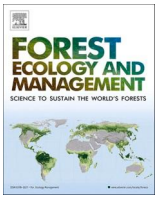
Forest Ecology and Management 532 (2023) 120820

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco



Eight-year field performance of backcross American chestnut (*Castanea dentata*) seedlings planted in the southern Appalachians, USA

Stacy L. Clark [a,*], Scott E. Schlarbaum [b], Arnold M. Saxton [c], Steven N. Jeffers [d], Richard E. Baird [e]

[a] Southern Research Station, U.S. Department of Agriculture Forest Service, 2505 E. J. Chapman Drive, Rm 427 Plant Biotech Bldg., Knoxville, TN 37996-4563, USA
[b] School of Natural Resources, The University of Tennessee, 2505 E. J. Chapman Drive, Rm 427 Plant Biotech Bldg., Knoxville, TN 37996-4563, USA
[c] Animal Science Department, The University of Tennessee, 208C Brehm Animal Science Building, Knoxville, TN 37996-4574, USA
[d] Department of Plant and Environmental Sciences, Clemson University, 105 Collings Street, Clemson, SC 29634, USA
[e] Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, Box 9655, Mississippi State, MS 39762, USA

ARTICLE INFO

Keywords:
Cryphonectria parasitica
Chestnut blight resistance
Genetic family
Growth pattern analysis
Restoration
Phytophthora cinnamomi
Phytophthora root rot
Seedling quality

ABSTRACT

The American chestnut (*Castanea dentata*) is imperiled due primarily to two diseases caused by nonnative pathogens, notably chestnut blight and Phytophthora root rot (PRR) caused by *Cryphonectria parasitica* and *Phytophthora cinnamomi*, respectively. Field reintroduction trials are important to test trees bred for blight resistance under forest conditions to quantify survival and field performance in managed stands that represent future restoration sites. We planted 513 pedigreed bare-root (1–0) nursery seedlings into an even-aged regeneration harvest in the Blue Ridge Mountains of eastern Tennessee, USA to test effects of breeding, genetics, and seedling size class on field performance, including survival, total growth, incremental growth (a measure of growth efficiency), and height growth patterns. Mortality was highest in the first two years after planting, and PRR symptoms coupled with soil and root assays that tested positive for *P. cinnamomi* suggested this disease was a significant contributor to mortality. Chinese chestnut (*C. mollissima*), known to be resistant to *P. cinnamomi* and *C. parasitica*, had the highest survival (96 percent) compared to American chestnut (34 percent) and hybrid generations, including the BC_3F_3 generation (41 percent). Less advanced hybrid generations, such as the BC_1F_3 and BC_2F_3, had the greatest total height and ground-line diameter (GLD) and height growth efficiencies. BC_3F_3 generation seedlings were associated with higher-than-expected rates of nominal height growth patterns (i.e., flat growth rates over time or early mortality) compared to Chinese chestnut seedlings. Results, however, indicated the breeding program was successfully integrating desired American chestnut growth traits into the hybrid genome while transferring an intermediate level of resistance from the Chinese chestnut. We identified superior and inferior BC_3F_3 families that can help inform breeding and restoration efforts. Visually grading seedlings before planting affected survival and growth of generations and genetic families differently, indicating that refinement of nursery production and restoration plantings should consider genetic effects. Across generations, seedling size grading improved total height and GLD growth that would save resources necessary for competition control and animal browsing protection, but large-size seedlings also exhibited lower *C. parasitica* resistance rankings over time, probably owing to earlier bark fissuring. PRR probably affected outcomes, particularly for hybrid and American chestnut seedlings that have very low levels of *P. cinnamomi* resistance. Managers could expect advanced breeding generations to perform similarly to American chestnut in growth through the critical stage of early stand development. More durable resistance to *C. parasitica* and mitigation of PRR through better site selection and/or resistance screening will improve future restoration efforts.

* Corresponding author.
E-mail addresses: stacyl.clark@usda.gov (S.L. Clark), tenntip@utk.edu (S.E. Schlarbaum), asaxton@utk.edu (A.M. Saxton), sjffrs@clemson.edu (S.N. Jeffers), reb58@msstate.edu (R.E. Baird).

https://doi.org/10.1016/j.foreco.2023.120820
Received 10 November 2022; Received in revised form 12 January 2023; Accepted 17 January 2023
Available online 2 February 2023
0378-1127/Published by Elsevier B.V.

1. Introduction

Chestnut (*Castanea*) species have provided important ecological services for millennia worldwide, such as hard mast and soil stabilization, and cultural values, such as food and heating sources (Clark et al., In review). The American chestnut [*Castanea dentata* (Marsh.) Borkh.], native to eastern North America, is an iconic tree species with historically important ecosystem functions and utilitarian values, but much of its traditional ecological knowledge as a mature component in eastern hardwood forests is unknown (Barnhill-Dilling and Delborne, 2019). The species was geographically widespread until the beginning of the 19th century, with a range of over 81 million ha (Little, 1977) and was a minor to dominant component in most upland forest types of the eastern United States (Braun, 1950; Russell, 1987). American chestnut comprised approximately 25 percent of the total timber volume in the southern Appalachian Mountains and 50 percent of timber volume in second-growth forests of New England and the mid-Atlantic region in the early 20th century (Frothingham, 1912; Saucier, 1973; Russell, 1987). Ecological services such as high water-use efficiency, soil nutrient cycling, and food for apex predators like black bear (*Ursus americanus*) (Bauerle et al., 2006; Rhoades, 2007; Clark et al., 2019a) were provided by this species. A versatility of forest products from American chestnut included rot-resistant lumber for construction and fencing, tannin for leather, nuts for a global food commodity market, and food for domesticated and undomesticated animals (Ziegler, 1920; Lutts, 2004).

Nonnative exotic diseases have reshaped forests globally (Pyšek et al., 2020; Poland et al., 2021), and American chestnut was perhaps one of the most ubiquitous and ecologically important tree species to be extirpated as a forest canopy dominant. A root pathogen, *Phytophthora cinnamomi* Rands, first introduced into North American in the early- to mid-1800s, killed American chestnut trees in low elevation forests with poorly drained or clayey soils and in riparian areas (Anagnostakis, 1995). A second disease, chestnut blight, caused by *Cryphonectria parasitica* (Murr.) Barr, was introduced into North American from Asia around the late 1800s and reduced this dominant forest tree species in upland sites to remnant understory sprouts by the mid-20th century (Anagnostakis, 2012). Other exotic pests such as Asiatic oak weevil (*Cyrtepistomus castaneus* Roelofs), Asian gall wasp (*Dryocosmus kuriphilus* Yasumatsu), and spongy moth (*Lymantria dispar* L.) have plagued the American chestnut to varying degrees (Payne et al., 1975; Cooper and Rieske, 2007; Case et al., 2016), but chestnut blight (syn. chestnut bark disease) caused the most widespread and severe reduction of the species throughout its range (Anagnostakis 1995).

Traditional breeding to produce trees with chestnut blight resistance has been proceeding since the early 1900s, and biotechnology programs have been proceedings since the early 2000s (Burnham et al., 1986; Anagnostakis, 2012; Newhouse et al., 2014; Westbrook et al., 2020). A limited number of studies using trees bred for resistance to chestnut blight have been established in the last decade in forest, plantation, and mine reclamation reintroduction trials (Clark et al., 2014; Pinchot et al., 2017; Thomas-Van Gundy et al., 2017; Skousen et al., 2018; Gilland and McCarthy, 2014). Genetically modified (GMO) trees are currently under federal regulatory control and have not yet been approved for public release although limited testing of GMO trees has been conducted (Powell et al., 2019; Newhouse and Powell, 2020). The backcross breeding technique integrates genes from Asian chestnut species into American chestnut through traditional breeding with the goal of improving pathogen resistance while maintaining desirable growth and form characteristics associated with the American chestnut (Burnham et al., 1986; Anagnostakis, 2012). Even though Chinese chestnut (*C. mollissima* Blume) is highly resistant to *C. parasitica*, it typically grows slower with inferior form compared to American chestnut, making it relatively undesirable for forest restoration plantings (Fei et al., 2012). Linkages and effects of disease-resistant genes to phenotypic traits, such as growth form and competitive ability, have not been adequately studied in plantings using traditionally bred chestnut trees.

Traditional breeding probably offers the most feasible pathway for widespread reintroduction of American chestnut on public lands, at least in the short term (Clark et al., 2020). The most advanced hybrid generation (BC_3F_3) from North American breeding programs is currently being tested and has exhibited cankering severity levels (i.e., response to *C. parasitica* infection after inoculation) more like the American chestnut than the Chinese chestnut (Steiner et al., 2017; Westbrook et al., 2020). BC_3F_3 generation trees in the oldest forest reintroduction trials, however, exhibited levels of resistance closer to the Chinese chestnut than to American chestnut (Thomas-Van Gundy et al., 2017; Clark et al., 2019b). Unlike orchard tests, trees in forest reintroduction trials were not inoculated, and disease symptoms may be comparatively delayed and not uniformly distributed among trees. Multiple forest field trials over many years will need to be conducted to determine long-term durability of disease resistance across various environmental conditions as blight resistance is affected by the interaction of the pathogen genotype, genetics of the host, and the abiotic and biotic environment. Frosts/freezes, animal browsing, insect herbivory or damage, amount of available sunlight, and drought can all effect the incidence and severity of blight symptoms and pathogen resistance (Griffin, 1992, 2000; Anagnostakis, 2001). Adequate quantities of advanced breeding material have recently become available for widespread testing across the native chestnut range (Westbrook et al., 2020), and the oldest field trials are now into their second decade of development (Clark et al., 2014; Thomas-Van Gundy et al., 2017; Skousen et al., 2018).

In addition to chestnut blight resistance, tree growth and persistence under adverse forest conditions are important goals for restoration of American chestnut (Jacobs et al., 2013). Trees must compete with native vegetation and survive multiple stressors (e.g., drought, late-season frost, deer browsing) after planting to establish self-perpetuating populations. Nursery cultural practices, such as seedling grading, has been shown to affect field performance of Fagaceae species, including American chestnut (Dey et al., 2008; Clark et al., 2016), and scarcity and relatively high value of genetic material (Clark et al., 2014; Steiner et al., 2017) provides support for refined silvicultural approaches, like seedling grading to improve planting success. Similarities or deviations between BC_3F_3 and American chestnut trees in growth response to seedling grading treatments should be evaluated over the long-term across the species range. Preliminary results indicated BC_3F_3 generation seedlings had slower growth than American chestnut seedlings mainly due to one or two poor performing BC_3F_3 families (Clark et al., 2016; Thomas-Van Gundy et al., 2017). Additionally, planting larger seedlings increased early mortality, but also increased seedling growth efficacy of American chestnut and BC_3F_3 chestnut seedlings compared to planting smaller sized seedlings at the same time. Long-term effects of using quality-graded seedlings of American chestnut are not known.

Successful forest reintroduction will require additional knowledge than is currently available on the ability of traditionally bred seedlings to survive, remain resistant to chestnut blight, and grow competitively over the long term. Faster growing trees were infected by *C. parasitica* sooner than slower growing trees after eight growing seasons (Clark et al., 2019b), but trees that grow faster under full-sun conditions may have better response to infection over time (Griffin et al., 1991). Temporal dynamics in growth will also affect a tree's ability to compete with native vegetation, escape deer browse, and receive adequate sunlight to sustain physiological functions (Pinchot et al., 2017). A tree that has increasing growth rates over time may have the best competitive ability and probability to early fruit production, while a tree that has nominal early growth followed by exponential growth may be able to better respond to disturbance events and overcome suppression that limits survivability (Stimm et al., 2021). Breeding to reduce pathogenic effects on temporal growth dynamics of traditionally bred chestnut trees has not been tested. Growth pattern analysis in a related oak species, *Q. rubra* L., found differences among genetic families that were not found in traditional analysis of variance models (Pinchot et al., 2018).

In this study we examined eight-year field performance of hybrid American chestnut trees from different backcross generations (i.e., BC_1F_3, BC_2F_3, BC_3F_2, and BC_3F_3) and their associated parental species (American and Chinese chestnuts) planted in a regeneration harvest in the Blue Ridge Mountains of eastern Tennessee, USA. Our specific objectives were to quantify differences in survival, growth, and *C. parasitica* resistance among *a prior* treatments (seedling size class, breeding generation or species, and genetic family) and to examine temporal height growth patterns among treatments and effects from *C. parasitica* infection. We have several specific research hypotheses related to eight-year performance of hybrid breeding generations and parental species currently being evaluated in long-term field trials (Clark et al., 2014):

1. Hybrid generations and parental species will differentiate in field performance metrics:
   a. Chinese chestnut will have relatively low survival and slow growth but exhibit the highest blight resistance,
   b. Less advanced generations (BC_1F_3, BC_2F_3) will have blight resistance similar to the BC_3F_3 generation but survival and growth intermediate between the two parental species.
   c. BC_3F_3 will exhibit higher survival, more positive long-term growth dynamics, and higher blight resistance than the American chestnut over time.
   d. American chestnut will exhibit relatively fast initial growth followed by dieback and mortality related to chestnut blight.
2. Genetic differences within each breeding generation or parental species will facilitate identification of poor or superior performing genetic families:
   a. BC_3F_3 families with slower growth will also exhibit higher blight resistance.
3. Seedling size at the time of planting will affect field performance outcomes:
   a. Large-size seedlings will exhibit improved growth compared to small-size seedlings as plants age.
   b. Larger-size seedlings will have reduced *C. parasitica* resistance compared to small-size seedlings.

