Regionally Adapted Seed Orchards within TACF's State Chapters

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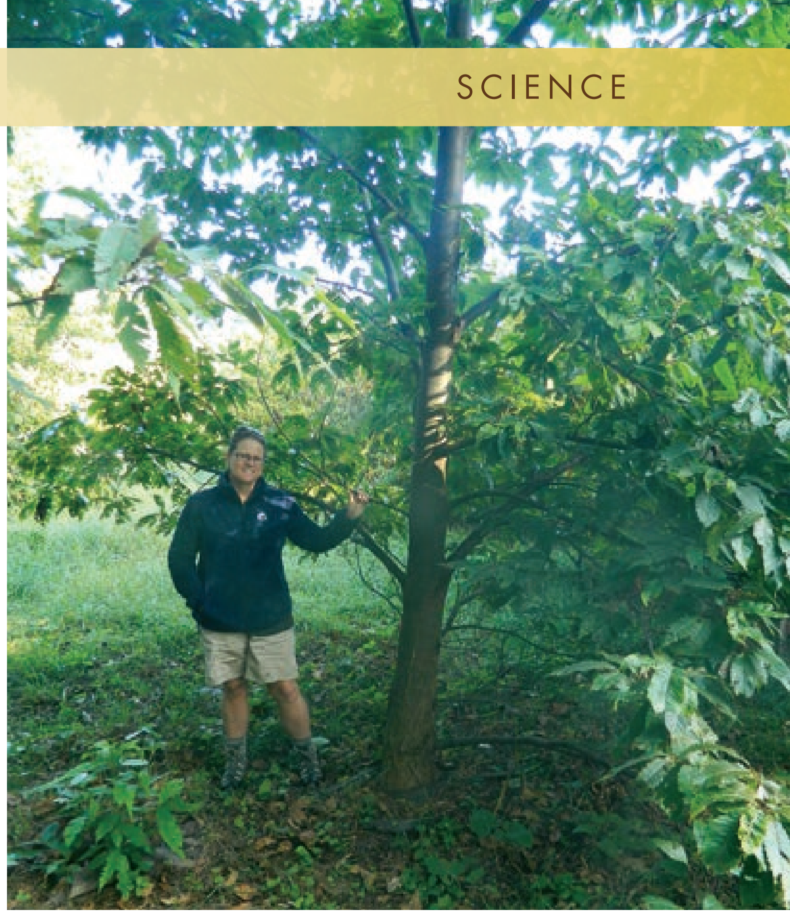

Sara Fitzsimmons stands next to one of the first PA-TACF regionalized B_3F_2 selections at the Penn State Arboretum. Planted in 2003, this tree exhibits vigorous callusing of inoculation points, which is an indication of a high level of blight resistance. Photo by Jeff Donahue

Several of TACF's Chapters have now planted **seed orchards**, also known as Legacy Tree Orchards,[1] and are one step away from producing regionally adapted, potentially blight-resistant American chestnuts. Seed orchards have been established in the Maine, Massachusetts/Rhode Island, Pennsylvania, Indiana, Maryland, Tennessee, and the Carolinas Chapters.

As Chapters begin seed orchard development, TACF volunteers and members are asking some astute questions about how to establish and maintain these plantings. Here we provide general information and updates to the original article on seed orchard establishment, published in *The Journal* in 2002 and 2003.[2] Additional information may be found online at http://ecosystems.psu.edu/research/chestnut/breeding/orchard-design, or by contacting a TACF Regional Science Coordinator.

Introduction to Seed Orchards

Forestry seed orchards can serve many purposes, including progeny testing (a way to determine parental quality), genetic gain (improving trait[s] of interest), capture of diversity, and seed production.[3] The purpose will determine both the design of a seed orchard and the mating design used to create the trees planted there. Although TACF's B_3F_2 seed orchards aim to serve each of those purposes, the most important among them is to capture the maximum amount of genetic diversity recovered by TACF's breeding with native American chestnuts trees.

