# *American Chestnut: First-Year Field Viability*

Master of Forestry Project

Department of Forestry and Environmental Resources

North Carolina State University

Alexander Warren, Master of Forestry

Under the direction of

Drs. Joseph Roise, Rachel Cook, Jodi Forrester

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# Contents

## INTRODUCTION:

The story of the American chestnut (*Castanea dentata*) is well known among foresters. In what may go down as one of the most ecologically impactful events in modern North American history, the fungal pathogen known today as chestnut blight (*Cryphonectria parasitica*) was first discovered in our hemisphere in 1904 at the Bronx Zoo in New York City, most likely from nursery stock of Japanese chestnut that was carrying the disease (Anagnostakis 1997). It is with the effects and treatment of this disease that many foresters and natural resource professionals of various disciplines are concerned, and around which the experiment to be detailed in the following paper is focused.

Before the turn of the 20th century, American chestnut was one of the most dominant woody species in eastern North America, comprising approximately 25% of all overstory hardwood trees in the southern Appalachians in particular ("Natural Range"). It had an extensive range, with over four billion individuals covering over 200 million acres of forest stretching from the Deep South all the way to Maine ("Natural Range"). Not only was it a keystone species due to the high value placed on its nuts and browse by wildlife, but it was also prized by humans owing to the quality of its wood, its rapid growth habit, large size, and prolific fruit production ("History").

Today, the same forests once dominated by this tree have a much different look. The blight moved swiftly: by 1940, in only a matter of decades, almost all mature chestnut trees in the United States had been killed by the disease ("History"). Entering through fissures that occur in the bark of adolescent trees as they grow, *C. parasitica* quickly causes a sharp pH drop (Powell et al. 2019) as it develops under the bark, eventually killing the cambium and effectively girdling the tree (Anagnostakis 2000). It is not a process easily or frequently survived. The highly infectious pathogen is now so widely distributed across the continent that it is next to impossible to grow an American chestnut to maturity without inviting an attack. This is in part due to the ability of the blight to persist on dead wood for a long time, as well as the huge reservoir of wild American chestnuts in the former range, especially in the mountains of Tennessee and North Carolina (Fitzsimmons). In addition, some oak species can serve as hosts, albeit largely unaffected, much like Chinese and Japanese chestnut. The disease can also persist via its role as a soil saprophyte, although for how long and in what state remains unclear (Fitzsimmons).

The scenario is not as bleak as it may seem, however. Chestnut, as a species, continues to endure through a peculiar set of circumstances, described by many as "functional extinction:" although the aboveground portion of the tree is killed by the blight, the roots survive and are able to send up new shoots (Anagnostakis 2000). The process then repeats as the new stem ages and fissures, allowing the disease to once more infiltrate through the bark and cause another dieback. It is in this way that American chestnut persists, through an ostensibly endless cycle of death and resprouting; never attaining maturity, but never being completely eliminated by the blight.

It is this dire ecological situation that motivates the ongoing restoration efforts dedicated to reestablishing chestnut to its former status as a dominant overstory tree; efforts conducted in some form since about the 1930s. The undisputed national leader in these exploits is an organization by the name of The American Chestnut Foundation (TACF), which employs cutting-edge tactics centered around genetic manipulation with the goal of increasing the resistance of our native chestnut to the effects of infection by chestnut blight. The ACF played a key role in the development and implementation of the experiment to be detailed in the following pages. Their core strategies and methodology for achieving their aims will be of particular relevance.

The long-term goals of this project are to establish a population of American chestnut at NC State University's G.W. Hill Forest, comprised of individuals from fourteen distinct genetic families of improved B3F3 backcross hybrid American x Chinese chestnut trees. In establishing this population, the objective is to grow these improved seedlings for a number of years and then inoculate them with the blight in order to test each genetic family's individual resistance (or lack thereof) to infection, and ultimately death. In doing so, the idea is to provide The American Chestnut Foundation with additional data corroborating their genetic manipulation efforts of American and Chinese chestnut. It is the belief of many that the data from this experiment and many others like it will be the cornerstone of the knowledge that revives American chestnut as a viable overstory tree in North America while simultaneously retaining as much of its original genetic makeup as possible.

## TACF Strategies:

The approaches to ecological restoration undertaken by The Foundation are divided into three categories and united under the codename *3BUR*: Breeding, Biotechnology, and Biocontrol united for Restoration ("Using Science"). It is with the first of these three approaches that this project, and indeed the bulk of the ACF's efforts, are concerned. However, they are each briefly highlighted below in an effort to lend some context to their mission as a whole.

### B1 – Breeding:

The "traditional" approach at the core of TACF's day-to-day activities, the breeding program focuses on crossing individual American chestnuts and Chinese chestnuts and selecting for the specimens that display the greatest blight resistance. These operations are carried out at over 500 orchards across sixteen TACF chapters and have, four generations of trees later, yielded a genetically diverse population of hybrids from which the seeds involved in this project were taken. While the bulk of this research has been concerned with improving blight survival rates, it is now moving to also integrate resistance to the root rot-causing pathogen *Phytophthora cinnamomi*, a major source of mortality for American chestnut populations ("Using Science").

### B2 – Biotechnology:

The biotechnology program focuses itself on transgenics, or the practice of artificially inserting genes from one species into another in hopes of achieving some desired effect in the recipient. In the case of American chestnut, that goal is blight resistance. Specifically, a gene found in wheat that produces oxalate oxidase (OxO) is being introduced, as it has been found to substantially bolster blight tolerance. In addition to this promising find, TACF is investigating the possibilities of relatively new gene-manipulation technologies such as CRISPR ("Using Science").

## B3 – Biocontrol:

Primarily used on small scales to extend the lifespan of individual orchard specimens, the biocontrol approach utilizes a technique called hypovirulence to infect the blight fungus itself with a virus, thus reducing its capacity to mortally wound a tree. The main idea behind this strategy is to enable the natural defenses of American chestnut to stop a blight infection, much like a human immune system would stop and expunge disease-causing pathogens ("Using Science").

## Selected Literature:

1) *Eight-year blight (Cryphonectria parasitica) resistance of backcross-generation American chestnuts (Castanea dentata) planted in the southeastern United States*:

This study, authored by Stacy Clark, Scott Schlarbaum, Arnold Saxton, and Richard Baird, concerns itself with the short- to medium-term blight resistance of three plantings of FF3 backcross hybrids. They found that after eight years, almost 40 percent of planted chestnuts exhibited symptoms of blight infection, with hybrid crosses showing significantly lower resistance than pure Chinese chestnut of a similar age. They also found that the hybrid crosses exhibited markedly higher blight resistance than pure American chestnut. Both of these findings are consistent with expectations, implying that TACF's backcross program is effective, but not wholly so. They also determined that planting site had a significant effect on a particular breeding family's resistance to blight infection, although none of the mortality involved in this experiment was ostensibly blight-related. Finally, they determined that tree basal diameter at age 8 was positively associated with blight infection, a finding that corroborates the current knowledge of the pathway by which chestnut blight is able to infect individual trees.

2) *Toward development of silvical strategies for forest restoration of American chestnut (Castanea dentata) using blight-resistant hybrids*:

In this piece, Douglass Jacobs examines strategies for re-introducing blight-resistant chestnut hybrids to the landscape on a large scale. In order to do this, he focuses on the known silvics of the species in order to develop pragmatic silvical guidelines for real-world reintroduction in forest ecosystems. He notes the soil preferences of the species, which tend toward well-drained sandy soils aligning with results found and detailed later in this paper (specifically those of site 3). He documents the ability of American chestnut to survive for prolonged periods in the shade yet respond quickly to canopy openings and the accompanying high light levels that result. Mentioning several other aspects of American chestnut's ecology and silvics, all pointing toward the species' remarkable competitive ability, he recommends several reintroduction strategies, prominent among which is afforestation in mine reclamation plantings. Finally, he notes several challenges for successful chestnut restoration, among which is of course the blight, but which also include public acceptance of hybrid chestnut, *Phytophthora*, and exotic insects such as the Gypsy moth (*Lymantria dispar*), which has shown to perform very well on American x Chinese chestnut hybrids in recent years.

3) *<u>American Chestnut Growth and Survival Five Years after Planting in Two Silvicultural Treatments in the Southern Appalachians, USA</u>*:

This study, conducted by Stacy Clark, Henry McNab, David Loftis, and Stanley Zarnoch, all with the USDA Forest Service Southern Research Station, concerned itself with the same metrics as those involved in this project, albeit with a slightly different timeline. Specifically, they examined the effects of two different silvicultural regimes on height growth, root collar diameter growth, and survival after five years. The regimes, a two-age shelterwood treatment and a midstory-removal treatment, created different light conditions for the chestnut seedlings, but interestingly had an insignificant effect on survival, which averaged 67%. The high survival in this study is likely due to a combination of partial-shade forest conditions preventing the rapid growth of pioneer species, as well as the geographic location being ideal for chestnut growth (Bent Creek Experimental Forest near Asheville, NC). Also factoring into the high survival was aggressive treatment of competition, mostly in the form of stump sprouts, with herbicide: an observation of particular relevance later in this report. The researchers found differences in height and diameter after 2, 3, and 5 years, but these were not statistically significant for either treatment, suggesting that chestnut is able to perform well in a variety of light environments. Other important conclusions included the relatively superior competitive ability of American chestnut compared to other Fagaceae species, as well as their findings that blight incidence was not more prolific in open areas when compared to understory sites.

4) *<u>Establishment of American chestnuts (Castanea dentata) bred for blight (Cryphonectria parasitica) resistance: influence of breeding and nursery grading</u>*:

This study, authored by many of the same people involved in the previous studies, concerns itself entirely with the four-year survival and growth and three-year competitive ability of pure American, pure Chinese, and two families of hybrid (BC2F3) chestnut planted at three different light levels in the Daniel Boone National Forest in Kentucky. The experiment determined that survival was affected mostly by genetic makeup, and not by light levels, although higher light levels were correlated with faster growth, as is to be expected. At the end of the study, Chinese chestnut had by far the highest survival (over 90%), while pure American and one family of hybrid chestnut had very low survival rates, similar to the ones experienced in this project (21% and 27%, respectively). The other hybrid family had intermediate survival, just over 50%. Arguably the most important finding of this study, and perhaps the most relevant to the aims of TACF, was that blight incidence was less than 10% across all seedlings by year four, while *Phytophthora* was present in every soil sample taken across all sites. This implies that the majority of seedling mortality was caused by the pathogen, as Chinese chestnut is so much more resistant to it than American chestnut. As mentioned previously, efforts are already underway by TACF to breed for *Phytophthora* resistance.

5) *<u>Establishment of American chestnuts (Castanea dentata) bred for blight (Cryphonectria parasitica) resistance: influence of breeding and nursery grading</u>*:

The final piece covered in this section is again written by many of the same authors, including Stacy Clark and Scott Schlarbaum. Their experiment is arguably the most similar to the one with which this paper is concerned: they tested early field performance of B3F3 backcross hybrids, albeit with the difference that the site used was a highly productive southern Appalachian one. They also used a two-aged regeneration harvest, contrasting with the clearcut planting pursued in this project. A secondary objective of the study was to test the efficacy of common visual nursery grading techniques that grade seedlings by size prior to planting. The study found that backcross hybrid seedlings had comparable survival to pure American chestnut at age four, but with smaller heights and root collar diameters. By year four, blight incidence in the backcross hybrids was similar to that of Chinese chestnut at the same age, and lower than that of American chestnut. Interestingly, larger seedling classes selected as a result of visual grading had a higher incidence of stem dieback and a slightly lower survival than smaller seedling classes; however, they also grew faster, exhibiting the same year three height as smaller seedlings did in year four. All of these findings could have potential implications in future restoration efforts, especially in ones similar in nature to this project (i.e. a large number of planted seedlings with noticeable and possibly significant differences in size prior to planting).

## PROJECT DESIGN:

This project, intended to be a long-term, multi-year endeavor passed periodically from student to student, began in January 2018 under the supervision of Dr. Joseph Roise of NC State University. The duties of the project were initially handled by Rachel Jessup, and then in early 2019 were passed to the author of this paper (Alex Warren). The objectives of the project are as follows:

*Short-term Objective*: Test 14 different American chestnut families that will be received from TACF for family differences in germination, survival, and growth.

*Medium-term Objective*: Evaluate the 14 different families for blight resistance, survival, and growth associated with 3 levels of herbicide treatment.

*Long-term Objective*: Evaluate the 14 different families' long-term survival, blight resistance, and growth and yield. Intended to be done every ten years.

As this project is in its earliest stage, the chief concern of this paper was with the short-term objectives. In addition to planting as many seedlings as possible in accordance with the experimental design, the specific aim of this experiment was to answer the following questions: First and most importantly, what are the effects of genetic family on first-year field survival? Secondary research questions are: what are the effects of site, planting date, planting method, and genetic family on survival? Thirdly, what are the effects of these variables on root collar diameter and height?

## Experimental design:

A randomized complete block design (RCBD) (Grant) was used for the planting of the seedlings, similar to the one shown below in Figure 1.

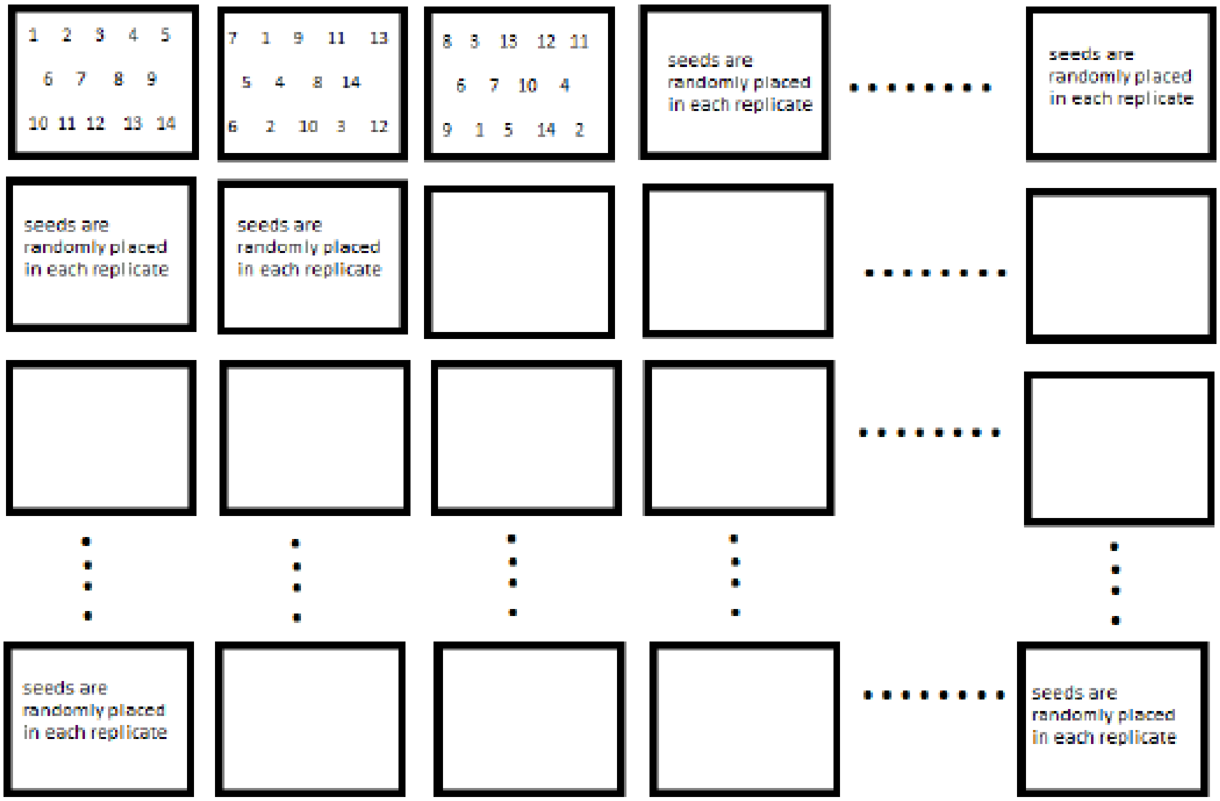


*Figure 1*. An example of a randomized complete block design. Each box represents a replicate (rep). Numbers 1-14 inside of each box are the different genetic families. These are distributed randomly within each rep, with one representative of each family appearing once. Rows of unimproved seedlings planted around each rep act as buffers between different treatments; this includes one unimproved seedling in each rep's middle row for symmetry and evenness.

The block design used in this experiment was identical to the one depicted in Figure 1, with seven sets of three reps (represented by boxes above) planted at three different sites at NC State's G.W. Hill Forest. Each set of three reps, hereafter referred to as a "block," was planted so that each rep within a block adhered to one of three spacing schemes: 5'x12', 7'x12', and 9'x12'. Buffer trees were planted around each block and between each rep within blocks as described above in Figure 1. The spacing schemes are part of a long-term objective, but had no impact in this study, as none of the chestnut seedlings had begun root or canopy competition with one another at their young age.

The RCBD method was chosen with the intent that additional treatments could be applied to individual reps in the future, such as herbicide or fire treatments, after the seedlings had become established and allowed for more growth and survival data to be collected.

## METHODS:

The procedure of this experiment can be subdivided into four steps: 1) plant chestnut seeds in containers and tend them for one year as they grow into seedlings; 2) plant the seedlings at each of three predetermined sites; 3) record survival, height, and root collar diameter of every improved seedling; and 4) perform basic statistical analysis on the measurements obtained. What follows will be a largely chronological documentation of every aspect of the experiment's methodology, from 2018 to present.

### Seeds:

In January of 2018, The American Chestnut Foundation sent genetically improved American x Chinese chestnut hybrids in plastic bags labeled by family to NC State, with 60 seeds to a bag. The fourteen families all followed a specific naming scheme: a letter and a digit (such as W1, for example), followed by two numbers. The initial letter refers to the parcel from which the individual came: "D" seedlings came from the Duncan farm and are of the Clapper source of resistance, and "W" seedlings came from the Wagner farm and are of the Graves source of resistance: both are parcels owned by TACF in Meadowview, VA. "Clapper" and "Graves" are both named first-generation backcross trees that were chosen by researchers in the mid-1900s to begin the backcross program for TACF. The numbers in each family name refer to positioning within TACF's planting scheme. The numbers paired with the initial letter refers to the block in which the individual was planted within a plot (9-10 blocks per plot). The middle number refers to the plot (30 plots), and the ending number refers to the tree's position in the plot (from 1-150) (Fitzsimmons). These elements were separated by dashes. The family names are as follows:

- D2-10-3
- D3-7-33
- D4-27-78
- D4-29-10
- D5-28-88
- W1-19-19
- W1-31-60
- W2-20-147
- W2-27-99
- W4-14-67
- W4-28-38
- W4-32-87
- W5-24-75
- W8-19-26

*Table 1*: Naming codes of improved crosses.

| PARCEL: | BLOCK: | PLOT: | POSITION: |
|---|---|---|---|
| **DUNCAN** | 2 | 10 | 3 |
| **DUNCAN** | 3 | 7 | 33 |
| **DUNCAN** | 4 | 27 | 78 |
| **DUNCAN** | 4 | 29 | 10 |
| **DUNCAN** | 5 | 28 | 88 |
| **WAGNER** | 1 | 19 | 19 |
| **WAGNER** | 1 | 31 | 60 |
| **WAGNER** | 2 | 20 | 147 |
| **WAGNER** | 2 | 27 | 99 |
| **WAGNER** | 4 | 14 | 67 |
| **WAGNER** | 4 | 28 | 38 |
| **WAGNER** | 4 | 32 | 87 |
| **WAGNER** | 5 | 24 | 75 |
| **WAGNER** | 8 | 19 | 26 |

In addition to the improved chestnuts, TACF also sent a large number of unimproved American chestnut seeds for use as buffers between the improved specimens. These seeds were B3F3 crosses like those listed above but may or may not have come from selected parents. The buffer seeds came in two varieties, with assigned codes according to seed lot. The buffer families were:

- PA-B3F31733
- PA-B3F31734

In these codes, "PA" stands for Pennsylvania, where the seed was collected; "17" indicates that the seed was harvested in 2017; and "33" or "34" indicate that the seeds were in the 33rd or 34th lot collected, respectively. Only the maternal parents of these families were known; both are located in the American chestnut seed orchard at Penn State University's arboretum (Fitzsimmons).

The exact number of unimproved buffers was not recorded. Measurements for survival, height, and diameter were also not taken for these, as the focus of the experiment is to determine viability of selected crosses only.

## Scarification, initial planting, and germination*:*

All chestnuts (improved and buffer varieties) were cold scarified in order to improve germination rates upon initial planting. Nuts were placed in plastic bags, which were then filled with room temperature water and allowed to soak overnight. The following day, several dozen holes were pricked in each bag and water was allowed to drain out. After this, each bag was placed in refrigerated storage at 38°F and kept in this state until the initial planting date.

Over the course of two days (March 15th-16th, 2018), the crosses of improved families were planted in D-20L plastic pots in a peat/vermiculite/perlite soil mix provided by NC State Tree Improvement, for a total of 60 seeds per family. Student volunteers helped in the process of mixing soil and water. Seedlings were marked with metal identification tags indicating to which genetic family they belonged; the markings on these tags were by impression only and would often prove challenging to decipher, leading to difficulties in family identification at later phases. On March 19th, 2018, 1134 buffer seeds were planted. There was some degree of inconsistency in planting depths; this may or may not have affected germination. Individual germination was not recorded; however, on April 9th, 2018, crosses that had not germinated were reseeded.

## First-year watering and initial growth*:*

Immediately upon planting, hand watering began. Seedlings received first-year water in three phases. First, the seedlings were kept in the NC State Tree Improvement Co-op's heated greenhouse in the horticultural research field lab behind J.C. Raulston Arboretum in Raleigh, NC. Water was delivered by hand from March 15th to May 1st. Beginning on May 2nd, the seedlings were moved outside to a sprinkler system, where they were to remain until the end of the first growing season. On December 9th, after the seedlings had entered dormancy but before freezing temperatures began to threaten, they were moved to an unheated greenhouse nearby and regular, albeit less frequent hand watering was resumed. This stretch of dormant season watering was first undertaken by Rachel Jessup and was then passed to the author, who continued weekly until the first field planting.

The majority of test subject families were spread out in trays obtained from Stuewe & Sons, as well as handmade plywood/PVC pipe trays, such that no two seedling containers were directly adjacent to each other, in order to give them more space to grow. Buffer trees were not spread out in the same fashion. In both cases, neither the broadness of the leaves nor the speed of growth was anticipated, resulting in light competition between seedlings as well as some difficulty in watering. Many seedlings appeared drought-stressed upon repeated checkups during the growing season, owing likely to inconsistency in coverage of sprinkler watering or the overlapping leaves of neighboring seedlings preventing water from reaching the soil in their containers. However, in general, growth was rapid and robust. Heights and diameters were not recorded at the end of the first year's growth, which eliminates the possibility of knowing the exact second-year growth.

On January 12th, 2019, an overall initial survival count was performed. Dead chestnut specimens were removed from their containers and trays were organized by genetic family. Watering duties were also taken over by the author hereafter. A count of surviving seedlings for each family after one year was taken, with the exception of one: W4-28-38, which was somehow omitted. Survival data were also collected for buffer trees as well as specimens whose genetic identity was unknown, owing either to missing or illegible identification tags. As the chestnuts were dormant, survival was determined not by the presence of leaves, but by giving each stem a gentle tug. If the seedling was separated easily from its soil, it was determined to be dead, counted, and removed from the experiment. Because there was a degree of imprecision in this method, a concurrent count of "questionable" seedlings was taken in order to accommodate those specimens that did not readily present themselves as alive or dead. The data obtained from this initial count are presented in the appendix.

## Site overview and field planting:

Planting of the year-old chestnut seedlings occurred in several stages between February 19th and April 20th, 2019. Seedlings were planted utilizing various methods and by a large number of different volunteers. As stated before, three sites in three different locations at NC State University's G.W. Hill Forest were used as planting spaces. Soil samples were taken from each of these sites and sent to a lab at Clemson University to test for the presence of *Phytophthora cinnamomi* and were determined to be free of the pathogen. Three other sites were dropped from consideration because of *Phytophthora.* All were recently clear-cut sites. These three sites, labeled and hereafter referred to as sites 1, 2, and 3, are depicted in the following maps. Figure 2 demonstrates the size, shape, and location of each site relative to one another in a close-up view of G.W. Hill Forest. Figure 3 depicts the location of the three sites within Durham County, NC. Note that these sites have been without trees for many years.

