Nursery performance of American and Chinese chestnuts and backcross generations in commercial tree nurseries

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The American chestnut [Castanea dentata (Marsh.) Borkh.] was decimated by an exotic fungus [Cryphonectria parasitica (Murr.) Barr] in the early 1900s. Breeding efforts with American and Chinese chestnuts (C. mollissima Blume) produced putatively blight-resistant progeny (BC3F3) in 2007. We compared two nut size classes for differences in seedling quality of bare-root stock grown in commercial nurseries. We compared the BC3F3 generation to parental species and other generations. Nuts in the large size class produced taller trees than nuts in the small size class, but sizing nuts prior to sowing did not reduce variability in nursery seedling size. Results indicate that overall seedling quality could be improved by culling small nuts, but seedling uniformity would only be improved by culling seedlings before planting. We recommend refinement of restoration efforts to match seedling size to site type and planting goals. BC3F3 chestnuts differed from Chinese chestnuts in 67% of tests, and were different than American chestnuts in half the tests, indicating not all American traits were recovered in this early phase of seedling development. Family differences within the BC3F3 generation were most apparent for mean nut weight, and only one BC3F3 family differed from other BC3F3 families in seedling growth characteristics.

Introduction

The American chestnut [Castanea dentata (Marsh.) Borkh.] was one of the most abundant and important tree species of the eastern deciduous forest of North America for thousands of years, until decimated by chestnut blight, a disease caused by an exotic fungus [Cryphonectria parasitica (Murr.) Barr].¹ The fungus probably arrived on imported Japanese chestnut [C. crenata Siebold & (Zucc.)] nursery stock in the late 1800s,² and spread rapidly, killing mature trees and reducing chestnut populations throughout the natural range to recurring sprouts in the forest understorey by the mid-twentieth century.¹ To date, efforts to restore American chestnut have largely included development of blight-resistant material through traditional breeding techniques with resistant Chinese (Castanea mollissima Blume) or Japanese chestnut,³–⁶ biological control through hypovirulence of chestnut blight,⁷,⁸ breeding low-to-moderate levels of resistance using pure American parents,⁹ and early steps towards genetic transformation.¹⁰,¹¹

The American Chestnut Foundation (TACF) is a non-profit organization attempting to restore this species using a backcross breeding programme that was first initiated in the mid-1980s, but was based on previous decades of trial and error using various lines of resistance.⁵,¹² In theory, the first putatively blight-resistant generation, the BC3F3 generation, is 94% American chestnut, 6% Chinese chestnut and is predicted to have the desired phenotypic characteristics of the American chestnut parent while maintaining blight resistance of the Chinese chestnut parent.⁵ After several decades of breeding work in the early twentieth century that ultimately failed,¹² and then nearly three decades of using a backcross breeding technique, TACF orchards produced sufficient material for field testing of the BC3F3 generation in 2007. In addition to assessing blight resistance, a vital research problem in chestnut restoration is to determine whether seedlings will recover American chestnut growth and morphology or if they will carry traits that resemble the Chinese parental species.¹³ American chestnuts are the preferred species for growth form and nut production when compared with the Chinese species that has a non-timber form (i.e. low branching with no clear leader, short height growth), less desirable nuts for human consumption and is generally not competitive in a natural forest planting.³,¹⁴,¹⁵ Recovery of American genes for desired growth and fruiting characteristics will be important to long-term restoration goals.
Successful restoration of Fagaceae species through artificial regeneration requires improved seedling quality at planting to overcome vegetation competition and animal browse pressure.\textsuperscript{16–18} The relationship between seedling quality and seedling response to silvicultural treatments and site quality has only received limited testing with American chestnut.\textsuperscript{19–22} Despite the fact that American chestnuts have been grown in nurseries and planted since colonial days in the USA,\textsuperscript{23} no experiments have tested American chestnut growth in the nursery using pedigreed material in a replicated design, and there has been no research on seedlings from the BC\textsubscript{3}F\textsubscript{3} generation, due to lack of availability. A single study has tested the effects of nursery seedling quality on field survival and growth of American chestnut.\textsuperscript{24} They found that chestnut could grow to a large size in the nursery (>1 m) had high variability in growth within and among genetic families and also had strong correlations between root-collar diameter (RCD) growth and other growth variables.

