TITLE: AMERICAN CHESTNUT RESEARCH IN THE SOUTHERN REGION

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NARRATIVE

Justification for proposal

American chestnut (*Castanea dentata*) restoration is of national importance as an ecological keystone species, a food source for humans and wildlife, a versatile tree for wood-products, and its popularity in the public. Restoration will require that putative blight-resistant strains from TACF are properly tested in forest settings to determine if they can survive harsh conditions, compete with natural competitors, and maintain blight resistance to ultimately reproduce in the wild. Approximately 16% of BC_3F_3 progeny currently produced through the TACF breeding program will have blight resistance comparable to the Chinese chestnut (*Castanea mollissima*) (Hebard 2012). TACF predicts the majority of progeny will have high levels of blight resistance by 2023 (Steiner, personal communication[1]). The USDA Forest Service is currently testing this material from TACF in forest field tests across the southern Appalachians, and has completed the only research to date studying nursery production of chestnut planting material (Clark et al. 2009, 2012a). More work is needed, however, to make reliable predictions for how BC_3F_3 progeny will survive, compete, and grow once progeny with stable blight resistance are released for general reforestation. Additional alternatives to bare-root nursery production, including containerized seedlings also needs to be tested to ensure restoration of the species is conducted using the most advanced techniques possible.

Southern Research Station, Research Work Unit 4157 has led the most comprehensive and multidisciplinary research to date to understand barriers and mechanisms controlling forest restoration of the species on National Forest System (NFS) lands in the Southern Region (Region 8). Resources for this research are extremely limited and are growing more limited in the face of declining federal budgets. The Forest Service is one of the most important partners in chestnut restoration at a national level due to our ability to conduct long-term research and provide appropriate land for restoration plantings using material from breeding programs like TACF. The research proposed herein is consistent with the recent Memorandum of Understanding signed by TACF and the Forest Service in 2010, and will further strengthen the partnership between these two agencies.

Current state of collaborative research and management on NFS lands

SRS-RWU-4157, in collaboration with the University of Tennessee, has planted 4,389 chestnut seedlings from TACF in 11 forest test plantings and 2 seed orchard plantings in the Southern Region since 2009 (Table 1). Approximately 70% of trees planted were from the BC_3F_3 generation. Methods for establishment, measurement descriptions, and analysis of data have been previously described in a 2010 TACF proposal submitted and approved for funding. Since planting establishment, we have collected annual data on survival, deer browse, stem dieback, and blight on each tree planted. We have collected annual growth and competition data for trees planted at most locations. We have collected annual bud-break phenology on trees planted at four locations. We recently submitted a paper to *Forest Science* that summarizes a portion of the

[1] Steiner, K. Announced at The American Chestnut Summit. October 21, 2012

most recent results from our 2009 and 2010 plantings (Clark et al. submitted). This paper represents the largest and most comprehensive research in North America using the most advanced chestnut material currently available. Previous publications outlined the preliminary status of plantings and the potential barriers associated with American chestnut restoration (Clark 2012, Clark et al. in press, 2012b).

Our work has shown that chestnut is a fast-growing species that can be competitive on high-quality forest sites where yellow-poplar (*Liriodendron tulipifera*) is present. The main barriers we have encountered are deer browse to smaller seedlings and root rot disease from *Phytophthora cinnamomi.* Deer browse effects can be mitigated by planting larger seedlings, using fencing, tree shelters or repellants. We hypothesize the root rot disease was transported from the commercial tree nursery to the planting sites (Crandall et al. 1945), and is causing moderate to severe mortality, depending on planting location. During the past two years, we have partnered with Clemson University to conduct soil and seedling testing within and surrounding each planting area to confirm or reject the hypothesis that the root rot disease is coming from the nursery. Our research shows that chestnut nursery seedlings did contain the disease prior to planting and that planting site conditions (e.g., soil texture, water holding capacity, elevation) are having a significant effect on the ability of the root rot disease to cause tree mortality.

One of the methods to mitigate for this disease is to plant disease-free seedlings using container-grown seedlings. In 2013, we initiated a container-tree study to test effects of container size and family on tree physiology and morphology within the Root-Production Method (RPM®) at Forrest Keeling Nursery in Missouri. We hope to continue this work with additional BC_3F_3 families and seedlings.

