Appendix 1 - Orchard Definitions

Orchard plantings require coordination with TACF science staff, and/or the state chapters to determine need, as well as available material and local logistical support. These planting types should not be offered to prospective hosts as anything more than a possibility before confirming that they are desired in a given location.

Breeding Orchard

Definition: A breeding orchard is typically comprised of 3rd or 4th backcross generation trees from TACF's breeding program (though they could be earlier generations for newer sources of resistance). These orchards are established as the need arises at the Meadowview Research Farms, and within the state chapter breeding programs.

Size range required: At least 1 acre, often more.

Number of trees planted: Typically, 300-500, but somewhat flexible.

Timeline required of planting: 10-15 years.

Seed Orchard

Definition: A seed orchard is comprised of B3F2 intercross generation trees from TACF's breeding program. After making breeding selections, the seed orchard produces B3F3 chestnuts through open-pollination. These orchards are established as the need arises at the Meadowview Research Farms, and within the state chapter breeding programs.

Size range required: 1 acre/block; more than one block may be planted on a site (complete blocks only, partial blocks are not useful).

Number of trees planted: one block is ultimately comprised of 3,000 trees (planted over time).

Timeline required of planting: 30-45 years.

Germplasm Conservation Orchard (GCO)

Definition: An American chestnut GCO is comprised of American chestnut sources of interest. The native sources of the trees, as well as the layout and size of these orchards can vary widely, offering greater flexibility than other types of orchards. A Germplasm Agreement is not required for this type of planting.

Size range required: Typically, ½-1 acre.

Number of trees planted: Design and number of trees is flexible. A common standard for GCOs is 10 different sources of trees, with 10 representatives of each, for a total of 100 trees.

Timeline required of planting: 10-15 years, or as long as the host will continue to maintain the planting.

Where does the planting material come from? American nuts are typically collected by the state chapters, or other TACF members. They may be stored and distributed by the chapters, Regional Science Coordinators, or shipped to Meadowview for storage and distribution.

Backcross Conservation Orchard

***Definition*:** A backcross conservation orchard (BCO) is a collection of backcross chestnuts that have been assessed for blight resistance and found to be lacking. These trees are not appropriate to use for further breeding in a seed orchard but represent unique wild-type American chestnut germplasm and should be conserved for transgenic diversification, PRR breeding, or other uses.

Size range required: Typically, ½-1 acre.

Number of trees planted: Design and number of trees is flexible. Following the GCO model of 10 different sources of trees, with 10 representatives of each, for a total of 100 trees, would be one option.

Timeline required of planting: 10-15 years, or as long as the host will continue to maintain the planting.

Where does the planting material come from? Backcross nuts are harvested from breeding orchards, after the selection process has been completed. They may be stored and distributed by the chapters, Regional Science Coordinators, or shipped to Meadowview for storage and distribution.

Progeny Test

Definition: A progeny test orchard is comprised of B3F3 sources and used to test the quality of B3F2 breeding selections. Progeny testing of the B3F2 selections at Meadowview is underway, though it is still unclear the extent to which state chapters will need to take this on with material from their local breeding programs. A progeny test orchard may aim to observe performance long-term without intervention, or may be inoculated and rated to provide more timely results.

Size range required: At least 1 acre, often more.

Number of trees planted: Highly variable, 300 – 2000.

Timeline required of planting: 7-30 years.

Transgenic Planting

Definition: *Note: this planting type is not yet available*. Transgenic plantings contain transgenic trees developed by the SUNY-ESF lab, which are currently in the process of obtaining registration with EPA, USDA, and APHIS such that the trees may be released and made available to the public. At this point however, all transgenic plantings are conducted at a small number of USDA approved sites, including Meadowview, VA, SUNY-College of Environmental Science and Forestry, NY, and the University of New England, Saco, ME.

Appendix 2

VT/NH Chapter Specific Science Plan – 2021 and beyond

This document is meant as a template to guide your chapter's breeding program based on The American Chestnut Foundation's national science objectives. We are not expecting *all* chapters to participate in *all* of the activities outlined in this document. Please work with your regional science coordinator to refine your chapter's priorities based on individual chapter resources, enthusiasm for specific objectives, and stage in the breeding program.

