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Survival, Growth, and Physiology of American, Chinese, and Hybrid Chestnut Seeds Out-planted in Established Chestnut Plantations

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Abstract

The American chestnut is functionally extinct in its former range from chestnut blight introduction. To reintroduce this foundation species, The American Chestnut Foundation is attempting to create blight-resistant American-Chinese chestnut hybrids. Much of the ecological research has used out-planted, nursery seedlings, with little known about seedling establishment, often the most challenging stage in a tree's life cycle. This study examined the survival, growth, and photosynthetic characteristics of American, Chinese, and F_1 hybrid chestnut seeds planted in corresponding chestnut, and a silver maple (control) stand to determine establishment in conspecific and non-conspecific stands. If parent trees harbor distinct ectomycorrhizal communities, conspecific seedlings might benefit from connecting into these networks. Steady-state light response curves were measured to estimate photosynthetic parameters. Growth parameters (leaf, stem, root, shoot, and total mass; number of leaves and leaf area; stem basal diameter, and root:shoot ratio) of harvested seedlings were analyzed using two-way ANOVAs with seed-type and stand as factors. Where interactions were not significant, one-way ANOVAs were used to assess stand and seed-type effects. Maximum photosynthetic capacity was highest for seedlings in the American, lowest in the maple, and intermediate in the Chinese and hybrid stands, likely as a result of significantly higher light in the American stand. There were few and inconsistent significant interactions but stand and seedling-type had significant effects. In general, seedlings in the American stand had larger basal diameter, stem length, and shoot mass while the Chinese seedlings had larger basal diameter, stem length, shoot mass, despite germinating almost a month after the other seeds. ANOVA of ectomycorrhizal colonization found the American and Chinese stands had significantly higher inoculation frequency, while linear regression found that root and shoot mass increased with inoculation. Ectomycorrhizal inoculation varied by mature stand genotype, but with limited effects on conspecific seedling growth in monotypic plantation environments.

Introduction

The American chestnut (*Castanea dentata* (Marshall) Borkh.) was a foundation species throughout the hardwood forests of the Appalachian Mountains and eastern United States, driving ecosystem dynamics, nutrient cycling, and wildlife populations (Davis 2005; Laport et al. 2022). Following the introduction of chestnut blight (*Cryphonectria parasitica* (Murrill) Barr) this species became functionally extinct, shifting ecological dynamics within the forests (Palliet 2003; Kane et al. 2019). Before this decimation, the American chestnut was estimated to cover over 8 million hectares across the southeast and comprised anywhere from 25-33% of the total canopy cover in the area. American chestnut wood tended to grow fast, straight, and was rot-resistant when harvested, making the sale of its timber extremely valuable to the early Appalachian economy. Chestnuts were also used by both humans and animals alike, providing consistent nut-crop for wild game species, food for herbivorous insects, and feed for hogs (Davis 2005).

Chestnut blight is a fungus that infects the inner bark and cambium of developing trees, inhibiting nutrient transport, stunting growth, and killing the above ground stem (Palliet 2003; Dalgleish et al. 2023). By the 1960s the American chestnut had been completely extirpated from its native range, leaving gaps in the deciduous forest ecosystem and local economy (Brown et al. 2014). The slower maturation to production of edible seeds with higher deterrence (i.e. tannins and thick shells) among remaining tree species in the area, such as oaks (*Quercus* spp.) and hickories (*Carya* spp.), led to decreases in resource availability, harming consumer populations in the region (Palliet 2003; Dalgleish and Swihart 2011).

The American Chestnut Foundation (TACF) has led chestnut restoration efforts by working to develop blight-resistant trees phenotypically indistinguishable from the original American chestnut through the hybridization of American and Chinese (*Castanea mollissima* Blume) trees (Diskin et al. 2006; Clark et al. 2023). The Foundation's Backcross Breeding Program has created a series of hybrid American-Chinese trees, ranging from 50-94% American Chestnut. These trees are produced by backcrossing sequential hybrid generations as a means of retaining as many American chestnut traits as possible. All backcrossed generations are inoculated with blight fungi in order to measure blight tolerance and aid in selection of resistance in subsequent chestnut trees

(PA-TACF 2016; Figure 1). This project focused on the growth of American, Chinese, and their F_1 (50%) seeds in order to best represent the range of American-Chinese genetic variations and avoid higher parental contribution from either genotype.

