The American Chestnut Foundation
2nd Science Review
August 8-11, 2006

Introduction

Over 150 years ago, Louis Pasteur said that chance favors the prepared mind. A review of a scientific program prepares the minds of those involved to not only have a well-formulated plan but to also recognize those opportunities that present themselves along the way. Much of the value of a scientific review is in the preparation - summarization of the pertinent information obtained to date on which to base future work, development of a plan for the next steps in the process, projection of needs in terms of information and resources, and presentation of the pertinent points to a review team. This advanced information helped us to focus on the mission of TACF which is to restore the American Chestnut to the original range in the eastern United States by developing, testing, and distributing a chestnut tree with the basic American Chestnut traits and a reasonable degree of resistance to chestnut blight.

The TACF Science Review Team was composed of Lauren Fins, Professor of Forest Genetics, University of Idaho, Robert McIntosh, Honorary Professor, Plant Breeding Institute, University of Sydney, Ron Phillips, Regents Professor of Plant Genetics, University of Minnesota, and Glen Stanosz, Professor of Plant Pathology, University of Wisconsin-Madison. TACF staff included Dr. Frederick V. Hebard, Dr. Robert L. Paris, Dr. Paul H. Sisco, Ms. Sara F. Fitzsimmons, and Ms. Cornelia C. Pinchot. TACF members included Dr. Kim Steiner, Dr. Albert Ellingboe, Dr. Cameron Gundersen, Dr. J. Hill Craddock, Mr. K.O. Summerville, Dr. William MacDonald, Dr. Safiya Samman, Dr. C. Dana Nelson, and Richard S. Will. Their participation was of great assistance to the review team.

The process for this review of the American Chestnut Foundation's science activities involved the delivery in advance of extensive documentation of progress to date and several publications that reviewed the history of TACF and goals for the future. We were privileged to visit a forest site with mixed forest species, including chestnuts in various stages of their life cycle. We also learned about canker formation caused by the chestnut blight and examined the growth pattern of the fungus under the bark. We then observed the research material at the three primary research sites (Wagner, Price, and Price-annex farms). This was followed by key staff orally presenting their research program largely from a breeding and genetics perspective. We also learned about the activities of the various TACF state chapters and how they relate to the mission of TACF. In addition, the review panel informally interviewed Richard Will, Finance Committee Chair, Al Ellingboe, Science Director, and Kim Steiner, Vice Chair Science. The Review Panel greatly appreciated the excellent review of the program presented by the TACF staff and volunteer members.

The format of this report is to provide a brief listing of some of the current strengths that represent significant progress in the program since the 1st TACF Science Review in 1999, to present a list of our recommendations, and then to discuss various issues related to breeding, genetics, adaptation and environmental variation, efficiency, scientific communication, resources, and germplasm release and commercialization.

Current strengths: The Review Panel was encouraged by the presence of several talented scientists with noticeable enthusiasm toward the TACF mission, the development of third backcross F2 materials (called B3F2), initiation of a study on new sources of resistance, generation of considerable molecular marker mapping information, the careful decisions on artificial inoculation procedures and modifications based on experience, the development of pertinent research projects by outside scientists, the significant NSF grant to advance the molecular genetics of the chestnut species, the publication of progress, and the maturation of the chapter concept. The enthusiastic state chapters are important in many ways, including the evaluation and contribution of genetic materials.

Recommendations

The Review Panel recommends:

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.

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.

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

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

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.

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.

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.

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

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

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

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

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

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.

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

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

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

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

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

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.

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

Breeding

Currently, there continues to be three presumed sources of resistance in the Clapper, Mahogany/Graves and Nanking sources. While genetic studies indicate that the Mahogany source may contain 2 or 3 partially dominant resistance genes, it is not known if the other sources are genetically different from Mahogany, or from each other. This should be determined by the recently approved NSF grant in which Chestnut will serve as the basic model. In addressing individual resistance sources the review panel endorses the current program and procedures in breeding for resistance using the initial three sources of resistance and to the controlled distribution of B3F3 populations. Feedback of data on the performance of such trees across environments and their blight responses under natural infection conditions, where possible, will be essential.