Chapter Specific Science Plan - Outline

Objective 1. Maximize blight resistance in chapter breeding programs

I. Increase stringency of selection in BC_3/BC_4 orchards

1. Inoculate and make phenotypic selections per existing protocol.
   - May 2021: Inventory and assess inoculation potential of Otter Brook Farm and Old Gurdy Farm breeding orchards.
   - Field season 2021: Work to reinvigorate Lake St. Catherine breeding orchard and navigate relationship with VT State Parks in wake of Denis Lincoln's retirement.
   - Spring 2021: Plan a work day at Merck Forest breeding orchard in collaboration with new ED Rob Terry.
   - June 2021: Initial ratings of 2020 inoculations in the High Shelter Farm breeding orchard and mark worst trees for rogueing.
   - Spring or Fall 2021: Make initial selections from 2019 inoculations in Valley View – Pine breeding orchard.

2. Reassess selected BC_x trees that were inoculated at least two years ago for their long-term field resistance (See Appendix A for metrics).
   - June 2021: Assess selected trees in the Sheiling Forest breeding orchard, coordinate with F1 assessments (see II. 1 on page 3).

3. All orchards/trees must be in *dentata*Base!
   - This is complete, though updated inventories are welcome for any breeding orchards.

4. Collect young leaves for genotyping BC_x trees selected in 2017 or later
   - April/May 2021: Collect young leaves from selections and nice F1 trees marked in Sheiling Forest breeding orchards. Also coordinate with F1 collections (see II. 1 on page 3).

5. Work with regional science coordinators to use phenotype and genotype data to increase stringency of selection in BC_x orchards and refine BC_xF_2 planting decisions.

   Jared Westbrook made preliminary predicted selections based on genotype and phenotype data. These data may be used to compare with what has been planted in existing seed orchards and also revisit selections over the summer to determine if these predictions seem to hold up or make sense. This is a first pass – nothing set in stone.

   Below are preliminary predicted selections from Jared – April 2021.

Orchard	Pollen	source	TreeID	Am	Recommendation
Valley View - Lower	AL50	Graves	VT-VV07-R2T3	0.851518171	BCO/Rogue
Valley View - Lower	AL50	Graves	VT-VV07-R5T19	0.867805699	BCO/Rogue
Valley View - Lower	AL50	Graves	VT-VV07-R5T6	0.87505372	BCO/Rogue
Valley View - Lower	AL50	Graves	VT-VV07-R1T9	0.898457785	BCO/Rogue
Valley View - Lower	AL50	Graves	VT-VV07-R3T15	0.915643723	BCO/Rogue
Valley View - Lower	DV189	Graves	VT-VV07-R3T4	0.912499292	BCO/Rogue
Valley View - Lower	DV189	Graves	VT-VV07-R4T20	0.931006569	BCO/Rogue
Valley View - Lower	DV189	Graves	VT-VV07-R4T18	0.962911553	BCO/Rogue
Valley View - Lower	DV189	Graves	VT-VV07-R3T2	0.967726741	BCO/Rogue
Valley View - Lower	DV189	Graves	VT-VV07-R2T6	0.985153094	BCO/Rogue
Valley View - Lower	DV189	Graves	VT-VV07-R1T3	0.994527694	BCO/Rogue
Valley View - Upper	AB247	Graves	VT-VV05-R3T23	0.882159214	BCO/Rogue
Valley View - Upper	AB247	Graves	VT-VV05-R8T7	0.884323806	BCO/Rogue
Valley View - Upper	AB247	Graves	VT-VV05-R7T11	0.885550496	BCO/Rogue
Valley View - Upper	AB247	Graves	VT-VV05-R7T4	0.896132745	BCO/Rogue
Valley View - Upper	AB247	Graves	VT-VV05-R4T30	0.995890357	BCO/Rogue
Valley View - Upper	AB247	Graves	VT-VV05-R5T6	0.996908602	BCO/Rogue
Valley View - Upper	AB419	Graves	VT-VV05-R2T25	0.859617283	BCO/Rogue
Valley View - Upper	AB419	Graves	VT-VV05-R6T28	0.996210628	BCO/Rogue
Valley View - Upper	AB419	Graves	VT-VV05-R8T24	0.996358369	BCO/Rogue
Valley View - Upper	AB419	Graves	VT-VV05-R5T26	1	BCO/Rogue
Valley View - Upper	BG531	Graves	VT-VV05-R9T19	0.997573252	BCO/Rogue