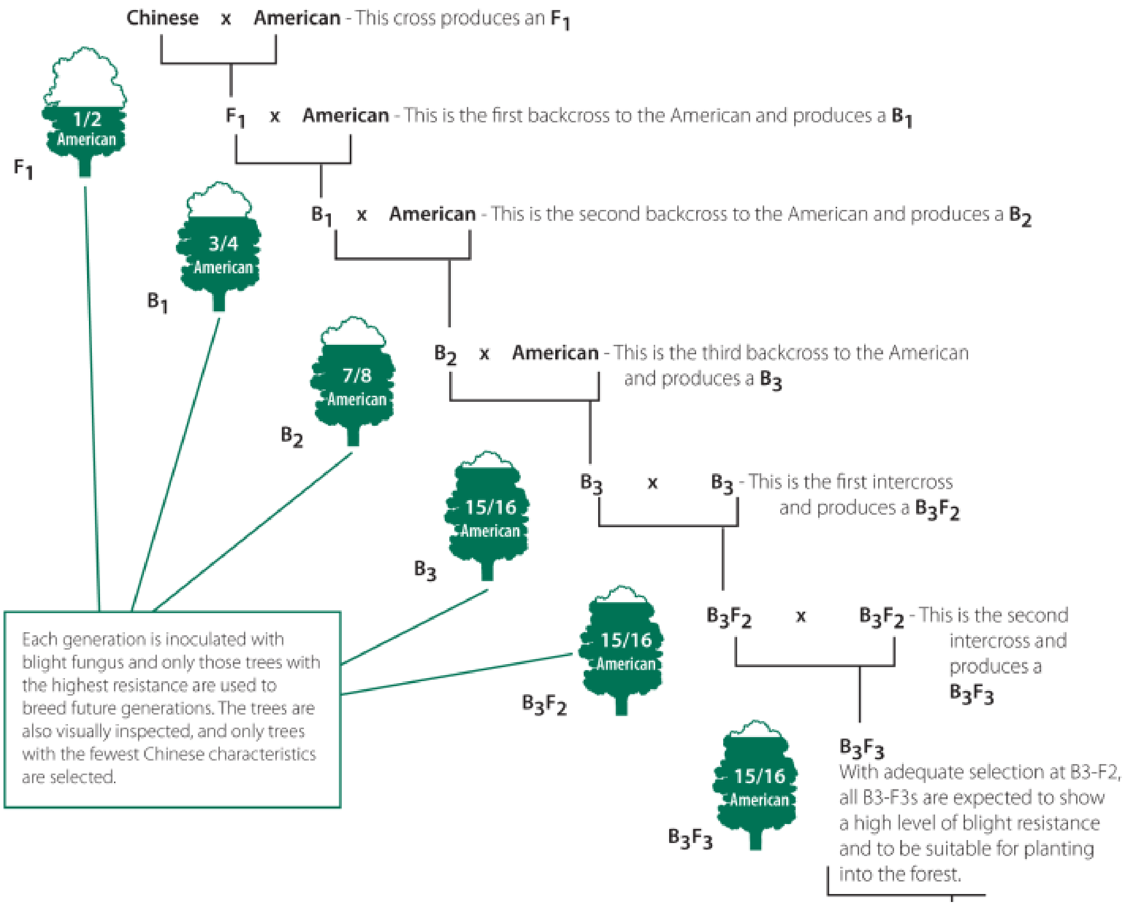

Figure 1. Hybridization methods employed by TACF's Backcross Breeding Program to produce hybridized American-Chinese chestnut trees ranging from 50% American (F_1) to 94% American (B_3F_2 and B_3F_3). Initial figure retrieved from PA-TCAF's Backcross Breeding Program via: https://tacf.org/wp-content/uploads/2016/09/Backcross-Breeding-Program.pdf (2016).

Chinese chestnuts have a stronger blight resistance than American chestnuts as a result of co-evolution with the pathogen. However, Chinese trees are unable to fill the same ecological niche due to variations in growth morphology and physiology (Laport et al. 2022). Compared to the American trees, the Chinese chestnut grows in a shorter multi-stemmed pattern, making them an inefficient canopy species. Chinese chestnuts also tend to be significantly less shade-tolerant, making them poor competitors in understory growth (Boyd and Craddock 2012). Additionally, much of the previous research on backcross hybrid chestnuts has been on out-planted, pot-grown seedlings from nurseries (Brown et al. 2014; Knapp et al. 2014; Clark et al. 2023). Little is known about natural regeneration dynamics from seed in chestnut species outside of general growth trends based on environmental conditions. For instance, American and B_3F_3 hybrid chestnuts growing in light environments above 800 μmol m^{-2} s^{-1} displayed increases in carbon assimilation, quantum efficiency, and maximum photosynthetic rate when compared to Chinese and other hybrid species (Knapp et al. 2014).

One important component of successful seedling establishment is the development of beneficial fungal symbioses and the formation of common mycorrhizal networks (CMN) with mature trees. Previous research into the role of these CMNs has analyzed ectomycorrhizal communities (ECM) found only within mixed forest stands (Walker et al. 2005; D'Amico et al. 2014). Little is currently known about the role of these CMNs on seedling growth in stands with remnant American chestnut populations.

This project was part of a larger study to investigate ECM communities and their effects on chestnut seedling establishment. A reciprocal seedling transplant was used to assess the ECM community composition in pure stands of American, Chinese, and F_1 hybrid chestnuts and colonization patterns of seedlings of these three chestnut lineages planted in corresponding pure stands of each chestnut type, and a non-ECM control stand of silver maple (*Acer sacharrum* L.). We expect that if the different chestnut types harbor different mycorrhizal communities, then seedlings might perform better when planted in conspecific stands because of the chestnut type-specific ECM and CMNs.