2. Methods

2.1. Experimental material

Nuts from hybrid trees were obtained in autumn 2008 from The American Chestnut Foundation (TACF) Research Farm in Virginia, USA (Table A1). Hereafter, a genetic family refers to seedlings derived from nuts collected from a single open-pollinated or controlled-pollinated tree. The American chestnut families were from open-pollinated mother trees growing wild in southeast Virginia, USA. The Chinese chestnut family was from a controlled pollination of a Chinese chestnut mother tree at the TACF Research Farm. Families from hybrid generations were produced through backcross breeding (Burnham et al., 1986; Anagnostakis, 2012; Clark et al., 2016). Nuts from an individual American chestnut or hybrid families were putative half-siblings, a result of open-pollinated crosses between female (known genetic identity) and male (unknown genetic identity) parents. Hereafter, the term 'generation' collectively refers to the backcross breeding generation (BC_1F_3, BC_2F_3, BC_3F_2, or BC_3F_3), pure American chestnut, or pure Chinese chestnut collection trees. After collection, nuts were stored in slightly moistened sphagnum moss in plastic bags at 4 °C to stratify until sowing.

Nursery production and growth were described by Clark and others (2012). After lifting from the nursery in February 2010, each seedling was assigned an individually numbered tag to ensure that family identification was maintained. Lateral roots were clipped to within 15 cm of the main taproot to facilitate planting and to prevent root-tip dieback. Seedlings from each family were visually assessed and equally divided into two seedling size classes (small or large) as previously described for a similar study (Clark et al., 2016). Size class distinctions were based on overall tree size with an emphasis on root-collar diameter because of this variable's high correlations with other seedling variables in previous studies of Fagaceae species (Clark et al., 2000; 2009; 2012a). Size class sorting also reduces potential bias associated with selecting larger seedlings first during sorting into experimental designs (Pinto et al. 2011).

2.2. Study areas

Site selection and site preparation treatments were designed to be like those that would be conducted by the United States Department of Agriculture Forest Service, National Forest System once chestnut blight-resistant seedlings become widely available (Clark et al., 2014). Seedlings were planted on the Cherokee National Forest within the Blue Ridge Mountain physiographic section in Tennessee (N35° 56′ W-82 56′) (Bailey et al. 1994). Soils were classified as Brasstown loam 12–20 percent slopes; soils are deep and well-drained, derived from loamy residuum weathered from metasedimentary parent material (NRCS, 2020). Prior to harvest, stands were dominated by mature (80- to 100-years old) eastern white pine (*Pinus strobus* L.) and hardwoods, primarily white oak (*Quercus alba* L.), chestnut oak (*Q. prinus* L.) and scarlet oak (*Q. coccinea* Muenchh.). Site index (base age 50) for white oak was 24 m. Elevation was approximately 460 m above sea level (ASL), mean annual precipitation averaged 152 cm, and mean annual air temperature was 13° C. Slopes averaged 10 to 20 percent from horizontal, and aspect direction of the planting area was North (5°). Natural American chestnut sprouts were found at the site, indicating that the planting area was well-suited for American chestnut.

The stand was harvested using a shelterwood-with-reserve regeneration treatment, which creates a two-age stand and provides adequate sunlight to regeneration (Nyland, 1996). Commercial timber harvesting was completed in November 2009. After harvest, basal area averaged 4.6 m^2/ha, consisting of primarily oak and white pine. An herbicide (triclopyr) treatment was applied just prior to planting to treat undesirable species [i.e., species except oak, hickory, American chestnut, or cherry (*Prunus* L.) species] originating from stump sprouts that were at least 3 cm in diameter at breast height (DBH). An additional herbicide treatment was applied in April 2016 (just prior to the seventh growing season) using a triclopyr basal bark treatment to treat competitors within a 45-degree cone of interference above the planted chestnut seedlings.

2.3. Experimental design and planting methodology

We used an *a priori* experimental design to empirically test treatment effects of nursery seedling size class, generation/species, and genetic family, on survival, growth, and blight resistance. We used a resolvable incomplete block design with single tree plots with a nested, factorial treatment arrangement. Incomplete blocks were grouped together to constitute one complete replication of each treatment combination (i.e., resolvable). This design was used to accommodate the relatively high number of family by size class treatment combinations ($n = 34$). Each incomplete block consisted of six trees that represented six different treatment combinations. Treatment combinations within each incomplete block were arranged using the procedure Optex in SAS software (SAS, 2013) to maximize the ability to test for generation/species, family, and seedling size class differences.

Family was treated as a fixed effect nested within the fixed effect of breeding generation/species. Identifying family and generation/species as fixed effects allowed computation of means and differences among means, which can be compared to results from previous orchard inoculation tests (Hebard et al., 2012). Furthermore, the number of families within a generation tested was relatively low ($n \leq 6$) due to the lack of available material in the TACF breeding program at the time, making

extrapolation of results as a random variable to the general population of the breeding program difficult. Seedling size class was a fixed effect cross-classified with generation/species and family.

Seedlings were planted on a 2.5 m by 2.5 m spacing using modified JIM GEM® KBC bars (modified to increase bar width to 30 cm) on 1 March 2010. Tree bark protectors (A.M. Leonard Company™) were placed around all seedlings immediately following planting to protect from deer browsing. The protectors were 1.5 m in height, 10 cm in diameter, had a 2 cm by 2 cm rigid plastic mesh, and were held in place using metal rebar and cable ties. The mesh protectors were preferred over a closed shelter to avoid increasing temperature and moisture conditions around the tree that could encourage pathogen infection (Minter et al., 1992; Ponder, 1995, Ward et al., 2000). The protectors were left on the trees for the first two years after planting or until the tree grew above the top of the protector. Deer browsing to the terminal bud could sometimes occur on the seedlings after the tree protectors were in place because the stem grew through the 2 cm mesh; however, shelters were maintained yearly to position terminal buds inside the tree protector.

2.4. Data collection

Stem height (nearest 1 cm) and ground-line diameter (GLD) (nearest 0.1 mm) were measured on all seedlings after bud set was complete (October-March). Stem height was measured immediately after planting and for the first eight years, and GLD was measured immediately after planting and for years 3–8. GLD was not measured in the first two years after plantings because tree protectors restricted access to the ground-line. Height was measured from the base of the tree to the tallest live bud, perpendicular to the ground, using a telescoping height pole. GLD was measured along the shortest width of the stem, when stems were non-circular in shape, where the tallest live stem emerged from the litter layer using a digital or manual dial caliper.

C. parasitica infections were identified as ellipsoid–shaped cankers that was sometimes accompanied by bark discoloration, fissuring, or orange stromata protruding through the bark (Berry, 1959; Griffin and Elkins 1986), and we tallied cankers in the late summer/early fall of each growing season. To confirm the cankers were result of *C. parasitica* infections, two bark samples (5 × 5 mm) were removed along outer margins of questionable cankers, which primarily consisted of bark wounds with no visible stromata or probable cankers that appeared to be in the early stages of development. Bark samples were taken to the laboratory for further evaluation. Generally, cankers with abundant stromata were not sampled. A total of 79 cankers were sampled for confirmation. Bark samples were placed into microtiter plates so that they could later be identified as to specific cankers and locations on a tree. All samples were stored at 4 °C in the laboratory until processed using isolation procedures (Baird, 1991). The bark samples were surface disinfected in 0.525% w/v sodium hypochlorite solution for 10 min, rinsed in sterile distilled water and cultured onto glucose-yeast extract medium (GYE) in 10 × 1.5 cm Petri plates for 10-d at room temperature (20 °C) while exposed to overhead fluorescent light and diffused sunlight. Colonies were then transferred to potato dextrose agar (PDA, Difco Lawrence, KS). Colony morphology was verified after 10-d incubation at 20 °C under fluorescent light. When colony morphologies were uncertain, molecular sequencing was conducted using ITS primers and sequencing methods as described previously (Baird, 2014).

Between August and December 2011, non-random sampling was used to collect representative tree and soil samples where PRR was suspected to be affecting planted seedlings. Sampled seedlings were recently dead (i.e., dead within 6 months) or dying and had characteristic symptoms of PRR [i.e., wilted or necrotic foliage, decayed roots, and black to dark brown lesions in the root crown that occasionally extended above the soil line into the lower stem (Crandall et al., 1945; Sharpe, 2017)]. Not all trees with symptoms consistent with PRR were sampled. A total of 28 seedlings were removed (15 BC_3F_3, seven BC_3F_2, two BC_1F_3, and four American), and soil samples from the planting area associated with 16 of these seedlings were collected. In addition, soil samples were collected in June 2013 from six random locations within the study site (at least 1 m distant from any planted seedling), and four soil samples were collected outside the study site (10 m from the edge of the planting located upslope, downslope, and to either side). Soil samples (approximately 1 L) were composed of 5–10 subsamples collected to a depth of 15–20 cm with a soil knife that was disinfested with a 25-percent bleach solution between samples. All samples were sent to the laboratory to determine if *Phytophthora* spp. were present. Diagnostic and detection methods were similar to those described by Sharpe (2017). Briefly, *Phytophthora* spp. were isolated from seedling samples by embedding pieces of tissue (~5 × 5 mm) from lesions on the roots and lower stem into PARPH-V8A selective medium (Jeffers, 2015a); isolation plates were placed at 20 °C in the dark for 3 to 7 days and observed daily for characteristic mycelia of *Phytophthora* spp. Single hyphae from suspected colonies were transferred to fresh PARPH-V8A and then to 5% clarified V8 juice agar (cV8A; Jeffers 2015b) to obtain axenic cultures. *Phytophthora* spp. were recovered from soil samples using a standard baiting bioassay (Ferguson and Jeffers, 1999; Meadows et al., 2011); 100 ml of soil was flooded with 200 ml of distilled water in a 400-ml plastic container, and camellia and rhododendron leaf disks (5-mm diameter) were floated on the surface for 3 days at room temperature (22 to 25 °C). Leaf pieces were moved to PARPH-V8A and then to cV8A to isolate axenic cultures of *Phytophthora* spp. All isolates were saved in a permanent collection on cV8A in glass vials stored at 15 °C in the dark. Isolates were identified based on standard morphological and molecular characteristics (Erwin and Ribeiro, 1996; Gallegly and Hong 2008; Abad et al., 2019).

2.5. Resistance to Cryphonectria parasitica

We ranked blight cankers according to disease severity symptoms: 4 = no visual evidence of disease symptoms; 3 = cankers were in early phases of development or were exhibiting signs of resistance, including a lack of orange stromata and usually accompanied by slight swelling at the site of infection; 2 = cankers had abundant orange stromata with the main terminal of the tree killed above the point of infection; and 1 = trees were assumed to have died from *C. parasitica* infection (Clark et al., 2019b). Although we did not measure canker lengths, this qualitative ranking system closely resembled that of the system used following stem inoculations (Hebard, 2005). If the tree's primary stem died-back completely from *C. parasitica* infection and produced a new primary sprout, a yearly resistance ranking value of 2 was recorded for the first and second year of the new sprout, but a value of 4 was assigned thereafter until new pathogen infection occurred. If the tree died before *C. parasitica* infection was identified, yearly resistance ranking was recorded as missing data for the year of mortality and each year thereafter.

We produced an overall resistance value by summing the number of years the tree lived before being infected (1–9) with the number of years the tree lived before death that preceded *C. parasitica* infection (1–9). In this analysis, 'year' refers to the number of growing seasons after planting (1–8), with year 9 assigned if the tree was alive in year 8 and/or pathogen infection was never detected. If a tree lived all 8 years and was never infected, an overall resistance of 18 was recorded (i.e., 9 + 9). A tree was assigned an overall resistance of 17 if it was infected in year 8 and lived all eight years (i.e., 8 + 9). If the tree died and evidence of chestnut blight was never recorded, overall resistance was recorded as missing data. Overall resistance ranged from 2 (tree died in year 1 from pathogen infection in year 1) to 18 (tree did not develop blight symptoms and lived all eight years).

2.6. A priori statistical analysis

We used general linear mixed models (GLM) to analyze if dependent

variables (survival, height, GLD, yearly resistance ranking, and overall resistance,) were affected by *a priori* fixed effects (generation, size class, and family). The survival model was the normal approximation to the binomial with very stable results. Nonlinear models were not stable if all variables were included (c.f., Clark et al., 2016). Models for GLD did not include the first and second year after planting because tree-bark protectors prohibited access to the ground line (described above). Models for GLD also did not include trees identified with *C. parasitica* infections within 20 cm of the base because reaction to the *C. parasitica* infection often creates a swollen base that would bias GLD measurements.

Replication and incomplete block within replication were random effects in GLM models. Year was used as a repeated measure in all models except for overall resistance (because only one value over the life of the tree is calculated), and we used an autoregressive covariance structure (Littell et al. 1998). We compared corrected Akaike information criterion (AICc) values for other covariance structures (compound symmetry, unstructured, spatial power) and determined the autoregressive structure had the smallest AICc values and was the most parsimonious model. Normality assumptions of residuals were assumed if Kolmogorov-Smirnov D-statistic was$<$0.10 or the Shapiro-Wilke estimate was$>$0.9. Homogeneity of variance assumptions were tested for each model by examining plots of residual versus predicted values and determining if residuals were within 5-fold of each other. Height and GLD were square-root transformed and we applied a quadratic transformation to overall resistance to meet normality and homogeneity of variance assumptions of residuals. The Kolmogorov–Smirnov D-statistic was 0.38 for yearly resistance ranking indicating a deviation of normality; however, yearly resistance is essentially rank-transformed. Therefore, data were analyzed using the above linear models to produce non-parametric results (Conover and Inman, 1981) like a previous study (c.f., Clark et al., 2019b). We computed specific comparisons among least-squares (LS) means using Tukey's HSD (honestly significant difference) mean separation.