We can use previous studies on Fagaceae species, such as oak, \textit{Quercus} \textit{L.}, to make inferences regarding American chestnut response to nursery and silvicultural practices. This assumption is based on the similar growth strategies exhibited by chestnut and oak in greenhouse studies,\textsuperscript{25} and the close phylogenetic relationships between the two genera.\textsuperscript{26} Most research shows that oak seedling development was positively related to acorn size or weight,\textsuperscript{27–29} particularly when genetic parameters were controlled.\textsuperscript{30,31} However, some research did not support intraspecific relationships between acorn size and seedling height\textsuperscript{32} or it showed differences too small to be of practical use.\textsuperscript{33}

Seedling quality at planting will affect subsequent field performance, specifically seedlings larger in height and RCD generally outperform smaller seedlings after several years in the field.\textsuperscript{34–36} Increasing numbers of chestnuts bred for blight resistance will become available,\textsuperscript{37} and understanding how to improve seedling quality at planting will become paramount for successful restoration. Restoration of American chestnut will require planting of the species throughout its native range, a large extent consisting of medium to high-quality sites that will have deer-browsing (\textit{Odocoileus virginianus}) pressures and fast-growing competing vegetation of native species.\textsuperscript{38–39} Production of high-quality and uniform seedlings for planting with growth advantages over natural competition and browse pressure will ultimately improve efficiency and success of forthcoming reintroduction efforts. To date, testing seed size effects on nursery seedling development and testing for variation in growth parameters within and among genetic breeding lines of American chestnut and hybrids bred for blight-resistance have not been conducted.

We used experimental material from several genetic families within American and Chinese parental species and within various breeding generations, including the first putatively blight-resistant generation produced by TACF (BC\textsubscript{1}F\textsubscript{3}). It is also important to identify genetic differences within the BC\textsubscript{3}F\textsubscript{3} generation as a way to maximize growth potential of the species, as has been shown with other hardwoods,\textsuperscript{40} and to identify families that are retaining undesirable Chinese characteristics. The genetic material used in this study was limited due to the relatively low numbers of nut-producing mother trees at TACF orchards. Despite the limitations in available genetic material, the nuts used in this study reflect 100 years of chestnut breeding efforts by the United States Department of Agriculture, the Connecticut Agricultural Experiment Station and the TACF\textsuperscript{5,12,40} to produce blight-resistant American chestnuts. This study represents the first examination of morphological differences within and among families of the BC\textsubscript{3}F\textsubscript{3} generation and the first to compare the BC\textsubscript{3}F\textsubscript{3} generation to the parental species and other less advanced generations.

The goal of this study was to characterize high-quality chestnut seedlings that have been bred for blight resistance and to examine cultural practices that will improve seedling quality and reduce seedling variability prior to planting in the field. Our objectives were to test chestnut breeding material in commercial forest nurseries to: (1) determine effect of nut size class on nursery seedling quality, (2) test for similarities and differences in growth characteristics among the different breeding generations and parental species and (3) to test for growth differences among genetic families of the BC\textsubscript{3}F\textsubscript{3} generation.

Materials and methods

Experimental material

The experimental material for this study came from TACF’s research orchards in Meadowview, VA, USA.\textsuperscript{5} Nuts were collected in September 2007 and in September 2008, stored for ~4 months in slightly damp sphagnum moss at 3°C in closed plastic bags, 2 ml in thickness and sowed on 16 January 2008 and 29 January 2009. For this study, a genetic family is defined as progeny from a single open-pollinated mother tree in the orchard. We assume that progeny are a result of crosses between female (known genetic identity) and male (unknown genetic identity) parents within the respective orchard; we recognize that pollen from outside the orchard could contaminate mother trees, but pollen contamination events are probably rare because seed orchards of each generation are isolated.\textsuperscript{12} Hereafter, the term breeding generation refers to the specific series of crosses and intercrosses using the blight-susceptible American chestnut and blight-resistant Chinese chestnut, and parental species refers to the American or Chinese chestnuts. For this study, we used 24 families from two parental species and four breeding generations (Table 1).\textsuperscript{13} One Chinese, two BC\textsubscript{0}F\textsubscript{1}, two BC\textsubscript{2}F\textsubscript{1} and one BC\textsubscript{3}F\textsubscript{3} families (D1) had adequate seed availability during both years of seed collection; the remaining 18 families produced nuts for only 1 year of seed collection (Table 1).