a. Use and remove unselected BC_x trees if pollen contamination may occur.
b. If the unselected trees are not contaminating open pollination now, there is no need to cut these trees down.
c. Preserve unselected trees with unique American germplasm for future crossing with transgenic trees.

For this chapter, very few lines have been selected from and none of the phenotypic selections have been pulled out by Jared's model as selections (unique to this chapter in New England). That said, since a small number of these trees have been represented in the chapter's three seed orchards, the current recommendation is to challenge them with inoculation as planned and see if the results agree with these predictions. Also, several of the trees genotyped at 98-99% American. These are essentially American trees and could be used to conserve these sources in GCO's for transgenic diversification.

6. Intercross and plant progeny from BC_x selections that have resistant phenotypes and inherited >5% of their genome from Chinese chestnut.
   - Put this on hold until new selections are available, genotyped, and phenotyped from the Sheiling Forest and Valley View – Pine breeding orchards.

II. Incorporate new sources of blight and phytophthora root rot resistance

1. Select hybrid chestnut parents with blight resistance, phytophthora root rot resistance, and timber-type form

a. Potential new sources of resistance include, but are not limited to F_1, BC_1 and BC_2 trees descended from sources other than 'Clapper' and 'Graves.' Consult your regional science coordinator for locations of candidate parents in your region.

b. Phenotype those individuals for long-term field resistance (See Appendix A for metrics).

- Spring 2021: Phenotype Russell-Abbott trees with Will Guinn (NH DFL) for blight resistance.
- May/June 2021: Phenotype nice F1s in Sheiling Forest breeding orchard. Then emasculate so the initial selections can open-pollinate.

c. Phenotype for timber-type form (metrics to be determined).

- Phenotype Russell-Abbott trees with Will Guinn (NH DFL), and Sheiling Forest F1s, assuming metrics are available.

d. Collect leaves from candidate parents for genotyping to infer species ancestry.

- April/May 2021: Coordinate this with Will Guinn (NH DFL) for Russell-Abbott trees – send him a kit and instructions for three trees/samples.
- April/May 2021: Coordinate with Curt Laffin and Bill Widmer (VT/NH-TACF), and AJ Dupere (NH DFL) for Sheiling Forest F1 trees – send them kit and instructions or join them for the collection.

e. Work with your regional coordinator to select new sources of resistance based on results from a. through d.

2. Cross new sources of resistance with selected BC_3 and BC_4 descendants of 'Clapper' and 'Graves'

This on hold for 2021 until above phenotyping and genotyping complete for Russell-Abbott and Sheiling Forest sources, and any additional sources identified.

a. Aim to incorporate three to five additional sources of resistance into your seed orchards.
b. Cross each new source of resistance with selected 'Clapper' and 'Graves' BC_3 or BC_4 trees.
c. Generate a minimum of 150 seeds per cross so that you have enough seed to plant a single plot from each new source of resistance in each seed orchard block.
d. If you generate more than 150 seeds per cross, we can screen the excess seeds for root rot resistance.
e. We suggest using whatever tree is larger as the female[1] to maximize the number of seeds per cross.

[1] Male sterility may result if you use the BC_3/BC_4 tree as the female parent and the F_1 or BC_1 parent as the male parent. Male sterility arises due to genetic incompatibilities between chloroplasts inherited from American chestnut and nuclear genes inherited from Chinese chestnut. The chloroplasts are inherited from the maternal parent.

f. Chapters may also send seeds to Meadowview, Penn State or another location for small stem assays to preselect a subset of trees with elevated blight resistance for planting in seed orchards.