2.7. Post-hoc exploratory analyses

Height growth efficiency was analyzed using indicator variable linear regression. We modelled annual height growth as a function of height at the beginning of the growth interval. This incremental growth analysis has been shown to be superior to analyses of mean relative growth rates because it equalizes the basis of comparisons to size at the beginning of the measurement interval (South 1995). The effects of generation, nursery seedling size class, and *C. parasitica* presence/absence were tested as possible predictor variables for inclusion in the final model. Negative height growth values due to stem dieback were entered as zero. Differences in parameter estimates between growth curves of the categorical predictors (generation, size class, blight occurrence) were tested using contrast statements with Bonferroni adjustments. For the *post-hoc* GLMM and linear regression models, we selected variables to include in the final models by first testing candidate predictor variables and using model building techniques to select the most parsimonious models. We tested for multicollinearity, linearity, and interactions, and we compared several candidate models. Final models included predictors that were significant ($P < 0.05$) and had the lowest corrected Akaike information criterion (AICc) value.

Temporal height growth patterns were derived by developing regression models for each tree, adopted from methods described by Pinchot et al. (2018). Each tree was tested for significant linear (L) and quadratic (Q) effects of year after planting (year and year^2, respectively) on stem height using Type I sums of squares. Trees were categorized into one of nine possible growth pattern categories: linear and quadratic effects that were either negative (N), neutral (0), or positive (P). For example, a PL-PQ model had significantly positive linear and quadratic effects, indicating positive growth that increased over time; a 0L-0Q model had non-significant linear and quadratic effects, indicating a tree had nominal growth or early mortality. The frequency of growth patterns across generations, blight occurrence, and nursery seedling size groups were compared using chi-square analyses, and differences among specific growth pattern categories or predictor variables were computed with Bonferroni adjustments. Trees that died the first growing season ($n = 153$) were not included in this analysis because of inadequate data needed to compute model parameters.

3. Results

3.1. Survival

Generations differentiated in survival, and mean differences were significant beginning in the second growing season (Table 1 and Fig. 1). Chinese chestnut had higher survival than most generations over time, with $<$5 percent of mortality by the eighth growing season. American chestnut had similar survival (34 percent in year 8) to hybrid generations, except the BC_2F_3, which had the highest survival among hybrid generations (61 percent in year 8). Mortality was highest during the first and second years for hybrid generations and American chestnut when 18 to 44 percent of trees died. Annual mortality rates generally decreased after the second growing season, ranging from $<$1 percent to 12 percent, and stabilized from years 6 to 8 for the BC_3F_3 generation (44 to 41 percent, respectively).

Families differentiated in survival rates (Table 1). The BC_2F_3 family SA330 had lower survival (33 percent) than SA417 (77 percent), and BC_3F_3 family D8 had lower survival (9 percent) than families D1 (60 percent) and D6 (49 percent) (Table 2). Most other families had similar survival means if they were from the same generation or species (family means for all years are in Table A2).

The main effect of size class and its interactions with years or generations were not significant, but size class interacted with family to affect survival (Table 1). Most of the American chestnut, BC_3F_2, and BC_3F_3 families had $>$10 percent higher survival for small-size seedlings than large-size seedlings. The less advanced generation families from the BC_1F_3 and BC_2F_3 generations along with the Chinese chestnut families had either negligible differences ($<$5 percent difference) or a 9 to 27 percent higher survival for large-size seedlings than small-size seedlings (Table 3). Although some of these specific mean differences were relatively large, none were significant in year 8.

3.2. Growth differences among treatments

Maximum tree height and GLD were 12.5 m and 12.8 cm, respectively (data not shown). Generation affected height and GLD differently within specific years (Table 1), and the only significant growth differences were between the BC_1F_3 (478 cm height and 50.0 mm GLD) and the BC_3F_3 generations (293 cm height and 34.9 mm GLD) in the eighth growing season (Table 4). The Chinese chestnut was generally smaller in GLD over all eight years and smaller in height in the first four years after planting, although these differences were not significant. The BC_3F_3 generation was the only generation to have a decrease in height and GLD from year 7 to year 8 (-50 cm height and $-$6.6 mm GLD).

Although the main effects of family and interactions with year were significant for both height and GLD (Table 1), families within the same generation did not differentiate in height or GLD until year 8 (family means for all years are in Table A2). BC_3F_3 family D1 had larger trees (589 cm height and 56.5 mm GLD) than families D6 (325 cm height and 18.8 mm GLD) and D8 (370 cm height and 9.3 mm GLD); family D7 had larger GLD (59.4 mm) than families D6 and D8, but its height (443 cm) did not significantly differ from any other BC_3F_3 family (Table 2). BC_3F_3 families D6 and D8 were the only families to decrease in height (-187 cm and $-$165 cm, respectively) and GLD (-17.5 mm and $-$19.7 mm, respectively) from years 7 to 8.

Size class had the second highest contribution to variation in the height and GLD models (Table 1). Large-size seedlings increased their height advantage over small-size seedlings from the time of planting

Table 1
General linear models for survival, height, ground-line diameter (GLD), yearly resistance ranking, and overall resistance ranking. Italicized *p*-values < 0.05 were significant. Yr = Year, Gen = Generation/Species, SC = Seedling size class, Fam = Genetic family.

Effect	Survival		Height		GLD		Yearly resistance ranking		Overall resistance ranking	
	F Value	*p*	F Value	*p*	F Value	*p*	F Value	*p*	F Value	*p*
Gen	15.47	*<0.0001*	0.93	0.4603	3.83	*0.0021*	10.43	*<0.0001*	7.78	*<0.0001*
Yr	22.75	*<0.0001*	114.78	*<0.0001*	257.67	*<0.0001*	22.57	*<0.0001*	–	–
Gen*Yr	2.14	*0.0001*	2.95	*<0.0001*	3.49	*<0.0001*	3.32	*<0.0001*	–	–
SC	0.32	0.5741	20.22	*<0.0001*	45.29	*<0.0001*	21.39	*<0.0001*	20.58	*<0.0001*
Gen*SC	1.48	0.1944	1.20	0.3062	2.92	*0.0132*	2.27	*0.0463*	3.03	*0.0123*
SC*Yr	0.78	0.6026	2.85	*0.0038*	1.29	0.2583	4.97	*<0.0001*	–	–
Gen*SC*Yr	0.94	0.5622	1.26	0.1266	2.14	*0.0003*	1.75	*0.0042*	–	–
Fam(Gen)	7.24	*<0.0001*	1.96	*0.0305*	3.67	*<0.0001*	3.05	*0.0005*	2.4	*0.0091*
Yr*Fam(Gen)	1.35	*0.0240*	2.36	*<0.0001*	2.73	*<0.0001*	1.70	*0.0002*	–	–
SC*Fam(Gen)	2.12	*0.0177*	3.05	*0.0006*	2.78	*0.0016*	1.31	0.0612	1.48	0.1619
SC*Yr*Fam(Gen)	0.94	0.6216	1.91	*<0.0001*	2.37	*<0.0001*	1.23	0.0981	–	–

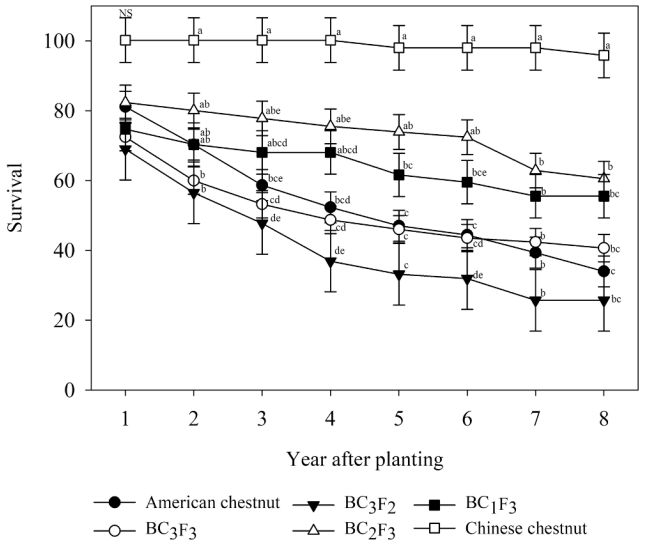


Fig. 1. Survival least-squares means for each generation or parental species over eight growing seasons. Letters denote differences among means within a year (Tukey's HSD *p*-value < 0.05; NS = differences were not significant).

(156 cm versus 93 cm) to year 8 (443 cm versus 333 cm), such that large-size seedlings were similar in size at year 4 (316 cm) to small-size seedlings in year 8 (333 cm). Size class interacted with generation to affect GLD but not height. Size class did not affect GLD of American chestnut seedlings, but large-size seedlings of BC_3F_3 and Chinese chestnut were 9.9 and 12.1 mm larger than small-size seedlings, respectively, across all years. Within a specific year, however, size class differences within a generation were typically not significant (size class means for each generation and year are in Table A3). Similarly, within families, large-size class seedlings had larger mean eight-year heights and GLDs than small-size seedlings for 67 and 80 percent of the time, respectively, but these specific mean differences were only significant for one family, NB35, in year 8 (Table 3).

3.3. Incremental height growth

Height of the prior year (*p*-value < 0.0001), generation (*p*-value = 0.2575), their interaction (*p*-value = 0.0105), and blight occurrence (p-value < 0.0001) were included in the final regression model to predict annual height growth increment. The effect of seedling size class and its interactions with other variables were not included in the final regression model due to low explanatory power (*p*-value > 0.49). The only significant differences between generational growth curves were between American chestnut, BC_1F_3, and BC_2F_3 generations. American chestnut had reduced annual height growth increment (adjusted *p*-value = 0.0225 for specific comparisons to both BC_1F_3, and BC_2F_3 generations), and this reduction became more pronounced as height of the prior year increased (Fig. 2). No other generations differed from each other (adjusted p > 0.687). *Cryphonectria parasitica* infection reduced incremental growth curves for all generations/species similarly by −18.5 cm (Parameter estimates for generations/species can be found in Table A4).

Table 2
Least-squares family means (and associated standard errors) for eight-year survival, height, ground-line diameter (GLD), and yearly and overall resistance to *Cryphonectria parasitica*. Letters within columns denote differences among family means (Tukey's HSD test *p*-value < 0.05).

Generation	Family	Survival (percent)	Height (cm)	GLD (mm)	Yearly resistance (1-4)	Overall resistance (2-18)
American chestnut	BH	46 (5.8) bcd	371 (25.6) ab	42.8 (2.82) abc	3.1 (0.11) bc	15.6 (0.03) bc
	HK	36 (6.8) bcd	344 (33.5) ab	36.3 (3.57) bcd	2.9 (0.15) abc	15.4 (0.04) bc
	Plummer	20 (9.5) cd	339 (52.4) ab	38.3 (5.69) abcd	2.1 (0.19) c	12.8 (0.08) c
BC_3F_3	D1	60 (6.5) abc	589 (58.2) a	56.5 (3.41) ab	3.7 (0.13) cd	17.4 (0.03) ab
	D6	49 (13.5) abc	325 (39.8) b	18.8 (4.40) cd	3.4 (0.29) abcd	17.1 (0.06) abc
	D7	45 (9.6) abcd	376 (33.0) ab	59.4 (5.66) ab	3.2 (0.19) abcd	16.3 (0.05) abc
	D8	9 (9.3) d	370 (23.5) b	9.0 (4.41) d	3.0 (0.41) abcd	16.4 (0.11) abc
	D9	33 (7.2) cd	443 (53.7) ab	36.5 (3.98) bcd	3.6 (0.18) bcd	17.1 (0.04) ab
	D10	48 (7.9) bcd	379 (32.4) ab	47.0 (3.85) abc	3.1 (0.15) abc	15.7 (0.04) abc
BC_3F_2	CH283	28 (6.8) cd	519 (116.3) ab	55.0 (6.10) ab	2.7 (0.17) ab	14.6 (0.05) bc
	CH526	23 (16.3) abc	513 (31.7) ab	50.5 (11.97) abcd	3.8 (0.40) abcd	18.0 (0.07) abc
BC_2F_3	C240	72 (11.4) abc	325 (39.8) ab	34.7 (4.31) bcd	3.7 (0.19) bcd	16.7 (0.05) abc
	SA330	34 (6.5) cd	376 (33.0) ab	39.8 (3.52) abcd	3.1 (0.14) abc	15.2 (0.04) bc
	SA417	76 (6.6) ab	370 (23.5) ab	42.1 (2.58) abc	3.6 (0.11) cd	17.1 (0.02) ab
BC_1F_3	NB1	36 (8.7) bcd	589 (58.2) a	58.7 (5.86) a	3.6 (0.22) bcd	16.9 (0.05) abc
	NB35	75 (8.7) abc	379 (32.4) ab	42.0 (3.52) abc	3.6 (0.15) bcd	17.2 (0.03) ab
Chinese chestnut	XLQUN5	96 (6.4) a	376 (21.6) ab	36.7 (2.17) bcd	4.0 (0.10) d	18.0 (0.02) a

Table 3
Survival, height and ground-line diameter (GLD) least-squares means for size class and family in the eighth growing season. An asterisk indicates differences between size classes within a family (Tukeys HSD p-value < 0.05). Means for families D8 and CH528 were not calculated due to low sample size.

