Response of TACF Research Farm Staff to the recommendations of the 2006 Science Review Panel

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In 2006, a panel of four scientists reviewed the TACF breeding program, and formulated 20 recommendations for our consideration. The full report can be found on the Board of Directors page at http://www.acf.org/. For those without access to that page, the report also is deposited at http://www.acffarms.org/papers/. Other publications to which references may be encountered in this document also can be found at these sites.

The recommendations are listed below in bold-face type and the response to each one immediately below it, in plain face type.

1. Continuation of the current breeding program to the B6 generation, and the interim limited distribution of B3-derived populations as a means of assessing American Chestnut characteristics, variability and adaptation, and blight assessment levels over a range of environments.

This recommendation appears to endorse our current breeding plan as outlined in the "Ten-Year Plan" (see websites above). That plan envisions advancing at least 15 sources of blight resistance to third backcross, but only one source to sixth backcross, in the immediate future (the next 10 years or so). The Science Review Panel's recommendation can be implemented for additional sources at the end of that period.

With regard to this timetable, it also is important to note that it would be best, in our opinion, to follow the basic blueprint of the backcross method of plant breeding, as outlined by Briggs, and later by Briggs and Allard, in their series of texts, "Introduction to Plant Breeding." That blueprint includes making an intercross generation at third backcross before continuing on to sixth backcross. The reason offered by these authors is to increase the chances for recombination in both parents, as, by third backcross, linkage drag is an important factor in recovery of the recurrent type. The first three cycles of backcrossing are primarily the time when entire chromosomes from the non-recurrent parent are discarded that lack genes for the trait of interest. The fourth through sixth cycles are when the blocks of DNA surrounding the genes of interest are whittled down in size. Most of our sources of blight resistance will only be entering B_3-F_2 ten years from now, so we will not be able to move on to sixth backcross in the immediate future, for most sources of resistance.

In practice, the program takes a rather broad view of the stages of backcrossing, considering third and fourth backcross trees, for instance, to be operationally at the same level of backcrossing.

One element of the "Ten-Year Plan" which is not included in this recommendation is to move on from B_3-F_2 to B_3-F_3 or B_3-F_4 seed orchards, if it is necessary to improve blight resistance or other traits. That would essentially be occurring before B_6-F_2 seed orchards were in production.

There also is the question of how large is an "interim limited distribution." If B_3-F_3 trees perform well in initial forest tests, we would limit their distribution to 500,000 to 1,000,000 acres, which is a very small fraction of the original habitat of the American chestnut tree, but should be sufficient to put enough selective pressure on the blight fungus for resistance-breaking races to evolve if they can. This would entail planting about 250 to 500 million seedlings and nuts, at 500 plants per acre.

2. The collection of molecular genetic information that will support identification of current and future resistance sources and the selection of the American Chestnut genetic background in future breeding endeavors.

This is an ongoing project that has been underway since 1990. It fortunately was greatly accelerated by the recent acquisition of a large grant from the NSF, due to the admirable efforts of Dr. Paul Sisco, our Southern Regional Science Coordinator.

Based on the previous mapping work, the Research Farms have been collaborating with Shiv Hiremath of the Forest Service and with John Carlson of Penn State to select for American type at third backcross. The two groups are using different, complementary methods.

Also important to "the collection of molecular genetic information to identify current and future sources of blight resistance" is the development of pedigrees permitting suitable analysis. That is a primary reason why Bob Paris was hired.

In practice, it is our intent to use a two-pronged approach to identifying current and future sources of blight resistance using both classical techniques with pedigrees as well as molecular techniques. Previously, we relied too heavily on molecular techniques and have not progressed in this effort as much as we had hoped.

3. TACF and its Chapters formalize a Germplasm and Commercialization Strategy for identification, distribution, release and benefit-sharing of genetically improved populations.

