence and activity of oxalate oxidase. SUNY-ESF's transgenic American chestnuts have been transformed with the oxalate oxidase (OxO) gene. The gene product converts oxalic acid (the blight's main virulence agent) into hydrogen peroxide and carbon dioxide. The first assay employed was the OxO histochemical assay which directly stains tissues where oxalate oxidase is being expressed. This is a quick and easy test for screening new OxO plant sources and also ensures the transgenic events are expressing OxO before resistance assays. The second assay employed was the oxalic acid tolerance assay. Leaves were soaked in a 50mM solution of oxalic acid for 24 hours. The percent necrotic area was measured and compared to the still living tissue. This compared the transgenic event's ability to withstand oxalic acid to American and Chinese controls.

Other plant sources are being screened for OxO to show that transgenic trees are not adding anything new to the environment. Matthews tested old switch panic grass (*Panicum virgatum*). Endosperm of the grass highly expresses OxO. He also tested Virginia wildrye (*Elymus virginicus*) which had localized expression in the seed.

The following is a list of other grasses being tested for OxO.

- *Andropogon gerardii*-Big bluestem
- *Bouteloua curtipendula*-Sideoates grama
- *Carex stipata*-Wild awlfruit sedge
- *Schizachyrium scoparium*-Little bluestem
- *Scirpus cypernus*-Woolgrass
- *Sorghastrum nutans*-Yellow Indian Grass

Chinese chestnut does not have a known gene that converts oxalic acid directly like oxalate oxidase or oxalate decarboxylase that gives it resistance to not only the pathogen but to the acid itself. A pathway has been proposed that converts oxalic acid into carbon dioxide. This pathway was discovered in *Arabidopsis* and is outlined below.



Oxalate oxidase colorimetric quantitative activity assay. A quantitative OxO activity assay will be used to measure concentrations of OxO in the transgenic tissues (root, shoot, nut, and stem) and compared with concentrations of OxO in native species containing the gene. Seventy-five mg of ground plant tissue (roots, shoots, leaves) is immersed in quantitative assay solution for 2 h and then compared against a standard curve of purified OxO.

- OxO is attached to plant cell walls meaning extraction solution and cell debris need to be taken into account.
- OxO breaks down the oxalic acid substrate in the QAS producing a quantitative violet color read at 555nm.

- Non transgenic control and reaction controls are included in assay.

His conclusions were:

- Histochemical assays allow quick and easy testing to ensure transgenic clonal lines are expressing OxO as they should to ensure further resistance assays are accurate. Also tested for new plant sources of OxO. And finally, the assay can show where OxO is being expressed within the plant tissues.
- Oxalic acid tolerance assay is a quick test that allows approximate resistance to be assessed without sacrificing trees for small stem assays and on a much shorter time scale.
- Quantitative activity assay assays allow for quantitative enzyme activity to be measured as well as OxO concentration in transgenic tissues as well as native OxO sources. This will be important to compare with relative RNA expression data and also is vital in the regulatory approval process.

Allison Oakes, SUNY-ESF

***Ex vitro* rooting.** Oakes has been working on her post-doctoral research, which primarily concerns improving *ex vitro* rooting of micropropagated American chestnut plantlets. After finding that *ex vitro*-rooted plantlets handily out-performed *in vitro* rooted plantlets in acclimatization survival and plantlet quality, she has switched over the production to the better, cheaper, and faster production method. She is currently investigating multiple variables to optimize the procedure, including rooting substrates, substrate soaks, rooting hormone dips, temperature, light, and treatment length.

Erik Carlson, SUNY-ESF

Prospects for CRISPR/Cas9 in the American chestnut research and restoration project.

The CRISPR/Cas9 genome editing system has become a powerful tool in the field of biology. This programmable endonuclease system originated as a form of immune system to viral infection in bacteria, Cas9 specifically from *Streptococcus pyogenes*. Through genetic engineering, CRISPR/Cas9 can be used as an *in vivo* genome editing tool. Guide RNAs (gRNA) direct the Cas9 endonuclease to specific sequences in the target genome, where the Cas9 conducts a double-stranded break adjacent to a protospacer adjacent motif (PAM). Utilizing a vector construct with a dual gRNA sequence, as well as a donor DNA sequence flanked by PAM sequences, it is possible to achieve a targeted gene knockin by taking advantage of the cellular process of homology-directed repair (HDR). The donor DNA in this case would be the wheat gene *OxO*, which has been shown to instill resistance to Chestnut blight (*Cryphonectria parasitica*) in transgenic American chestnut (*Castanea dentata*). In current transgenic lines, the *OxO* insertion is hemizygous, and therefore is only inherited by ~50% of offspring. By using the CRISPR/Cas9 insertion method, it is possible to target opposing chromosomes and achieve a homozygous insertion. This would amount to 100% inheritance of blight resistance by the offspring, both from the nuts produced on the tree, as well as the nuts that result from any pollination. The importance of increased inheritance of blight resistance cannot be overstated, as it would accelerate breeding and restoration efforts by a significant margin. Any homozygous blight resistant trees planted in the forest or in seed orchards would potentially produce blight resistant offspring for several decades, ensuring generations of

blight-free American chestnut for many years to come. If successful, this technique could serve as a starting point for future restoration projects involving genetic engineering solutions for other threatened tree species.

Anna Conrad, Forest Health Research and Education Center, University of Kentucky

Chemical fingerprinting: an alternative approach for screening hybrid chestnut for disease resistance. Conrad talked about plant-derived chemicals and tree defense mechanisms. Plant specialized (secondary) metabolites are one way trees defend themselves against pests and pathogens.

- Present before (constitutive and after (induced) infection)
- Have many modes of action:
 - Toxic or anti-microbial
 - Within plant signal following infection
- Phytochemicals have been identified as markers of disease resistance in other forest pathosystems
 - Can chemical fingerprinting be used to identify disease resistant/susceptible chestnut?

Chemical fingerprinting and chemometrics:

- Chemical fingerprints (CF) include the entire suite of metabolites in a given sample
 - Individual compounds are not separated or quantified
 - Fingerprints are used to distinguish between different groups
- Chemometrics is multivariate statistical analysis of chemical data
 - Focus on identifying chemical differences between groups
 - Examine association with quantitative trait

Chemical fingerprinting methods include:

- Fourier-transform infrared (FT-IR) spectroscopy
- Measures changes in molecular absorption of IR radiation and vibrations
 - Molecular structure impacts absorption and vibrations
 - Mid-IR region (700 - 4000 cm^{-1})
 - Benchtop and handheld devices are available
- Raman spectroscopy
 - Measures the exchange of energy after molecules are irradiated with a laser
 - Analogous to FT-IR spectroscopy

Research objectives

- Evaluate if chemical fingerprinting can be used to distinguish between chestnut hybrids that vary in disease susceptibility
 - Chestnut blight assay
 - PRR assay

Methods included:

Blight assay

- Tissue and phenotypic data from 2015 small stem inoculation experiment was provided by Jared Westbrook (TACF)
 - Stem lesions lengths and blight ratings
 - Tissue collected before the inoculation
- Stem tissue extracts were evaluated from
 - American and Chinese chestnut seedlings
 - Seedlings from 21 BC3F3 hybrid families
 - 0-3 individuals per blight rating group were evaluated for each hybrid family
 - Two hybrid training data sets included Clapper and Graves

Conrad indicated that American and Chinese chestnut chemical fingerprints differ. Soft independent modeling of class analogy (SIMCA) can be used to discriminate between stem extracts of American and Chinese seedlings. Also, FT-IR can estimate blight lesion length for the Clapper training set.

Conclusions for the FT-IR analysis and blight susceptibility include:

- Chinese and American chestnut CF's differed.
- There was no clear relationship between blight phenotype and CF across all 21 hybrid families examined.
- There was a strong positive correlation between measured and predicted blight lesion length for 'Clapper' training set.
- Partial least squares regression can distinguish between 'Clapper' hybrids that vary in blight susceptibility.
- No clear relationship between CF and blight phenotype for 'Graves' training set.

PRR assay

- Tissue and phenotypic data came from a study on chestnut genetics and PRR resistance and was provided by T. Zhebentyayeva.
- The assay:
 - Analyzed foliar tissue collected from 2 families: NK4 and HB2.
 - 40-50 individuals were analyzed per family.
- Tissue was collected in 2014 prior to inoculation with PRR.
- Phenotypic data included PRR ratings.
- Ratings were assigned based on severity of root lesions (0=no lesion' 3=dead plant).
- SIMCA (soft independent modeling of class analogy) was used to assess chemical

fingerprints within the HB2 family. This program uses principal component analysis on a whole dataset in order to identify groups of observations. Group 3 (dead plants) could be differentiated from group 2 plants (lesions on tap root).

FT-IR and PRR susceptibility conclusions:

- There was no clear separation between PRR rating groups when HB2 and NK4 CF data were analyzed together.
- SIMCA could be used to distinguish between HB2 individuals that differed in susceptibility to PRR.
- There was a weak relationship between CF and rating groups 1 and 3 for the NK4 family.

Future research directions include:

- Optimize and validate existing predictive models
- Collect CF data from additional individuals
- Sample mother trees and determine if CF can be used to predict progeny disease susceptibility
- Test additional chemical fingerprinting methods (e.g. Raman spectroscopy)

Conrad detailed a handheld Raman spectrometer. It is a Rigaku Progeny analyzer that is battery powered. It analyzed samples either directly (e.g. piece of leaf tissue) or indirectly (i.e. through containers such as plastic tubes). This device has the potential for more high-throughput analysis.

OBJECTIVE 2. To evaluate biological approaches for controlling chestnut blight from the ecological to the molecular level by utilizing knowledge of the fungal and hypovirus genomes to investigate the mechanisms that regulate virulence and hypovirulence in C. parasitica

Angus Dawe, Mississippi State University

Previous graduate students – Mona Pokharel (completed 2016), Xiaoping Li (completed 2016)

Current personnel at MSU:

Graduate students – Didi Ren, Soum Kundu

Research Associate – Gisele Andrade

Developing a re-annotated genome sequence to facilitate transcriptomics analyses and gene identification. This work has begun as an extension of a project to examine the function of the Vib-1 protein. Previously, we have reported a knockout of the gene encoding this protein and noted the different phenotypes of increased pigmentation, sporulation and reduced virulence, as well as a failure of vegetative incompatibility-mediated programmed cell death between strains different at the *vic-4* locus. In order to understand what the mutant phenotype means, and potentially identify downstream targets, Illumina next generation transcriptome sequencing technology was used to profile the variation of expression patterns between mutant and wild-type strain. With 170 million 50bp high-quality RNA-seq reads obtained from Illumina, TopHat was used to align them against *C.parasitica* genome sequence .fasta file and its genome annotation .gff file to identify exon-exon splice junctions. HTSeq was then used to take above output files to generate a list of reads count per transcript. DeSeq R package, an implementation of negative binomial distribution was used to normalize the HtSeq output and indicate significant changed transcripts and its corresponding visualized plots, like a heat map, MA plot and PCA plot. Then, Gage R package was used to statistically calculate a integrated *p* value of all transcripts, which are in one KEGG pathway and provide a visualized expression pattern contrast between mutant and wild-type strain.

However, it is impossible to fully analyze current RNAseq and further ChIPseq data with the currently available annotation of the genome from *C. parasitica* (from 2009) because it lacks both mRNA and gene structure predictions. Now, by using MAKER (a configurable genome annotation pipeline), we have added these additional gene features into a newer version of the genome annotation (Table 1).

Feature components listed in the new and old version genome annotation.

Old version (2009)	New version (In progress)
<ul style="list-style-type: none"> • exon • CDS 	<ul style="list-style-type: none"> • gene • mRNA • exon • CDS • three_prime_UTR • five_prime_UTR

We are now able to optimize the genome annotation by comparing current six different genome annotation processes with abundant data. As this phase is completed we will generate a more complete genome annotation for *C. parasitica* that will provide better information for future transcriptomic analyses and gene identification.

Polyamine metabolism and hypovirus infection. In *C. parasitica*, infection with hypovirus has been shown to alter various metabolic pathways. One such pathway is the synthesis of the polyamines, putrescine and spermidine, which are required for growth and development of the fungus. While the function of polyamines in various cellular processes has been extensively studied in other fungi, less is known about the effects of viral infection on polyamine metabolism. This study demonstrated the significantly higher accumulation of spermidine in virus-infected mycelium in comparison to uninfected tissue by thin layer chromatography. To understand the possible molecular mechanism for this differential accumulation, we investigated different catalytic enzymes and regulatory components involved in the biosynthesis of polyamines. The enzyme that catalyzes an initial (rate-limiting) step for polyamine biosynthesis is ornithine decarboxylase (ODC). Western blot analyses of ODC showed higher abundance in virus free than compared to the virus infected strain. ODC is subject to a complex post-translational regulatory pathway through inhibition by an antizyme AZ. When examined by western blot, we observed the abundance of AZ was at a level that corresponded to the level of ODC and, therefore, we hypothesized that be the cause of the elevated spermidine levels in the absence of the virus. Given that this pathway is a single route-synthesis, where formation of different polyamines occurs only via ornithine, we also investigated another protein, S-Adenosylmethionine decarboxylase (SAMDC) that supplies a key component, the aminopropyl moiety, in the conversion of putrescine to form spermidine. In this case, we observed a higher SAMDC accumulation in the virus infected than the virus free strain, thus permitting increased synthesis of spermidine even though the accumulation of ODC is paradoxically lower. Therefore, this study provides a mechanistic model to explain the observed differences in polyamine accumulation following virus infection.

Didi Ren, Mississippi State University

LysM proteins and *C. parasitica* virulence. By examining genome sequence data, *C. parasitica* was found to contain five putative proteins containing LysM motifs (2014 report). These motifs have been recognized using information from the organism's genome portal. Of relevance to this study is the potential of these proteins to act as an effector protein, which plays a role in the virulence of certain pathogens. Recent findings provided evidence of LysM containing proteins

in two other fungal plant pathogens, *Cladosporium fulvum* and *Magnaporthe oryzae*, which are secreted during the initial fungal infection of the plant. It has been determined that these LysM containing proteins are able to bind to chitin, competing with the plant's pattern recognition receptors, therefore helping to overcome the host's defense response. Knockouts of four of these genes have been created, but only one showed significant reduction of virulence, a phenotype also coupled with a strong vegetative growth defect. However, one, called LM12, when eliminated, resulted in a modest increase in virulence (2015 report). Further analysis of this strain appears to show that the cell volume of the knockout is increased, although this preliminary data requires confirmation. Additional studies planned include development of mutantions in LM12 that will prevent glycosylation to test whether this modification is important for the protein's role, and to identify potential roles for the other LysM proteins in fungal behavior.

Donald Nuss, University of Maryland, Institute for Bioscience and Biotechnology Research, Shady Grove Campus (now adjunct at West Virginia University)

Engineering super mycovirus donor strains of chestnut blight fungus by systematic disruption of multilocus vic genes. Transmission of mycoviruses that attenuate virulence (hypovirulence) of pathogenic fungi is restricted by allorecognition systems operating in their fungal hosts. We report the use of systematic molecular gene disruption and classical genetics for engineering fungal hosts with superior virus transmission capabilities. Four of five di-allelic virus-restricting allorecognition [vegetative incompatibility (*vic*)] loci were disrupted in the chestnut blight fungus *Cryphonectria parasitica* using an adapted *Cre-loxP* recombination system that allowed excision and recycling of selectable marker genes (SMGs). SMG-free, quadruple *vic* mutant strains representing both allelic background of the remaining *vic* locus were then produced through mating. In combination, these super donor strains were able to transmit hypoviruses to strains that were heteroallelic at one or all of the virus-restricting *vic* loci. These results demonstrate the feasibility of modulating allorecognition to engineer pathogenic fungi for more efficient transmission of virulence-attenuating mycoviruses and enhanced biological control potential.

Field testing of super donor formulation. A site containing significant numbers of infected chestnut sprouts was identified in the Savage River Forest in Garrett County, Maryland near Grantsville. Three research plots were established on 12 July 2016 with the assistance of the Savage River State Forest staff. American chestnut trees in each plot were numbered, as were the infections on each stem. Three plots were established within the site. In one plot, all cankers were treated with the Super Donor formulation. Two similar plots were treated comparably with: 1) a slurry containing hypovirus-infected strains without the vegetative compatibility gene deletions; or, 2) a water agar slurry without fungus.

Protocol:

- Identify canker on flagged trees or newly identified trees in plot. Number trees.
- Outline canker with sharpie and number canker.
- Measure length and width of canker. Also measure circumference at site of canker and the distance of canker (middle) to ground.
- Sample cankers with bone marrow device in four spots for later recovery of *C. parasitica*.

- Make punch holes around canker about 2 inches apart, leaving cardinal points free.
- Fill holes with treatment slurry and cover holes with masking tape.

