Progress Report – February 2019: Conservation and collection of *Castanea dentata* germplasm in the South

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Narrative Summary

The American chestnut (*Castanea dentata* Borkh.) has been devastated by the exotic invasive pathogens *Cryphonectria parasitica* (Murr.) Barr and *Phytophthora cinnamomi* Rands to which it has no resistance. The American Chestnut Foundation (TACF) has developed an interspecific backcross breeding program to introgress disease resistance from Asian chestnut species, primarily *Castanea mollissima*, into *C. dentata* populations. The genetic base of this program must be expanded to be successful in the long run. Southern populations harbor greater genetic diversity and possibly rare alleles. Conservation of these diverse populations will widen the genetic base of the TACF breeding program and strengthen efforts to restore the species. We propose vegetative propagation through grafting in order to collect and conserve southern populations of *C. dentata*. We have located and collected scionwood from 28 American chestnuts (four of which are new to the breeding program) across 9 sites in Tennessee and Alabama. Four types of rootstocks (*C. dentata, C. mollissima*, and F1 and B3F2 hybrids) were chosen to account for possible graft incompatibility, although compatibility was not measured in this study. The whip-and-tongue and bark-flap grafting techniques were used depending on scion-rootstock diameter. Nine of the 28 individuals were grafted to 155 rootstocks, and 40 grafted plants have survived the first growing season. These container-grown grafted plants will be conserved *ex situ* in a nursery where, released from competition for light, they can produce flowers. To date, two individuals have produced male flowers at three and five months after grafting and the pollen collected is in cold storage for use this summer year. The eventual production of female flowers from these novel Southern sources will enable us to capture cytoplasmic genes as well.

Objectives

SHORT-TERM: The short-term goal of this proposal was to capture new and/or under sampled sources of American chestnut throughout the Southeast and accelerate the rate of flower production for breeding. Scionwood was collected from a total of 9 sites across Tennessee and Alabama during the winter of 2017-2018 (December – February). Grafting was performed between May and June 2018. At this point, 9 individuals have been conserved through grafting, four of which had not been conserved through the TACF breeding program.

LONG-TERM: The long-term goal is to incorporate genes from the grafted specimens into the breeding program to broaden its genetic base. New breeding lines of *C. dentata* will facilitate the addition of new sources of blight and root rot resistance to the restoration effort. Two grafted plants flowered in the nursery, and pollen was collected from one. Although both of these individuals had been known to flower, this result represents the ability of grafted plants to flower in favorable conditions, allowing easier collection and potential pollination.

Materials and Methods

SAMPLING: Under-sampled populations of *C. dentata* in the South were targeted using the county-by-county range map of conserved trees in the TACF breeding program (Figure 1). Counties with fewer than 10 conserved trees were considered under-sampled. Specific locations of wild trees were solicited through a member–wide announcement to the Alabama, Georgia, Kentucky, and Tennessee Chapters of TACF in addition to SERNEC (Southeast Regional Network of Expertise and Collections). Identified locations were to be visited in Fall 2017 to confirm location and species identification and again in Winter 2017 – 2018 during winter dormancy to collect dormant scionwood for grafting in Spring 2018.

COLLECTION: During winter dormancy, scionwood was collected and cut to the length of a standard gallon bag and rolled from bottom to top to remove excess air. While in the field, samples were kept in a cooler with ice packs. Upon return from field, samples were stored in the crisper drawer of a standard refrigerator (0 – 1°C).

ROOTSTOCK SELECTION: Although this study is not designed to test graft compatibility, to account for potential graft incompatibility, a variety of rootstocks were selected (*C. dentata, C. mollissima*, and F1, B1F1, B3F2 hybrids).

GRAFTING: When rootstocks showed signs of active growth, each ortet was grafted to each rootstock. Graft technique (whip-and-tongue, bark-flap, or cleft) was selected based on diameter of rootstock and scionwood. Ramets were maintained in pots in the UTC nursery and treated with fertilizer and fungicide to promote growth and reduce pathogen pressure.

Results

SAMPLING: The location information was garnered from 13 sites: 11 from TACF Chapter-wide announcement (6 in AL, 4 in TN, 1 in KY), and 2 from SERNEC. Only one site was in a county with greater than 10 conserved trees in the TACF breeding program.