Methods

Study Area

This study was conducted at TACFs Meadowview Research Farm in Meadowview, VA from April - August 2025. The Meadowview Research Farm was established by TACF in 1989 for seed production within their Backcross Breeding Program and supports a range of conservation efforts (VA-TACF). This farm is located in the Blue Ridge Mountains within the southeastern region of the native American chestnut range (TACF; Figure 2). The American stand was in a small, cleared area between rows of American chestnuts. Most of the American trees were immature resprouts due to die-back from blight infection. The Chinese stand was in a thicket of large, mature, Chinese chestnut trees with a gap in the canopy that allowed variable light to reach the plot directly. The F_1 hybrid stand was in a partially cleared stand of mature F_1 hybrid chestnut trees with an incomplete canopy that allowed for consistent filtered light to reach the plot. Finally, the non-ECM control plot was located directly underneath a mature silver maple tree, in a cleared area away from any chestnut or other trees, allowing for a high and consistent light environment.

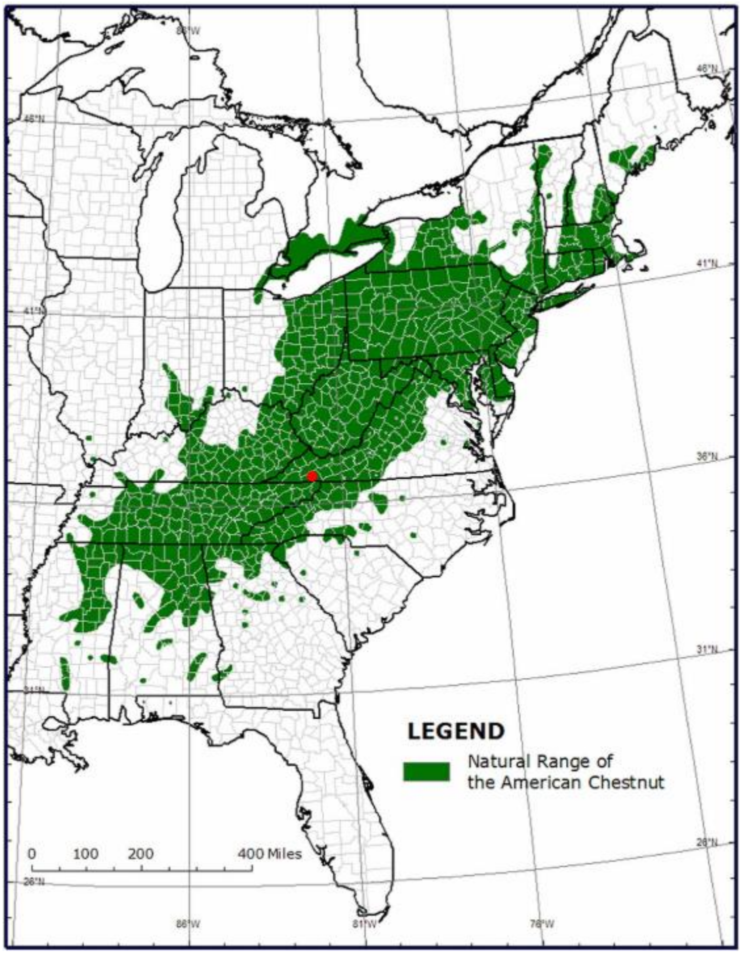

Figure 2. Location of the Meadowview Research Farm (denoted by red dot) within the natural range of the American chestnut. Initial figure retrieved from The American Chestnut Foundation's website via: https://tacf.org/american-chestnut-range-map/#:~:text=American%20Chestnut%20Native%20Range,of%20the%20American%20Chestnut%20page. (2024).

Data Collection

Seed Monitoring and Light Conditions

Sixty-six seeds of each type (American, Chinese, and F_1 hybrid) were randomly planted in mature chestnut (American, Chinese, and F_1 hybrid) and control (sugar maple) stands in a three by four factorial design in order to bait fungi for ECM colonization during initial seedling establishment (Walker et al. 2005; Caruso et al. 2021). This initial planting was done on April 30, 2025 with the help of student volunteers and TACF staff at Meadowview Research Farm.

American and F_1 hybrid seeds were sourced directly from TACF, but the Chinese seeds were purchased from Sheffield's Seed Company, a third party seller, due to low supply at

TACF. A total of 198 seeds were randomly planted in a 10 x 20 grid with 0.33 m spacing in each stand. The American plot was already inside a large deer-exclusion fence, but chicken wire was added around maple, F_1 hybrid, and Chinese plots to prevent deer herbivory. Seeds were planted in holes dug roughly 15 cm into the ground, before being re-covered with soil and encased in a plastic tube secured with a stick to prevent seed predation (Figure 3). The survival and growth of the planted seeds were monitored monthly from May - July 2025 in order to assess seed germination (shoot presence) and the number of leaves present.