Number of cankers treated in each plot

Super donor formulation	40
Cytoplasmic hypovirulent formulation	33
Water agar	31

A preliminary assessment of canker length was made on 16 Sept. 2016, two months after challenge. Canker (L+W)/2 was 0.24 cm, 2.6 cm and 4.4 cm for super donor, cytoplasmic hv and water agar, respectively. On 16 Sept. 44 new cankers were detected among all three plots; they were sampled and treated with the respective treatment slurry.

Mark Double, West Virginia University

Introduction of hypoviruses at West Salem, Wisconsin (in cooperation with D.F. Fulbright and A.M. Jarosz, Michigan State University; and, A. Davelos Baines, University of Wisconsin-LaCrosse). The stand of American chestnut in West Salem became infected with chestnut blight in the late 1980s after 100 years of blight-free growth. Hypovirus introduction (individual canker treatment) was conducted from 1992-1997 (700 cankers on 133 trees received inoculum). From 1998-2002 hypovirus introduction was halted. In 2001, due to a large increase in the number of cankers in the stand, twelve permanent plots were established in three regions of the stand representing differing levels of disease: Disease Center; Front; and, Beyond the Front. Hypoviruses were reintroduced in 2003; annual treatment has continued through 2016. Approximately 25% of the trees in each plot are untreated to assess tree-to-tree spread of hypovirulent strains.

Hypovirus spread has been assessed annually by analyzing isolates of *C. parasitica* that arise from bark samples. Hypovirulent isolates are recovered most readily from treated cankers followed by non-treated cankers on treated trees. Hypoviruses have spread less effectively to untreated trees. Since 1992, a total of 3,467 cankers have been identified in the 12 plots. Three-hundred, twelve cankers on living trees were sampled in July 2016; 82 were newly discovered.

General observations:

- When the 12 permanent plots were established in 2001, there were 517 living stems included in the study. As of 2016, 54% of the original stems in the Disease Center plots remained alive compared to 24% and 8% in the Disease Front and Beyond the Disease Front plots, respectively. Some loss of stems may be attributed to the harsh winters of 2013-14 and 2014-15.
- Chestnut sprout populations have increased significantly as the mortality of the original stems has resulted in additional light reaching the understory. Sprout survival is for the Disease Center, Disease Front and Beyond the Disease Front is 36%, 40% and 31%, respectively.
- Vegetative compatibility type WS-1 continues to be the dominant vc type in the stand although its frequency has decreased from 100% in 1995 to 84% in 2015. WS-2 and WS-3 were found at rates of 4% and 7%, respectively.

OBJECTIVE 3. To investigate chestnut reestablishment in orchard and forest settings with special consideration of the current and historical knowledge of the species and its interaction with other pests and pathogens

Laurel Rodgers (and students Fawzia Bhatti and Dillon Richardson), Shenandoah University

Using Illumina sequencing to analyze endophyte populations in the American and Chinese chestnut trees. The purpose of this grant was to determine whether Illumina sequencing can be an effective tool for surveying the endophyte population within American and Chinese chestnut trees. To accomplish this task we wanted to directly compare traditional Sanger sequencing to Illumina sequencing. Two bark samples were taken side-by-side from the trunk of a chestnut tree. One sample was used to grow and isolate endophytes growing in the tree. Sanger sequencing was used to identify these isolated cultures. DNA was extracted directly from the second sample and sent to the UNC-Chapel Hill genomics facility for Illumina sequencing. We wanted to determine whether the endophytes we identified as growing within the tree could also be identified by Illumina sequencing.

Two summer research students worked on this project from mid-June through mid-August. These same students are currently receiving course credit to help complete the project. Thus far, we have collected eleven paired samples from an American chestnut tree and eleven paired samples from a Chinese chestnut tree at the TACF plots located at Blandy Experimental Farm in Boyce, VA. These trees are located side-by-side and therefore have been exposed to the same local environmental conditions. The students have successfully cultured, isolated, and identified the endophytes (by Sanger sequencing) growing in each tree. Based on their results and the total DNA isolated in each sample, six of the paired samples were selected to be analyzed for Illumina sequencing. The Illumina results from the UNC-Chapel Hill sequencing facility are pending.

The table below summarizes our Sanger sequencing results. We identified seven species of fungi that were unique to the American chestnut and three that were unique to the Chinese chestnut tree. There were two that were found in both the American and the Chinese chestnut trees. The numbers in parenthesis indicate the number of samples that each fungus was isolated from. A few fungi samples have been difficult to sequence, and therefore have not been identified. We are working on alternate DNA extraction methods in order to confirm their identity.

Identification of Fungi from American and Chinese Chestnuts		
American Chestnut	Chinese Chestnut	American and Chinese Chestnut
<i>Hypoxyylon rubiginosum</i> (1)	<i>Alternaria alternata</i> (1)	<i>Pestalotiopsis</i> genus (3,3)
<i>Sodariomycetes</i> genus (1)	<i>Diaporthaceae</i> family (1)	<i>Epicoccum nigrum</i> (1,1)
<i>Lecythophora</i> genus (1)	<i>Fusarium</i> genus (2)	
<i>Diplodia seriata</i> (1)		
<i>Cladosporium cladospriedes</i> (1)		
<i>Leptosphaerulina chartarum</i> (1)		
<i>Pseudopestalotiopsis theae</i> (1)		

Though this initial study was not designed to compare the endophyte population between the two chestnut species, some interesting observations can be made. These two trees were growing side by side, yet we have only identified two species growing in both trees. Our sample size will need to be expanded in order to determine whether the differences are an accurate representation of endophyte communities growing in the American and Chinese chestnut trees.

Fred Hebard, The American Chestnut Foundation, Meadowview

Prolonged survival of blight by American chestnut. Longer periods of survival in clearcuts and shelterwoods are associated with release of young chestnut sprouts from competition 5 to 10 years after the initial cut, as proposed by Gary Griffin. It was thought release from competition would simulate the situation in Europe, where, after 1-2 cycles of sprouting, blighting, cutting and resprouting, hypovirulence became prevalent. In the US, most chestnut sprouts die within 10 years after clearcutting or other harvest method that lowers residual basal area to 4.6-6.9 square meters per hectare. However, in some clearcuts, a few trees survive—usually characterized by highly swollen, apparently superficial cankers ("big, ugly"). To promote and sustain flowering near Meadowview, chestnut sprouts in clearcuts are released from competition in order to increase exposure to sunlight. In association with continued release from competition at a few sites, but not most, Hebard has seen prolonged survival of sprouts for up to 32 years after clearcutting. Survival is very site specific.

Throughout the natural range of American chestnut, there are in excess of 50-100 trees that have survived blight for long periods, in excess of 10 years, and grown large, in excess of 25 cm in diameter at breast height (dbh). These are not necessarily associated with release from competition. These are known to occur at sites in North Carolina, Tennessee, Kentucky, Ohio, West Virginia, Virginia, Maryland, Pennsylvania, New Jersey and Connecticut. Some of these trees exceed 120 cm in dbh. From most trees that are surviving blight, strains of *C. parasitica* with reduced virulence can be isolated. In some, heritable resistance has been detected in addition to reduced virulence. The ubiquity of reduced virulence makes it difficult to ascribe a cause to survival. This is a fluid situation that merits continued monitoring and further exploration of causal factors.

There also are large American chestnuts throughout the range that have escaped infection rather than survived it, primarily on the fringes of the range. It is thought that these escapes have no resistance to blight and have survived due to inadequate concentrations of blight inoculum to infect them at a younger age. Most die from blight before they reach 60 cm in dbh.

Sandra Anagnostakis, The Connecticut Agricultural Experiment Station

Important stuff at The Connecticut Agricultural Experiment Station. After 50 years, Anagnostakis retired from CAES and moved to Massachusetts. She continues to work at CAES two days a week. Anagnostakis has been the registrar for cultivars; Greg Miller has agreed to take over this duty.

CAES has a treasure-trove of many items—books and records from the USDA, notes about forest pathology, file drawers of USDA breeding records, etc. People have been working on chestnuts at CAES since 1930. Many of the items held at CAES are detailed below.

1. USDA Plant Importation records: in a wooden credenza, main floor of the Library in the entryway, has the original sources of plant materials imported into the U.S.
2. USDA Chestnut Records: in a wooden card file cabinet, third floor of the Library, has all the records of where imported chestnut trees/seeds were sent, organized by state, also has records of surveys for chestnut blight disease. Files for CT, NY, MA, PA and RI currently in small plastic file boxes in SLA book case (in office).
3. Breeding records of CAES chestnut work: on a labeled library shelf, wooden bookcase, main floor of the library.
4. Theses relating to chestnuts: on a labeled library shelf, wooden bookcase, main floor of the library.
5. Photographs and Negatives: on the microfilm cabinet, west side of the third floor of the Library, photos taken by Plant Introduction expeditions and early USDA chestnut work
6. Hansborough Herbarium of fungi: third floor of the Library, south side, in boxes
7. Fungal Studies books VERY IMPORTANT, VERY VALUABLE: on shelves near the Hansborough Herbarium, third floor of the Library (DeWei Li has taken some to Windsor)
8. Card file of the Plant Pathology reprint collection: in a metal card file, north side of the third floor of the Library
9. Two small notebooks with the key to the Plant Pathology reprint collection file: on the reprint card file cabinet, third floor of the Library
10. Plant Disease Surveys, 1916 to 1948: metal card file on top of the Plant Pathology reprint collection, in a metal card file, third floor of the Library
11. CAES Fungal Herbarium: metal cases, south side of the third floor of the Library (including "books" of Rabenhorst), some of this material is not available anywhere else in the world
12. Index to the CAES Fungal Herbarium: card file next to the metal cases of the Herbarium, third floor of the Library

Abbreviations and names used in the following documents:

Lockwood Farm

- NH New Hybrid orchard, #7 on the map
 HH Humphrey Hill orchard, #9 on the map
 RH Rocky Hill orchard, #13 on the map, original female tree number in parentheses
 i.e., RH(5) R1T4 is tree #4 in Row 1 whose mother was tree 5 in Rocky Hill, CT
 see Anagnostakis, S. L., and J. Kranz. 1987. *Phytopathology* 77:751-754.

Chestnut Plantation at Sleeping Giant

- SL South Lot
 CC Chinquapin Corner
 WL West Lot
 WRPL West Red Pine Lot
 SpL Spring Lot

Two important field plots

Sara Cunningham orchard

A lady with several houses (all over the world), Miss Cunningham bought the land in Dayville because it had a beautiful view of the sunset. She built a house, and left the running of the property to her farm manager, Mr. George Harrington. She named the property "Quinequack Farm" because of the noise of the ducks from the nearby Quinebaug River. In 1926 she requested Chinese chestnut trees from the USDA, and they sent 67 seedlings of the import #58602.

This importation was a mixture of seeds sent by J. H. Reisner of the University of Nanking who said that he was *"asking for seven or eight pounds of the chestnuts from each locality.Strains of fruits and nuts have been developed in a community for hundreds of years; in some cases possibly thousands of years. It is very common to hear the Chinese say the variety or strain of fruit or nut which does well in a small local community is not adapted to other situations.I am hoping to get something to you that will prove hardy and resistant."*

The seedlings were widely distributed in the U.S. (there are records of 7,826 trees being sent out). Unfortunately, no records were kept of the origin in China of each small lot, so no correlations can be made between survival and origin. There are also #58602 trees in the planting at Nathan Hale State Forest, and in Stamford on Scofieldtown Rd. (now "Georgian Court").

George Harrington inherited Quinequack Farm when Sarah Cunningham died, and showed R. A. Jaynes the small trees that had grown up in adjacent fields from seed from the original trees. Jaynes wrote a paper on this "naturalization" in 1965. Anagnostakis looked at the trees again in 1992, and found 28 of the original trees still alive, and significant naturalization. There are no American chestnut trees in the area.

When the land was sold in 1995, a conservation easement area containing the chestnut trees, with a twenty-five foot wide access easement, was given to the Town of Killingly. Seed from the original trees was collected for several years and given to the State Nursery. These were grown for two years and sold to landowners in the 'wildlife packet.'

DIRECTIONS:

I-395 to exit 93, Rt. 101 West, on the right just after a package store in a log cabin is Lake Rd., right (north) on Lake Rd., just past two houses is the new development, the access easement is on the left side of the development (along a line of trees), Chestnut trees are at the back of the property, about 500 feet in from Lake Rd.

Nathan Hale

The State of CT acquired the property which is now the Nathan Hale State forest in 1946. It originally totaled 850 acres of land in the towns of Coventry and Andover and is now 1,529 acres. It is named for the Revolutionary War Hero, Nathan Hale, who was born and lived in the house on the property, which is now a museum. The owner who sold the land to the state tried to restore it to a state comparable to that which existed during Hale's boyhood, when most of the land was cleared and grazed by sheep and cattle. During the 1930's he allowed it to revert to forest for timber products and wild life. He also established plantations of trees including white, red, and Austrian pine, arborvitae, hemlock, Douglas fir, white and Norway spruce, and tulip poplar. Since 1946, 19 acres of open field have been planted to white pine, red pine, spruce, hemlock, and Douglas fir, and a limited area seeded directly to oak. All of these plantations were weeded, and some pruned and thinned. The CT Department of Energy and Environmental Protection (DEEP)

has posted its 10 year plan for this forest property at http://www.ct.gov/deep/cwp/view.asp?a=2697&q=322868&deepNav_GID=1631.

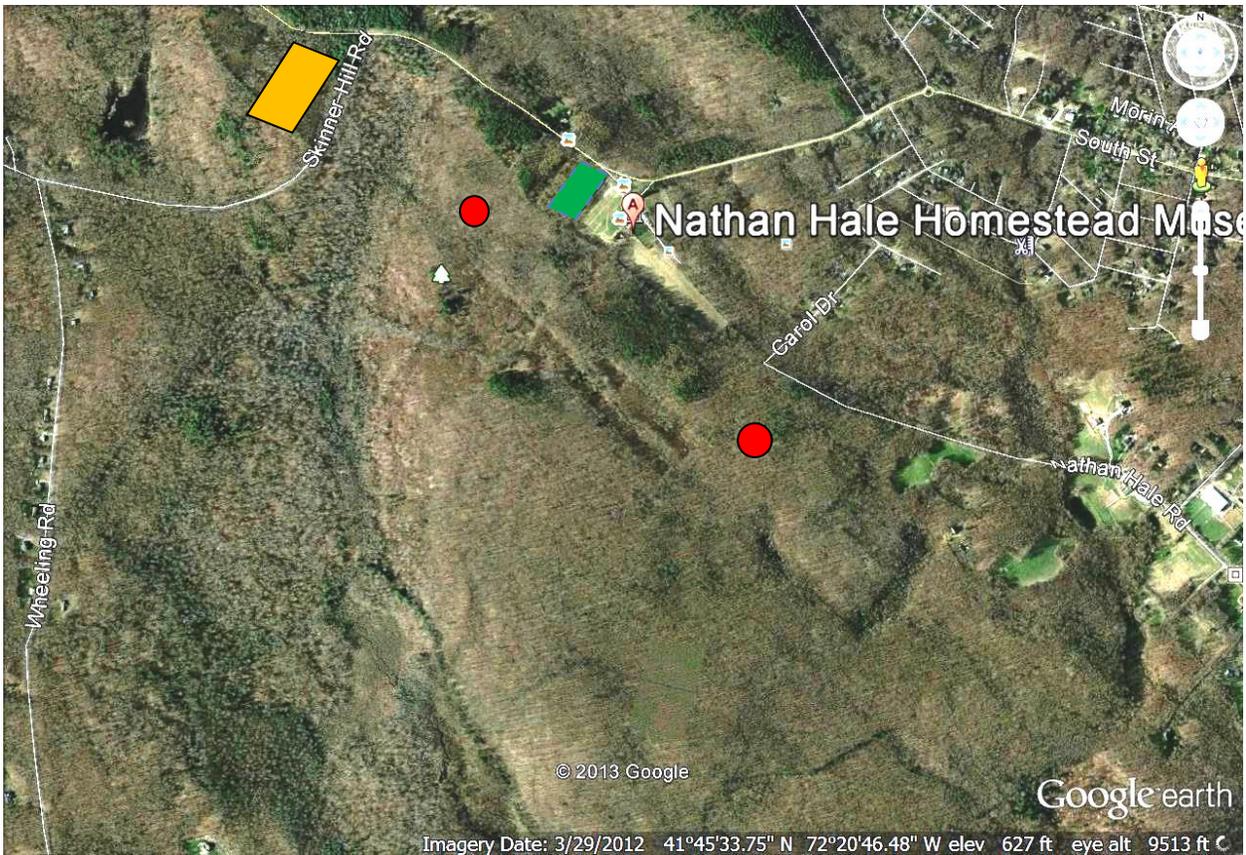
In the spring of 1951 the CT DEEP in cooperation with the USDA and The Connecticut Agricultural Experiment Station planted two plots, about one mile apart, with chestnut trees. These were areas with mature forest, and competing trees were girdled. The USDA contributed Chinese chestnut seedlings (*Castanea mollissima*) from the Savannah, GA Plant Introduction Station planting of chestnut importation #58602 from Nanking, China. The seed had been purchased from Prof. J.H. Reisner, in the Forestry Department at the University of Nanking in 1924. Savannah seed was collected from four numbered trees, grown in the nursery there, and three year old seedlings sent to CT. There were 43 planted in Plot 1 and 41 in Plot 2, and mother-tree designations were noted. The Connecticut Agricultural Experiment Station (CAES) provided 182 seedling hybrids from 42 kinds of crosses. In Plot 1, 57 hybrids of 19 kinds were planted, and in Plot 2, 125 hybrids of 26 kinds were planted. These hybrids were made using the species *C. dentata*, *C. mollissima*, *C. crenata*, *C. sativa*, and *C. pumila* and their hybrids, all growing in CAES plantations. The plots were occasionally cleared of brush and trees measured by CAES staff. In 1963, there were 67 trees alive in Plot 1 and 9 had died and sprouted. In Plot 2, 92 trees were alive and 19 had died and sprouted. No clearing or brush treatment has been done since then. In 1991 there were 34 trees alive in Plot 1 and 36 in Plot 2. A rough count in Plot 1 in 2013 yielded 21 live chestnuts, few seedlings and little sign of sprouting native *C. dentata*. In Plot 2 in 2013, there were many large chestnuts and abundant sprouts and seedlings in the understory. No attempt was made to identify these understory chestnuts, but some were certainly *C. dentata* based on their morphology.

