

a. Project Title: Conservation and collection of *Castanea dentata* germplasm in the South: Development of an accelerated Chinese-American chestnut hybridization method to increase genetic diversity in the breeding program for resistance to *Cryphonectria parasitica* and *Phytophthora cinnamomi*

b. Summary:

We request funds to develop an accelerated, graft-based, germplasm conservation method to increase the number naturally occurring American chestnut individuals into the ongoing backcross breeding program. Scionwood will be collected from American chestnuts throughout the Southeast from individuals not suited for traditional pollination breeding methods (sexually immature, equipment/man-hour limitations). This will allow the potential introduction of additional lines of resistance and add genetic diversity. American scions will be grafted to Chinese rootstocks. Grafted plants will be exposed to varied temperature and photoperiod in growth chambers to expedite flowering. Pollen collected from the treated trees will be used for hybridization.

c. Principle investigator and institutional affiliation:

Trent Deason and Dr. Hill Craddock
University of Tennessee Chattanooga
Department of Biology, Geology, and Environmental Science

d. Duration of project:

August 2017 – May 2018

e. Total amount requested:

\$3,515

f. Short and long term goals:

The short term goal of this proposal is to capture new and/or under sampled sources of American chestnut throughout the Southeast and accelerate the rate of flower production for breeding. 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 resistance, to both *C. parasitica* and *P. cinnamomi*, to the restoration effort.

g. Narrative:

Introduction: The American chestnut, *Castanea dentata*, is susceptible to a fungal blight (*Cryphonectria parasitica*) as well as to Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi*. Conversely, the Chinese chestnut (*Castanea mollissima*) has shown resistance to both (Anagnostakis 2001, Burnham 1988). Previous research has produced viable Chinese-American chestnut hybrids that capture the disease resistance from the Chinese, while maintaining the morphological characters of the American (Burnham 1988, Diskin 2006). However, the primary breeding method requires locating flowering American chestnuts, which is difficult, effectively limiting the number of individuals in the gene pool (Fei 2007). Most individuals in surviving *C. dentata* populations never bloom because they are in the understory (Paillet 2002). Even when conditions are ideal, breeding wild trees is time consuming and involves repeated visits to the field in order to place and retrieve pollination bags to collect

pollen and/or seeds. This method is complicated when field conditions, such as terrain and distance from roads, make it difficult to collect pollen and nuts efficiently. I propose a graft-based method that focuses on collecting scionwood, rather than pollen or seeds, from naturally occurring American chestnut trees. Collection of scionwood is not dependent on the sexual maturity of the plant; even trees that are not blooming can be thus brought into the breeding program. American scions grafted to Chinese rootstocks can be planted in the field, in a germplasm conservation orchard, or maintained in containers. Container grown trees can be manipulated in ways that may accelerate the development of flowers. The increased temperature and photoperiod conditions in a lighted greenhouse, or growth chamber may expedite flowering and shorten the time to pollen collection (Sanz-Pérez 2008). If these Southern American clones produce seeds, we also capture their cytoplasmic genes, and possibly rare alleles, that will further enhance the genetic diversity of the breeding program and conservation efforts.

Background/Significance: The natural history of American chestnut, *Castanea dentata*, and its demise, have been thoroughly reviewed (Roane et al., 1986; Anagnostakis, 1987). In the eastern hardwood forests, and particularly in the mountains of the Southeast, American chestnut was important economically and culturally, possibly more than any other one tree in its range (Ashe, 1911). In 1904, a fungal blight, later described as *Cryphonectria parasitica*, was documented on chestnut in the Bronx Zoological Park, New York. Despite prevention measures, chestnut blight spread rapidly, decimating American chestnut throughout the entirety of its range (Roane et al., 1986; Anagnostakis, 1987). Early efforts to find blight-resistant cultivars failed, which gave way to a backcross breeding program developed by TACF designed to introgress disease resistance from *C. mollissima* into surviving *C. dentata* (Burnham 1988). This ongoing program has had considerable success in development of both potentially blight resistant lineages and maintaining morphological characteristics of American chestnut (Diskin 2006).

Chestnut breeding could be advanced by including graft-based propagation methods in order to collect individuals previously excluded due to reproductive immaturity and/or geographic inaccessibility. Expanding the current breeding program to include regionally sourced, grafted American chestnuts will provide potential development of additional resistant lineages as well as conserve regional variation of germplasm and nuclear and cytoplasmic diversity.