Generation	Family	Size class	Survival (percent)	Height (cm)	GLD (mm)
American chestnut	BH	L	47 (8.4)	400 (38.6)	45.2 (4.20)
		S	45 (7.9)	350 (33.1)	41.1 (3.68)
	HK	L	26 (9.5)	308 (49.6)	31.3 (5.02)
		S	47 (9.8)	370 (43.5)	40.2 (4.81)
	Plummer	L	9 (13.5)	291 (83.2)	40.9 (10.04)
		S	30 (13.5)	387 (68.9)	37.8 (6.94)
	D1	L	57 (9.3)	558 (47.3)	67.6 (5.42)
		S	64 (9.1)	470 (40.8)	46.9 (4.12)
BC_3F_3	D6	L	37 (19.0)	186 (67.5)	31.8 (8.78)
		S	61 (19.0)	155 (50.9)	11.0 (4.29)
	D7	L	45 (12.8)	595 (74.0)	72.8 (8.26)
		S	45 (14.2)	295 (58.0)	46.4 (7.33)
	D9	L	25 (9.5)	480 (66.2)	58.2 (7.36)
		S	40 (11.0)	196 (38.2)	21.7 (4.07)
	D10	L	53 (11.0)	319 (40.1)	42.0 (4.73)
		S	42 (11.4)	420 (53.2)	57.0 (6.31)
BC_3F_2	CH283	L	11 (9.5)	533 (103.5)	68.4 (11.92)
		S	45 (9.5)	364 (43.1)	42.6 (4.73)
BC_2F_3	C240	L	85 (16.0)	399 (56.5)	44.7 (6.45)
		S	58 (16.1)	248 (52.9)	24.7 (5.33)
	SA330	L	32 (9.1)	357 (46.7)	39.3 (5.21)
		S	35 (9.1)	385 (44.7)	39.2 (4.56)
	SA417	L	77 (9.1)	443 (35.0)	47.0 (3.76)
		S	75 (9.5)	303 (30.1)	37.4 (3.41)
BC_1F_3	NB1	L	18 (12.8)	316 (82.7)	49.7 (10.45)
		S	55 (11.8)	598 (63.2)	52.4 (5.95)
	NB35	L	84 (12.3)	555* (52.6)	60.1* (5.67)
		S	66 (12.3)	228* (36.1)	26.8* (4.00)
Chinese chestnut	XLQUINS	L	100 (9.1)	457 (33.0)	45.4 (3.35)
		S	91 (8.9)	304 (26.9)	28.9 (2.67)

3.4. Growth pattern analyses

Out of the nine possible height growth patterns, trees fell into seven categories; no trees fell into the categories of nominal growth following by positive growth (0L-PQ) or increasingly negative growth (NL-NQ) (Fig. A1). Only one tree fell into the negative linear growth category (NL-0Q) category, so it was placed into the 0L-NQ category because both of these categories represented negative growth. The B_3F_2 and B_3F_3 generations were pooled together in subsequent chi-square analysis (cumulatively referred to as B_3 generation) because they had similar growth pattern distributions. Pooling reduced the model chi-square from 40.5 with 25 df to 39.7 with 20 df. The final analysis indicated the generations were associated with different growth pattern distributions (p-value = 0.0054). Across all generations, the majority of trees (61 percent) were classified as having positive growth without dieback (PL-0Q or PL-PQ), and a minority of trees were classified into growth patterns involving dieback (0L-NQ, NL-0Q, PL-NQ, NL-PQ; cumulatively 19 percent).

Table 4
Height and ground-line diameter (GLD) least-squares means for each generation or parental species across eight growing seasons. Letters denote differences among means within a year (Tukey's HSD p-value < 0.05).

Year	American	B_3F_3	B_3F_2	B_2F_3	B_1F_3	Chinese
	Height (cm)					
0	146 (9.1) a	130 (7.5) a	121 (16.7) a	125 (9.5) a	107 (11.0) a	104 (11.2) a
1	157 (9.7) a	148 (8.5) a	148 (19.4) a	130 (9.9) a	125 (12.6) a	112 (11.7) a
2	214 (11.9) a	208 (10.8) a	201 (23.5) a	182 (12.0) a	183 (16.0) a	149 (13.4) a
3	241 (13.5) a	241 (12.6) a	231 (26.8) a	228 (13.7) a	223 (18.5) a	185 (15.0) a
4	280 (15.6) a	287 (15.1) a	293 (33.3) a	269 (15.2) a	271 (21.0) a	244 (17.2) a
5	276 (16.5) a	290 (16.1) a	333 (37.9) a	294 (16.1) a	322 (23.8) a	292 (18.8) a
6	293 (17.8) a	322 (18.4) a	360 (41.3) a	304 (16.6) a	363 (26.1) a	331 (20.1) a
7	308 (19.4) a	343 (20.0) a	421 (48.6) a	330 (17.9) a	420 (29.0) a	362 (21.0) a
8	351 (22.9) ab	293 (19.2) b	480 (54.8) ab	356 (19.1) ab	478 (31.6) a	376 (21.6) ab
	GLD (mm)					
0	15.2 (0.95) a	14.1 (0.79) a	13.1 (1.77) a	13.9 (1.02) a	12.1 (1.20) a	11.4 (1.20) a
3	26.1 (1.38) a	26.8 (1.25) a	28.7 (2.87) a	24.5 (1.41) a	25.9 (1.90) a	19.1 (1.55) a
4	32.0 (1.69) a	34.6 (1.61) a	39.4 (3.81) a	30.5 (1.62) a	33.1 (2.26) a	25.5 (1.79) a
5	33.0 (1.85) a	36.4 (1.79) a	42.8 (4.30) a	32.9 (1.73) a	39.2 (2.61) a	29.0 (1.91) a
6	31.5 (1.89) a	38.7 (2.03) a	45.7 (4.96) a	33.6 (1.79) a	40.6 (2.77) a	30.3 (1.96) a
7	34.4 (2.09) a	41.5 (2.24) a	48.6 (5.49) a	37.1 (1.97) a	45.2 (3.03) a	34.6 (2.10) a
8	39.1 (2.47) ab	34.9 (2.13) b	52.7 (5.99) ab	38.8 (2.08) ab	50.0 (3.29) a	36.7 (2.17) ab

The BC_3 and BC_2F_3 generations were each associated with different growth patterns than the Chinese chestnut (adjusted p-values = 0.0131 and 0.0446, respectively), but pairwise comparisons between other generations were not significant (adjusted p-value > 0.2125). The BC_3 generation had a higher proportion of trees (28 percent) in the growth pattern that represented nominal growth or early mortality (0L-0Q) compared to the Chinese chestnut (2 percent) (Table 5). Each generation had relatively similar proportions in the positive linear (PL-0Q) (41–62 percent) and the increasingly positive growth pattern (PL-PQ) (10–18 percent).

The nominal growth/early mortality growth pattern (0L-0Q) was associated with different generations when compared to two other growth pattern categories, the NL-PQ (adjusted p-value = 0.0415) and the PL-PQ (adjusted p-value = 0.0002), but pairwise comparisons between the other growth patterns were not significant (adjusted p-values > 0.0727). Trees in the 0L-0Q growth category were disproportionally from the BC_3 generation (48 percent) while having lower-than-expected associations with Chinese chestnut (1 percent). The growth pattern of dieback followed by positive growth (NL-PQ) had a disproportionally high number of Chinese chestnut trees (44 percent).

Trees with blight symptoms were more likely to be categorized into

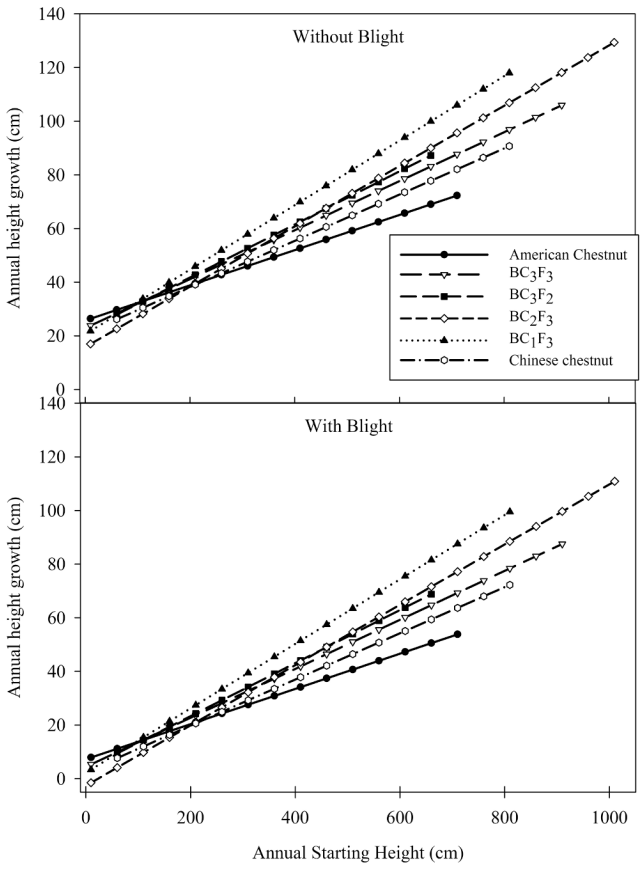


Fig. 2. Incremental height growth curves for each generation or parental species for trees with and without blight. The American chestnut had significantly reduced growth curves compared to the BC_1F_3, and BC_2F_3 generations (adjusted p-values = 0.0225), and no other differences among generational growth curves were significant. Trees with blight had significantly reduced incremental height growth (−19 cm) compared to trees without blight (p-value < 0.0001).

negative growth patterns compared to trees without disease symptoms (model chi-square = 28.74 with 5 df, $p < 0.0001$). For example, trees with blight symptoms were overrepresented in the nominal growth/early mortality pattern (0L-0Q) (35 percent), but only 3 percent of trees with blight symptoms had increasing growth over time (PL-PQ). Trees with 0L-0Q growth pattern were overrepresented by diseased trees (43 percent), compared to the positive linear growth pattern (PL-0Q) (23 percent; adjusted $p = 0.0261$) or the increasingly positive growth pattern (PL-PQ) (6 percent; adjusted $p < 0.0001$). Size class had no effect on growth pattern distributions (model chi-square = 3.855 with 5 df, p-value = 0.5705).

3.5. *Cryphonectria parasitica* resistance

Yearly resistance rankings and overall resistance rankings were affected by generation, genetic family, and size class, and most interactions were also significant (Table 1). Generations differentiated in overall resistance and began to differentiate in yearly resistance in year 4. American chestnut had lower yearly resistance (3.3–2.7 for years 4–8, respectively) and overall resistance (14.6) than the Chinese chestnut, which had perfect resistance (4.0 for yearly resistance in years 4–8 and 18.0 for overall resistance) (Fig. 3). The lower yearly resistance of the BC_3F_3 generation trees (3.9–3.3 for years 4–8, respectively) was not significantly different from the Chinese until year 8. The BC_3F_3 trees had higher overall resistance (16.7) and higher yearly resistance than the American chestnut in years 4–8. Hybrid generations had similar overall and yearly resistance to each other. The BC_1F_3 and BC_3F_2 were the only hybrid generations that did not significantly decrease in yearly resistance rankings from the time of planting until year 8.

Family effects were significant for overall and yearly resistance (Table 1), but specific differences among hybrid families within the same generation were not significant (Table 2). Families began to differentiate in yearly resistance starting in year 4 (family means by year are in Table A2). BC_3F_3 families D1 and D9 had higher overall (17.4 and 17.1, respectively) and year 8 resistance (3.7 and 3.6, respectively) than American chestnut family, Plummer (12.8 for overall resistance and 2.1 for year 8 resistance) (Table 2). All BC_3F_3 families had similar overall resistance (ranging from 15.7 to 17.4) to the Chinese chestnut (18.0), but family D10 had significantly lower year 8 resistance (3.1) than the Chinese chestnut (4.0). The American chestnut families also differed from each other in year 8 when family BH had higher yearly resistance (3.1) than Plummer (2.1).

Large-size seedlings had significantly lower yearly resistance (3.5) and overall resistance (15.8) than small-size seedlings (3.9 and 17.4, respectively) across all years, generations, and families. Across generations, size class differences in yearly resistance were significant beginning in year 4 (data not shown) and increased over time until year 8

Table 5
Chi-square values for generations or parental species associated with each growth pattern group (asterisks indicate significant cell values at p-value < 0.05). Row percent represents the percent of trees from each generation associated with a growth pattern, and column percent represents the percent of trees from each growth pattern associated with a generation. The BC_3F_2 and BC_3F_3 generations were combined and are displayed as the BC_3 generation. For specific growth pattern distributions refer to Fig. A1.