In 1956, an orchard of 158 hybrid chestnut trees (from 15 different crosses) was planted adjacent to the Hale Homestead building near Plot 1. This orchard was kept clear of competing vegetation for about 50 years, but no maintenance has been done for several years. In 1976 trees were pruned, and 104 with poor nut production removed. The remaining 54 trees have continued to produce abundant seed, and seedlings are present in the open areas around the orchard.

Less than ½ mile to the north west of Plot 1, an area with abundant native *C. dentata* was clear-cut in the winter of 1990-1991. Sixty sprout clumps of *C. dentata* in about ¼ acre were treated with our biological control for chestnut blight disease, a mixture of *C. parasitica* strains with hypovirulence virus. Treatments were done in the fall of 1992, spring and fall 1993, and spring 1995. Competing vegetation was cut in the spring of 1998 and a treatment was done in the fall of that year. The last measurements, in 2004, showed that the hypovirus was still present and many *C. dentata* had reached flowering size. No treatment was done in an adjacent area of the clear-cut, and 33 sprout clumps are monitored for evidence of spread of the biocontrol.

The DEEP has designated the area with Plot 2 (Compartment 10) as an "Old Forestland Management Site" where no clearing will be done and stand succession will be allowed to occur naturally without silvicultural disturbance. Plot 1 is in Compartment 6 where there are no restrictions on management.

Which of the chestnut species and hybrids planted in this forest have survived for 60 years? Have any of them produced seed/seedlings that are now established in the understory? Have the previously abundant native *C. dentata* crossed with any of these planted trees and produced seed/seedlings into the understory?



The large rectangle in the NW of the picture is the area that was clear-cut in 1990/1991 for a study of biocontrol of chestnut blight disease on native *C. dentata*. The small rectangle near the cleared field is the area where an orchard of hybrid chestnut trees was planted in 1956. The circle near that is Plot 1 and the circle near Carol Drive is Plot 2.

Lockwood Farm, Corner of Kenwood Ave.



CHESTNUTS (and Friends) AT LOCKWOOD FARM, September 2016

Donald Jones, Hans Nienstaedt, Richard Jaynes, and Sandra Anagnostakis

1. KENWOOD AVENUE

- by gate: *Castanea mollissima*
USDA import FP#7275, planted 1939 [peroxidase AB]
- next west *Castanea mollissima*
cultivar '**Bartlett**' grafted 1939 [peroxidase AA+]
- next west *Castanea mollissima*
USDA import FP#7284, planted 1939 [peroxidase BB]
- next west *Castanea mollissima*
USDA import FP#7273, planted 1939 [peroxidase BB]

FARM, CENTER

2. near barns: *Castanea dentata* American Orchard

four rows of 18 seedlings from Michigan and Wisconsin, planted 1976
when 2 and 3 yrs old, R1T1 is at the NE corner
[all Santamour peroxidase AA]

27 trees from the American Chestnut Cooperator's Foundation planted 2007

original stem old sprouts young sprouts, lots of dieback North

Tree 1	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18	<input type="checkbox"/>	ACCF NC Champ	<input type="checkbox"/>	<input type="checkbox"/>
19	Smith Middle School, CT	cross #6-07 American tag #24712	ACCF Pacman	ACCF VT 1

20	ACCF NC Champ best	cross #6-07 American tag #24713		Smith Middle School, CT
21	cross #6-07 American tag #24714	ACCF VT 1	Turkey, C. sativa	ACCF Loudon
22	ACCF NC Champ	ACCF VT 1		Turkey, C. sativa
23	ACCF Thompson	ACCF Thompson	Turkey, C. sativa	Burton 27a, GA 2011
24	ACCF Thompson	Smith Middle School, CT	Smith Middle School, CT	Sault St. Marie tree from Canada
25	ACCF JEB	ACCF JEB		
	Row 4	Row 3	Row 2	Row 1

3. east, center `Scientist's Cliffs" graft (doesn't look like *dentata*) called "American," from Glenndale, MD, land of G.F. Gravatt, also known as FP1000, graft about 1959 [Santamour peroxidase AA]

4. SE corner (*Castanea mollissima* x *C. dentata*) x *C. dentata*

two clones of hybrid 'Clapper' from a USDA cross in 1946, grafted here about 1960. This was: "M16" = PI#34517, Tientsin, China (1912) crossed with an "American" in MD known as FP 555, and the hybrid crossed again with FP 555 (original now dead), these two clones are not cross-fertile [peroxidase AA and AA], (stump sprout there too!)

5. North and East of 'Clapper', on the road Commercial chestnuts

	<u>S</u>	<u>Center</u>	<u>N</u>
W	'Colossal' x 'Lockwood'	'Colossal' x 'Lockwood'	'Colossal' [perox. BB]
C	'Colossal' x 'Lockwood'	'Colossal' [perox. BB]'	'Colossal' x 'Lockwood'
E	'Colossal' x 'Lockwood'	'Colossal' x 'Lockwood'	'Colossal' x 'Lockwood'

6. East of 'Clapper', on the road, between grapes Commercial chestnut seedlings

	<u>S</u>	<u>Center</u>	<u>N</u>
W	Dunston	Dunston	Dunston
C	Dunston	Dunston	Dunston
E	Dunston	Dunston	Dunston

7. NEW HYBRIDS, just WEST of the American orchard

R1T1 is at the SE corner

R1T1	Fred Blankenship hybrid 2013	
R1T2	<i>(C.dentata x C. crenata) x C. dentata</i>	BC1
	cross #3-09 NH R1T11 x RH(5) R2T4 (see Rocky Hill Americans, #13)	
R1T3	<i>C. ozarkensis x C. crenata</i> , NH R2T2 x WL R34T6, 2011	F1
R1T4	<i>C. ozarkensis x C. crenata</i> , NH R2T2 x WL R34T6, 2011	F1
R1T5	<i>C. dentata x C. crenata</i> , HH R1T6 x WL R34T6	F1
R1T6	<i>(C. crenata x C. ozarkensis) x (C. ozarkensis x C. crenata)</i>	F2
	cross #7-02, SpL R7T61 x SpL R8T63, male fertile 3 nuts/bur	
	(SL R7T7 x RgammaT3) x (RalphaT2 x Early Jap Δ)	
R1T7	<i>C. ozarkensis x C. crenata</i> , NH R2T2 x WL R34T6, 2011	F1
R1T8	<i>(C. crenata x C. ozarkensis) x (C. ozarkensis x C. crenata)</i>	F2
	cross #7-02, SpL R7T61 x SpL R8T63, male fertile but little pollen	
	(SL R7T7 x RgammaT3) x (RalphaT2 x Early Jap Δ) 3 nuts/bur	
R1T9	<i>C. ozarkensis x C. crenata</i> , NH R2T2 x WL R34T6, 2011	F1
R1T10		
R1T11	<i>C. dentata x C. crenata</i>	F1
	cross #14-91, RH(5) R2T5 (farm) x WL R34T6 (Plantation), male sterile, Excellent resistance, shed nuts by mid-September, poor apical dominance	
R1T12-13		
R1T14	<i>C. dentata x C. crenata</i>	F1
	cross #11-91, RH(11) R2T7 x WL R34T6 (Plantation),	

male sterile, form poor, resistance good, early nuts, **seedlings have good roots**, good apical dominance (peroxidase AB)

R1T15 (*C. crenata* x *C. ozarkensis*) x (*C. ozarkensis* x *C. crenata*) **F2**
cross #7-02, SpL R7T61 x SpL R8T63, male fertile
(SL R7T7 x RgammaT3) x (RalphaT2 x Early Jap Δ)

R1T16 *C. dentata* x *C. crenata*, HH R1T6 x WL R34T6 **F1**

R1T17 'Colossal' x 'Lockwood' 2014 (also planted in the Commercial Orchard #5)

R1T18 (*C. dentata* x *C. crenata*) x *C. dentata* **BC1**
cross #3-09 NH R1T11 x RH(5) R2T4

R1T19 *C. ozarkensis* x *C. crenata*, NH R2T2 x WL R34T6, 2011 **F1**

R2T1

R2T2 *C. ozarkensis* x *C. ozarkensis*
cross #8-02, CC RetaT4 x CC RgammaT3 (both Arkansas)

R2T3 *C. dentata* x *C. henryi* **F1**
cross #9-09 RH(5) R2T5 x WL R32T1

R2T4

R2T5 *C. ozarkensis* x (*C. henryi* x *C. ozarkensis*) **BC1**
cross #6-06, RgammaT4 x SpL R5T37, male fertile, **one nut per bur**

R2T6 *C. ozarkensis* Arkansas 1 from Steve Bost 2013, Nat. d-w Scott County, AR, Ouachita National Forest, o.p.

R2T7 *C. ozarkensis* x *C. crenata*, HH R2T1 x WL R34T6, 2011 **F1**

R2T8 *C. dentata* x *C. henryi*, HH R1T6 x WL R32T1 2011 **F1**

R2T9 *C. ozarkensis* Arkansas 2 from Steve Bost 2013, Nat. d-w Scott County, AR, Ouachita National Forest, o.p.

R2T10

R2T11 'Colossal' x 'Lockwood' 2014

R2T12 *C. ozarkensis* Arkansas 2 from Steve Bost 2013, Nat. d-w Scott County, AR, Ouachita National Forest, o.p.

R2T13 *C. dentata* x (*C. pumila* x *C. crenata*)
cross #22-94, RH(14) R2T9 x SpL R7T14 (Plantation, Δ), resistance good, male sterile

R2T14 *C. dentata* x *C. Henryi* **F1**
cross #9-09 RH(5) R2T5 x WL R32T1

R2T15

R2T16

R2T17-19 'Eaton' x *C. ozarkensis* (OK), Kenwood orchard x HH R2T1, 2013

R3T1-6 'Colossal' x *C. ozarkensis* (AR), commercial orchard x HH R2T2, 2013

R3T7 WATER LINE

R3T8-13 'Colossal' x *C. ozarkensis* (AR), commercial orchard x HH R2T2, 2013

R3T14 *C. ozarkensis* x *C. henryi*, NH R2T2 x WL R32T1, 2011 **F1**

R3T15-16 'Colossal' x *C. ozarkensis* (AR), commercial orchard x HH R2T2, 2013

R3T17 'Colossal' x 'Lockwood', 2014

R3T18 *C. ozarkensis* x *C. henryi*, NH R2T2 x WL R32T1, 2011 **F1**

R3T19 'Colossal' x *C. ozarkensis* (AR), commercial orchard x HH R2T2, 20

8. TURKISH CHESTNUT TREES directly south of the New Hybrids

R1T1 is at the SE corner, at the road

- R1T1 #24771, Eastern Turkey, Artvin Province, Collection 1, 2007, perox. AB
- R1T2 #24772, Eastern Turkey, Artvin Province, Collection 1, 2007, perox. AB
- R1T3 Eastern Turkey, Artvin Province, Collection 1, 2007, perox. AB
- R1T4
- R1T5 Eastern Turkey, Artvin Province, Collection 1, 2007, perox. BB
- R1T6 #24770, Eastern Turkey, Artvin Province, Collection 1, 2007, perox. BB
- R1T7
- R1T8 Eastern Turkey, Artvin Province, Collection 2, 2007, perox. AB
- R1T9
- R1T10 Eastern Turkey, Artvin Province, Collection 2, 2007, perox. AB
- R1T11
- R1T12 #24776, Eastern Turkey, Artvin Province, Collection 2, 2007,
- R1T13
- R2T1 Eastern Turkey, Artvin Province, Collection 3, 2007, perox. BB
- R2T2
- R2T3
- R2T4 #24778, Eastern Turkey, Artvin Province, Collection 3, 2007, perox. AB
- R2T5
- R2T6 Eastern Turkey, Artvin Province, Collection 4, 2007, perox. BB
- R2T7 #24777, Eastern Turkey, Artvin Province, Collection 3, 2007, perox. AA
- R2T8 Eastern Turkey, Artvin Province, Collection 4, 2007, perox. AA+
- R2T9 #24781, Eastern Turkey, Artvin Province, Collection 4, 2007, perox. BB
- R2T10 Eastern Turkey, Artvin Province, Collection 4, 2007, perox. AB
- R2T11 #24784, Eastern Turkey, Artvin Province, Collection 4, 2007, perox. AB
- R2T12 #24783, Eastern Turkey, Artvin Province, Collection 4, 2007, perox. AB
- R2T13 Eastern Turkey, Artvin Province, Collection 4, 2007, perox AB
- R3T1
- R3T2
- R3T3
- R3T4
- R3T5
- R3T6
- R3T7
- R3T8 #24786, Eastern Turkey, Artvin Province, Collection 5, 2007, perox. AB
- R3T9 #24789, Middle Black Sea region, Turkey, Ordu Province, collection 6, 2007,
perox. AB
- R3T10
- R3T11 Middle Black Sea region, Turkey, Ordu Province, collection 6, 2007,

perox. BB

R3T12 *C. ozarkensis* x *C. henryi*, NH R2T2 x WL R32T1, 2011 **F1**
R3T13 *C. ozarkensis* x *C. henryi*, NH R2T2 x WL R32T1, 2011 **F1**

9. HUMPHREY HILL

along the north end, small trees

FL pum. FL pum. MD pum. MD pum FL pum. FL pum. MD pum. *C. henryi*? MD pum. *C. henryi*?