How Many Selections to Use

In TACF's mainline backcross breeding plan, a minimum of six generations is needed to create a blight-resistant American chestnut. The seed orchard is the fifth of those generations. The parents of that generation, the fourth generation, are called B_3 trees[4] and are planted in orchards referred to as **breeding orchards**. Our Chapters have planted nearly 250 breeding orchards, representing approximately 50,000 trees. Over 300 native American chestnuts have been used as direct parents of those B_3 trees.

Within that fourth generation, each one of those 300+ native American chestnut trees is represented in what we call a **family line**. Assuming that blight resistance is controlled in an incompletely dominant fashion by three major genes, 12.5% of the nuts planted in each one of those B_3 family lines should exhibit moderate blight resistance, and could be selected for parenting seeds to plant in the seed orchards. The number of trees that are actually selected for further breeding depends on several factors and, within a given line, can vary from one to as many as ten.

Although several factors are considered when selecting for further breeding, the most important is blight resistance. The number of trees selected, whether nearby non-selected chestnut trees could contaminate the B_3F_2 cross, as well as how many family lines are present in a given orchard dictate whether we use open or controlled pollination techniques to produce the B_3F_2s that are to be planted in the seed orchard.

Open Pollination vs. Controlled Pollination

We plant A LOT of trees in our seed orchards (Figure 1). The main reason for this is that there is a very low statistical probability of obtaining a highly blight-resistant tree from this fifth-generation cross. Again, assuming that blight resistance is controlled in an incompletely dominant fashion by three major genes, only one out of every 64 seeds planted can have the potential for

