Where to Start? Aligning Products with Capabilities

Maybe some metrics:

Risk: Use a scale of 0 to 10, where 0 is virtually certain, 10 is virtually impossible, and so on.

Importance: Use a scale of 0 to 10, where 0 is of no importance or value, and 10 is necessary.

Time: Estimate project lengths in years (or fractions thereof).

Cost: Estimate project costs in millions of $.

Who has the expertise and capability to do the work?

Who does the work? Is it TACF staff, volunteers, academic partners, small commercial organizations, or large sophisticated industrial organizations? Or a combination, led and managed by those with the expertise.

What kind of work is required: Basic Research, Applied Research, Technical Development, Horticultural Development, Production?

Note: Most of these metrics are not directly addressed in this draft.

Accomplishments

In the past few years, TACF has made great strides in implementing its strategic plan of 2017. Some of the most notable of these are:

Developed complete genomic references for both American and Chinese chestnut with Hudson/Alpha.

Used those reference genomes to understand the complexity of blight resistance, and to essentially prove that continued back-cross breeding is not likely to yield hybrid chestnuts suitable for restoration.

Used the reference genomes as a basis for genotyping trees in TACF's orchards. Genotyping revealed that many trees identified as advanced hybrids were in fact out-crosses to Chinese chestnut.

Based on genotype and phenotype data, heavily culled TACF's orchards of hybrid back-cross trees with inferior blight resistance, as well as Chinese out-crosses. This greatly improved the stock within the foundation's network of chapter, regional, and national hybrid orchards.

With SUNY/ESF, advanced the progress of blight-resistant transgenic American chestnuts towards use in chestnut restoration.

Expanded and standardized the testing and selection of hybrid trees for ink root-rot disease caused by Phytophthora cinnamomi.

Initiated the establishment of pure American chestnut orchards for future use in transgenic breeding.

Scope of Research and Development Programs and Proposals

Over the past several years, and more intensively over the last year, a wide variety of research and development programs have been proposed to further the foundation's task of producing American chestnuts with enough disease resistance to be used in restoration efforts. These proposals range from eminently practical and useful improvements in farm operations to the cutting edge (and sometimes a bit beyond) of scientific inquiry using the most advanced biotechnology. Proposals have come from TACF staff, members of TACF's Science and Technology Committee, academic partners, and advanced biotechnology advisors.

There is a developing consensus of how we should proceed or think about the next research steps for the foundation. Here is a list of the documents that should be considered at this point:

(1) Debby's Powerpoint Vision document of July 2021.
(2) Jared's 3 Buckets discussion (extracted from email) of July 2021, or cells E1-E22 from the Jared's Excel spreadsheet on the implementation of TACF's strategic plan.
(3) Sarah's spring 2021 presentation to the Science and Technology Committee meeting.

Caveats

No proposed product discussed here should be considered as the final perfect product. Early products will have deficiencies and may even fail over the long time horizons required for landscape level species restoration.

Continued development of various, hopefully improved products is required. Long-term testing and monitoring of deployed products is also required. The overall hope is that as varieties of products are introduced, they contain more and more fit, and a more diverse set of genetics to enable American chestnut to survive, reproduce, and thrive in the eastern North American forests. It's very likely that the genetics introduced over time may complement each other in restoring the species.

All distinctions between demonstration trees and restoration trees have been removed. The only difference is in genetic diversity, deficiencies in which will be removed over time as our programs develop and our production expands.

Objectives and Key Results (with Minimal Acceptable Products)

Key to nomenclature: "O" represents OBJECTIVES, which is what should be achieved. "KR" represents CRITICAL RESULTS which are the how the objective will be achieved. This is a hierarchical schema (and all such schema break down at some point), where critical results (KRs) of one level become objectives (Os) of another level. (This is a schema used by some venture capitalists, large successful technical companies, and very large non-profits).

O1: Develop and produce large populations of genetically diverse American chestnuts that are resistant to chestnut blight and Phytophthora root-rot suitable for restoring American chestnut as a key important species in eastern North American forests.

KR1.1: Develop and produce large populations of diverse American chestnuts that are resistant to chestnut blight. Within 3 years produce 1,000s of seedlings, within 6 years 10,000s of seedlings, and within 10 years 100,000s to 1,000,000s of seedlings. (Within 40 years, have plantations that can yield wood and nut products for consumption.)

KR1.2: Develop and produce populations of diverse American chestnuts that are resistant to Phytophthora root-root and chestnut blight. Within 15 years produce 100s of seedlings, within 20 years 1000s of seedlings, and within 25 years 10,000s of seedlings.

KR1.3: Develop critical technologies and horticultural capabilities to support future product improvement and testing.

KR1.4: Continued monitoring and use of advanced biotechnology to improve the products in KR1.1 and KR1.2.

OBJECTIVE 1.1
Develop and produce large populations of diverse American chestnuts that are resistant to chestnut blight.