Research Plan:

I propose a regionally focused, graft-based propagation method for collecting American chestnut individuals not suited for traditional pollination breeding and individuals not easily pollinated due to factors such as equipment access, geography and/or sexual immaturity. Areas for scionwood collection will be divided into four regions: 1) southeast Tennessee/northwest Georgia, 2) south-central Tennessee/northern Alabama, 3) northern-central Tennessee/southwestern Kentucky, and 4) western Tennessee/northern Mississippi. Selection sites will be chosen using university herbarium data and GIS prediction models based on bedrock composition, soil type, and dominate land use data (Alexander 2003, Fei 2007). Trips will be made to each identified location in winter of 2017-2018 to collect scionwood, which will be grafted and grown the following spring 2018.

It is important to note that this study is not designed to measure graft compatibility, but only to employ the most widely accepted chestnut grafting techniques in order to increase success of the graft. Scionwood collected from naturally occurring *C. dentata* (ortets) will be

grafted to *C. mollissima* rootstocks and grown ex situ. Under normal greenhouse/nursery conditions, 200 Chinese chestnut rootstocks have already been grown during spring/summer of 2017. Another 200 rootstocks will be planted in 2018. We will use the whip and tongue and/or bark-flap grafts (Craddock and Bassi, 1999). Container-grown, grafted plants (ramets) will be grown under increased temperature (heated greenhouse/growth chamber) and photoperiod (artificial lighting) in order to expedite budburst and development of inflorescence (Huang 1994, Sanz-Pérez 2008). Of the scions collected, a portion of the ramets will be used in a control group to be grown under standard greenhouse/nursery conditions. This will serve as a base line comparison for the effectiveness of increased temperature and light exposure on reducing the number of days to produce flowers and pollen. We hope to collect scionwood from at least ten ortets from four sampling regions, and to graft at least ten rootstocks per ortet.

I predict that increased light and temperature conditions will produce flowers and pollen on successful grafts sooner than the control group. Data will be collected through regular observations of both groups and due to the potential for small sample sizes, it will be analyzed through a Fisher's exact test to show any statistical significance on reduction of flowering time.

References

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- 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
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Huang, H, Norton JD, Boyhan GE, Abrahams BR. 1994. Graft compatibility among chestnut (*Castanea*) species. *Journal of American Society of Horticulture Science* 119(6):1127–1132. *Journal of American Society of Horticulture Science*

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

Roane, MK, Griffin, GJ, and Elkins, JR. 1986. Chestnut blight, other *Endothia* diseases, and the genus *Endothia*, St. Paul, MN.

Sanz-Pérez, V, Castro-Díez P, Valladares F. 2009. Differential and interactive effects of temperature and photoperiod on budburst and carbon reserves in two co-occurring Mediterranean oaks. *Plant Biology* 11 (2): 142-151. doi:10.1111/j.1438-8677.2008.00119.x.

h. Timeline

August - October 2017	December - February 2017-2018	March - April 2018	May 2018
Scout locations of naturally occurring American chestnut in each region	Return to sample sites to collect scionwood	Graft scions to Chinese rootstocks, test group begin growth chamber conditions	Manuscript in preparation

i. How results will be measured and reported

A financial report and final outcomes will be submitted to TACF upon completion of the project. The results will, we hope, be presented at scientific conferences as well as published in reviewed scientific journals.

j. Breakdown of how funds will be spent

Identified American chestnut habitat/locations will be visited once each during the summer and fall of 2017 in order to locate healthy individuals and update any previously recorded data, then each site will be revisited in winter of 2017-2018 to collect scionwood. Mileage is based on two trips per collection region (one for scouting and one for scion collection) at an estimated 300 mi roundtrip, at the University of Tennessee Chattanooga mileage rate. Pots and fertilizer will be required to grow rootstocks and will be ordered through existing suppliers. A grafting kit and supplies will be required, including a knife, sharpening stone, and grafting tape. Consumables, such as ice/ice packs and sealable plastic bags will be required on collecting trips in order to maximize scion survival. Student salary is based on work required in the greenhouse/nursery, growth chamber, and grafting; calculated at \$10 per hour, six hours a week, for 10 weeks from March to May 2018.