The goal of our research program is to 1) provide progeny test results to TACF to help them in their breeding efforts, 2) to provide important recommendations to managers for the proper production of chestnut for planting and the correct silvicultural procedures to manage chestnut, and 3) to provide predictions for American chestnut survival and adaptability in the forest. Since the inception of this work, our research has popularized the work of TACF, the Forest Service, and other partners in the research community and in the general public. Our research has been published in several magazines/newspapers (*CompassLive, USA Today, University of Tennessee Land and Life, Treasured Forest Magazine*), and scientific publications (*Forestry*, Biennial Southern Silvicultural Research Conference proceedings, International Chestnut Symposium proceedings), and we have been invited to speak at over a dozen venues, including, university seminars (Purdue, The University of Tennessee, The University of the South), lay organization events (Winchester, TN Rotary Club, Elk River Forestry Association), and professional group meetings (Society of American Foresters, Western North Carolina Timber Conference).

Proposed Activities

Our research program is multidisciplinary through partnership building with entomologists, physiologists, pathologists, silviculturists, and tree improvement specialists. We are not seeking funds for the extended multidisciplinary research program of our existing plantings. A portion of requested funds will be used for continuation of the ‘core’ measurements we have collected since planting establishment. These core measurements are vital to the larger multidisciplinary effort by providing baseline information for understanding the trees response or relationship to mechanisms controlling survival and growth.

The requested funds would provide resources to continue data collection of plantings for one year. We plan to collect measurements on the 13 plantings we have established using TACF material (Table 1). We visit each planting site at least once annually, and we visit some sites 3 times or more each year. Tree mortality is related to a variety of causes, and frequent observations are required to truly understand mechanisms affecting tree mortality. To date, blight infection has been low (2 to 12 percent of trees, depending on location), and survival has been significantly related to non-blight effects, including seedling size at planting (Clark et al., submitted). Some plantings have succumbed to root rot disease and we will only collect limited data on those plantings due to low survival (Table 1).

Core measurements of the planted tree include the following: stem height, ground-line diameter, diameter at breast height (dbh, for seedlings $\geq$4 cm), stem dieback, deer browse, and chestnut blight characteristics. From 2009 to 2012, we collected data on blight occurrence on each tree, but our assessments were restricted to presence/absence on live trees. Blight was conservatively identified; positive identification was only made if *Cryphonectria parasitica* stromata were present or mycelial fans in the cambium could be seen with a hand lens (Griffin and Elkins 1986). In summer 2013, we incorporated more refined measurements and testing to ascertain field blight resistance than has been previously conducted. Measurements of blight now include location of canker on stem (height from base of stem to lower edge of canker), vertical length of canker, estimated circumference of canker (percent of bole affected), presence/absence of fruiting bodies, and general appearance of canker (cracking, swelling, sunken, etc.). For cankers where blight identity is in question, we will collect samples in the field and grow cultures for positive identification of blight strains and potential hypovirulent strains in the laboratory. We will use The University of Tennessee's Tree Improvement Program facilities to conduct these blight assays.

For every tree planted, we assess survival at least once per year. For some plantings, we measured bud-break phenology. In the early spring of each growing season, each tree was assigned two bud-break rankings using methods adapted from West and Weins (1971). We visited each site once when bud-break was approximately half-way completed across the planting location. For the terminal bud, we assigned a ranking from 0-5: 0-Bud dormant and no sign of breaking, 1-Bud displays silver/green tip, 2-Bud green and no leaves unfolding, 3-Bud expanding, leaves unfolding from bud but no internodes visible, 4-Internodes visible, leaves hanging but not enlarged, and 5-Internodes visible, leaves enlarged. We also assigned a bud-break ranking to the entire tree by first assigning the ranking described above to the most advanced bud on the tree, and then estimating the percent of the seedling (to the nearest 10%) that had developed to this stage. For example, a seedling that had the most developed bud with a ranking of 3, and had 41-50 percent of the buds on the tree at this stage would receive a 3.4 bud-break ranking for the entire tree.

For some plantings, we also collect measurements on the natural vegetation competition within a 1.3 m radius surrounding each planted seedling: species of tallest competitor <4 cm dbh (i.e., understory), species of largest competitor $\geq$4 cm dbh (i.e., midstory), height of tallest competitor <4 cm dbh, dbh of largest competitor $\geq$4 cm dbh, species of most abundant competitor <4 cm dbh, and species of most abundant competitor $\geq$4 cm dbh.

A portion of funds allocated will be used to grow container chestnut seedlings produced through the RPM® method to determine effects of container size on field survival and growth. Based on our preliminary findings from work conducted this summer, we will select two container sizes to test up to six BC_3F_3 families, at least one American family, and one Chinese

family. Experimental material for this work will be requested through Region 8 of the Forest Service, who receives material directly from TACF. We will request 600 BC_3F_3 seedlings, 100 American chestnut seedlings and 100 Chinese chestnut nuts for use in this study. Nuts will germinate and seedlings will be grown for one year in 2014 at the Forrest Keeling nursery using the RPM® method. After dormancy and just before planting, seedlings will be measured for root weight, stem height, and root-collar diameter. Seedlings will be outplanted in 2014 (autumn) or 2015 (spring) to test effects of container size on growth and container size x family interactions.