COLLECTION: Logistical issues prevented the Fall 2017 visit (except Cannon, Co, TN) and resulted in the collection of a non-target species, *Castanea pumila* var. *alabamensis* (5 individuals). However, winter identification and knowledge of local guides allowed collection of 33 individuals (28 *C. dentata*, 5 *C. pumila* var. *alabamensis*) from 9 of the 13 sites (6 AL, 3 TN), across 5 AL and 3 TN counties (Figure 2).

GRAFTING: 14 of 33 ortets were grafted to each rootstock type, amounting to 155 grafted plants total. 13 ortets have survived to the present (40 ramets – 26% survival).

FLOWERING GRAFT: Two ramets produced catkins 3 and 5 months after grafting. Pollen was collected and cold stored (0°C) for breeding in Summer 2019. One ortet had not be conserved previously and represents a new individual conserved into the TACF breeding program.

GENOMIC SAMPLING: Grafted trees maintained in the nursery allow for concentration of genomic diversity, thus removed the logistical burden of sampling. Leaf samples were collected from all 14 grafts in support of the TACF landscape genomics study.

Conclusion

IMPLICATIONS ON CONSERVATION: Asexual propagation through grafting is a low-tech, non-invasive method for conservation of potentially rare American chestnut germplasm (Craddock and Bassi 1999). Particularly, when *in-situ* conditions prevent trees from reaching sexual maturity, graft-propagation allows release from light competition and disease pressure to promote flowering (Paillet 2002). Collection of pollen from grafted trees allows conservation of genetic resource that were previously unavailable or difficult to access.

REDUCED LOGISITCS: Breeding wild trees is time consuming and often limited by access (terrain and distance from roads) and involves repeated visits to the field. Conversely, scionwood collection for grafting is significantly less strenuous. Flowering grafted trees maintained in a nursery, representing a wide geographic range, reduce the logistical burdens of breeding, which may increase the number of crosses made in a given season.

FUTURE DIRECTIONS: GIS modeling can be applied to better target additional areas of collection (Fei et. al. 2007). Additionally, grafted trees maintained in pots can be grown in growth chambers under increased light to expedite flowering. Inducing flowering would allow earlier pollen collection to be made available to breeders (Baier 2012).

Final Financial Report

Breakdown of Funds Spent	
Item	
Student Salary	$610.00
Travel	$327.12
Media Processing – Printing charges	$1.01
Shipping Charges	$25.46
Supplies	$2550.85
TOTAL	$**3,514.44**