Note 1.1.1: Due to the recent dramatic success in producing pollen from seedlings within one year using high light chambers, it is possible to expand the production of OxO genetically modified trees and counter the founder effect by breeding trees for 4 or more generations within a 5-year time horizon.

Note 1.1.2: It is highly unlikely that continued breeding of pure back-cross hybrids either by continued back-crossing, intercrossing, or recurrent selection will yield hybrid American chestnuts suitable for use in restoration. Furthermore, hybrid trees are almost entirely unlikely to produce successful progeny in restoration plantings from breeding with surviving American chestnuts.

Recommendation: The foundation should stop supporting the production of hybrid American chestnuts for direct use in restoration plantings. Rather, all hybrid orchards should be used as parent orchards for transgenic trees, or for genetic studies. The number of supported orchards should be reduced to regional or state orchards with a focus on quality vs. quantity.

KR1.1.1: Using the diversity within back-cross hybrid orchards and high-light intensity production of pollen from OxO seedlings, develop and produce large populations of diverse American chestnuts that are resistant to chestnut blight. These trees will be 31/32 (97%) American, and half of them will carry and propagate the blight resistance trait. Furthermore, their progeny will be capable of breeding with surviving American chestnuts to produce even more diverse localized population over time. Within 3 years produce 1,000s of seedlings, within 6 years 10,000s of seedlings, and within 10 years 100,000s to 1,000,000s of seedlings. We can improve production in out years by developing homozygous OxO open-pollinated production orchards. (Within 40 years, have plantations that can yield wood and nut products for consumption.)

KR1.1.2: Using American chestnuts from germ-plasma conversation orchards, and high-light intensity production of pollen from OxO seedlings, develop and produce large populations of diverse American chestnuts that are resistant to chestnut blight. These trees will be 100% American, and half of them will carry and propagate the blight resistance trait, and their progeny will be capable of breeding with surviving American chestnuts to produce even more diverse localized populations over time. Within 7 years produce 1,000s of seedlings, within 12 years 10,000s of seedlings, and within 15 years 100,000s to 1,000,000s of seedlings by developing homozygous OxO open-pollinated production orchards. (Within 50 years, have plantations that can yield wood and nut products for consumption.)

Note 1.1.3: We should think about developing partnerships with outside companies to produce our products for us. In the near future, all production starts with our chapter, regional, and national orchards.

KR1.1.3: Develop additional genetically modified founder American chestnuts using Agrobacterium-mediated OxO transformations for blight resistance using a small number of American chestnut targets from geographically diverse parts of the tree's range. Then use these genetically modified trees as in KR1.1.2. Timeline is extended by 3 to 5 years. Additional cost is about $1,000,000 or more.

KR1.1.4: (Stretch) Develop 100s of identically genetically modified founder American chestnuts using CRISPR-like directed OxO transformations for blight resistance using a large number of American chestnut targets from geographically diverse parts of the tree's range. Then use these genetically modified trees as in KR1.1.2. Timeline is identical to KR1.1.3 (3 – 5 years beyond KR1.1.2) since genetic diversity is built into the founder lines and the number of generations required to dilute the founder effect is reduced to 0. Not currently feasible with available technology. Additional cost is millions or more. Might be feasible within 3 to 7 years.

KR1.1.5: (Stretch) Using technology developed for KR1.1.4, and extreme automation, develop production facilities for 100,000s of identically genetically modified founder American chestnuts with OxO genes for blight resistance, and then grow 1,000,000s to 10,000,000s of trees directly in tissue culture (clones of the 100,000 founders). Not feasible with available technology. Addition cost is many, many millions or more without partners in technology. Might be feasible in 10 years.

KR1.1.6: (Stretch) Using technology developed for KR1.1.4, use all genetic knowledge to develop a set of cis-genes for blight resistance in American chestnut, and produce many variants of trees with these cis-genes. Choose the best for production as in KR1.1.5. Not feasible with available technology or knowledge. Addition cost is millions to tens of millions. Might be feasible in a decade or two.

Objective 1.2
Develop and produce populations of diverse American chestnuts that are resistant to Phytophthora root-root and chestnut blight.

KR1.2.1: Use the population of Graves hybrid American chestnuts identified by Joe James with some Phytophthora root-root resistance as a basis for a population of trees also with blight resistance by pollinating this sub-population with OxO pollen. Within 15 years produce 100s of seedlings, within 20 years 1000s of seedlings, and within 25 years 10,000s of seedlings. Aggressive work in developing orchards could raise the production levels by factors of 2 to 10.

Note 1.2.1: This long timeline may be surprising when compared to the timelines under O1.1. Our current genetic models of Graves-hybrid-type Phytophthora resistance show that we have resistance based on the homozygosity of 3 or 4 controlling genes. When we breed even homozygous PC resistant trees with OxO trees, we produce F1 heterozygous trees. It takes another two generations to produce true-breeding F3 trees homozygous for the PC controlling genes. Since both male and female flowers are required, between 5 and 10 years per generation are required to produce useful trees.