Because of the limited size of the program, three sources of resistance were as much as could be handled in the past, but with the addition of a second breeder, the search for additional resistance sources should be actively pursued taking advantage of molecular marker information becoming available from the genetics program. Given the varied success of resistance breeding covering a wide range of plant species over the past century, it is likely that no single resistance source will provide the long term durability of resistance needed for the present program aimed at the re-establishment of the American Chestnut to its former geographical range.

In order to maintain a high level of genetic variation in an obligate outbreeding species, particular attention has been given to a strategy of crossing to an array of different local surviving American Chestnut trees in order to preserve genetic variation and avoid inbreeding depression. Considerable attention has also been given to the strategy of detailed partial diallelic intercrossing of the B3F1 and B3F2 plants that will produce the populations of adequate size and genetic variability for future distribution. This strategy could perhaps be simplified by a factorial crossing strategy that targets the maintenance of all genes in the population rather than all genotypes (see later figure). In this case all trees are involved in crosses but not in all combinations. A factorial design is logistically more efficient in that each tree is used only as a female, or as a male, parent.

Pollen of plants developed in the core program has been provided to Chapters for crossing with locally surviving trees in the relevant regions. Eventually comparisons of genotypes developed in the different regions should provide information on both local and more general adaptation.

The Review Panel endorses the continuation of backcrossing to the B6 generation for at least 1 or 2 resistance sources. However as molecular marker data become available

increased emphasis should not only be given to the possibility of using closely linked markers for the resistance genes, but also to the identification of genome-wide "American" Chestnut alleles for selection of the American genetic background (or for selection against Chinese or other genetic backgrounds). As always in the case of linked, rather than 'perfect' markers, field testing is essential to ensure that resistance is present and adequately expressed. It is therefore important that molecular testing strategies be such that data will be accumulated to demonstrate future efficiencies in the discovery and introduction of further resistance sources to the program.

Selection for blight resistance:
The blight response testing strategy is based on the concurrent use of a highly aggressive strain and a less aggressive strain. Differences in the responses allow the determination of different levels of expression. The efficiency of the procedure will be fully assessed only when populations can be grown under "natural' conditions. As this may take several years it is important that selected materials be distributed as soon as possible as the findings will serve as a basis for the future. Widespread testing will provide data on the stability of resistance across environments and also the possibility of pathogenic races in the pathogen.

While hypovirulence caused by infection of the pathogen by viruses leads to reduced aggressiveness by infected pathogen isolates, practical use of the phenomenon currently appears to be limited to horticultural and garden situations where hypovirulent cultures can be used to inoculate existing cankers. There is no evidence for preferential spread of hypovirulent strains, or to widespread infection of existing cankers by hypovirulent types, leading to reduced disease levels and increased survival of individual trees in forest situations.

Transgenics:
In view of the long-term objectives of the program to re-instate the American Chestnut to forests, a watching brief needs to be given to the potential of transgenics. This potential has many dimensions including the cloning and insertion or resistance genes from both related and more distant species, and the up-regulation of degrading enzymes, such as chitinases, and defense-related proteins already present in host plants, but not expressed at sufficient levels to give protection against invading pathogens. In any case such initiatives may not give complete protection and may need to be used in conjunction with partial resistances coming from the conventional breeding program.

Genetics

Molecular genetics knowledge of the Chestnut has greatly increased in the last few years. The development of RAPD and AFLP maps has provided information on the various linkage groups. The development and mapping of more user-friendly markers such as SSRs (Simple Sequence Repeats) is underway and will be important to the program. The identification of six BACs (Bacterial Artificial Chromosomes) with one marker sequence known to be linked to disease resistance and two BACs with another marker sequence also linked to resistance is an important development. The new NSF grant will provide many advances for Chestnut molecular genetics and, with guidance from key TACF staff,

should provide several opportunities to advance the breeding program. The NSF program should provide information on gene expression that would not otherwise be a part of the TACF activities. Special attention should be paid to the literature of species expected to be highly co-linear with the Chestnut; such publications can inform the chestnut program in many different ways. The molecular genetics program at TACF should have objectives that will advance the breeding program.