C. pumila from North Florida, planted July 2011, *C. henryi*? Schumacher, pl. 2013

R1T1 is at the NE corner

R1T1 *C. dentata* x (*C. ozarkensis* x *C. sequinii*) "Windsor Nice #1"
moved here from Windsor in 2006 one nut/bur!
cross #19-93, RH(3) R4T2 x WL R29T14 (#23-60)

R1T2 *C. sativa*
from Bursa, Turkey; wild population #010, seed 1990, planted 1991
(peroxidase AA)

R1T3 *C. ozarkensis* x *C. ozarkensis*
cross #8-02, CC RetaT4 X CC RgammaT3 (both Arkansas)

R1T4-5 *C. sativa*
from Bursa, Turkey; wild population #018, seed 1990, planted 1991
(peroxidase AA, AA)

R1T6 *C. dentata*
Roxbury #2 op: open-pollinated seedling from tree #2 in a group of
American chestnut sprouts on Painter Hill Rd. in Roxbury, CT seed 1988, planted
1989, no leaf hairs (peroxidase AA)

R1T7-8 *C. crenata*
open pollinated '**Japanese Giant**' from Rochester, NY, seed 1990,
planted 1991 (peroxidase BB)

R2T1 *C. ozarkensis* Ouachita National Forest, OK, planted 2004

R2T2 *C. henryi* o.p. WL R32T1 o.p. 2009 (has 3 nuts per bur, male fertile)

R2T3-4 *C. sativa*
from Bursa, Turkey, wild population #018, seed 1990, planted 1991
(peroxidase AA, & AA)

R2T5 *C. sativa*
European (Black Forest, Germany), seed 1984, planted here 1988
(peroxidase AA)

R2T6 *C. dentata*
seed 1984 from E. Wisniewski, Norwich, CT, planted 1988 no leaf hairs
(peroxidase AA)

R2T7 *C. dentata*

- Rox 2 op, see R1T6, no leaf hairs (peroxidase AA)
- R2T8 *C. mollissima*
Chinese, cultivar '**Orrin**', planted 1963
- R3T1 *C. mollissima*
Chinese, cultivar '**Kuhling**' PI #108552, "K'uei Lee"
from Louis Gerard Nursery in Illinois, planted 1961
- R3T2 *C. sativa*
from the Cavcas Biosphere Reserve, seed 1993, planted 1994
collected by Fred Paillet (peroxidase AA)
- R3T3 *C. sequinii* x *C. sequinii*, HH R4T2 x SL R8T4, 2011
- R3T4 *C. sativa* hybrid
looks like a European X Japanese hybrid, seed from E. W. Morse,
Grandview, Washington, 1944 as "various unidentified" nuts, moved
here 1952
- R3T5 *C. mollissima*
"wild Chinese" from Dr. Liu Liu, Nanjing, seed 1992, planted 1994 [perox AB]
- R3T6 *C. dentata*
American, seed 1985 of Watertown III X Watertown I, trees in upstate
New York thought to have some blight resistance, seedling from W.
Mac Kentley, St. Lawrence Nurseries, Potsdam, NY, planted 1989, no leaf hairs
(peroxidase AA)
- R3T7 *C. mollissima*
Chinese, cultivar '**Abundance**' from Louis Gerard Nursery in Illinois,
planted 1963
- R3T8 *C. dentata*
seedling of Watertown III x Watertown I, see notes R3T6, planted 1989
(peroxidase AA)
- R4T1 *C. pumila*
from Empire Chestnut Co., 2000 (peroxidase AA)
- R4T2 *C. sequinii* x *C. sequinii*
cross #4-98 of SL R8T4 x SL R2T16, planted 1999, perox. A+BB
- R4T3 '**Scientists' Cliffs**' x *C. dentata*
'**Scientists' Cliffs**' x Roxbury #5, planted 1990, no leaf hairs
- R4T4 *C. mollissima* x *C. mollissima*
cross #15-90, '**Mahogany**' SL R1T15 x '**Tiger Paw**' SL R9T2,
planted 1991, very late blooming, (peroxidase AA+),
- R4T5 *C. crenata* X *C. sativa*
'**AW 74**' Japanese x European "natural hybrid" from near Brive, France
(1946) sent by Solignat as a graft, buried in-arch resulted in rooting,
hybrid now on its own roots planted 1961
- R4T6-7 *C. dentata*
American, Watertown III x Watertown I, see notes R3T6, planted 1989
no leaf hairs (peroxidase AA)

R4T8 [(*C. crenata* x *C. sativa*) x *C. dentata*] o.p.
'Lockwood'
 seedling from SL R4T3 (Plantation) open pollinated, 1946:
 SL R4T3 is **'Hammond-'86'**, from cross #86-31: Its male parent was a tree near Washington, DC (FP 551). Its female parent was the east branch of a tree managed by P. Hammond, Syosset, Long Island, New York, (estate of Bronson Winthrop). The Long Island tree was grafted with two leaders: one with a single nut in each bur, (east branch) and the other with three nuts per bur (west branch; broken off in Hurricane Gloria, 1985). A peroxidase test was done on material from the surviving east branch in 1994, and it was AB, proving that it was not pure Japanese (as Hammond assumed) but a hybrid. We believe that the east branch was Japanese X European and the west branch (probably the root stock) was Japanese. The hybrid **'Hammond-'86'** has good blight resistance. **'Hammond-'86'** was open pollinated in 1946 (probably by Japanese), and seedling **'Lockwood'** was planted here about 1957 (peroxidase BB)

R5T1-2 *C. alnifolia*
 Florida chinquapins from Lafayette County (50 miles NW of Gainesville, FL) in an oak-pine forest with sandy soil, collected by R. D. Wallace, Chestnut Hill Nursery, planted 1995, bloom late and seed rarely matures, not winter hardy (peroxidase AA & AA)

R5T3-6 American persimmons
 These were originally grafted with Asian cultivars, but all of the grafts died. The American seedlings were collected near Aurora, Arkansas in 1937

TOP OF HUMPHREY HILL

10. DENSE PLANTING OF AMERICAN CHESTNUT TREES

C. dentata, from the Wexford County Soil and Conservation District, Michigan, 226 seedlings planted at the top of the hill in April, 1981, used mixtures of hypovirulent strains, last treatment 1988. The tree in the NW corner used in crosses in 1988 is peroxidase AA

11. HYBRID CHESTNUT TREES (South of the Dense Michigan tree Planting)

R3T1 is on the North-East corner

- R3 T1, 2 **DW1 = 'Hope', and DW2**, same origin as **'Little Giant'** (below)
 T3 "*C. dentata*" from Schlarbaum, #90027, from State Nursery 2002 (mycorrhizae)
 T4 #24785, Eastern Turkey, Artvin Province, Collection 5, 2007
 T5 "*C. dentata*" from Schlarbaum, #90027, from CT State Nursery 2002 (mycorrhizae)
 T6 Eastern Turkey, Artvin Province, Collection 5, 2007
 T7 "*C. dentata*" from Hibben (Lasden Arboretum, CT) via Schlarbaum, #90025, from CT State Nursery 2002 (mycorrhizae)
 T8 *C. crenata* Bee & Thistle o.p., CT State Nursery 2002

12868 13 AA	12004	12882 ozarkensis
12855 14 AA	12030	...
12862	12013	...
...	12019 30 AA	...
12847 15 AA
...	12021	12967
12861 16 AA	12006	...
12853 17 AA
...
...	12031	...
...
...
...	Row 7 next WEST row	13072
...	Cross 4, 2003	
#7.5-03	HH R1T1 x 'Little Giant'	...
#7.5-03	#4-03 Wn nice*LG	...
#7.5-03
12848 18 AA
12832 19 AA	#14-04 Wtn x Rox	12881
13032 20 AA	#4-03	12975
...	#14-04 Wtn X Rox	...
12022 21 AA	#4-03 Wn nice*LG	...
...	#4-03	12909
12819 22 AA	#4-03	
...	#4-03	
..	#4-03	
13377 23	#16-04 K Art x LK	
...	#4-03	
13260 24 AA	#18-06	
13255 25 AA	#18-06	
13259 26 AA	#18-06	
...	#8-06	
...	#7-06	
...	#12-06	
...	#9-06	
12033 27 AA	#9-06	
12016 28 AA	#10-06	
12018	#10-06	
12014	...	
12010 29 AA	...	
12017	12982 ozarkensis	
...		

<u>Row 8 next WEST</u>	...
#4-03 HH R1T1 X LG	#8-03
#4-03	#8-03
#4-03	#8-03
#4-03	#8-03
#4-03	...
#4-03	...
#4-03	...
#4-03	#8-03
...	#8-03
#4-03	...
#4-03	#8-03
#4-03	#8-03
...	...
...	#8-03
...	...
#6-03 BC3 (C)RH R3T20	<u>and 14 #17-03 trees</u>
#6-03	<u>Morris (Merribrook)</u>
#6-03	<u>Stamford</u>
#7-03 BC3 (C)RH R3T14	...
#7-03	...
...	...
...	...
...	...
...	...
...	...
...	...
...	...
#7-03	...
...	...
...	...
...	...
#7-03	...
#7-03	...
...	...
#7-03	...
#7-03	...
#7-03	...
#7-03	...
#7-03	...
#7.5-03 BC3(C)RHR4T14	
#7.5-03 BC3 (C)	
...	
#8-03 BC3 (C)	

Row 9 (west of Row 8) NW corner

#16-04 K Art x Lk

#16-04

#16-04

#16-04

#16-04

#16-04

#16-04

Rock pile

#29-06 DW2 x 'Lockwood'

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

#29-06

Big rock

#12-04 Rox x S8

#12-04

#12-04

#12-04

#12-04

#12-04

#12-04

#12-04

#12-04

#12-04

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#12-04
#12-04
#12-04
#12-04
#23-04 Library o.p.
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04
#23-04

Row 10, next West

1. HH ozarkensis x HH R4T7 Watertown American (2008 seed)
3. HH ozarkensis x HH R4T7 Watertown American (2008 seed)
4. HH ozarkensis x HH R4T7 Watertown American (2008 seed)
5. HH ozarkensis x HH R4T7 Watertown American (2008 seed)

Bee and Thistle o.p.

C. crenata trees in rest of row

Row 11, next West

HH ozarkensis x SpL R7T61 **BC1** (crenata*ozarkensis) cross #6-08 (16 alive)
[WL R7T7 x Ark. RgammaT3]

Row 12, next West

1-6 HH R1T6 x HH R4T7 Rox x Watertown
7-9 KA *Lock x HH R4T7, #16-04 x Watertown
10-36 #7-08 HH oz x SpL R8T62 **BC1**
oz x oz*J Δ
(24 alive)

13. WEST OF THE ROCK, ROCKY HILL ORCHARD

Seed was from a wood-lot in Rocky Hill, CT in 1985 (open pollinated) from numbered female trees, planted at Smith College in a seed bed. Trees were transplanted to Lockwood Farm in the spring of 1988, and replacements for dead trees moved in the spring of 1989.

R1T1 (at the SE corner) RH #2 op, peroxidase AA

R1T2-3 RH #3 op, both peroxidase AA

R1T4-7 RH #5 op, all peroxidase AA

R1T8 RH #11 op, peroxidase AA

R1T9-10 RH #14 op, both peroxidase AA

R1T11 RH #26 op, peroxidase AA

R2T1 RH #2 op, peroxidase AA

R2T2 RH #3 op, peroxidase AA

R2T3-6 RH #5 op, all peroxidase AA

R2T7 RH #11 op, peroxidase AA

R2T8 RH #5 op, peroxidase AA

R2T9 RH #14 op, peroxidase AA

R2T10-11 RH #26 op, both peroxidase AA

R3T1-3 RH #3 op, all peroxidase AA

R3T4 RH #5 op, peroxidase AA

R3T5-6 RH #5 op, both peroxidase AA

R3T7 RH #3 op, peroxidase AA

R3T8 RH #14 op, peroxidase AA

R3T9 RH #5 op, peroxidase AA

R3T10 RH #3 op, peroxidase AA

R3T11 RH #26 op, peroxidase AA

R4T1-2 RH #3 op, both peroxidase AA

R4T3-6 RH #5 op, all peroxidase AA

R4T7 RH #11 op, peroxidase AA

R4T8 RH #14 op, peroxidase AA

R4T9 RH #3 op, peroxidase AA

R4T10 RH #11 op, peroxidase AA

THE ROCKY HILL PLANTING WAS EXTENDED IN JUNE 1996

R3T13-14 [(*Castanea dentata* x *mollissima*) x *C. dentata*] x *C. dentata* **BC2**
cross #7-95, NH R2T10 x RH(14) R3T8
[SL R10T12 x RH(5) R4T3]

R3T216-17? [(*Castanea dentata* x *mollissima*) x *C. dentata*] x *C. dentata* **BC2**
cross #7-95, NH R2T10 x RH(14) R3T8
[SL R10T12 x RH(5) R4T3]

R4T12 [(*Castanea dentata* x *mollissima*) x *C. dentata*] x *C. dentata* **BC2**
cross #7-95, NH R2T10 x RH(14) R3T8
[SL R10T12 x RH(5) R4T3]

14. SOUTH OF THE ROCK

Row 1 Tree 1 is in the north-east corner

R1	T1,2 T3,4 T3-13 T14-15 Lanz, (Japanese?) o.p. T16-18 Szego (Long Island) seguine*dentata x dentata*crenata	
R2	T1-2 <i>C. dentata</i> x [<i>C. dentata</i> x [(<i>C. crenata</i> x <i>sativa</i>) x <i>C. dentata</i>]] cross #9-95, RH(14) R1T10 X NH R2T3 (ex ' Hammond-86 ') T3 T4 [(<i>C. dentata</i> x <i>mollissima</i>) x <i>dentata</i>] x <i>dentata</i> cross #9-99, NH R2T10 x Walbridge, OH American T5 unknown <i>Castanea</i> from Rau in Washington State T6 T7-8 <i>C. dentata</i> x [<i>C. dentata</i> x [(<i>C. crenata</i> x <i>sativa</i>) x <i>C. dentata</i>]] cross #9-95, RH(14) R1T10 x NH R2T3 (ex ' Hammond-86 ') T9 T10 <i>C. dentata</i> x [<i>C. dentata</i> x [(<i>C. crenata</i> x <i>sativa</i>) x <i>C. dentata</i>]] cross #9-95, RH(14) R1T10 x NH R2T3 (ex ' Hammond-86 ') T11-13 unknown <i>Castanea</i> from Rau in Washington State T14 T15-16 <i>C. dentata</i> x [<i>C. dentata</i> x [(<i>C. crenata</i> x <i>sativa</i>) x <i>C. dentata</i>]] cross #9-95, RH(14) R1T10 x NH R2T3 (ex ' Hammond-86 ') T17 T18 <i>C. dentata</i> x [<i>C. dentata</i> x [(<i>C. crenata</i> x <i>sativa</i>) x <i>C. dentata</i>]] cross #9-95, RH(14) R1T10 x NH R2T3 (ex ' Hammond-86 ')	BC3 BC2 BC3 BC3 BC3
R3	T1 [(<i>C. dentata</i> x <i>mollissima</i>) x <i>dentata</i>] x <i>dentata</i> cross #9-99, NH R2T10 x Walbridge, OH American T2-4 root sprouts of seedling 'Little Giant' T5 T6 [(<i>Castanea dentata</i> x <i>mollissima</i>) x <i>dentata</i>] x <i>dentata</i> cross #9-99, NH R2T10 x Walbridge, OH American	BC2 BC2
R3	T7 T8 <i>C. "henryi"</i> II, (probably not pure <i>henryi</i>) from Liu Liu, Nanjing, 1991 Botanical Garden (root sprouts only, 2000) T9-12 <i>C. henryi</i> I, from Liu Liu, Nanjing Botanical Garden, 1991	
R4	T13-14 [(<i>C. dentata</i> x <i>mollissima</i>) x <i>dentata</i>] x <i>dentata</i> cross #9-99, NH R2T10 x Walbridge, OH American	BC2
R5	T1-2 [(<i>C. dentata</i> x <i>mollissima</i>) x <i>dentata</i>] x <i>dentata</i> cross #9-99, NH R2T10 x Walbridge, OH American T3 <i>Castanea dentata</i> x ' Lockwood ' cross #13-94, RH(11) R1T7 x HH R4T8 T4 <i>Castanea dentata</i> x ' Lockwood '	BC2

	cross #11-94, RH(5) R1T5 x HH R4T8	
T5-6	[(<i>C. dentata</i> x <i>mollissima</i>) x <i>dentata</i>] x <i>dentata</i> cross #9-99, NH R2T10 x Walbridge, OH American	BC2
T7	STUMP	
T8-9	<i>C. dentata</i> x ' Lockwood ' cross #11-94, RH(5) R1T5 x HH R4T8	
T10-13	[(<i>C. dentata</i> x <i>mollissima</i>) x <i>dentata</i>] x <i>dentata</i> cross #9-99, NH R2T10 x Walbridge, OH American	BC2
T14	<i>C. dentata</i> x (<i>C. dentata</i> x <i>mollissima</i>) cross #25-94, RH(5) x NH R3T5	BC1
T15	[(<i>C. dentata</i> x <i>mollissima</i>) x <i>dentata</i>] x <i>dentata</i> cross #9-99, NH R2T10 x Walbridge, OH American	BC2

15. Nut Planting South of the Rock

South East

Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9
Chestnut Sleeping Giant failed graft	'Colossal' x henryi 2011	'Colossal' x henryi 2011 1c	'Colossal' x henryi 2011	'Colossal' x henryi 2011	'Colossal' x henryi 2011	'Colossal' x henryi 2011	Persian Walnut 'Hansen'	
'Colossal' x henryi 2011	'Eaton' x henryi 2011	PERSIAN WALNUT 'Somers'	PERSIAN WALNUT 'Hansen'	BLACK WALNUT 'Grundy'	'Colossal' x henryi 2011	PERSIAN WALNUT 'Hansen'	PERSIAN WALNUT 'Broad-view'	
'Colossal' x henryi 2011	'Eaton' x henryi 2011	'Colossal' x henryi 2011	'Eaton' x henryi 2011	BLACK WALNUT 'Vander-sloot'	'Eaton' x henryi 2011	'Eaton' x henryi 2011	'Eaton' x henryi 2011	
HH R1T6 x C. henryi 2011			GA 31 C. henryi (GA) 2011	GA 30 C. henryi (GA) 2011	GA 30 C. henryi (GA) 2011	GA 30 C. henryi (GA) 2011	'Eaton' x henryi 2011	
PERSIAN WALNUT 'Broad-view'	BLACK WALNUT 'Grundy'?		GA 30 C. henryi (GA) 2011	C. henryi? Schm. 2013	C. henryi? Schm. 2013	CHEST-NUT 'Hartman' 17-8	C. henryi? Schm. 2013	HEART-NUT 'Rhodes'
X	CHEST-NUT 'Orrin'	CHEST-NUT 'Eaton'	CHEST-NUT 'Lenoir'	PERSIAN WALNUT 'Broad-view'	C. henryi? Schm. 2013	C. henryi? Schm. 2013		