We separated nuts from each family into two equally divided size classes, large and small, by visually assessing the nut size. We split each size class into two equally divided replications by number and weight to the nearest 0.1 g. The 2007 seed crop was sown at the Georgia Forestry Commission’s Flint River Nursery near Byronville, GA, USA. The 2008 seed crop was similarly sown at the East Tennessee State Nursery in Delano, TN, USA. Hereafter, seedlings will be referred to by the US postal abbreviation for the nursery in which they were grown [Georgia nursery (GA) or Tennessee nursery (TN)].

Experimental design

We used a nested, split-plot treatment arrangement to determine whether nursery (or year nuts were collected), generation-parental species, genetic family and nut size class affected nut weight and seedling growth. We used a randomized complete block design with sampling to arrange the experimental units for each nursery. Nursery was a fixed factor, and generation was a fixed factor nested within nursery because different generations were sown at each nursery. Family was a fixed whole plot factor nested within generation because we used different families within each generation. The nut size class was a fixed split-plot factor within family. Individual nuts or...
<table>
<thead>
<tr>
<th>Parental species or generation</th>
<th>GA nursery (nuts collected 2007)</th>
<th>TN nursery (nuts collected 2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Family</td>
<td>Number of nuts sown</td>
</tr>
<tr>
<td>100% American</td>
<td>GMNew</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>PLIS</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Towers1</td>
<td>51</td>
</tr>
<tr>
<td>100% Chinese</td>
<td>CD</td>
<td>277</td>
</tr>
<tr>
<td>BC1F2 (75% American)</td>
<td>NB1</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>NB35</td>
<td>151</td>
</tr>
<tr>
<td>BC1F3 (88% American)</td>
<td>SA330</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>SA417</td>
<td>152</td>
</tr>
<tr>
<td>BC1F3 (94% American)</td>
<td>CH283</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>CH526</td>
<td>102</td>
</tr>
<tr>
<td>BC1F3 (94% American)</td>
<td>D1</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>D3</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>D4</td>
<td>148</td>
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<tr>
<td></td>
<td>D5</td>
<td>147</td>
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<tr>
<td></td>
<td>D6</td>
<td>146</td>
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<td></td>
<td>D7</td>
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<td></td>
<td>D8</td>
<td>98</td>
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<tr>
<td></td>
<td>D9</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>D10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>D11</td>
<td>99</td>
</tr>
</tbody>
</table>
nursery seedlings within the nut size class seed lot were samples. All nut size class treatments within each family were replicated twice, except for some families that could only have one replication due to lack of available material (Table 1). Each of the two replications was grouped into a block to account for environmental differences within the nursery seed beds (e.g. slope of the ground, distance from watering source). Nursery was analysed as a fixed effect in this model because we did not choose nursery from a random sample of available nurseries. We chose them based on our experiences with these two particular nurseries using protocols that favoured growth of high-quality hardwood seedlings for planting. A total of 46 seed lots, each representing a distinctive generation/parental species, family, nut size class and replication/block were sown by hand at the GA nursery. Forty-eight seed lots were similarly sown at the TN nursery.