Mapping:
Applications of the molecular genetics tools to breeding should include the mapping of disease resistance genes in the current and future materials. This activity should result in the tagging of disease resistance loci with flanking markers allowing selection at the seedling stage and information on whether disease resistance genes from various sources are different. Such gene tags should allow the selection of recombination events. Some of the original mapping populations should be maintained since data for additional traits may be obtained later and entered into the database for QTL analysis. The advanced backcross materials available also provide powerful mapping opportunities. Using backcross lines, only a few plants are required to detect linkage of polymorphic markers to the genes for the trait under selection. The concept is that the allele of a marker uniquely present in the donor parent will only be present in backcross lines if it is linked to a trait under selection; the number of backcrosses and the number of independent backcross lines affect the probability of linkage and the numbers required. The results are more complicated, however, when several traits are under selection.

Molecular genetic marker uses:
Molecular markers also should be employed to detect the presence of outcrosses. Such early detection will reduce the amount of confusing results, and in the long run save space in the nursery. Outcrossing in controlled crosses for genetic studies can cause major problems. Outcrossing in the breeding program may or may not be of major importance. However, the elimination of outcrosses is a form of quality control that can only be helpful.

Recovery of the genotype of the recurrent parent (American) can be accelerated by the use of molecular genetic markers. This approach coupled with the backcrossing breeding scheme may give materials that are essentially the equivalent of one or two generations beyond normal expectations via standard breeding. Every generation saved in Chestnut tree breeding represents major savings in time and in accomplishing the ultimate objectives.

Fingerprinting the breeding materials can be important in the description and identification of lines. Such information also will be important in any form of intellectual property protection that TACF decides to pursue. This application of molecular markers requires an understanding of the allelic variation present in Chestnut populations and the selection of a set of clear markers (perhaps 20) that are unique to these Chinese and American materials.

Chromosome translocation tests:
The molecular genetic map derived from Chinese x American crosses indicates the presence of 11 instead of 12 linkage groups. The B and E linkage groups appear to be combined to form a B/E linkage group. The AFLP mapping results of this Chinese x American cross, involving Mahogany as a resistance source, gave evidence for heterozygosity of a chromosome translocation between linkage groups B and E. These linkage groups, known to be independent from earlier studies with intraspecific crosses, formed one linkage group in the Chinese x American interspecific cross. This is not unexpected since these materials represent different species that have become independently diversified through evolution. The new B/E linkage group is quite large and, therefore, could have a major effect on genetic segregation in the progeny.

In addition to the finding of a B/E linkage group, segregation distortion was observed. About 30% of the loci in the above cross showed segregation distortions, and a large portion involved loci on the B/E linkage group. At least one "aneuploid" plant has been observed based on the presence of more than two alleles per locus. These results would be expected if the translocation had a break near the end of a chromosome. The greatest distortion should be near the breakpoints and the most prevalent allele depends on which side of the breakpoints that the locus resides. Analysis of the distortion data may give an indication of the position of the breakpoints.

The third point of interest is that disease QTLs (genes) have been mapped in this cross to linkage groups B, E, and F. The genes on the B/E linkage group are about 25 map units apart. Unusual genetic behavior of the key chromosomes with disease resistance genes may result in unexpected breeding results including difficulty in obtaining highly resistant progeny. Translocation heterozygosity can lead to reduced recombination depending on the chromosome pairing relationships, the transmission of duplicate-deficient gametes and/or the frequency of alternate disjunction in the translocation heterozygote. In addition, if one or more genes for the selected American traits resides on the B and or E linkage groups, then reduced recombination may make it difficult to obtain both disease resistance and the American type. The use of molecular markers to detect appropriate recombination events may be important in such a circumstance. Some of the selected American traits such as pubescence is not on the B/E linkage groups but genes for other American features probably reside on this chromosome making selection of resistance in the progeny more difficult.

Cytology of meiosis in the F1 should reveal the presence of the translocation(s) by exhibiting a ring-of-four or, perhaps more likely in this case, a chain-of-four chromosomes plus 10 chromosome pairs. Pollen sterility also should exist in the heterozygote (F1 or members of later generations) of between 25 and 50%. If duplicate-deficient gametes are produced, one might also observe smaller pollen but well-filled with starch.