Budget

<u>Item</u>	<u>Cost (\$)</u>
Mileage	1150
Pots	950
Potting Medium	550
Fertilizer	100
Grafting Kit	110
Grafting Supplies	25
Consumables	30
UTC Student Salary	600
TOTAL	\$3,515

k. Brief C.V. for each Principal Investigators

Curriculum Vitae for Trent Deason

Education

B.S. Environmental Science, Minor: Biology. In Progress. University of Tennessee at Chattanooga GPA: 4.0/4.0

A.A.S. Electronic Systems Technology. Community College of the Air Force

Awards and Distinctions

2017 University of Tennessee at Chattanooga Environmental Science Award
2017 University of Tennessee at Chattanooga SGA Outstanding Senior Award
2015 – 2017 Dean's List
2015 USAF Commendation Medal
2014 NCO of the Quarter
2013 USAF Achievement Medal
2013 USAF Airmen Leadership School Academic Achievement Award
2013 NATO Service Medal
2012 USAF Achievement Medal
2011 Airman of the Quarter

Professional Memberships

The American Chestnut Foundation

Recent Professional Service

October 2016 – Present Planting, pollination, assessment of disease severity in chestnut trees, and other work performed in cooperation with the American Chestnut Foundation.

July 2009 – July 2015 Honorable service in the United States Air Force as a Radio Frequency Transmission Systems technician and supervisor, performed and led numerous communication system repairs, maintenance, and operations.

Curriculum Vitae for J. Hill Craddock

UC Foundation Davenport Professor in Biology
The University of Tennessee at Chattanooga
Department of Biology, Geology, and Environmental Science
615 McCallie Ave. Chattanooga, TN 37403

a. Professional Preparation

Università di Torino	Turin, Italy	Pomology	Dott. Ric. 1992
Oregon State University	Corvallis, OR	Horticulture	M.S, 1987
Indiana University	Bloomington, IN	Biology & Fine Arts	B.A., 1983

b. Appointments

2008 – present Professor, University of Tennessee at Chattanooga
1999 – 2007 Associate Professor, University of Tennessee at Chattanooga
1996 – 1998 Assistant Professor, University of Tennessee at Chattanooga
1994 – 1996 Postdoctoral Research Associate, Tennessee State University
1993 – 1994 Postdoctoral Fellow, Università di Torino
1989 – 1992 Graduate Research Assistant, Università di Torino
1984 – 1987 Res. Assistant, USDA-ARS National Clonal Germplasm Repository

c. Publications (*relevant to current proposal*)

1. Georgi, L., **Craddock, J.H.**, Bevins, D., Kling, R., and Hebard, F. (2014) Grafting chestnuts. *J. Amer. Chestnut Foundation* 28(1): 20-23.
2. Sisco, PH, TC Neel, FV Hebard, **JH Craddock**, and J Shaw. (2014) Cytoplasmic male sterility in interspecific hybrids between American and Asian *Castanea* species is correlated with the American D chloroplast haplotype. *Acta Hort.* 109:215-222.
3. Perkins, M.T.* and **J.H Craddock**. (2014). The effect of phosphite fungicides on mycorrhiza formation in a family of BC4 Chinese-American chestnut seedlings. Technical Report to USDA NE-1333: Biological Improvement of Chestnut through Technologies that Address Management of the Species, its Pathogens and Pests. 6-5 September 2014, La Crosse, WI.
4. Shaw, J., **Craddock, J.H.**, and Binkley, M.A.* (2012). Phylogeny and Phylogeography of North American *Castanea* Mill. (Fagaceae) Using cpDNA suggests Gene Sharing in the Southern Appalachians (*Castanea* Mill., Fagaceae). *Castanea* 77(2):186-211.
5. **Craddock, J.H.** (2009) USA. Pages 168-174 in: Avanzato, D. (ed.) Following Chestnut Footprints (*Castanea* spp.) - Cultivation and Culture, Folklore and History, Tradition and Uses :: Sulle Orme del Castagno - Coltura e Cultura, Folclore e Storia, Tradizioni e Usi. *Scripta Horticulturae* (ISHS) Number 9. 175 pp. ISBN 978-90-6605-632-9
6. **Craddock, J.H.** (2007) Chestnut Relatives. In: Bolgiano, C. (ed) *Mighty Giants: An American Chestnut Anthology. Images from the Past, and The American Chestnut Foundation.* 296 pp. ISBN 978-1884592485
7. **Craddock, J.H.** (2006) Chestnut Breeding in the United States. Pages 109-128 in: *Tree Breeding Researches: for the next half century. Proc. International Symposium for the 50th Anniversary of KFGR & the 20th Anniversary of the late Dr. SK Hyun.* June 15-16, 2006, Forest Seed Research Center, Suanbo, Korea. KFRI.

