## TESTING BLIGHT-RESISTANT AMERICAN CHESTNUTS

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Purpose of the task force (by our interpretation): To develop recommendations for testing the form, adapt-ability, botanical characteristics, and durability of resistance of chestnut progenies intended for release and deployment as 'blight-resistant American chestnuts.'

## Assumptions

• The goal of TACF is to bring blight resistance into wild, naturally regenerating populations of Castanea dentata in Appalachian forests and, by doing so, restore the species to its former role. Achieving this goal requires the use of non-native alleles because the genome of Castanea dentata is deficient in naturally occurring alleles for strong resistance. Thus, the specific objective of the breeding program of TACF is to produce backcross trees that will fall within the range of morphological, developmental, and ecological characteristics of Castanea dentata as understood from monographs and voucher specimens. It is anticipated that the B 3 F 3 generation may meet this o b j e c t i v e

• The goal of TACF will not be achieved by replacing the existing millions of surviving American chestnuts with B 3 F 3 trees. Plantings may never be established in some rather large blocks of the chestnut range, such as the Shenandoah and Smoky Mountain National Parks. Also, natural regeneration from planted trees, with accompanying natural selection and the potential for hybridization with native chestnut, will play large roles in achieving the goal of TACF. Finally, it expected that additional sources of blight resistance must be incorporated into the breeding program, and it is expected that additional backcross generations may be warranted to achieve a higher average proportion of American chestnut alleles in the genome.

• For these reasons, future breeding will be required to bring new sources of resistance into the breeding pool and carry the existing backcrosses to additional generations. Also, it is expected that future breeding programs will place ever increasing emphasis on regional adaptation by employing local, autochthonous sources of American chestnut parents.

• The purpose of testing the B 3 F 3 generation is to determine how well we have progressed toward our goal at a stage that is expected to yield a tree that bears strong resemblance to Castanea dentata and has good resistance to blight. In other words, the principal objectives of testing are to determine 1) to what degree the B 3 F 3 resembles American chestnut, especially in a natural forest setting, and to what degree Asiatic characteristics (other than blight resistance) may remain, 2) to what degree the B 3 F 3 is resistant to blight, and 3) how long resistance persists in B 3 F 3 p l a n t a t i o n s . Subsidiary objectives are to determine if there are differences in performance among the progeny sets of different B 3 F 2 parents, to measure the extent of genotype x environment interaction, and to identify differences in regional adaptability as suggested by growth, survival, and phenology.

• Although test plantations might later be converted to seed orchards, test plantations should not be designed with the purpose in mind of converting them to seed orchards at a later date. The creation of seed orchards should be pursued separately from testing.

• Testing should precede public distribution or sale of seed with implied genetic qualities.

• Future breeding and deployment efforts should be guided by test results.

## TASK FORCE RECOMMENDATION S:

• Testing will involve finding and preparing planting sites, getting labeled trees from TACF, planting them, mapping their locations, protecting them, and measuring them. It is expected that test plantations will be arranged, installed, maintained, and measured by chapter volunteers and other cooperators.

- Given the goal of the breeding program, testing shall be done on:
  - naturally forested sites (which may be temporarily devoid of trees because of recent harvest),
  - on soils that are considered suitable for American chestnut, and
  - in close (a few hundred yards maximum) proximity to existing sprouts of American chestnut.

This latter requirement, which may not always be possible to meet, ensures that the soils are appropriate for American chestnut, provides the opportunity of directly comparing phenology of backcross trees as one indicator of environmental adaptation, and provides the opportunity to determine whether resistance alleles from TACF backcrosses can move into other American chestnut by natural crossing.

• To the extent possible, test sites shall be scattered throughout the original distribution of Castanea dentata, or at least the region of its greatest abundance (Massachusetts to North Carolina and Tennessee). Although B 3 F 3 material now under development was derived

from central Appalachian provenance of American chestnut, it is important to learn about regional adaptability in this species and in the likely products of the TACF breeding program.

• Test sites should be evaluated and approved prior to planting by someone from TACF. Corner coordinates shall be established by accurate GPS measurement.

• Cooperators shall agree to the use of standard protocols for the design and measurement of test plantations. It may be desirable and even necessary to underwrite these tests with research grants from TACF.

• Test plantation design shall conform to the following criteria:

- 8- x 8-foot spacing between trees (square grid arrangement),
- minimum of 25 single-tree replications of each experimental unit ("treatment") (see below), and
- completely randomized design. Each test plantation shall contain the following experimental units:
- pure American chestnut of local provenance at double replication (minimum 50),
- one or more designated Asian chestnut varieties at double replication (minimum 50),
- a core set of at least five B 3 F 3 families (open-pollinated progenies of B 3 F 2 lines) common to all plantations, and
- additional B 3 F 3 families as availability and space allow, including if possible families from different regional breeding programs.
- advanced generation backcrosses (other than B 3 ) if available.

Plantation sites shall have minimal overstory (30 sq. ft./acre basa area), and clearcut sites will be generally preferred. Existing green vegetation shall be sprayed with glyphosate (or similarly acting herbicide) in a meter circle around each planting spot in advance of planting. Directed applications of glyphosate (with seedlings shielded) shall be used after plantation establishment as needed to exclude serious competition from herbaceous and woody plants. The site shall be protected from deer if deer browsing is likely to be serious.

All records of test plantation establishment, including a map of the design, detailed directions to the location, contact information for ownership, date of establishment, and contact information for the person responsible, shall be sent to a central office to be designated by TACF.

Cooperators shall commit to making annual (initially) measurements of the following:

- height,
- diameter,
- survival,
- form (index to be developed),
- severity of cankering (0 = none),
- date of bud burst,
- date of flowering,
- date of fruit maturation,
- date of fall coloration,
- abundance of male and female flowers (0 = none), and
- presence and nature of other serious insect or disease injury.

For reference, the dates of phenological events (bud burst, flowering, fruit maturation, and fall coloration) should also be recorded for nearby native trees. It is highly recommended that plantations be visited several times during the growing season, especially during the first few years. Measurement protocols will be developed and distributed to cooperators. Cooperators may record additional information if they desire. In order to allow measurements of naturally occurring rates of disease progress, trees will not be artificially inoculated. Other plantings in orchard settings will be tested for blight resistance by artificial inoculation. Cooperators shall verbally commit to plantation care and measurement for a minimum five-year period. All data shall be sent annually to the central office designated by TACF. Cooperators shall be free to publish data from their test plantations. The central office will annually prepare a report on the progress and performance of all test plantations. It is impossible to specify a precise duration for the usefulness of these test plantations. A minimum of three to five years' evaluation will be required for preliminary conclusions about relative performance. Fairly definitive conclusions about some aspects of performance should be available within ten years. With time, the value of each additional year's duration will diminish, but never quite to zero. A key question to be answered is whether backcross hybrid chestnuts will have the ability to grow to dominant canopy height in competition with naturally occurring trees. A definitive answer to this question will require per-haps two or three decades of testing, but crown form and the pattern of early height growth can be useful predictors.

Test plantations will be thinned at an appropriate time after crown closure in order to maintain reasonable access with measuring poles without modifying too much the natural progression of forest stand development. When fairly definitive conclusions are possible regarding the relative performance of backcrosses vs. Asian chestnuts, a decision will have to be reached on whether to remove all Asian chestnuts from the test plantation. The purpose of this removal would be to remove a source of major pollen contamination from the planting.