16. Grafted Butternuts planted May 2012, April 2013, updated 5 June 2013

South East corner

	PA 25-9	PA 64-4		PA 18-4	PA 18-4
	VT Richmond A-19			PA 18-4	PA 64-4
	IA 12004	BUT BS #1	PA 61-1	PA 64-4	PA 25-4
VT Williston #4		PA 61-1			PA 61-1
BUT RS #1		PA 5-8	VT Williston #2		
VT Williston #1	VT Fox Run #1	VT St. Albans #3	PA 10-2		
	PA 64-1	VT St. Albans #3			MOCA 17
PA 64-6		PA 59-6		PA MOCA 17	PA 10-9
VT St. Albans #2	IA 021001	PA 17-1	PA 10-2	PA 10-8	VT Williston#2
PA 17-1		BUT BS #1	PA 10-9	PA 10-2	

17. Elm trees

From Gene Smalley, planted spring 1992

east side	south end						
20	<i>U. parvifolia</i>	2213	2213	2245-9	2245-9	2245-9	2245-9
19	all	"	2244-1	2245-3	"	"	2250-1
18	this	"	"	"	"	2245-10	"
17	row	"	"	"	"	"	"
16		"	"	"	"	"	"
15		"	"	"	"	"	"
14		"	"	"	"	"	"
13		"	"	"	"	"	"
12		"	"	"	2245-8	"	"
11		2276-1	"	"	"	"	"
10		"	2233-1	"	"	2247-3	"
9		"	"	2245-2	"	"	"
8		"	"	"	"	"	"
7		"	"	"	"	"	"
6		"	"	"	"	"	"
5		"	"	"	"	"	2245-5
4		"	"	"	"	"	"
3		"	"	"	"	"	"
2		"	"	"	"	"	"
1		"	"	"	"	"	"

18 Fagaceae Genetics Project

2006

- Crossed 'Mahogany' X 'Nanking' pollen (*Castanea mollissima* X *C. mollissima*)
 - WL R1T15, PI# 70315 X Greg Miller pollen, PI# 108552
 - 277 nuts sent to F. V. Hebard, Meadowview, VA
 - Some seedlings returned (bare-root) in 2008, but none survived transplanting
- Crossed Spring Lot R4T52 X SpL R4T31
 - (*C. mollissima* X *C. dentata*) X (*C. mollissima* X *C. dentata*)
 - ('Mahogany' X Roxbury, CT #1) X ('Mahogany' X Roxbury, CT #4)
 - 74 nuts sent to F. V. Hebard, Meadowview, VA

2007

- Crossed 'Mahogany' X 'Nanking' pollen (as above)
 - 304 nuts planted in the greenhouse, CAES
 - seedlings tagged/numbered and individual leaves sent to T. Kubisiak in Saucier, MS for DNA
 - seedlings planted at Lockwood Farm (CAES), Hamden, CT in 2008
- Crossed SpL R4T52 X SpL R4T31
 - (*C. mollissima* X *C. dentata*) X (*C. mollissima* X *C. dentata*)
 - ('Mahogany' X Roxbury, CT #1) X ('Mahogany' X Roxbury, CT #4)
 - 77 Nuts planted in the greenhouse, CAES
 - Seedlings given to F. V. Hebard, Meadowview, VA in 2008
- Crossed SpL R4T31 X SpL R4T52
 - (*C. mollissima* X *C. dentata*) X (*C. mollissima* X *C. dentata*)
 - ('Mahogany' X Roxbury, CT #4) X ('Mahogany' X Roxbury, CT #1)
 - 58 Nuts planted in the greenhouse, CAES
 - Seedlings given to F. V. Hebard, Meadowview, VA in 2008

2008

- Crossed 'Mahogany' X 'Nanking' pollen (as above)
 - 70 nuts planted in the greenhouse, CAES
 - seedlings tagged/numbered and individual leaves sent to T. Kubisiak in Saucier, MS for DNA
 - seedlings planted at Lockwood Farm (CAES), Hamden, CT in 2010
 - trees 10 ft apart in rows 10 ft apart
- Crossed SpL R4T52 X SpL R4T31
 - (*C. mollissima* X *C. dentata*) X (*C. mollissima* X *C. dentata*)
 - ('Mahogany' X Roxbury, CT #1) X ('Mahogany' X Roxbury, CT #4)
 - 1 Nut planted in the greenhouse, CAES
 - Seedling given to F. V. Hebard, Meadowview, VA in 2009
- Crossed SpL R4T31 X SpL R4T52
 - (*C. mollissima* X *C. dentata*) X (*C. mollissima* X *C. dentata*)
 - ('Mahogany' X Roxbury, CT #4) X ('Mahogany' X Roxbury, CT #1)
 - 10 Nuts planted in the greenhouse, CAES

The first chestnuts planted on this site, other than native trees, were set out by Dr. Arthur H. Graves in March of 1930. At that time Dr. Graves was a curator at the Brooklyn Botanic Garden, and this was his family's land. He received those first Asian chestnuts from the USDA in Beltsville, Maryland.

About 1939 Dr. Donald F. Jones, Chief of the Genetics Department at The Connecticut Agricultural Experiment Station, became interested in the chestnut breeding program, and participation by the Experiment Station in the project was begun.

In 1949, Dr. Graves sold 8.3 acres of land to the Sleeping Giant Park Association, reserving its use for The Connecticut Agricultural Experiment Station for tree breeding. Then in 1950 the Park Association gave five pieces of land (including the 8.3 acres) to the State for Sleeping Giant State Park, reserving the use of the 8.3 acres for the Experiment Station. Since that time, the chestnut project has been administered by The Experiment Station with the cooperation of the Sleeping Giant State Park Rangers. Dr. Graves actively continued his work with chestnuts until his death in December, 1963.

SOUTH LOT

East side of Chestnut Lane

Row B is nearest Chestnut Lane, trees are numbered from north to south

RBT5 *Castanea* (NOT *dentata*)?

bark graft V205 of tree from **Scientists' Cliffs**, MD, on land of F.

Gravett, (probably *sativa*), stock Japanese, graft 5-V-62 [peroxidase AA+]

RBT8 *Castanea henryi*

from R. C. Ching, Lu-Shan Botanic Garden, Han-Po-Kou, Lu-Shan,

Kiu Kiang, China, 4000 ft above sea level, planted 1935 (not winter-hardy)

RBT10 *Castanea crenata* (X *sativa* ?) X *dentata*

Cross #238-31 1B113 (called the "**Smith hybrid**"), female parent was R. S. Smith, Oyster Bay, Long Island, New York, purchased as 'Japanese Giant' from a nursery near Rochester, NY, Graves said this was "evidently of hybrid nature," it was 35 years old in 1929, one foot dbh, and had only one nut per bur, male parent was a tree in Washington, DC, F.P. 551 (*dentata* on Beall's land) fuzzy veins top and bottom, very few stellate hairs [SLA peroxidase AB]

RBT11 'Eaton' seedling planted 1973

RBT13 complex hybrid planted 1958

Cross #24-55, 'Sleeping Giant' X 'Toumey'

[C X (JE*A)] X [C X (JE*A)]

RBT16 large leaf, stellate hairs, unknown

R1T3 *Castanea mollissima*

USDA #70315, hardy trees from northeastern China, seed purchased 1926 by J.H.

Reisner, Nanking University, planted 1930 [peroxidase AB]

R1T6,7 unknown

R1T9 'Eaton' seedling

R1T15 *Castanea mollissima*

- `Mahogany' USDA #70315, hardy trees from northeastern China, seed purchased 1926 by J.H. Reisner, Nanking University, planted 1930 [Santamour peroxidase AB]
- R1aT11 complex hybrid cross #17-36, planted 1937
 graft of 'S8' X Smith hybrid, (*pumila**J) X (JE*A), few stellate hairs, fuzzy buds, green twigs, hairs top mid-vein
- R2T1-10 complex hybrid, HH ('Little Giant'?) X 'Eaton'
 Cross #1-70
- R2T11 *Castanea mollissima* X *sequinii*
 Cross #17-34, female parent was R1T12, USDA #70315, male parent R3T8 [peroxidase AB]
- R2T12 DEAD *Castanea crenata* seedling, Higashiyama, Kuriyama, Hokkaido wild seed, planted 2001
- R2T13 "Eaton" seedling
- R2T16 *Castanea sequinii*
 USDA #70317 seed 1926, seedling planted 1930, F.A. McClure #700, Chiuhywashaan, Anhwei, called "Mo lut tsz" [Santamour peroxidase BB]
- R3T1-7 complex hybrid, HH ('Little Giant'?) X 'Eaton'
 cross #1-70
- R3T8 *Castanea sequinii*
 USDA #70317 seed 1926, seedling planted 1930 (see R2T16) [peroxidase BB]
- R3T9 complex hybrid, HH ('Little Giant'?) X 'Eaton' cross #1-70
- R3T11 Earl Douglas hybrid
- R3T12 *Castanea crenata* seedling, Higashiyama, Kuriyama, Hokkaido wild seed, planted 2001
- R3T15 cross #13-55, Denmark #4 X (*mollissima* R1T12 X *sequini*, cross #17A-34)
- R3T16 *Castanea mollissima*
 from R. C. Ching, Lu-Shan Botanic Garden, Han-Po-Kou, Lu-Shan, Kiu Kiang, China, 4000 ft above sea level, had survived -15°F, planted 1935 [peroxidase AA]
- R4T3 *Castanea (crenata* X *sativa*) X *dentata*
 graft spring 1948 of R4T10, `Hammond-'86' on *C. crenata* forest type (USDA 1930); R4T10 was cross #86-31, female parent was the east branch of P. Hammond, Syosset, Long Island, New York, estate of Bronson Winthrop, probably a hybrid of Japanese X European, a grafted tree with two leaders: one (east branch; peroxidase test in 1994: AB) with a single, and the other (west branch; broken off in Hurricane Gloria, 1985) with three nuts per bur, good blight resistance, male parent was a tree in Bell, MD, (FP#551). graft flowering, blighted, and inarched 1957 [SLA peroxidase AB]
- R4T5 *Castanea mollissima* X *dentata*
TRIPLOID, cross #86-34, female parent was SL R1T4 *mollissima* USDA #70315, male parent F.P. 551; tree listed as sterile in 1957 but a sprout had both male

- and female flowers 1988, and set filled nuts, leaves much smaller than triploid part
- R4T13 cross #13-55, Denmark #4 X (*mollissima* R1T12 X *seguini*, cross #17A-34)
- R5T3 *Castanea mollissima* X [(*crenata* X *sativa*) X *dentata*]
Cross #327B-37, SL R1T12 x SL RBT12
- R5T4 *Castanea mollissima* hybrids, Earl Douglas, NY 1974
- R5T6-9 *Castanea mollissima* hybrids, Earl Douglas, NY
- R5T10 complex hybrid, A (Rox.5) X C*JA, planted 1951
cross #46-48
- R5T13 *Castanea mollissima* X [(*crenata* X *sativa*) X *dentata*]
`**Sleeping Giant**' (=C2), #276A'-37, female parent was R1T12 *mollissima* USDA #70315, male parent was RBT12, Smith hybrid, cross 233A' 1931 (Oyster Bay, NY X FP#551, Washington, DC) [Santamore peroxidase BB]
- R5T14 *Castanea crenata* seedling, Higashiyama, Kuriyama, Hokkaido wild seed, planted 2001
- R6T2 'Hammond '86' open pollinated, 1941
- R6T5 *Castanea (pumila* X *crenata*) X *crenata*
graft #V222 in 1963 of R16T1 `**Essate-Jap**' (=C1) on *crenata*, this was R2T1, ['S8' of Van Fleet (*pumila* X *crenata*), grafted tree (?) or seedling planted 1930] crossed in 1934 with Japanese forest-type USDA #78626 seedling planted 1930
- R6T6 *Castanea mollissima* X [(*crenata* X *sativa*) X *dentata*]
graft on *crenata*, 1957, of R59T39 `**Toumey**' (=C5), MJ X Smith hybrid, R8T6 X RBT12
- R6T11 *Castanea* sp
- R6T12-13 *Castanea crenata* seedling, Sakurayama, Kuriyami, Hokkaido wild seed, planted 2001
- R6T15 [(*Castanea crenata* X *C. pumila*) X *C. crenata*] X *C. dentata*(?)
cross #4-55 'S8'*J X A (suspect!) (peroxidase AB)
- R6T16 'C9' X 'Clapper' cross #2-72
- R7T3 *Castanea* hybrid
graft #V119, 1957, of R3AT44 `**C-3**'
'S8' X 'MI', SL R2T1 X SL R8T7
- R7T6 *Castanea mollissima* X [(*crenata* X *sativa*) X *dentata*]
graft on *crenata*, 1957, of R59T39 `**Toumey**' (=C5), 'MJ' X Smith hybrid (R8T6 X RBT12)
- R7T7 *Castanea crenata*
USDA #78626, seed 1929, wild tree #748, Oguriyama, Chitose Mura, Naka, Tsugaru Gun, Amori Ken, Japan (Santamour peroxidase BB)
- R7T8 *Castanea [mollissima* X (*crenata* X *dentata*)] X [(*mollissima* X *dentata*) X *dentata*]
'C-9' X 'Clapper' cross #4-70, planted 1972, called `**Hamden**'
- R7T9 *Castanea crenata* X [(*crenata* X *sativa*) X *dentata*]

- Cross #81-42, Folk WL R15T2 X 'Hammond '86', perox. BB
- R7T10 (*Castanea dentata* X S8) X *crenata* & *mollissima*
Cross #23-47, R8T9 X R1T7 & R1T6, male sterile, 25 chromosomes in nuts (1958 data)
- R7T11 *Castanea crenata* X *dentata*
Cross #22-35, female parent SL R10T5 "Mammoth"; USDA #76873 ('MJ'), male parent "Clapper (F.P. 555) and No. Spring", male sterile (peroxidase AB)
- R7T12, 13 *Castanea crenata* seedling, Sakurayama, Kuriyami, Hokkaido wild seed, planted 2001
- R7T15 *Castanea* [*mollissima* X (*crenata* X *dentata*)] X [(*mollissima* X *dentata*) X *dentata*]
'C-9' X 'Clapper' cross #4-70, planted 1972
- R8T1 unknown
- R8T4 *Castanea sequinii*
(formerly R4T2) USDA #70317 seed 1927, seedling planted 1930 (see R2T16) [peroxidase BB]
- R8T5 *Castanea crenata*
from Col. E. Thompson, RI, parents brought from Korea after the war, planted 1993
- R8T6-9 *Castanea crenata* seedling, Higashiyama, Kuriyama, Hokkaido wild seed, planted 2001
- R8T11-15 *Castanea mollissima*
seed from Helen Foster Snow in 1972 Wen Chia Shih, Liu Yanghsien in Hunan from trees planted by Mao Tse-tung in 1929
- R9T2 *Castanea mollissima*
USDA #78744, 'Tiger Paw' FP 'MCH' collected by Peter Liu from the Fa Hua Ssu Temple near Peiping, Hopei, China [peroxidase AB]
- R9T4 *Castanea* hybrid
graft of SL R5T13 'Sleeping Giant' (C2), 1956
- R10T9 POSSIBLY one of the original Mintern hybrids, *C. crenata* X *dentata*
since notes say there was a label that said "JA 19-33"
- R10T10 *Castanea dentata* X (*pumila* X *crenata*)
Long Island cross #25 (or #60A) -35, female parent from Half Hollow Hills, Melville, LI, male parent probably SL R2T3, 'S8' of Van Fleet, grafted on Japanese [peroxidase AB]
- R10T11 *Castanea mollissima* X *sequinii*
Cross #17B-34, female parent R1T12 USDA #70315, male parent called "everbearing sequine"
- R10T12 *Castanea dentata* X *mollissima*
Long Island cross #58-35, very different morphology from R10T10, male parent probably USDA #70315 from SL R1, male sterile, catkins form but don't open [peroxidase AB]
- R11T9 'S8' X unknown, 1937

- R11T10 unknown chinquapin, called "C-55" in 1958
 R11T14 *Castanea mollissima*
 Hobson, Jasper, GA, PI #36666 o.p., seed 1938, planted 1943
 R12T9 *Castanea mollissima*
 Hobson, Jasper, GA, PI #36666 o.p., seed 1938, planted 1943
 R12T10 *Castanea crenata* X (*crenata* X *dentata*)
 Cross #48-42, Japanese from Mr. Folk, WL R15T2 X Mintern SL R2T4
 R13T12 *Castanea mollissima* X *dentata*
 Cross #263A-37, SL R1T2 X Pennsylvania & North Spring

Chinkapin Corner (southeast corner of the South Lot)

