DRAFT – 3/21/12
Seed Orchard Questions

I. Pre-Seed Orchard:
Making B3 or B4 selections and crosses

There are some common questions we hear about breeding orchard activities that lead up to seed orchard installation.

1. **How many trees should there be in a backcross line to expect a good selection?**
   The goal is to create 100 backcross nuts per line (Hebard, 1994). Assuming a 3-gene inheritance model, the absolute minimum, statistically, to get one selection is roughly 8 (based on a 12.5% probability of producing a fully heterozygous individual) (Klug et al, 2006). But this is for resistance only (no environmental interaction, etc) and in the field things never work out as neatly as they do on paper. Realistically, you want to have about 30 or 40 at a minimum, but 50 or 60 is even better. Some should still show good resistance. Generally we end up inoculating about 50% of what was originally planted. Having more trees to inoculate and select for resistance just gives you more trees from which to select for American character - the more trees, the better.

   a. **What if there are fewer trees? Is it relative? How should this be implemented?**
      It is relative (Hebard 1994, 2005). The goal is to have 20 lines, but is that absolutely necessary? Probably not. If the chapter has 15 good lines from each of which you have one good selection, you should be fine but this is a "best guess" and we need to investigate further. There are, however, several factors that need to be weighed, not just how many lines. You also need to see how much time is involved between all the lines you made. You don't want to have your first and last lines be 20 years apart. So overshoot the 20, make some backups, and expect some failures, but don't fret too much about it.

      Follow-Up:
      20 lines is the goal but what if you do have fewer lines - what are the concerns as the number of lines decreases? Is there an absolute minimum that should be adhered to?

2. **What if there are more than 20 backcross lines to pick from?**
   Great! Good Job. Yes, include the lines in the seed orchard design. There will be many family differences, but by design a seed orchard should maximize the intercrossing through open pollination. Ideally every family would be represented - this will maximize diversity.

   a. **Should we include extra lines/plots in the seed orchard?**
      Yes, include the lines in the seed orchard design (as above).

   b. **What if we have more than 20 lines/families, should we use all of them or aim for a specific number, and if so, how do we pick the ones to use?**
      You want to rank on resistance and American character. Certainly if a family gives markedly worse progeny than other families, then all the progeny from that family should be discarded. But if it has some individuals that appear to have levels of resistance similar to selections in other families, then those selections should be retained, or a suitable number

with the most American morphological traits. For instance, unless all the trees, including F1 controls, in an orchard only had a blight resistance rating of "4," then you should try to pick the best trees. But if only the F1s had ratings of "3," you should discard all the progeny in that orchard. This could be quantified by measuring canker lengths, but we generally don't have the capacity to do that.

c. How do we make the cut-off or determination?

Prioritize based on resistance first, then form and American character. If that isn't enough look at the general health and vigor of the tree. Remember too that backup selections can be cut and allowed to re-sprout (see Section II: Seed Orchard Planning).

3. How many selections do we keep per backcross line? Is it two? Is it four?

At Meadowview we generally try to keep the same number of selections for each line in an orchard if B3-F2s are being produced by open pollination, which is not always possible. Usually 3-4. It's usually limited by the number of trees per line.

Follow-Up:

How many selections should we keep per backcross line per orchard? And how many should we keep across orchards?

4. When choosing the number of backcross selections, does it matter if there are two lines or 10 lines in the breeding orchard?

It doesn't matter for controlled crosses, other than fewer lines makes controlled crossing more difficult as it narrows your choices. Fewer lines than 4-5 also lessens yield per orchard with open pollination. We usually prefer 4-5 lines per breeding orchard for open pollination. More lines can lead to more inbreeding IF only one male dominates, but that is very unlikely, especially with more lines.

Follow-Up:

But what if you only have one or two lines in a breeding orchard? Do the same recommendations apply?

a. Does it depend on whether open or controlled pollination is planned?

Yes. If you're doing controlled, you can do different plots w/ the different selections. If you're doing open, it has little effect on inbreeding (Hebard, 2002 (a) - discussion of Figure 1). The only caveat is the loss of yield per orchard.

Follow-Up:

How many parents do we ideally want to put together? This is a difficult and will likely require analyzing several specific scenarios to address the best approach for a given circumstance.

5. Should we plan for open or controlled pollination in the breeding orchards?

Open pollination, preferably. However this may not be possible in every orchard. Even when open pollination is possible one could bring in additional selected pollens to mix up diversity, however this does significantly increase the amount of effort required to produce B3F2s.

a. Are there times when one is more appropriate than the other?

Open is always best because of the increased yield and reduced labor, but if sources of resistance are mixed in the orchard then controlled pollination is probably preferable to emasculating trees in one of the sources. Controlled would also be an option if there were non-selected flowering trees in the orchard. Controlled pollinations permit one to capture all the genetics of each parent with five progeny per line rather than nine with open, but we feel the need for increased yield and less labor now outweigh the size advantage. Additionally, more is always better here, in reducing the risk of catastrophic failure.

Some orchards contain trees, beyond breeding selections, that should not be removed. Making controlled crosses allows for more than just breeding selections to remain in place. But this can require more effort, and reduce the amount of crossing between lines.

Follow-up:

Doesn't this also depend on the number of lines in an orchard, as well as the number of selections/line? What are the most appropriate options for some of the scenarios our Chapter orchards frequently face? And if we use a combination of approaches, how do we make and track those decisions? Would a matrix be helpful? Perhaps an explanation of the statistics of open-pollination would be useful here (any sources for that?).

b. What about crosses that can't be made because selections are not in the same orchard?

