THE EFFECT OF SITE QUALITY ON PERFORMANCE OF AMERICAN CHESTNUT (CASTANEA DENTATA) SEEDLINGS BRED FOR BLIGHT (CRYPHONECTRIA PARASITICA) RESISTANCE

Title presented at workshop:
INFLUENCE OF SITE QUALITY ON BLIGHT RESISTANCE AND GROWTH OF PLANTED HYBRID CHESTNUT SEEDLINGS

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ABSTRACT

Efforts to produce American chestnuts (Castanea dentata) resistant to chestnut blight fungus (Cryphonectria parasitica) have spurred an interest in reintroducing the species to managed forests. Understanding how site characteristics impact chestnut performance will inform appropriate site selection. This study was designed to evaluate the impact of site quality on survival, growth, competitive ability, and blight resistance durability of American, Chinese, and three families of backcross hybrid chestnuts. Chestnuts were planted in xeric, intermediate, and sub-mesic sites in Pennsylvania. Three years after planting, survival was 86 percent across all treatments. American chestnuts were taller on intermediate than xeric sites, and two backcross hybrid families were taller on intermediate as compared to mesic sites. Intermediate sites may offer enough soil moisture to optimize growth, without the intense competition characteristic of more mesic sites. Incidence of blight infection was too low to assess differences among treatments, though is expected to increase over time.

INTRODUCTION

Introductions of non-native invasive pests and pathogens have caused the loss or decline of an increasing number of tree species globally (Campbell and Schlarbaum 2014, Loo 2008, Santini et al. 2013). These losses can cause significant alterations to ecosystem processes and functions and threaten forest resilience to future pressures (Ellison et al. 2005, Flower et al. 2013). In response, there is a growing interest to identify or breed populations of tree species resistant to their respective pests and pathogens (Sniezko 2006), with the goal of reintroducing these species to managed landscapes. Reintroducing extirpated tree species may restore altered ecosystem dynamics, functions, and services, thereby enhancing resilience, and contributing to larger ecosystem restoration goals (Knight et al. 2011, 2017). Understanding the durability of resistance (Sniezko, 2006) and performance of improved genotypes in field settings (Clark et al. 2014, Seddon 2010) is essential for successful species reintroduction, however long term studies on these considerations are limited (Thompson et al. 2006).

Extensive efforts have gone into developing American chestnuts (Castanea dentata) that are resistant to chestnut blight disease (caused by the fungus Cryphonectria parasitica), and more recently ink disease (caused by the oomycete, Phytophthora cinnamomi), (Anagnostakis 2012, Steiner et al. 2017). The principal strategy has involved hybridizing American chestnuts with blight resistant chestnut species (primarily Chinese chestnut, C. mollissima), followed by repeated backcrossing and intercrossing to recover American chestnut traits (Anagnostakis 2012, Hebard 2005). More recently, The American Chestnut Foundation, one of the primary organizations working to develop blight-resistant American chestnuts, has incorporated the use of genomic selection to accelerate the breeding program (Steiner et al. 2017).

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Once blight-resistant American chestnuts are available, planting them into managed forests will be the next step toward restoring the species. Understanding the interacting effects of genotype and environment on long-term performance and durability of resistance of planted chestnuts will inform operational reintroduction of the species, including site selection. Selecting sites for American chestnut reintroduction that optimize growth, competitive ability, and blight control will help maximize limited resources available for restoration efforts. Site characteristics may affect these metrics of establishment success differently, however (Griffin et al. 2006). For example, Rhoades et al. (2009) found that planted American chestnut had better growth rates and lower incidence of blight on mesic than xeric sites 2 years after planting. McNab (2003), however, found that planted chestnuts were outcompeted by faster growing species on mesic sites, a trend that Griffin et al. (1991) also noted for naturally occurring chestnut sprouts growing on mesic sites. Bauman et al. (2014) found that site preparation treatments that led to greater growth of planted chestnut also yielded higher blight infection rates, by decreasing the time required for bark splits and subsequent infection. Gao and Shain (1995) found that chestnut blight canker expansion on American chestnut stem segments was negatively related to moisture availability, which suggests blight resistance may be influenced by soil moisture availability in situ. Here we present early results from a study to evaluate the long-term impact of site quality on field performance and resistance durability of hybrid backcross American chestnuts planted in recent shelterwood harvests.