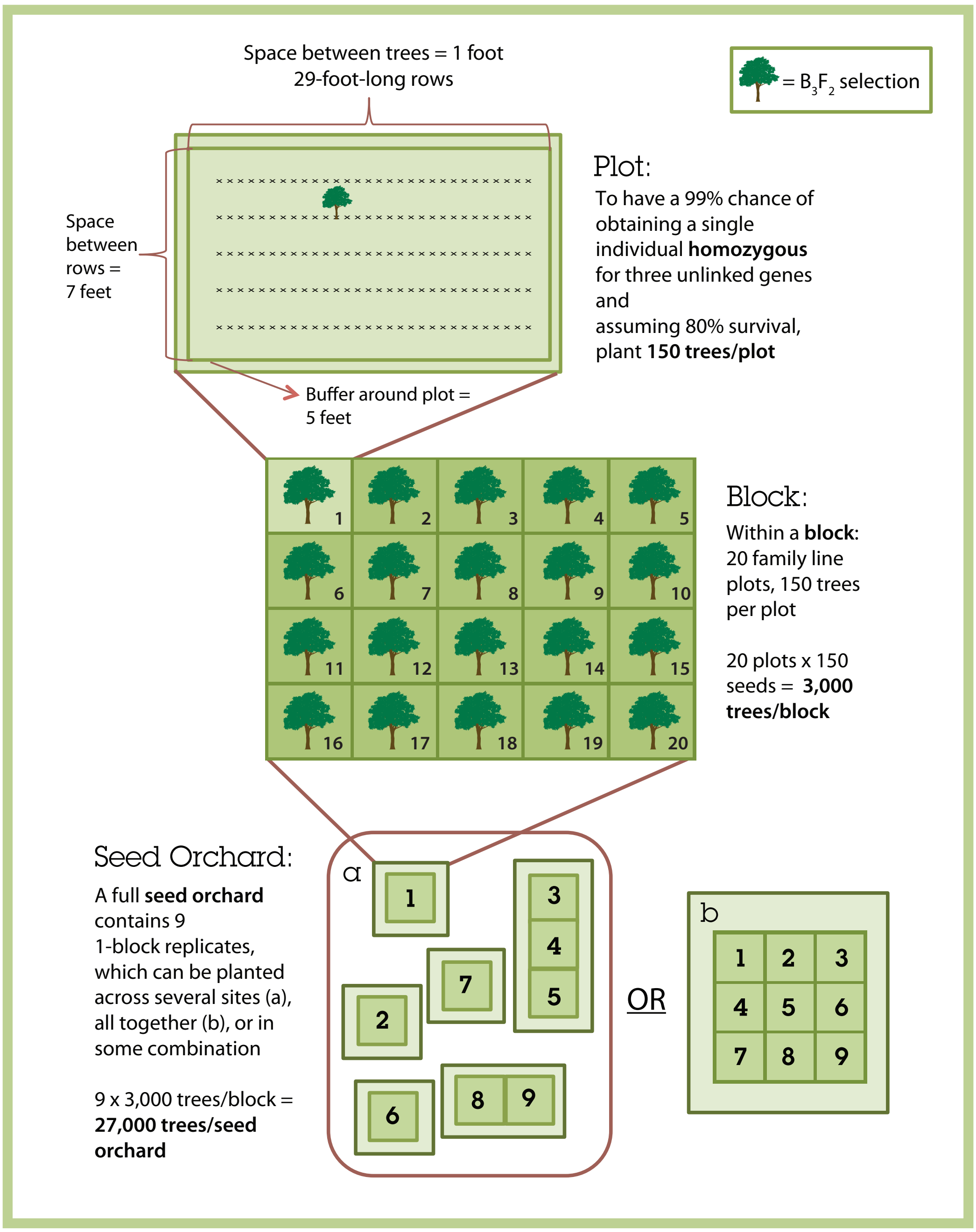

Figure 1. Seed orchard layout. How do we find that one homozygous tree per plot? See caption for Figure 2.

♂ pollen

♀ pistil

	RRR	RRr	rRR	RrR	Rrr	rrR	rRr	rrr
RRR	RRRRRR	RRRRRr	RrRRRR	RRRrRR	RRRrRr	RrRrRR	RrRRRr	RrRrRr
RRr	RRRRrR	RRRRrr	RrRRrR	RRRrrR	RRRrrr	RrRrrR	RrRRrr	RrRrrr
rRR	rRRRRR	rRRRRr	rrRRRR	rRRrRR	rRRrRr	rrRrRR	rrRRRr	rrRrRr
RrR	RRrRRR	RRrRRr	RrrRRR	RRrrRR	RRrrRr	RrrrRR	RrrRRr	RrrrRr
Rrr	RRrRrR	RRrRrr	RrrRrR	RRrrrR	RRrrrr	RrrrrR	RrrRrr	Rrrrrr
rrR	rRrRRR	rRrRRr	rrrRRR	rRrrRR	rRrrRr	rrrrRR	rrrRRr	rrrrRr
rRr	rRRRrR	rRRRrr	rrRRrR	rRRrrR	rRRrrr	rrRrrR	rrRRrr	rrRrrr
rrr	rRrRrR	rRrRrr	rrrRrR	rRrrrR	rRrrrr	rrrrrR	rrrRrr	rrrrrr

Figure 2. Punnett square showing all the combinations of alleles for blight resistance and susceptibility that could be inherited from two B_3 parents heteroyzgous at each of three loci for resistance. As can be seen in the upper left-hand corner, only one combination is fixed for resistance alleles (R) at three loci. It should be noted that we currently select our trees by examining canker expansion, which is the phenotype. Using only phenotypic data, it probably will not be possible to distinguish the one genotype with six alleles for blight resistance (in red) from those with five (orange) or four (yellow). We'll discuss this problem in a future issue of *The Journal*.

inheriting all six alleles for blight resistance[5] from their two B_3 parents (Figure 2); that is about a 1.5% probability of obtaining a suitable selection from any given seed.

Based on previously published calculations,[6] we estimate that at least nine B_3F_2 selections per family line will be required to capture most of the allelic diversity contained within the B_3 parent, which is why we plant nine complete seed orchard **blocks**, or **replications**. These nine blocks may be planted all together in one location, or they may be split across as many as nine locations. Assuming we have 20 distinct family lines (the goal for most Chapters working with a single source of resistance), and 150 trees per family line plot, using open pollination we need to plant 3000 trees per block.