Growth pattern		American	BC_3	BC_2F_3	BC_1F_3	Chinese	No Blight	Blight
0L-0Q	Chi-Square	0.88	4.06*	0.21	2.20	7.23*	3.22	9.55*
	Row Percent	27	48	19	4	1	57	43
	Column Percent	25	28	18	9	2	16	35
0L-NQ/NL-0Q	Chi-Square	0.10	0.26	0.00	0.69	0.07	0.74	2.20
	Row Percent	25	29	21	14	11	61	39
	Column Percent	9	7	8	12	7	6	12
NL-PQ	Chi-Square	0.50	1.40	1.95	0.03	7.35*	0.24	0.71
	Row Percent	33	11	0	11	44	89	11
	Column Percent	4	1	0	3	9	3	1
PL-NQ	Chi-Square	0.05	0.37	3.54	0.20	1.90	0.51	1.50
	Row Percent	24	28	38	7	3	86	14
	Column Percent	9	7	14	6	2	9	4
PL-0Q	Chi-Square	0.66	0.01	0.44	0.21	2.05	0.08	0.22
	Row Percent	19	35	19	11	16	77	23
	Column Percent	41	48	42	53	62	49	44
PL-PQ	Chi-Square	0.11	1.51	0.93	0.35	0.49	2.47	7.32*
	Row Percent	20	24	28	12	16	94	6
	Column Percent	13	10	18	18	18	17	3

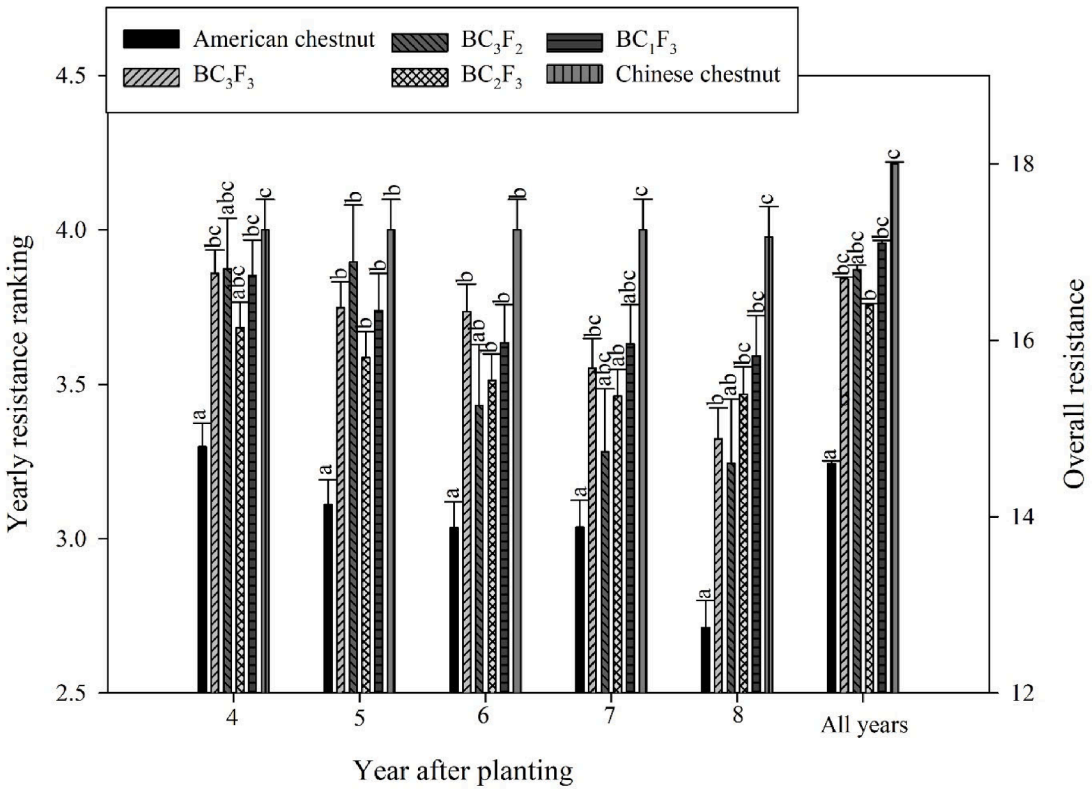


Fig. 3. Yearly blight resistance rankings (left y-axis) and overall resistance ranking (right y-axis) least-squares means for years 4–8 for each generation or parental species. Letters denote differences among means within a year (Tukey's HSD p-value < 0.05). Yearly resistance rankings ranged from 1 (death caused by blight) to 4 (no blight), and overall resistance ranged from 2 (blight killed tree in the first year) to 18 (no blight detected after 8 years). Differences among generations were not significant for years 1–3 (data not shown).

when large-size seedlings had lower resistance (3.1) than small-size seedlings (3.7). Within generations, size class only affected yearly resistance for the BC_2F_3 and BC_3F_2 generations, such that large-size seedlings were 0.7 and 1.7 lower in year 8 resistance, respectively, compared to small-size seedlings (means for each generation and year are in Table A3). Similarly, only the BC_3F_2 generation had significant mean differences in overall resistance between the large- (14.1) and small-size seedlings (18.0). Size class interactions with family were not significant for yearly or overall resistance.

3.6. Isolation of Phytophthora species

Based on morphological and molecular characteristics, *P. cinnamomi* was the only species of *Phytophthora* isolated from the 28 plant and 26 soil samples submitted for diagnosis. The pathogen was isolated from 20 of the 28 plant samples collected: 11 out of 15 BC_3F_3 (73 percent), 5 out of 7 BC_3F_2 (71 percent), both BC_1F_3 (100 percent), and 2 out of 4 American (50 percent) seedlings; American chestnut seedlings usually had severe root rot symptoms, which can compromise pathogen recovery. *Phytophthora cinnamomi* was recovered from all 16 of the soil samples collected from planting spots associated with symptomatic trees—including five samples collected around seedlings from which the pathogen was not isolated. Soil samples were not collected around the other three seedlings that tested negative for *P. cinnamomi*. This pathogen also was recovered from 6 of the 6 (100 percent) random soil samples located within the planting area and 1 of the 4 (25 percent) of the soil samples collected outside the planting area (only the upslope sample was positive).

4. Discussion

4.1. Comparative success of chestnut reintroduction

This study represents one of the longest-term datasets for American chestnut reintroduction test plantings in North America but was of relatively short duration given the average rotation age (ca. 60–70 years) of American chestnut grown for timber production in pre-chestnut blight forests or species maximum longevity of several hundred years (Mattoon, 1909; Ashe, 1911). Contemporary American chestnut reintroduction is generally aimed towards long-term ecological restoration with goals centered around obtaining reproductive maturity to successfully repopulate forests over decades (Jacobs, 2007; Clark et al., 2014). The stand initiation phase of stand development, as studied in this experiment, is crucial to our understanding of the future success of restoration plantings, as this phase represents the time that species differentiate in growth to determine future canopy dominance (Oliver, 1981), particularly in upland hardwood stands (Loftis, 1990). Survival of advanced hybrid material (BC_3F_3) was lower than generally considered acceptable after 8 years (41 percent) and was substantially lower than previously reported for advanced hybrid material planted in southern Appalachian forests [70 percent reported after eight years in Clark and others (2019b) and 85 percent reported after three growing seasons in Brown and others (2022)] and northern Appalachian forests [87 percent reported after four years in Pinchot and others (2022)]. Survival was like other studies [4-year survival of 59 to 88 percent reported by Thomas-Van Gundy and others (2017) and 4-year survival of 10–65 percent reported by Pinchot and others (2017)] that reported confirmed or potential Phytophthora root rot (PRR) related mortality. The 12 to 40 percent mortality documented in the first two years was probably related primarily to PRR because symptoms consistent with this disease were previously documented (Clark et al., 2014). Samples of

affected root systems and associated forest soils were positive for PRR with *P. cinnamomi* isolated from 20 (71 percent) out of 28 seedlings submitted for evaluation in 2011 (Sharpe, 2017). This pathogen was also recovered from 23 (88 percent) out of 26 soil samples assayed in 2011 and 2013, which demonstrated the widespread occurrence of *P. cinnamomi* in soils at the study site. The relatively high survival of Chinese chestnut seedlings (96 percent) compared to other hybrid generations and American chestnut also strongly suggests PRR related mortality in susceptible material because Chinese chestnut is highly resistant (Jeffers et al., 2009). PRR resistance is unrelated to *C. parasitica* resistance, and breeding for this nonnative disease is in the early stages for American chestnut (Westbrook et al., 2019). The occurrence of PRR in our study site, the potential transfer of PRR from nurseries to planting sites (Wamishe et al., 2008), in addition to PRR related mortality documented in other chestnut test plantings (Thomas-Van Gundy et al., 2017) reveals a substantial challenge for future research and restoration efforts for chestnuts globally, but particularly in southeastern North American or Mediterranean forests where *P. cinnamomi* is not limited by prolonged periods of cold temperature. Increasing temperatures worldwide from climate change is also predicted to exacerbate this problem (Gustafson et al., 2022), but mitigation by selecting sites with appropriate soils or landform shapes within the susceptible PRR range has not been studied and was outside the scope of our experiment.

Seedling survival had the largest decreases during the first two years after outplanting, and growth was relatively stagnant in years 1 and 2, probably as a function of a planting shock. Larger-size seedlings from some of the American chestnut, BC_3F_2, and BC_3F_3 families were disproportionally affected, although size class across families and generations did not contribute to lower survival. Root systems, undercut and disturbed at the time of lifting, may not be able to support the physiological demands of above-ground structures after outplanting, particularly for larger-size seedlings (Struve and Jolly, 1992; Struve et al., 2000). Course roots were found to be a relatively large source of nonstructural carbohydrates that maintain cellular processes in American chestnut seedlings (Montague et al., 2022), and the loss of course and fine roots during lifting, packing, and planting leads to planting shock, especially for larger trees (Struve et al., 2000). Planting shock was similarly documented in a related study of American chestnut (Clark et al., 2009) and in a planting of 1–0 bareroot nursery seedlings from the same seed orchard as the current study (Clark et al. 2016). Planting shock commonly occurs in Fagaceae species, typically resulting in a loss of 5 to 10 percent of seedlings, depending on local site conditions and nursery seedling physiology and morphology (Thompson and Schultz, 1995; Clark et al., 2015), but determining cause of death for any individual tree is problematic (Franklin et al., 1987). The increase in planting shock related to larger-sized seedlings is typically overcome with improved competitive abilities and growth over the long term (Struve et al., 2000; Clark et al., 2016), which is what we observed in this study.

Surviving hybrid trees were capable of relatively fast growth for planted seedlings, averaging 20–46 cm of annual height growth or 163–371 cm in total height growth over eight growing seasons. American chestnut was historically reported as a fast-growing species following disturbances, growing 120 to 180 cm in the first year as sprouts from seedlings or stumps (Mattoon 1909), but growth rates of planted hybrid seedlings have only recently been evaluated. Seedlings were taller (244–293 cm) and larger in GLD (26–39 mm) after four years compared to seedlings planted from similar seed sources and breeding lines in nearby companion plantings (height ~ 110–250 cm and GLD ~ 20–30 mm as reported after four years in Clark et al., 2016), perhaps because seedlings were slightly larger in our study at the time of planting. Similarly, seedlings in our study were larger but had comparable relative growth to advanced backcross material planted in Pennsylvania, USA using containerized seedlings after six growing seasons (height ~ 90–180 cm and GLD ~ 15–23 mm reported in Pinchot et al., 2022) and compared to 1–0 bareroot seedlings from similar seed sources planted on a xeric site in western North Carolina [height ~ 125–160 cm and 23–30 mm in GLD reported by Brown and others (2022)]. Relatively poor survival and growth were reported after seven years on mine reclamation sites for hybrid seedlings [survival ~ 28–56 percent and height ~ 26–86 cm reported by Skousen and others (2018)], but relatively high five-year survival and growth was reported when soil remediation measures were employed (survival ~ 58–70 percent, height ~ 169–180 cm, and GLD ~ 29–30 mm). Collectively, these studies indicate that backcross chestnut hybrid seedlings are capable of relatively fast growth after planting across a range of site conditions and stock types, excluding perhaps the most degraded sites such as unripped mine sites. Despite our relatively fast growth rates, we suspect that growth at our planting may have been slowed by PRR or other pests such as the Asiatic oak weevil, a nonnative insect documented at the study site disproportionally on BC_3F_3 seedlings (Case et al., 2016). Unfortunately, causal mechanisms affecting survival and growth are difficult to determine in these 'real-world' test plantings.

4.2. Field performance considerations for breeding programs

We documented differences in growth, growth patterns, and chestnut blight resistance among breeding generations, lending partial support to our first research hypothesis. Field performance of chestnut seedlings was largely affected by chestnut blight pressure, occurrence of PRR, and/or hybrid vigor. Contrary to our expectations (research hypothesis 1a) Chinese chestnut had the largest proportion of seedlings of any generation in the positive growth categories (PL-0Q or PL-PQ; 80 percent). This species' total height, GLD, and incremental height growth curves were comparatively low, but mean differences were not significant. Chinese chestnut was disproportionately likely to have dieback early followed by increasingly positive growth (NL-PQ), but only 9 percent of seedlings fell into this category, probably not greatly affecting the overall yearly height means. Chinese chestnut generally has poorer growth responses than American chestnut or interspecific hybrids in eastern North American forests (Schlarbaum et al., 1994; Clark et al., 2016), but our results and results from others indicate that PRR may allow Chinese chestnut to keep pace with or even outgrow interplanted chestnut species (Pinchot et al., 2017; Thomas-Van Gundy et al., 2017) even though Chinese chestnut can be infected by *P. cinnamomi* and sustain mild symptoms (Jeffers et al., 2009; Sharpe, 2017; Westbrook et al., 2019).

The less advanced BC_1F_3 and the BC_2F_3 generation seedlings outperformed the more advanced BC_3F_3 and BC_2F_3 hybrid seedlings in survival and growth while hybrid generations exhibited similar resistance levels to each other, contrary to research hypothesis 1b. These results could be a function of PRR pressure or a function of hybrid vigor that has not been documented in previous studies (Clark et al., 2016; Skousen et al., 2018). Theoretically, the BC_1F_3 and BC_3F_3 generations are 75 percent and 94 percent American chestnut, respectively (Hebard, 2001), but inheritance of American chestnut genes has been lower than expected (Westbrook et al., 2020). Hybrid vigor and higher *P. cinnamomi* resistance would be expected in generations with more Chinese ancestry, and these less advanced hybrid seedlings had the most efficient growth that became more pronounced over time, as indicated by the incremental growth curves (South, 1995). *C. parasitica* resistance levels did not interact with generation or chestnut species and could not explain this improved growth efficiency.