Controlled pollination would allow for the inclusion of pollen from flowering lines in different locales, which may be necessary. You want an even number of parents contributing to producing an even number of progeny so you should select whatever method that will help you accomplish that goal. If controlled pollination is used, we do recommend a circular mating design (referenced below in Section I: 6a)

Follow-up:

But what about the increased mixing of genes provided by a pollen mix? Is this not a concern? Wouldn't this be a way to better mimic the situation at Meadowview?

c. Are controlled crosses preferred if they are possible (i.e. the Chapter can "afford" to make them)?

Not really. It will depend more on how many lines and how many selections w/in a line are at any given orchard. A higher amount of production is needed to complete seed orchards in a reasonable amount of time. Open pollination will give this, however it is not always possible. In Meadowview there are lines that are way behind because of lack of yield. Some of these lines may be dropped from the program for that reason.

d. Many orchards contain lines that were planted in different years and may not all begin flowering at the same time. Wouldn't controlled pollination allow you to take advantage of those selections that are flowering and maximize their use?

Again, something else to consider with all the rest of the factors described above. If it is just one line, yes. Otherwise emasculate the others. Some question has been raised about

unintentional selection for early flowering; however we have not seen evidence that this is a concern.

6. **How do we choose which controlled crosses to make?**
   a. **Should these be 1 x 1 line crosses?**
   We recommend a circular mating design: A x B, B x C, C x D…, U x A.

   Follow-up:
   But this is a logistical challenge for the Chapters – wouldn't a pollen mix help you to get to the same end with less effort? If so, it would be best to mix all selected females with all possible males, assuming you can get the right pollens from the right trees at the right time for pollination. The logistics are still not ideal but should be simpler than a circular mating design.

   b. **Can we maximize variability w/ pollen clouds?**
   You could but practically this method may not be feasible with chestnut. We don't know of any good way to generate pollen clouds. This has been done on a small basis in pine orchards, but pine produces a lot of pollen. It probably wouldn't be worthwhile until the Seed Orchard trees had lots of flowers.

7. **How should crosses be noted? We will know the mother and is it likely there are only a known number of possible fathers? Should this be tracked? How?**
   In control pollinations, crosses are indicated as Female ID x Male ID (ladies always first in such nomenclature). In open pollinations: Female ID x OP. If a pollen mix (also known as a polymix) is used in controlled pollinations, then the mix of fathers would be documented in your files, and have an ID assigned, and the cross indicated as Female ID x PM 246. If you can't say which specific males pollinated a tree, the cross needs to indicate an OP.

   Follow-Up:
   Pollen mixes still present an issue for tracking, analysis and logistics – we need to address this further.

II. Seed Orchard Planning:
Selecting a site and planning for planting

What are the considerations for selecting the best site for a seed orchard?
***Disclaimer*:** we have tried to include the best information available regarding land-use and cooperative agreements, as well as provide practical considerations for deeds and land ownership. That said, TACF Chapters should seek independent legal counsel before entering into any legal agreement.

1. **How secure should the land be?**
   The land needs to be secure enough that the trees will have sufficient time to grow as large as they need to for each tree to produce a substantial quantity of nuts. To do this, the land's "security" should include the protection of the trees for a determined number of years – we are currently estimating that seed orchards will require a 45 year commitment.

Any "Final Selection of Suitable Land" should involve some type of Land Use Agreement, Lease, or Contract that should spell out the specifics of timeframe of land use, ownership of genetic material (GPA), and requirements of landholder and TACF (more in Section II: 3). The process of where to locate a site with adequate long term capacity should be evaluated carefully and planned well into the future. Each Chapter should try to identify key partners or contacts that may be willing to cooperate or have the land base capacity for future seed orchard location.

2. Is there a preferred type of land ownership?

No. Private and public could both work, as long as the land stays protected. The expectation for a B3-F2 seed orchard is a 45-year commitment.

The ownership type will likely be a blend of both public interest and large landowner interest that has a history or background of working the land. The question may come down to ***who has the resources (operational or financial), personnel, and long term commitment*** to see this project through for at least 45 years initially, and maybe longer.

Land owned in perpetuity may be the most secure for such a long-term planting. This is most common for land trusts and other private land-holding conservation groups, educational institutions or for public lands. Land trusts and other conservation groups may be the most secure, especially if their reason for owning the land is to preserve it. Universities can be great partners, but land conservation is not their primary focus and it is best to explore the specific parcels being offered and weigh them against the likely need for a parking lot or dorm space (and/or the types of agreements they'd be willing to sign).

Private land should not be out of the question, but it is good to know what the plans for land-ownership are in the event something happens to the current owner. Private land with a conservation easement or restriction that allows for chestnut orchard activities would be a good fit, as long as the land the planting occurs on falls within the easement area. Private landowners would likely require financial assistance and technical assistance for the project to succeed long term.

3. What kind of land-use agreements should we look/ask for?

A separate type of agreement will definitely be needed with specifics clearly outlined so both parties will know what they are getting into and what type of activities they are obligated to perform or assist with. It's likely that an Orchard Manager will need to be assigned or designated to be the lead contact to coordinate and oversee any activities. An official signed agreement will be needed.