Most of the implementation of this recommendation will be the duty of the Board of Directors of TACF and TACF's development staff. A critical question that needs to be addressed promptly is whether TACF will attempt to protect B_3-F_3 nuts. Staff recommends that B_3-F_3 nuts be regarded as a released product and not be protected by a germplasm agreement, but rather, if any protection using the legal system is used, that they be patented. The most important protection we can envision is for TACF to dominate the market in blight-resistant chestnuts. This could be achieved by: providing an overwhelming supply of nuts at reasonably prices; by constantly improving our

material –from B_3-F_2 to B_3-F_3 and from B_3 to B_6-- coupled with vigorous publicity, so that we are always providing the "latest and greatest;" by forging close partnerships with the forestry community, both public and private; and by use of the trademark system.

4. A close collaboration of the TACF staff with the NSF grant participants in order to encourage research that will advance the breeding program.

This is occurring. Paul Sisco is a co-PI on the proposal and Fred Hebard one of the senior personnel.

5. That the TACF staff follow the literature dealing with species that are collinear with the Chestnut since much of that information will be as valuable as if acquired with the Chestnut.

We will endeavor to continue following the literature. Library service is a budget item in the farm budget that frequently is not exceeded, but long-distance library services are only now advancing to the point where most journals are online, so care should be taken not to reduce this item.

6. Disease resistance genes be mapped from current and future genetic sources with flanking markers identified in order to follow the resistance genes in a limited marker-assisted selection program.

Mapping the genes for blight resistance is one of the objectives of the NSF-grant work. It is unclear to the farm staff whether marker-assisted selection for resistance would be effective, as it might be difficult to identify all the genes for resistance or the correct genes. A test program of simultaneous selection with markers and disease screening would be needed. The funds and personnel to conduct this program are not in hand (see recommendation 14).

7. To the extent possible, long-term maintenance of mapping populations be a priority to facilitate subsequent gathering of data on other traits deemed important.

We respectfully disagree with the review committee regarding the value of this recommendation. Most of the current mapping populations are Chinese chestnut or fairly simple crosses of Chinese and American chestnut. We are not particularly interested in traits of the Chinese chestnut tree, rather the American chestnut tree. Pure American chestnut trees would die from blight before much data could be collected. Implementation of this suggestion would necessitate vegetative propagation of these populations, probably by stooling, as the French are doing with their mapping population of chestnut.

We plan to maintain the mapping populations being generated for the NSF-grant work, but, for the above reasons, we do not feel it would be worthwhile to collect forest-performance data from them and to try to relate it to molecular marker information. That will await the arrival of B_3-F_3 nuts, especially pedigrees from controlled pollinations

whose parents chosen for interesting polymorphisms. Identifying these polymorphisms is Objective C.5 of the "Ten-Year Plan."

8. The advanced backcross material be used in mapping studies since mapping procedures can be more efficient with such materials.

This is currently one of the objectives of the NSF-grant work.

9. Involvement of molecular markers for detecting outcrosses, accelerating the backcross program, and fingerprinting for ID and protection purposes.

As indicated above for Recommendation 2, we have ongoing programs on selection for recurrent type. For fingerprinting, a large component of the NSF-grant work is to genotype B_3-F_2s. This information should be useful in identifying fingerprint markers and outcrosses.

10. Testing for the presence of one or more chromosome translocations in the current breeding materials and the new sources of resistance.

We are initiating a contract with the Southern Institute of Forest Genetics of the U.S. Forest Service to pursue this study.

11. Testing for population differences across species range and use of the information to inform breeding and deployment strategies.

We are initiating a common-garden study of American chestnut and straight backcross trees to do this. Additionally, Objective C.5 of the "Ten-Year Plan" is to perform common-garden studies with B_3-F_3 plants.

12. Establishing field tests to determine levels of field resistance to blight and other potential problems using the most advanced generation of trees.

This is Objective C.2 of the "Ten-Year Plan."

13. That TACF continues to engage members by asking them to be involved in the testing program and to provide feedback, similar to beta sites and/or rose testing.

We intend to engage chapters in the common-garden studies and in some field tests.

14. Adding at least a half-time position for database and web management and a lab technician for molecular genetics applications.