To have a 99% chance of obtaining those nine replications from any one family line, we have to grow 1080 trees from which to select. Assuming 80% survival of seeds planted, that means we should plant a minimum of 1350 seeds for each family line. If we break up those trees into the nine blocks, that works out to planting 150 seeds per family plot (Figure 1).[7]

Controlled pollinations will allow less than 3000 seeds per block to be planted, but appropriate designs for such seed orchards are too complicated and numerous to describe here in detail. TACF's Regional Science Coordinators will work with Chapter volunteers and land managers to recommend the most appropriate mating and planting designs based on the number of B_3 selections and their locations. It is highly desirable that the selected mating and planting design be followed for any given seed orchard and all its component blocks. Otherwise, if pollination methods are mixed, it would be best to have each family line represented by 150 nuts, or 3000 trees per block.

With each family line being represented by anywhere from 1 to 10 B_3 parental selections, we have to choose which parents to use for any given cross, especially if using controlled pollination. We prioritize use of selections both by blight-resistance performance and also by American traits such as form, growth, and other species-specific characteristics. Because chestnuts reliably sprout from the root collar, backup selections can be clearly marked, pruned back, and allowed to re-sprout, just in case primary selections fail for one reason or another. It is better to use two or three parental selections within each family line rather than only one.

Layout, Maintenance, and Selection for Blight Resistance

One unique feature of TACF's B_3F_2 seed orchards is the layout. Based on the very low statistical probability of obtaining a highly blight-resistant tree from this intercross, many trees have to be planted in order to obtain a suitable selection (Figure 1). In the original design, 150 seeds from a given family line are planted in a five-row **plot** at a spacing of one foot between trees, and 7 feet between rows (Figures 3). This very tight spacing is used to achieve a final spacing of 30-35 trees per acre after all selections for blight resistance and other traits of interest are complete.

With the trees planted so closely together, TACF staff and volunteers must act quickly to perform selections. Inoculation with the blight fungus, then, occurs when the trees reach 1" in diameter at 12" above the ground.

This may occur as early as the second growing season! With the added stress from neighboring competition and being inoculated at such a young age, the trees have a more difficult time fighting off the intentional blight infection we use as our main selection tool.

Work is currently being done to quantify the effect of spacing and early inoculations. Meanwhile, there are several ways to alleviate some of the stress. One is to do a **staggered inoculation**, wherein we do an initial inoculation at age two or three with only a weakly pathogenic strain of the blight fungus, SG2-3. Based on the results of this inoculation, we can cull more than 50% of the trees in that plot. We then give the trees two more growing seasons and finalize selection with the strongly pathogenic strain of the blight fungus, EP155. Further culling after an initial screening with SG2-3 can also be based on the incidence and severity of naturally occurring cankers. Still another way to handle the stress imposed by narrow spacing and inoculation when

LAND OWNERSHIP

The life span of a TACF seed orchard can be as long as 45 years. There are no guarantees that any planting site will be available for that time period, and across TACF's Chapters the ownership of seed orchard blocks will be a combination of both public and private. The decision on where to plant ultimately comes down to who has the resources, personnel, and long-term commitment to see the project through to completion.

Planting a seed orchard plot on a Small Woodland Owners Association of Maine (SWOAM) property in Winthrop, ME, in 2013. Photo courtesy of Kendra Gurney

Land owned in perpetuity may be the most secure for such a long-term planting. This is most common for land trusts, educational institutions, and public lands. Of these, land trusts and other conservation groups may be the most secure. Universities can be great partners, but land conservation is not their primary focus and the possibility for the area to become a parking lot, dorm space, or used for other future development should be considered.

Private land is another option but it is good to explore long-term plans for ownership and management. A **conservation easement** or **restriction** that allows for chestnut orchard activities can be a good fit. Whenever possible, Chapters and volunteers should work with various types of agreements to ensure long-term stability of orchard management.