Laboratory and field methods

Prior to sowing, we recorded the weight of each individual nut that was to be sown to the nearest 0.1 g. Nuts had not yet germinated at the time of sowing at both nurseries. At both nurseries, chestnuts were sown at a density of 65 per m² in prepared seed beds consisting of four rows, 15 cm apart. To reduce variability in available sunlight reaching each seedling, only the inner two rows were sown with chestnuts and the outer two rows were sown with white oak (Quercus alba L.) for the 2007 nut crop and with Nuttall oak (Q. nuttallii Palmer) for the 2008 nut crop. Seed lots were separated by 0.8 m. Chestnuts and acorns were covered with ~3 cm of pine bark mulch immediately after sowing. Nursery beds were fertilized from May through August of each year according to prescriptions developed by Kormanik et al. and irrigated as needed. At each nursery, the seedlings were grown for one growing season and lifted in the following February. Seedlings were undercut to a depth of 30 cm and lifted using a Fabro machine lifter. In May 2009 at the East Tennessee Nursery, we noticed a complete lack of germination in all of the Chinese seed lots, which we attribute to seed desiccation. We also noticed symptoms of disease caused by Phytophthora cinnamomi in portions of the nursery beds for seedlings at the East Ten- nessee Nursery in June 2009. This fungal pathogen is widespread in the southeastern USA, and can destroy chestnut nursery seedlings and chestnut plantings in years with conducive weather and/or site condi-
tions. Symptoms included chlorosis or wilting of leaves with associ-
ated black lesions on root systems. All seedling beds were subsequently treated with a fungicide, aluminium tris(0-ethyl phos-
phonate) (Aliette WDG), at a rate of 2.2 kg ha⁻¹ in early June 2009 to control for the disease. Nursery personnel destroyed a few diseased trees within the bed during the growing season. Less than 10 trees in total were removed and impacts on the overall study were judged to be minimal.

After lifting, we measured seedlings for total height (nearest 1 cm) from the root collar to the top of the tallest terminal bud. The root collar is defined as the transition zone between the above-ground and below-ground portion of the stem at the ground-line of the seedling. We measured RCD (nearest 0.1 mm), using digital calipers, and we counted the number of first-order lateral roots (FOLRs). An FOLR is defined as a lateral stem beginning at or near the root collar and extending at least half the length of the main stem.

Statistical analysis

We did not include diseased or damaged seedlings in any statistical analyses, and we excluded seedlings with missing tap roots in statistical analysis for the number of FOLR. We conducted t-tests to determine whether pre-treatment differences existed between replications in mean nut weight for each size class within a family. We did not include the TN nursery Chinese nuts or seedlings in the analysis due to complete lack of germination of this family. We used mixed model ANOVA and Dan-

Results

Survival and nut weight

Pre-sowing differences between replications in nut weight were not significant (P ≥ 0.10), demonstrating that bias did not occur in splitting nut size classes into two replications. All families except CH526, D6 and D11 had >50% survival (Table 1). Chinese seed lots at the TN nursery did not germinate, so they were not included in the estimates of survival. Seedlings at the GA nursery had 87% survival, and seedlings at the TN nursery had 57% survival overall. American families had the best overall survival at both nurseries; BC₁F₃ families D6 and D11 had the lowest survival.

The nut weight was highly variable, ranging from 1.0 to 17.7 g (Table 2). All main effects and their interactions for the nut weight were significant sources of variation (Table 3), indicating that we were successful at visually distinguishing two size classes of nuts that differed in weight, and that generation/parental species and family differences existed. Nuts in the large size class weighed 1.2–2.0 g more than nuts in the small size class, for the 2008 and 2007 nut collections, respectively (Table 2). Interactions were because small nut size classes of the Chinese parent and BC₁F₃ generations/families had heavier
mean nut weights than large nut size classes of other genera-
tions/families (Figure 1).
Nuts from the Chinese and BC1F3 generations had significantly
heavier weights and were more variable in weight compared
with other generations (Table 4). Nuts from the BC3F3 generation
were different in weight than the American parental species, but
the trend depended on the year seed was collected. The BC3F3
generation had significantly lower weight than both parental
species and all generations for nuts collected in 2007, but had
the second highest weight compared with other generations
and parental species, including the American parent, for nuts col-
lected in 2008. All generations and parental species with two or
more families exhibited family differences in the mean nut
weight.