Cytological and pollen sterility studies should be done on all new sources of resistance. A cross free of a translocation(s) would be desirable. A written plan would be useful describing the crosses of new resistance sources to be made and the expected results

under various hypotheses. Mapping of the resistance genes in the new sources should be initiated.

Adaptation and Environmental Variation:
High levels of genetic variation among populations are typical of forest tree species that have broad geographic distributions, particularly when their natural ranges occur over steep environmental gradients. Because much of this variation is adaptive, it is critical to understand its distribution and patterns, which should then play a key role in selection and breeding programs and deployment strategies. Phenological traits, such as the date of bud set and the onset of dormancy, are good examples because they are under strong genetic control and can be closely related to adaptation to cold tolerance.

Long-distance movement of trees, particularly from south to north, may place the trees at risk of damage by cold temperatures in late spring or early fall. In reverse, movement from north to south may reduce height growth because trees cease to grow earlier in the season than locally adapted materials.

Although most have been lost to blight, numerous naturally regenerated American Chestnut remnant trees remain in forested areas throughout the original species distribution. These trees can provide the seeds needed for studies of inherent variation across the species range. Such studies can be conducted as *common garden studies* on seedlings over a period of only a few years once the seeds have been collected. Dates of bud break, bud set, and the onset of dormancy, height and diameter growth, and other potentially differentiating traits can be assessed and compared among samples from different populations under relatively uniform environments, such as forest nurseries or farm fields. Such juvenile tests may not have high accuracy in predicting all of the fastest growing trees over a rotation, but they are excellent tools for detecting and differentiating among populations that are genetically different from one another.

This information should then be used to group trees in the breeding program and also in distributing and deploying the material. Ultimately the question that needs to be addressed is how far genetic material can be moved safely, that is, without diminishing adaptation and/or growth and survival potential. This would be an excellent opportunity for a graduate student project.

Testing for Field Resistance:
The inoculation procedure for testing for resistance to blight seems to be working well, ensuring the infection of virtually all trees included in the tests. However, experience with other species suggests that field performance may be considerably different from performance in test environments, potentially better, potentially worse.

We favor the establishment of field tests of the same materials as are included in the inoculation trials. Ideally these materials would be the same genotypes, produced through cloning of embryos or with tissue cultured plantlets, rooted cuttings or grafts as cloning would allow the parallel testing of the same genotypes that are included in the

inoculation trials. We understand that cloning Chestnut is apparently quite difficult, so field testing of the genotypes from the same families is the next best alternative. Family testing would provide information on overall performance of the family as a whole and may be quantified in percentages, for example.

Knowing the levels of field resistance will help the group to provide information to users on real expectations for relatively long-term survival for each generation of selection and breeding. These materials can also serve as archives for genetic variants that may be of use in future studies. Field resistance studies should be well-designed, and replicated in time and space. These tests will also provide opportunities to select individuals for other specific traits that may confer resistance – perhaps some bark characteristics or branching traits.

Engaging Members:
Although we do not believe the genetic materials generated to date are ready to be released to the general public, or even to members broadly, members are likely to be excited about cooperating with TACF to help test their advanced materials for field resistance. The program could be structured similarly to "rose trials" where members are asked to establish seedlings under specified conditions and to collect information for submiission to the foundation. This is also similar to the use of Beta Sites in the software industry where co-operators are asked to test the technology and report on problems and/or their satisfaction with the product. Tested materials should be given names that indicate their "experimental" or temporary status until they have been proven to be resistant at high levels and under high risk conditions for infection.

The Beta Site approach has the advantage of engaging members in hands-on work (thereby helping to maintain their enthusiasm) and provides them with materials sooner than if they had to wait for proven resistant genotypes. It also has the advantage of distributing field resistance tests over a broader area for relatively low cost.

The primary challenge with this approach is to maintain contact with the co-operating members and ensure their submission of good data to the project. This may take considerable effort by a staff member and/or by technically competent chapter members.