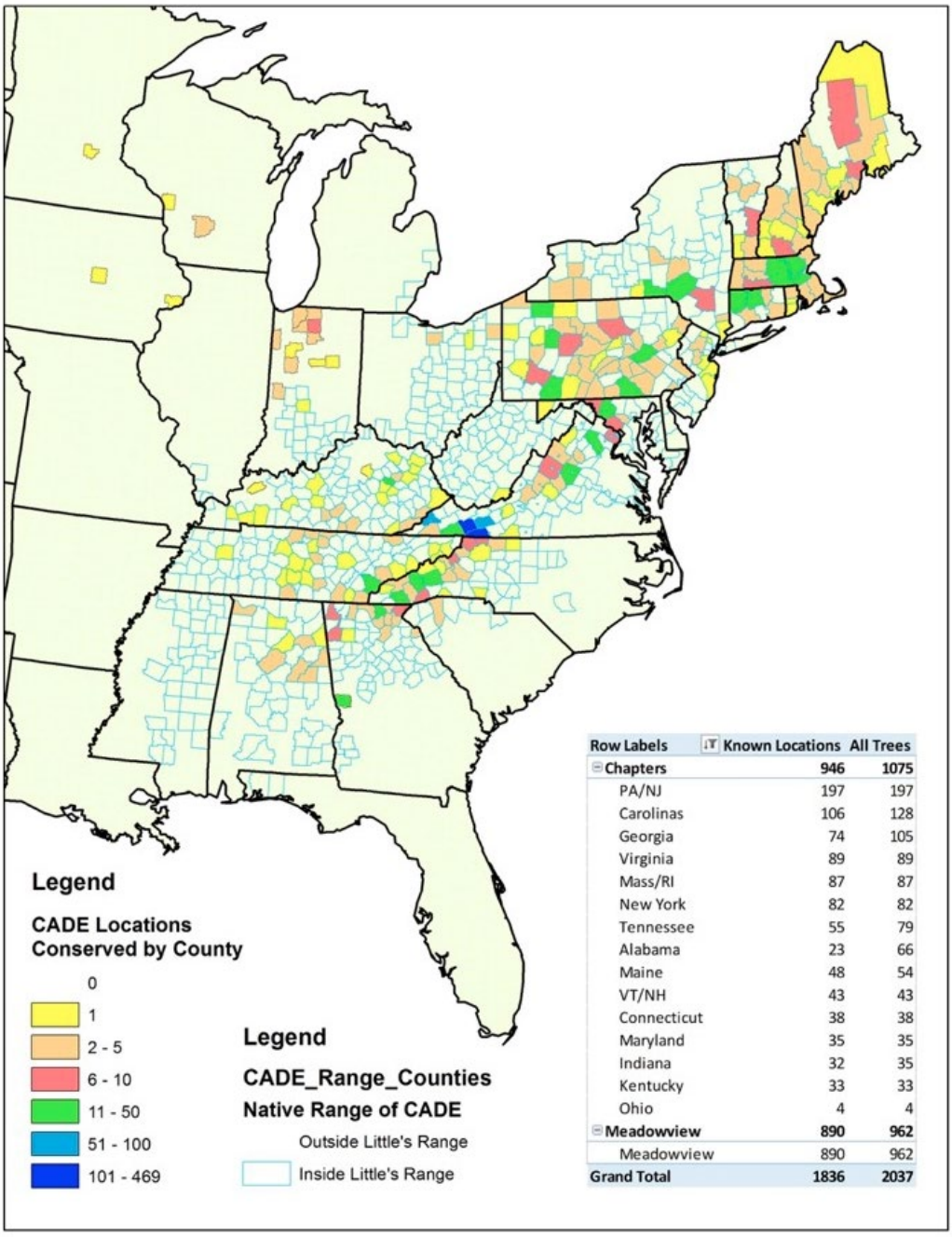
Supplies included:

- Pots: Rootmaker Stuewe and Sons

- Grafting Knife
- Grafting wax
- Pruning shears
- Chinese chestnut (*C. mollissima*) Rootstocks