Figure 3. Randomized seedling layout for the F_1 hybrid stand. Seeds were planted with protective plastic cones within to prevent herbivory and predation. Colored flags represent American (blue), Chinese (pink), and F_1 hybrid (yellow) seeds. Plots in all stands were randomized in a similar manner.

The first monitoring trip was made May 29, 2025 and found consistent growth and germination of the American and F_1 hybrid seeds, but limited germination of the Chinese seeds across all four stands. It was determined that the outsourced Chinese seeds were likely improperly stored leading to a lack of cold stratification needed to induce proper germination and would need to be re-planted. The second monitoring trip was made June 13, 2025, and any Chinese seeds that had failed to germinate were replanted with a new seed sourced from TACF. The third monitoring trip was completed over July 7 - 8, 2025, and found considerable damage at the Chinese stand. It is assumed that an animal was able to pass through the fencing and dug up a significant portion of the planted seeds. The final monitoring trip was made July 22 - 24, 2025.

Steady-State Leaf Gas Exchange

The light environment was quantified July 7 - 8 2025 using a line quantum sensor (LiCor LI-191R, LiCor Biosciences, Lincoln, NE) measured over each row of seedlings (n = 20) for all stands except maple where n = 10, because of equipment failure. We constructed steady-state light response curves using two portable photosynthesis systems (LI-6400XT, LiCor Biosciences, Lincoln, NE). light response curves were fit using mathematical models from Marshall and Biscoe (1980) and Thornley and Johnson (1990) using a macro in MS Excel to estimate photosynthetic parameters: maximum photosynthetic capacity (A_{max}), dark respiration (R_d), quantum yield (QY), and light compensation point (LCP). Measurements were made on three randomly selected seedlings from each seed x stand combination. All gas exchange measurements were taken during two campaigns in July (7 - 8 and 22 - 24 2025) on days with similar weather. For each curve, a fully expanded leaf was selected and cleaned of any dirt or residual dew, before the leaf cuvette was attached. CO_2 was maintained at 420 ppm, relative humidity was maintained within 70 - 75%, and leaf temperature was held within 1.5 - 2 °C of the ambient temperature throughout the duration of the photosynthetic measurements. Leaves were allowed to equilibrate to chamber photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1} before light response curves were initiated. Once equilibrated, light was decreased stepwise to darkness (1200, 1000, 750, 500, 250, 100, 75, 50, 25, 10, 0 μmol m^{-2} s^{-1}), allowing leaves to equilibrate at each light level before a datapoint was collected.

Harvest and Biomass Assessment

A subset of five seedlings (including those on which light response curves were measured) from each seed-stand combination (n = 60) were harvested on August 13 2025, with the soil volume around the roots collected to preserve fine root tips. Seedlings were then bagged, labeled, and transported back to UNC Asheville where they were held in the fridge to preserve quality until processing. All harvested seedlings were excised into leaves, stems, and roots for characterization and biomass processing. Leaves for each seedling were counted, and total leaf area was measured using a leaf area meter (LI-3000C, LiCor Biosciences, Lincoln, NE). Stem basal diameter and total stem length were measured. After processing, leaves and stems for each seedling were dried individually at 60 °C for 48 h before being weighed. Specific leaf mass (g/cm^2) was calculated. Root systems were gently cleaned of all soil and particulate matter before fine root tips were excised and processed separately for mycorrhizal colonization. To do this, one hundred root tips from each plant were randomly surveyed for mycorrhizal presence to calculate an average inoculation frequency for each plant. Prior to ECM processing the main root system of each seedling was dried at 60 °C for 48 h before being weighed. After ECM processing of fine roots, these were dried separately, weighed and added to the main root mass, in order to get total root mass (g).

Data Analysis

Because of low sample size (n = 3), photosynthetic parameters (A_{max}, R_d, QY, LCP) were compared among stands, seedlings, and seedlings within each stand (seed-by-stand) using separate one-way analysis of variance (ANOVA). Light was compared among stands using a one-way ANOVA. Growth parameters (leaf mass, leaf count, total leaf area, specific leaf mass, stem basal diameter, total stem length, stem mass, shoot mass, root mass, root-shoot ratio, and total mass) were first compared using a two-way ANOVA with stand, seedling, and their interaction as factors. For parameters that had no significant interaction, separate one-way ANOVAs were used to test the effect of stand and seedling individually. For parameters with significant interactions, a separate one-way ANOVA was used to test for seedling differences by stand. Inoculation frequency was analyzed using a two-way ANOVA with stand and seed-type as the interaction factors. Because there was no significant interaction, separate one-way ANOVAs were used to analyze seedling and stand effects. For all one and two-way ANOVAs means were compared using Tukey's *post-hoc* test where significant differences occurred. Additionally, seedling mass was aggregated by section (leaf, stem, root) and chestnut stand (American, Chinese, F_1 hybrid) and analyzed against inoculation frequency using linear regression. All analysis was performed using SAS Enterprise Guide v7.15 HF9 (SAS Institute Inc. 2017).

