Note: this first page might serve as the introduction to the overall revised SP

TACF STRATEGIC PLAN
2022

During the past 100 years, chestnut blight, caused by the introduced fungus, *Cryphonectria parasitica*, killed an estimated four billion American chestnut (*Castanea dentata*) trees and pushed the iconic species into a state of functional extinction. Human activities inadvertently triggered the American chestnut's demise–and now scientific innovation and private philanthropy under the leadership of The American Chestnut Foundation offer the best chance to save it.

The American chestnut tree was a vital component of eastern North American ecosystems, its rural economy, and our cultural history. Before the blight, chestnut was an important food source for a wide variety of wildlife and a valuable cash crop for communities from Maine to Alabama. As reliable and productive as the American chestnut tree was, the species now exists in forests primarily in a state of stunted vegetative regeneration from surviving root systems, and the surviving population continues to dwindle. TACF's effort to restore American chestnut is arguably the most ambitious species rescue mission ever attempted.

Our Vision is a robust eastern forest restored to its splendor.

Our Mission is to return the iconic American chestnut to its native range.

Our Scientific Objectives over the next decade are:

To develop sufficient resistance to two major diseases of American chestnut to allow trees to thrive under the high disease pressure caused by the continued presence of these pathogens in our forests. As a first priority, we focus on chestnut blight caused by *Cryphonectria parasitica* while giving second priority to *Phytophthora* root rot (PRR) caused by the invasive soil pathogen *Phytophthora cinnamomi*. Potential sources of resistance to aid in this work include Chinese and other Asian chestnut species as well as transgenes.

To characterize, preserve, and utilize the genetic diversity of existing American chestnut trees for research and to ensure that the developed disease resistance is incorporated into these environmentally-adaptable, diverse populations.

To develop the capacity to create forest plantings of genetically diverse and disease-resistant seedlings capable of sustained population growth and expansion across the broad and ever-changing landscape of our eastern North American hardwood forests.

THE STRATEGIC SCIENCE PLAN

EXECUTIVE SUMMARY

Objectives 2022-2032

The American Chestnut Foundation's (TACF) mission is to restore the American chestnut (*Castanea dentata*) to its native range from Maine to Mississippi. The scientific objectives for this revised Strategic Plan continue to place developing resistance to chestnut blight as its first priority, with a lesser priority given to resistance to *Phytophthora* root rot (PRR), also called ink disease. PRR is of regional importance in the southern range and a growing problem in previously unaffected central and northern areas. Another objective is to characterize, preserve, and utilize the genetic diversity found in the existing American chestnut population for research and to ensure development of environmentally-adaptable, disease-resistant populations. Finally, we will continue to develop the capacity to scale up and deliver genetically diverse and disease resistant trees capable of sustained population growth and expansion across the American chestnut's native range.

Current State of Our Resources and Research

Resources. TACF has succeeded in recent years to develop reliable and steady income from more than 5,000 members, donors and foundations. Approximately 94% of TACF's revenue comes from private philanthropy. This has allowed TACF to support essentially all of its proposed research activities, to continually update its major breeding and performance trial facility at Meadowview Research Farms, and to employ a small team of scientists with skills in quantitative genetics, bioinformatics, forest restoration and ecology, tree breeding and project and facility management. TACF has benefitted immensely from a cadre of thousands of enthusiastic volunteers who serve as a unique and valuable resource as they support our horticultural and research efforts on more than 500 orchards through our 16 state chapters. These volunteers are guided by four TACF-funded regional science coordinators. We also greatly benefit from collaboration with other colleagues who engage in research on chestnuts at universities such as SUNY-ESF, University of New England in Maine, Penn State, West Virginia University, Virginia Tech, Berry College, Clemson, Purdue, the University of Tennessee Chattanooga, and the University of Georgia.

Research on Disease Resistance. Our science team has brought the power of genomics—the study of the genetic material of an organism via molecular approaches---to support our future efforts including breeding. Collaboration with the HudsonAlpha Institute for Biotechnology has resulted in the notable achievement of the complete DNA sequencing and annotation of the genomes of one American and two Chinese chestnut trees. In the five years since our 2017 Strategic Plan, we have made extensive use of this resource and also improved methods for evaluating and rating resistance to chestnut blight and PRR.

With regard to blight resistance, thirty-six years of effort involving planting and selection among hundreds of thousands of trees and a backcross breeding strategy to integrate resistance genes from Chinese chestnut into American chestnut, has resulted in approximately 500 trees that we refer to as our "best hybrids" that contain on average only ~12% Chinese chestnut DNA yet have inherited intermediate blight resistance similar to 50/50 Chinese-American hybrids. A handful of these advanced hybrids also exhibit the desired American chestnut character along with intermediate blight resistance. Long-term field trials are required to determine if progeny from crosses among our backcross selections have sufficient blight resistance and competitive ability for sustainable growth in the wild. Further genetic analysis confirms our results from backcross breeding that blight resistance is a complex process controlled by many more than the previously-hypothesized two or three genes. Unexpectedly, TACF has also seen evidence in the backcross breeding program that certain Large Surviving American (LSA) chestnuts used as parents have conveyed superior levels of blight resistance to their progeny compared to the progeny of typical wild

American chestnuts. Neither the heritability nor a possible mechanism of resistance in these surviving trees is understood at this time, but they provide another interesting object for research.