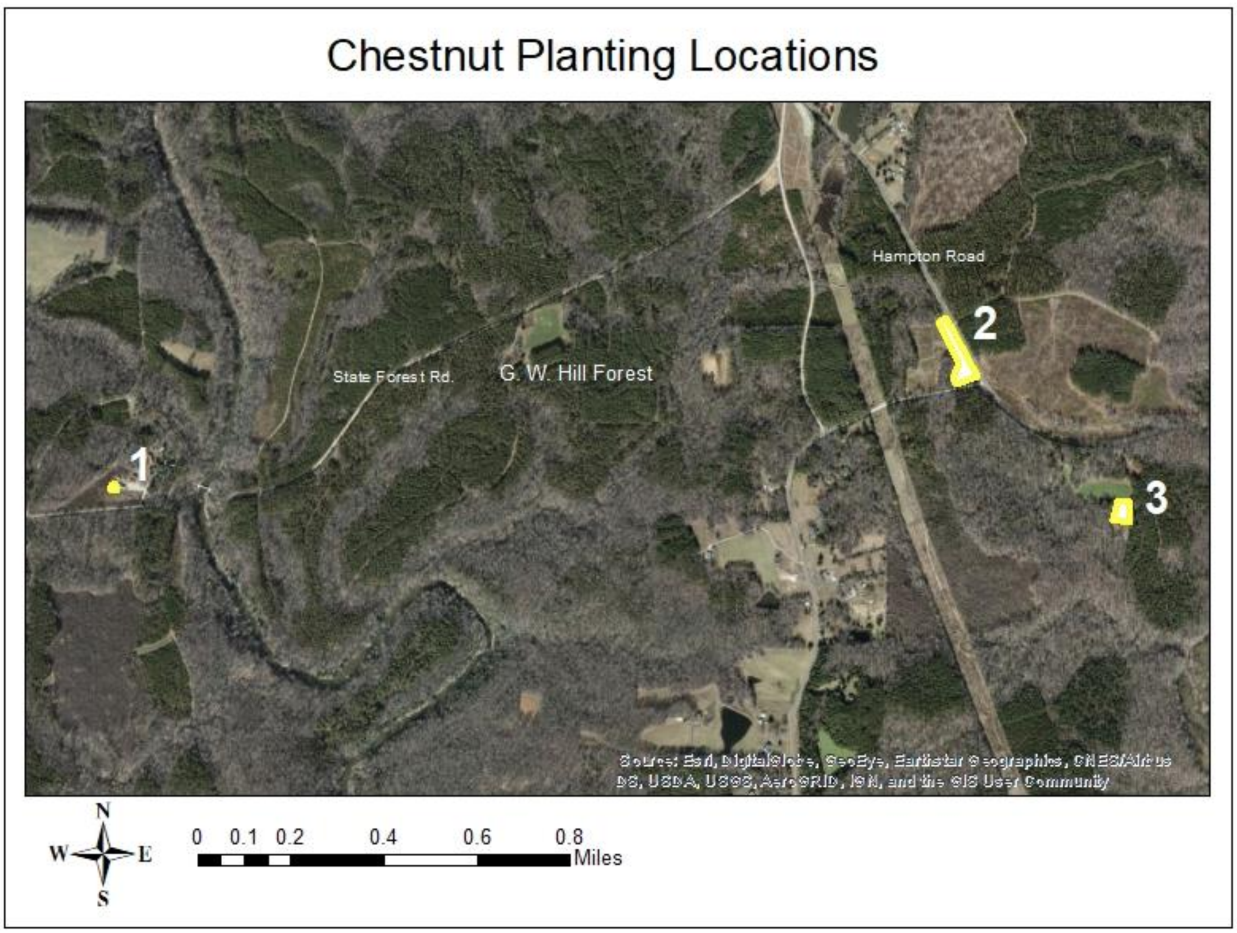


*Figure 2*: Aerial photograph of American chestnut planting sites at G.W. Hill Forest. Sites are outlined in yellow and labeled according to site number.

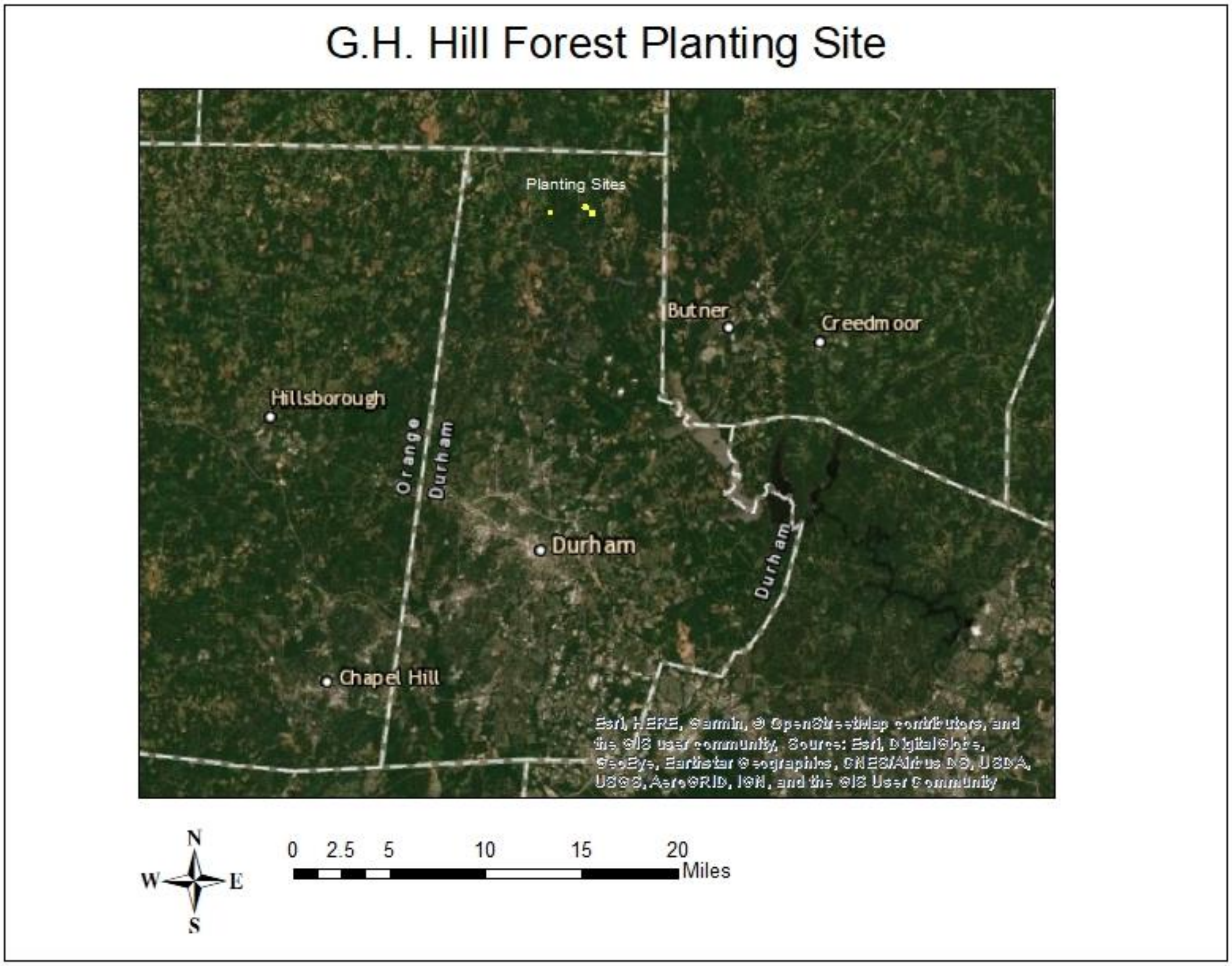


*Figure 3*: Location of American chestnut planting sites within northern Durham County, North Carolina. Planting sites highlighted in yellow.

## Description of sites and site preparation:

Site 1, the smallest of the three, contained one experimental block. Located directly adjacent to the Camp Slocum complex at Hill Forest, this site was burned before planting but the remaining standing vegetation (mostly grasses and forbs) were not cleared. The site was mown regularly throughout the summer of 2019 and boasted the lowest level of competition for the chestnuts as a result. The soil type at site 1 was a Georgeville silt loam (GeC), 6 to 10% slope ("Soil Map").

Site 2, the largest, contained four experimental blocks, as well as several improved seedlings that were planted randomly around the final block to be planted and were not included in the official data for this study. Located alongside Hampton Road, this site was drum-chopped by Elizabeth Snider prior to planting, but vegetation control was not implemented, resulting in a high incidence of competition. The soil type at site two was a Herndon silt loam (HrB), 2 to 6% slope ("Soil Map"). Although this is a well-drained soil series, Site 2 was often saturated, indicating poor drainage. This site features the largest variation in planting date and planting method, as well as the experimental block with the highest survival across the entire experiment. The position of site 2 in relation to site 3 is depicted in Figure 4 below, with site 2 in the upper left.


*Figure 4*: Relative position of planting sites 2 (upper left) and 3 (lower right) at Hill Forest.

Site 3 contained two complete blocks and was located off of Hampton Road, adjacent to a food plot maintained by deer hunters. There was no site preparation, although a portion (approximately one-third) of each block planted at site 3 is located on a contiguous patch of land with substantially reduced vegetation due to some kind of management. However, the exact use of this land is unclear. A closeup of site 3 is included below. The aforementioned managed land is the horizontal strip in the center. The soil type at site 3 is a Cecil fine sandy loam (CfB), 2 to 6% slope ("Soil Map").

*Figure 5*: Close-up view of planting site 3 located at the old Mangum Homestead on Hill Forest.

## Field planting:

All sites were marked for planting in the same way: utilizing a Keson open reel measuring tape, pin flags were placed in the ground at regular intervals to indicate planting locations such that each experimental block contained three reps laid out in linear, sequential fashion. Each rep within a block contained seedlings planted at one of three spacings: 5'x12', 7'x12', and 9'x12', with 7'x12' always located in the middle. Each block was planted with buffer trees around its periphery and between its individual reps, as described earlier. All seedlings were outfitted with a protector tube, secured around the main stem with a bamboo stake and a zip tie. Pin flags and metal identification tags remained after planting.

Field planting began at Site 1, with one complete rep planted on February 19th and the remaining two planted on March 14th. The first rep was planted at the north end of the site at a spacing of 9'x12', and the remaining two were planted at spacings 7'x12' and 5x'12', moving north to south. 44 improved and 53 buffer trees were planted, for a total of 95. Site 1 was the only planting location in which shovels were used in addition to dibble bars, which ostensibly affected the way soil was packed around planted seedlings. The effect of this method is described in further detail in the results section. In addition, slight deviations in the RCBD method became evident while assessing seedling survival: one buffer separating replicates was missing and was replaced by an improved seedling; and an additional improved seedling was planted in the 5'x12' replicate on top of that, resulting in 44 total improved seedlings in the block, as opposed to the intended 42.

Site 2 was planted in four phases, with one experimental block completed on each of March 15th, 16th, 17th, and April 20th, 2019. With 42 improved seedlings to each, the total number planted at site 2 is 168. These blocks shall hereafter be referred to as blocks 1, 2, 3, and 4, respectively, in order of their planting dates. The site was planted from north-northwest to south-southeast, with block 1 being the furthest north, and block 4 the furthest south. Spacings for replicates in blocks 1-3 ran from widest (9'x12') to narrowest (5'x12') in the same direction. An initial mistake in planting buffer trees between the first and second block resulted in a double row of unimproved seedlings between the two, a pattern that was kept in order to maintain continuity at the site. Blocks were laid out in a linear and sequential fashion, as mentioned above. Block 3 was not perfectly in line with blocks 1 and 2 and was instead flagged out angling slightly away from Hampton Road: a measure intentionally taken in order to avoid planting in a strip of heavy brush between the road and the cleared planting area. All seedlings in the first three blocks, including buffers, were planted using dibble bars. It should be noted that the metal tag identifying to which genetic family each seedling belonged was misplaced for one seedling in block 2; this individual was recorded as "unknown" for the purposes of statistical analysis and in the appendix.

On April 20th, the final day of planting, a large group of volunteers planted an additional experimental block (block 4) at site 2 with some key distinctions from other blocks previously planted at site 2. First, this block was planted at a slight angle: away from the road and from block 3, similar to the relationship between blocks 2 and 3. Second, the spacing pattern was reversed from the previous three blocks, meaning that the replicates from blocks 3 and 4 closest to each other were both planted at a spacing of 5'x12'. Third and perhaps most importantly, block 4 was planted entirely using a Stihl BT 45 Earth Auger. Individual holes were drilled using the auger, with volunteers following behind and planting seedlings into these holes. This allowed for not only remarkable efficiency, but also uniformity for each newly planted chestnut. As will be demonstrated later in this report, this planting method had by far the highest survival rate, despite the late date at which block 4's seedlings were planted (April 20th is far outside the recommended planting window in the Piedmont of North Carolina). The fourth and final distinction resulted directly from the third: as block 4 was completed with unexpected rapidity, there was ample time for several additional seedlings to be planted in the surrounding area. Lacking the space necessary to properly plant another full block, several extra improved seedlings were planted at random around the periphery of blocks 3 and 4, also in holes drilled with the auger. These were not counted, nor were their positions recorded for the experiment, as they did not conform to the prescribed RCBD approach outlined earlier. It was this final day of planting that perhaps bore the most significance for the aim of restoring American chestnut to the landscape, for reasons that will be examined in later sections.

Site 3 was planted in its entirety in the time between the plantings of blocks 3 and 4 at site 2. Planting at site 3 took place over two days: the first block (block 1) was planted on the western side of the site on March 19th, 2019, and the second (block 2) was planted on the eastern side on March 26th, for a total of 84 improved crosses. Both blocks were planted running north to south, with the furthest spacing scheme (9'x12') located at the north end and the closest (5'x12') at the south end. All seedlings at site 3 were planted using dibble bars. The metal identification tags of three seedlings, two in block 1 and one in block 2, were misplaced during planting, and thus were recorded as "unknown" in statistical analysis and the appendix as before.

## Measuring survival, height, and root collar diameter:

After all seven experimental blocks of seedlings were in the ground, they were left untreated for the entirety of the 2019 growing season, with the exception of the seedlings at site 1, which enjoyed regular mowing to keep competition levels low. Seedlings were not measured at the time of planting. A much more intensive first-year silvicultural approach was originally intended but proved outside the realm of possibility for logistical reasons, not the least of which was lack of human resources. This resulted in an abundance of competing vegetation, which in turn caused significant difficulty in recording growth and survival data, as the seedlings were quite often overtopped and hidden within competing grasses and foliage. Even so, each genetically improved seedling was counted and measured individually by hand.

Initial field survival was recorded on September 21st and 23rd, 2019. Site 1's overall survival was taken on the 21st; sites 2 and 3 were completed on the 23rd. Mortality was determined by the presence of green foliage. If no foliage was present, the seedling in question was recorded as dead. Hand maps were drawn of each replicate, and each seedling was marked according to its survival. These maps can be found in the project notebook that is meant to accompany this paper and any further work on this experiment, as well as in the appendix. Owing to the difficulty in traversing sites 2 and 3, as well as the challenges of locating the often-overtopped seedlings, growth measurements were not recorded until over a month later, on October 26th, 2019. Using the same hand-drawn survival maps, the total height and root collar diameter of each surviving seedling were recorded, both in millimeters. Heights were measured to the nearest millimeter, while diameters were measured to the nearest hundredth of a millimeter.

Seedlings that were determined to be dead at the time of these recordings were not included. Some seedlings that had green foliage at the time of the initial survival count no longer had any when attempting to measure them and were subsequently counted as dead. Height measurements were taken with a meter stick at the highest point of the main stem. In cases where the main stem was bent, angled, or drooping, it was held up against the meter stick to determine total height. This was not done if there was a severe bend in the main stem, however. Diameter measurements were taken using a pair of electronic microcalipers checked out from NC State's College of Natural Resources equipment room. Two measurements were taken at the root collar of each seedling at 90° angles and averaged to determine diameter. Neither buffer trees nor the additional improved seedlings planted around block 4 in site 2 were measured, nor was survival taken for these seedlings.

## Statistical analysis:

All data for the three measurable metrics involved in this study were entered into Microsoft Excel to produce relevant statistical and visual analysis. The first step in this process was to produce a comprehensive database of every individual improved seedling involved in the experiment. To do this, a number was given to each seedling on the hand-drawn maps mentioned above. These numbers were used as identifiers and are intended to be used similarly in the future as a reference. The sequence repeated by block, or in other words, the seedlings in each complete experimental block were numbered starting from zero, a method intended to avoid unwieldy numbers. The potential identification issue of repeat numbers is rectified by the site, block, and spacing (i.e. replicate) being recorded concurrently. The database can be found in the appendix attached to this report.

The next step was to record survival. The total count of living and dead seedlings was entered by site, replicate, and spacing. A total survival count across all sites was included in this step. Next, the evaluation method of the effect of the outlined independent variables on the dependent variables was determined.

For the effects of genetic family, site, planting date, and planting method on seedling survival, the analysis was relatively simple: using PivotTables in Excel, the number of surviving individuals as well as the total number of seedlings planted were lined up adjacent to the appropriate independent variable. The totals were converted into percentages, and bar graphs were produced displaying the results. Bar graphs with error bars were also constructed for the height and diameter metrics, in addition to more detailed analysis introduced in the following paragraph.

The impacts of the four variables on diameter and height were slightly more complex to analyze: the effect of genetic family required the use of the ANOVA single factor test, which will be explained in greater detail below; and the effect of site required the use of Two-Sample T-Tests (assuming unequal variances). The effect of planting date on seedling diameter was not considered, nor was the same effect of planting method. It was decided that the analysis of planting date was of relative unimportance, as the goal of the study is to identify how to manipulate certain variables in order to achieve greater viability of experimental chestnut crosses, and an earlier planting date would always be chosen with this goal in mind if possible. Planting method's effect on growth metrics was not analyzed due to an issue of sample size: the vast majority of seedlings were planted using dibble bars, and, although survival proved to be significantly higher using a power auger, the limited number of repetitions using both it and shovels influenced the decision to omit it as a variable for consideration.

For the effect of site on height and diameter, a relatively simple process was followed. Instead of attempting to prove superiority or inferiority of one site compared to another in its effect on growth, the focus instead was to demonstrate significant difference. Towards this end, for each of the two growth metrics, columns were constructed and organized by site. Every individual measurement recorded for that site was listed in the column. Heights, as mentioned before, were measured to the nearest millimeter, while diameters were measured to the nearest hundredth of a millimeter. To analyze these data against each other, a Two-sample T-Test assuming unequal variances was chosen in order to accommodate the differences in the sizes of the sample populations, and an alpha of 0.05 was used. Sites 1, 2, and 3 were all tested against each other using the T-Test available in Microsoft Excel, and P-Values were examined to determine statistical significance and whether the null hypothesis (that there was no difference between samples) could be rejected. The results of these tests will be examined in the results section.

The most complex and labor-intensive statistical analysis process in this experiment was to determine differences between genetic families in their effects on seedling growth. As there were so many more genetic families than sites, it was not efficient to perform t-tests on each pair of families in order to determine the presence of statistically significant differences. Instead, a combined approach using an ANOVA single-factor test in conjunction with T-Tests was used.

First, columns were created for each family and the measurements for height and diameter were individually included in these columns as before. Next, the families were separated into three groups of four such that the four families with the lowest average heights or diameters were placed together, the four with the next-highest averages were placed together, and the four with the highest averages were placed together. Two families, D3-7-33 and D4-27-78, were not included in this methodology due to their having no surviving seedlings. This allowed for twelve families to be analyzed, which in turn allowed for evenly sized groups of four for the analysis.

Once the families were organized this way, the ANOVA single-factor test was performed on each group in turn. This test was selected because it allows for the analysis of means across multiple populations. Regardless of whether any significant difference was found within groups, Two-sample T-Tests assuming unequal variances were then performed to determine the presence of significant differences across groups. In order to do this, one representative genetic family from each group was selected to be tested. In all cases, this was the family with the highest sample size (number of seedlings). By following this procedure, the representative groups of seedlings (one could call them height and diameter classes) were able to be tested against each other in the same way that sites 1, 2, and 3 were, as described above. The findings of these methods are displayed in the Results & Discussion section.

It should be noted that the focus of this phase of the experiment was on survival, not growth, of individual seedlings. Although a great deal of meaningful analysis was not possible due to the scope and timeframe of the project, T-Test data was included as the simplest means of determining early differences between families in survival and growth.

## RESULTS AND DISCUSSION:

### Overall survival:

Unfortunately, first-year field survival was much lower than was anticipated. Out of 295 total improved seedlings planted (minus those randomly planted outside the RCBD structure on April 20th), only 57 displayed green foliage when checked at the end of the growing season, for a total survival rate of 19.32 percent. There are a variety of factors likely at play for this, including drought, poor site quality, excessive competition, deer browse, and improper/variable planting due to the many different people involved in each phase of planting. It is also likely that the timing which the seedlings were planted contributed to the high mortality, although it should be noted that block 4 at site 2 was planted the latest of all (some seedlings had already leafed out in their containers at this point) and yet enjoyed the highest survival rate. It is difficult to pinpoint which of these interacting factors played the most significant role, but the scope of this experiment includes analysis only on the four variables mentioned in the methods section: site, genetic family, planting date, and planting method.

## Survival by site:

Seedling survival was best at site 3, with approximately 21% of specimens showing foliage when checked in September. Curiously, it was lowest at site 2, even though site 2 contained the block with the highest survival rate in the entire experiment, block 4, at about 36%. Competition observed was heavy and overtopped seedlings at both sites 2 and 3, almost certainly contributing to mortality; however, site 1 enjoyed vegetation control throughout the first growing season and displayed a survival rate very similar to that of site 2. Owing to the small sample sizes, especially for sites 1 and 3, few conclusions can be drawn concerning the interaction of site and survival.

A graph (Figure 8) was included below that excludes the positive effect of the use of a power auger on site 2's survival in an attempt to more accurately portray the effects of soil type on seedling survival. Although there were many other variables involved, the soil at site 2, a Herndon silt loam, appeared to be the poorest environment for chestnut growth after correcting for the effect of the power auger. Site 3's Cecil fine sandy loam performed the best, with site 1's Georgeville silt loam in between the two. The relatively superior performance of seedlings on the sandier soil of site 3 is in accordance with Douglass Jacobs' findings described earlier in this paper in *Toward development of silvical strategies for forest restoration of American chestnut (Castanea dentata) using blight-resistant hybrids*.