III. Select the 1% most blight resistant trees in seed orchards (BC_3F_2 and BC_4F_2 generations)

1. Include American chestnut, F_1, and Chinese chestnut controls in seed orchards.
   - On hold until new planting planned for existing seed orchards.

2. Inoculate with SG2,3 when most trees are greater than 1 inch in ground line diameter. Rogue the most blight-susceptible trees.
   - Spring 2021: inventory and measure diameters of four initial seed orchard plots at Fox Forest and estimate expected date to begin inoculations.
   - Spring or Fall 2021: inventory Essex FPR and Tom Rush seed orchards.

   Following steps on-hold until seed orchard inoculations begin

3. After 2+ years, re-inoculate with a more pathogenic strain of *C. parasitica* (Weekly or EP-155). Further cull seed orchard plots to < 20 trees per plot.
4. Conduct small stem assays on a subset of your selection candidates for the purpose of developing genomic prediction models for progeny blight resistance. Collect ~ 50 BCxF3 seeds from 50 – 100 BCxF2 trees and send to Meadowview (or another location) for small stem assays to assess resistance of seed coming from orchard.
5. Collect young leaves for genotyping the trees whose progeny are in the small stem assay.
6. Collect young leaves from remaining selection candidates for genomic selection. Cull the orchard down to the 1% most blight resistant trees based on genomic data.

Objective 2. Conserve a range-wide collection of wild American chestnuts to prepare for diversifying transgenic blight-tolerant American chestnuts

1. Take inventory of survival in your current germplasm conservation orchards
   - Field season 2021: Inventory Plymouth – South Main St GCO, either spring or fall ideal. Track any replacement plantings/updates in *dentata*Base.

2. Scout and find Wild Type American chestnuts.
   a. Record locations in TreeSnap or with Tree Locator Form. Collect samples for species ID/verification.
   b. Flag or mark trees that you want to revisit in winter for scion collection
   - Goal to identify 5 new sources for use in GCOs. While grafting support is uncertain and no one in the chapter knows how to graft, priority should be on finding flowering or fruiting new trees.

3. Collect nuts in the fall
   - June/July 2021: Plan controlled American pollinations as needed/possible.
   - Fall 2021: Aim to collect 30-50 nuts/new source. Ensure any new sources harvested have been sampled for ID and properly documented in TACF's *dentata*Base.

4. Collect scion wood in winter
   - Winter 2021: Scions could be collected and sent on to PSU/Purdue or other locations as grafting support is available. Work with RSC to determine priority for sources and the support available for grafting.

5. Conserve trees in germplasm conservation orchards, with biocontrol (for Chapter conservation goals, see Appendix B):
   a. Mud-packing
   b. Mixed strains of hypovirulent *C. parasitica*
   c. Super donor strains*
   - Spring 2021: Plant new sources in Rutland – Allen St GCO.
   - Spring 2021: document any other new GCO plantings – coordinate with Doug McLane a/o VT/NH-TACF's biotech or American location committees.
   - Develop and update a table of VT and NH American sources planted/represented to track progress toward goal of 35 new sources. (Table below is missing Rutland – Allen St GCO. Will be updated.)