Additional staffing is an ongoing process at the Research Farms as we bootstrap our operation to a fully-fledged research site.

15. That TACF utilize the broader scientific community's meetings, such as AAAS, to network and disseminate information on the "Chestnut Story."

Thus far, we have only reported at discipline-specific meetings, such as those of the American Phytopthological Society, the Society of American Foresters, the Ecological Society of America, and the Plant Genome Meeting. We will endeavor to report at more general meetings, such as AAAS.

16. That TACF host internal "working" meetings for everyone involved in hands-on work in all chapters.

Generally, these are conducted at the chapter and regional level and at national meetings. We also have had one meeting of all the chapters exclusively devoted to such issues.

17. The use of small non-overlapping independent factorials (SNIFs) for mating design in breeding program.

This recommendation arose from a misunderstanding between Research Farm Staff and the Review Panel. Farm staff were modeling inbreeding of B_3-F_2 nuts as disconnected partial diallels, to approximate the actual production under open pollination, under which SNIF mating designs would not be possible. Should controlled pollinations be used for production of B_3-F_2s, we will use SNIF designs.

18. The use of clonal material when appropriate for seed orchard establishment and for other studies that support the research objective of TACF.

We have a small greenhouse to propagate trees by grafting, which Bob Paris is using this spring. In the past, we have rooted cuttings from basal stem sprouts or micropropagated same. We have never used stooling, which is the preferred method in Europe for propagation of chestnut on their own roots.

19. The examination of responses of superior selections to a broader range of strains of the chestnut blight pathogen (including hypovirulent strains), as well as responses to less aggressive inoculation techniques and natural infection.

We broadened our culture collection before the review last summer, isolating from severe cankers on Chinese chestnut trees in Washington County, VA, near the Research Farms, and from severe cankers on F_2 and B_1-F_2 selections. We have started to test these isolates for general and specific virulence.

In conjunction with these isolations, we also evaluated canker severity on F_2 and B_1-F_2 selections. The severity index (percent of cankered stem) was a composite measure of resistance to infection and resistance to colonization.

With Bill MacDonald and colleagues, we have been installing a study of the response of trees with various levels of resistance to hypovirulent strains of the blight fungus for the past four years.

Our selections also will be exposed to a broader range of strains of the blight fungus when they are tested in the forest.

We are continuing tests of methods for evaluating blight resistance in young seedlings and attempting to develop a method for assessing resistance to infection more rapid than long-term field performance. Our current screening method measures resistance to colonization.

20. Increased availability of land and equipment sufficient to support immediate and future progress in the breeding program and seed orchard development.

Subsequent to the science review, TACF was fortunate to be able to purchase 25 acres adjacent to its main Price Research Farm. That land should provide sufficient planting space to accommodate our core research for the next 7-10 years. We also need to acquire a third seed orchard for the 'Nanking' source of blight resistance. For this, we are negotiating to use some land that Emory and Henry College recently acquired two miles from our Wagner Research Farm. Bob Paris and Fred Hebard presented a letter to the President of Emory and Henry College in March concerning this. We do not expect to be able to make firm plans for another year, but we are keeping the ball rolling on this.

We need a laboratory, as the review panel pointed out. A more pressing priority is a maintenance shop, where equipment can be worked on in the winter during cold weather. A prioritized list of additional equipment and instruments needed in the next few years follows, with estimated cost.

Item	**Cost**	**Year Needed**
Extend irrigation system to new farm	$15,000	2007
Routine overhaul, existing bucket truck	17,000	2007
Seed storage, near term	10,000	2007
Gas chromatograph, used	10,000	2007
Additional bucket truck	50,000	2007
Microscope	17,000	2007
Maintenance shop	120,000	2007
Chassis for existing bucket truck	10,000	2008
Transfer hood	5,000	2008
Track hoe	35,000	2008
Replace cab tractor	30,000	2008
Replace backhoe	35,000	2008
Seed storage, long term	50,000	2009
Bulk harvesting equipment	100,000	2009
Laboratory, equipped	2,000,000	2009