- R-alphaT2 *Castanea ozarkensis*, Garfield, Centon Co., Arkansas, planted 1936
 R-bettaT1 *Castanea pumila*, G. Miller, Carrollton, OH, planted 2003
 R-bettaT2 *Castanea alnifolia*
 R-gammaT3-4 *Castanea ozarkensis*, Garfield, Centon Co., Arkansas, planted 1936
 R-deltaT1 *Castanea ozarkensis*, Garfield, Centon Co., Arkansas, planted 1936
 R-epsilonT3 *Castanea ozarkensis*, Garfield, Centon Co., Arkansas, planted 1936
 R-etaT4 *Castanea ozarkensis*, Garfield, Centon Co., Arkansas, planted 1936

WEST LOT

West side of Chestnut Lane, North end of property

- R13T2 *Castanea crenata* o.p.
 nuts from H.N. Folk, Brielle, NJ, 1930 parents purchased as 'Japanese Giant' from a nursery near Rochester, NY, prize nuts, NNGA
 R13T4 *Castanea (pumila X crenata) X crenata*
 Cross #5C-34 which was SL R2T1, [S8 of Van Fleet, *pumila X crenata*, grafted on Japanese] crossed with a Japanese forest-type USDA #78626 in SL R6T11
 R13T6 *Castanea crenata* o.p.
 nuts from H.N. Folk, Brielle, NJ, 1930 parents purchased as 'Japanese Giant' from a nursery near Rochester, NY [peroxidase BB]
 R13T9 *Castanea dentata*
 nut from Thomson, Ashville, NC [peroxidase AA]
 R13AT1 *Castanea mollissima*
 nut from J.B. Gable, Stewartstown, PA 1938 [Santamour perox. BB]
 R13AT8 *Castanea mollissima*
 nut from J.B. Gable, Stewartstown, PA 1938
 R14T3 *Castanea mollissima* X [*crenata sativa*] X *dentata*
 Cross #338C-37, SL R1T4 X Hammond '86 (SL R4T10)
 R14T8 *Castanea crenata* o.p.
 nut from H.N. Folk, Brielle, NJ, 1930 [Santamour peroxidase BB]
 R14T9 *Castanea dentata*
 nut from Thomaston, PA 1933 [peroxidase AA+]

- R14AT1 *Castanea crenata* x *dentata* #95-34, SL R1T7 x American (Washington)
FP 551
- R15T5-7,10 *Castanea mollissima*
selected Chinese
- R16T1 *Castanea (pumila X crenata) X crenata*
'Essate-Jap' (=C1), cross #9-34 which was SL R2T1, ['S8' of Van Fleet, *pumila X crenata*, o.p.] crossed with a Japanese forest-type USDA #78626 in SL R6T11, dense stellate hairs [Santamour peroxidase BB]
- R16T8-9 *Castanea mollissima*
selected Chinese
- R16T11 *Castanea pumila X asheii*
Cross #14-61, R-epsilonT4 X West Spring
- R17T5-6 *Castanea mollissima*
selected Chinese [Santamour peroxidase AB, BB]
- R17T9-10 *Castanea pumila X alnifolia*
Cross #15-61, R-epsilonT4 X R-betaT3
- R17T11 *Castanea pumila X seguinii*
Cross #12-61, R-deltaT4 X SL R3T8, three nuts/bur, perox. AB
- R18T4 *Castanea sativa X crenata*
Cross #17-51; Villa Colombo X GH-4 pollen (USDA)
- R18T6 *Castanea mollissima X [(crenata X sativa) X dentata]*
Cross #32-51, SL R1T15 (Mahogany) X SL R4T4 (Hammond '86 graft)
- R20T11 *Castanea* hybrid
Cross #18-51, J*JA X J ('M38')
- R20T12 *Castanea dentata X mollissima*
Cross #1-89, American (farm) R1T7 X Chinese WL R37T7
- R20T14-19 *Castanea* hybrid
Cross #67-61, AC*J X C
- R21T7 *Castanea [(crenata X sativa) X dentata] X mollissima*
Cross #50-51, Hammond '86 grafts at R3T3 and R4T4 X 'Mahogany' at R1T15
- R23T1 *Castanea* hybrid
Cross #63-60, R13AT7 ("Denmark") X American R17T7 from J.J. McKenna, PA 1938, planted 1960 [Santamour peroxidase AB]
- R23T10?
- R23T14-17 *Castanea ozarkensis X mollissima*
Cross #18-61, REpT3 X Burbank's 'Miracle' R8T7 graft, planted 1965
- R23T19 *Castanea dentata X seguinii*
Cross #37-61, female parent was American, Roxbury, CT #5, male parent was SL R3T8
- R24T9??
- R25T5 *Castanea alnifolia X ozarkensis*
Cross #55-60, R-betaT3 X R-alphaT2
- R25T9??

- R25T14-17 *Castanea ozarkensis* X *mollissima*
Cross #18-61, REpT3 X Burbank's `Miracle' R8T7 graft, one nut per bur [Santamour peroxidase AB, AA, x, x]
- R25T10 S8*J X S8*J #2-51 multiple stems
- R26T8 *Castanea* hybrid
Cross #77-51, C*S X C*S, SL R2T11 X SL R10T11
- R26T12 *Castanea* hybrid
Cross #77-51, C*S X C*S, SL R2T11 X SL R10T11
- R27T14 *Castanea mollissima*
Cross #121-60, R1T9 selfed
- R27T15-16 *Castanea ozarkensis* X *alnifolia*
Cross #21-60, R-gammaT3 X R-betaT3 planted 1965
- R27T17-18 *Castanea* hybrid
Cross #62-60, R13AT3 "Lindholm" (Denmark) X Roxbury #1
- R27T19 *Castanea dentata* X *pumila*
Cross #41-61, Roxbury #1 X R-epsilonT4
- R28T7 *Castanea mollissima* X [(*crenata* X *sativa*) X *dentata*]
graft V57 of R5T13 `Sleeping Giant' (=C2), 1953
- R29T1 *Castanea* hybrid
Cross #87-60, E(?) X J
- R29T3 *Castanea* hybrid
Graft V59 X J XJ*A
- R29T5 *Castanea* hybrid
Cross #15-53 of (*crenata* X *dentata*) X *mollissima*
- R29T7 *Castanea* hybrid
Graft V56 of C5
- R29T9 *Castanea* hybrid
Cross #63-60, R13AT7 "Lindholm" (Denmark) X R17T7 (*ashei*)
- R29T11 unknown *mollissima*
[Santamour peroxidase AA]
- R29T14 *Castanea ozarkensis* X *C. seguinii*
Cross #23-60, R-alphaT2 X R3T8 [Santamour peroxidase AB]
- R29T16 *Castanea henryi* X *ozarkensis*
Cross #4-60, R32T1 X R-gammaT3 [Santamour peroxidase AB]
- R29T17 *Castanea ozarkensis* X (*crenata* X *sativa*)
Cross #37-60, R-gammaT3 X M82 (graft V84) Solignat says latter "not vigorous or blight resistant, large nuts, flavor not very sweet"
[Santamour peroxidase AB]
- R29T19 *Castanea dentata* X *seguinii*
Cross #47-60, Roxbury #3 X R3T8
- R30T9 & 11 *Castanea ozarkensis* X *dentata*
Cross #35-60, R-gammaT3 X Roxbury #1 nuts one or two per bur, and one per bur

- R30T13-14 *Castanea dentata* X *ozarkensis*
Cross #67-60, Roxbury #1 X R-gammaT3 nuts one or two per bur,
and one, two, or three per bur [Santamour peroxidase AA, x]
- R30T15-16 *Castanea dentata* X *ashei*
Cross #68-60, Roxbury #1 X West Spring, nuts one per bur and
three per bur [Santamour peroxidase AA, AA]
- R30T17-(18) *Castanea ozarkensis* X *henryi*
Cross #5-60, R-gammaT3 X R32T1 [Santamour peroxidase AB, BB]
**lower leaf few long simple hairs on all veins, glands, appressed stellate hairs
between veins, top leaf few long simple hairs on veins**
- R30T19 *Castanea mollissima*
Mahogany at R1T15, selfed, #122-60

WEST LOT

FIRST TREE ON RIGHT AT TOP OF NORTH PATH

- R32T1 *Castanea henryi*
USDA #104058 (FP #HE), Hsiaohsing, Anhwei Prov, planted 1935
[Santamour peroxidase AB, SLA peroxidase BB]
- R32T4 *Castanea ozarkensis* X *henryi*(?)
Cross #2-58, R T3 X Graves tree
- R32T6 *Castanea mollissima*
FP 530, from Tientsin, purchased in a San Francisco market, down
in hurricane Gloria 1985
- R33T3 *Castanea* hybrid
graft of J X J*A, Cross #48-55, New Jersey tree X Hammond '86

In the small triangle near R32T2

- △ 'Early Jap'
Based on location, this could be "GM" which is PI #104014 from Temple
Forest, Koyasan, Wakayana-Ken Japan (33* lat.), seed 1934, Graves got
seedling in 1935
- R34T1 *Castanea crenata*
stump sprouts of unknown Japanese
- R34T2 unknown *Castanea* [peroxidase AB]
- R34T4 *Castanea* hybrid
Cross #18-55, C*S X J(prize nuts) #18-55
- R34T6 *Castanea crenata*
USDA #104016, Japanese GO, Numakunai Eirinsho, Ippoimura,
Iwate-gun, Iwate-ken, Japan, planted 1935 [peroxidase BB]
- R36T4 *Castanea* hybrid
Cross #16-55, C X J*A, 'Mahogany' X 'Mintern'
- R37T4 & 7 *Castanea mollissima*

USDA #104061, Chinese MAU, "Tall Chinese" `Lui An', Chekiang Province, China (28-32 deg. latitude), Peter Liu; seed lot reported to be 47 to the pound and with easy pellicle removal, planted 1935 [T7 is peroxidase AA]

R38T2,3,4,6

Castanea mollissima

USDA #104063, Chinese MAW "Large Chinese" `Kuei Lee', Hsin Teng, Chekiang Province, China, Peter Liu; seed lot reported to be 40 to the pound and with poor pellicle removal, planted 1935 [T3 is peroxidase BB]

WEST RED PINE LOT

West of the northern part of the West Lot

R2T1

Castanea hybrid

Cross #72-51, CJA X CJA

R2T10 *Castanea* hybrid

Cross #72-51, CJA X CJA

R3T2 & 8

Castanea hybrid

Cross #69-51, CJA X CJA

R4T9

C. (mollissima X dentata) X (crenata X dentata)

Cross #66-51, R1T15 and R1T7, Americans both F.P. #551 "Beall's" from Bell, MD, this tree looks very Chinese!

R5T11 *Castanea* hybrid

Cross #48-51, C X JA

R6T2

Castanea hybrid

Cross #35-51, J X JA, 'M38' X J(prize nuts)*A [peroxidase AB], "handsome tree, bearing well

R6T10-11

Castanea mollissima X sativa

Cross #29-51, Chinese R1T12 X European, Villa Colombo

R7T5

Castanea hybrid

Cross #18-55, CS X J (which was the "prize nuts" J)

R8T10 *Castanea* sp. [root sprouts of grafted tree]

R9T1

Castanea crenata X dentata **NOT**

graft of Litchfield R1T12 which is #65-39, SL R1T7 X American, spring lot perox. BB

R10T2 *Castanea* hybrid

Cross #43-53, C X JA, 'Mahogany' X 'Minturn'

R10T11(sprouts), 12, 13, 15 *Castanea (dentata X pumila*crenata) X mollissima*

Cross #40-53, SL R10T10 X R1T3, planted 1964 ?

R11T1 *Castanea* hybrid

Cross #18-55, CS X J (which was the "prize nuts" J), tall tree

R11T8 *Castanea* hybrid

Cross #63-52, CJA X CJA

R11T16

*Castanea (dentata X pumila*crenata) X mollissima*

Cross #40-53, SL R10T10 X R1T3, planted 1964?, small sprouts 2003

- R13T1 *Castanea (mollissima X dentata) X dentata*
 Cross #37-53, (Chinese R1T15 X F.P. 551; cross #105B-34) at SL R2T8 X Bowman,
 Clinton Corners, NY, this is the tree called 'Graves', perox. AB
- R14T12 *Castanea* hybrid
 Cross #18-55, CS X J (which was the "prize nuts" J)

WEST WEST LOT
West of the southern part of the West Lot

- R1T1 [SE corner of block]
C. dentata *X crenata*
 Cross #28-89, R3T17 X Cheshire 'Parsons' Japanese'
- R1T2 *C. dentata* *X [(crenata X sativa) X dentata]*
 Cross #16-89, R2T18 X SL RBT10 'Smith'
- R1T4 *C. dentata X [(crenata X sativa) X dentata]*
 Cross #16-89, R2T18 X SL RBT10 'Smith'
- R1T5 & 7 (*C. dentata x [pumila*crenata]*) *X dentata*
 Cross #42-89, SL R10T10 X Mich
- R1T9-14 (*C. dentata x mollissima*) *X dentata*
 Cross #31-89, SL R10T12 X Mich
- R2T2 *C. dentata* *X crenata*
 Cross #8-91, R4T12 X WL R34T6
- R2T3-4 *C. dentata X [(crenata X sativa) X dentata]*
 Cross #16-89, R2T18 X SL RBT10 'Smith'
- R2T6 (*C. dentata x [pumila*crenata]*) *X dentata*
 Cross #42-89, SL R10T10 X Mich
- R2T8 *C. dentata X crenata*
 Cross #28-89, R3T17 X Cheshire 'Parsons' Japanese'
- R2T9,14 (*C. dentata x mollissima*) *X dentata*
 Cross #31-89, SL R10T12 X Mich
- R2T15 *C. dentata x crenata*
 Cross #28-89, R3T17 X Cheshire 'Parsons' Japanese'
- R3T1 *C. dentata X crenata*
 Cross #29-89, R3T16 X Cheshire 'Parsons' Japanese'
- R3T6-7 (*C. dentata x [pumila*crenata]*) *X dentata*
 Cross #42-89, SL R10T10 X Mich
- R3T9-10,14 (*C. dentata x mollissima*) *X dentata*
 Cross #31-89, SL R10T12 X Mich
- R3T15 *C. dentata X crenata*
 Cross #28-89, R3T17 X Cheshire 'Parsons' Japanese'
- R4T3 *C. dentata X [(crenata X sativa) X dentata]*
 Cross #16-89, R2T18 X SL RBT10 'Smith'
- R4T6-7 (*C. dentata x [pumila*crenata]*) *X dentata*

R4T8 Cross #42-89, SL R10T10 X Mich
C. dentata X *crenata*

R4T9-14 Cross #28-89, R3T17 X Cheshire 'Parsons' Japanese'
 (*C. dentata* x *mollissima*) X *dentata*

R5T2 Cross #31-89, SL R10T12 X Mich
C. dentata X [(*crenata* X *sativa*) X *dentata*]

R5T6 Cross #16-89, R2T18 X SL RBT10 'Smith'
C. dentata X *crenata*

R5T9 Cross #17-91, R4T10 X WL R34T6
C. dentata X *crenata*

R5T10-12 Cross #17-91, R4T10 X WL R34T6
 (*C. dentata* x *mollissima*) X *dentata*

R6T4 Cross #31-89, SL R10T12 X Mich
C. dentata X [(*crenata* X *sativa*) X *dentata*]

R6T5 Cross #16-89, R2T18 X SL RBT10 'Smith'
C. dentata X *crenata*

R6T6 Cross #24-89, R3T17 X Cheshire 'Parsons' Japanese'
 (*C. dentata* x [*pumila***crenata*]) X *dentata*

R6T7 Cross #42-89, SL R10T10 X Mich
C. dentata X *crenata*

R6T8 Cross #17-91, R4T10 X WL R34T6
C. dentata X *crenata*

R6T9 Cross #28-89, R3T17 X Cheshire 'Parsons' Japanese'
C. dentata X *crenata*

R6T11 Cross #17-91, R4T10 X WL R34T6
 (*C. dentata* x *mollissima*) X *dentata*

R6T11 Cross #31-89, SL R10T12 X Mich

SPRING LOT

North of the South Lot on the east side of Chestnut Lane

R1T1-70 *Castanea* hybrids, mostly 'Sleeping Giant' op

R2T1-61 *Castanea* hybrids, mostly op

R3T1-49 *Castanea* hybrids, mostly op

R3T50, 52, 55, 56, 57 *Castanea crenata*
 Cross #39-59, SL R7T7 X R7T5 (both USDA-PI #78626)

R3T61-65 *Castanea crenata* X *seguini*
 Cross #41-59, R7T7 (USDA-PI # 78626) X R2T16 (USDA-PI #70317)

R3T66 *Castanea seguini* op

R4T16 *Castanea pumila* var *ashei*
 "North Spring Chinquapin"