Establishment:
Although some work has begun to address the challenge of establishment of Chestnuts in prepared areas or forests (two papers in the 2004 meeting proceedings are noted), success is likely to prove very challenging. Nuts will be subject to predation and both nuts and seedlings will suffer damage or death from a variety of naturally occurring and introduced pathogens and insects. Effects of vegetative competition are likely to be severe. Members and supporters of TACF need to develop a realistic appreciation of the practical challenges involved in establishment and recruitment of young trees. Additional applied research on techniques to facilitate establishment under the conditions likely to be encountered in the reintroduction process is greatly needed.

Efficiency

Mating Designs:

The mating design in the Chestnut breeding program is used primarily to produce families from which to select individuals for forward selection. The largest number of independent families that can be generated from X number of individuals is X/2, which is most efficiently accomplished by single pair matings. So 20 parent trees are crossed 1 on 1 in 10 crosses to produce 10 new families, none of which are related to each other unless their parents were related. However, with the low numbers of seeds produced per cross, it might be wise to mate each candidate with more than one other selection. Regardless of how many crosses and combinations are made, the maximum number of unrelated crosses of the 20 parents is still 10, if the starting number is 20 unrelated parent trees.

The most efficient mating design from a logistical standpoint is the SNIF design (Small, Non-overlapping Independent Factorials) using the smallest groups (2 X 2). See example below and comparison with disconnected half-diallels.

Disconnected Half-Diallels

	1	2	3	4	5	6	7	8
1		X	X	X				
2			X	X				
3				X				
4								
5						X	X	X
6							X	X
7								X
8								

Small Disconnected Factorials

	1	2	3	4	5	6	7	8
1								
3	X	X						
4	X	X						
5								
6								
7					X	X		
8					X	X		

For 8 parents, the diallels require 12 crosses to generate 4 independent families and 4 of the trees are used as both males and females, which entails extra field visits to the trees.

For the same number of parents, the factorials require only 8 crosses, results in the same number of independent families (4), but each tree is used as only a female or a male and is crossed with only 2 other parent trees. This arrangement decreases the number of visits to half of the parent trees, reduces the number of pollen lots that must be tracked and reduces the opportunities for error in labeling since each tree is used in a maximum of two crosses. For these reasons, the factorial design is highly superior to the diallel.

Clonal Propagation:

Selective use of clonally propagated Chestnut materials would facilitate achievement of some research objectives and increase efficiency of effort and land use. Although tissue techniques for multiplication of chestnut are not now available, their development by external groups with expertise in that field should be encouraged. In the interim, grafting is possible. Examination of the variation in responses of multiple individuals of the same genotype to inoculation with one or more isolates at one location would be possible. Alternatively, responses of single genotypes in different regions would allow evaluation of genotype by environment interactions (local adaptation and response to local pathogen pressure or "field resistance"). Responses of various genotypes to cultural practices and conditions in establishment would also be facilitated. Most immediately, however, possible development of grafted seed orchards is again suggested. This could allow for consolidation of selections, resulting in more efficient land use and a reduction in the rate of increase of maintenance inputs including labor.

Inoculation:

Effectiveness of any disease resistance screening method is measured by achievement of the differential response between resistant and susceptible plants. The currently used procedure involves wounding and inoculation of field-grown trees with agar plugs bearing mycelia of the pathogen (repeated on the same tree for each of two strains of varying aggressiveness) and appears to be successful. However, this method is very aggressive, tipping the balance heavily in favor of the pathogen and disease development, and often resulting in death. Events that might occur during natural (albeit through small wounds) infection are by-passed and mechanisms that might operate at more natural levels of "inoculum potential" may be overwhelmed.

Given the identification of individuals and families with a range in both response to inoculation and field performance over at least several years, examination of responses of such material to more natural/subtle inoculation methods should be pursued, using field tests, as recommended above, or by monitoring infection and survival in operational plantings. Results might allow more rapid identification of younger material for further breeding. Responses might also reveal more information about speed or variability in successful response of trees to a broader range of pathogen strains, and better reflect field resistance under natural infection and realistic inoculum pressure.