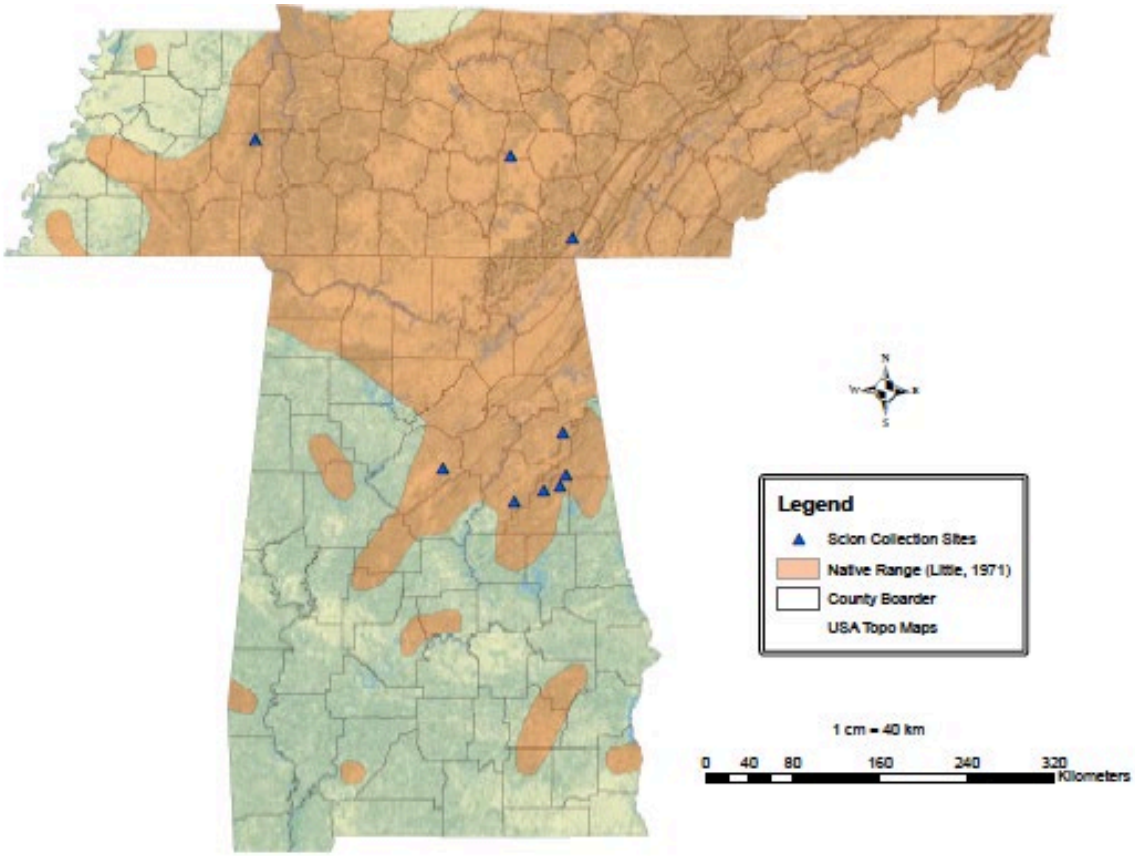
Figures

Figure 1: *Castanea dentata* conserved county-by-county provided by TACF



Row Labels	Known Locations	All Trees
Chapters	**946**	**1075**
PA/NJ	197	197
Carolinas	106	128
Georgia	74	105
Virginia	89	89
Mass/RI	87	87
New York	82	82
Tennessee	55	79
Alabama	23	66
Maine	48	54
VT/NH	43	43
Connecticut	38	38
Maryland	35	35
Indiana	32	35
Kentucky	33	33
Ohio	4	4
Meadowview	**890**	**962**
Meadowview	890	962
Grand Total	**1836**	**2037**

Figure 2. *Castanea dentata* Scionwood Collection sites in Tennessee and Alabama over Little's (1971) Historic Range



References

Baier, K, Maynard, C, Powell, W. 2012. Chestnuts and Light. Journal of the American Chestnut Foundation. 3(26): 8-10

Craddock, JH and Bassi, G. 1999. Effect of clonally propagated interspecific hybrid chestnut rootstocks on short-term graft compatibility with four cultivars of Italian "Marrone." Acta horticulturae 494 (7): 207

Fei, S, Schibig J, Vance M. 2007. Spatial habitat modeling of American chestnut at Mammoth Cave National Park. Forest Ecology and Management 252 (1): 201-207. doi:10.1016/j.foreco.2007.06.036

Paillet, FL. 2002. Chestnut: history and ecology of a transformed species. Journal of Biogeography 29:1517–1530.

Published Works and Presentations

1. Deason, T. (2018). Conservation and collection of *Castanea dentata* germplasm in the South. *Departmental Honors Thesis.* https://scholar.utc.edu/honors-theses/146
2. Deason, T. and Craddock, J.H. (2018). Accelerated, grafted-based germplasm conservation targeting under-sampled and genetically diverse American chestnut populations allows rapid introduction of rare adaptive alleles into TACF breeding program. Poster. 35th Annual meeting of The American Chestnut Foundation, Huntsville, AL, October 26, 2018.
3. Deason, T. and Craddock, J.H. (2018). Conservation and collection of American chestnut germplasm in the South. Technical Report to USDA NE-1333: Biological Improvement of Chestnut through Technologies that Address Management of the Species, its Pathogens and Pests. September 7-9 2018, State College, PA.
4. Invited Lecture. Conservation and collection of *Castanea dentata* germplasm in the South. Fall Plant Sale, Reflection Riding Arboretum and Nature Center. 21 Sept. 2018. https://reflectionriding.org/www/cal/september/2018/event/32:1537585199/fall-native-plant-sale-chattanooga