Wild American Tree Name	Short Code	County	EPA Eco-Region	GCO_1	GCO_2	GCO_3
Rumney1 (Buffalo Rd)	NH-RM001	Grafton	58n White Mountain Foothills	Saco - Wild Meadow Farm (ME)	Dover-Foxcroft (ME)	Plymouth
Rumney2 (Buffalo Rd - small)	NH-RM002	Grafton	58n White Mountain Foothills	Saco - Wild Meadow Farm (ME)	Dover-Foxcroft (ME)	
Milford4 (The Reserve)	NH-MF004	Hillsborough	59h Gulf of Maine Coastal Plain	Saco - Wild Meadow Farm (ME)	Dover-Foxcroft (ME)	
Colchester2 (Lavigne Rd1)	VT-CC006	Chittenden	83b Champlain Lowlands	UVM HRC		
Wilton2 (Gage Rd)	NH-WL002	Hillsborough	58g Worchester/Monadnock Plateau	UVM HRC		
Canaan2 (Hall Rd - Little)	NH-CA002	Grafton	58q Sunapee Uplands	UVM HRC	Plymouth	
Eaton2 (Rockhouse North Tree)	NH-EN002	Carroll	58r Sebago-Ossipee Hills and Plains	UVM HRC		
Eaton4 (Barnyard)	NH-EN004	Carroll	58r Sebago-Ossipee Hills and Plains	UVM HRC		
Eaton5 (Ridge Rd - McKenzie)	NH-EN005	Carroll	58r Sebago-Ossipee Hills and Plains	UVM HRC		
Freedom1 (Paul Ellis - Fogg Ridge)	NH-FR001	Carroll	58r Sebago-Ossipee Hills and Plains	UVM HRC	Plymouth	
Freedom6 (Paul Ellis - Granite)	NH-FR006	Carroll	58r Sebago-Ossipee Hills and Plains	UVM HRC		
Freedom7 (Paul Ellis - Granite)	NH-FR007	Carroll	58r Sebago-Ossipee Hills and Plains	UVM HRC		
Windsor1 (VELCO)	VT-WN001	Windsor	58o Northern Connecticut Valley	UVM HRC		
Berlin2	VT-BE002	Washington	58l Northern Piedmont	UVM HRC		
Burlington4 (Ethan Allen Park1)	VT-BT004	Chittenden	83b Champlain Lowlands	UVM HRC		
Merrimack5 (Hospice House - North)	NH-MM005	Hillsborough	59h Gulf of Maine Coastal Plain	UVM HRC		
Pelham3 (Con Com)	NH-PL003	Hillsborough	59h Gulf of Maine Coastal Plain	UVM HRC		
Plymouth2 (Rogers St)	NH-PY002	Grafton	84a Cape Cod/Long Island	Plymouth		

*Note: while not yet available, the super donor strains of hypovirus hold the most promise. They are still regulated and require a permit to release, but work is under way to assess their regulatory status and we are hopeful they may be used by our program in the future.

Objective 3. Combine resistance to chestnut blight and Phytophthora root rot

1. Collect a minimum 50 nuts from untested Graves lines and other resistance sources. Send seed to Meadowview research farms.
   - No Graves B3F2 seeds harvested in 2020.

2. Identify potential orchard sites where *P. cinnamomi* is present in the soil. Send soil samples to Dr. Steve Jeffers at Clemson University, who will test the soil for *P. cinnamomi*.
   - N/A: PRR not yet in New England, that we know of.

Objective 4. Plant reintroduction trials to determine if current levels of disease resistance and American chestnut characteristics are sufficient for restoration

1. TACF staff will allocate specific families and work with collaborators to design experimental plantings.
   Current experimental plantings and partners:
   - Fay's Meadow/GMNF, Goshen, VT – USFS and UVM
   - UVM Forest Sciences Lab, South Burlington, VT – USFS and UVM
   - 2nd College Grant, Milan, NH – USFS and UVM
   - Talcott Woods/Catamount Family Forest – USFS and UVM (planned for 2021 or 2022)
   - Jericho Research Forest, Jericho, VT - UVM
   - Vincent State Forest, Weare, NH – NH DFL
   - Maynard Forest, Gilsum, NH – Monadnock Conservancy
   - Beaver Brook Association, Hollis, NH – Beaver Brook Association

   Smaller demonstration/trial plantings:
   - Branch Hill Farm – Milton, NH
   - Fairlee Town Forest – Fairlee, VT

2. TACF is looking for collaborators who will install, manage, and collect data on the plantings. Chapters may help us identify landowners and agency cooperators to manage these restoration plantings.

Additional Chapter Projects

Please include any additional chapter science projects that do not fall within the above objectives.

Chapter Specific Science Plan - Explanation

The chapter specific science plan explanation provides greater details and clarifications for the current breeding strategy and protocols. Again, please work with your regional science coordinator to refine your chapter's priorities.

Objective 1. Maximize blight resistance in chapter breeding programs

The American Chestnut Foundation's traditional breeding program was built upon research which suggested that blight resistance was a relatively simple trait, controlled by only 2 to 3 genes. Through decades of backcrossing and, now, integration of genomic analysis, we have learned that blight-resistance is a complex trait that is controlled by many genes. As a consequence, backcrossing to American chestnut has partially diluted blight resistance. TACF science staff has outlined steps to maximize blight resistance in chapter breeding programs in light of the recent findings.