Expected output

Results from this study will be disseminated via publications, presentations, and websites (http://www.srs.fs.usda.gov/uplandhardwood/research-topics/duplicates/american-chestnut.html). Through this research, we will continue to publish results in peer-reviewed journals and the TACF journal, and provide presentations to professional and lay organizations. We will provide consultations to TACF and others as requested. We expect to publish at least 2 more peer-reviewed research articles in 2014 in addition to the submitted article. Fred Hebard, Chief Scientist with TACF, will be co-author on these articles, as the material used in this research represents his life's work. We expect blight incidence to increase on trees in 2014, and we will be able to provide TACF with better information on blight resistance of the first line of BC_3F_3 trees to be planted in field tests.

TIMELINE

The resources requested will be utilized from October 2013 through October 2014. Dormant season measurements (growth of planted tree, blight assessments, survival, deer browse, stem dieback) will be conducted from November 2013 through January 2014. Bud-break measurements (blight assessments, survival, deer browse, stem dieback) will be conducted in April or May 2014. Growing season measurements (competition measurements in 1.3 m radius around planted tree, blight assessments, survival, deer browse, stem dieback) will be conducted from late July through September 2014. Additional site visits may be necessary to collect soil samples for *Phytophthora* studies or to conduct additional blight surveys.

BUGET

We are requesting $9,850 to support activities associated with this research program (Table 2).

Explanation of Budget

Contributed costs include a portion of the scientist salary. Other contributed costs include supplies for field measurements (write-in-rain paper, height pole, calipers, safety equipment, flagging, tags, flags) and blight assay equipment for the laboratory. Some travel for the scientist will be incurred to visit planting sites and to conduct technology transfer (presentations or training). Forest Service vehicles will be used to visit sites and associated costs are estimated. Requested costs include a cost reimbursable agreement with the University of Tennessee to hire and provide support to a summer student worker for approximately 12 weeks to assist in data collection, data entry, and data quality control. Approximately $6,000 will be used to pay for the student worker salary, and $500 will be used to pay for the student worker to travel to the planting sites. Requested costs include supply costs for the containers that Forrest Keeling will use to grow the chestnut seedlings (estimated at $0.40 per container from Stuewe and Sons).

Table 1. Planting locations and measurements taken at each location.

Planting Location	**Number of trees planted**	**Year planted**	**Survival data taken annually**	**Seedling growth measurements taken annually**	**Bud-break phenology measurements taken annually**	**Competition measurements taken annually**
Clinch Ranger District, George Washington and Jefferson National Forest	369	2009	Yes	Yes	Yes	Yes
Tusquittee Ranger District, Nantahala National Forest	344	2009	Yes	Yes	Yes	Yes
Nolichucky Ranger District, Cherokee National Forest	442	2009	Yes	Yes	Yes	Yes
Beech Creek Seed Orchard, Nantahala National Forest	244	2009	Yes	Yes	No	No
Clinch Ranger District, George Washington and Jefferson National Forest	579	2010	Yes	Yes	Yes	Yes
Nolichucky Ranger District, Cherokee National Forest	513	2010	Yes	No	No	No
Beech Creek Seed Orchard, Nantahala National Forest	161	2010	Yes	Yes	No	No
Cheoah Ranger District, Nantahala National Forest (Shelterwood harvest site)	302	2011	Yes	Yes	No	Yes
Cheoah Ranger District, Nantahala National Forest (Midstory removal site)	284	2011	Yes	Yes	No	Yes
Eastern Divide Ranger District, George Washington and Jefferson National Forest (Shelterwood harvest site)	254	2011	Yes	Yes	No	Yes
Eastern Divide Ranger District, George Washington and Jefferson National Forest (Midstory removal site)	305	2011	Yes	Yes	No	Yes
Watauga Ranger District, Cherokee National Forest (Shelterwood harvest site)	304	2011	Yes	Yes	No	Yes
Watauga Ranger District, Cherokee National Forest (Midstory removal site)	288	2011	Yes	Yes	No	Yes

Table 2. Budget for American chestnut project

Resource	**Requested cost**	**Contributed cost**	
		Forest Service	**University of Tennessee**
Scientist Salary		51,000	
Technician Salary			44,500
Supplies	350	1,500	500
Travel		2,000	4,000
Vehicle costs including gas		2,400	
University of Tennessee amendment to existing cost reimbursable agreement	6,500		
Contract with Forrest Keeling Nursery	3,000		
Total	**9,850**	**55,500**	

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