R4T18-21 *Castanea* op

R4T23-24 *Castanea mollissima* X *dentata*

- Cross #45-59, SL R11T14 (Jasper, GA) X American (Bristol, CT) [F. Hebard reports T23 good resistance] -T23 is perox. AB
- R4T26 *Castanea mollissima* X *dentata*
Cross #37-59, SL R11T4 (Jasper, GA, PI #36666 o.p.) X American (Roxbury, CT, #4 (west))
- R4T27 *Castanea dentata*
Roxbury, CT tree #4 (west) open pollinated
- R4T31 *Castanea mollissima* X *dentata*
Cross #36-59, 'Mahogany' SL R1T15 X American (Roxbury, CT, #4 (west)) [peroxidase AB]
- R4T33-34 *Castanea* op
- R4T37 & 39 *Castanea dentata*
American, Roxbury, CT, #1 (east) open pollinated [peroxidase AA]
- R4T43 unknown *Castanea*, very large, looks Japanese, perox. AB
- R4T49, 52, 54 *Castanea mollissima* X *dentata*
Cross #35-59, 'Mahogany' SL R1T15 X American (Roxbury, CT, #1 (east)) [peroxidase AA, AA, AB]
- R4T55-67 *Castanea* hybrids, mostly op
- R5T1-31 *Castanea* hybrids, mostly op
- R5T33 *Castanea henryi* X *ozarkensis*
Cross #3-59, WL R32T1 X RepsilonT3
- R5T38-52 *Castanea* hybrids, mostly op
- R5T61 & 67 *Castanea crenata* X root stock (?)
Cross #8-60, SL R7T7 X WL R15T6
- R6T10 graft '**Redwing**', V196, in 1963 [this is on the edge of the driveway]
- R6T21-26 *Castanea crenata* X root stock (?)
Cross #8-60, SL R7T7 X WL R15T6
- R6T29-31 *Castanea crenata* X *seguini*
Cross #45-60, SL R7T5 (USDA-PI #78726) X SL R3T8 (USDA-PI #70317)
- R6T35 *Castanea crenata* X *henryi*
Cross #28-60, Early Jap (triangle) X WL R32T1, tall, no stellate hairs, very good resistance
- R6T37 perox. BB
- R6T41,42,44,45,58,49 *Castanea ashei* X *crenata*
Cross #81-60, West Spring X WL R14T8 (Folk Japanese, Briel, NJ, op), T45 tall, rest short, flat stellate hairs, short simple hairs, glands, one nut/bur
- R6T52 *Castanea ashei* op
West Spring
- R6T57-58 *Castanea ashei* X *henryi*
Cross #78-60, West Spring X WL R32T1
- R6T65 *Castanea pumila* X *crenata*
Cross #16-60, RdeltaT2 X Early Jap (triangle), [perox. AB]
- R7T13 *Castanea mollissima*
'**Nanking**' V218 graft, April 1963 [tree is on the edge

of the driveway]

- R7T14 *Castanea pumila* X *crenata*
Cross #16-60, RdeltaT2 X Early Jap (triangle)
- R7T15,21,25 *Castanea ozarkensis* X *mollissima*
Cross #50-60, RgammaT3 X SL R1T6 (USDA-PI #70315)
- R7T29-30 *Castanea mollissima* (?) X (*crenata* X *sativa*)
Cross #41-60, WL R15T6 X HH R3T2 (was M15 from France)
- R7T31 & 33 *Castanea mollissima* (?) X *crenata*
Cross #7-60, WL R15T6 X SL R7T7
- R7T44,46,47,48 *Castanea ozarkensis* X *ashei*
Cross #32-60, RgammaT3 X West Spring
- R7T59,61 *Castanea crenata* X *ozarkensis*
cross #27-60, SL R7T7 X RgammaT3, [perox. BB and AB]
- R8T43 as above
- R8T62 *Castanea ozarkensis* X *crenata*
Cross #25-60, RalphaT2 X Early Japanese (triangle)
- R9 Hybrids, op

HYBRID SLOPE

North of the Spring Lot on the east side of Chestnut Lane

These trees have a lawn around them, next to swimming pool

south end

R62T43 Cross #83-39 *mollissima* R1T9 X Hammond 99A-33, C X JA **C7**

south and east

R61T48 Cross #253-37, *mollissima* 'Mahogany' R1T15 X Smith RBT12, C X JEA
C4

east and north

R59T39 Cross #138A-37 *mollissima* MJ R8T6 X Smith RBT12, C X JEA, **C5**,
'Toumey'

east

R57T35 Cross #261-38 *C. seguinii* R4T2 X *C. alabamensis*

east

R53T2 *C. mollissima*, Simpson, China, 1941

east

R51T31 Hammond '86 open pollinated, 1940, JEA op, **C6**

east

R50T42 Cross #55-40 *C. mollissima* MI R8T4 (NOT BAGGED) x *dentata*,
Monroe

Hill Craddock, University of Tennessee, Chattanooga

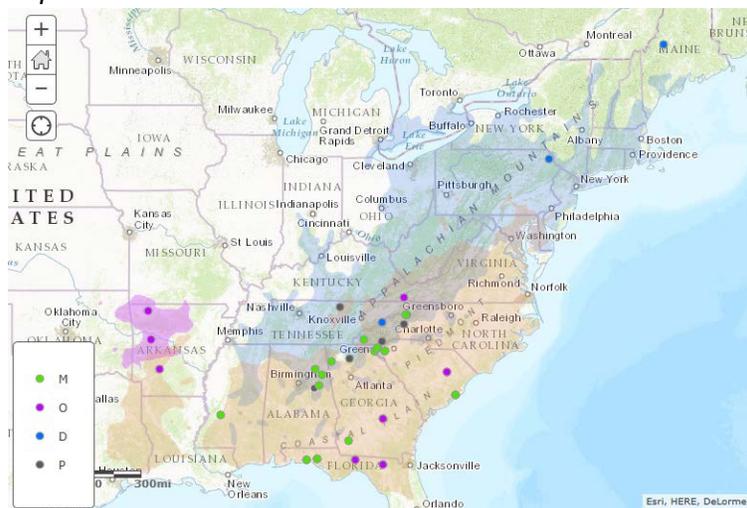
The Chattanooga Report. Craddock reported on two student projects.

1. Taylor Perkins is working on chloroplast DNA phylogeography of the North American *Castanea*. Perkins is looking at chloroplast markers to determine if there is any gene flow between *Castanea* species. There is natural hybridization between *C. pumila* and *C. dentata* and it is ongoing. Perkins wants to know how many species of *Castanea* there are—lumpers say there are only two species (*pumila* and *dentata*). Perkins pulled 23 taxa from the literature and he wants to know if some of the variation in chloroplast are associated with some of the 23 taxa. *C. alabamensis* is a hybrid as it has simple and stellate trichomes. Perkins is asking:

- What is the frequency of hybridization and introgression in American chestnuts and chinquapin populations?
- By sampling many *C. dentata* and *C. pumila* growing in sympatry, and sequencing multiple cpDNA loci, we can be more confident that hybridization, rather than incomplete lineage sorting, is the cause of shared chlorotypes in some cases
- We then ask, “do *C. dentata* and *C. pumila* growing together at a particular location exhibit higher sequence similarity with each other than with conspecifics in other populations?”
- Document *C. dentata* with non-D cytoplasm for breeding purposes
- Does the cpDNA phylogeny agree with the current taxonomy? (e.g., are some basionyms, like *C. alabamensis*, valid?)
- Ultimately, we will also need sequence data from the nuclear genome to test taxonomic hypotheses.

Perkins’ methods were:

- Sequence 6 noncoding cpDNA loci in accessions from throughout the ranges of *C. dentata*, *C. pumila*, and *C. ozarkensis*
 - More loci than previous studies may provide more resolution to cpDNA phylogeny
- More samples per locality compared to previous studies
 - Increase chances of documenting “chloroplast capture” where *C. dentata* and *C. pumila* co-occur



Geographic distribution of cpDNA haplotypes. Shading indicates the distribution of three North American *Castanea* taxa: Blue = *C. dentata*; red = *C. pumila*; purple = *C. ozarkensis*. Haplotype **D** represents all *C. dentata*; **P** represents *C. pumila*; **O** is *C. ozarkensis* found mainly on the gulf coast, coastal plain;. **M** represents mixed haplotypes, restricted to the southern Appalachians (middle TN, Alabama)—no M north of southwestern Virginia. Dots indicate sample sites of the present study and provenance of 13 plants sequenced at 6 loci from Shaw et al. (2012). Dot colors correspond to the four clades of haplotypes (D, O, M and P). In previous studies by Dr. Fenny Dane’s group, and in earlier studies by the UTC group, O haplotypes were found mainly in *C. pumila* and *C. ozarkensis* in Arkansas, with a few plants documented in Florida and Virginia. They have documented O haplotypes in northern Florida (Suwannee Lake = FL S1 and S2), in southern Georgia (Little Ocmulgee State Park = GA L1 and L2), and in the Coastal Plain of South Carolina (Woods Bay State Park = SC WB1 and WB2). The SC population of both *C. pumila* and *C. dentata* are identical at 6 loci. Perkins believes this is evidence of recent introgression.

2. Anna Clair Robison—she is working on measuring *Phytophthora* resistance phenotypes in segregating testcross families of hybrid American chestnuts. She used the 0-3 (0=healthy root; 1=lesions on feeder roots; 2=lesions on tap root; 3=dead) rating system for PRR. Her results of PRR trials (in tubs) were:

Least resistant families

- UTC2 (47.5% resistant)
- UTC3 (48.3% resistant)

Most resistant families

- UTC 1 (85.2% resistant)
- UTC 12 (83.3% resistant)
- UTC14 (73.5% resistant)

The sources of resistance of the families was 15 different Chinese or Japanese lines (no ‘Nanking’ or ‘Mahogany’ served as sources of resistance).

Robinson took the survivors (the ones rated “0” from the tub study and outplanted them at Lake the Allatoona orchard, part of GA-TACF. Her results were:

<u>Type</u>	<u>Alive</u>
American	13%
B3F2	13%
Chinese	77%
B3F3	21%
F1	0%
GA B1	14%
TN B1	70%
<u><i>C. henryi</i></u>	<u>100%</u>

Craddock reported that they now have thousands of trees planted using the Meadowview method. Their problem is finding landowners who are willing to plant 10 acres to chestnut.

Aleurodiscus oakesii is a fungus that causes smooth patch on white oak but Craddock found it on chestnut.

Lynne Rieske-Kinney, University of Kentucky

Asian chestnut gall wasp (ACGW). Rieske-Kinney reported on an extension of Ignazio Graziosi's Ph.D. work on fungal lesions on galls formed by ACGW. Graziosi performed a series of experiments where he isolated the fungus, performed Koch' Postulates and he was able to infect galls with the fungus. He showed that fungal infection caused 100% gall mortality and <1% parasitoid mortality. The fungus was identified as the *Colletotrichum acutatum* species complex. This fungus causes anthracnose on many plants; it also caused blossom end rot of chestnut, a problem for chestnut growers. There are strains in this disease complex that are pathogenic on plants and others that are pathogenic on the ACGW galls.

Colletotrichum acutatum has been reported on:

- Brazilian citrus scale
- Elongate hemlock scale
- Asian chestnut gall wasp



Little in common; all with sedentary phase

Defining *C. acutatum* is a mess. Resequencing generated closest match to *C. acutatum fiorinae*, the elongate hemlock scale entomopathogen.

The Northern Nut Growers are interested in this fungal species complex and they provided Rieske-Kinney with a small grant to look at fungal samples from blossom end rot and infected galls. She is isolating and extracting DNA and sequencing. She is comparing plant pathogenic vs entomopathogenic strains to see if there any difference in enzymatic activity—are there some specific to gall wasp?

With the assistance of Dr. Lisa Vaillancourt, a *Colletotrichum* specialist at U.K, they inoculated ACGW galls with 14 *C. acutatum* strains + H2O control

- Apple, blueberry, corn, sorghum, strawberry, tomato, and more
- Elongate hemlock scale fungus
- Symptomatic chestnut
- Asymptomatic chestnut (endophyte?)
- ACGW fungus
- Evaluated effects on gall wasp and parasites

Conclusions are:

- *C. fiorinae* and *C. gloeosporioides* from chestnut have the potential to colonize gall wasps to varying degrees.
- Of all the *C. acutatum* isolates, those isolated from ACGW seem to be the most aggressive.
- Parasitoids seem to be unaffected by the *C. acutatum* isolates
- Opportunistic hypothesis
- Potential for some degree of specialization

Gary Micsky, Penn State Cooperative Extension, Educator/PA-TACF Volunteer

Objectives:

I. To develop and evaluate blight resistant chestnut trees for food and fiber through traditional and molecular technologies that incorporate knowledge of the chestnut genome

II. To investigate chestnut reestablishment in orchard and forest settings with special consideration of the current and historical knowledge of the species and its interaction with other pests and pathogens

III. To develop and utilize a network of trained volunteers who can be informed and mobilized electronically to assist in multiple chestnut restoration activities

Program: Leadership and Volunteer Development; Natural Resource and Environmental Management. NE 1333 participants and TACF are valued and effective partners in my Natural Resources Extension education programming. Collaborations with NE 1333 and TACF personnel and resources continue to be critical to the success in expanding outreach to new audiences and have enhanced the quality of existing Extension programming.

Methods:

- Training workshops and field experience
- Extension newsletters, press releases, woodland owner association newsletters
- Grower/Site evaluations
- Pest Surveys

Evaluation Process:

- Number of 2016 demonstration orchards maintained (N=3)
- Number of 2016 breeding orchards maintained (N=2)
- Number of 2016 on-site test plantings established (N=11)
- Volunteers trained to assist in controlled pollinations (N=2)
- Number of volunteers trained in 2016 (N=34)
- Volunteer hours reported
- Volunteers requesting to join chestnut listserv (N=17)
- Chestnut vigor/survival on Site Assessment Plots
- Number of requests for information on chestnut – related topics October 2015 - September 2016, Telephone, Email, and Face-to-Face Contacts (N=126)

Volunteer Role:

- Tree ID, pollination, record keeping, culture & aftercare, program delivery
- Host research/demonstration plots
- Collect/supply genetic material
- Assist in TACF and other research activities as needed
- Advisory Committees and Volunteer Recruitment

Identifying Potential Sites/Growers (Objective III). Participants at 2016 “Grower Schools” were provided with 10 open pollinated seed and asked to provide baseline data regarding their success or failure in growing chestnut seedlings on their site. 80 open pollinated seed and 15 seedling trees were distributed to 8 individuals. One additional participant of a previous school received 20 F1 hybrid seeds to establish an F1 orchard near Corry, PA. Follow-up surveys utilizing the Chestnut Chatter listserv will be sent out in late September 2016.

Survey will be used to determine: 1) grower commitment; 2) site suitability for future plantings. Baseline data will include: % seed surviving, height of seedlings, weed and pest controls, tree protection, and problems encountered as of September 2016. Class participants donated \$120.00 to support PA-TACF and Extension chestnut restoration efforts.

06.3.16 Orchard Inspection & Pruning Clinic, Lake Erie Grape Research Station, Erie County PA . 10 participants (Objectives I, II, & III)

Concerns. Demonstration orchard trees exhibited multi-stem growth, and interference/damage from protective tubes and wire cages. Exploration of chestnut as an alternative crop is among reasons justifying the demo orchard. As such, aesthetic appearance is important in generating acceptance and support of this orchard.

Participants received instruction on both positive and negative consequences of pruning chestnut species from Extension Urban Forestry Educator and Certified Arborist Scott Sjolander and Renewable Natural Resources Extension Educator Gary Micsky.

Objectives included documentation of:

- Effects of removing multiple stems
- Differences in disease occurrence compared to non-pruned control trees
- Aesthetic impact
- Pest ID
- Extension Educator G. Micsky and PA Bureau of Forestry Service Forester J. Scheib examined/sampled several NW PA chestnut trees exhibiting unusual decline. These trees were determined to be weakened by accumulating factors including: poor subsurface drainage, drought stress, Asian Gall Wasp, root rots, and record-breaking late May freezing temperatures in the years preceding dieback. PSU Plant Disease Clinic identified presence of the fungus *Botryosphaeria* sp. This canker-causing fungus is associated with branch dieback on many susceptible woody ornamentals. Several of the trees examined across Mercer County PA have or are currently close to death. Many of these trees are older (50+ years) trees planted by homeowners to replace American chestnut. Most are unidentified Chinese cultivars.
- Outreach Efforts (Objectives I, II, & III)
- *"American Chestnut Restoration"* continues as a State-wide Program for Penn State Extension presented by the Renewable Natural Resources Team
- *"Chestnut Chatter"* an Extension mailing list developed in 2008 and adapted to a Penn State listserv in 2009 accommodates the need to quickly notify approximately 160 trained volunteers of program activities such as: pollination schedules, orchard plantings, harvest dates, and other labor intensive activities. (Objective III.)
- The Penn State Extension newsletter *"The Woodlander"* informs over 1200 subscribers throughout western Pennsylvania and eastern Ohio. The Fall 2015 edition informed and invited subscribers to attend the 2015 TACF Annual Meeting/Schatz Tree Genetics Colloquium held in State College, PA. Similarly, the Summer 2016 edition informed readers on the impact of Brood V of the 17 year Cicada emergence on several Washington County Pennsylvania TACF chestnut plantings.