**METHODS**

**Study Area**

We planted chestnut seedlings at 15 northern hardwood forest sites distributed throughout a 6500-km² area of northern Pennsylvania, USA, spanning from Warren County to the west and Potter County to the east. Major tree species found at the sites include red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), black cherry (*Prunus serotina*), and American beech (*Fagus grandifolia*), with northern red oak (*Quercus rubra*), eastern hemlock (*Tsuga canadensis*), birch (*Betula* spp.), and white ash (*Fraxinus americana*) found in lesser abundance. The 15 sites used in this study were selected from a larger set of 25 sites (Royo et al. 2016, 2017) to capture variation in soil moisture availability. To do this we calculated the integrated moisture index (IMI), (Iverson et al. 1997) for all 25 available sites. The integrated moisture index combines GIS-derived topographic and soil features of the landscape into a single index that models potential soil moisture capacity (Iverson et al. 1997). We then ran a cluster analysis to group sites based on their IMI values (PROC CLUSTER, SAS Institute Inc. 2011). The analysis distributed the 25 sites among four clusters, one containing only one site with the highest IMI value (most mesic), which we merged with the cluster with the next highest IMI values to produce three clusters representing xeric, intermediate and sub-mesic site types. We chose five sites within each cluster to maximize variability across treatments. The integrated moisture index for the selected xeric sites averaged 31.7 (range 29.4–34.7), 40.3 for the intermediate sites (range 39.5–40.8), and 46.9 for the sub-mesic sites (range 43.6–55.6).

In 14 of the 15 sites, managers conducted the initial cut of a shelterwood sequence to reduce stand relative density (the residual relative density was 31–61.5 percent and the residual basal area was 13.9–26.1 m²/ha) and applied broadcast herbicides (tank mix of glyphosate and sulphometuron methyl), (Marquis et al. 1992) to control interfering plant species in the mid- and understory layers 3–6 years prior to planting. The site that did not receive a harvest treatment was dropped from the analysis, leaving four sites in the intermediate treatment and five each in the xeric and sub-mesic treatments.

**Experimental Materials**

Four-hundred and eighty seven chestnut seedlings; 83 American, 84 Chinese, and 320 backcross hybrid chestnuts (Anagnostakis 2012, Hebard 2005) from three families were used in this study (table 1). The backcross hybrid chestnuts were from the BC₃F₂ generation—second generation of the third backcross—or were crosses between
BC3F1 and BC2F1 parents. All chestnuts were from open-pollinated seed collections made in the fall of 2013 and grown for 1 year at commercial or State tree nurseries. The American chestnuts were collected from one mother tree in Maryland and grown at the Kentucky State tree nursery (Grassy Creek, KY). The Chinese chestnuts were collected from multiple mothers from one orchard and grown at the Forrest Keeling Nursery (Elsberry, MO). The backcross hybrid American chestnuts (three families, table 1) were collected at the Windsor Locks orchard of the Connecticut Agricultural Experiment Station (Anagnostakis 2012) and grown at the Vallonia Nursery in Vallonia, IN (fig. 1A). All seedlings were lifted as 1-0 bare-root seedlings in the early spring of 2015 and stored in a cold room (~1° C). The seedlings were processed for planting in March, with roots trimmed to 15 cm from the main tap root to facilitate planting (fig. 1B). Seedlings were planted April 10–14, 2015 with a Jim Gem KBC© bar, modified by adding 5 cm to each side of the blade, creating a blade 20 cm at the top, tapering to the tip.

**Experimental Design and Data Collection**

Site type (xeric, intermediate, sub-mesic) was analyzed as a completely randomized design. Chestnut species and hybrids were analyzed as one treatment factor, called “family”, with five total treatment levels (table 1). Within each site type replicate, the chestnut seedlings were arranged using an incomplete block design, each

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**Table 1—Chestnut species/generations and families used in this study**