Results

Steady-State Light Response Curves

Mean light response curves show some variation among seed types (Figure 4). However, there were no significant differences among photosynthetic parameters among seedling types. Stand-type had significant effects on some parameters (Figure 5). Maximum photosynthetic rate (A_{max}) was significantly higher in the American, intermediate in the Chinese and F_1 hybrid, and lowest in the maple stand (F = 5.07, df = 3, p = 0.0055; Figure 5A). There were no significant differences in A_{max} among seedling types in individual stands. Dark respiration (R_d) was significantly higher in the F_1 hybrid, intermediate in the

American and Chinese, and lowest in the maple stand (F = 3.62, df = 3, p = 0.0235; Figure 5B). Seedling by stand interaction showed R_d was significantly higher in American, intermediate in F_1 hybrid, and lowest in Chinese seeds when planted in the Chinese stand (F = 9.16, df = 2, p = 0.0150), but there were no differences among seed types in other stands. Quantum yield (QY) was significantly higher in the Chinese and American, intermediate in the F_1 hybrid, and lowest in the maple stand (F = 6.23, df = 3, p = 0.0019; Figure 5C). There were no significant differences in QY among seedling types in individual stands. Light compensation point had no significant difference based on stand-type alone (F = 1.55, df = 3, p = 0.222; Figure 5D). Seedling by stand analysis found LCP was significantly higher in F_1 hybrid seeds and lowest in American and Chinese seeds when planted in the F_1 hybrid stand (F= 98.02, df = 2, p < 0.001). Ambient light was highest in the American stand and did not differ in the remaining stands (F= 18.65, df = 3, p < 0.0001; Figure 6).

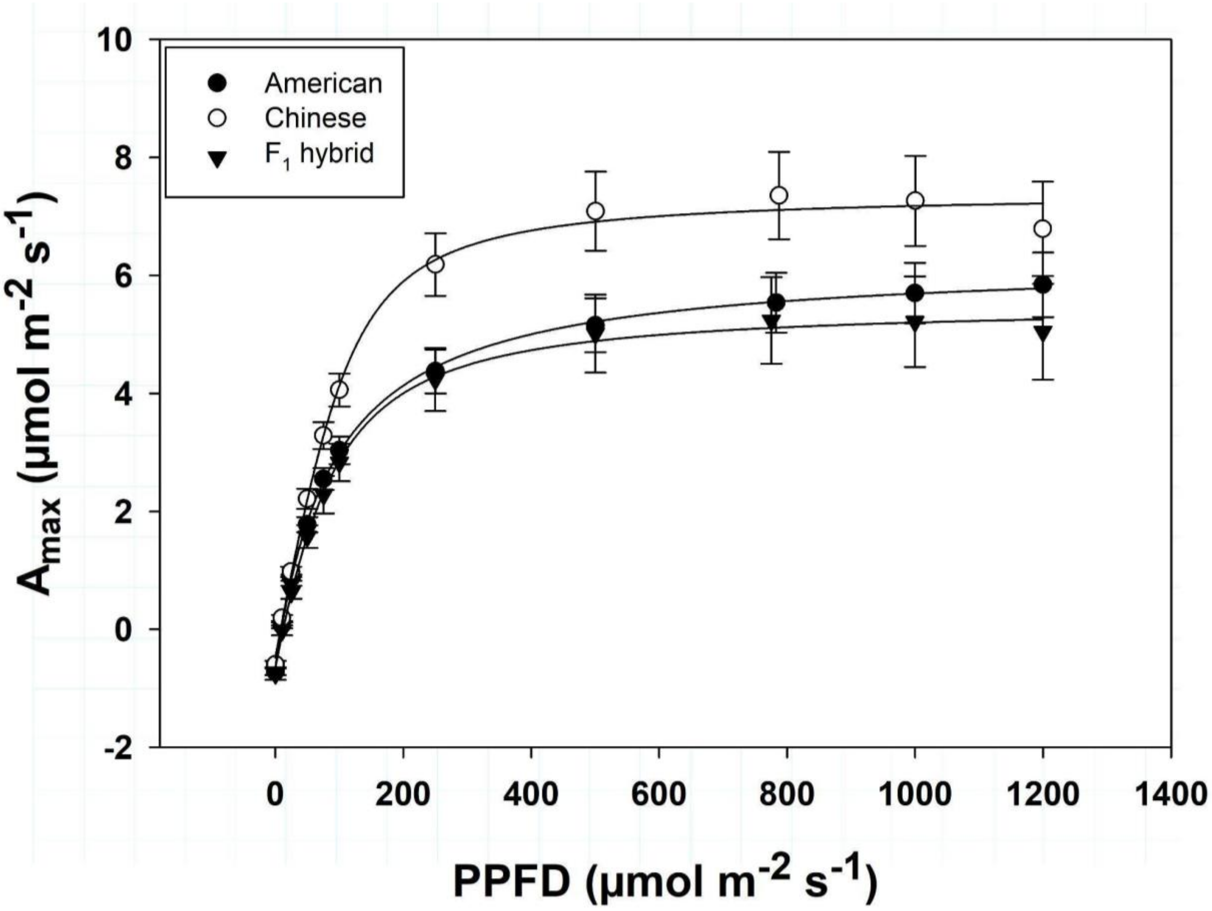

Figure 4. Representative light response curves constructed from mean American, Chinese, and F_1 hybrid chestnut seed data averaged across all four stands at descending photosynthetic photon flux density (PPFD). Points represent the mean ± SE of 12 trees.