Note 1.2.2: The genetic diversity in the ultimate product is controlled by the diversity of the initial population, and the diversity remaining within the population after three generations of selection. The final population of trees will effectively not pass any of its PC resistance to any other population of chestnuts, wild or otherwise.

Note 1.2.3: It would be very nice to have trees that produce female flowers within a year or so planting.

Recommendation: The product represented by KR1.2.1 is a genetic dead end. There are no follow-on projects that can extend or improve this product. Other than using Graves hybrid PC resistance trees for genetic information, the foundation should seriously consider not pursuing this specific goal for mass production, except for demonstration level plantings.

Note 1.2.4: The genetic diversity for PC resistance represented with the Graves-hybrid trees is likely to be a very small sample of the overall PC resistance within other species of Castanea because that trait has not been selected for over 100 years in American chestnut breeding.

Note 1.2.5: Research groups from Europe have detected much more effective PC resistance genes in Japanese chestnuts and other species.

Note 1.2.5: PC is perhaps the most destructive plant pathogen to both agricultural crops and native species known. PC resistance is being actively researched in many species world-wide. Other species of

Phytophthoras have caused major problems as well (P. infestans – potato blight; P. ramorum – sudden oak death).

KR1.2.2: Actively follow and cooperate with research groups world-wide to discover and develop PC resistance genes for use within American chestnut. No time or money estimate.

KR1.2.3 (Stretch) Use our knowledge of PC resistance genes within Graves-type hybrids to develop a cassette of genes to be introduced into American chestnut for PC resistance. Without further technology or partners, a decade or more and ten or so million $.

KR1.2.4 (Stretch) Use our knowledge of PC resistance genes from all sources along with CRISPR-like technology to develop a cassette of cis-genes to be introduced into American chestnut for PC resistance. A decade or more and tens of millions of $.

OBJECTIVE 1.3
Develop critical technologies and horticultural capabilities to support future product improvement and testing.

Note 1.3.1: A very glaring lack in TACF's capabilities is in vegetative (cloning) propagation of plants. This means that we cannot take remote samples from the field and grow them in orchards, and nor can we reproduce many clonal copies of plants for extensive testing.

KR1.3.1: Develop horticultural and field techniques to propagate American chestnuts from field samples (roots, leaves, stems, etc). Could take up to 2 years of intensive work by plant growth scientists and horticulturalists to develop field protocols, chemical (hormones and inorganic salts, pH, etc) treatments, and greenhouse protocols. Cost estimated to be less than $500,000 up to $2,000,000.

KR1.3.2: Develop American chestnut germ-plasma conversation orchards. There are several critical issues to be decided here: (1) where to place them or it --- for example far outside the range of blight; (2) how to propagate them; (3) how many of them or the population in each do we need. And: rather than going out and collecting nuts from wild rare trees, use the work of KR1.3.1 to propagate anything.

KR1.3.3: Develop similar techniques to propagate American chestnuts from single cells to plants using tissue culture techniques. 2 to 4 years and $1,000,000 to $4,000,000.

KR1.3.4: (Semi-stretch) Hone Agrobacterium-mediated transformations using techniques from KR1.3.2 to beyond 1 or 2% success. Even a small improvement here can result in a 20x improvement.

KR1.3.5: (Stretch) Develop directed CRISPR-like transformation protocols for insertion or deletions of genes from American chestnut.

Note 1.3.2: Here should we talk about gene discovery. Once we have complete genomes (American, Chinese, hybrids), should we not be investing in some type of advanced analysis (comparative genomics) in an attempt to identify the specific genes responsible for both blight and PRR resistance. If we could ID these genes, they could be inserted into the American chestnut to create a cisgenic American chestnut. We have reason to believe that these type products may not be heavily regulated in the future.

<u>OBJECTIVE 1.4</u>

Continued monitoring and use of advanced biotechnology to improve the products in KR1.1 and KR1.2.

Note 1.4.1: It is very unlikely that hypovirulence will prove to effective in restoring American chestnut, but may have some use in preserving American germ-plasma orchards.

<u>KR1.4.1:</u> Cease all active research into hypovirulence, but actively monitor outside research results.

<u>KR1.4.2:</u> Actively explore American chestnut sites in the triangle of Georgia, North Carolina, and Tennessee where the most genetic diversity of chestnuts is likely to be found. Preserve that diversity, but realize that those trees will not disappear due to climate change in the next 100 years. (See papers that can be referenced).

<u>KR1.4.3:</u> All gene discovery and gene expression work we can imagine. ……. This includes all RNA-seq and gene-knock out that can be thought of. $$$$ and needs to be justified to the products

End Notes: Continual monitoring of advances in the field genetics will require a lot of networking especially with Big Ag and Pharma. This is a big job that requires specific skills. Where should this go?

Do we have a complete understanding of EXACTLY what's been done to genetically alter Eucalyptus, poplar, and now, orange trees (the Greening Disease)? Anything to learn here?

ALL OF THIS WOLD BE PART OF THE TACF SCIENCE PLAN.