*Table 2*: Seedling survival by site.

| *Site* | *Surviving* | *Total Planted* | *Percent Surival* |
|---|---|---|---|
| *1* | 8 | 44 | 18.18% |
| *2* | 30 | 168 | 17.86% |
| *3* | 18 | 84 | 21.43% |

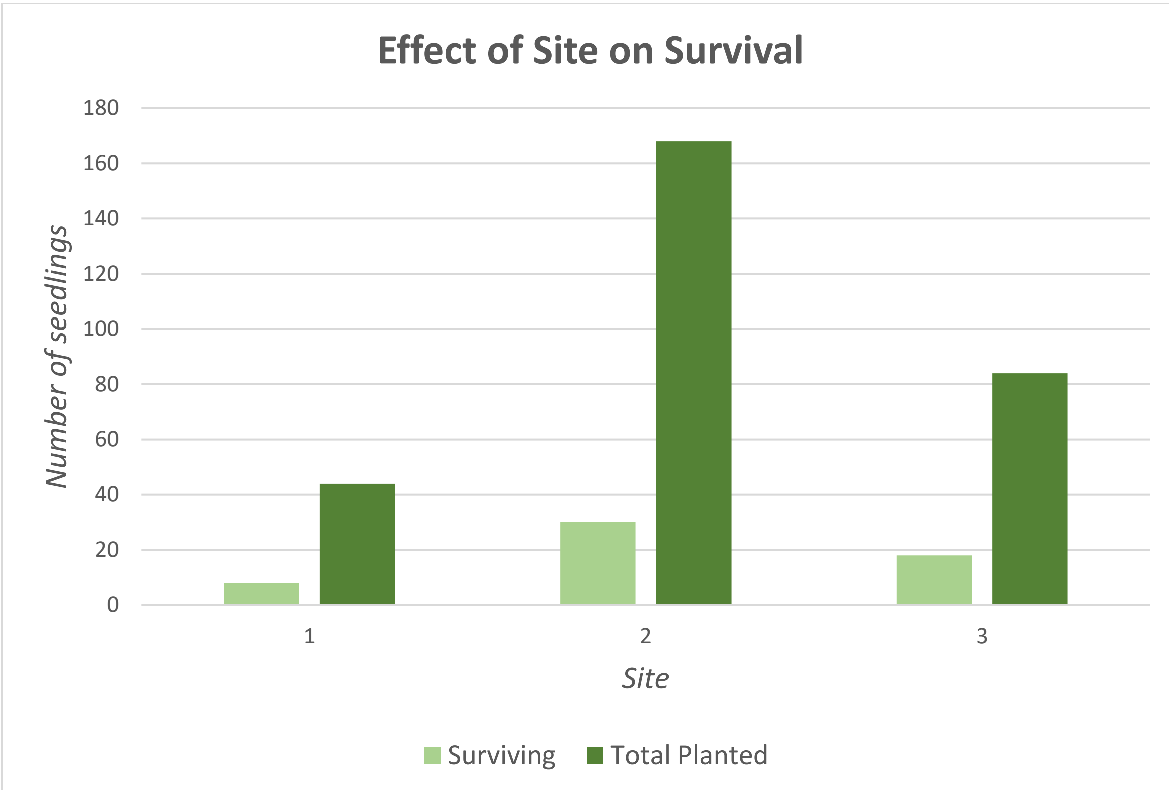


*Figure 6*: Total number of seedlings planted and total number surviving by site.

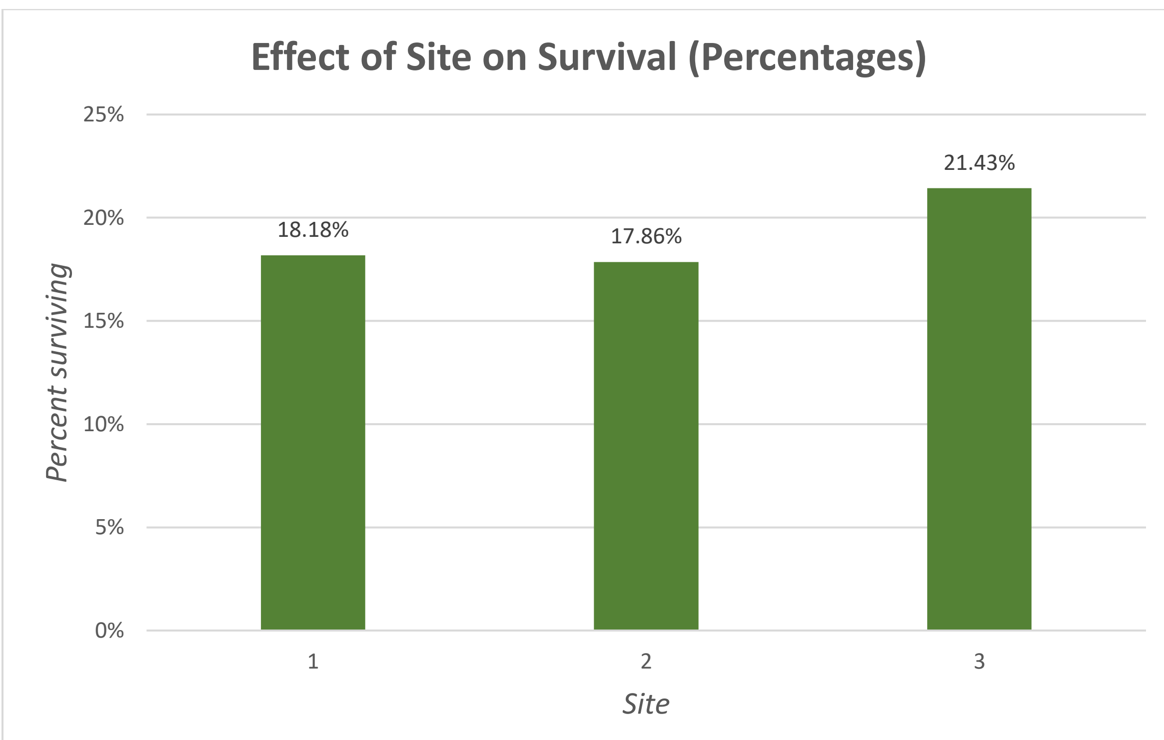


Figure 7: *Total percentage of surviving seedlings at each site.*

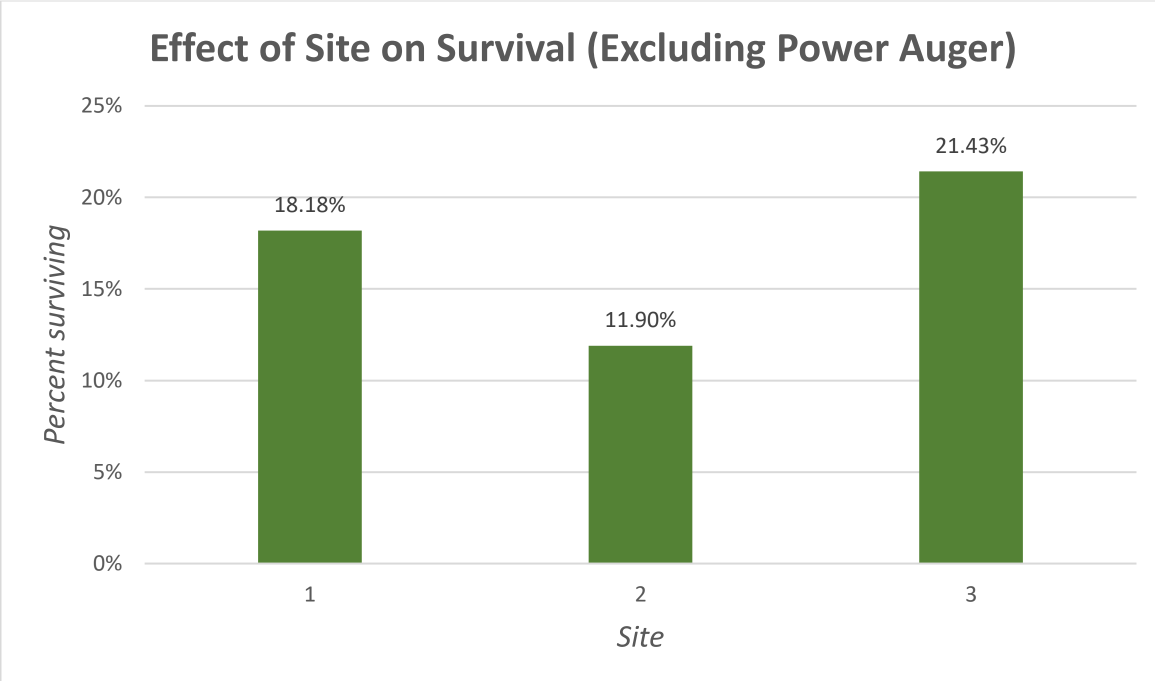


*Figure 8*: Total percentage of surviving seedlings at each site, excluding seedlings planted by power auger in block 4 at site 2.

## Survival by family*:*

Arguably the most important variable affecting survival rates for the purposes of this experiment, genetic family displayed considerable variability across all sites. While noting that a vast array of additional interacting factors likely contributed to the individual survival rates of each cross, the zero percent survival rate of crosses D3-7-33 and D4-27-78 are likely to have significant viability implications in future experiments, as are the 30, 33, and 44 percent survival rates of W1-19-19, D2-10-3, and W5-24-75, respectively. Interestingly, D-7-33 and D4-27-78 also had noticeably low survival rates in the initial survival count that was recorded before field planting. That data can be found in the attached appendix. It is unclear whether this is correlated with those families' low field survivals, however. Nothing definitive is known about these families that would set these apart from the others. Included in Table 2 below are also the statistics for the four seedlings whose genetic identities were lost in some way. Also evident in the table is the disparity in planting totals for each genetic family. Although one seedling from each family was intended to be planted in every replicate, this was not the case. Many reps contained repeat seedlings, while others were missing one or more families. The reasons for this are unclear, but the most likely include: duplicate or excluded seedling families in planting trays, errors by volunteers, and/or misreading of identification tags. However, trends in survival for each family remain.

*Table 3*: Seedling survival by family.

| *Family* | *Surviving* | *Total planted* | *Percent Survival* |
|---|---|---|---|
| *D2-10-3* | 7 | 21 | 33.33% |
| *D3-7-33* | 0 | 19 | 0% |
| *D4-27-78* | 0 | 15 | 0% |
| *D4-29-10* | 3 | 21 | 14.29% |
| *D5-28-88* | 2 | 17 | 11.76% |
| *Unknown* | 2 | 4 | 50% |
| *W1-19-19* | 6 | 20 | 30% |
| *W1-31-60* | 2 | 22 | 9.1% |
| *W2-20-147* | 5 | 24 | 20.83% |
| *W2-27-99* | 5 | 22 | 22.73% |
| *W4-14-67* | 3 | 19 | 15.79% |
| *W4-28-38* | 4 | 22 | 18.18% |
| *W4-32-87* | 2 | 23 | 8.7% |
| *W5-24-75* | 12 | 27 | 44.44% |
| *W8-19-26* | 3 | 20 | 15% |

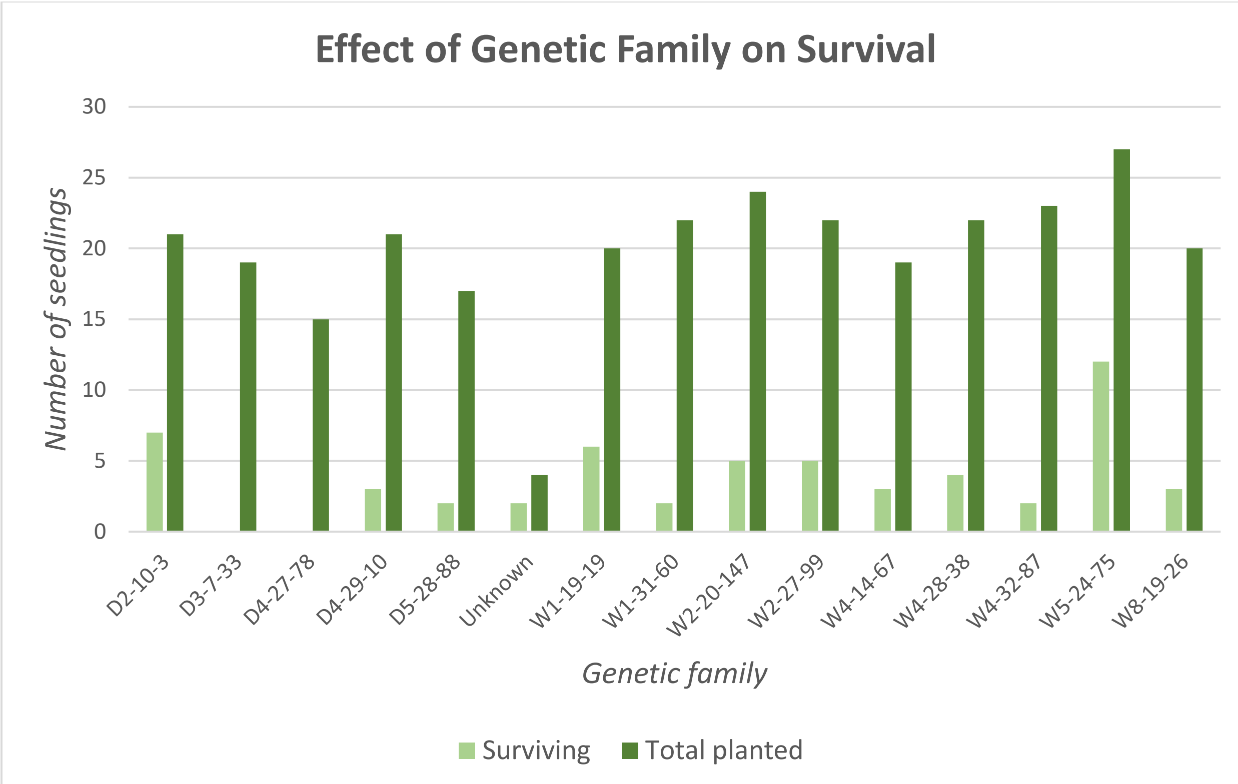


*Figure 9:* Total number of seedlings planted and total surviving in each family.

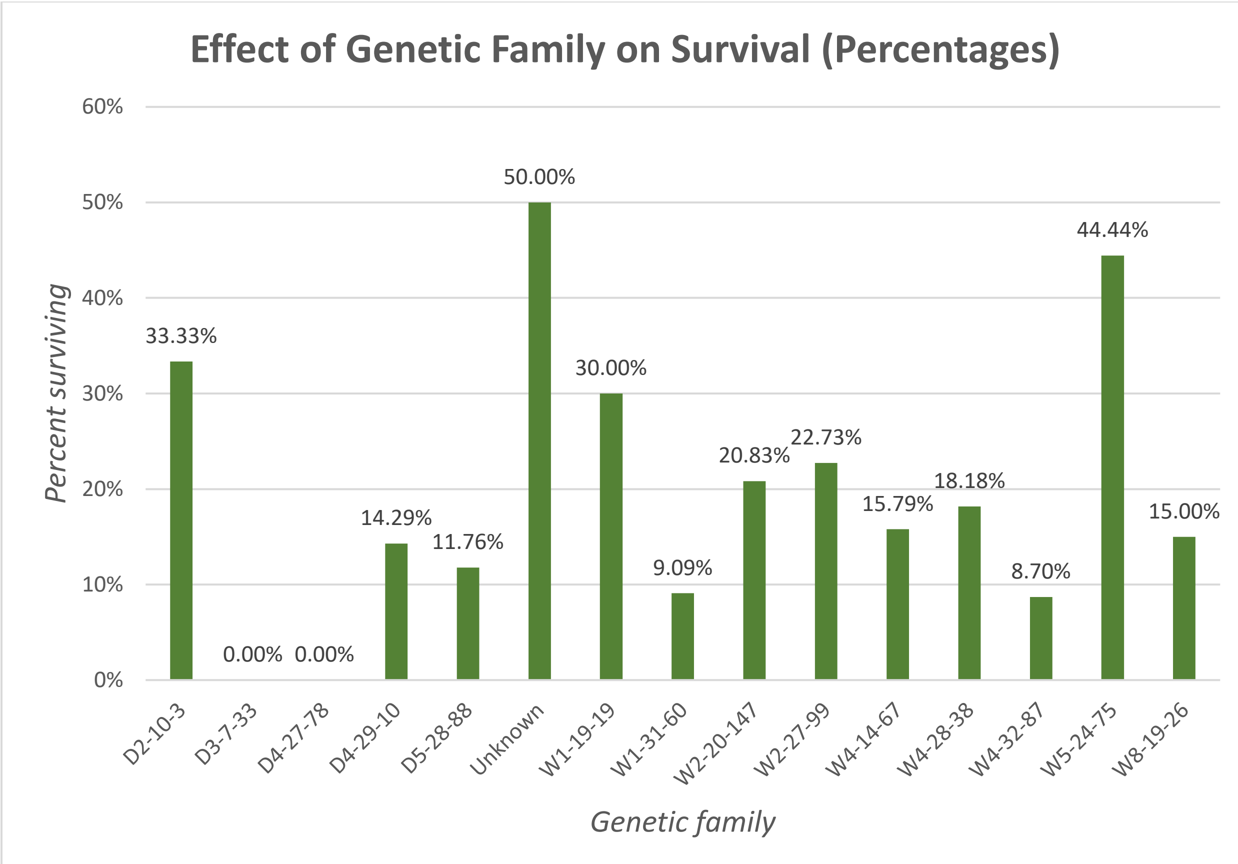


*Figure 10:* Total percentage of surviving seedlings in each family.

## Survival by planting date:

A seemingly uninteresting statistic to monitor, especially for those concerned with viability of experimental chestnut plantings, planting date should be expected by most reasonable land managers to correlate negatively with survival: the less time a seedling is afforded to take root and establish in its new environment, the more likely it would be to fail. However, the data below show that there is essentially no discernible relationship between the two, suggesting that planting date was in fact one of the less important factors in determining seedling mortality. A possible explanation for this phenomenon could be the containerized nature of the seedlings themselves, which could (and very well may) have provided a significant buffer against the stresses of planting that bareroot seedlings would otherwise experience in a similar scenario. Regardless, like the effect of site, it is difficult to draw conclusions about mortality from only the available planting date data alone.

*Table 4*: Seedling survival by planting date.

| *Planting Date* | *Surviving* | *Total Planted* | *Percent Survival* |
|---|---|---|---|
| *2.19.20* | 2 | 16 | 12.5% |
| *3.14.20* | 6 | 28 | 21.43% |
| *3.15.20* | 9 | 42 | 21.43% |
| *3.16.20* | 2 | 42 | 4.76% |
| *3.17.20* | 4 | 42 | 9.52% |
| *3.19.20* | 13 | 42 | 31% |
| *3.26.20* | 5 | 42 | 11.9% |
| *4.20.20* | 15 | 42 | 35.71% |

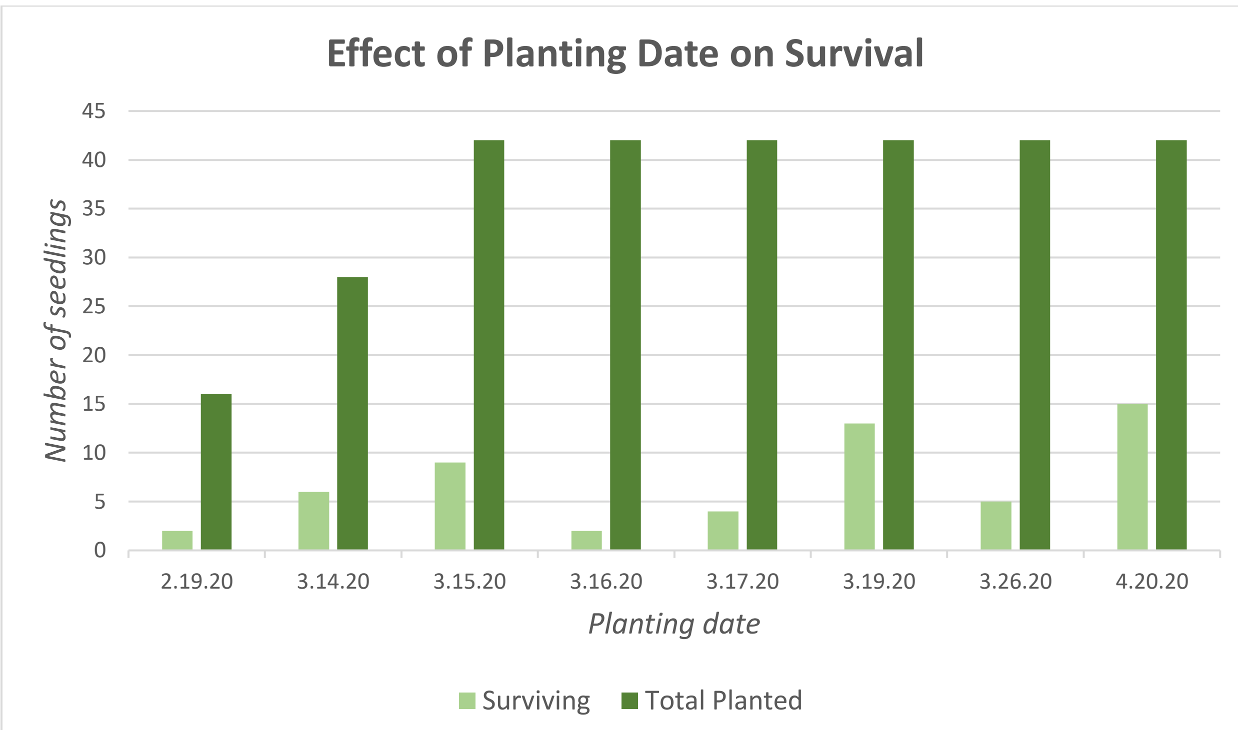


*Figure 11*: Total number of seedlings and total surviving from each planting date.

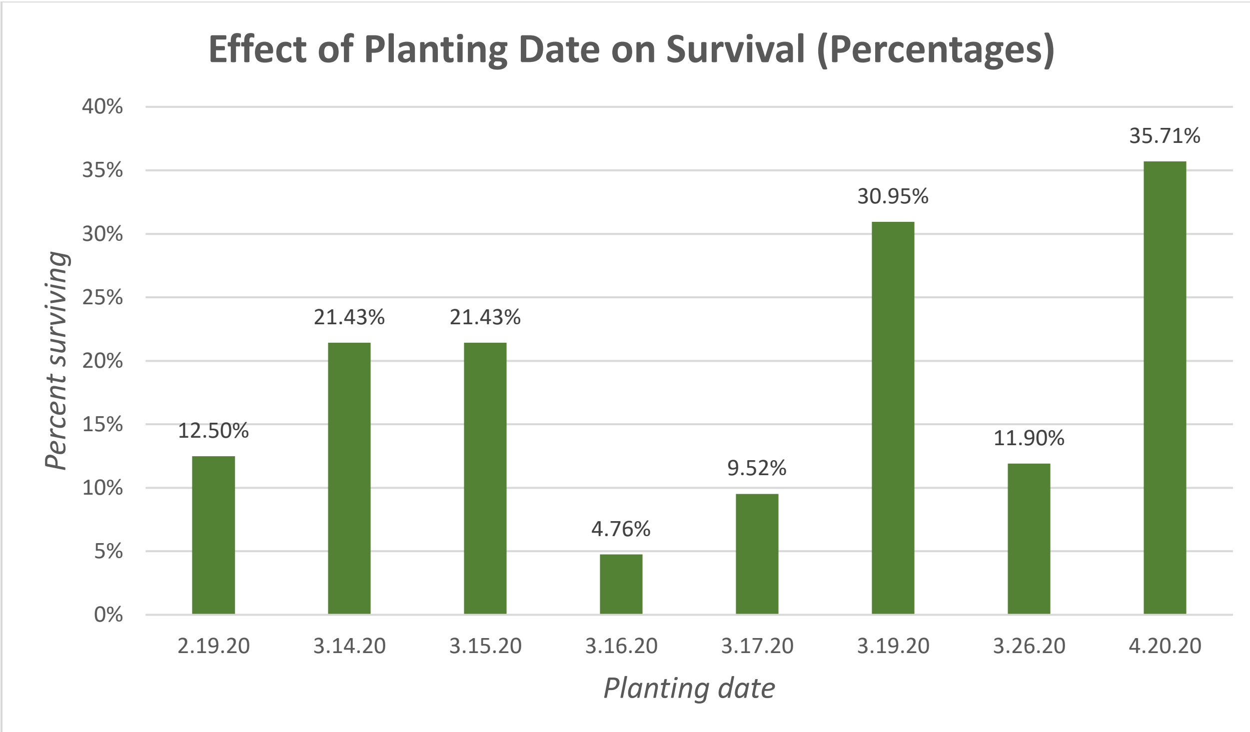


*Figure 12*: Total percentage of surviving seedlings from each planting date.

## Survival by planting method*:*

Planting method, like many, if not all of the previously discussed variables in this section, is almost certainly not entirely responsible for the differences in survival depicted in the figures below. However, it can be reasonably assumed that the power auger method did in fact result in a measurably and statistically significant difference in mortality. While the survival rates for dibble bar planting alone and shovels and dibble bars together hovered around a similar figure, the block planted via power auger essentially doubled the seedlings' survival rates. This is arguably one of the most important conclusions that can be drawn from this experiment so far. It is likely that the dibble planting method, especially at the hands of such a large and diverse array of planters, many of whom were probably not sufficiently experienced to make a perfect plant every time using the tool (especially considering the difficult, compacted soil conditions often encountered), did not allow the seedling's root system to extend properly from the potting mix in its individual container. There are two probable reasons for this: one, over-compaction of the surrounding soil; and two, the formulation of air pockets in the improperly compacted soil surrounding the container and root mass. By the same token, it is unlikely that the use of shovels in addition to dibble bars at site 1 was the deciding factor in improving survival from 15.7% in the dibble-only method to 18.2%, meager as that increase was. It is likely the benefits of regular mowing that provided that slight uptick in survival. The power auger, for its part, seemed to be able to drill a sufficiently large hole in which to plant a seedling (a recurring problem with dibbles), as well as one that had well-tilled soil through which roots could presumably easily penetrate.