Approximately 57% of the variation in seedling height could
be explained by nut weight and its interaction with nursery
(P < 0.001), but the slope of this regression line for the TN
seedlings was not significantly different from zero. Using the
GA nursery data, the regression model predicted an increase of
3 cm height for every 1 g increase in the mean nut weight
(P < 0.0001, \( R^2 = 0.57 \), Figure 2a). The mean nut weight could
not be used to explain variation in nursery seedling height
when using generation/parental species or nut size class as an
interaction term in the regression models. None of the regression
models had a significant relationship between the mean nut
weight and RCD. Slope interaction terms between mean nut
weight and nursery, generation/parental species, and size class
were not significant in explaining the variation in the number
of FOLR. Pooled data for the mean nut weight, however, did
explain the variation in the number of FOLR (Figure 2b;
\( P = 0.0002 \)). For every 2 g increase in the mean nut weight, seed-
lings were predicted to gain one FOLR, although this model had
low predictive power (\( R^2 = 0.14 \)).

**Seedling growth and morphology**
Seedlings were highly variable in size, ranging from 10 to 262 cm
in height, from 2.0 to 31.4 mm in RCD and from 0 to 48 in FOLR
number (Table 2). The strongest correlation coefficients were
between height and RCD, and correlations between height
and number of FOLR and between RCD and FOLR number were
singly lower (Table 5). For the TN seedlings, height and RCD

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**Table 2.** Descriptive statistics (standard errors in parentheses) of nut weight and seedling growth characteristics and least-squares mean differences between nut size classes and nursery/year of nut collection.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Nut weight (g)</th>
<th>Height (cm)</th>
<th>RCD (mm)</th>
<th>Number of FOLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursery</td>
<td></td>
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<td></td>
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<tr>
<td>Generation (nursery)</td>
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</tr>
<tr>
<td>Family [generation (nursery)]</td>
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<tr>
<td>Size(nursery)</td>
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<tr>
<td>Size x generation(nursery)</td>
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<td></td>
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<tr>
<td>Size x family[generation (nursery)]</td>
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</tr>
</tbody>
</table>

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1Generation denotes both breeding generation and parental species.
had weak and negative correlations with missing tap root occurrence, and height had a weak and negative correlation with stem forking. For both nurseries, stem forking had weak, but positive relationships with missing tap root and with the FOLR number.

Interactions with nut size class were not significant for generation/parental species or family effects for any seedling growth variable (Table 3). Large nut size classes had 5–7 cm taller heights than small nut size classes, depending on nursery (Table 2). Large and small nut size classes had similar standard errors and ranges in seedling heights, and the largest seedling in the study originated from a nut classified as small (Figure 3). RCD and number of FOLR were not affected by nut size class, but RCD differences were approaching significance (Table 3).

Generation/parental species affected height and RCD growth, but not number of FOLR (Table 3). Rankings of generation/parental species depended on the nursery in which the seedlings grew (Table 4). The BC3F3 generation had smaller heights compared with the American parent at the TN nursery. At the GA nursery, however, all generation/parental species had similar heights to the American parent, except the BC1F3 generation, which was taller. At the GA nursery, the BC3F3 generation and the American parent were smaller in RCD than the Chinese and BC1F3 generation seedlings. American chestnuts and BC3F3 generations had weak and negative correlations with missing tap root occurrence, and height had a weak and negative correlation with stem forking. For both nurseries, stem forking had weak, but positive relationships with missing tap root and with the FOLR number.

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Family differences were significant for height, but not for RCD or number of FOLR (Table 3). BC3F3 families did not have significant differences in height growth at the GA nursery (Table 4). The largest family difference at the TN nursery for any generation/parental species was between BC3F3 families D10 (168 cm) and D6 (79 cm). In fact, D6 was smaller in height than every other BC3F3 family at the TN nursery, and no other family differences within this generation were significant at either nursery. Family distributions of height indicated that uniformity within a family was similar for both large and small nut size classes (Figure 3). Although the interaction between nut size class and family was not significant, it did appear that some families had more trees with taller heights in small nut size classes than in large nut size classes (Figure 3, D9 family).

In the logistic regression analysis, the nut size class did not affect the chances in stem forks or missing tap roots, and this term was removed from the final regression model. The model fit was good according to the Hosmer-Lemeshow goodness-of-fit test statistic ($P > 0.30$ for stem forks and $P > 0.14$ for missing tap roots). Chinese chestnut and BC1F3 generation seedlings had the highest odds of stem forking and missing tap roots compared with the BC3F3 generation seedlings (Table 6). American chestnut seedlings had significantly lower odds than BC3F3 generation seedlings of stem forks (1.9 times) and missing tap root (3.1 times). If the model was conducted using Chinese chestnut seedlings as the missing dummy variable, all other generation/parental species had significantly lower odds of having stem forks, and all other generation/parental species, except BC1F3 had significantly lower odds of having a missing tap root.