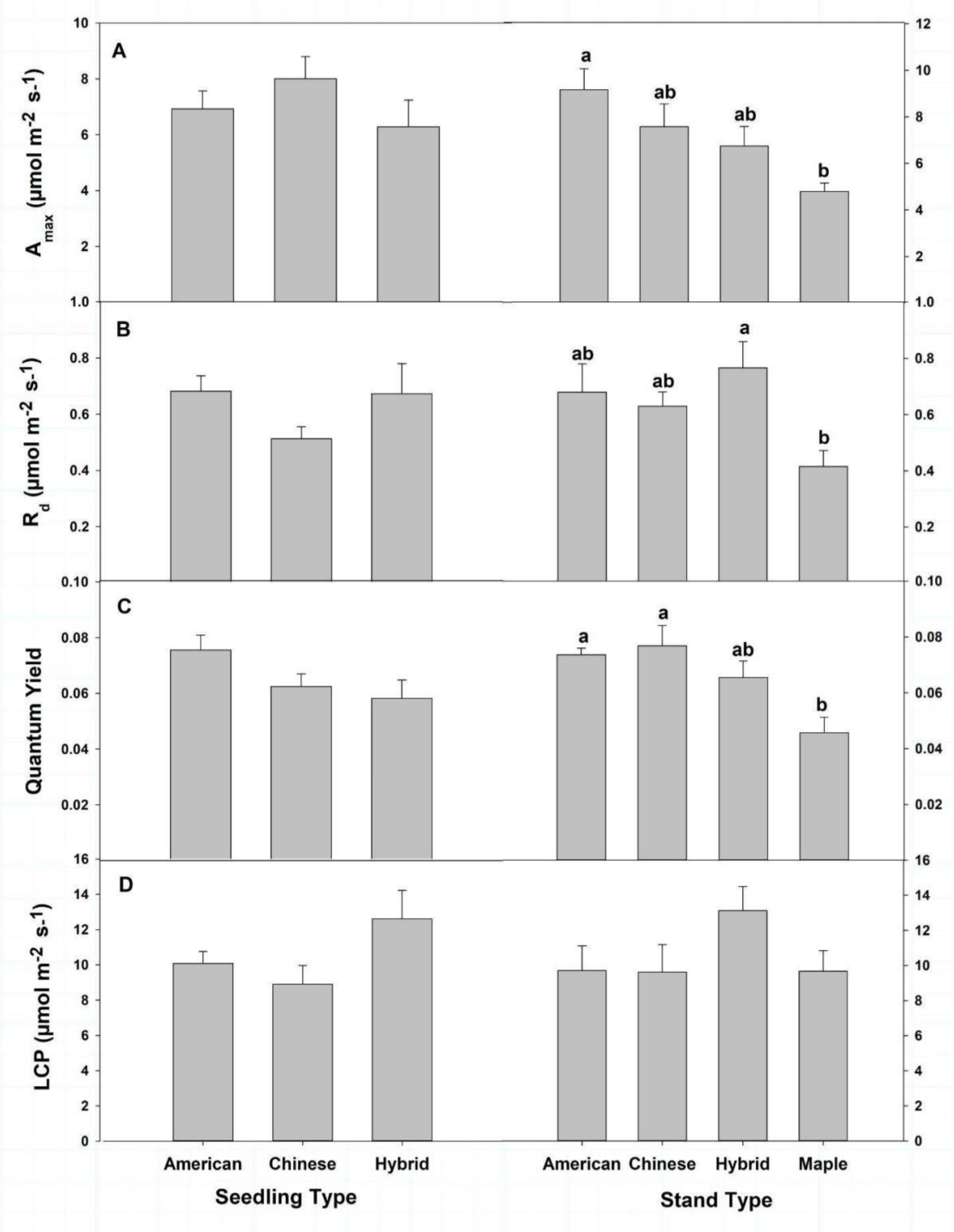

Figure 5. Mean + SE maximum photosynthesis (A_{max}: A), dark respiration (R_d: B), quantum yield (QY: C), and light compensation point (LCP: D) for seed and stand-type. Bars with different letters represent significant differences between means ($p < 0.05$).