I. Increase stringency of selection in BC_3/BC_4 orchards and incorporate additional sources of resistance

Some trees in the BC_3/BC_4 generations that were selected based on subjective canker ratings are likely to have inferior blight resistance due to inaccuracies in selection compounded over multiple generations. We would like to identify and remove these inferior selections to improve the average blight resistance of the progeny of the remaining BC_3/BC_4 trees. We would like all chapters to work with their regional coordinators to phenotype their current backcross selections (BC_1, BC_2, BC_3, and BC_4 generations) for traits indicative of long-term blight resistance/susceptibility. TACF national is currently genotyping chapter backcross trees to determine how much of their genome was inherited American v. Chinese chestnut. We will pair the phenotype and genotype information to determine which of the current backcross selections have moderate resistance and which have inferior resistance. We recommend conserving the inferior backcross trees for their American chestnut diversity, but not using them as parents in the breeding program.

II. Incorporate new sources of blight and phytophthora root rot resistance

Once chapters have made more stringent selections in backcross orchards, we recommend crossing some of your BC_3/BC_4 selections with earlier generation hybrids (F_1, BC_1, BC_2, or other complex hybrids) that descended from Chinese chestnut sources of resistance other than Clapper or Graves. The purpose of these crosses is to bring additional genes for blight and root rot resistance from novel Chinese chestnut sources into the breeding program. These trees will have more Chinese chestnut ancestry, but will serve as a safeguard against the dilution of blight resistance that occurred through backcrossing to American chestnut. We recommend planting three to five plots containing progeny of these earlier generation crosses in each block of your existing BC_xF_2 seed orchards. After selecting for blight resistance, we recommend making additional selection for American chestnut genome ancestry, leaf characteristics, and timber-type form among the progeny from earlier generation crosses. Once selection is complete, these earlier generation crosses will interbreed with BC_xF_2 selections in your seed orchard.

Additional selection in ensuing generations may be required to find an optimal balance between blight tolerance and American chestnut characteristics.

III. Select the 1% most blight resistant trees in seed orchards (BC_3F_2 and BC_4F_2 generations)

We intercross the BC_3/BC_4 selections to improve blight resistance in the following generation (BC_xF_2). The BC_xF_2 progeny potentially inherit genes for blight resistance from both BC_3/BC_4 parents. However, due to the dilution of blight resistance through multiple generations of backcrossing to American chestnut, we expect that the selected trees in BC_xF_2 seed orchards will have intermediate blight resistance. In the previous generation (BC_3/BC_4), we recommended increasing the stringency of selection and conducting a limited number of controlled crosses between BC_3/BC_4 selections and F_1 and BC_1 trees to improve the average level of resistance in BC_xF_2 seed orchards. Next, we outlined steps to accurately select the 1% blight resistant trees in BC_xF_2 seed orchards. By culling all but the most blight resistant trees, we will maximize blight resistance of the seed coming from these orchards. Blight resistance may be further improved through additional generations of selection in orchards or naturally in the forest.

Objective 2. Conserve a range-wide collection of wild American chestnuts and diversify the transgenic blight-tolerant American chestnut population

Breeding transgenic blight-tolerant American chestnuts with susceptible wild-type (WT) trees is potentially an efficient method to rescue the genetic diversity and adaptive capacity of the American chestnut population for large-scale restoration. We would like to chapters to conserve a total of 1,000 WT American chestnuts in germplasm conservation orchards (including current collections) to prepare for outcrossing and diversifying transgenic populations. If federal regulatory approval is granted to release transgenic trees, we would like to chapters and TACF staff to outcross transgenic trees to wild trees over three to five generations to increase regional adaptation and minimize inbreeding in transgenic blight tolerant populations (see Westbrook et al., 2019 for more details on the plan to diversify transgenic populations). Getting started on germplasm conservation now gives us time to find new sources American chestnuts, develop our skills with graft propagation, and test the efficacy of hypovirulence and other methods to keep blight-susceptible American chestnuts healthy for use in breeding.