TACF is now incorporating multiple strategies to improve the disease resistance of trees to be reintroduced to the forest. In addition to improvement of these best hybrids, one very different approach is the highly promising transgenic chestnut referred to as Darling 58---D58 for short--- developed by the team at the American Chestnut Research and Restoration Project at the State University of New York's College of Environmental Science and Forestry (SUNY-ESF). D58 relies upon insertion of a wheat gene that encodes an enzyme, oxalate oxidase (OXO), that degrades the potent fungal toxin oxalic acid. D58 shows measurable resistance to blight and is now under safety review for deregulation by the federal government. A longer-term approach of TACF--- still in its early phases---aims to identify the specific resistance genes present in the Chinese chestnut genome through advanced molecular approaches.

Genomic analysis of our breeding population has identified regions in the Chinese chestnut genome that are strongly associated with resistance to PRR. This offers promise that few major genes may be involved and likely explains the better results we have had in selecting for PRR resistance using the same backcrossing strategy as used for blight resistance. Though still in need of refinement, we are confident that future genome-assisted breeding should result in selection of acceptable PRR-resistant hybrid trees.

Germplasm Characterization and Preservation. In a collaboration with scientists having expertise in landscape genomics at Virginia Tech, we now have DNA sequence information for hundreds of American chestnut trees from throughout the species' natural range. Analyses of these sequences show that the existing American chestnut population can be subdivided into three geographically distinct sub-populations—northeastern, central and southwestern. In addition to providing valuable leads toward future work on genetic control of adaptation to climate and soils, the southwestern sub-population has been highlighted for preservation as it is both the most genetically diverse and the most threatened with extinction. This is consistent with its being the only sub-population that represents all the diversity that existed in ancient American chestnut and was preserved through surviving the last glaciation period 12,000 years ago.

Preservation of germplasm for research and eventual restoration efforts offers many challenges. For example, our volunteers and staff have made impressive efforts to conserve progeny of over 500 genetically diverse trees in our many germplasm and backcross conservation orchards yet, predictably, older American chestnut trees eventually succumb to blight. We will continue to reinforce and integrate the conservation of the most valuable and diverse germplasm in this way while also adopting additional approaches.

Strategic Goals for the Next 3-5 Years

To consolidate our current progress through more rigorous quantification of blight resistance in our best-performing backcross hybrids, in LSA trees, and in transgenic D58, and to attempt to achieve acceptable PRR resistance through continued backcrossing of Chinese germplasm into American chestnut trees. For D58, we will conduct rigorous field trials at greenhouse, orchard and forest locations to quantify the efficacy against blight of D58 in the short, medium and long term, and to ensure there are no harmful, unintended effects as a result of insertion of the OXO gene. Assurance of acceptable resistance without serious penalties will allow us to move forward over the next 3-5 years toward some first-generation restoration efforts with this line of transgenic trees assuming regulatory approval is granted. The history of transgenic crops shows that the first version of a transgenic is but the first step on a continuing journey toward ever-better versions, and D58 is viewed in the same light. The SUNY-ESF team is already beginning assessment of another OXO transgenic line called DarWin, which expresses the OXO gene only in tissues where infection has occurred.

Combining different forms of resistance. It is likely that each of our best hybrids has only a subset of the many resistance genes in Chinese chestnut. Starting with genomic and phenotypic analyses of the best blight-resistant hybrids, we can design pairs of crosses of trees that contain differing chromosomal regions of Chinese chestnut DNA thus increasing the chance of obtaining additive resistance. Similarly, we will cross hybrids with D58 to see if these differing forms of resistance are additive. We will also increase the number of crosses between PRR-resistant and hybrid and transgenic blight-resistant trees to protect our population from the growing threat of PRR. Provided that some blight resistance is confirmed in the LSA trees, we will include them in our breeding program, and eventually compare the mechanism of resistance with that found in Chinese chestnut.

To ensure that disease resistance is integrated into environmentally-adaptable, genetically-diverse germplasm. TACF-supported development of DentataBase has created an online platform for accurate documentation of all of our chestnut germplasm and merits continuing support and improvement. Multiple strategies will be employed for the long-term conservation of American chestnut's remnant population diversity. We will continue to supplement the genetic diversity in our breeding populations with special focus on collections from the southern and southwestern portions of the native range. Interbreeding these trees with D58 will help ensure the preservation of this germplasm by protecting the trees from blight. We anticipate that the OXO gene may perform better in some American chestnut genetic backgrounds than in others.