8. **Craddock, J.H.**, Alexander, M.T.,* Alexander, S.H.,* Bramblett, J.L., and Worthen, L.M.* (2005) Chestnut cultivar evaluations in Tennessee: orchard establishment and early germplasm characterization. *Acta Hort.* 693: 465-467.
9. Alexander, M.T.,* Worthen, L.M.,* and **Craddock, J.H.** (2005) Conservation of *Castanea dentata* germplasm of the southeastern United States. *Acta Hort.* 693: 485-490.
10. Bassi, G. and **Craddock, J.H.** (1999) Performance and description of the introduced chestnut cultivar 'Colossal' in Cuneo province, northwest Italy. In: Salesses, G. (ed.) Proc. 2nd International Chestnut Symposium, Bordeaux, France. *Acta Hort.* 494: 317-318.
11. **Craddock, J.H.** and Bassi, G. (1999) Effect of clonally propagated interspecific hybrid chestnut rootstocks on short-term graft incompatibility with four cultivars of Italian "Marrone". In: Salesses, G. (ed.) Proc. 2nd International Chestnut Symposium, Bordeaux, France. *Acta Hort.* 494: 207-212
12. Craddock, J.H. and **Bassi, G.** (1999) Introduction into Italy of eight *Castanea mollissima* cultivars from China. In: Salesses, G. (ed.) Proc. 2nd International Chestnut Symposium, Bordeaux, France. *Acta Hort.* 494: 319-321.
13. **Craddock, J.H.** (1998) Chestnut Resources in North America. *Annual Report of the Northern Nut Growers Association* 89: 19-30.
14. Bounous, G., Paglietta, R., Bellini, E. and **Craddock, J.H.** (1995) Il miglioramento genetico del castagno: situazione, obiettivi e metodi. *Frutticoltura* 57(11): 63-73.
15. **Craddock, J.H.**, Ferrini, F., Mattii, G.B., Nicese, F.P. and Pellegrino, S. (1991) Ricerche per l'individuazione di impollinatori del "Marrone di Chiusa Pesio." *Frutticoltura* 53(12): 61-63.

* denotes student author

d. Service

- The American Chestnut Foundation, Board of Directors (1997-2014), V.P. and Chair, Science Cabinet (1997-2001), Chair Awards Committee (current)
- Highlands Biological Station, Board of Scientific Advisors (2009-2012)
- Reviewer/referee for *Castanea*, *Journal of the American Society for Horticultural Science*, *HortScience*, and *J. American Chestnut Foundation*
- Reflection Riding Arboretum and Nature Center, Board of Directors (2000-2014)
- City of Chattanooga Tree Commission (2011-present)
- U.S. Dept. Agriculture Regional Project NE1033: Biological Improvement of Chestnut through Technologies that Address Management of the Species, its Pathogens and Pests (was NE-1015 from 2008-2013), 2017 Meeting Chair (2016 –present)
- U.S. Dept. Agriculture Regional Project NE-140 (Secretary 1999; Chairman 2000)
- International Society for Horticultural Science, Member, Reviewer
- Northern Nut Growers Association, Member, Reviewer, Editor
- Beta Beta Beta Biological Honor Society, Faculty Advisor

3. Public Outreach

- Led public classes at the Reflection Riding Arboretum and Nature Center including "The Fungi" and "Tree ID" as part of the core course offerings for the Certificate of Native Plants program (2013-2014).

- City of Chattanooga Tree Commission, planned and coordinated a series of lectures and volunteer workdays for the “Take-Root Chattanooga Citizen Forester” program (2010-2015).
- City of Chattanooga Tree Commission, helped plan and coordinate the “If Trees Could Sing” installation and launch party, in collaboration with The Nature Conservancy, Coolidge Park (2016).
- Coordinated the led the training workshops Appalachian Trail Mega Transect Project, in collaboration with The American Chestnut Foundation, Nantahala Outdoor Center, (2008-2010).
- Numerous public lectures and radio and newspaper interviews about restoration of the American chestnut (1996-present).