<table>
<thead>
<tr>
<th>Chestnut species/hybrid generation</th>
<th>Family</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>American chestnut</td>
<td>American</td>
<td>83</td>
</tr>
<tr>
<td>Chinese chestnut</td>
<td>Chinese</td>
<td>84</td>
</tr>
<tr>
<td>BC3F1 backcross hybrid</td>
<td>W1-100</td>
<td>83</td>
</tr>
<tr>
<td>BC3F1xBC3F1 backcross hybrid</td>
<td>W3-20</td>
<td>84</td>
</tr>
<tr>
<td>BC3F1xBC3F1 backcross hybrid</td>
<td>W4-75</td>
<td>153</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>487</td>
</tr>
</tbody>
</table>

*All chestnuts in the Windsor orchard except for one family are male-sterile, therefore, the father family is known, even though the crosses were open-pollinated.*

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*Figure 1—(A) backcross hybrid chestnuts growing at Vallonia Nursery (Vallonia, IN), and (B) Trimming the roots of and tagging the lifted seedlings at the Tennessee Tree Improvement Center (Knoxville, TN). (Forest Service photo by Cornelia Pinchot)*
block containing four seedlings from different family treatments. At each site, between 34 and 36 chestnut seedlings were planted within a 0.42 ha deer exclosure. Seedlings were planted in grids, 3.7 m spacing within and 6 m spacing between rows.

Seedling height and ground-level diameter (GLD) were measured at the time of planting and towards the end of the first three growing seasons (August or September, once the buds were set) since planting. Mortality and incidence of chestnut blight was also assessed at this time.

A camera equipped with a hemispherical lens was used to evaluate canopy openness above each planted chestnut seedling during the 2017 (year 3) field season. To evaluate competing vegetation, a 2.6 m diameter competition plot was centered on each chestnut seedling and competition data on height and species of the tallest understory woody plants (DBH <10 cm) collected towards the 2017 growing season.

All analyses for this study were processed using SAS 9.3 software (SAS Institute 2011). Seedling height and GLD were analyzed using a mixed-model analysis of covariance (ANACOVA) to determine significant differences among the fixed effects of site type, chestnut family, and their interactions for year 3 after planting. Initial height, GLD, and percent canopy openness were tested as covariates in the ANACOVA models for height and GLD, respectively. Generalized linear mixed model with binomial distribution was used to analyze third year survival (1 = alive, 0 = dead) and dominance of the seedlings. Seedlings that attained at least 80 percent of the height of the tallest competitor within the competition plot were defined as dominant (Spetich et al. 2002). Data were checked for homogeneity of variance and normality. Unequal variance was added to the model if the Akaike Information Criterion (AICc) was significantly improved. Least-significant-difference tests were performed to identify differences among means (α = 0.05). Incidence of blight was too low to analyze for statistically significant differences among treatments.

**RESULTS**

**Survival**

Three growing seasons after planting, 86 percent (±2) of the chestnut seedlings were alive. There was no difference in survival among site types (P = 0.99), while family did differ (P = 0.01). Chestnuts in the W4-75 backcross family had greater survival (99 percent ± 2) than all other chestnuts except those in the W1-100 backcross family (97 percent ± 2). All other chestnuts were statistically similar in survival (and did not differ from the W1-100 backcross family); 87 percent ± 4 for the W3-20 backcross family; 84 percent ± 5 for Chinese, and 83 percent ± 5 for American chestnuts. The interaction between site and family treatments was not significant (P = 0.24).

**Height and Ground Level Diameter**

Height after three growing seasons did not differ among site or family treatments (P = 0.20, P = 0.19, respectively) (fig. 2), and averaged 162 cm across all treatments. However, the interaction between the two treatment factors was significant (P = 0.03) (fig. 3). American chestnuts were taller on intermediate than xeric sites; while W1-100 and W4-75 backcross hybrid families were taller on intermediate than sub-mesic sites. There were no differences in height for W3-20 backcross hybrid or Chinese chestnuts across the site types. Height at planting and percent canopy openness were both significant covariates in this model (P < 0.0001 and P = 0.001, respectively). Ground level diameter after three growing seasons was similar across the site types (P = 0.41), but differed among families (P < 0.0001). Diameter was greatest for backcross hybrid families W1-100 and W4-75 (17.5 mm ± 0.8 and 17.4 mm ± 0.7, respectively). Diameter was similar among the remaining families; 14.7 mm ± 0.8 for W3-20, 14.6 mm ± 0.8 for American, and 13.7 mm ± 0.8 for Chinese chestnuts. The interaction between site and family treatments was not significant (P = 0.23). Ground level diameter at planting and percent canopy openness were both significant covariates in the model (P < 0.0001 for each).
Figure 2—Chestnut seedlings in a xeric (A), intermediate (B), and sub-mesic (C) sites 3 years after planting. (Forest Service photo by Cornelia Pinchot)

Figure 3—Mean height (± standard error) of chestnut families among site types. Different letters indicate height differences among treatments (α = 0.05).