TACF's Germplasm Agreement (GPA) is a good place to start. The GPA is a type of Material Transfer Agreement (MTA), which is an agreement for transfer of proprietary material from one entity to another. In addition, there are some other types of agreements that are worth developing, depending on the situation:

There is a desire for TACF to develop standard language for including chestnut breeding/planting activities in a conservation easement or restriction.

i. **Chapter Orchard Agreement** – some chapters have developed an additional agreement that outlines their expectations for a hosted planting. This may cover the time frame, which party is

responsible for what in terms of financial support or labor, access rights, etc. It would be a good idea to have such an agreement looked over by a lawyer before asking anyone to sign it. If you don't know anyone who would do this for the chapter, contact your RSC or the TACF HQ for assistance. Your RSC should also be able to provide example agreements from other chapters.

ii. **Memorandum of Understanding (MOU)** – an MOU is often a good idea when partnering with another organization for hosting an orchard or planting. In general, an MOU includes a description of each organization and their mission and outlines each organization's responsibilities. Some organizations will be happy to sign an MOU for the entire time-frame of the planting, but in some cases, especially when dealing with Universities or State/Federal agencies, an MOU may be written to be reviewed and renewed on a regular interval, such as every 5 or 10 years. In these cases it is somewhat of a judgment call as to whether the project will be secure over the long term. **Follow-up**: Can we develop a standard TACF MOU?

iii. **Cooperative Research and Development Agreement (CRADA)** – a CRADA is a working agreement between two entities for a joint research and development effort. The agreement spells out the responsibilities of both entities and the proprietary rights of each entity to the results of the joint effort. This may be more appropriate than an MOU when partnering with a University of other research institution, especially if there is more to the arrangement than just hosting the planting.

iv. **Conservation Easement** – an easement is the right to use someone else's land, usually for a specific purpose. A conservation easement is typically given by a landowner to an individual, a land-protection organization or government agency. A conservation easement usually restricts certain land uses, such as development or commercial use, and once signed follows the land even if it changes hands. An easement might also include a clause for automatic termination if the easement is no longer being used for the stated purpose. For example, if the purpose was to provide a seed orchard site and the seed orchard is no longer in use, the easement could terminate. It is probably best for a conservation easement to be given by the landowner to a conservation organization, rather than TACF, but if requested TACF could provide appropriate language for ensuring that any existing or planned chestnut activities are covered under the conditions of the easement. **Follow-Up**: We can think about drafting a couple of standard or example easements – one for orchard work and one for active forest management/restoration planting…this is in the works.

v. **Conservation Restriction (CR)** – From John Mirick, lawyer and MA/RI Chapter Board member: "A CR is a restriction on the use of property, given by a landowner to a governmental or qualifying charitable entity. Most CRs are given in perpetuity. A CR spells out what is allowed and what is prohibited. The legal requirements vary a little from state to state, but generally there are four parties to a CR: the landowner who gives the CR, the recipient of the CR, the city/town in which the land is located, and the state in which the land is located. All parties have to agree that the CR is for an appropriate purpose, and have to agree on the language. Easements and CRs may be gifted, purchased or a combination of a gift and a purchase."

vi. **Lease Agreement** – A lease agreement is commonly used in agriculture and may be a good approach for TACF orchards. In the right situation land could be leased (ideally) for a nominal fee ($1/year) and extended period of time. Laws pertaining to lease agreements will vary by state and TACF is working to develop a set of considerations that can be used when drafting a

lease agreement for a seed orchard (or any TACF chestnut planting). It is recommended that chapters seek legal counsel in their state and use the considerations TACF will provide to develop a lease agreement that will be appropriate for a seed orchard.

4. Do we need a deeded easement?

Need may be a strong word, but it could be an option to consider. From John Mirick, lawyer and MA/RI Chapter Board member: "A deeded easement is a bit of a loose term because any good attorney is going to recommend/require that the easement be recorded so that it is a matter of public record, and any future owner of the land subject to the easement has formal notice of the easement. Sometimes, easements are reserved in deeds (such as a right of way to backland)."

TACF should first try to work with cooperative partners that have the land base and merely provide the intellectual capital and financial assistance for installation and periodic maintenance or scheduling of activities. This will be more cost efficient than tying up money for land. We can always re-evaluate the need at a later date if we have trouble finding suitable sites or willing partners.

5. Should we own the land?

TACF does not generally recommend owning land, but will not dictate what chapters choose to do. Purchasing land outright is a big expense, however accepting the donation of land may be worth careful consideration. Donated land should not be accepted just because it is offered, but if it would supply a useful site for long-term planting it is worth exploring the options.

There are liability issues with owning land – this is something each chapter should consider carefully before pursuing. The rules for landowner liability will vary by state. If land ownership is desired, a liability insurance policy is encouraged. This may cost a little on an annual basis but may be better than paying an attorney if a suit is ever filed.

If the land is being donated, putting a seed orchard on it would be an option. TACF ownership should not be sought out or required though. TACF ownership of the land would help with the long-term protection of the site, but would bring additional costs and liability issues. TACF is an organization based on cooperative efforts with other organizations/agencies/educational groups and this seems to have served the organization well in the past. This sort of cooperation with seed orchards should work as well.

In addition, the practical issue of owning land, especially as a non-profit, can be tricky in terms of long-term planning. What happens if somewhere down the road the chapter goes inactive (not an expectation, but a possibility)? What happens to the land then? Does it go to TACF? If the land is owned by another non-profit with a proven history, like a land trust, it may be used by the chapter without a need for a contingency plan for the land ownership.