Research hypothesis 1c was not fully supported because the BC_3F_3 generation trees had higher-than-expected proportion of trees with nominal growth or early mortality and average total height, total GLD and incremental height growth. BC_3F_3 generation trees did not exhibit higher survival or better growth than the American chestnut, as we predicted, but BC_3F_3 trees did have higher disease resistance rankings than the American chestnut for the last four growing seasons and in overall resistance. The BC_3F_3 trees are currently exhibiting *C. parasitica* resistance levels intermediate between American and Chinese chestnuts

(higher than American chestnut, but lower than Chinese chestnut), which aligns with orchard tests (Steiner et al., 2017; Westbrook et al., 2020). The BC_3F_3 generation trees had overall resistance closer to the Chinese chestnut than the American chestnut, results that are similar to a companion study (Clark et al., 2019b), but durable blight resistance is yet to be determined.

Research hypothesis 1d was only partially supported. American chestnut seedlings did not exhibit significantly larger height and GLD growth at any point in time, as expected, compared to hybrid species or the Chinese chestnut. American chestnut's incremental height growth curves were also lower compared to those in the BC_1F_3 and BC_2F_3 generations. These results deviate from previous studies that found faster initial growth in American chestnut compared to advanced hybrid seedlings and/or Chinese chestnut (Clark et al., 2016; Brown et al., 2022). The American chestnuts had the lowest levels of resistance, an expected outcome (Griffin, 2000). Their relatively slow growth compared to that in previous studies could be related to PRR and/or to the relatively high chestnut blight pressure that caused dieback and resprouting in this older study (c.f., Clark et al., 2016, 2019).

The majority of trees in each generation or parental species fell into the positive growth categories (PL-0Q or PL-PQ) (54–80 percent of trees in each generation/species), indicating most trees exhibited the fast growth desired to produce small diameter wood products or future sawtimber, regardless of ancestry. However, other growth patterns may be more desirable for ecological, disease resistance, or cultural benefits. For example, faster growth has been correlated to increased blight levels (Reynolds and Burke, 2011), but also to increased blight management with hypovirulence (Griffin et al., 1991). Non-timber forest products such as nut production may also differ based on growth patterns as documented in European chestnut (Beccaro et al., 2020). Extrapolation of growth patterns observed in this study should be conducted with caution, and additional research is needed as results may vary depending on disease pressure, edaphic site conditions, browse pressure, age, and specific genetics tested. For example, a breeding population of Chinese chestnut was found in Connecticut, USA, that has not been found anywhere else in North America, and is thought to be spreading because of limited seed predation, land-use history, and shallow soils (Miller et al., 2014).

We identified one high-performing family (D1) and two low-performing families (D6 and D8), supporting hypothesis 2. The slower-than-expected growth of the BC_3F_3 generation trees could be partially explained by the two poor performing families that lowered the generational mean. The D6 and D8 families also had relatively low survival rates either early after planting (D8, see Table A2) or decreasing rates over time (D6), which may have contributed to the higher-than-expected nominal growth pattern distribution found in the BC_3F_3 generation. Family D1 had relatively high survival, large heights and GLDs, and relatively high disease resistance levels. These results deviate slightly from those previously reported by Clark and other (2016) that found D1 had average height and GLD after four years and average resistance after eight years (Clark et al., 2019b). Chestnut blight from *C. parasitica* has challenged more trees in this study than previously reported at the 8-year-old companion plantings (c.f., Clark et al., 2019b), which may result in significant family differentiation compared to sites with less fungal infections reported overall. Family growth rates of Fagaceae trees can change over time (Kriebel et al., 1988), and family rankings in this study have shifted from growing seasons 4 to 8 years; family D1′s comparative growth rates improved over time (Table A2). This family has not yet been culled from the TACF breeding orchard, while poor performing family D8 has been culled (Jared Westbrook, personal communication), demonstrating an important cohesion between field tests and orchard tests (Steiner et al., 2017; Westbrook et al., 2020). Family D7 was also culled from the TACF orchard probably due to its relatively low resistance levels in orchard tests. This family also had relatively low resistance levels in our field test, although specific mean differences were not significant. Family D9 is the only other BC_3F_3 that has been culled from the TACF orchard to date, but in our study, it showed relatively fast growth and moderately high *C. parasitica* resistance levels (3.6/4.0 for year 8 resistance and 17.1/18.0 for overall resistance).

Environmental interactions with the host plant and the chestnut blight pathogen can create discrepancies among testing sites (Clark et al., 2016; Steiner et al., 2017), and ideally families will be selected that perform equally well across multiple test plantings over the long-term. However, the majority of culling decisions are being made based on phenotypic selections and not based on forest field trials or progeny tests that could take decades (Steiner et al., 2017). Families D1 and D7 were also tested in West Virginia, USA forest field trials, but results were too early to make valuable comparisons (Thomas-Van Gundy et al., 2017).

We did not provide support for hypothesis 2a. The slower growing BC_3F_3 families were not negatively related to higher blight resistance or vice versa. This correlation has been a concern for breeding programs historically (Jaynes 1978) and has been partially corroborated by recent tests (Westbrook et al., 2020). PRR may have confounded our results, however, by eliminating trees from families with lower *P. cinnamomi* resistance (Westbrook et al., 2019).

4.3. Influence of size class grading

Our results support research hypothesis 3 because we found a positive relationship between seedling size at the time of planting and subsequent field performance. Overall height and GLD were improved through size grading, supporting hypothesis 3a. Small seedlings at age 8 were similar in height to large seedlings at age 4 indicating that tree height could have been improved by a factor of four years if only large-size seedlings were planted. These results agree with similar studies of Fagaceae species that showed improved growth with seedling size grading (Dey et al., 2008; Clark et al., 2015; Clark et al., 2016). In addition to growth advantages that improve competitive status (Spetich et al., 2002; Johnson et al., 1986), taller seedlings also have reduced deer browsing (Oswalt et al., 2006). Size grading, however, remains relatively undefined and difficult to implement due to the high variability in Fagaceae nursery seedling quality across years, provenances, and nurseries (Dey et al., 2008; Clark et al., 2012a; Pinchot et al., 2015).

Our results suggest support for hypothesis 3b because large-size class seedlings had lower *C. parasitica* resistance than small-size class trees beginning around age 4, but this effect varied by breeding generation or species. The effect of seedling size on blight resistance was only significant for the BC_2F_3 and BC_3F_2 generations (Table A3) and not significant for any individual family. The lack of significant effects of seedling size on American chestnut may be because GLD was relatively similar between size classes for this species in most years (Table A3). These results differ from similar studies that found size grading increased GLD and/or height in American chestnut seedlings (Clark et al., 2012b; Clark et al., 2016). Alternatively, Chinese chestnut's high *C. parasitica* resistance probably negated any seedling size impact. Larger sized seedlings tend to produce more bark fissures and branch stubs that *C. parasitica* spores can enter and infect trees with low levels of resistance (Griffin and Elkins, 1986; Griffin et al., 1991). Our results partially agree with a companion study that found size class did not interact with generation to affect *C. parasitica* resistance (Clark et al., 2019b), but we did not examine the relationship between GLD and the probability of *C. parasitica* infection.

Our results point to the need to better integrate genetics, nut size, and seedling size to refine nursery production and restoration practices. Many morphological traits within chestnut species are correlated and can be selected for in breeding programs (Tuğ et al., 2022) although genetic variability of many traits remains relatively untested for most chestnut species (Mellano et al., 2012). Nut size (Clark et al., 2012a; Pinchot et al., 2015) and seedling size at planting (Clark et al., 2016) affect subsequent growth and competitive abilities after planting, and

these factors are partially controlled by genetics (Detlefsen and Ruth, 1922; Clapper, 1954). Height and GLD did not improve (significantly or non-significantly) for large-size seedlings compared to small-size seedlings for 20 to 33 percent of families, indicating this practice may not be warranted for all genotypes. Selecting for genetic traits that reduce variability in seedling size might improve desired seedling morphology or physiology, which would benefit restoration efforts and increase efficiencies associated with planting, competition control, and deer browse mitigation (Grossnickle and MacDonald, 2018).

4.4. Management implications

Chestnuts bred for *C. parasitica* resistance were primarily challenged by PRR, which reduced survival of hybrid generations and American chestnut and probably confounded the ability to determine breeding and/or genetic effects on growth. This disease is predicted to worsen in Europe and North America with climate change (Brasier, 1996; Gustafson et al., 2022) and will continue to be a primary hindrance to chestnut management and restoration until PRR resistance is incorporated into chestnut breeding programs (Westbrook et al., 2020; Fernandes et al., 2022). Not all sites where reintroduction trials have occurred have been affected (Bauman et al., 2014; Clark et al., 2016; Brown et al., 2022) because *P. cinnamomi* is not ubiquitous and evenly distributed in forest soils, and long-term survival can be affected by environmental conditions (Erwin and Ribeiro, 1996; Meadows and Jeffers, 2011). Other challenges remain—such as animal browse (Pinchot et al., 2022) or nonnative insects (Case et al., 2016). Planting seedlings from nurseries or areas in the nursery with well-drained sandy soils and testing soils before planting for the presence of *Phytophthora* spp. should help manage this disease (Crandall et al., 1945; S.N. Jeffers, personal communication), but there currently is no effective treatment for landscape-level plantings.

Resistance to *C. parasitica* was intermediate for the most advanced breeding generation, BC_3F_3 and was trending lower annually starting in year 4 after planting. Overall resistance is expected to increase in the TACF breeding orchard with further phenotypic and genomic selections and associated culling in orchards (Steiner et al., 2017), but the relatively new discovery of polygenic inheritance is challenging testing and selection efforts (Westbrook et al., 2020). We expect resistance in our planting to continue to decrease over time if results from orchard inoculations transfer to forest field trials.

It is not clear what level of blight resistance and American chestnut phenotypic inheritance is required for successful reintroductions and restorations (Westbrook et al., 2020), but our results suggest less advanced hybrid generations like the BC_1F_3 are capable of desirable annual growth and incremental growth for the first eight years after planting. This growth advantage may be a result of hybrid vigor or related to higher PRR resistance. We expect this advantage to be relatively ephemeral based on previous observations of Chinese chestnuts or less advanced crosses (Burnham et al., 1986; Schlarbaum et al., 1994). Competitive abilities have not been evaluated and reproduction has not yet occurred, which are important metrics in future evaluations of Fagaceae plantings (Spetich et al., 2002; Steiner et al., 2017). We saw no deviations between the BC_3F_3 and the American chestnut in any metric we tested, other than *C. parasitica* resistance, indicating the breeding program is successfully integrating desired growth traits into the hybrid genome while transferring disease resistance. Once disease resistance is durable, managers could expect the advanced breeding generations to perform similarly to American chestnut in terms of growth metrics, at least through the early critical part of the stand initiation phase of development.

Genetic differences revealed at least one superior family (D1) that exhibited relatively fast growth while maintaining acceptable levels of *C. parasitica* resistance. Two poor performing families were also identified (D6 and D8). Comparisons to companion studies are difficult as results are mostly premature (Thomas-Van Gundy et al., 2017; Clark et al., 2019b), but general trends in growth and pathogen resistance were found across the southern Appalachian region of the USA (Clark et al., 2016).

Managers could save time and be more efficient by grading seedlings for quality using nursery-tested visual grading criteria (Clark et al., 2000). While this might decrease the time to *C. parasitica* infection, it may also improve competitive ability and resource allocations that can assist in controlling chestnut blight over the long-term (Griffin, 1989). Size grading unequally affected height and GLD among generations and families, however, indicating that refinement of nursery production should incorporate a genetic component. This may be difficult given the degrading infrastructure and programmatic support for nurseries and tree improvement programs (Wheeler et al., 2015; Haase, 2022).

The American chestnut remains an iconic tree species whose restoration using traditional breeding has found broad public appeal but comes with other biological, cultural, and technological challenges (Jacobs et al., 2013; Clark et al., 2014; Clark et al., 2020). Our study provides information that can assist breeding programs and forest managers with expectations and considerations on field performance once *C. parasitica*-resistant material becomes widely available for reforestation. This study was limited in its evaluations and was challenged by PRR but provides a practical examination of hybrid chestnuts planted into commonly used silvicultural treatments on representative sites in the Appalachian Mountain region, within the heart of the American chestnut's former range in North America. Future research should include development of models for competitive success in forest field plantings, integration of genomic ancestry, and evaluations of different silvicultural treatments, site types, and stock types.

Funding

This work was partially supported by The American Chestnut Foundation Grants Program [collection Agreement Nos. 10-CO11330134–055, 11-CO-11330134–136, 14-CO-11330134–004]; the U.S. Department of Agriculture, Forest Service; The Highlands Biological Station's Grant-In-Aid program; and the University of Tennessee, Tennessee Agricultural Experiment Station.