- “ Penn State Ag Progress Days” August 15, 16, 17, 2016
- Conduct tours of PA-TACF/PSU breeding orchards
- Staff exhibit and recruit new memberships in TACF 28 contacts
- “2016 Mercer/Lawrence County Country Tour” September 17-18
- Provided educational information to 28 interested individuals relating to TACF and SUNY-ESF restoration efforts in cooperation with the Mercer County Woodland Owners Association and the PA WoodMobile
- Timeline and impact of additional Volunteer Recruitment, Development, and Utilization (Objective III)
- Sept.-Oct. 2015 Recruited 3 participants for TACF Annual Meeting/Schatz Tree Genetics Colloquium
- 10.02.15 Open pollinated chestnut harvest & processing: 5 volunteers, 16 volunteer hours
- 10.16.15 Open pollinated chestnut harvest & processing: 2 volunteers, 8 volunteer hours
- 10.23.15 Registration desk and support, TACF Annual Meeting/Schatz Tree Genetics Colloquium, University Park, PA (2 volunteers)
- 10.24.15 Registration desk and support, TACF Annual Meeting/Schatz Tree Genetics Colloquium (2 volunteers)
- 04.01.16 Inspection of Smith Orchard, Jefferson County, PA
- 04.02.16 PA/NJ TACF Spring Meeting, Harrisburg; Collect 2016 grower school and orchard supplies
- 04.14.16 Inventory at Forest County Extension Demo Orchard
- 04.23.16 “American Chestnut Site Selection and Aftercare Workshop” Northeast, PA 8 participants
- 05.02.16 Chestnut Restoration Program for BSA Troop 86, Mercer, PA, 16 participants, 7 volunteer hours (Objectives I& II)
- 05.06.16 Establish F1 Breeding Orchard at Fulkman Farm, Pulaski, PA, 2 participants, 6 volunteer hours
- 05.07.16 Eagle Scout Court of Honor, Mentor for Ryan Hamilton’s Chestnut Restoration Project
- September 2016 Open pollinated chestnut harvest & processing: 8 volunteers; Total service hours pending
- 09.20.16 Inspection/evaluation of BC1, BC4, OP American, F1 hybrids, and Chinese trees at Freeman Tree Farm, St. Petersburg, PA and a scheduled meeting with PA Bureau of Forestry, Freeman Tree Farm, Mercer County Woodland Owners Association (MCWOA), and the Woodland Owners of Clarion and Allegheny Valley (WOCV) to develop a July 8, 2017 Educational Field Day to explore American chestnut restoration efforts in NW PA.
- Activities scheduled for 2017 resulting from Multi-state initiatives begun in 2016: Our network of trained Pennsylvania chestnut restoration volunteers has been asked and has agreed to assist the Ohio Chapter of TACF in planting 100 of the “Restoration 1.0” seedlings at Mosquito Lake State Park in Cortland, Ohio in early Spring 2017.

Steve Jeffers, Clemson University (submitted report)

In collaboration with: Chestnut Return Farm (Seneca, SC), The American Chestnut Foundation (TACF), and the USDA Forest Service

Background Information. Our research focuses on Phytophthora root rot (PRR) of American chestnut and its hybrids, which is caused by *Phytophthora cinnamomi*. While the story of chestnut blight and efforts to overcome this disease have been the subject of much public attention and numerous research efforts, much less consideration has been given to PRR on American chestnut, which also is devastating. While stems killed by chestnut blight may re-sprout and survive if conditions are favorable, PRR destroys the root system, killing the tree. Fortunately, *P. cinnamomi* is not present over the entire range of the American chestnut. The first field plantings in South Carolina of TACF hybrid backcross chestnut seedlings suffered up to 50% mortality from PRR even before these seedlings could be challenged by the chestnut blight fungus. Fortunately, Chinese chestnut also is resistant to *P. cinnamomi*, and genes for resistance to this oomycete pathogen are present in a proportion of hybrid seedlings that have been selected for resistance to *Cryphonectria parasitica*.

It is generally believed that *P. cinnamomi* is native to southeastern Asia and, perhaps, South Africa. It is believed that this pathogen was introduced accidentally into the coastal region of the southeastern U.S. in soil or on the roots of containerized plants imported from Asia in the late 1700s; it then spread inland along with the human population. During the mid-1800s, devastating losses to American chestnut and native chinkapin trees (*Castanea pumila*) in the Coastal Plain and Piedmont regions throughout the southeastern U.S. have been attributed to PRR, with widespread death of trees occurring long before chestnut blight arrived in North America. Phytophthora root rot was first reported on American chestnut in 1932 and later confirmed over the next 10-15 years.

P. cinnamomi is one of the most economically important plant pathogens worldwide. It is known to attack over 1000 host plants, and it has been speculated that several thousand more plant species are susceptible in Australia alone. *P. cinnamomi* has caused numerous destructive diseases on agricultural, ornamental, and native plants around the world: dieback of eucalyptus trees and numerous understory species in forests of western Australia; mortality of oaks in Mexico; little leaf disease of shortleaf pines and root rot of Fraser fir Christmas trees in the southeastern U.S.; heart rot of pineapple and root rot of ohia trees in Hawaii; blight and canker of macadamia trees; root rot of avocado; root rot and trunk cankers on many hardwood and conifer trees; and root and crown rot on many different nursery and landscape plant species—particularly camellia and members of the Ericaceae (e.g., azalea, heath, Pieris, Rhododendron, etc.). *P. cinnamomi* is considered to be a fairly uniform clonal population in most regions where it occurs, with the A2 mating type dominating most local populations and phenotypic and genotypic variation relatively low. However, over the years, pathogenic variation within this species has been reported. Recently, we have identified genotypic variation that correlates with phenotypic variation in a population of *P. cinnamomi* from ornamental plants.

P. cinnamomi limits the range where American chestnut trees can be planted and grown. American chestnut appears to have no resistance to this pathogen while Chinese chestnut and other Asian chestnut species are known to be resistance. Through our annual screening efforts—a collaboration among my lab at Clemson University, Dr. Joe James at the

Chestnut Return Farm in Seneca, SC, and colleagues at TACF—we have detected resistance to *P. cinnamomi* in a small percentage of hybrid backcross seedlings from the TACF breeding program. This shows that resistance to *P. cinnamomi* in American chestnut can be improved with inter-species breeding. Resistance to both *C. parasitica* and *P. cinnamomi* will be required for successful restoration of American chestnut in forests in the southern portion of its original range.

Screening for resistance to *P. cinnamomi*. We are in the 13th year of screening TACF hybrid backcross seedling families for resistance to *P. cinnamomi* at the J.B. James Chestnut Return Farm in the piedmont region of South Carolina. The basic protocol is as follows. Germinated seeds of American, Chinese, and hybrid chestnut are planted outside in April in 570-L plastic tubs filled with a soilless container mix (Fafard 3B). The tubs are infested 12 to 14 weeks after planting with a mixture of two isolates of *P. cinnamomi* originally recovered from diseased chestnut seedlings growing at the study site. After infestation, the container mix in each tub is brought to saturation at least once while plants are actively growing to encourage disease development. In December, each seedling is uprooted and scored for mortality and PRR severity (using a standard 0-3 scale) by visually examining the roots and lower stem.

In these tests, *C. dentata* seedlings consistently have been susceptible and died, and *C. mollissima* seedlings consistently have been resistant and survived. From 2004-2015, over 200 hybrid families have been tested from generations that ranged from F₁ to BC₄. The 2016 test is in progress with ~1220 seeds planted; these seeds are from 21 hybrid families from TACF, two families tested previously, and our control families (American and Chinese). Families with seedlings resistant to *P. cinnamomi* have occurred each year, but the number of resistant seedlings and PRR severity ratings varied considerably among families—depending on which families were being screened. Paul Sisco and Jared Westbrook at TACF are summarizing our data to determine the best sources of resistance.

We continue to collaborate Dr. Tatyana Zhebentyayeva here at Clemson, Paul Sisco and Jared Westbrook at TACF, Bert Abbott's research group at the University of Kentucky to identify genes associated with resistance to *P. cinnamomi*.

***Phytophthora* spp. in chestnut soils in the eastern USA.** We continue to cooperate with TACF personnel to assay soil samples for *Phytophthora* spp. Soil samples are collected around chestnut trees with *Phytophthora* root rot and from actual or potential chestnut planting sites in eastern forests and landscapes. Since 2004, we have assayed several hundred soil samples and collected numerous isolates of *P. cinnamomi* and some isolates of other species of *Phytophthora* from these sites. These isolates have been added to the permanent collection of *Phytophthora* spp. maintained by our lab. Eventually, we will investigate the diversity in the population of *P. cinnamomi* naturally occurring in eastern forest soils.

Diversity and pathogenicity of species of *Phytophthora* associated with hybrid American chestnut seedlings. Currently, there is an MS graduate student, Ms. Suzette Sharpe, working with me to investigate the diversity of *Phytophthora* spp. associated with root rot on American, Chinese, and hybrid chestnut seedlings (developed by TACF) that were planted in test plots in forest sites in three southeastern states: NC, VA, and TN. These plots are part of a research project being conducted by Dr. Stacy Clark with the USDA Forest Service. Since 2011, over 600 samples have been received, and 242 Isolates of *Phytophthora* spp. have been recovered from roots and soil. The two main objectives of this project are to: (1) Identify and

characterize all isolates of *Phytophthora* spp. based on morphological, physiological, and molecular traits; and (2) determine pathogenicity of all species of *Phytophthora* associated with American chestnut using Koch's postulates.

Results from this project have been very interesting and enlightening. For over 80 years, only *P. cinnamomi* has been associated with American chestnut. However, we have recovered *P. cinnamomi* (primarily), *P. cambivora*, *P. heveae* (infrequently), and several isolates of *Phytophthora* sp. that have yet to be identified. Sequencing of rDNA-ITS and several other genes is being conducted to verify identities of these species. We have determined the mating type of all heterothallic isolates and screened all isolates for sensitivity to the fungicide mefenoxam. In pathogenicity tests on American chestnut seedlings, *P. cinnamomi* was most aggressive—as expected—but the other species of *Phytophthora* also were capable of colonizing roots and causing disease. We are in the process of completing this project, but it appears that there are species of *Phytophthora* other than *P. cinnamomi* that are capable of causing Phytophthora root rot on American chestnut.

Business Meeting

Administrative advisor, Bradley Hillman conducted the business meeting. NE-1333 is in the fourth year of the current 5-year project (Oct 2013-Oct 2018). Hillman asked the group what they would like to see happen after 2017. Hill Craddock is chair-elect for the 2017 meeting. The termination date of the project is 20 Sep 2018, so the 2018 meeting must be held prior to 01 October. John Carlson agreed to host the meeting at State College in 2018. Carlson noted that Sara Fitzsimmons' orchards will be another year older and thus Penn State would be a good site for the 2018 meeting. Fred Hebard moved that John Carlson be elected as chair for 2018. Paul Sisco seconded the motion. Carlson was elected unanimously.

The project has been ongoing since 1982. Hillman indicated that it takes a lot of effort to write a project renewal. Lynne Rieske-Kinney wrote the most recent renewal and she indicated it takes a full two months to write a project renewal. The purpose of a multistate project is for real collaboration and cooperative grants. If the group decides not to renew, we simply become a coordinating committee. The advantage of renewing the project is that a portion of Hatch funds must be spent on these types of projects. Some experiment directors use Hatch funds for salaries and travel. Those participants who benefit the most are at land-grant institutions (Carlson, Gold, Hillman, Jarosz, Jeffers, MacDonald/Kasson, Riesky-Kinney, Schlarbaum). Hillman feels that someone at a land grant institution should rewrite the project. Any member of NE-1333 can rewrite the project (all the members listed in Appendix E); however, those not at land grant institutions cannot benefit financially. Fred Hebard indicated that he is retired and he can do a lot of the writing but an experiment station member should be the main author.

Riesky-Kinney indicated that she could not have written the last project without significant assistance. Thus, all members of the project should help write their section of the renewal. At some point, there needs to be an agreement on objectives and everyone can then write their section as per the new objectives. There should be communication of potential objectives via email. Powell indicated that in many of the objectives, we are no longer "investigating" but we are now "implementing".

The request to rewrite a project is due March 2017. Hillman will coordinate with Hebard and will provide him the forms to submit the request. Thus, Hebard will be the main contact for the project renewal.

Laurel Rodgers asked if anyone can be a formal participant. Hillman stated that anyone can be an official member; they just have to be added to Appendix E.

Hill Craddock will assume duties as the chair in 2017; he will host the meeting 8-9 Sept 2017 at the Cataloochee Ranch in Maggie Valley, NC.

The meeting adjourned at 11:30 am followed by tours of the SUNY-ESF greenhouses and field plantings.

*Respectfully submitted,
Mark Double
West Virginia University
November 2016*

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Milestone Accomplishments

- A study involving CHV-1/EP713, CHV-1/Euro7 and newly characterized virus CHV-1/EP721 reported in *J. Virol*, **86**:12933-12939, 2012, revealed unexpected variations in the transcriptional activation of the RNA silencing pathways and in virus-mediated symptom expression in the absence of the RNA silencing pathway. A robust level of antiviral RNA silencing of CHV-1/Euro7 and CHV-1/EP721 was inferred in wild-type *C. parasitica*, as evidence by the increase in viral RNA accumulation in the $\Delta dcl2$ strain, in the apparent absence of significant induction of *dcl2* transcript accumulation. The increase in CHV-1/EP721 RNA accumulation in the $\Delta dcl2$ strain was not accompanied by the debilitating growth phenotype observed for CHV-1/EP713 and CHV-1/Euro7 infections. Moreover, the difference in the virus-mediated $\Delta dcl2$ debilitating phenotype could be mapped to a viral coding domain. These results challenge the previous view that the $\Delta dcl2$ debilitating phenotype is due simply to highly elevated levels of viral gene expression in the absence of the RNA silencing pathway. While providing new insights into the interactions between mycoviruses and host RNA silencing antiviral defense, the combined results also suggest a higher degree of complexity than previously anticipated.
- A thorough analysis of the four *C. parasitica* RNA-dependent RNA polymerase (*rdr*) genes was completed during this reporting period. Disruption mutants were made for each of the *rdr* genes independently. Double ($\Delta rdr1/\Delta rdr3$ and $\Delta rdr2/\Delta rdr3$) and triple ($\Delta rdr1/\Delta rdr2/\Delta rdr3$) mutants were also made to overcome potential problems of functional redundancy (a quadruple mutant was not prepared because *rdr4* appears to be a pseudo-gene). None of the *rdr* disruption mutants displayed any growth or morphology phenotypes that differed from the wild-type strain either with or without hypovirus infection. Deletion of the *rdr* genes also failed to result in detectable changes in transposon expression or hypovirus recombination activity. We conclude that *rdr* genes in *C. parasitica* do not have significant roles in RNA silencing as part of defense responses against mycoviruses or transposons or have a significant role in viral RNA recombination as we have shown previously for *dcl2* and *agl2*.
- A simple and efficient system was developed by adapting the Cre-*loxP* recombination system for unlimited recycling of the limited number of available selectable marker genes (SMGs). The successful application of this method to *Metarhizium robertsii* suggests potential use for optimizing reverse-genetics analysis in a broad range of filamentous fungi.
- Mutational analyses of the infectious CHV-1/EP713 infectious cDNA clone defined the requirements for autocatalytic cleavage of papain-like leader proteases p29 and p48 and the functional importance of autoproteolysis in the context of hypovirus replication. The studies also exposed an alternative p48 processing pathway independent of the encoded papain-like protease activities.
- In order to effectively determine the vegetative incompatibility genetic structure of *C. parasitica* field populations, we designed PCR primer sets that selectively amplify and distinguish alleles for each of the six known diallelic *C. parasitica* *vic* genetic loci. PCR assay results were validated using a panel of 64 European tester strains with genetically

determined *vic* genotypes. Analysis of 116 *C. parasitica* isolates collected from five locations in the eastern United States revealed 39 unique *vic* genotypes and generally good agreement between PCR and tester strain coculturing assays in terms of *vic* diversity and genotyping. The availability of molecular tools for rapid and precise *vic* genotyping significantly improves the ability to predict and evaluate the efficacy of hypovirulence and related management strategies.

- The identification of *vic* genes and adaptation of the Cre-*loxP* recombination system in previous years allowed us to systematically disrupt multilocus *vic* genes and excise exogenic genes to generate strains of the chestnut blight fungus able to transmit hypovirulence to strains with genotypic differences at any or all of the defined *vic* loci. The results demonstrate the feasibility of modulating fungal allorecognition to promote transmission of virulence-attenuating mycoviruses for enhanced biocontrol potential. These “Super Donor” strains are currently being tested in USDA permitted field trials in collaboration with Bill MacDonald, Mark Double and Matt Kasson from West Virginia University.