**Discussion**

Seeding quality was high for both nurseries according to hardwood standards developed for planting in even-aged silvicultural harvests. Improvements in root biomass of chestnut and BC1F3 generations had the highest odds of stem forking and missing tap roots compared with the BC3F3 generation seedlings (Table 6). American chestnut seedlings had significantly lower odds than BC3F3 generation seedlings of stem forks (1.9 times) and missing tap root (3.1 times). If the model was conducted using Chinese chestnut seedlings as the missing dummy variable, all other generation/parental species had significantly lower odds of having stem forks, and all other generation/parental species, except BC1F3 had significantly lower odds of having a missing tap root.

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**Figure 1** Nut weight and standard error bars of each generation/parental species and nut size class for the GA nursery (a) and the TN nursery (b).
Table 4. Least-squares means differences (standard error in parentheses) in nut weight, height and RCD for each breeding generation/parental species and genetic family within each nursery/year of nut collection.

<table>
<thead>
<tr>
<th>Nursery and year of nut collection</th>
<th>Generation or parental species</th>
<th>Nut weight mean</th>
<th>Height mean</th>
<th>RCD mean</th>
<th>Family</th>
<th>Nut weight mean</th>
<th>Height mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA, 2007 Chinese</td>
<td></td>
<td>8.7 (0.09)A</td>
<td>100 (0.22)DEF</td>
<td>15.3 (0.74)BCD</td>
<td>CD</td>
<td>8.7 (0.09)B</td>
<td>100 (0.22)GHIJ</td>
</tr>
<tr>
<td>GA, 2007 BC1F3</td>
<td></td>
<td>8.8 (0.09)A</td>
<td>104 (0.15)DE</td>
<td>14.1 (0.55)CD</td>
<td>NB1</td>
<td>8.6 (0.13)B</td>
<td>102 (0.22)GHIJ</td>
</tr>
<tr>
<td>GA, 2007 BC2F3</td>
<td></td>
<td>5.5 (0.03)B</td>
<td>90 (0.15)EF</td>
<td>13.5 (0.55)DE</td>
<td>SA330</td>
<td>4.7 (0.04)G</td>
<td>92 (0.10)HJKL</td>
</tr>
<tr>
<td>GA, 2007 BC3F3</td>
<td></td>
<td>3.2 (0.03)H</td>
<td>91 (0.12)EF</td>
<td>12.5 (0.44)EF</td>
<td>D1</td>
<td>2.6 (0.08)N</td>
<td>94 (0.41)GHIJK</td>
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<tr>
<td>GA, 2007 BC3F2</td>
<td></td>
<td>4.0 (0.05)E</td>
<td>144 (0.15)AB</td>
<td>17.5 (0.58)A</td>
<td>SA17</td>
<td>4.2 (0.10)H</td>
<td>125 (0.24)BCDEFG</td>
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<tr>
<td>GA, 2007 D1</td>
<td></td>
<td>2.8 (0.04)M</td>
<td>133 (0.12)BC</td>
<td>16.1 (0.44)AB</td>
<td>D1</td>
<td>3.0 (0.04)L</td>
<td>135 (0.23)ABCDEF</td>
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<tr>
<td>GA, 2007 D2</td>
<td></td>
<td>6.2 (0.08)C</td>
<td>151 (0.14)A</td>
<td>17.4 (0.55)A</td>
<td>Bell Hollow</td>
<td>4.2 (0.04)H</td>
<td>137 (0.22)ABCDEF</td>
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<tr>
<td>GA, 2007 D3</td>
<td></td>
<td>3.9 (0.05)J</td>
<td>79 (0.35)JKL</td>
<td>154 (0.41)ABC</td>
<td>High Knob</td>
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<tr>
<td>GA, 2007 D4</td>
<td></td>
<td>3.4 (0.03)G</td>
<td>151 (0.14)A</td>
<td>17.4 (0.55)A</td>
<td>Plummer2</td>
<td>3.4 (0.08)K</td>
<td>164 (0.41)A</td>
</tr>
</tbody>
</table>