Characterization of pathogen populations:
Performance of material that is ultimately deployed will be influenced by the population of the pathogen encountered. Effective resistance at one location may not be expressed when other populations of the pathogen are encountered at another. Also, superficial canker development and survival of trees at some locations may be a function of hypovirulence in the Chestnut blight fungus population at a given site. Provision of host material with a degree of resistance (that supports hypovirulence effectively) may very well be the key to a greater contribution of hypovirulence as a natural and perhaps long-lasting means of suppression of damage. Thus, consideration should be given to providing or encouraging support for appropriate investigators to characterize pathogen populations both across the range of Chestnut and particularly in areas of deployment of materials from the TACF breeding program.

Scientific Communication

Database:
The Panel support the establishment and maintenance of a web-based database. We are concerned that current staff will not have time or resources to do this work and suggest that a second half-time position be added. The primary responsibility of that half-time person would be to build and maintain the web-based database and ensure its user-friendliness.

Scientific Meetings and Symposia:
While enthusiasm for Chestnut remains high, this is an opportune time to showcase the TACF program. One possibility that would cost little, but provide great exposure is a 90- or 180-minute symposium on Chestnut at AAAS meetings. As a large cadre of reporters generally attends the meetings, a line-up of 3-6 good presentations on the history of Chestnut and the genetic work that has been conducted to date, would well serve the objective of excellent public relations and public education. Other ideas for dissemination of information and good exposure are to "piggy-back" with other forest science or forest genetics and tree improvement meetings, including the Southern Forest Tree Improvement Committee meetings, National Science Foundation, North American Forest Biology Workshop, and SAF Conventions.

Working meetings:
In addition to the scientific meetings suggested above, it might be useful to hold an annual meeting that would include all of the hands-on people employed by or involved with TACF and the state chapters. Logistical and operational issues would be the focus of the meeting. This will help to maintain relative uniformity of test establishment, data collection and entry, and general operations.

Resources

Land:

Land must become increasingly available to advance the breeding program and further develop seed orchards. Although some increased efficiency in current land use is possible, additional land of appropriate qualities for good Chestnut establishment and growth appears to be needed. Whether this can be obtained by land swap, long-term lease, or purchase, it should be acquired soon. This land needs to be as convenient to existing farms as possible to allow efficient use of equipment and personnel.

Personnel:

The current research team consists of very capable personnel who are both passionate and serious about their role in achieving the ultimate goal of TACF. Other personnel will be required to exploit progress in internal and external research. Collaboration with external groups might be most appropriate to achieve a specific objective for which specialized expertise and equipment are required and available in an external program (e.g., cytological research). Routine utilization of molecular marker technology, however, could be accomplished on site by TACF personnel. Addition of a skilled laboratory technician with experience in molecular methodology and designation of the appropriate team member as their supervisor is recommended.

Equipment:

A significant improvement in the rudimentary laboratory space and equipment would also greatly enhance the activities of the research team, and they should be allowed to develop a lab modernization and equipment plan. The laboratory, not for basic research, should allow exploitation of results from molecular studies as the latter provide information and tools useful to the breeding program. Though it need not be large or elaborate, a clean, well-lighted, adequately powered, climate controlled space is required. Equipment should include a laminar flow hood, incubator(s)/freezers, and modern dissecting and compound microscopes. To exploit the benefits of molecular marker analysis, equipment and space for DNA extraction, and polymerase chain reaction amplification, and gel documentation are required. Laboratory space for pollen work is required, and provisions might be necessary for separation of this activity from other laboratory space to avoid DNA contamination.

Although other major equipment needs appear to be met, continued progress in provision of space for equipment storage is necessary. In addition, provision of an additional bucket truck should be considered. Whether by purchase, long-term lease, or recurring seasonal lease, availability of this equipment would increase efficiency personnel at critical times in the breeding program which is core to the goal of the foundation.

Germplasm release and commercialization

While not part of the formal presentation, the Review Panel saw it imperative that TACF develop a deployment policy on release and distribution of blight resistant Chestnut material. The panel was later given a policy statement that addressed many of its concerns; we fully endorse the six guidelines outlined in the document.

The questions of adherence to such guidelines, ownership of germplasm, and benefit sharing between TACF and the Chapters must be more clearly resolved. A simple, perhaps sequential population/cultivar naming system should be adopted. Naming systems that attempt to identify pedigree histories and other details quickly become abbreviated and confused.