Growth Parameters

Results of a two-way ANOVA found limited seed-by-stand interactions for the growth parameters. Leaf area showed a marginally significant interaction between stand and seed-type (F= 2.27, df = 6, p = 0.0524) while specific leaf mass (SLM) showed a more prominent stand and seed-type interaction (F = 2.75, df = 6, p = 0.0199). One-way ANOVA on seedling types in individual stands found Chinese seeds had the highest SLM followed by F_1 hybrid and American seeds when planted in the maple stand (F = 9.22, df = 2, p = 0.0037). Additional seed-by-stand ANOVA found American seeds had the highest leaf area followed by Chinese and F_1 hybrid seeds when planted in the Chinese stand (F = 4.67, df = 2, p = 0.0317). Overall, there was not a consistent pattern in seedling-by-stand interactions.

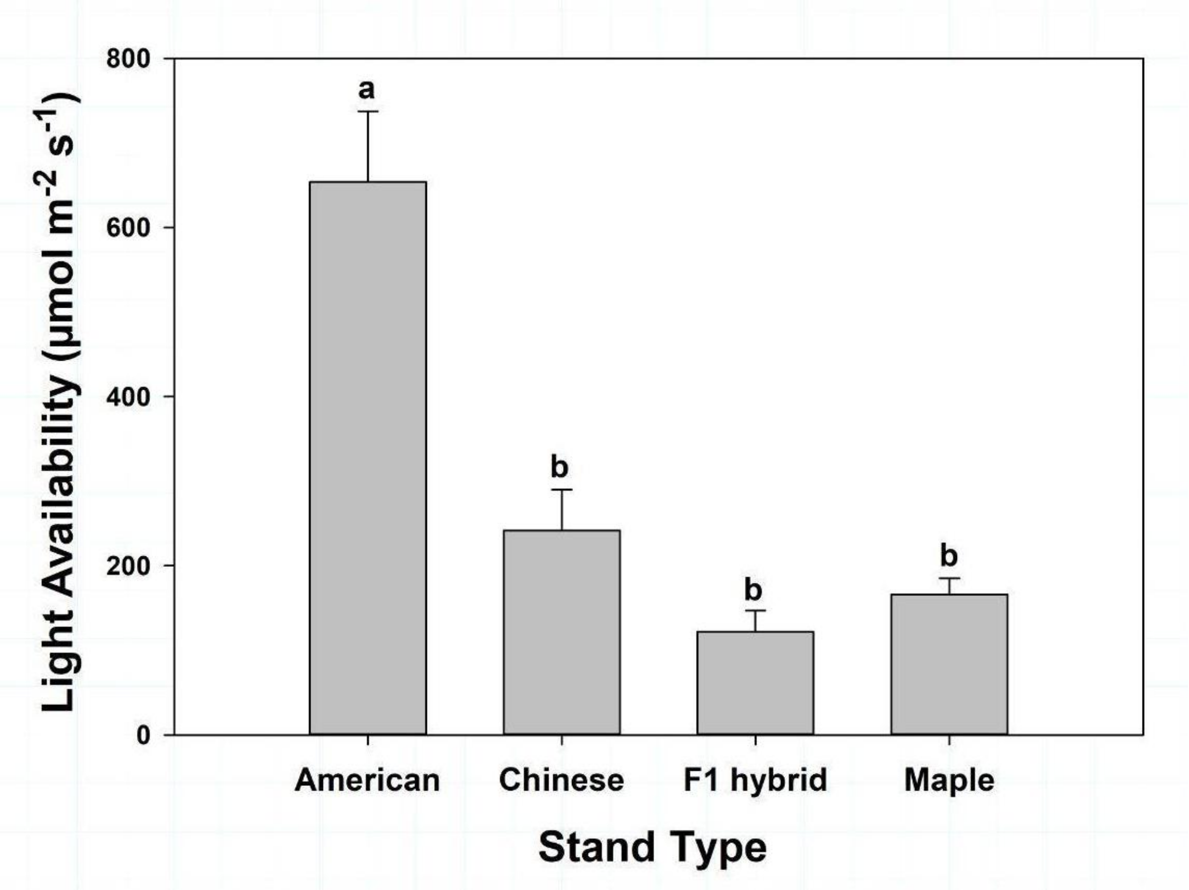

Figure 6. Mean + SE light availability by stand-type. Bars with different letters represent significant differences between means ($p < 0.05$).

Results of a one-way ANOVA found the American and maple stands had significantly higher values for most growth parameters (leaf mass, leaf count, total leaf area, specific leaf mass, stem basal diameter, total stem length, stem mass, shoot mass, root mass, and total mass; Table 1) than the Chinese and F_1 hybrid stands. Chinese seeds had significantly higher values for most growth parameters (leaf count, specific leaf mass, stem basal diameter, total stem length, stem mass, shoot mass, root mass, and total mass; Table 2) than the American, F_1 hybrid, and maple seeds. Overall, Leaf mass and leaf area only showed significant stand-type effects (Table 1), while root-shot ratio only showed significant seed-type effects (Table 2).

Table 1. Mean ± SE growth parameters for each stand-type. Letters denote significantly different values within a parameter at $p < 0.05$.