Means followed by same letter within each breeding generation and family are not significantly different.
Our results are in agreement with research conducted on Chinese chestnut that found seedling height differed among nut size classes that were visually distinguished.51 Oak species have also shown positive relationships between height and seed size,27,28,30,32 but this relationship has not been previously tested in Castanea species of North America. Since chestnut has been shown to have an intermediate shade tolerance, with the best growth in full-sun conditions.20,49,52 successful field establishment will require seedlings to remain competitive with the fastest growing natural vegetation on site. Additionally, hardwood seedlings that are already taller than deer browse height (~1.3 m) will have a significant advantage over small seedlings if planted in areas with high deer populations.17 The preliminary results of chestnut test plantings using seedlings from this study indicate deer preferred to browse on shorter seedlings.53

The results concurred with past studies of northern red oak (Quercus rubra L.) that found seed weight can affect nursery height and this relationship appears to be under genetic control.29,30 Too few families were studied to calculate genetic gains or heritability in this study, but a previous study noted that the nut size in F1 hybrid chestnuts was heritable.5 Similar to our results, Detlefsen and Ruth16 found that paternal trees of American chestnut were visually different in nut size and shape, and they found that first-generation hybrids with Japanese chestnuts had the largest variation. Our results indicate that genetic family will have the strongest effect on nut weight and, to a lesser extent, seedling height. Identification of BC3F3 families with superior growth was not possible in this study due to too few differences among families within this generation and too few families studied. Family differences may be inconsequential at this early stage, compared with differences due to seed sizes or other unknown elevation or geographic influences.29,30

Small nut size may be partially dominant over large nut size in F1 hybrid chestnuts,3 and nut size may be controlled by multiple genes.54 A potential problem could arise if selecting for larger size nuts in the B3F3 generation leads to indirectly selecting for seedlings that have retained other Chinese chestnut morphological traits. Studies have not been conducted in determining whether nut size/weight is linked to other genes found in Chinese chestnut (e.g. blight resistance, non-timber form).

Increasing seedling uniformity at planting is desired in addition to improving overall seedling size; if seedlings can be grown to a uniformly adequate size, then predictions of planting success and planting efficiency will increase. Our results indicate seedlings would not have been more uniform if small-sized nuts were culled prior to sowing, but the overall height would have increased. In previous studies, the within-family growth variation was high for chestnut and oak species grown in commercial nurseries,22,30,42 and variation in bulked seed where genotype was unknown was also high.31 Thus, improving nursery production efficiency by culling seed instead of culling seedlings does not seem possible for American chestnut or blight-resistant hybrids unless better controls for seed moisture loss and seed damage can be instituted.

Due to the large variation in seedling size at lifting, we recommend that seedlings are graded into different size classes based on RCD after lifting. Visually separating seedlings using RCD has been proposed in other studies involving oak and chestnut, and is easily transferable to management in the nursery.35,42 Chestnuts from advanced breeding generations are highly valued due to decades of breeding work and research,5,35,42 and culling seedlings may be undesirable. Managers can match the seedling size to site quality and goals of the planting. For example, the largest seedlings can be planted on the most competitive sites or on sites where early nut production is desired.18 Limited
resource material could also be retained by growing small nuts in separate seed lots with a more intense fertilization regime to improve the seedling size or for planting on different site types.

The results suggested TACF accomplished its goal to reduce the Chinese chestnut contribution in advanced generations. BC2F3 generation chestnuts performed differently than Chinese chestnuts in 67% of ANOVA and logistic regression statistical tests. In 5 of 10 statistical tests, however, the BC3F3 generation also differed from American chestnut. We would expect some similarities between American and Chinese chestnut species in morphology, but the results do raise some questions regarding recovering gene expression of American characteristics expressed in the backcross generations. Of particular interest is the higher probability of stem forking, a characteristically Chinese chestnut trait, in the BC3F3 generation when compared with the American chestnut parent.