*Table 5*: Seedling survival by planting method.

| *Method* | *Surviving* | *Total Planted* | *Percent Survival* |
|---|---|---|---|
| *Dibble Bar* | 33 | 210 | 15.71% |
| *Power Auger* | 15 | 42 | 35.71% |
| *Shovel and Dibble* | 8 | 44 | 18.18% |

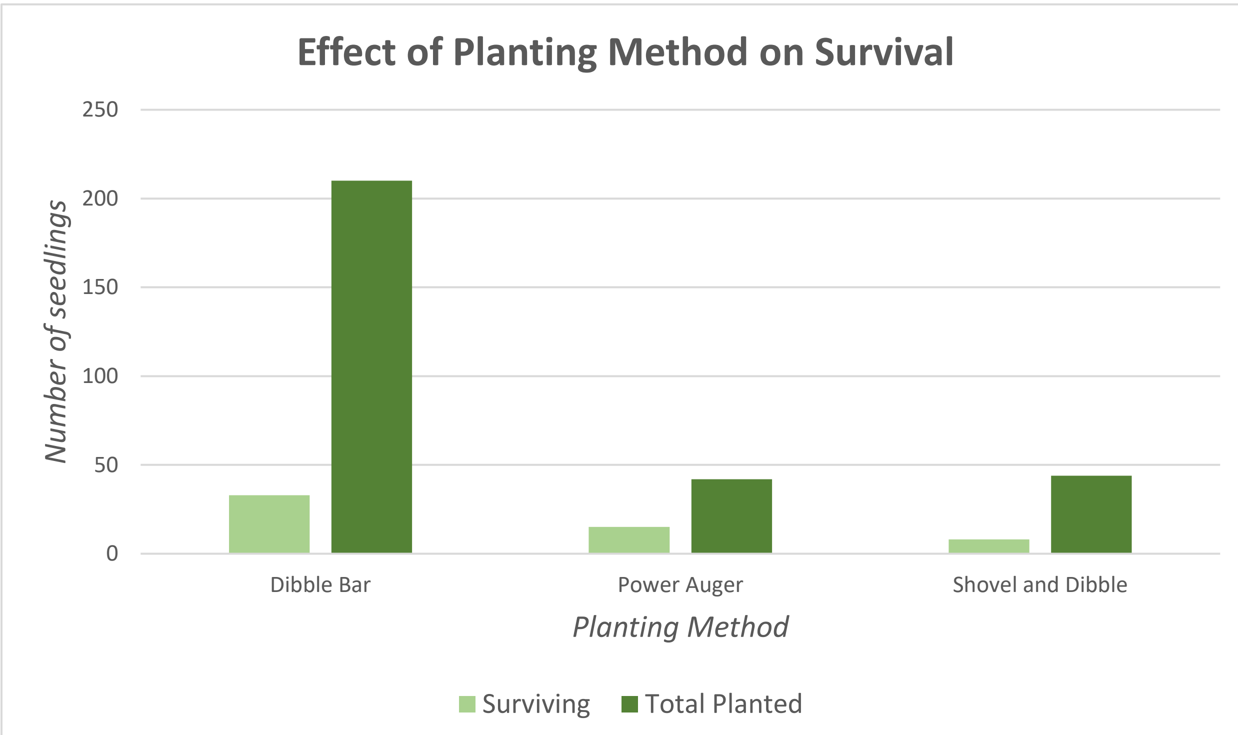


*Figure 13*. Total number of seedlings planted and total surviving using each planting method.

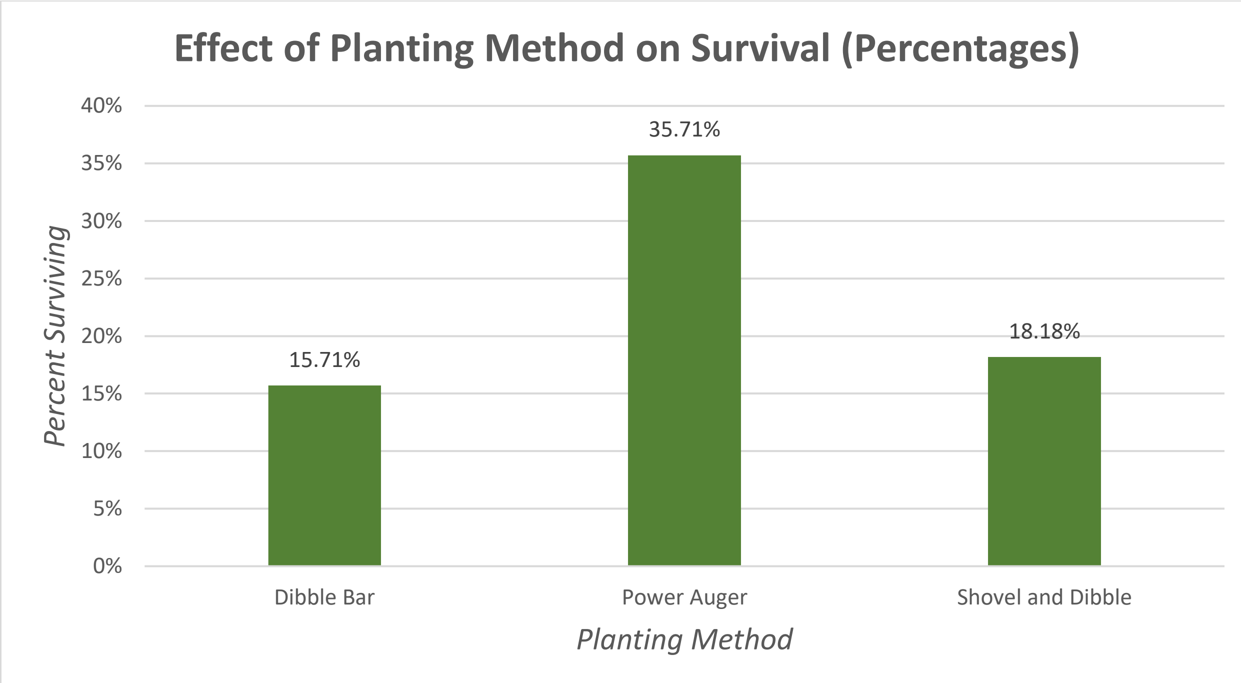


*Figure 14*: Total percentage of surviving seedlings using each planting method.

## Effects of planting site on seedling growth:

As will be demonstrated in the figures and tables below, planting site had a seemingly negligible effect on overall seedling growth, both in height and in diameter. While site 2 had the highest overall height and site 3 the largest diameter at the end of the first field growing season, subsequent T-Tests revealed that there was no statistical significance in the differences between any of the sites whatsoever. As stated earlier in the report, no measurements were taken at the end of the first growing season (post-germination in containers). As there is no way to know the growth of the seedlings specifically in the first field season, the height and diameter measurements taken in October may not reflect on the productivity of the individual sites on which seedlings were planted.

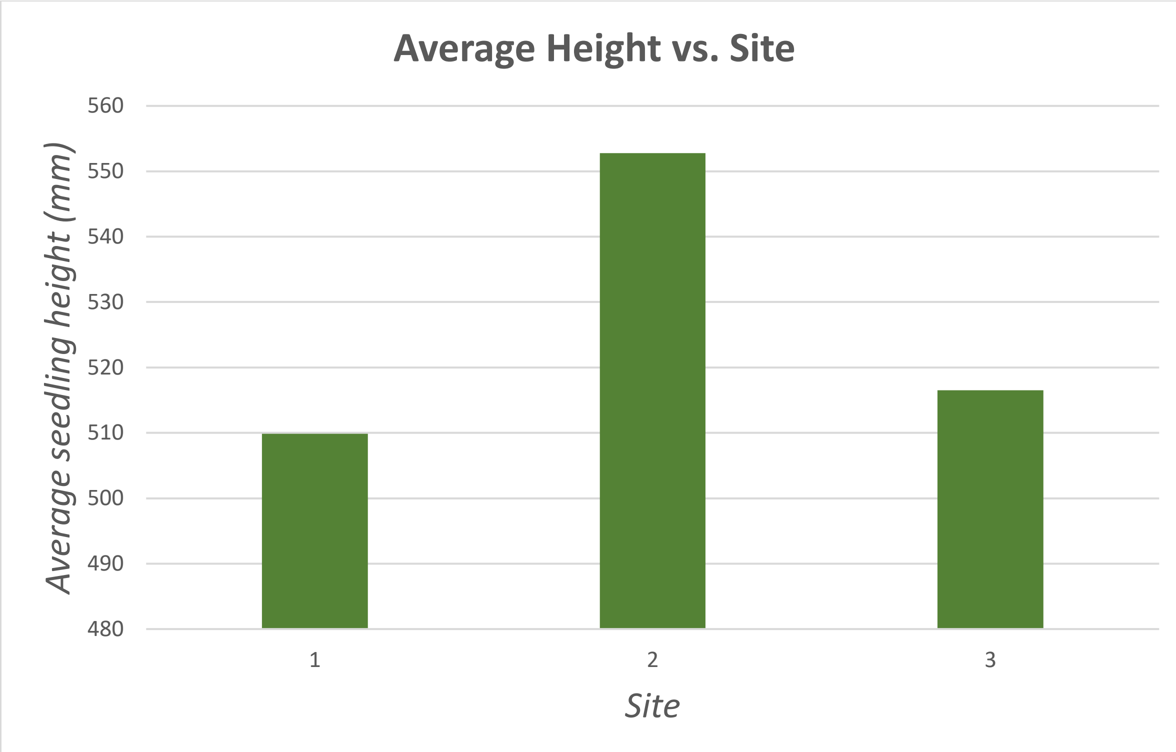


*Figure 15*: Average seedling height (in millimeters) at each planting site.

Site 2 had the largest average height at 552.8mm, while site 1's was the lowest at 509.8mm. Although there is no overlap in the visible error bars of site 2 with sites 1 and 3, the T-Tests below demonstrate that this difference is not significant.

*Table 6*: Testing for difference between sites 1 and 2. P-values for one- and two-tail tests at the 0.05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Site 1* | *Site 2* |
|---|---|---|
| Mean | 509.9 | 552.8 |
| Variance | 15236.6964 | 20153.3379 |
| Observations | 8 | 30 |
| Hypothesized Mean Difference | 0 | |
| df | 12 | |
| t Stat | -0.8456811 | |
| P(T<=t) one-tail | 0.20713789 | |
| t Critical one-tail | 1.78228756 | |
| P(T<=t) two-tail | 0.41427577 | |
| t Critical two-tail | 2.17881283 | |

*Table 7*: Testing for difference between sites 2 and 3. P-values for one- and two-tail tests at the 0.05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Site 2* | *Site 3* |
|---|---|---|
| Mean | 552.8 | 516.5 |
| Variance | 20153.3379 | 13580.6176 |
| Observations | 30 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 41 | |
| t Stat | 0.96118676 | |
| P(T<=t) one-tail | 0.17104647 | |
| t Critical one-tail | 1.682878 | |
| P(T<=t) two-tail | 0.34209294 | |
| t Critical two-tail | 2.01954097 | |

*Table 8*: Testing for difference between sites 1 and 3. P-values for one- and two-tail tests at the .05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Site 1* | *Site 3* |
|---|---|---|
| Mean | 509.875 | 516.5 |
| Variance | 15236.6964 | 13580.6176 |
| Observations | 8 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 13 | |
| t Stat | -0.1284758 | |
| P(T<=t) one-tail | 0.44986937 | |
| t Critical one-tail | 1.7709334 | |
| P(T<=t) two-tail | 0.89973875 | |
| t Critical two-tail | 2.16036866 | |

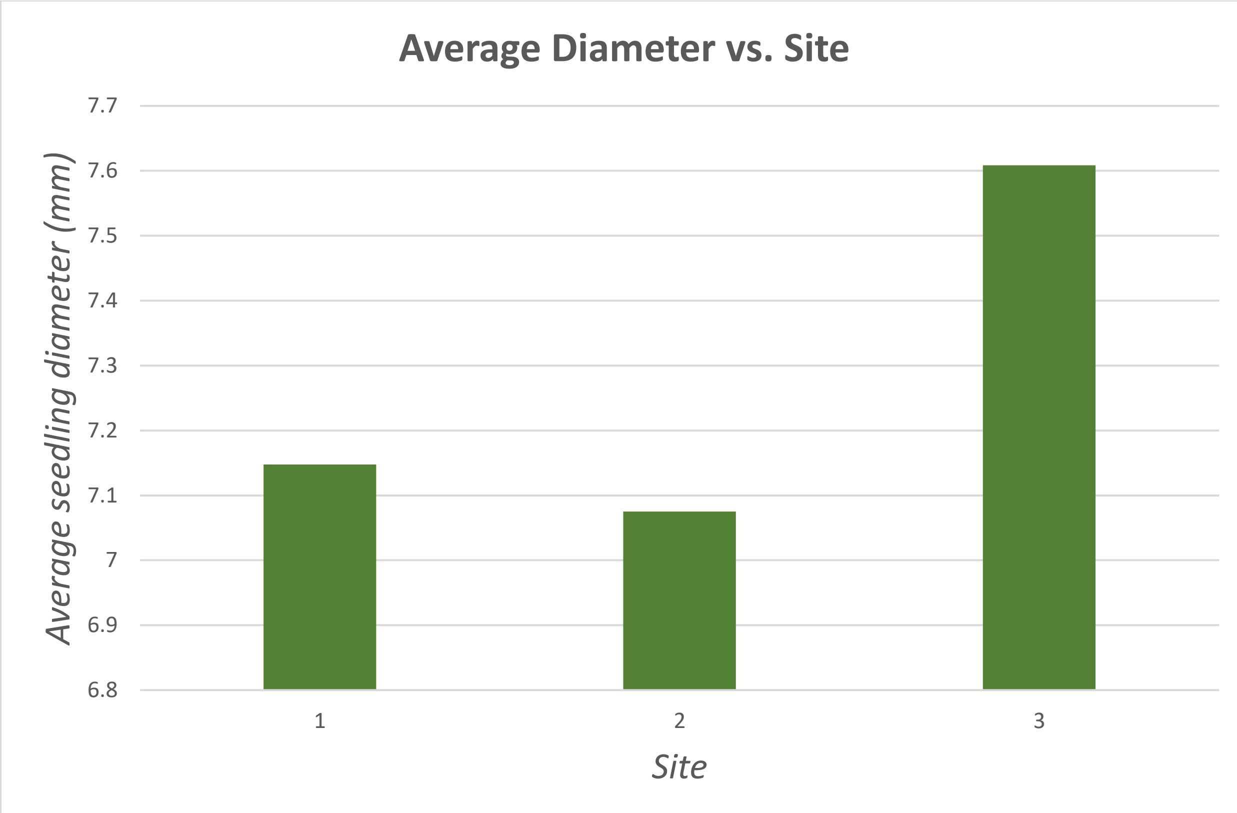


Figure 16: Average seedling root collar diameter (in millimeters) at each planting site.

The average diameter of seedlings at site 3 was the highest at 7.6 mm, while site 2's was the lowest at 7.08mm. The differences in absolute terms between average diameters was much smaller than for height, although this is to be expected from young seedlings and even woody plants in general. Similar to before, although the error bar of site 3 shows no overlap, the T-Tests below demonstrate that the differences between sites are not significant, and thus no conclusions can be drawn about the effect of site on either growth metric for this experiment.

*Table 9*: Testing for difference between sites 1 and 2. P-values for one- and two-tail tests at the .05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Site 1* | *Site 2* |
|---|---|---|
| Mean | 7.15 | 7.07 |
| Variance | 2.0445 | 2.1909 |
| Observations | 8 | 30 |
| Hypothesized Mean Difference | 0 | |
| df | 11 | |
| t Stat | 0.13170978 | |
| P(T<=t) one-tail | 0.44879601 | |
| t Critical one-tail | 1.79588482 | |
| P(T<=t) two-tail | 0.89759203 | |
| t Critical two-tail | 2.20098516 | |

*Table 10*: Testing for difference between sites 2 and 3. P-values for one- and two-tail tests at the .05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Site 2* | *Site 3* |
|---|---|---|
| Mean | 7.07 | 7.61 |
| Variance | 2.1908 | 1.8570 |
| Observations | 30 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 38 | |
| t Stat | -1.2777038 | |
| P(T<=t) one-tail | 0.10455234 | |
| t Critical one-tail | 1.68595446 | |
| P(T<=t) two-tail | 0.20910469 | |
| t Critical two-tail | 2.02439416 | |

*Table 11*: Testing for difference between sites 1 and 3. P-values for one- and two-tail tests at the .05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Site 1* | *Site 3* |
|---|---|---|
| Mean | 7.15 | 7.61 |
| Variance | 2.0445 | 1.8571 |
| Observations | 8 | 18 |
| Hypothesized Mean Difference | 0 | |
| df | 13 | |
| t Stat | -0.7694081 | |
| P(T<=t) one-tail | 0.22770216 | |
| t Critical one-tail | 1.7709334 | |
| P(T<=t) two-tail | 0.45540431 | |
| t Critical two-tail | 2.16036866 | |

## Effect of genetic family on seedling growth*:*

With more interesting implications on seedling growth, both within the scope of this experiment and the aims of the American Chestnut Foundation in general, and with mixed results compared to those with site in terms of statistically significant differences, the effect of genetic family was analyzed according to the approach outlined at the end of the Methods section. As stated before, families were grouped by average growth (height or diameter) and then ANOVA was used to test for differences between families within those groups. In no case was there found to be a difference within a group using ANOVA. For this reason, one representative family (the family with the largest population size) was chosen to represent each group and tested against other representative families to determine statistically significant differences between family groups. No differences between groups were found for diameter growth, but such differences were indicated for height growth.

Presented below are the following:
1. Bar graphs depicting average heights and diameters by genetic family
2. The family groupings, low to high, based on average height and diameter of each family
3. ANOVA test results for each group
4. T-Tests between representatives from each group.

*Height:*

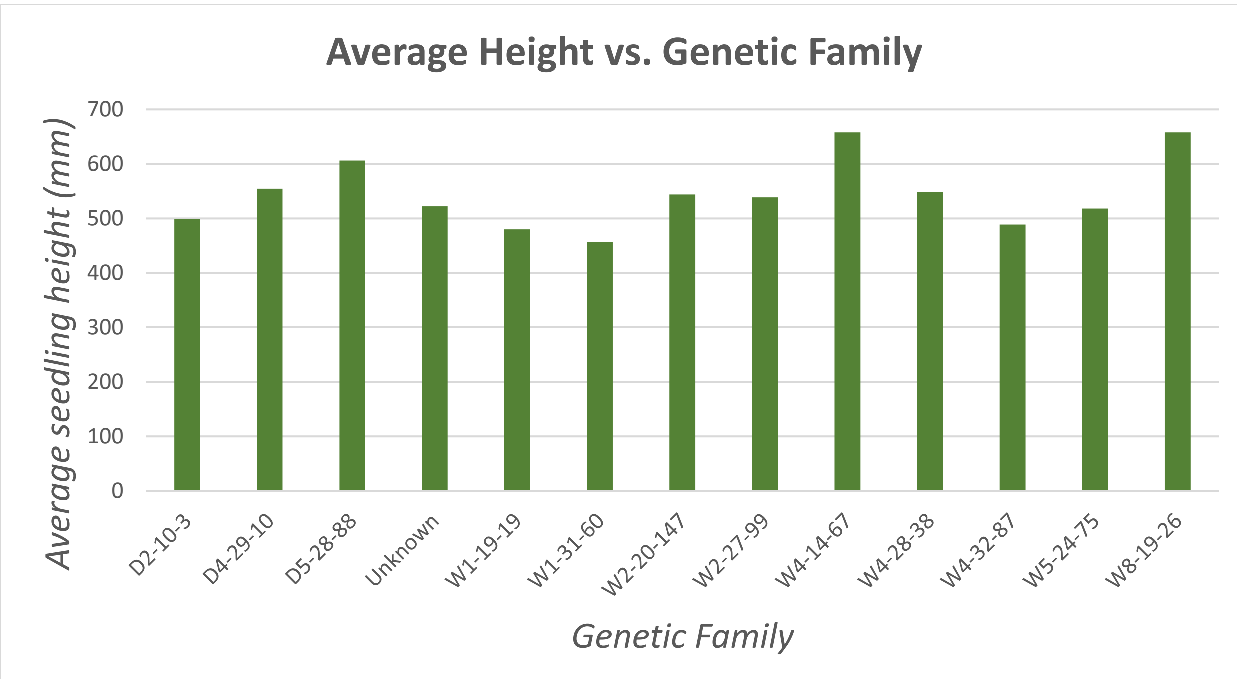


*Figure 17*: Average height (in millimeters) of seedlings from each of the fourteen improved crosses, with the exceptions of D3-7-33 and D4-27-78, neither of which had any surviving individuals available to measure.

Average heights across families varied considerably. Families with notably good growth include D5-28-88, W4-14-67, and W8-19-26; while families displaying notably inferior growth include W1-19-19, W1-31-60, and W4-32-87. It should be noted that a certain degree of selection bias is likely present in average heights across families. When planting seedlings in the field, trays (each intended to contain one seedling from every family) were selected individually by hand; this may have led to trays with larger- and healthier-looking seedlings on average being preferentially selected. Since seedlings were already one year old at the time of field planting, growth differences had already manifested themselves and were likely generally identifiable by planting tray. Further, it is important to mention that the above heights are not first-year field height growth, but rather total height after two years. Since heights and diameters were not measured after one year of container growth, it is impossible to determine exactly how much growth took place in the field.

## Family groupings (by average height) and accompanying ANOVA tests:

Low Height Families:

| | | | |
|---|---|---|---|
| W1-31-60 | W1-19-19 | W4-32-87 | D2-10-3 |

*Table 12*: ANOVA results for the low height group. In ANOVA Single Factor tests, if F > F crit, the null hypothesis is rejected. The null hypothesis for these tests is that the means differ significantly within family groups. Thus, the null hypothesis here cannot be rejected, and we conclude that there is no significant difference among means.

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 3086.22549 | 3 | 1028.74183 | 0.04778301 | 0.98552995 | 3.41053364 |
| Within Groups | 279882.833 | 13 | 21529.4487 | | | |
| | | | | | | |
| Total | 282969.059 | 16 | | | | |

Medium Height Families:

| | | | |
|---|---|---|---|
| W5-24-75 | W2-27-99 | W2-20-147 | W4-28-38 |

*Table 13*: ANOVA results for the medium height group. As F crit > F, the null hypothesis is not rejected. There is no significant difference among means.

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 4339.03846 | 3 | 1446.34615 | 0.08903406 | 0.96531265 | 3.04912499 |
| Within Groups | 357387 | 22 | 16244.8636 | | | |
| | | | | | | |
| Total | 361726.038 | 25 | | | | |

High Height Families:

| | | | |
|---|---|---|---|
| D4-29-10 | D5-28-88 | W4-14-67 | W8-19-26 |

*Table 14*: ANOVA results for the high height group. As F crit > F, the null hypothesis is not rejected. There is no significant difference among means.