Follow-up:

What ***would*** happen if a chapter owned land and then dissolved? What if a chapter, currently incorporated as separate 501(c)(3) organizations, dissolves to come under the umbrella of TACF – what would happen to any land owned?

6. **Are there other issues we should be aware of? What situations might get us into trouble down the road?**

   Watch out for agreements that require the chapter to pay for maintenance or restrict access, these could cause problems down the road, especially with leadership shifts over the long-term. No matter the type of land, some type of written agreement or long-term management plan should be developed to help deal with things like leadership turnover or land changing hands. In addition, investigate other easements or rights to the property (ex. power lines, mineral rights). A conservation easement would generally protect against this, but any other rights to the property should still be fully explored before beginning a seed orchard. The last one has the potential to have the biggest impact.

   Related to the scope/scale of a seed orchard project, also be mindful of trying to take on more than can realistically be done by unpaid volunteers. A lack of leadership or direction to guide future decisions or activities could be an issue.

7. **How long will it take to fill-in the seed orchard?**

   It really depends on how long it takes to make all your selections and get seed from them.

   PA-TACF started their orchard in 2002 and is only about ¼ of the way done because they had such a lag in B3 production between 1996 and 2000. They'll probably be done filling in the orchard by 2016.

   At Meadowview, the Duncan Farm is still not finished after 10 years. Early on the first lines will be highly represented. Also at Meadowview are two lines that are being control pollinated because the trees are located at a different farm. These lines are taking much longer and are severely behind in finishing the assigned blocks.

   a. **What are the implications of only adding 1-2 lines to the seed orchard (block) each year?**

      The main implication is stalling production of good, diverse B3F3s. But there's really nothing we can do about that, is there?

   b. **Will it cause problems if you are ready to screen lines in the ground before the orchard (block) is filled in?**

      No. Or, at least, it should not.

8. **How are other Chapters approaching seed orchards?**

   ME – Seed orchard sponsors. From Glen Rea, ME-TACF President: "We are looking for sponsors of the seed orchards and we will name the orchards for the sponsor. A five acre orchard will cost $10,000, and a 1 acre orchard will be $5,000, and this money will be used to pay for the maintenance of the orchards."
   MA/RI – List of bullet points for seed orchard land-use agreements, compiled by John Mirick (Board member and legal counsel), and plans to develop a draft agreement from those bullet points.
   PA – Penn State and private landowners
   IN – Purdue

There is a nice presentation and other seed orchard planning resources available at: http://sfr.psu.edu/public/chestnut/breeding/orchard-design

Follow-up:
Would a case study be a useful appendix? Besides costs, what else would you want to add to a case study? The whole process of what the chapter went through to get the orchard set up? Maps of the seed orchard layout?

9. **How can TACF facilitate the sharing of knowledge and information to educate and train Chapters as well as interested participants?**
   a. Regional Meetings with Updates and Planned Activities
   b. Bi-Yearly Teleconference for Field Volunteers
   c. Visit working seed orchards, such as Meadowview

   It might also be useful to keep a running list of where there are seed orchards so that chapters entering into the process will know who they could contact to bounce ideas off of and see what other chapters have done. This could also be set up as part of the Annual Meeting's Chapter Meeting.

10. **If not all seed orchard blocks are to be in one place (land availability issues are likely) what are acceptable ways of breaking out replicates?**
    a. **How small can a block be?**
       One acre is a good estimate when looking for sites. Each block should include a plot of 150 trees for each breeding line in a resistance source. In theory there should be 20 lines, meaning 20 plots. However in practice many chapters have bred additional back-up lines and may end up planting 23 plots, or 25 plots, depending on how many lines produce good selections. It may be possible to fit 20 plots in a space closer to 0.75 acres, but plan on a full acre for each block.

    b. **Are single block orchards ok? Would two blocks at a minimum be preferable?**
       Single-block orchards are often recommended because of the amount of work involved with roguing, at least using the "pluck with backhoe" method. **Really**, this varies based on cooperators and resources available. Single orchard manager should be able to take on one or two blocks. More blocks than that should only be attempted by cooperators that have a larger infrastructure in place to help manage the planting.

    c. **Are there other ways to break down the size of the seed orchard? Must all lines be represented in each block?**
       Every effort should be made to represent every line in each block in order to equalize their contributions to subsequent generations. This is because unequal representation of lines increases inbreeding. Despite your best efforts, however, some lines may never be fully represented. One should evaluate these incomplete (see Section I: 1 for more information regarding the number of lines needed.)

       The lay-out of each block should be consistent and blocks next to each other should not have plots of the same line adjacent to one another (Hebard 2002b).

d. Is it better to split up blocks for security reasons? Or to increase partnerships?

Splitting up the blocks does allow for more partnerships and preservation of material, should something like a natural disaster, pest infestation or land use change impact the trees in a given orchard. That said, finding partners willing to care for a few blocks will significantly reduce work. A private landowner may be able to take on one or two blocks and a large, professional cooperator may be able to take of four or five blocks, but more than that may be too much to manage (Section II: 10b). It is not recommended to lump more than half of the blocks in one place. Therefore, both approaches may be needed. This just gives a little more protection against unknown forces or factors that you can't control. Having similar material at two different locations may help to mange risks.

11. Is it possible to re-use breeding orchards for seed orchard blocks?

This is not recommended. It is possible to re-use a breeding orchard for a seed orchard, but it is much simpler and more straight-forward to find a fresh planting site for the seed orchard plantings.

a. What are the concerns?