Identification of superior generation/parental species and families depended on the nursery, indicating that environmental (e.g. climate, soil, weather) by genetic interactions were apparent in this study. Testing differences between advanced generations and the preferred American parent may be difficult if interactions between generation/parental species and nursery site conditions are real. Inferences on breeding efforts of American chestnut should be made with caution regarding the seedling performance in commercial tree nurseries until more families can be repeatedly tested, and nut viability can

Table 6. Logistic regression model for probability of stem forking and missing tap roots.

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>Stem forking</th>
<th>Missing tap root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter estimate</td>
<td>P-value</td>
</tr>
<tr>
<td>Intercept</td>
<td>-3.0122</td>
<td>&lt;0.0001</td>
</tr>
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<td>GA nursery</td>
<td>0.5751</td>
<td>0.0009</td>
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<td>Chinese</td>
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<td>BC1F3</td>
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</tr>
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<td>&lt;0.9898</td>
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<td>BC3F2</td>
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<td></td>
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<tr>
<td>American</td>
<td>-0.6571</td>
<td>0.0052</td>
</tr>
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</table>

Figure 3 Height distribution for each nut size class for all seedlings from each nursery and for seedlings in families D4 and D9.
be better identified and controlled. The low amount of replication for each family could also be a contributing factor to the discrepancies in performance between nurseries. Additional replication would be desirable, but is limited by current seed source availability.5

A potential, yet untested, explanation for the high amount of variation in survival and growth within family/nut size seed lots could be related to cotyledon damage from weevils (Coleoptera: Curculionidae) prior to sowing in the nursery. In northern red oak, weevil-damaged northern red acorns had reduced RCD and stem height.56 Effects of nut damage to Castanea species on subsequent seedling development has not been well studied, but chestnut was highly susceptible to weevil damage in early hybridization studies,24 and insect damage to American chestnut was widely reported in early forestry reports.37 If large nut size classes were targeted over smaller nut size, then we postulate that large nut size class seedlings would have similar ranges in height as small nut size class seedlings (Figure 3); however, larger acorns were not targeted over smaller acorns in a study with northern red oak.32 Despite these unknown variables, our study is probably representative of conditions in most southern tree orchards and nurseries.

New knowledge was discovered in this study that will require further testing. The differences between the BC3F3 generation and the American parent seen at both nurseries do raise some concerns in regards to gene expression of a breeding line that is predicted to maintain blight resistance, but in other regards, behave like the American parent. More testing is needed before definitive differences or similarities can be determined. The relationship between FOLR number and stem forking has never been reported for any hardwood species. We initially suspected that late-season frosts may have caused stem forks, but that theory was eliminated after examining weather data from the nurseries; no late season frosts were reported during the time seedlings would have emerged (mid-April through late May). We suspect that seedlings with stem forks had more leaf area than seedlings with only one primary stem, and more leaf area resulted in a more productive root system.59

Another new characteristic we examined was the occurrence of missing tap root. We interpret the occurrence of missing tap roots as a negative characteristic for seedling performance due to its negative relationship with height and RCD in TN nursery seedlings, and that it was related to the Chinese genotype in the logistic regression. Seedlings with missing tap roots are more difficult to plant due to their spreading root system (S.L. Clark and S.E. Schlarbaum, personal observation), and we hypothesize that this characteristic could lead to poor performance in the field. Data are currently being collected from field plantings to investigate this hypothesis. Missing tap root occurrence may be under genetic control, or it could be due to the relationship between the nut size and planting depth. The larger nut that is associated with Chinese chestnut may be more prone to damage of the emerging radical at germination because larger nuts would indirectly be sown at a shallower depth, leaving them more prone to freezing/thawing while overwintering in the beds.

We established a similar nursery study in 2011 and installed 11 experimental plantings since 2009 using material studied in this paper with individual tree identity retained in the field.53 Results from these studies will be used in reforestation efforts of the National Forest System of the U.S. Department of Agriculture, Forest Service by providing recommendations on cultural practices to improve seedling quality and determine how that translates into field performance. Breeding programmes at TACF and Connecticut Agricultural Experiment Station5,57 can also use these results to refine breeding efforts and to better understand how phenotypic traits are being recovered in backcross generations.

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Conflict of interest statement
None declared.

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