Objective 3. Combine resistance to chestnut blight and Phytophthora root rot

American chestnut is highly susceptible to the soil borne pathogen, *Phytophthora cinnamomi*, which causes root rot that kills plants. The range of *P cinnamomi* is limited by prolonged freezing temperatures. Historically, this pathogen has affected American chestnuts in the southeastern U.S. As winters warm, *P. cinnamomi* is spreading north and is expected to reach New England by 2080. Combining resistance to phytophthora root rot with resistance to chestnut bight is essential for restoring the American chestnut.

The American Chestnut Foundation is collaborating with Clemson University and the U.S. Forest Service to screen American chestnut backcross hybrids for resistance to *P. cinnamomi*. To date, most of the *P. cinnamomi* resistance screening has been conducted on American chestnut backcross families from Southwest Virginia and some of these families inherited moderate to high levels of resistance to *P. cinnamomi* from Chinese chestnut. To increase the climate adaptability of backcross trees selected for *P. cinnamomi* resistance, TACF would like to screen backcross families from TACF's chapter breeding programs ranging from Maine to Georgia. Annually, for the next 5 to 10 years we plan to screen up to 4,800 backcross seedlings

for resistance to *P. cinnamomi* at the U.S. Forest Service Resistance Screening Center in Asheville, NC. We expect that approximately 5% of the seedlings will survive infection. Survivors with resistance will be planted at field sites where *P. cinnamomi* is present in the soil. Once these resistant trees grow large enough to flower, they will be bred with backcross or transgenic trees with resistance to chestnut blight to combine resistance to the two diseases. Chapters can get involved in the *P. cinnamomi* resistance screening effort simply by sending seeds from backcross trees to Meadowview to screen for root rot resistance at the U.S. Forest Service Resistance Screening Center. We ask for assistance from chapters in the South in identifying field sites with *P. cinnamomi.*

Objective 4. Plant reintroduction trials to determine if current levels of disease resistance and American chestnut characteristics are sufficient for restoration

We will assess restoration trials planted in the last decade and plant new restoration trials with our best material to date to determine if intermediate blight resistance is sufficient for backcross trees to compete and reproduce in Eastern forests. With sufficient blight resistance, it is our hope that natural selection will continue to improve blight resistance and competitive ability of backcross populations in the forest. In preparation for large-scale reintroduction, we would like to plant reintroduction/restoration trials to test how varying silvicultural treatments influence survival and blight resistance. For restoration plantings, TACF staff will allocate specific families and work with collaborators to design experimental plantings. However, TACF is looking for collaborators who will install, manage, and collect data on the plantings. Chapters may help us identify landowners and agency cooperators to manage these restoration plantings.

Appendix A

Phenotype backcross selections that have been inoculated at least two years following controlled inoculations to assess their long-term field resistance using the following metrics:

- Is the main stem alive? Y/N
- Are there any cankers on any stem that are larger than 15cm (about the size of your hand)? Y/N
- Is there exposed wood due to blight? Y/N
- Is there sporulation of the blight fungus? Y/N
- What percentage of the canopy is dead?
- What is the diameter at breast height of the largest living stem in inches?
- Are stump sprouts present? (Y/N)
- What is the level of callusing based on the following scale?
    I. Sunken – no callus
    II. Sunken – callus
    III. Swollen
    IV. Completely sealed over (not sunken or swollen)

Appendix B

See table for the number of new American sources to collect and conserve per Chapter in Germplasm Conservation Orchards (GCO's). This is a best guess at this point in time. These targets will change based on results from genomic analysis of genetic diversity and adaptation across the *C. dentata* range. Work with your regional science coordinator to ensure the sources you collect are representing the range of environmental variables within the species' native range in your state(s).

Chapter	**N sources**
AL	85
GA	85
TN	85
KY	85
NC/SC	85
VA	85
WV	85
MD	35
PA/NJ	85
IN	85
OH	35
CT	35
MA/RI	35
VT/NH	35
NY	85
ME	50
Total	**1075**