**Dominance**

Dominance was statistically similar across the site types (P = 0.33) see figure 4 for examples of dominant and suppressed seedlings), but differed both among families (P = 0.004) and the interaction between site and family treatments (P = 0.04). Dominance was greatest for backcross family W4-75 (42 percent) and family W1-100 (36 percent) and lowest for Chinese chestnuts (17 percent) (table 2). Family W4-75 was the only family to differ in dominance among site types;

<table>
<thead>
<tr>
<th>Chestnut family</th>
<th>Dominance after 3 years (%)</th>
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<tbody>
<tr>
<td>American</td>
<td>22 ± 7 BC</td>
</tr>
<tr>
<td>Chinese</td>
<td>17 ± 6 C</td>
</tr>
<tr>
<td>W1-100</td>
<td>36 ± 9 AB</td>
</tr>
<tr>
<td>W3-20</td>
<td>27 ± 7 BC</td>
</tr>
<tr>
<td>W4-75</td>
<td>42 ± 9 A</td>
</tr>
</tbody>
</table>

Note: Means followed by the same letter are not statistically different.
54 percent of W4-75 seedlings growing in xeric sites were dominant, compared with 60 percent (± 15) in intermediate sites, and 18 percent (± 9) in sub-mesic sites.

**Blight Incidence**

Chestnut blight was identified on 23 individual chestnut seedlings throughout the first three growing seasons; nine in xeric, and seven each in intermediate and sub-mesic sites. Blight was identified in three of five xeric, three of four intermediate, and four of five sub-mesic sites. Nine of the seedlings with blight symptoms were American chestnut, one Chinese, five W1-100, five W4-75, and three were W3-20 backcross hybrid chestnuts.

**DISCUSSION**

Early survival of planted chestnut seedlings was high across site and family treatments. Two of the backcross families (W1-100 and W4-75) demonstrated superior results in at least one performance metric (diameter, survival, and/or dominance). Differences in performance among chestnut families has been found in other studies evaluating outplanting performance in forested settings (Clark et al. 2016, Pinchot et al. 2017, Thomas-Van Gundy et al. 2017). Of greater interest is the significant interaction between site and family treatments for height and dominance. American chestnuts demonstrated inferior height in xeric, compared to intermediate sites, while two of the backcross hybrid families demonstrated inferior growth in sub-mesic sites. Intermediate sites may offer enough soil moisture to optimize growth, without the intense competition characteristic of more mesic sites (Loftis 1983). Continued monitoring of the seedlings over time is necessary to determine if differences across sites will become more pronounced across all families, or if there is indeed a genotype by environment interaction for these variables. While incidence of blight was too low for statistical analysis, more American chestnuts were infected than Chinese or backcross hybrid chestnuts. Incidence and severity
of blight infection will likely increase over time, as Hebard (1982) and Griffin (1989) have found for natural American chestnut sprout populations following canopy disturbance. Understanding if site and family treatments effect blight severity, particularly in the context of growth and competitive ability among treatments, will help inform a holistic American chestnut reintroduction strategy.

ACKNOWLEDGMENTS

We thank Michael French and The American Chestnut Foundation for providing the American chestnut seedlings and Bob Hawkins and Rob Winks, of the Indiana Division of Forestry, for growing the backcross hybrid chestnuts used in this study. We acknowledge the Allegheny National Forest, the Pennsylvania Bureau of Forestry, Bradford Water Authority, Forest Investment Associates, Generations Forestry, Hancock Forest Management, Landvest, and Kane Hardwoods for the use of their field sites. We thank Charles Vandever for assistance with data collection. Todd Hutchinson, Stacy Clark, and Tom Hall provided useful feedback on earlier versions of this paper.

LITERATURE CITED