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 21784.8485 | 3 | 7261.61616 | 0.38935286 | 0.76450296 | 4.3468314 |
| Within Groups | 130553.333 | 7 | 18650.4762 | | | |
| | | | | | | |
| Total | 152338.182 | 10 | | | | |

## T-Tests across groups:

*Table 15*: T-test between low group and medium group. Selected families: W1-19-19 and W5-24-75. P-values for one- and two-tail tests at the .05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Low Group* | *Med Group* |
|---|---|---|
| Mean | 480.17 | 518.25 |
| Variance | 7658.9667 | 9888.5682 |
| Observations | 6 | 12 |
| Hypothesized Mean Difference | 0 | |
| df | 11 | |
| t Stat | -0.8309393 | |
| P(T<=t) one-tail | 0.21183867 | |
| t Critical one-tail | 1.79588482 | |
| P(T<=t) two-tail | 0.42367734 | |
| t Critical two-tail | 2.20098516 | |

*Table 16*: T-test between medium and high groups. Selected families: W5-24-75 and W4-14-67. P-values for one- and two-tail tests at the .05 alpha level are able to reject the null hypothesis. There is a significant difference between groups.

| | *Med Group* | *High Group* |
|---|---|---|
| Mean | 518.25 | 657.67 |
| Variance | 9888.5682 | 3537.3333 |
| Observations | 12 | 3 |
| Hypothesized Mean Difference | 0 | |
| df | 5 | |
| t Stat | -3.1149928 | |
| P(T<=t) one-tail | 0.01320063 | |
| t Critical one-tail | 2.01504837 | |
| P(T<=t) two-tail | 0.02640126 | |
| t Critical two-tail | 2.57058184 | |

*Table 17*: T-test between low and high groups. Selected families: W1-19-19 and W4-14-67. P-values for one- and two-tail tests at the .05 alpha level are able l to reject the null hypothesis. There is a significant difference between groups.

| | *Low Group* | *High Group* |
|---|---|---|
| Mean | 480.17 | 657.67 |
| Variance | 7658.9667 | 3537.3333 |
| Observations | 6 | 3 |
| Hypothesized Mean Difference | 0 | |
| df | 6 | |
| t Stat | -3.5819462 | |
| P(T<=t) one-tail | 0.00580793 | |
| t Critical one-tail | 1.94318028 | |
| P(T<=t) two-tail | 0.01161586 | |
| t Critical two-tail | 2.44691185 | |

The results of the T-tests above, although somewhat simplistic, suggest that height differences between families belonging to the lowest height class and the medium height class hold no statistical significance. However, those differences between the medium and high height classes as well as the low and high height classes are significant. These data imply that families belonging to the top height class (D4-29-10, D5-28-88, W4-14-67, and W8-19-26) may be favorable in future experiments or restoration efforts in which rapid upward growth is a desired trait.

*Diameter:*

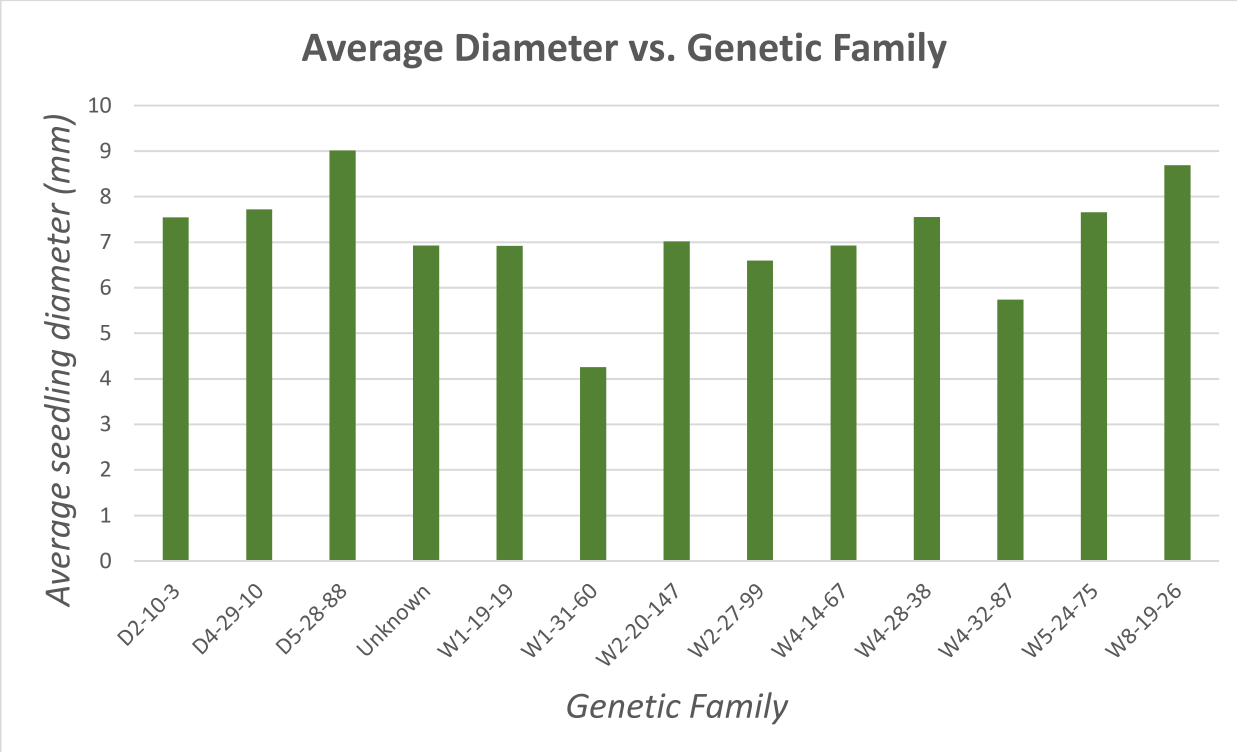


*Figure 18*: Average diameter (in millimeters) of seedlings from each of the fourteen improved crosses, with the exceptions of D3-7-33 and D4-27-78, neither of which had any surviving individuals available to measure.

As with average height, it is important to note that the above data do not show the exact diameter growth in the first field season; rather, they show the total average diameter after two growing seasons. The selection bias mentioned for height likely does not apply for diameter, as the differences are less obvious to the casual observer. The results for diameter were slightly different than those for height. Only two families, W1-31-60 and W4-32-87, display noticeably different average diameter from the rest of the improved crosses. Although these were members of the low average diameter group, the statistical tests showed that the differences between that group and the others were not statistically significant. However, such differences for these individual families could be found to be significant. The necessary scope and breadth of the tests needed to prove this precluded them from being included in this report.

## Family groupings (by average diameter) and accompanying ANOVA tests:

Low Diameter Families:

| | | | |
|---|---|---|---|
| W1-31-60 | W4-32-87 | W2-27-99 | W1-19-19 |

*Table 18*: ANOVA results for the low diameter group. As F crit > F, the null hypothesis is not rejected. There is no significant difference among means, even with the inclusion of the aforementioned families displaying poor diameter growth.

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 11.7681367 | 3 | 3.92271222 | 2.78484786 | 0.09067539 | 3.5874337 |
| Within Groups | 15.4945033 | 11 | 1.40859121 | | | |
| | | | | | | |
| Total | 27.26264 | 14 | | | | |

Medium Diameter Families:

| | | | |
|---|---|---|---|
| W4-14-67 | W2-20-147 | D2-10-3 | W4-28-38 |

*Table 19*: ANOVA results for the medium diameter group. As F crit > F, the null hypothesis is not rejected. There is no significant difference among means.

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 1.46832331 | 3 | 0.4894411 | 0.40341276 | 0.75266948 | 3.2873821 |
| Within Groups | 18.1987714 | 15 | 1.21325143 | | | |
| | | | | | | |
| Total | 19.6670947 | 18 | | | | |

High Diameter Families:

| | | | |
|---|---|---|---|
| W5-24-75 | D4-29-10 | W8-19-26 | D5-28-88 |

*Table 20*: ANOVA results for the high diameter group. As F crit > F, the null hypothesis is not rejected. There is no significant difference among means.

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 5.09077833 | 3 | 1.69692611 | 0.76980047 | 0.52759466 | 3.23887152 |
| Within Groups | 35.2699417 | 16 | 2.20437135 | | | |
| | | | | | | |
| Total | 40.36072 | 19 | | | | |

## T-Tests across groups:

*Table 21*: T-test between low and high groups. Selected families: W1-19-19 and D2-10-3. P-values for one- and two-tail tests at the .05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Low Group* | *High Group* |
|---|---|---|
| Mean | 6.92 | 7.54 |
| Variance | 1.1845 | 1.4503 |
| Observations | 6 | 7 |
| Hypothesized Mean Difference | 0 | |
| Df | 11 | |
| t Stat | -0.986680524 | |
| P(T<=t) one-tail | 0.172502719 | |
| t Critical one-tail | 1.795884819 | |
| P(T<=t) two-tail | 0.345005438 | |
| t Critical two-tail | 2.20098516 | |

*Table 22*: T-test between medium and high groups. Selected families: D2-10-3 and W5-24-75. P-values for one- and two-tail tests at the .05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Med Group* | *High Group* |
|---|---|---|
| Mean | 7.54 | 7.66 |
| Variance | 1.4503 | 2.3063 |
| Observations | 7 | 12 |
| Hypothesized Mean Difference | 0 | |
| df | 15 | |
| t Stat | -0.179144787 | |
| P(T<=t) one-tail | 0.430110692 | |
| t Critical one-tail | 1.753050356 | |
| P(T<=t) two-tail | 0.860221385 | |
| t Critical two-tail | 2.131449546 | |

*Table 23*: T-test between low and high groups. Selected families: W1-19-19 and W5-24-75. P-values for one- and two-tail tests at the .05 alpha level fail to reject the null hypothesis. There is no significant difference.

| | *Low Group* | *High Group* |
|---|---|---|
| Mean | 6.92 | 7.66 |
| Variance | 1.1845 | 2.3063 |
| Observations | 6 | 12 |
| Hypothesized Mean Difference | 0 | |
| df | 14 | |
| t Stat | -1.1868757 | |
| P(T<=t) one-tail | 0.12751328 | |
| t Critical one-tail | 1.76131014 | |
| P(T<=t) two-tail | 0.25502657 | |
| t Critical two-tail | 2.14478669 | |

Unlike the results for height, neither the ANOVA tests within groups nor the T-tests between group representatives yielded results that implied statistically significant differences between families. Thus, no meaningful conclusions can be drawn regarding desirability of particular families with respect to rapid diameter growth for future experiments or restoration efforts.

## CONCLUSION AND FUTURE RECOMMENDATIONS:

While there were many factors, not the least of which being the admittedly poor first-year survival of the chestnut seedlings in the field, that contributed to difficulties in carrying out the original aims of this long-term endeavor, there remain several important and useful conclusions regarding the approach to similar experiments in the future, as well as strengths and weaknesses of specific genetic crosses for use in such experiments. In effect, the results of this project so far demonstrate at least the viability (or lack thereof) of hybrid chestnut seedlings when planted in the North Carolina Piedmont and left unattended and untreated. While this is unlikely to be the favored approach for many chestnut restorationists, it nonetheless holds some value as a control experiment of sorts.

Although it is difficult to know for certain the causal relationships involved in the performance of specific genetic crosses in areas such as survival and growth, this experiment has hopefully laid some of the foundation for those relationships to be identified and explored further. A significantly larger sample size of improved seedlings coupled with a much higher survival rate would have almost certainly lent more weight to the predictive power generated by the activities outlined in this report. Nevertheless, those crosses that performed exceptionally well in the metrics with which this project is concerned may indeed prove to be viable options in The American Chestnut Foundation's ongoing mission to restore the species to the landscape, especially when coupled with some of the more successful practices described in the preceding paragraphs.

It remains evident that many corrective steps need to be taken for future experiments in order for them to succeed where this one did not, as evidenced by everything that has been presented until now. More regular watering, especially at sites 1 and 3, which were the drier sites, paired with fungicide application to newly germinated seedlings would likely have led to higher first-year survival in containers. This survival would mean nothing, of course, without the proper human resources to plant all available seedlings, or sufficient land in which to plant them, two attributes of efficacy that this experiment did not possess. A drastically earlier planting window likely would help similar projects in the future, as the first seedlings did not make it into the soil until February in this case, which ostensibly made the newly-planted seedlings less viable or resilient against competition. On the matter of competition, it should be highly recommended moving forward to aggressively control for competing vegetation in newly planted stands of hybrid chestnut, as thickets of sweetgum (*Liquidambar styraciflua*), *Rubus*, and various grasses and forbs proved to be a major problem for the test subjects, overtopping them in only one growing season. Further, supported by perhaps the most impressive display of viability improvement in the experiment, it is recommended to plant seedlings using a device such as a power auger that is able to create large planting holes while simultaneously alleviating the harmful physical qualities of possibly compacted soil. Use of such a tool gains even more in importance when planting is conducted with large groups of potentially inexperienced volunteers using implements such as dibble bars, which are easy to use but also equally easy to misuse.

It is an unfortunate consequence of the lower-than-expected vitality of the planted chestnut seedlings that many of the intended treatments to be implemented using the Randomized Complete Block Design likely will not have an opportunity to be carried out in a meaningful way at the G.W. Hill Forest site. Therefore, it is the recommendation of the author to any who may attempt a similar experiment in the future, to pay careful attention to all possible interacting mortality factors when planting hybrid chestnuts in the field. It is my hope that at least some of the concepts and findings from this phase of the experiment may go towards bolstering the strength of another, similar endeavor in the near future.

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| | |
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## APPENDIX:

This appendix contains an initial survival count of all seedlings prior to field planting and a complete database of every improved seedling involved in the experiment. Also included are the pictures of hand-drawn field maps previously mentioned in this report.

*Table 24*: Counts of surviving seedlings by family taken on January 12, 2019. "Questionable" means that it could not be determined with certainty if the seedling was alive. Note that family W4-28-38 is not present in the table. This is due to an inadvertent omission while recording survival.

| FAMILY | SURVIVORS | QUESTIONABLE |
|---|---|---|
| **D2-10-3** | 52 | 0 |
| **D3-7-33** | 33 | 0 |
| **D4-27-78** | 38 | 3 |
| **D4-29-10** | 45 | 1 |
| **D5-28-88** | 40 | 8 |
| **W1-19-19** | 51 | 5 |
| **W1-31-60** | 48 | 6 |
| **W2-20-147** | 36 | 0 |
| **W2-27-99** | 47 | 0 |
| **W4-14-67** | 57 | 2 |
| **W4-28-38** | N/A | N/A |
| **W4-32-87** | 53 | 2 |
| **W5-24-75** | 48 | 1 |
| **W8-19-26** | 46 | 3 |
| **BUFFER** | 663 | N/A |
| **UNKNOWN** | 9 | 0 |

*Table 25*: Complete list of every improved seedling involved in this study. In this case, “0” for survival means the seedling did not survive. Numbers in the “seedling” column correspond to those assigned in the hand-drawn site maps.

| SITE | BLOCK | SEEDLING | FAMILY | DATE | PLANTING METHOD | SURVIVAL | HEIGHT (MM) | DIAMETER (MM) | SPACING (FT) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 1 | 1 | W1-19-19 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 2 | W2-20-147 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 3 | W5-24-75 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 4 | D3-7-33 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 5 | W5-24-75 | 19-Feb | Shovel and Dibble | 1 | 317 | 5.96 | 12x5 |
| **1** | 1 | 6 | D4-29-10 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 7 | W4-32-87 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 8 | D2-10-3 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 9 | W2-27-99 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 10 | W4-14-67 | 19-Feb | Shovel and Dibble | 1 | 693 | 8.44 | 12x5 |
| **1** | 1 | 11 | W1-19-19 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 12 | W4-32-87 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 13 | D5-28-88 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 14 | W1-31-60 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 15 | W8-19-26 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 16 | W4-28-38 | 19-Feb | Shovel and Dibble | 0 | N/A | N/A | 12x5 |
| **1** | 1 | 17 | W2-20-147 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 18 | W2-27-99 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 19 | D3-7-33 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 20 | W4-14-67 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 21 | D4-29-10 | 14-Mar | Shovel and Dibble | 1 | 660 | 8.47 | 12x7 |
| **1** | 1 | 22 | W4-28-38 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 23 | D4-27-78 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 24 | W1-31-60 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 25 | W8-19-26 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 26 | W5-24-75 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 27 | D5-28-88 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 28 | W1-31-60 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 29 | W4-32-87 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 30 | W1-19-19 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x7 |
| **1** | 1 | 31 | D2-10-3 | 14-Mar | Shovel and Dibble | 1 | 431 | 6.21 | 12x9 |
| **1** | 1 | 32 | D3-7-33 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |
| **1** | 1 | 33 | D5-28-88 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |
| **1** | 1 | 34 | W5-24-75 | 14-Mar | Shovel and Dibble | 1 | 461 | 9.31 | 12x9 |
| **1** | 1 | 35 | W8-19-26 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 1 | 36 | W1-31-60 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |
| **1** | 1 | 37 | W4-28-38 | 14-Mar | Shovel and Dibble | 1 | 561 | 7.29 | 12x9 |
| **1** | 1 | 38 | W2-27-99 | 14-Mar | Shovel and Dibble | 1 | 486 | 5.74 | 12x9 |
| **1** | 1 | 39 | W4-32-87 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |
| **1** | 1 | 40 | W4-14-67 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |
| **1** | 1 | 41 | D4-27-78 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |
| **1** | 1 | 42 | D4-29-10 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |
| **1** | 1 | 43 | W2-20-147 | 14-Mar | Shovel and Dibble | 0 | N/A | N/A | 12x9 |
| **1** | 1 | 44 | W1-19-19 | 14-Mar | Shovel and Dibble | 1 | 470 | 5.76 | 12x9 |
| **2** | 1 | 1 | W4-32-87 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 2 | D3-7-33 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 3 | W1-19-19 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 4 | W4-32-87 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 5 | W5-24-75 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 6 | D2-10-3 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 7 | W8-19-26 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 8 | D4-27-78 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 9 | W1-19-19 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 10 | W2-27-99 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 11 | W1-31-60 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 12 | W2-20-147 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 13 | W4-28-38 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 14 | D4-29-10 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 1 | 15 | W4-32-87 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 16 | D3-7-33 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 17 | W1-19-19 | 15-Mar | Dibble Bar | 1 | 455 | 6.56 | 12x7 |
| **2** | 1 | 18 | W5-24-75 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 19 | W2-20-147 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 20 | W2-27-99 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 21 | W8-19-26 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 22 | D2-10-3 | 15-Mar | Dibble Bar | 1 | 400 | 5.87 | 12x7 |
| **2** | 1 | 23 | W2-20-147 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 24 | W4-28-38 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 25 | W4-32-87 | 15-Mar | Dibble Bar | 1 | 293 | 5.27 | 12x7 |
| **2** | 1 | 26 | D4-27-78 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 27 | D4-29-10 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 28 | W1-31-60 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 1 | 29 | W5-24-75 | 15-Mar | Dibble Bar | 1 | 651 | 9.75 | 12x9 |
| **2** | 1 | 30 | W4-28-38 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 1 | 31 | W8-19-26 | 15-Mar | Dibble Bar | 1 | 894 | 10.92 | 12x9 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **2** | 1 | 32 | W4-32-87 | 15-Mar | Dibble Bar | 1 | 685 | 6.21 | 12x9 |
| **2** | 1 | 33 | W2-27-99 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 1 | 34 | D4-29-10 | 15-Mar | Dibble Bar | 1 | 455 | 6.73 | 12x9 |
| **2** | 1 | 35 | D4-27-78 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 1 | 36 | W4-14-67 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 1 | 37 | W2-27-99 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 1 | 38 | D5-28-88 | 15-Mar | Dibble Bar | 1 | 640 | 9.06 | 12x9 |
| **2** | 1 | 39 | W2-27-99 | 15-Mar | Dibble Bar | 1 | 199 | 4.9 | 12x9 |
| **2** | 1 | 40 | W2-20-147 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 1 | 41 | D3-7-33 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 1 | 42 | D2-10-3 | 15-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 1 | D4-29-10 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 2 | W2-20-147 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 3 | Unknown | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 4 | W5-24-75 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 5 | D4-29-10 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 6 | W1-31-60 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 7 | W4-14-67 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 8 | W1-31-60 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 9 | D4-27-78 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 10 | W4-32-87 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 11 | W5-24-75 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 12 | W8-19-26 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 13 | D2-10-3 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 14 | D3-7-33 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 2 | 15 | D2-10-3 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 16 | D3-7-33 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 17 | D4-29-10 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 18 | W1-31-60 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 19 | D4-27-78 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 20 | W4-28-38 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 21 | D5-28-88 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 22 | W4-14-67 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 23 | W1-19-19 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 24 | W8-19-26 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 25 | W4-32-87 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 26 | W2-20-147 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 27 | W5-24-75 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 28 | W2-27-99 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 2 | 29 | D4-27-78 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |

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|---|---|---|---|---|---|---|---|---|---|
| **2** | 2 | 30 | D2-10-3 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 31 | W1-19-19 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 32 | W4-14-67 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 33 | W2-20-147 | 16-Mar | Dibble Bar | 1 | 486 | 7.93 | 12x9 |
| **2** | 2 | 34 | W4-28-38 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 35 | W1-31-60 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 36 | W2-20-147 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 37 | D5-28-88 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 38 | W4-32-87 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 39 | D4-29-10 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 40 | W2-27-99 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 41 | W8-19-26 | 16-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 2 | 42 | W5-24-75 | 16-Mar | Dibble Bar | 1 | 527 | 6.78 | 12x9 |
| **2** | 3 | 1 | D3-7-33 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 2 | W2-27-99 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 3 | W8-19-26 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 4 | W4-28-38 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 5 | D5-28-88 | 17-Mar | Dibble Bar | 1 | 572 | 8.97 | 12x5 |
| **2** | 3 | 6 | W4-28-38 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 7 | W2-20-147 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 8 | W4-14-67 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 9 | D2-10-3 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 10 | W5-24-75 | 17-Mar | Dibble Bar | 1 | 394 | 4.73 | 12x5 |
| **2** | 3 | 11 | W1-19-19 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 12 | W5-24-75 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 13 | D4-29-10 | 17-Mar | Dibble Bar | 1 | 549 | 7.97 | 12x5 |
| **2** | 3 | 14 | W1-31-60 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **2** | 3 | 15 | D5-28-88 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 16 | W1-31-60 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 17 | W2-20-147 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 18 | W2-27-99 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 19 | W4-32-87 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 20 | D2-10-3 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 21 | D4-27-78 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 22 | D4-29-10 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 23 | W4-14-67 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 24 | W1-19-19 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 25 | W5-24-75 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 26 | W8-19-26 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 27 | W4-28-38 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |

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|---|---|---|---|---|---|---|---|---|---|
| **2** | 3 | 28 | D2-10-3 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **2** | 3 | 29 | D2-10-3 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 30 | W1-31-60 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 31 | D3-7-33 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 32 | D4-27-78 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 33 | D4-29-10 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 34 | D5-28-88 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 35 | W4-14-67 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 36 | W4-32-87 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 37 | W5-24-75 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 38 | W2-20-147 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 39 | W1-19-19 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 40 | W4-28-38 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 3 | 41 | W2-27-99 | 17-Mar | Dibble Bar | 1 | 712 | 7.5 | 12x9 |
| **2** | 3 | 42 | W8-19-26 | 17-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 1 | W4-28-38 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 2 | W5-24-75 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 3 | D4-27-78 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 4 | D5-28-88 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 5 | W1-19-19 | 20-Apr | Power Auger | 1 | 449 | 6.08 | 12x9 |
| **2** | 4 | 6 | W4-28-38 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 7 | W8-19-26 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 8 | W4-14-67 | 20-Apr | Power Auger | 1 | 691 | 6.51 | 12x9 |
| **2** | 4 | 9 | W2-27-99 | 20-Apr | Power Auger | 1 | 664 | 8.16 | 12x9 |
| **2** | 4 | 10 | W2-20-147 | 20-Apr | Power Auger | 1 | 481 | 6 | 12x9 |
| **2** | 4 | 11 | W1-31-60 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 12 | D2-10-3 | 20-Apr | Power Auger | 1 | 515 | 8.99 | 12x9 |
| **2** | 4 | 13 | D3-7-33 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 14 | D4-29-10 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x9 |
| **2** | 4 | 15 | W4-32-87 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |
| **2** | 4 | 16 | W5-24-75 | 20-Apr | Power Auger | 1 | 608 | 6.29 | 12x7 |
| **2** | 4 | 17 | W8-19-26 | 20-Apr | Power Auger | 1 | 631 | 6.93 | 12x7 |
| **2** | 4 | 18 | W4-14-67 | 20-Apr | Power Auger | 1 | 589 | 5.84 | 12x7 |
| **2** | 4 | 19 | D4-29-10 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |
| **2** | 4 | 20 | D3-7-33 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |
| **2** | 4 | 21 | W2-20-147 | 20-Apr | Power Auger | 1 | 751 | 6.75 | 12x7 |
| **2** | 4 | 22 | W1-19-19 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |
| **2** | 4 | 23 | D5-28-88 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |
| **2** | 4 | 24 | D2-10-3 | 20-Apr | Power Auger | 1 | 536 | 8.59 | 12x7 |
| **2** | 4 | 25 | D4-27-78 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |

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|---|---|---|---|---|---|---|---|---|---|
| **2** | 4 | 26 | W1-31-60 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |
| **2** | 4 | 27 | W2-27-99 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |
| **2** | 4 | 28 | W4-28-38 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x7 |
| **2** | 4 | 29 | W5-24-75 | 20-Apr | Power Auger | 1 | 489 | 7.63 | 12x5 |
| **2** | 4 | 30 | W4-14-67 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **2** | 4 | 31 | D3-7-33 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **2** | 4 | 32 | W1-19-19 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **2** | 4 | 33 | D4-29-10 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **2** | 4 | 34 | W2-20-147 | 20-Apr | Power Auger | 1 | 464 | 6.2 | 12x5 |
| **2** | 4 | 35 | D2-10-3 | 20-Apr | Power Auger | 1 | 491 | 7.21 | 12x5 |
| **2** | 4 | 36 | W4-28-38 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **2** | 4 | 37 | W1-31-60 | 20-Apr | Power Auger | 1 | 711 | 5.34 | 12x5 |
| **2** | 4 | 38 | D5-28-88 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **2** | 4 | 39 | W4-28-38 | 20-Apr | Power Auger | 1 | 602 | 6.59 | 12x5 |
| **2** | 4 | 40 | W2-27-99 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **2** | 4 | 41 | W8-19-26 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **2** | 4 | 42 | W4-32-87 | 20-Apr | Power Auger | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 1 | D4-29-10 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 2 | W1-31-60 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 3 | W8-19-26 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 4 | D2-10-3 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 5 | W2-27-99 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 6 | W4-32-87 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 7 | W4-32-87 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 8 | D3-7-33 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 9 | W5-24-75 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 10 | W1-19-19 | 26-Mar | Dibble Bar | 1 | 370 | 6.57 | 12x5 |
| **3** | 1 | 11 | W2-20-147 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 12 | W4-28-38 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 13 | W2-20-147 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 14 | D4-27-78 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 1 | 15 | W1-31-60 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 16 | W4-28-38 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 17 | D5-28-88 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 18 | W2-20-147 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 19 | D4-29-10 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 20 | W4-32-87 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 21 | W1-19-19 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 22 | D3-7-33 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 23 | W5-24-75 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |

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|---|---|---|---|---|---|---|---|---|---|
| **3** | 1 | 24 | D4-27-78 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 25 | W4-14-67 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 26 | D2-10-3 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 27 | W2-27-99 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 28 | Unknown | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 1 | 29 | D4-27-78 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 30 | D4-29-10 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 31 | D2-10-3 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 32 | Unknown | 26-Mar | Dibble Bar | 1 | 411 | 6.04 | 12x9 |
| **3** | 1 | 33 | W5-24-75 | 26-Mar | Dibble Bar | 1 | 442 | 8.96 | 12x9 |
| **3** | 1 | 34 | W2-27-99 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 35 | D3-7-33 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 36 | W4-14-67 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 37 | W1-31-60 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 38 | D5-28-88 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 39 | W2-20-147 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 40 | W4-28-38 | 26-Mar | Dibble Bar | 1 | 431 | 8.34 | 12x9 |
| **3** | 1 | 41 | W4-32-87 | 26-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 1 | 42 | W8-19-26 | 26-Mar | Dibble Bar | 1 | 449 | 8.22 | 12x9 |
| **3** | 2 | 1 | W4-14-67 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 2 | W2-27-99 | 19-Mar | Dibble Bar | 1 | 631 | 6.67 | 12x5 |
| **3** | 2 | 3 | D4-29-10 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 4 | D3-7-33 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 5 | D2-10-3 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 6 | W4-28-38 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 7 | W4-32-87 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 8 | D5-28-88 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 9 | W1-19-19 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 10 | W8-19-26 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 11 | W5-24-75 | 19-Mar | Dibble Bar | 1 | 573 | 8.66 | 12x5 |
| **3** | 2 | 12 | W1-31-60 | 19-Mar | Dibble Bar | 1 | 203 | 3.18 | 12x5 |
| **3** | 2 | 13 | W5-24-75 | 19-Mar | Dibble Bar | 1 | 582 | 8.78 | 12x5 |
| **3** | 2 | 14 | W2-20-147 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x5 |
| **3** | 2 | 15 | W5-24-75 | 19-Mar | Dibble Bar | 1 | 572 | 7.23 | 12x7 |
| **3** | 2 | 16 | D4-29-10 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 17 | D2-10-3 | 19-Mar | Dibble Bar | 1 | 638 | 8.45 | 12x7 |
| **3** | 2 | 18 | W4-32-87 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 19 | W5-24-75 | 19-Mar | Dibble Bar | 1 | 603 | 7.81 | 12x7 |
| **3** | 2 | 20 | D5-28-88 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 21 | W1-19-19 | 19-Mar | Dibble Bar | 1 | 502 | 8.24 | 12x7 |

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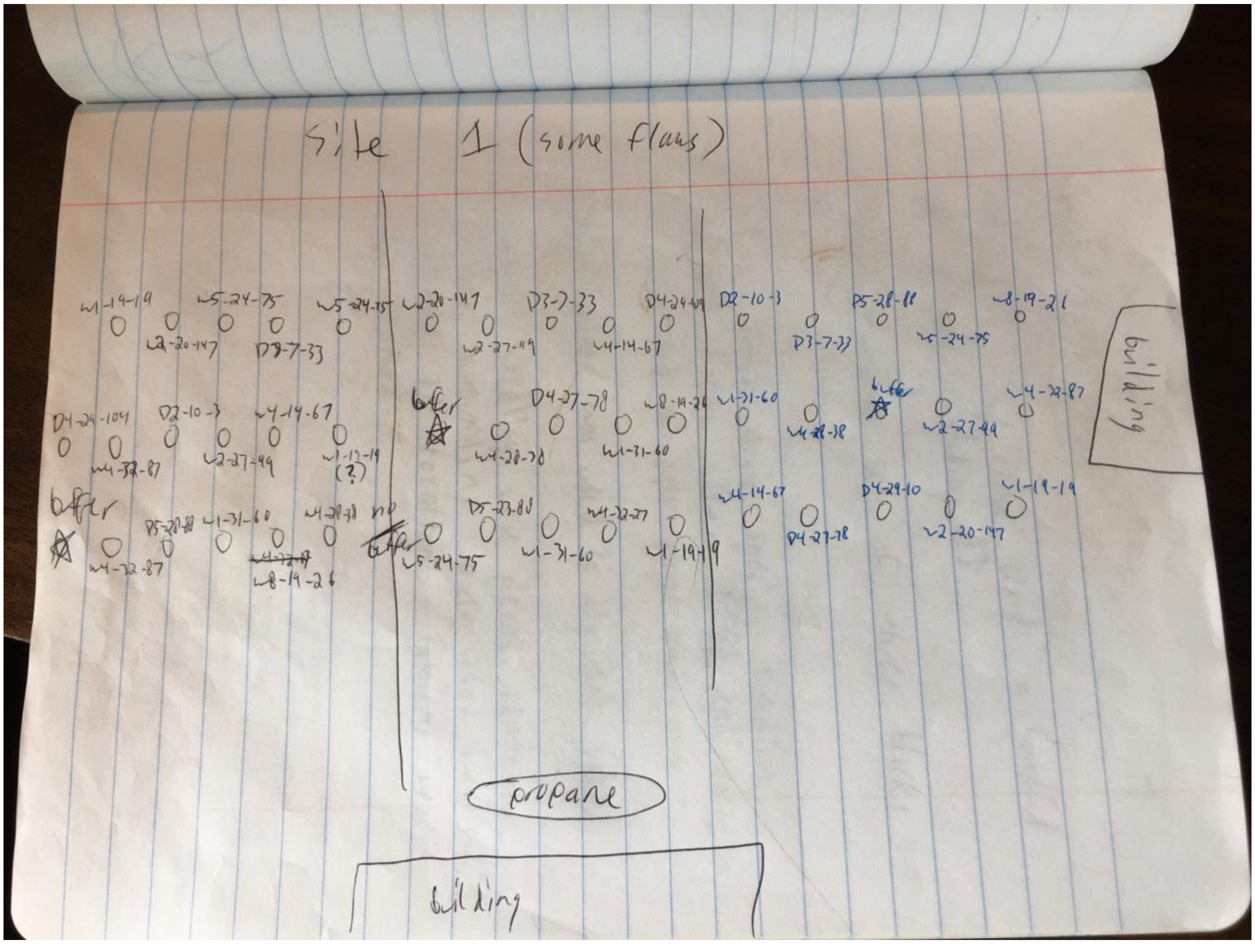
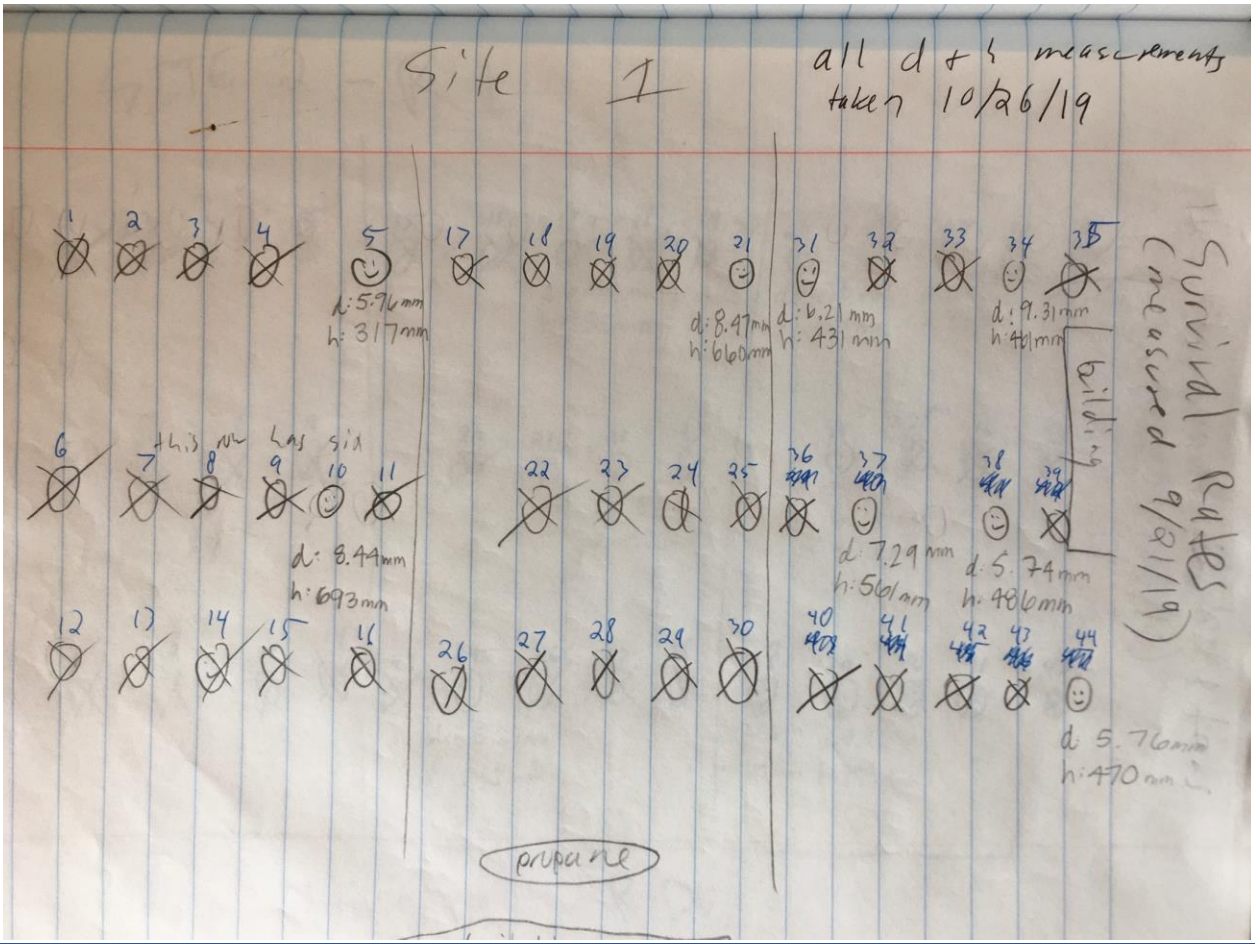
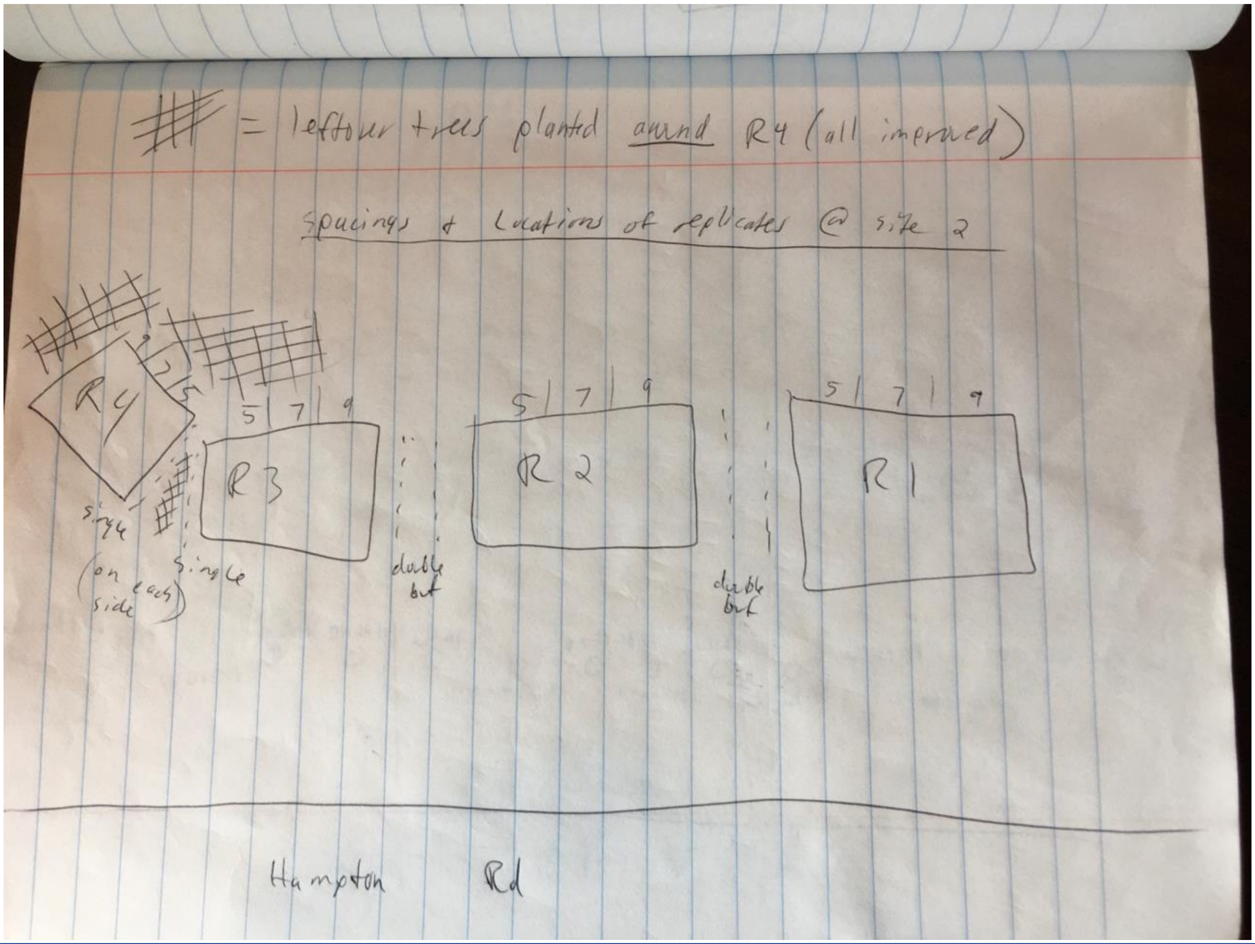
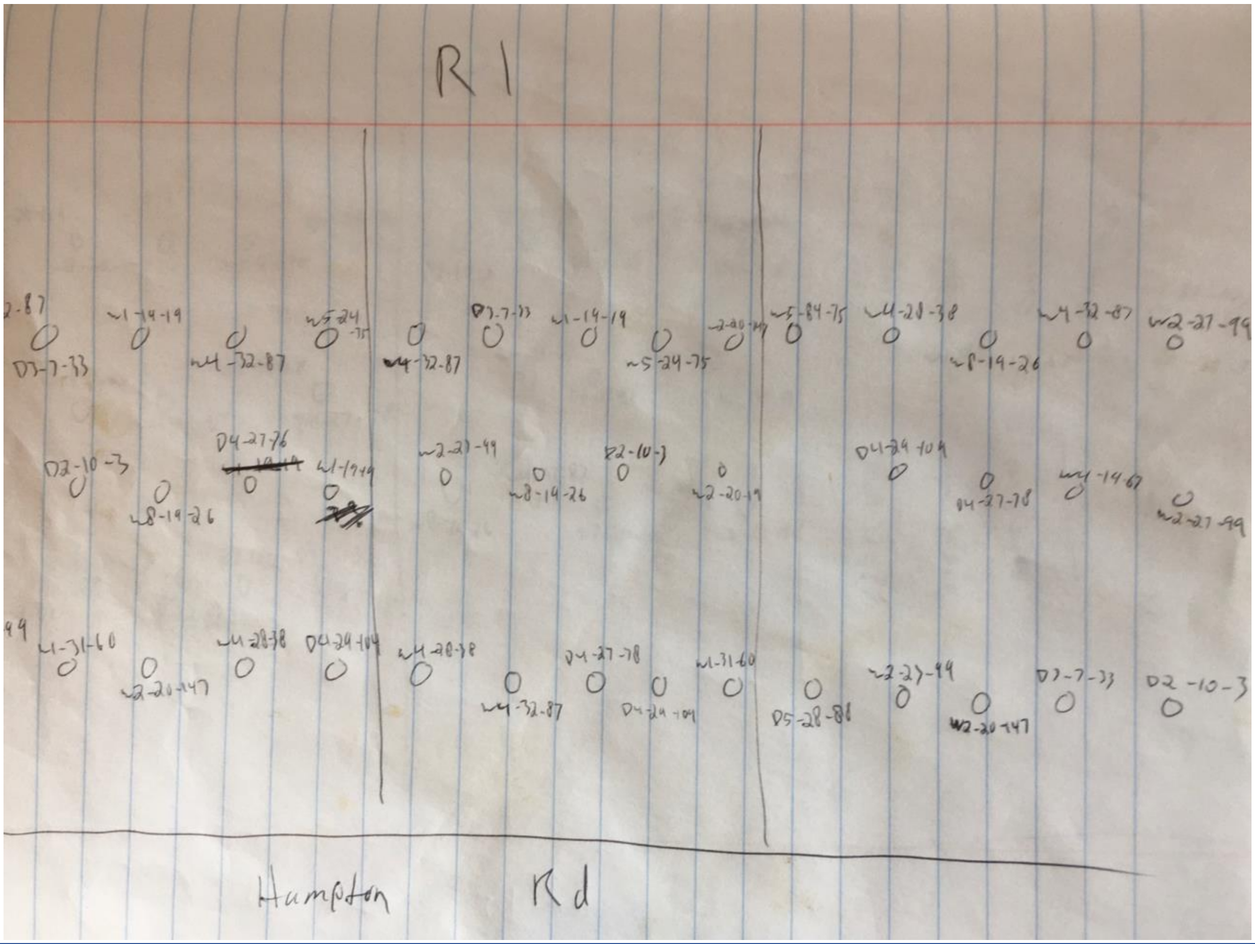
|---|---|---|---|---|---|---|---|---|---|
| **3** | 2 | 22 | D3-7-33 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 23 | W2-27-99 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 24 | W4-14-67 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 25 | W4-14-67 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 26 | W1-31-60 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 27 | W2-20-147 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 28 | W8-19-26 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x7 |
| **3** | 2 | 29 | W1-19-19 | 19-Mar | Dibble Bar | 1 | 635 | 8.29 | 12x9 |
| **3** | 2 | 30 | W5-24-75 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 31 | D5-28-88 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 32 | W8-19-26 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 33 | W5-24-75 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 34 | W4-32-87 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 35 | W4-14-67 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 36 | D2-10-3 | 19-Mar | Dibble Bar | 1 | 482 | 7.49 | 12x9 |
| **3** | 2 | 37 | W2-20-147 | 19-Mar | Dibble Bar | 1 | 539 | 8.22 | 12x9 |
| **3** | 2 | 38 | W4-28-38 | 19-Mar | Dibble Bar | 1 | 601 | 7.98 | 12x9 |
| **3** | 2 | 39 | W2-27-99 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 40 | W1-31-60 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 41 | D3-7-33 | 19-Mar | Dibble Bar | 0 | N/A | N/A | 12x9 |
| **3** | 2 | 42 | Unknown | 19-Mar | Dibble Bar | 1 | 633 | 7.82 | 12x9 |

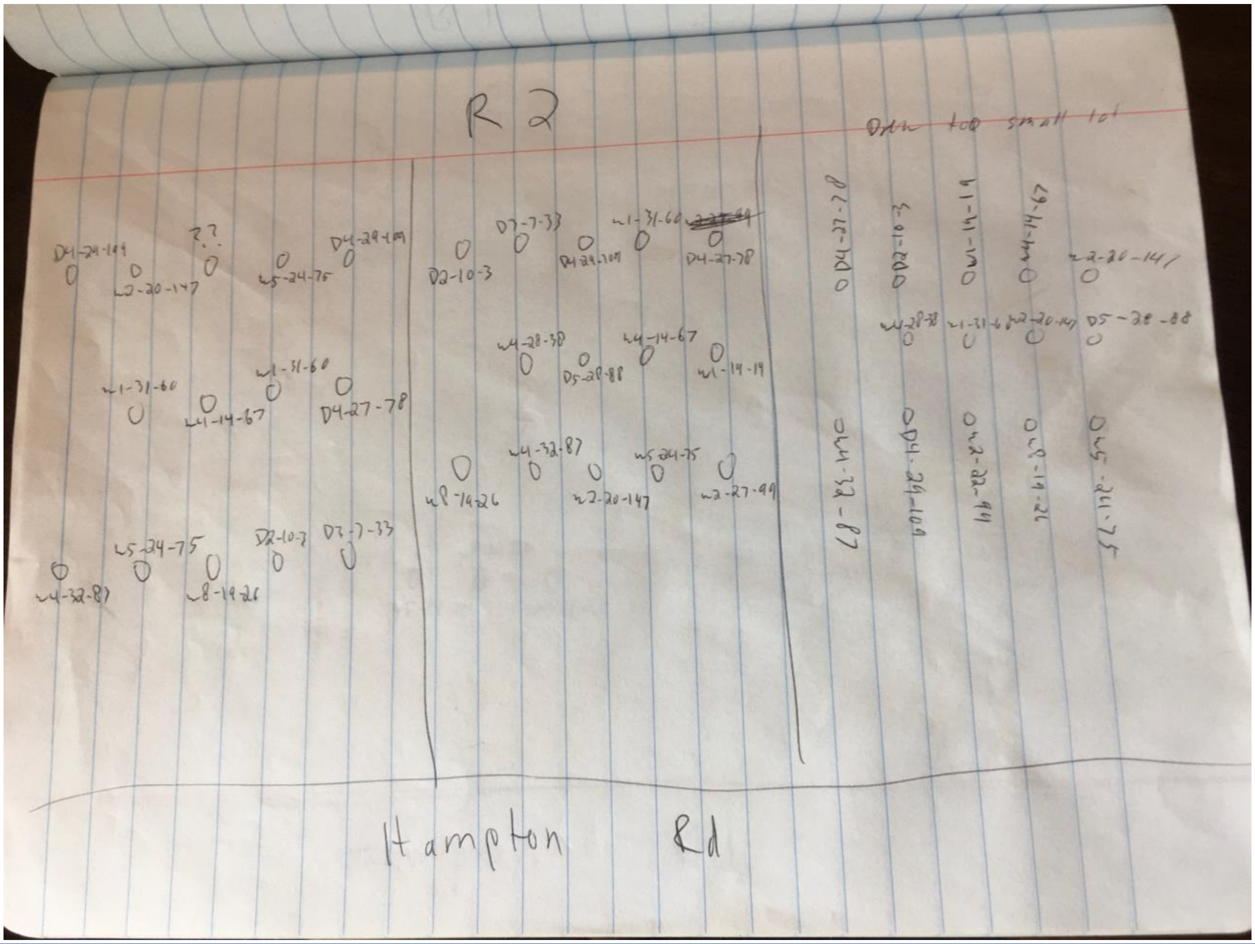
Hand-drawn maps are included below in order from Site 1 to Site 3. Blocks, seedling families, survival, height, and diameter are all recorded in the pictures.