No matter the type of land, a written agreement or long-term management plan should be developed to address leadership or ownership turnover. An orchard manager should be designated to coordinate and oversee any activities spelled out in the agreement. TACF's Germplasm Agreement (GPA) is a good place to start. In addition, there are other types of agreements to consider, depending on the situation. In all cases, TACF recommends seeking legal counsel to review any signed documents.

1. Chapter Orchard Agreement (COA) – We recommend this for every planting, as a way to assign responsibility for maintenance, financial support or labor, access rights, etc.

2. Memorandum of Understanding (MOU) – An MOU is often a good idea when partnering with another organization for hosting an orchard, as it outlines each organization's responsibilities. Some organizations will sign an MOU for the entire time frame of the planting, or it may be written to be reviewed/renewed at a regular interval.

3. Other legal documents – Conservation Easements (CE), Conservation Restrictions (CR), and Lease Agreements (LA) may also be considered as additional legal leverage when working with private lands.

young is to increase the spacing between trees from 1 foot to 2 feet. Of course, without narrowing the rows, this also increases substantially the size of the planting area. Ultimately, the decision on how to establish the site, if different from the original design, should account for all other aspects of maintenance. A Regional Science Coordinator can help with those decisions.

Where to Plant and Land Ownership

The first step in establishing a B_3F_2 seed orchard is to find a place to plant. Although the number of trees to be planted can seem daunting, the amount of effort needed to maintain such a planting need not be overwhelming. Because seed orchards can be broken down into single **blocks**, as little as an acre can be planted. Anywhere from 1500 to 3000 trees may be planted in that space, depending on the use of open or controlled pollination, discussed previously. Once selection and culling are completed, some 10 to 20 trees will remain in the space and should produce regionally adapted, highly blight-resistant American chestnuts for years to come.

Figure 3. The Nature Conservancy's Basin chestnut seed orchard in Phippsburg, ME, showing one-foot spacing within a single plot of B_3F_2s. Photo courtesy of The Nature Conservancy

Trees take a long time to grow, and this can often affect the locations where we can plant our orchards. Most breeding orchards can be completed in the span of 10-15 years, but seed orchards often require as long as 30-45 years to be fully planted with all family lines, culled to highly blight-resistant selections, and ultimately, to produce all the seed needed for progeny testing, reintroduction, and restoration plantings. Such an extended time period can affect the locations where seed orchards can be established, and there is really no one answer as to what type of land ownership yields the best results in the long term – all cases have advantages and disadvantages. Luckily, TACF has several tools that can help Chapters, their volunteers, and partnering landowners reach agreement on these long-term issues (*see sidebar on page 18*).

How You Can Help

Our Chapters are always looking for volunteers to plant new orchard locations. If you are interested in becoming a grower, or helping to maintain one of our seed orchards, please contact your local Chapter and/or Regional Science Coordinator to find out how to get involved.

ENDNOTES

1. Call TACF's Asheville office to find out more about naming rights to Legacy Trees.
2. Hebard, FV. 2002. Meadowview Notes 2001-2002. *Journal of The American Chestnut Foundation*. 16(1): 7-18; Hebard, FV. 2003. Meadowview Notes 2002-2003. *Journal of The American Chestnut Foundation*. 17(1): 7-14.
3. For an excellent introduction to both seed orchards and forest tree breeding, see Zobel, BJ and J Talbert. 1984. *Applied Forest Tree Improvement*. John Wiley & Sons, New York.
4. In actuality, many of our Chapters have produced B_4s rather than B_3s, or a mix of the two. Here, for simplicity, we refer to all of these as B_3s and their generation as the fourth.
5. When all alleles are the same for a given trait, that tree is considered to be homozygous, or fixed, for that trait.
6. Hebard, Meadowview Notes 2001-2002.
7. This design does not insure that each block will have a selection, only that nine selections will be obtained. Transplantation between blocks may be needed in a few cases.