CRediT authorship contribution statement

Stacy L. Clark: Conceptualization, Methodology, Resources, Writing – original draft, Visualization. **Scott E. Schlarbaum:** Conceptualization, Methodology, Resources, Writing – review & editing. **Arnold M. Saxton:** Methodology, Writing – review & editing. **Steven N. Jeffers:** . **Richard E. Baird:** Methodology, Resources, Investigation, Writing – original draft, Methodology, Resources, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

Acknowledgements

The authors would like to thank the U.S. Department of Agriculture, Forest Service Personnel: Barbara Crane, William Preston Durham, and Jim Stelick for their input and assistance in establishing, maintaining, and data collection and formatting. We would like to thank John Johnson, Ami Sharp, Jason Hogan, and Jesse Troxler from the University of Tennessee's Tree Improvement Program for their assistance in study establishment and field data collections. The Tennessee Department of Agriculture, Forestry nursery manager, John Conn, was instrumental in

growing quality seedlings. The American Chestnut Foundation's Fred Hebard coordinated the provision of chestnut experimental material. Inga Meadows, Clemson University, assisted with assays on soil and root samples for Phytophthora root rot. The findings and conclusions in this publication are those of the author(s) and should not be construed to represent any official USDA or U.S. Government determination or policy.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2023.120820.

References

Abad, Z.G., Burgess, T., Bienapfl, J.C., Redford, A.J., Coffey, M., Knight L., (2019). IDphy: Molecular and morphological identification of Phytophthora based on the types. USDA APHIS PPQ S&T Beltsville Lab, USDA APHIS PPQ S&T ITP, Centre for Phytophthora Science and Management, and World Phytophthora Collection. https://idtools.org/id/phytophthora/index.php (accessed 5 January 2023).

Anagnostakis, S.L., 1995. The pathogens and pests of chestnuts. Adv. Bot. Res. 21, 125–145.

Anagnostakis, S.L., 2001. American chestnut sprout survival with biological control of the chestnut-blight fungus population. For. Ecol. Manag. 152, 225–233.

Anagnostakis, S.L., 2012. Chestnut breeding in the United States for disease and insect resistance. Plant Dis. 96 (10), 1392–1403.

Ashe, W.W., 1911. Chestnut in Tennessee. Tennessee State Geological Survey Bulletin O-B 35, pp.

Baird, R.E., 1991. Growth and stromata production of hypovirulent and virulent strains of Cryphonectria parasitica on dead Quercus rubra and Acer rubrum. Mycologia 83, 158–162.

Bailey, R.G., Avers, P.E., King, T., McNab, W.H., 1994. Ecoregions and subregions of the United States (map). US Geological Survey, Washington, DC.

Baird, R., 2014. Diversity and density of the EM fungal community present in high elevation Fraser fir forests of Great Smoky Mountains National Park. N. Am. Fungi 9 (1), 1–21.

Barnhill-Dilling, S.K., Delborne, J.A., 2019. The genetically engineered American chestnut tree as opportunity for reciprocal restoration in Haudenosaunee communities. Biol. Conserv. 232, 1–7. https://doi.org/10.1016/j.biocon.2019.01.018.

Bauerle, W.L., Wang, G.G., Bowden, J.D., Hong, C.M., 2006. An analysis of ecophysiological responses to drought in American chestnut. Ann. For. Sci. 63, 833–842.

Bauman, J.M., Keiffer, C., McCarthy, B., 2014. Growth performance and chestnut blight incidence (*Cryphonectria parasitica*) of backcrossed chestnut seedlings in surface mine restoration. New For. 45, 813–828. https://doi.org/10.1007/s11056-014-9439-3.

Beccaro, G., Bounous, G., Tessa, G., Riqué, P.H., 2020. European Chestnut Traditional and High-Density Orchards. In: Beccaro, G., Alma, A., Bounous, G., Gomez-Laranjo, J. (Eds.), The Chestnut Handbook: Crop and Forest Management. CRC Press, Taylor & Francis Group, New York, p. pp..

Berry, F.H., 1959. Chestnut Blight and Resistant Chestnuts. USDA Farmers' Bulletin 2068.

Brasier, C.M., 1996. *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. Ann. Sci. For. 53, 347–358.

Braun, E.L., 1950. Deciduous forests of eastern North America. Blakiston, Philadelphia.

Brown, S.P., Clark, S.L., Ford, E., Jumpponen, A., Saxton, A.M., Schlarbaum, S.E., Baird, R., 2022. Comparisons of interspecies field performance of Fagaceae (*Castanea* and *Quercus*) planted in the southeastern United States with attention to soil fungal impacts on plant performance. For. Ecol. Manag. 525, 120569 https://doi.org/10.1016/j.foreco.2022.120569.

Burnham, C.R., Rutter, P.A., French, D.W., 1986. Breeding blight-resistant chestnuts. Plant Breeding Reviews 4, 347–397.

Case, A.E., Mayfield, A.E., Clark, S.L., Schlarbaum, S.E., Reynolds, B.C., 2016. Abundance and frequency of the Asiatic oak weevil (Coleoptera: Curculionidae) and defoliation on American, Chinese, and hybrid chestnut (Castanea). J. Insect Sci. 16 (1), 29, 1–8. https://doi.org/10.1093/jisesa/iew012.

Clapper, R.B., 1954. Chestnut breeding, techniques and results. I. Breeding material and pollination techniques. II. Inheritance of characters, breeding for vigor, and mutations. J. Hered. 45 (3), 201–208.

Clark, S.L., Schlarbaum, S.E., Kormanik, P.P., 2000. Visual grading and quality of 1–0 northern red oak seedlings. South. J. Appl. For. 24 (2), 93–97.

Clark, S.L., Schweitzer, C.J., Schlarbaum, S.E., Dimov, L.D., Hebard, F.V., 2009. Nursery quality and first-year response of American chestnut (*Castanea dentata*) seedlings planted in the southeastern United States. Tree Plant. Notes 53 (2), 13–21.

Clark, S.L., Schlarbaum, S.E., Saxton, A.M., Hebard, F.V., 2012a. Nursery performance of American and Chinese chestnuts and backcross generations in commercial tree nurseries. Forestry 85, 589–600. https://doi.org/10.1093/forestry/cps068.

Clark, S.L., Marcolin, E., Patrício, M.S., Loewe-Muñoz, V., n.d. In review. A silvicultural synthesis of sweet (Castanea sativa) and American (C. dentata) chestnuts. For. Ecol. Manage.

Clark, S.L., McNab, H., Loftis, D., Zarnoch, S., 2012b. American Chestnut Growth and Survival Five Years after Planting in Two Silvicultural Treatments in the Southern Appalachians, USA. Forests 3, 1017–1033. https://doi.org/10.3390/f3041017.

Clark, S.L., Schlarbaum, S.E., Pinchot, C.C., Anagnostakis, S.L., Saunders, M.R., Thomas-Van Gundy, M., Berrang, P.C., 2014. Reintroduction of American chestnut in the national forest system. J. For. 112 (5), 502–512. https://doi.org/10.5849/jof.13-106.

Clark, S.L., Schlarbaum, S.E., Schweitzer, C.J., 2015. Effects of visual grading on northern red oak (*Quercus rubra* L.) seedlings planted in two shelterwood stands on the Cumberland Plateau of Tennessee, USA. Forests 6 (10), 3779–3798.

Clark, S., Schlarbaum, S., Saxton, A., Hebard, F., 2016. Establishment of American chestnuts (*Castanea dentata*) bred for blight (*Cryphonectria parasitica*) resistance: influence of breeding and nursery grading. New For. 47 (2), 243–270. https://doi.org/10.1007/s11056-015-9512-6.

Clark, S.L., Schlarbaum, S.E., Clark, J.D., 2019a. Could restoration of the American chestnut remodel our wildlife landscape? The Wildlife Professional 13 (4), 52–56.

Clark, S.L., Schlarbaum, S.E., Saxton, A.M., Baird, R., 2019b. Eight-year blight (Cryphonectria parasitica) resistance of backcross-generation American chestnuts (Castanea dentata) planted in the southeastern United States. For. Ecol. Manag. 433, 153–161. https://doi.org/10.1016/j.foreco.2018.10.060.

Clark, S.L., Schlarbaum, S.E., Crane, B.S., Pinchot, C.C., Schaberg, P.G., Thomas-Van Gundy, M. (2020). Restoration of the American chestnut will require more than a blight-resistant tree. U.S. Dept. Agric. For. Serv. North. Res. Station, Gen. Tech. Rep., NRS-P-193. Madison, WI, pp. 38-40.

Conover, W.J., Inman, R.L., 1981. Rank trans-formation as a bridge between parametric and non-parametric statistics. Am. Stat. 35, 124–129.

Cooper, W.R., Rieske, L.K., 2007. Review of the historic and current status of the Asian chestnut gall wasp in North America. J. Am. Chestnut Found. 21 (2), 26–32.

Crandall, R.S., Gravatt, G.F., Ryan, M.M., 1945. Root disease of castanea species and some coniferous and broadleaf nursery stocks caused by *Phytophthora cinnamomi*. Phytopathology 35, 162–180.

Detlefsen, J.A., Ruth, W.A., 1922. An orchard of chestnut hybrids. J. Hered. 13, 305–314.

Dey, D.C., Jacobs, D.F., McNabb, K., Miller, G., Baldwin, V., Foster, G., 2008. Artificial regeneration of major oak (Quercus) species in the eastern United States - a review of the literature. For. Sci. 54 (1), 77–106.

Erwin, D.C., Ribeiro, O.K., 1996. Phytophthora Diseases Worldwide. APS Press, St. Paul, MN.

Fei, S., Liang, L., Paillet, F.L., Steiner, K.C., Fang, J., Shen, Z., Wang, Z., Hebard, F.V., 2012. Modelling chestnut biogeography for American chestnut restoration. Divers. Distrib. 18, 754–768. https://doi.org/10.1111/j.1472-4642.2012.00886.x.

Ferguson, A.J., Jeffers, S.N., 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83, 1129–1136.

Fernandes, P., Colavolpe, M.B., Serrazina, S., Costa, R.L., 2022. European and American chestnuts: An overview of the main threats and control efforts. Front. Plant Sci. 13, 951844 https://doi.org/10.3389/fpls.2022.951844.

Franklin, J.F., Shugart, H.H., Harmon, M.E., 1987. Tree death as an ecological process. BioScience 37, 550–555.

Frothingham, E.H., 1912. Second growth hardwoods in Connecticut. U.S Dept. Agric. For. Serv. Bulletin 96, 70 p.

Gallegly, M.E., Hong, C., 2008. Phytophthora: Identifying Species by Morphology and DNA Fingerprints. APS Press, St. Paul, MN.

Gilland, K.E., McCarthy, B.C., 2014. Performance and phenotypic variation of American Chestnut (*Castanea dentata*) hybrids on newly reclaimed mine sites in eastern Ohio, USA. Ecol. Restor. 32 (4), 379–387.

Griffin, G.J., 1989. Incidence of chestnut blight and survival of American chestnut in forest clearcut and neighboring understory sites. Plant Dis. 73, 123–127.

Griffin, G.J., 1992. American chestnut survival in understory mesic sites following the chestnut blight pandemic. Can. J. Bot. 70 (10), 1950–1956.

Griffin, G.J., 2000. Blight Control and Restoration of the American Chestnut. J. For. 98 (2), 22–27.

Griffin, G.J., Elkins, J.R., 1986. Chestnut blight. In: Roane, M.K., Griffin, G.J., Elkins, J.R. (Eds.), Chestnut blight, other Endothia diseases, and the Genus Endothia. The American Phytopathological Society, St. Paul, Minnesota, pp. 1–26.

Griffin, G.J., Smith, H.C., Dietz, A., Elkins, J., 1991. Importance of hardwood competition to American chestnut survival, growth, and blight development in forest clearcuts. Can. J. Bot. 69 (8), 1804–1809.

Grossnickle, S.C., MacDonald, J.E., 2018. Why seedlings grow: influence of plant attributes. New For. 49, 1–34. https://doi.org/10.1007/s11056-017-9606-4.

Gustafson, E.J., Miranda, B.R., Dreaden, T.J., Pinchot, C.C., Jacobs, D.F., (2022). Beyond blight: Phytophthora root rot under climate change limits populations of reintroduced American chestnut. Ecosphere 13(2), e3917. https:\\doi.org\10.1002/ecs2.3917.

Haase, D.L., (2022). Nurseries have a critical role in meeting forest restoration goals. In: Jain, T., Schuler, T.M. (Comps.), Foundational Concepts in Silviculture with Emphasis on Reforestation and Early Stand Improvement, The 2022 National Silviculture Workshop Proceedings. U.S. Dept. Agric. For. Serv. Rocky Mt. Res. Stn. Gen. Tech. Rep. Proc. RMRS-P-80. Fort Collins, CO, 3 pp. https://www.fs.usda.gov/treesearch/pubs/64691.

Hebard, F.V., 2001. Backcross breeding program produces blight-resistant American chestnuts (Virginia, US). Ecol. Restor. 19 (4), 252–254.

Hebard, F., 2005. The backcross breeding program of the American Chestnut foundation. J. Am. Chestnut Found. 19 (2), 55–78.

Hebard, F.V., Fitzsimmons, S.F., Gurney, K.M., Saielli, T.M., 2012. The Breeding Program of the American Chestnut Foundation. Acta Hort. 1019, 135–139.