The main concern is that there will be B3 or B4 selections remaining in the breeding orchard that will need to be worked around. While it is not imperative to keep these selections once enough seed has been harvested to fill the seed orchard, it is recommended to keep these trees if possible. If these selected trees are removed their germplasm may be lost. Such selections could be cut and allowed to re-sprout, or cloned and moved to a gene bank in another location, but this still adds a level of logistical complexity that is avoided with a fresh site. Another issue to watch out for is out-crossing. Any flowering trees remaining in the orchard could provide an unwanted pollen source. This is also a concern for adjacent orchards on the same site.

b. How can we address them? What would be needed to make this work?

The main goal of the breeding orchards is to produce B3F2 or B4F2 seed, so careful consideration of how close that goal is to complete is a good first step. It may be possible to plant "around" selected B3 or B4 trees during the early years of overlap. Once trees from both breeding generations are flowering, emasculation of the less desired generation is an option, though this can become a large task very quickly. Controlled pollinations are also an option, but this approach significantly increases the workload. Selected B3 or B4 trees may also be pruned at ground level and allowed to re-sprout - these should be clearly marked.

12. Is there a preference for a particular land-use when selecting a seed orchard site?

As always, proper site selection is the most important consideration when planting chestnut trees. A seed orchard site needs to be appropriate for growing chestnut. A seed orchard is planted on close spacing, so the trees are stressed, and they are inoculated when they are small, which introduces even more stress. In addition, the seed orchard's ultimate goal is to become a production orchard. So selecting a site where chestnuts will thrive is important. Do some research and ask questions about past land-use history. A small test planting a year or two before orchard installation is strongly recommended.

One important consideration in site selection is to have level land that bucket trucks can get over. Of course, this isn't always possible, and level land often is the least conducive to chestnut growth.

a. Should we look for certain types of vegetation?

Depending on current land use, look for species indicative of good chestnut sites – chestnuts themselves, or species that like acidic soils and/or do not tolerate wet sites. Stay away from wet-site indicators (sensitive fern for example). Some good indicator species include blueberry, mountain laurel, rhododendrons, white pine, white oak and of course, American chestnut sprouts.

b. Should we focus on fields/agricultural land?

There are pros and cons for both field and forested sites, therefore one is not recommended over the other. Just be aware of the main considerations for each type of site.

In a field site: access is often easy so large equipment like tractors or water tanks can get on-site. The biggest issue with a field site is the existing vegetation. Solid weed management strategies need to be in place, prior to and after planting. Also, a field site will not likely have the beneficial mycorrhizae found to help chestnuts get established. Including some forest soil at planting time may help in this regard.

On a forest site: access may be more difficult. If clearing was done recently there may be stump or rock removal needed as well. Remember, the spacing is tight in a seed orchard so on a forest site good site prep will be important. If access is difficult, strategies for getting water to the site or other equipment needed for future activities, like culling, should be considered.

Further considerations: For either type of site, if deer are an issue in the area fencing is strongly encouraged. The feasibility of fencing on a given site should be considered in the site selection process. Past land use and site history may be important variables for consideration as well. Suitable land that is already open or cleared is going to be the less costly option for a site that can be prepared more easily than taking a vegetated site.

c. Is it important to try to use different types of sites to test trees' response to different site conditions?

At this stage of breeding you want to make sure that the main focus of site selection is that the trees will be able to do well – the seed orchard is for production. Testing of response to different site conditions will be better accomplished at the B3F3 level. Due to the nature of how sites are generally found/offered to the chapters, it is likely that a variety will be used as they become available. Choosing different regions to focus on may be of interest as well. Important site selection characteristics are soil productivity, good terrain features that would allow equipment to operate easily, and good accessibility from a primary/secondary road. A site that is close to where equipment and supplies can be located or utilized would be better than a really remote site.

There is some selection for adaptation to a site. However, a main objective is to get as much genetic diversity into the B3-F3s as possible so that selection for adaptation to various sites can occur, and without undue depletion of the variation. It may be presumptuous of us to think we can specify the parameters of site type sufficiently to maximize diversity for site adaptation at B3-F2.

13. Should there be one seed orchard per Chapter (and per source of resistance)?

Regarding one seed orchard per Chapter – there doesn't seem to be a need for this, though this may ultimately be the wish of certain chapters to keep their local seed close at hand. This may also be a way for certain chapters to keep donors or valuable members closely involved with the organization. At first there should be one seed orchard per chapter. This will change with future breeding.

In the southern chapters there are trees with different types of chloroplast. In the northern states the tested trees all have the same chloroplast. So using the chloroplast type is the proposal for creating seed orchards in the South.

As for mixing sources of resistance, we do not want different sources mixed in a seed orchard because we want to get these trees homozygous for their source of blight resistance, as best as possible. Mixing is encouraged at the B3-F3 level and strongly discouraged at B3-F2.. We also do not want chapter selections mixed because we are trying as best as possible to capture the local adaptation in the chapters; outcrossing will destroy that adaptation for at least one generation.

14. What about regional seed orchards? Or provenance seed orchards?

Provenance seed orchards delimited by physiographic region rather than political boundaries are being considered by some chapters.