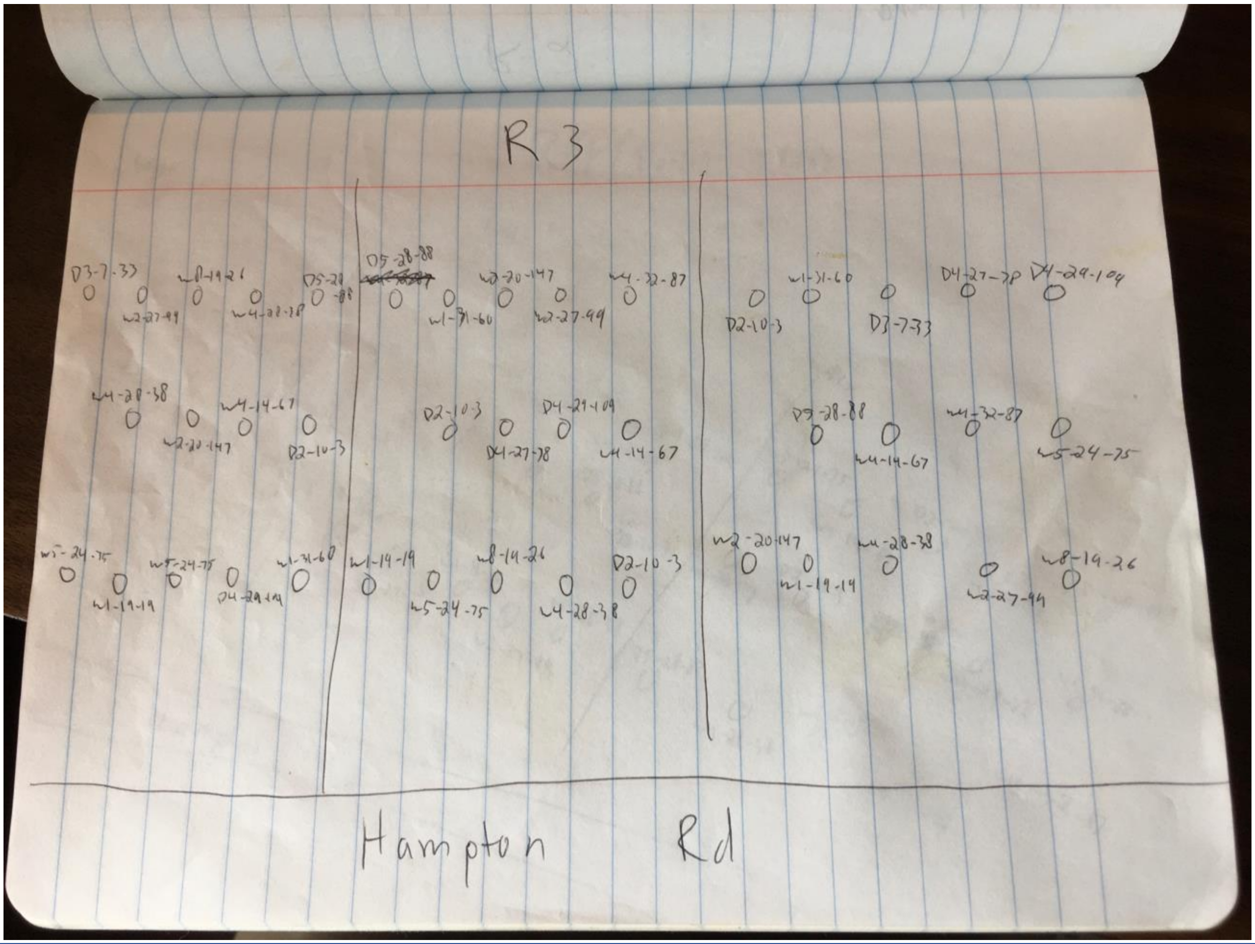

Site 1 (some flaws)
buffer
buffer
buffer
building
propane
building

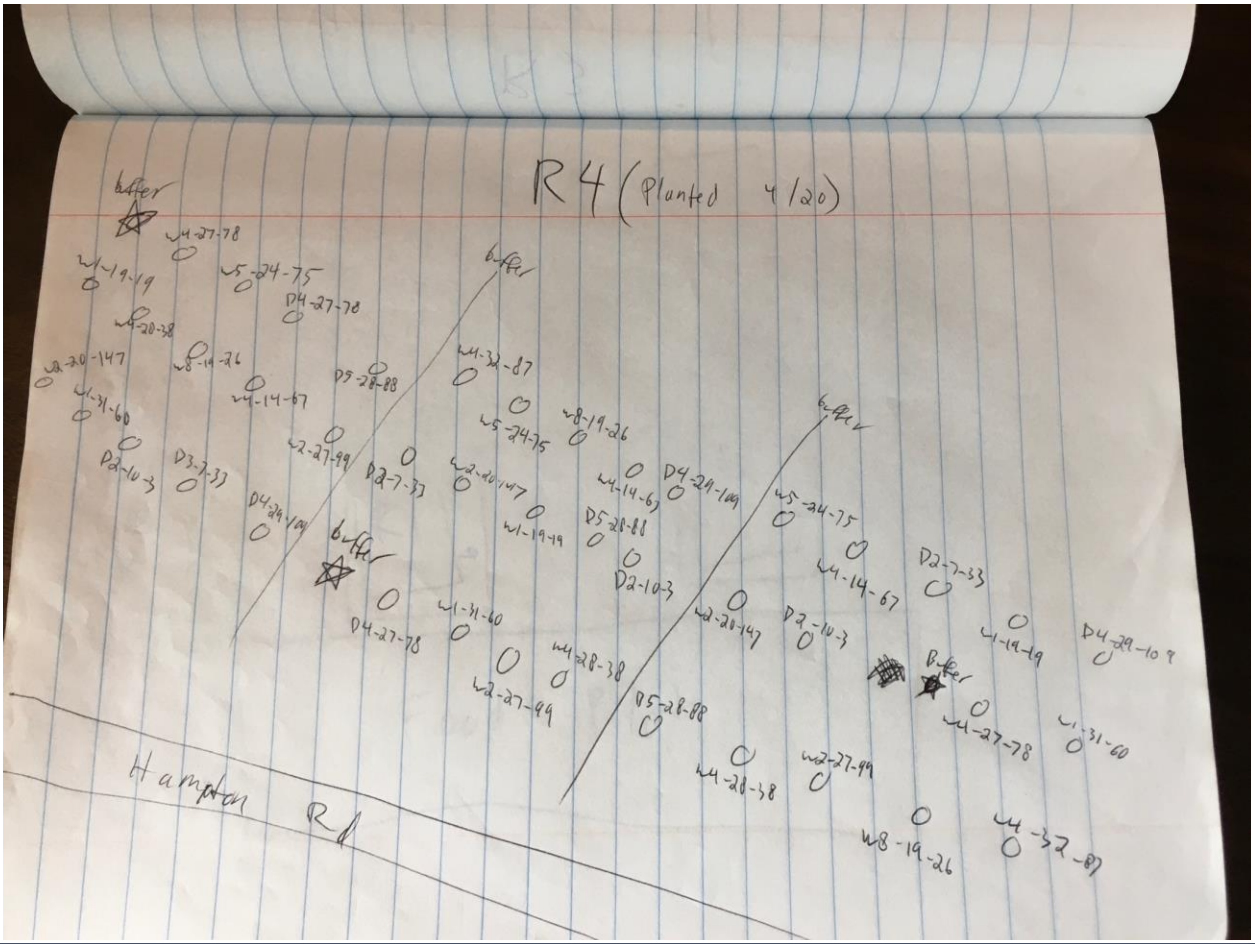

Site 1
all d + h measurements taken 10/26/19
Survival Rates (measured 9/21/19)
d: 5.96mm
h: 317mm
d: 8.47mm
h: 660mm
d: 6.21 mm
h: 431 mm
d: 9.31mm
h: 461mm
building
d: 8.44mm
h: 693mm
d: 7.29 mm
h: 561mm
d: 5.74mm
h: 486mm
d: 5.76mm
h: 470mm
propane

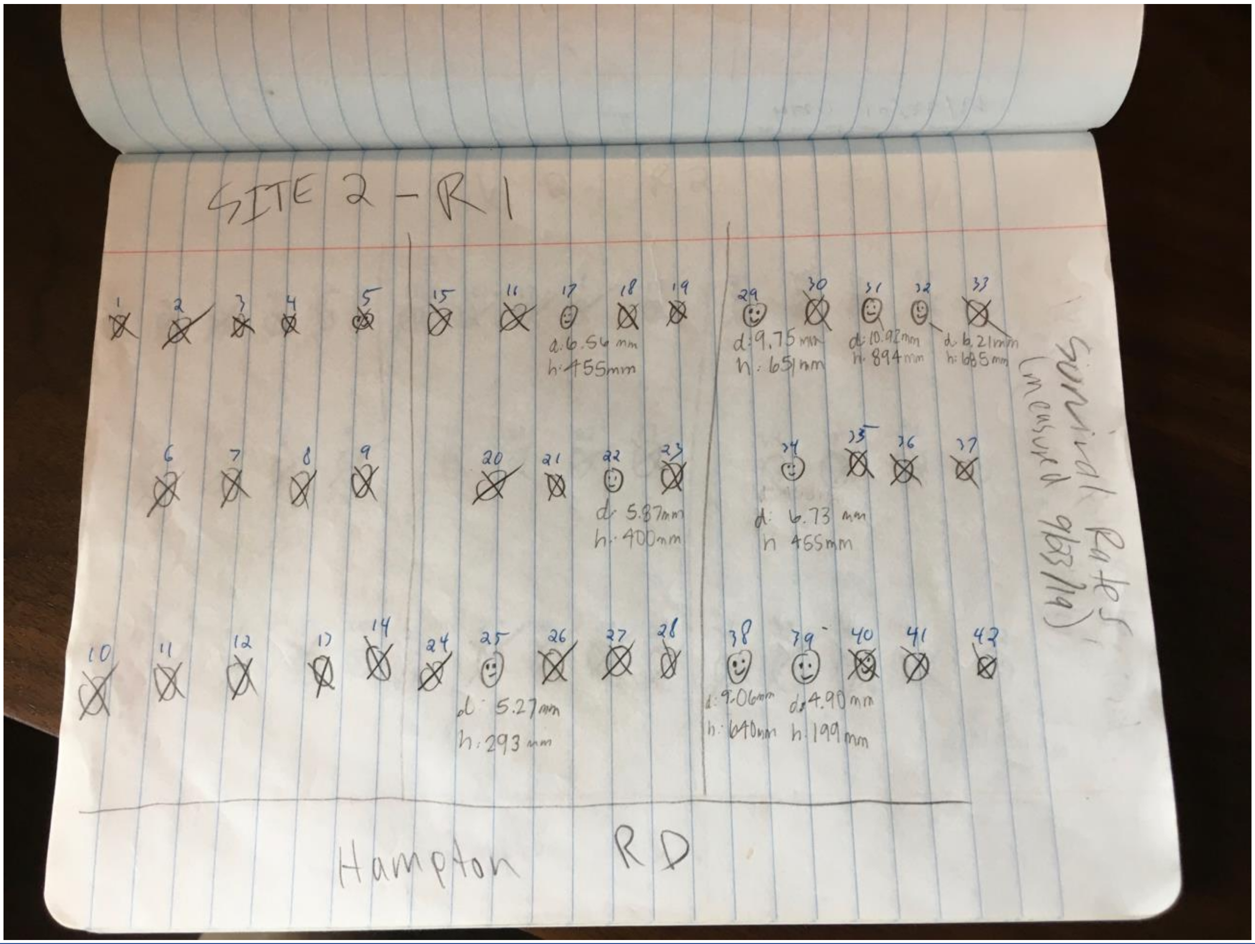

= leftover trees planted around R4 (all improved)
spacings + Locations of replicates @ site 2
R4
R3
R2
R1
5 | 7 | 9
single
(on each side)
single
double buf
double buf
Hampton Rd