Multiple approaches for conservation include more strategic site selection and maintenance of Germplasm Conservation Orchards (GCO); this will be coupled with preservation of frozen pollen that has been given increased focus due to the finding that high light treatment promotes production of catkins and pollen in young seedlings. We will support research to improve clonal propagation of precious material via rooting of scions since it is an attractive and simple option but not yet feasible for chestnut. Disease-resistant trees will have to be intercrossed with at least several generations of American chestnuts to create populations diverse enough to be suitable for restoration. Joint work between TACF and SUNY-ESF is already under way to broaden the diversity of the genetic background of D58 through crossing it with a broad collection of genetically-diverse American chestnuts. Integration of disease resistance into diverse backgrounds of American chestnuts will happen over time, and their deployment will occur on a rolling basis.

To build new partnerships for gene discovery. The strategies above all build on prior accomplishments by TACF and its partners. Even as we pursue them, we recognize the development of new methods and technologies that can accelerate and advance our work. The identification of particular Chinese or American chestnut genes or genomic markers associated with disease resistance could greatly accelerate our breeding program. The reference genomes TACF and its partners have developed are critically important new tools for such gene discovery. This type of work is knowledge-, resource- and labor-intensive, and we will seek new partnerships to develop and possibly lead projects aimed at better understanding the molecular relationship between the chestnut blight fungus and its hosts, with a view to finding specific genes involved in blight resistance or to develop novel forms of resistance. TACF will also continue to provide initial seed money to test new approaches through its modest external grants program. We are currently funding external research on clonal propagation, germplasm conservation, and improved methods for assessing blight resistance of potential parents.

To make hard choices if necessary. Our science team continues to develop a detailed implementation plan for the next 3-5 years. They have devised a decision process that sets quantitative goals for disease resistance necessary for moving forward any one of these approaches; if this is not met over a limited period of research for any particular approach, the approach will be discontinued.

To better define and seek ways to address current barriers to our research. We do not underestimate the enormous challenge involved in meeting these ambitious goals. Our strategy also includes an examination of the current barriers we face at all levels of our research due to the challenges posed by carrying out research on hardwood trees. Examples include long generation times, very low efficiency of clonal propagation, and for all steps in the development and testing of gene-edited trees. In Appendix A of our longer narrative, we outline a series of new projects that we deem necessary to overcome some of these barriers and to allow us to move forward aggressively in order to meet our new objectives.

To better clarify the important role of our member volunteers. Defining our current strategies and expanding our goals by no means excludes the important role played by our members and "citizen-scientist" volunteers. The work of volunteers and philanthropy from members have been essential pillars in the TACF model, which continues to show the promise of success in an endeavor that was abandoned by government agencies decades ago. Critical efforts at germplasm preservation, including the new emphasis on pollen conservation or transfer of the OXO gene into diverse germplasm, “best x best” breeding of select hybrids, the preparation for scaling up production of nuts from genetically diverse D58 and hoped-for first efforts at restoration, are all exciting goals for which our volunteers can make significant contributions. To this end, we look forward to more discussions of this overall plan with our local chapters.

To support an increase in budget commensurate with our scientific goals. Support for this more aggressive approach to our science will also require parallel efforts to substantially increase our revenues from all sources.

The Path Forward

This strategic plan describes our vision of the scientific efforts needed to create genetically-diverse, disease-resistant trees of American character. We are often asked, “When will you have trees good enough for restoration?” Our best answer for now is that such trees should have blight and/or root rot resistance at least as good, and preferably somewhat better, than 50/50 Chinese-American hybrids while displaying American character---the most critical being a capability to flower and produce seed in the forest canopies of the native American chestnut range. We now are engaged in a clearly-defined effort that indicates we are close to producing trees that meet these initial criteria. Further confirmation that we can produce such trees reproducibly over the next few years will position us to take the first steps toward restoration. Yet even as restoration begins, scientific efforts will continue with the aim of delivering better trees each year for many years to come.

The next major step—restoration---poses a very different set of challenges. With evidence that our science goals are attainable, TACF is working harder now to define the complex process needed for a progressive restoration effort that might begin its initial efforts within the next few years. Taking sources from breeding (pollen, nuts, seedlings), we propose a supply chain that moves forward to reproduction orchards and then to seedling nurseries where bareroot and containerized seedlings are labeled and distributed for planting. Along the way, issues of trademarking or variety protection, preparation of instruction materials for best locations and methods for initial plantings, recruiting a diverse workforce and procuring financial support will all need to be addressed. With an updated scientific strategy, a trained and dedicated force of volunteers, and a history of successful fund-raising, TACF is positioned to meet both the scientific and logistical challenges ahead.