State boundaries are poor representations of regional adaptability. Physiographic boundaries would better represent this adaptability, depending on what scale it was utilized. For example, some states such as Virginia have already designated certain areas "geographically separate" from other areas in the state and are using a different source of resistance for their breeding orchards in these areas. However, if one of these areas overlaps with a similar area in another state, there is no reason why these two could not be combined together. (If this idea of physiographic boundaries is used at a scale involving more than one state, it would be important that these boundaries should be determined by TACF or a qualified scientist and agreed upon by all chapters involved.)

For some areas, a physiographically-based seed orchard will equal a seed orchard shared by more than one state. During seed production, this could be very beneficial to those state chapters as it would minimize the amount of work each chapter would have to do over many smaller orchards, and concentrate all that work into one larger orchard (Section V: Seed Orchard Production). However, an orchard shared between different states might be difficult if these different chapters want their own seed orchard.

Also, note that logistical and political issues can arise with shared seed orchards (see Section II: 16, below)).

15. Is it more preferable to group lines by physiographic region? This is well-supported as a qualifier for regional adaptability for other species. Is this the case for chestnut?

It is not necessarily preferable to group lines by physiographic region, but can be done. Before doing this, though, Chapters will need to fully evaluate not only the scientific but also the practical logistics of doing such grouping.

The scientific literature does support regional adaptability for other species that includes Northern red oak, white pine, loblolly pine, shortleaf pine, black walnut. Perhaps there should be a

recommendation to the Science Cabinet to form a committee that looks further on this issue or draw conclusions from other hardwood species to make some general inferences or recommendations.

If a regional seed orchard is to work with chapter involvement, great care will need to be given to ensure that all chapters feel involved and important to the orchard work. If the orchards are regional based on physiographic boundaries, this may allow more than one seed orchard to overlap with a chapter.

16. **What are the mechanics of planning regional seed orchards? How could synchrony issues work between states at different stages of backcross breeding? How could responsibilities be fairly divided?**

    Responsibilities will include: maintenance, pollination, harvest, tracking of seed production and seed allocation, monitoring of orchard for health, supporting the relationship between TACF and the orchard landowner, public events/outreach, volunteer support and continued education. Chapters or volunteers closer to the orchard should be able to help more with the daily grind of the orchard. States involved with the orchard should come together for pollination and harvest. All should work towards fundraising and education within their own region and bring focus on the orchard/program. For fundraising events and/or educational events, the different chapters will need to consciously work together to ensure best results. No matter the location, it will need to be understood that every chapter involved in the regional orchard has an equal stake in its health and production, and a say in its maintenance and activities.

    For timing of these orchards, different states will most likely have seed available for planting in the orchard at different intervals. Equal space should be left for each state's seed allotment in the orchard. If this is not enough room for all the states, or if some states find that they have more seed than they have room for, at this time further seed orchards should be created and/or expanded upon. This will be the next question – once these orchards are filled, do chapters move on with local orchards, or create more regional/physiographical seed orchards? Any effort has to be planned well and have a project leader or Orchard Manager that keeps everyone informed and working together. When the time comes, there may be the need to have Chapter functions or events that work toward accomplishing key activities (planting, maintenance, pollination, harvest). Fundraising is going to be a big part of supporting the installation or periodic costs involved with keeping any orchard, particularly for private landowners.

17. **Should these seed orchards continue to be split between the various sources of resistance?**

    Yes. Intercrossing will happen in restoration plantings. However crossing sources in a controlled environment like Meadowview would be a good way to find out. (This has to be happening in Meadowview now, with open pollination, but a controlled test is needed - auxiliary plantings of source x source B3F2s can, and probably should, be tried.)

    a. **What about pyramiding resistance? If resistance loci may be variable would this be helpful? Is there evidence one way or another?**
    b. **Should we start having auxiliary orchards where the sources are planted together?**
    c. **What about planning for potential variations in thought on how resistance is controlled?**

III. Seed Orchard Installation:

Planting the seed orchard

Most chapters are experts at planting breeding orchards by the time they get to seed orchards. But are there any important differences to consider?

1. **Once you get the seed, in what arrangement should the lines be planted within a plot?**

   It's been done randomly at PSU to maximize diversity amongst our control pollinated lines. In Meadowview the plots are 38' x 38'. The tree takes up 29' x 28' of that and the rest is space for equipment (Hebard 2002 a and b). My answer to a spacing question in a backcross orchard is: what type of equipment do you want to get down the rows. It's the same thing here.

   In hindsight the space between should have been bigger.

   The plot maps are oriented to the south, so #1 is in the same position everywhere. We plant the seed until we run out and then start planting from that spot the next year to finish out the plot.

   The Meadowview seed orchard uses 1' x 7' spacing for seed orchard plots. Drip irrigation systems get in the way and are not recommended; overhead sprinklers from aluminum pipe sections would be workable, but sufficient water is needed. Spacing is too close for cages but shelters for the base of the trees should be used.

   a. **How is this impacted by using controlled pollination?**

   b. **How is this impacted by using a mixture of controlled and open pollinations?**

   c. **How should each plot be filled-in?**

      ? Not sure I understand the question

   d. **Is orientation of the plot important? Of the orchard?**

      I'd say no on this one. It's never really been a concern before?

2. **Do we need an irrigation system?**

   If proven needed in backcross orchards, then yes install one. This is going to add to the cost and could impact site selection if water source is not available. This may be beneficial for early growth & survival but not a requirement. If you are planning to pluck unselected trees with a backhoe as part of the future roguing, an irrigation system would probably not be worth installing and alternative watering methods should be explored.