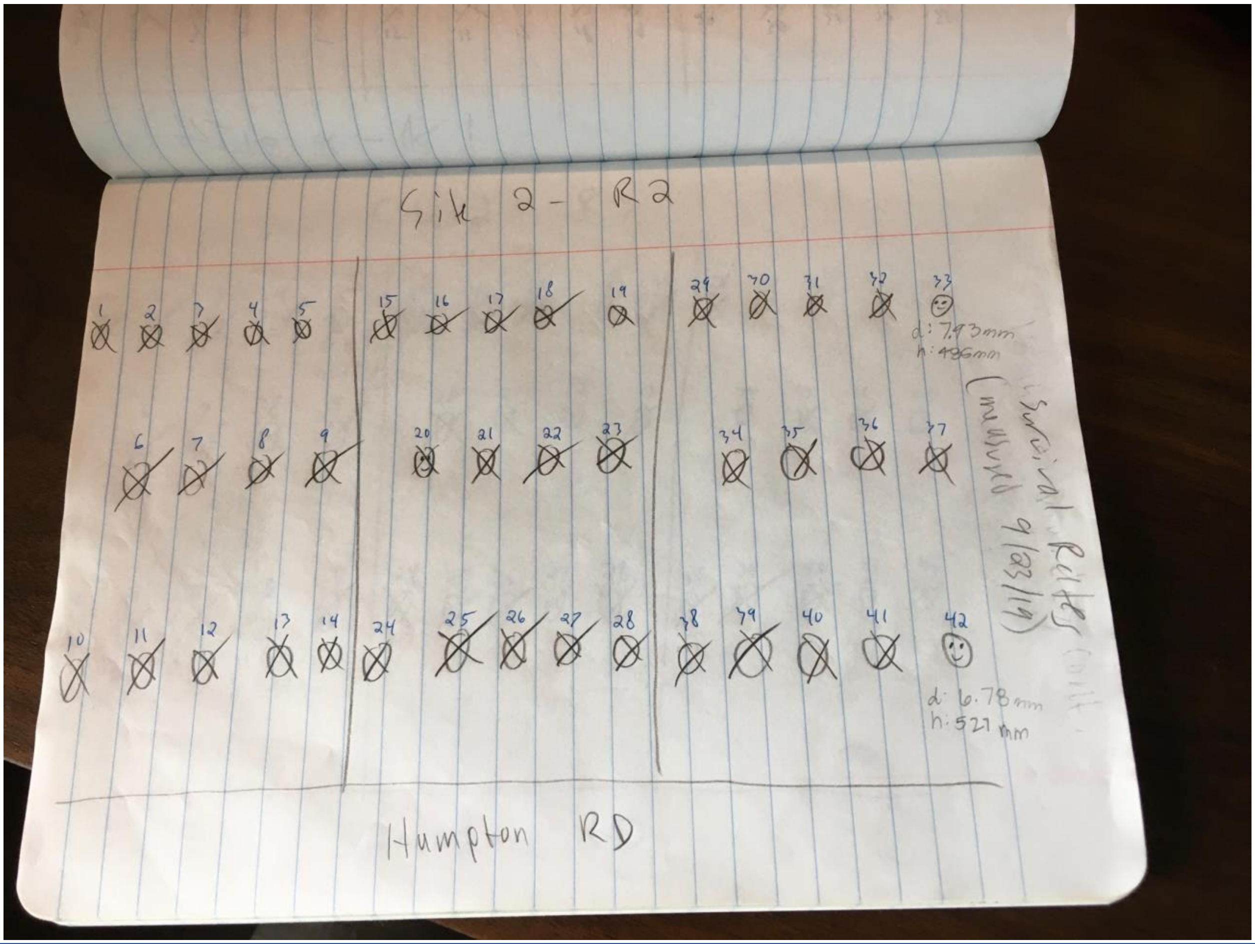

R1
Hampton Rd

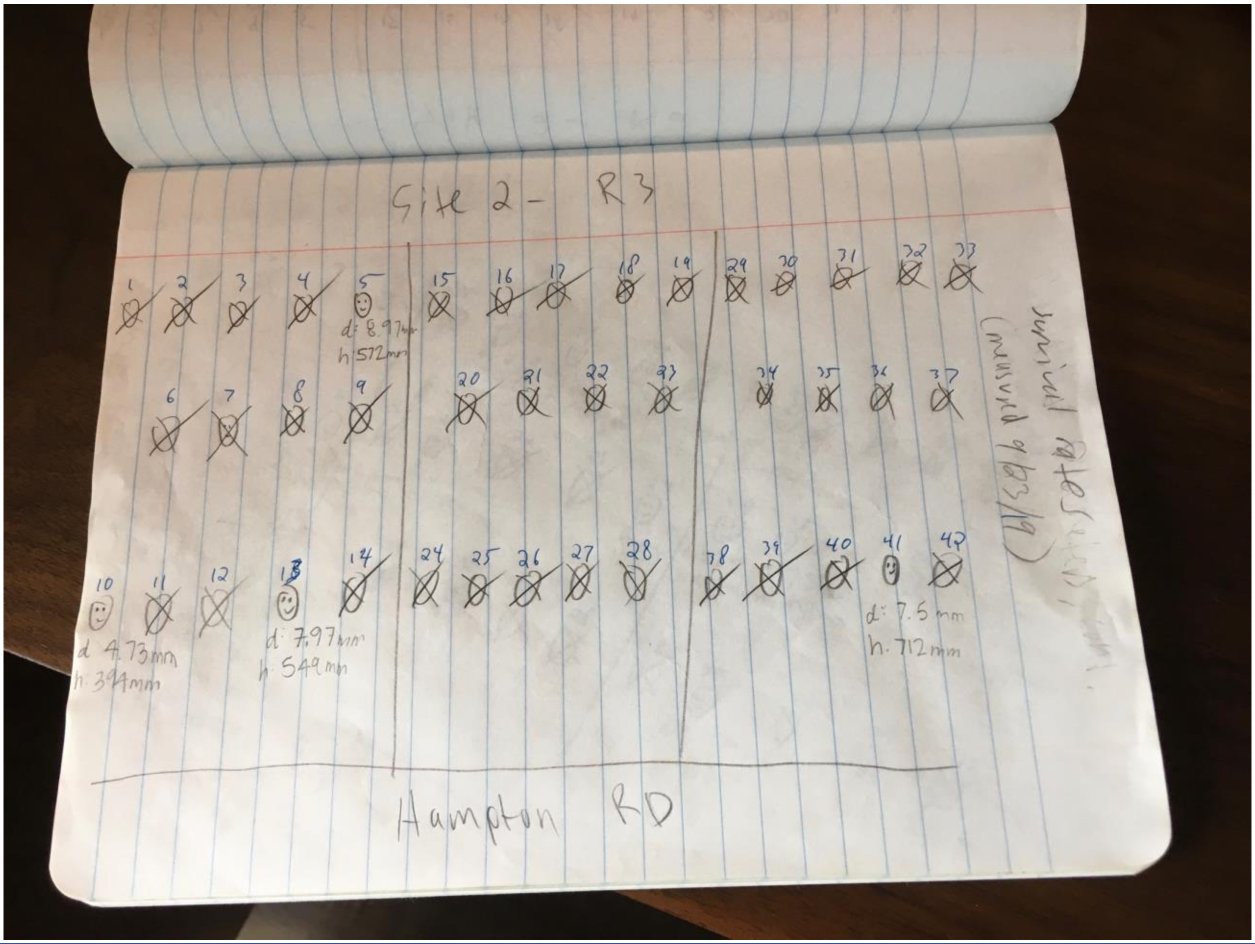

R 2
Hampton Rd

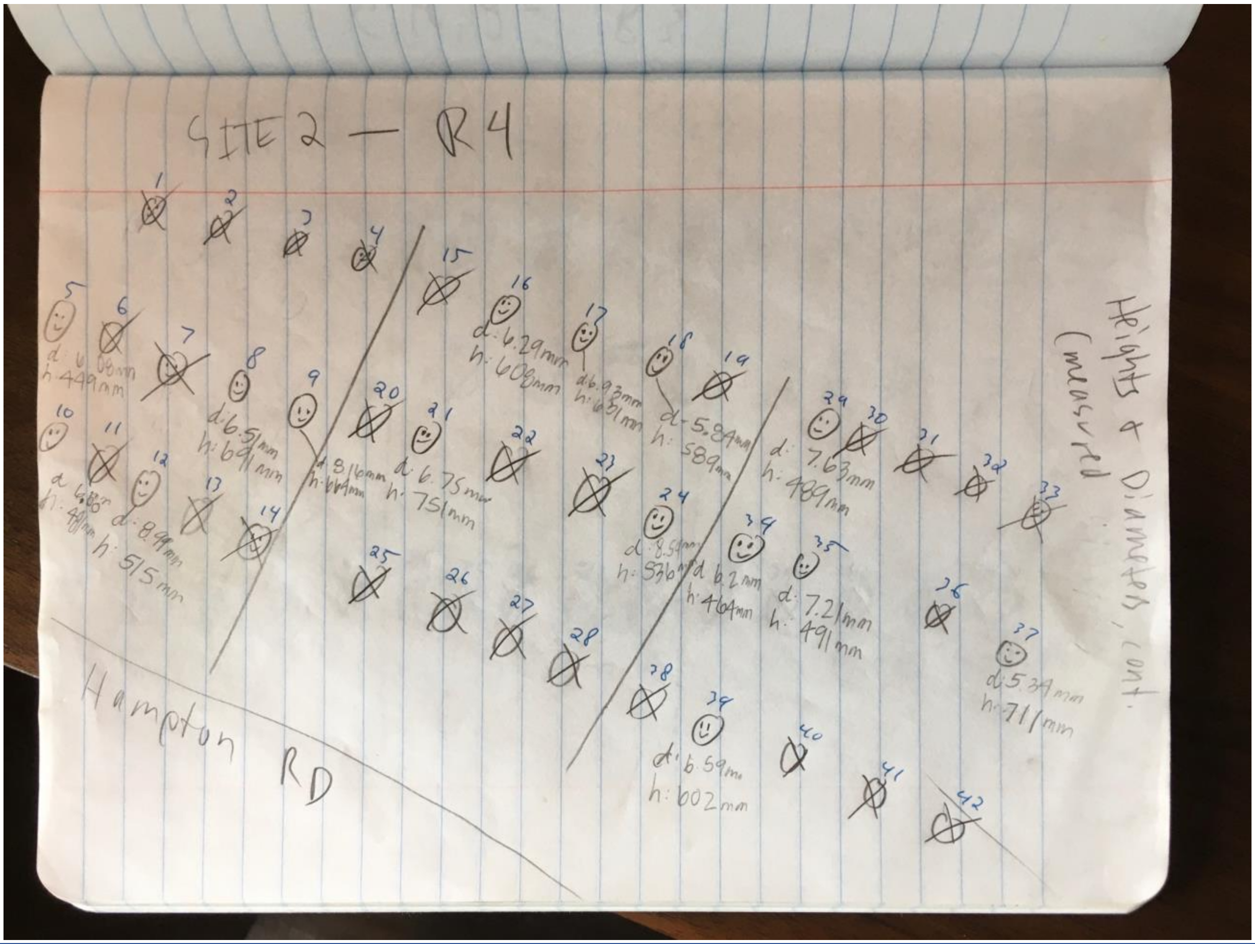

R3
Hampton Rd

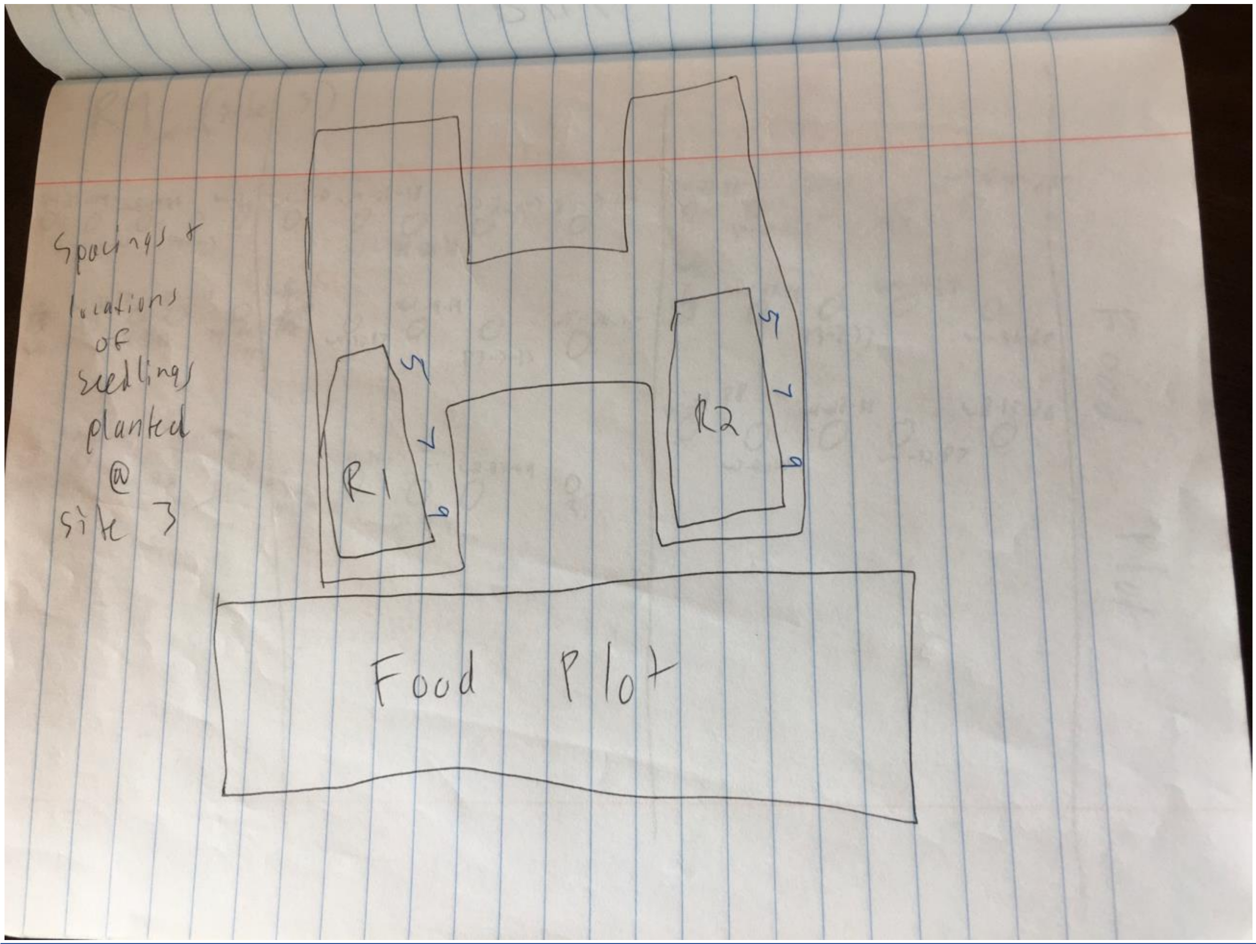

R4 (Planted 4/20)
buffer
W4-27-78
W1-19-19
W5-24-75
D4-27-78
W2-20-147
W8-14-26
W4-14-67
D5-28-88
W4-32-87
W1-31-60
W2-27-99
D2-10-3
D3-7-33
D2-7-33
D4-29-109
W8-19-26
W1-19-19
W4-28-38
Hampton Rd

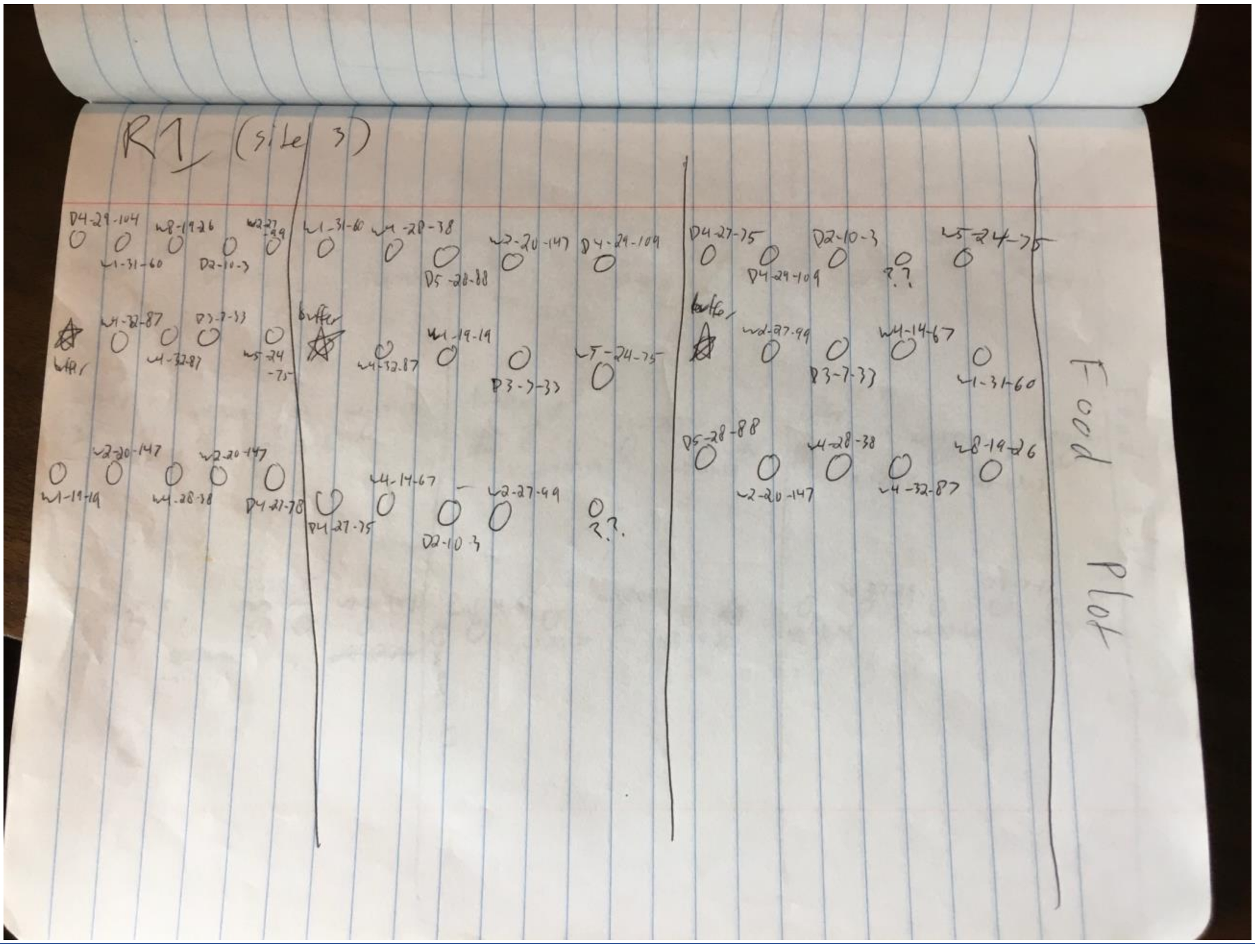

SITE 2 - R1
Survival Rates (measured 9/23/19)
d: 6.54 mm h: 455 mm
d: 9.75 mm h: 651 mm
d: 10.92 mm h: 894 mm
d: 6.21 mm h: 685 mm
d: 5.37 mm h: 400 mm
d: 6.73 mm h 455 mm
d: 5.27 mm h: 293 mm
d: 9.06 mm h: 640 mm
d: 4.90 mm h: 199 mm
Hampton RD

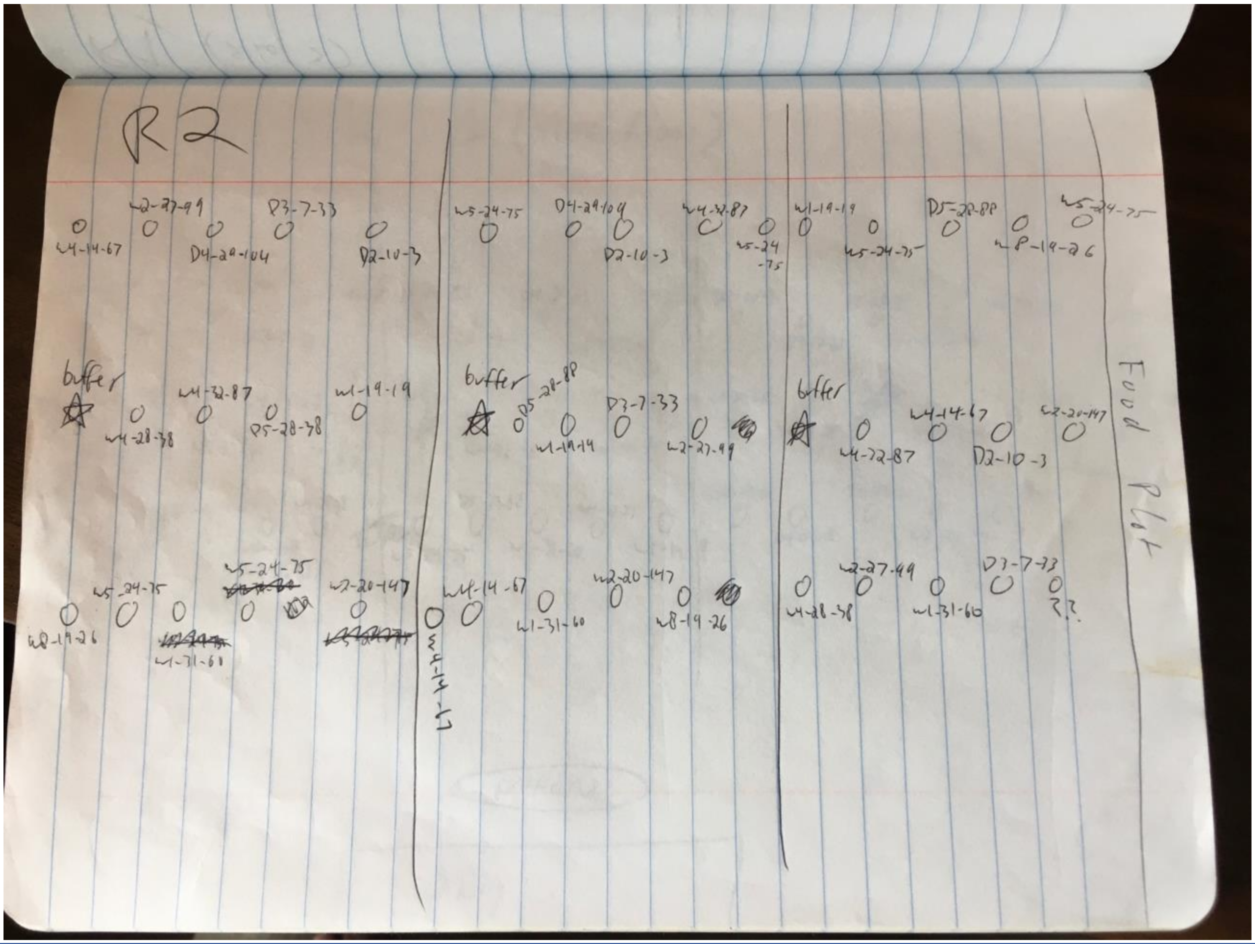

Site 2 - R2
d: 7.93mm
h: 486mm
d: 6.78mm
h: 527mm
Hampton RD

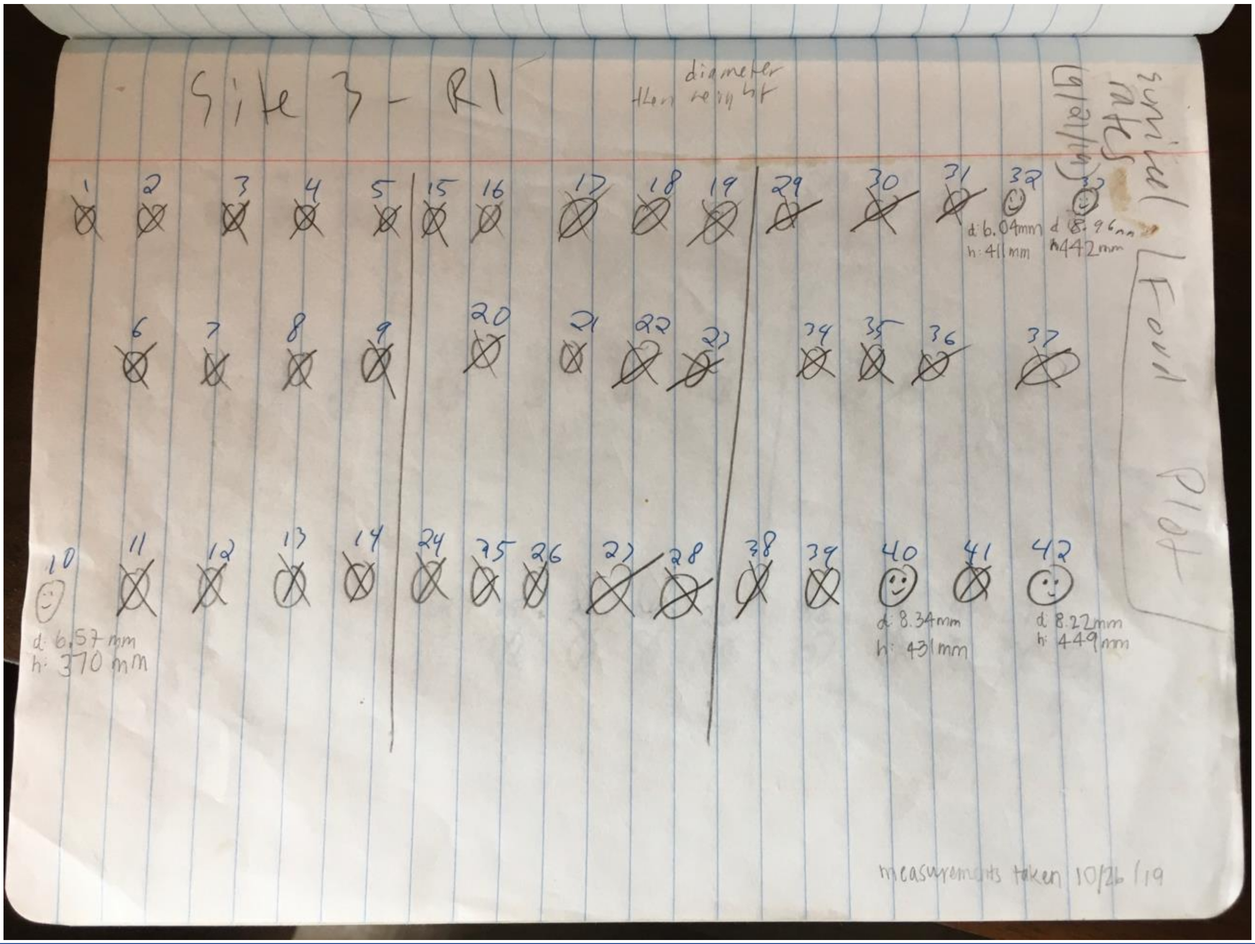

Site 2- R3
d: 8.97mm
h: 572mm
d 4.73mm
h: 394mm
d: 7.97mm
h: 549mm
d: 7.5 mm
h: 712mm
survival rates
(measured 9/23/19)
Hampton RD

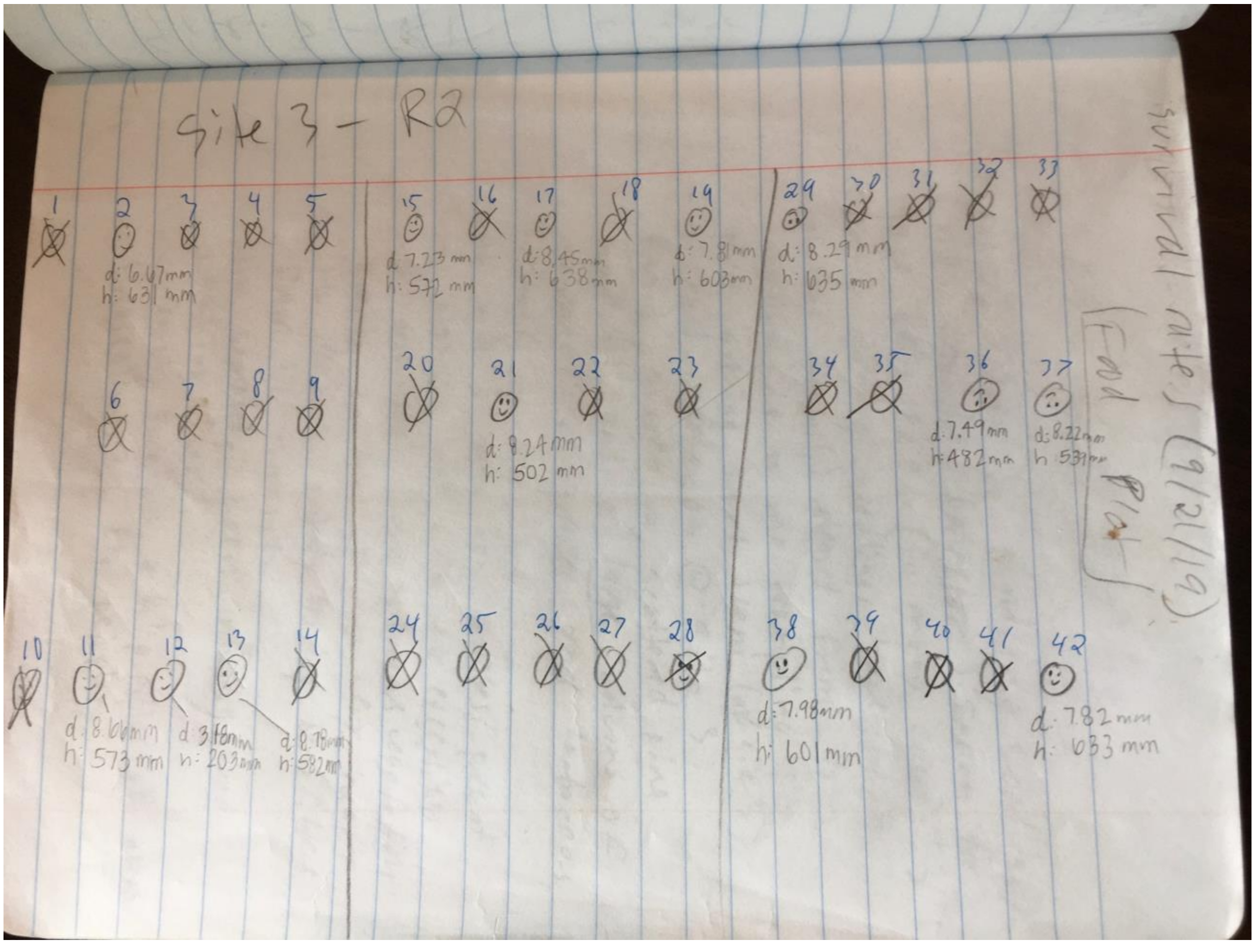

SITE 2 — R4
Heights + Diameters, cont.
(measured)
Hampton RD
d: 6.29mm
h: 608mm
d: 6.51mm
h: 691mm
d: 6.75mm
h: 751mm
d: 5.89mm
h: 589mm
d: 7.63mm
h: 489mm
d: 8.99mm
h: 515mm
d: 6.2mm
h: 464mm
d: 7.21mm
h: 491mm
d: 6.59mm
h: 602mm
d: 5.39mm
h: 711mm


Spacings &
locations
of
seedlings
planted
@
site 3
5
7
9
R1
5
7
9
R2
Food Plot


R1 (site 3)
buffer
Food Plot




Site 3 - R1
diameter
then height
survival rates (9/27/19)
d: 6.04mm
h: 411mm
d: 8.96mm
h: 442mm
Food Plot
d: 6.57mm
h: 370mm
d: 8.34mm
h: 431mm
d: 8.22mm
h: 449mm
measurements taken 10/26/19



Site 3 – R2
survival rates (9/21/19)
(Food Plot)
d: 6.67mm
h: 631 mm
d: 7.23 mm
h: 572 mm
d: 8.45mm
h: 638mm
d: 7.81mm
h: 603mm
d: 8.29 mm
h: 635 mm
d: 8.24 mm
h: 502 mm
d: 7.49mm
h: 482mm
d: 8.22mm
h: 531mm
d: 8.66mm
h: 573 mm
d: 3.18mm
h: 203mm
d: 8.78mm
h: 582mm
d: 7.98mm
h: 601mm
d: 7.82 mm
h: 633 mm

## WORKS CITED:

Anagnostakis, Sandra L. "Chestnuts and the Introduction of Chestnut Blight." *CT.gov*, The Connecticut Agricultural Experiment Station, Department of Plant Pathology and Ecology, 2020, portal.ct.gov/CAES/Fact-Sheets/Plant-Pathology/Chestnuts-and-the-Introduction-of-Chestnut-Blight.

Anagnostakis, Sandra L. "Revitalization of the Majestic Chestnut: Chestnut Blight Disease." *Aps.net*, The American Phytopathological Society, 2020, www.apsnet.org/edcenter/apsnetfeatures/Pages/ChestnutBlightDisease.aspx.

"Anova in Excel." *Excel Easy*, 2010, www.excel-easy.com/examples/anova.html.

Clark S.L, S.E. Schlarbaum, A.M. Saxton, F.V. Hebard "Establishment of American chestnuts (Castanea dentata) bred for blight (Cryphonectria parasitica) resistance: influence of breeding and nursery grading" New Forests. Vol. 47, 2016, pp. 243–270

Clark, S.L., S.E. Schlarbaum, A.M. Saxton, R. Baird. "Eight-year blight (Cryphonectria parasitica) resistance of backcross-generation American chestnuts (Castanea dentata) planted in the southeastern United States" Forest Ecology and Management, vol. 433, 2019, pp. 153–161

Clark, Stacy,H. McNab, D. Loftis, S. Zarnoch. "American Chestnut Growth and Survival Five Years after Planting in Two Silvicultural Treatments in the Southern Appalachians, USA" Forests, vol. 3, no. 4, 2012, 1017–1033

Fitzsimmons, Sara. "Re: Names of backcross families (and other questions)." Message to Alex Warren. 13 August 2020. E-mail.

Grant, Trudy. "The Randomized Complete Block Design (RCBD)." 2010, https://pbgworks.org/sites/pbgworks.org/files/RandomizedCompleteBlockDesignTutorial.pdf. PowerPoint presentation.

"History of the American Chestnut." *The American Chestnut Foundation*, The American Chestnut Foundation, 6 Jan. 2020, www.acf.org/the-american-chestnut/history-american-chestnut/.

Jacobs, Douglas F. "Toward development of silvical strategies for forest restoration of American chestnut (Castanea dentata) using blight-resistant hybrids" Biological conservation, vol. 137, no. 4, 2007, pp. 497–506

"Native Range Map of the American Chestnut." *The American Chestnut Foundation*, The American Chestnut Foundation, 6 Jan. 2020, www.acf.org/the-american-chestnut/native-range-map/.

Pinchot, C.C., S.E. Schlarbaum, S. L. Clark, A.M. Saxton, A.M. Sharp, C.J. Schweitzer, F.V. Hebard. "Growth, survival, and competitive ability of chestnut (Castanea Mill.) seedlings planted across a gradient of light levels" New Forests. Vol.48, 2017, pp. 491–512

Powell, William A., et al. “Developing Blight-Tolerant American Chestnut Trees.” *Cold Spring Harbor Perspectives in Biology*, vol. 11, no. 7, 2019, doi:10.1101/cshperspect.a034587.

USDA Natural Resources Conservation Service “Soil Map.” *Web Soil Survey*, websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx.

“Using Science to Save the American Chestnut Tree.” *The American Chestnut Foundation*, The American Chestnut Foundation, 27 July 2020, www.acf.org/science-strategies/3bur/.