Jaynes, R.A., (1978). Selecting and breeding blight resistant chestnut trees. In: MacDonald, W.L., Cech, F.C., Luchok, J., Smith, C. (Eds.), Proceedings of the American Chestnut Symposium. Morgantown, WV, pp. 4-6.
Jacobs, D.F., 2007. Toward development of silvical strategies for forest restoration of American chestnut (*Castanea dentata*) using blight-resistant hybrids. Biol. Conserv. 137, 497–506.
Jacobs, D.F., Dalgleish, H.J., Nelson, C.D., 2013. A conceptual framework for restoration of threatened plants: the effective model of American Chestnut *(Castanea dentata*) reintroduction. New Phytol. 197, 378–393.
Jeffers, S.N., James, J.B., Sisco, P.H., (2009). Screening for resistance to *Phytophthora cinnamomi* in hybrid seedlings of American Chestnut. In: Goheen, E.M., Frankel, S.J., (Tech. Eds.), Proceedings of the Fourth Meeting of IUFRO Working Party. U.S. Dept. Agric. For. Serv. Pac. Southwest. Res. Stn. Gen. Tech. Rep. PSW-221. Albany, CA, pp. 188-194.
Jeffers, S.N., (2015a). Protocol 07-04.1: PARP(H)-V8A. Laboratory Protocols for Phytophthora species. Amer. Phytopathol. Soc., St. Paul, MN. https://apsjournals.apsnet.org/doi/10.1094/9780890544969.07.04.1 (accessed 5 January 2023).
Jeffers, S.N., (2015b). Protocol 07-11.1: V8 agar (V8A) or broth. Laboratory Protocols for Phytophthora species. Amer. Phytopathol. Soc., St. Paul, MN. https://apsjournals.apsnet.org/doi/abs/10.1094/9780890544969.07.11.1 (accessed 5 January 2023).
Johnson, P.S., Dale, C.D., Davidson, K.R., 1986. Planting northern red oak in the Missouri Ozarks: a prescription. North. J. Appl. For. 3, 66–68.
Kriebel, H.B., Merritt, C., Stadt, T., 1988. Genetics of growth rate in Quercus rubra: provenance and family effects by the early third decade in the north central USA. Silvae Genetica 37 (5–6), 193–198.
Littell, R.C., Henry, P.R., Ammerman, C.B., 1998. Statistical analysis of repeated measures data using SAS proceedures. J. Anim. Sci. 76 (4), 1216–1231.
Little, E., (1977). Atlas of United States trees (Minor eastern hardwoods). U.S. Dept. Agric. For. Serv., Misc Pub 1342, Washington, DC.
Loftis, D.L., 1990. Predicting post-harvest performance of advance red oak reproduction in the Southern Appalachians. For. Sci. 36 (4), 908–916.
Lutts, R.H., 2004. Like Manna From God: The American Chestnut Trade in Southwestern Virginia. Environ. Hist. 9 (3), 497–525.
Mattoon, W.R., 1909. The origin and early development of chestnut sprouts. For. Quart. 7, 34–47.
Meadows, I.M., Jeffers, S.N., 2011. Distribution and recovery of *Phytophthora cinnamomi* in soils of mixed hardwood-pine forests of the south-eastern USA, New Zealand. J. For. Sci. 41S, S39–S47.
Meadows, I.M., Zwart, D.C., Jeffers, S.N., Waldrop, T.A., Bridges Jr., W.C., 2011. Effects of fuel reduction treatments on incidence of Phytophthora species in soil of a southern Appalachian Mountain forest. Plant Dis. 95, 811–820.
Mellano, M., Beccaro, G., Donno, D., Marinoni, D.T., et al., 2012. *Castanea* spp. biodiversity conservation: collection and characterization of the genetic diversity of an endangered species. Genet. Resour. Crop Evol. 59 (8), 1727–1741.
Miller, A.C., Woeste, K.E., Anagnostakis, S.L., Jacobs, D.F., (2014). Exploration of a rare population of Chinese chestnut in North America: stand dynamics, health and genetic relationships. AoB Plants 6, plu065-plu065. https:\\doi.org\10.1093/aobpla/plu065.
Minter, W.F., Myers, R.K., Fischer, B.C., 1992. Effects of Tree Shelters on Northern Red Oak Seedlings Planted in Harvested Forest Openings. North. J. Appl. For. 9, 58–63.
Montague, M.S., Landhäusser, S.M., McNickle, G.G., Jacobs, D.F., 2022. Preferential allocation of carbohydrate reserves belowground supports disturbance-based management of American chestnut (Castanea dentata). For. Ecol. Manag. 509, 120078 https://doi.org/10.1016/j.foreco.2022.120078.
NRCS, Natural Resources Conservation Service., (2020). Web Soil Survey. https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx/ (accessed 30 March 2020).
Newhouse, A.E., Powell, W.A., 2020. Intentional introgression of a blight tolerance transgene to rescue the remnant population of American chestnut. Conserv. Sci. Pract. e348, 8 pp. https://doi.org/10.1111/csp2.348.
Newhouse, A.E., Polin-McGuigan, L.D., Baier, K.A., Valletta,, et al., 2014. Transgenic American chestnuts show enhanced blight resistance and transmit the trait to T1 progeny. Plant Sci. 228, 88–97.
Nyland, R., 1996. Silviculture: Concepts and Applications, third edition. Waveland Press, Long Grove, IL.
Oliver, C.D., 1981. Forest development in North America following major disturbances. For. Ecol. Manag. 3, 153–168.
Oswalt, C.M., Clatterbuck, W.K., Houston, A.E., 2006. Impacts of deer herbivory and visual grading on the early performance of high-quality oak planting stock in Tennessee. USA. For. Ecol. Manag. 229, 128–135.
Payne, J.A., Menke, A.S., Schroeder, P.M., 1975. Dryocosmus kuriphilus Yasumatsu, (Hymenoptera: Cynipidae), an oriental chestnut gall wasp in North America. U.S. Dept. Agric. Coop. Econ. Insect Rep. 25, 903–905.
Pinchot, C.C., Schlarbaum, S.E., Clark, S.L., Saxton, A.M., Sharp, A.M., Schweitzer, C.J., Hebard, F.V., 2017. Growth, survival, and competitive ability of chestnut (*Castanea* Mill.) seedlings planted across a gradient of light levels. New For. 48, 491–512.
Pinchot, C.C., Clark, S.L., Schlarbaum, S.E., Saxton, A.M., Sung, S.J.S., Hebard, F.V., 2015. Effects of temporal dynamics, nut weight and nut size on growth of American chestnut, Chinese chestnut and backcross generations in a commercial nursery. Forests 6 (5), 1537–1556.
Pinchot, C.C., Hall, T.J., Saxton, A.M., Schlarbaum, S.E., Bailey, J.K., 2018. Effects of seedling quality and family on performance of northern red oak seedlings on a xeric upland site. Forests 9 (6), 351. https://doi.org/10.3390/f9060351.
Pinchot, C.C., Royo, A.A., Stanovick, J.S., Schlarbaum, S.E., Sharp, A.M., Anagnostakis, S.L., 2022. Deer browse susceptibility limits chestnut restoration success in northern hardwood forests. For. Ecol. Manag. 523, 120481 https://doi.org/10.1016/j.foreco.2022.120481.
Pinto, J.R., Dumroese, R.K., Davis, A.S., Landis, T.D., 2011. Conducting seedling stocktype trials: a new approach to an old question. J. For. 109 (5), 293–299.
Poland, T.M., Patel-Weynand, T., Finch, D.M., Miniat, C.F., Hayes, D.C., Lopez, V.M., 2021. Invasive Species in Forests and Rangelands of the United States: A Comprehensive Science Synthesis for the United States Forest Sector. Springer Nature, Switzerland.
Ponder, F.J., 1995. Shoot and root growth of northern red oak planted in forest openings and protected by treeshelters. North. J. Appl. For. 12 (1), 36–42.
Powell, W.A., Newhouse, A.E., Coffey, V., 2019. Developing blight-tolerant American chestnut trees. Cold Spring Harb. Perspect. Biol. 11 (7), a034587.
Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W., Essl, F., Foxcroft, L.C., Genovesi, P., 2020. Scientists' warning on invasive alien species. Biol. Rev. 95 (6), 1511–1534. https://doi.org/10.1111/brv.12627.
Reynolds, D.L., Burke, K.L., 2011. The effect of growth rate, age, and chestnut blight on American Chestnut mortality. Castanea 76 (2), 129–139. https://doi.org/10.2179/10-035.1.
Rhoades, C.C., 2007. The influence of American chestnut (Castanea dentata) on nitrogen availability, organic matter and chemistry of silty and sandy loams soils. Pedobiologia 50, 553–562.
Russell, E.W.B., 1987. Pre-blight distribution of Castanea dentata. Bull. Torrey Bot. Club 114, 183–190.
SAS Institute, Inc. (2013). SAS version 9.4. User's Guide. Cary, North Carolina.
Saucier, J. R. (1973). American chestnut - an American wood. U.S. Dept. Agric. For. Serv. FS-230, 3-6.
Schlarbaum SE, Anagnostakis SL, Morton MC. (1994). Evaluation of experimental chestnut plantings in Eastern North America. In: Double, M.L., MacDonald, W.L., (eds.) Proceedings of the International Chestnut Conference, West Virginia University Press, pp 52–66.
Sharpe, S.R., (2017). Phytophthora species associated with American, Chinese, and backcross hybrid chestnut seedlings in field sites in the southeastern United States. M.S. Thesis, Clemson University, Clemson, South Carolina, 130 pp.
Skousen, J.G., Dallaire, K., Scagline-Mellor, S., Monteleone, A., et al., 2018. Plantation performance of chestnut hybrids and progenitors on reclaimed Appalachian surface mines. New For. (Dordr.) 49 (5), 599–611. https://doi.org/10.1007/s11056-018-9643-7.
South, D.B., 1995. Relative growth rates: a critique. South Afr. For. J. 173 (1), 43–48.
Spetich, M.A., Dey, D.C., Johnson, P.S., Graney, D.L., 2002. Competitive capacity of *Quercus rubra* L. planted in Arkansas Boston Mountains. For. Sci. 48, 504–517.
Steiner, K.C., Westbrook, J.W., Hebard, F.V., Georgi, L.L., Powell, W.A., Fitzsimmons, S. F., 2017. Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen. New For. 48 (2), 317–336. https://doi.org/10.1007/s11056-016-9561-5.
Stimm, K., Heym, M., Uhl, E., Tretter, S., Pretzsch, H., 2021. Height growth-related competitiveness of oak (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.) under climate change in Central Europe. Is silvicultural assistance still required in mixed-species stands? For. Ecol. Manag. 482, 118780 https://doi.org/10.1016/j.foreco.2020.118780.
Struve, D.K., Jolly, R.J., 1992. Transplanted red oak seedlings mediate transplant shock by reducing leaf surface area and altering carbon allocation. Can. J. For. Res. 22, 1441–1448.
Struve, D.K., Burchfield, L., Maupin, C., 2000. Survival and growth of transplanted large- and small-caliper red oaks. J. Arboriculture 26, 162–169.
Thomas-Van Gundy, M., Bard, J., Kochenderfer, J., Berrang, P., 2017. Mortality, early growth, and blight occurrence in hybrid, Chinese, and American chestnut seedlings in West Virginia. in: Kabrick, J.M., Dey, D.C., Knapp, B.O., Larsen, D.R., Shifley S.R., Stelzer H.E. (Eds.), Proceedings of the 20th Central Hardwood Forest Conference. U. S. Dept. Agric. For. Serv. North. Res. Stn. Gen. Tech. Rep. NRS-P-167. Newtown Square, PA, pp. 222-239.
Thompson, J.R., Schultz, R.C., 1995. Root system morphology of Quercus rubra L. planting stock and 3-year field performance in Iowa. New For. 9, 225–236.
Tuğ, A., Hodžić, M.M., Ballian, D., 2022. Correlations among morphological traits of sweet chestnut (Miller) from Bosnia and Herzegovina. Folia Forestalia Polonica 64 (1), 49–57. https://doi.org/10.2478/ffp-2022-0005.
Wamishe, Y.A., Jeffers, S.N., Hwang, J. (2008). Monitoring for Phytophthora ramorum and other species of Phytophthora in nurseries and urban areas in the southeastern USA. In: Frankel, S.J., Kliejunas, J.T., Palmieri, K.M., (tech. coords.). Proceedings of the sudden oak death third science symposium, U.S. Dep. Agric. For. Serv. Pac. Southwest. Res. Stn. Gen. Tech. Rep. PSW-GTR-214. Albany, CA, 109-110.
Ward, J.S., Martin, P.N., Gent, G.R.S., 2000. Effects of planting stock quality and browse protection-type on height growth of northern red oak and eastern white pine. For. Ecol. Manag. 127, 205–216.
Westbrook, J.W., James, J.B., Sisco, P.H., Frampton, J., Lucas, S., Jeffers, S.N., 2019. Resistance to *Phytophthora cinnamomi* in American chestnut (*Castanea dentata*) backcross populations that descended from two Chinese chestnut (*Castanea mollissima*) sources of resistance. Plant Dis. 103, 1631–1634. https://doi.org/10.1094/PDIS-11-18-1976-RE.
Westbrook, J.W., Zhang, Q., Mandal, M.K., Jenkins, E.V., Barth, L.E., Jenkins, J.W., Grimwood, J., Schmutz, J., Holliday, J.A., 2020. Optimizing genomic selection for blight resistance in American chestnut backcross populations: a tradeoff with

American chestnut ancestry implies resistance is polygenic. Evolutionary Applications 13, 31–47. https://doi.org/10.1111/eva.12886.
Wheeler, N.C., Steiner, K.C., Schlarbaum, S.E., Neale, D.B., 2015. The evolution of forest genetics and tree improvement research in the United States. J. For. 113 (5), 500–510. https://doi.org/10.5849/jof.14-120.
Ziegler, E.A., 1920. Problems arising from the loss of our chestnut. For. Leaves 17, 152–155.