3. **Should we conduct a soil test?**

   Yes! Always conduct a soil test and check out the soil properties of the site on NRCS' Web Soil Survey. Pay close attention to any hydrology issues or impervious layers, like depth to bedrock or fragipan. This should definitely be a requirement. Better to find out early on if problems may arise than later on in the process. This may also be a helpful screening tool to aid in the decision making.

4. **Does the closer spacing have an impact on management options?**

   Yes, Meadowview bought new, smaller equipment for the seed orchards. But managing is the same: spraying weeds, fertilizing. The amount of work required by an orchard is determined much more by

its size than by the number of trees, except in the roguing stage. Not much else is different, though, other than inoculating sooner.

5. **What are the parts of the seed orchard plan that CANNOT be changed? Where is there flexibility?**

   Spacing probably shouldn't be changed too much. There is some flexibility with controls, though folks need to know the implications of putting them in. Inoculations have some amount of flexibility, but I'm noticing the staggered inoculations are almost required. The EP really does a number on the trees at such tight spacing.

   You can also split up blocks among cooperators, though that does reduce production at a given location. I think this is less of a concern at this stage, making sure the proper selections are being made here vs. worrying about production. Once proper selections are made, B3F3 progeny can be put into a larger, partner-run production orchard.

   Note, too, that B3F3s may start being put into production orchards. Production may soon get overwhelming for Chapters. Therefore, proper selection of good lines can be done at the Chapter level, but increased production can be done with nursery / commercial partners (sometime in the future).

   I'm sure there are others, but I'd like to get some of our other questions answered, especially as toward what goes in the orchard, before talking more flexibility options. As far as maintenance goes, whatever keeps the trees alive!!

IV. Seed Orchard Selection: Inoculation and selection procedure

As they plan for seed orchards, many chapters are just becoming comfortable with the inoculation and selection procedures for breeding orchards. How will the process in seed orchards differ?

1. **When do we inoculate? How big should the trees be? What do we inoculate with?**

   At Meadowview inoculation is during the third growing season. The trees are about 0.5-1.5in in diameter. We use a micro borer with SG2-3 only.

   At Meadowview, after two years of experience inoculating with both SG2-3 and Ep155, Ep155 was dropped to avoid killing the trees soon after inoculation. Using Ep155 we were effectively throwing out the baby with the bath water. The trees need to be inoculated at 2 years to avoid extremely high blight severity due to crowding in older trees. The spacing is chosen so that we have a residual density after selection of about 35 trees per acre, which has proven optimal for quite a few others species. We will have evidence on the efficacy of the current method before any seed orchards are even planted at chapters other than PA.

   For discussion of the PA Chapter's methods, see Section IV: 4, below.

2. **Will we stagger inoculation?**

   Yes, it is recommended it at this point. See Section IV: 4, below.

3. **Should we use controls?**

   Generally, controls are helpful when rating the resistance of inoculated trees and in the seed orchards Chinese controls would likely be the most useful. Using controls in the seed orchard will present some logistical challenges. For controls to be particularly useful, they should be left long enough to be used for comparison of the EP-155 inoculations, at which point they will provide a source of pollen contamination to other selected plots within the orchard. That said, there are still some unanswered questions about the use of controls in the seed orchards and if you wish to use them please work with your RSC to determine the best approach.

   a. **And if so, when should they be removed? Before they flower?**

      With the staggered inoculation and selection process used for seed orchards, there are possible pollen contamination issues with including Chinese controls. One way to avoid those is by emasculating the controls; however this is burdensome for volunteer growers.

   b. **Is there benefit to seeing what a Chinese canker looks like several years later, when started on such a small tree? Is this a good comparison for our trees?**

      There may be benefit to watching Chinese controls over time, as a comparison to the breeding trees. If this is desired however, it is important to emasculate the Chinese chestnuts so they do not provide pollen to any selections. As trees get large, this may become more difficult to keep up with and is not recommended for volunteer growers. Watching Chinese trees over time may be useful in a few instances but, for the most part, we just need them until that second, 365 day rating.

      We get a much more accurate measure of the resistance of individual trees by examining their progeny in properly controlled tests. Adequate differentiation can be obtained with SG alone. Make final determinations in progeny tests.

      Follow-up:

      Could we conduct a controlled test to help better answer this question?

   c. **What about leaving F1 male-sterile trees as controls/possible future sources of breeding lines?**

      F1s should have a similar level of resistance as the backcross trees but at the B3F2 level they probably are not as useful a comparison. If they are to be used as controls they should be used in concert with Chinese controls, which should be more useful and informative for making selection decisions. F1s would only tell you whether or not our trees are better than F1s. Guess it might be better than nothing, but it wouldn't tell you for sure whether or not you have "Chinese levels of resistance".

   d. **What about using B3 x Ch as seed orchard controls? With the B3 as the female parent these should be male-sterile and be more resistant than an F1. It seems we could use male-sterility for controls, especially when pollen-contamination is of particular concern.**

      This could be an option, though unsure if it would be worth making the pollinations? You avoid the pollen contamination issue, but Chinese still seems like a better comparison for rating resistance. B3 x Ch would probably segregate for male sterility somewhat and would

depend on which B3 is used and their background. Probably most would be sterile, but they would still need to be observed to make sure. Their resistance would not be full, so we'd have to keep that in mind when looking at cankers. We'd want our cankers to be better than the B3 x Ch.

4. **Will we leave one selection/intercross line? Two? Maybe two but one pruned at ground level? Having a back-up seems like a good plan.**
   Yes, saving backups is recommended. I've been leaving 2 selections in the orchard where I have 10 lines. Again, I think this depends on how many lines are in any given place and if you're going to use controlled vs. open pollination. Ideally one is the plan however if there are two that are equal, keep them both, you will have higher production.

   The selection process is gradual. There's the first round of heavy selection during the third growing season, with roguing after the season. Then gradually weed down to 1 to 5 trees per subplot. The final selection might be based on the result of progeny tests. When the trees are big enough to take up most of a subplot, one probably would not want another backup in that subplot. The replication of genotypes over the block would have to suffice.

   Discussion of Meadowview inoculation process: Seed orchard plots inoculated at beginning of third growing season, trees are ~ 0.5-1½" in diameter at the base. Use a micro-borer from a hobby shop, rather than cork borer. Inoculation made 8-10" from the ground and with SG 2-3 only to start. When to use EP-155 is less clear – natural inoculum load at research farm may be enough to finish the job. Use a 1-3 scale, keep the 1's, maybe 1.5's.

   Discussion of PA inoculation process: Seed orchard plots inoculated 3-4 years after planting, or whenever trees start to shade each other out (close spacing). SG 2-3 is used first and rogued 6-12 months later. Then allow first round selections to grow for a season and inoculate with EP-155 two full years after the initial inoculation. Rating scale of 1-5 used – 1-3.5's kept in fall, 1-3's in the spring (usually only ~20% trees left by spring).

   Protocol is still being refined and we should expect active adaptive management. There may be other blight-resistance assays to use or new technologies available in the future.

V. Seed Orchard Production: Harvesting local B3F3s

Producing local B3F3's will be a truly exciting accomplishment for the chapter breeding programs.

1. **How many trees (lines) should be flowering before harvesting useful seed?**
   Early seed could be used for testing purposes primarily.

   Follow-Up:
   Work with a population geneticist to determine how many breeding lines should be flowering before collecting seed to use for restoration planting. Simulation or outside opinion might be helpful.

2. **What size are trees expected to be when we start harvesting?**
    a. **Can we use ladders? Should we plan on bucket trucks?** Best not to wait for natural nut-drop.
    Ladders will work fine if you can reach the flowering part of the tree. If not, bucket trucks will be necessary. This will most likely be the case one these trees are fully mature and large enough to produce seed on a large scale.

    b. **Should harvest equipment access be a consideration included in site selection?**
    Yes. This is a large cost and a huge resource for the orchard. Depending on donated bucket truck time will not be efficient in the long term, when production is at its fullest. Places that have a resident bucket truck or access to one at short notice will allow flexibility and will make harvest easier.

3. **What do we do with the harvested seed?**
    In terms of storage – this should also be a consideration during site selection. Having the facilities on hand to store the harvested seed will make this an easier process when on a larger scale. To minimize work and make the process as streamlined as possible right from the beginning, the seed should have a determined home before harvest, and should be sorted prior to storage.

    As below (4.a), need to make sure all the seed are labeled properly. Huge amount of work!

4. **What are the possible complications of harvesting large quantities of seed?**
    a. **Processing, labeling, etc. will soon get to be a lot of work. How do we handle that?**
    De-burring, processing, sorting, labeling, storing, etc. will be a lot of work, even before production of the seed orchard is at its fullest. Seed orchards that have the facilities (or which are close by) capable of processing, sorting, packaging, labeling and storing the seed should be prioritized locations. There should also be professional oversight either on hand or close by to help organize the activities of TACF volunteers.

    b. **Harvested seed should be kept separate by tree so pedigree is known for testing.**
    The benefit of working with established nurseries, whether public or private, will be especially useful during the initial seed orchards. The expertise these nurseries have in raising large number of seed, plus the understanding of all the detailed mechanics of harvest and storage, will be very useful for chapter members learning the ropes for the first time.

5. **What is the TACF distribution policy regarding chapter-bred B3F3's? Will the chapter make a distribution plan for committee approval? Will TACF make additional requests for chapter-bred seed? Please be up-front so there are no surprises down the road.**
    TACF's board of directors has established a Distribution Policy for all B3F3 (and future generation) nuts, whether bred at Meadowview or by the chapters. The Distribution Policy is implemented by the Distribution Committee, who submits a distribution plan to the full board each year for approval. The policy is fairly straight-forward and states that all B3F3 (and future generation) nuts are the property of TACF and will carry the TACF trademark. As such, all distribution plans will go through the Committee. Specific to the state chapters, the policy states that the chapters will distribute B3F3 (and future generation) nuts under the direction of the Distribution Committee and that the chapters

will have their distribution plans approved by the Committee. As this policy is put into practice the Distribution Committee will review and revise or amend as needed.

Follow-up:
What will the policy be for the sale of chapter-produced B3F3 seed? We currently make them available in small number to our members for the cost of shipping and handling, but for large plantings value them around $250/nut. Would chapters get a share of any funds raised through nut sale?

6. **What are the plans for common garden tests? When would those be installed? How many chapters need to be producing B3F3s?**
   They'll be used to ID resistance and form. Eventually, more can be inter-planted and they'll be used as the first restoration plots as long as the material pans out to be high resistance and good form / survivability.

   a. **Add use of chapter-bred nuts to B3F3 testing protocol?**
      Look to final write-up of restoration plan to help answer this…no one has made a solid start at solving yet.

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