Stand						
	$F_{(3,60)}$	P Value	American	Chinese	F_1 Hybrid	Maple
Basal diameter (mm)	9.62	< 0.0001	5.5 ± 0.4^{a}	4.1 ± 0.3^{b}	3.5 ± 0.2^{b}	5.4 ± 0.3^{a}
Stem length (cm)	7.4	0.0003	59.7 ± 4.3^{a}	37.1 ± 2.8^{b}	35.3 ± 4.2^{b}	48.2 ± 5.0^{ab}
Stem mass (g)	9.33	< 0.0001	1.71 ± 0.25^{a}	0.67 ± 0.11^{b}	0.63 ± 0.12^{b}	1.36 ± 0.17^{a}
Total leaf count (#)	7.5	0.0003	14.60 ± 1.45^{ab}	10.40 ± 0.70^{b}	9.60 ± 0.88^{b}	16.00 ± 1.37^{a}
Leaf area (cm^2)	5.73	0.0017	427.8 ± 49.3^{ab}	283.7 ± 41.5^{b}	250.6 ± 37.1^{b}	466.2 ± 48.0^{a}
Leaf mass (g)	6.73	0.0006	2.39 ± 0.32^{a}	1.35 ± 0.2^{b}	1.2 ± 0.2^{b}	2.5 ± 0.3^{a}
Specific leaf mass (g/m^2)	4.78	0.0049	54.3 ± 2.0^{a}	46.6 ± 1.7^{b}	47.1 ± 1.5^{b}	53.6 ± 2.3^{ab}
Shoot mass (g)	7.91	0.0002	4.10 ± 0.56^{a}	2.02 ± 0.33^{b}	1.85 ± 0.33^{b}	3.84 ± 0.42^{a}
Root mass (g)	9.61	< 0.0001	1.73 ± 0.22^{a}	0.86 ± 0.15^{b}	0.87 ± 0.17^{b}	2.11 ± 0.25^{a}
Root-shoot ratio	2.23	0.0945	0.44 ± 0.03	0.43 ± 0.03	0.49 ± 0.05	0.57 ± 0.05
Total mass (g)	9.01	< 0.0001	5.82 ± 0.76^{a}	2.87 ± 0.47^{b}	2.71 ± 0.48^{b}	5.95 ± 0.63^{a}

Table 2. Mean ± SE growth parameters for each seed-type. Letters denote significantly different values within a parameter at $p < 0.05$.

Seed					
	$F_{(2,60)}$	P Value	American	Chinese	F_1 Hybrid
Basal diameter (mm)	14.38	< 0.0001	3.6 ± 0.2^{c}	5.7 ± 0.4^{a}	4.5 ± 0.2^{b}
Total stem length (cm)	8.38	0.0006	34.5 ± 3.4^{b}	56.0 ± 4.5^{a}	44.8 ± 3.1^{ab}
Stem mass (g)	6.38	0.0032	0.64 ± 0.12^{b}	1.46 ± 0.22^{a}	1.17 ± 0.14^{ab}
Total leaf count (#)	3.85	0.0027	11.15 ± 1.03^{b}	15.10 ± 1.24^{a}	11.70 ± 0.98^{ab}
Leaf area (cm^2)	1.86	0.1664	300.5 ± 47.4	377.9 ± 43.9	392.7 ± 35.0
Leaf mass (g)	2.36	0.104	1.42 ± 0.25	2.06 ± 0.28	2.09 ± 0.22
Specific leaf mass (g/m^2)	5.73	0.0054	45.8 ± 1.0^{b}	53.1 ± 2.2^{a}	52.2 ± 1.6^{a}
Shoot mass (g)	3.72	0.0302	2.06 ± 0.36^{b}	3.52 ± 0.49^{a}	3.27 ± 0.35^{ab}
Root mass (g)	6.61	0.026	0.84 ± 0.15^{b}	1.52 ± 0.20^{a}	1.81 ± 0.22^{a}
Root-shoot ratio	3.77	0.0289	0.44 ± 0.03^{b}	0.45 ± 0.03^{ab}	0.56 ± 0.49^{a}
Total mass (g)	4.66	0.0134	2.90 ± 0.50^{b}	5.04 ± 0.70^{a}	5.08 ± 0.54^{a}

ECM Colonization

Results of a two-way ANOVA found no significant interactions between seed and stand-type for inoculation frequency (F = 1.09, df = 6, p = 0.380). Additional one-way ANOVA found no significant differences in inoculation frequency between seed-type (F = 0.18, df = 2, p = 0.835), but did find that the American and Chinese stands had a significantly higher inoculation frequency, with the maple stand intermediate, and the F_1 hybrid stand lowest (F = 6.25, df = 3, p = 0.001; Figure 7). Addiitonal linear regression analysis showed root, leaf, and shoot mass increased significantly as inoculation increased (root: $r^2 = 0.0067$, p = 0.003; leaf: $r^2 = 0.074$, p = 0.016; shoot: $r^2 = 0.0803$, p = 0.0283; Figure 8). There was no significant relationship between inoculation and stem mass ($r^2 = 0.0532$, p = 0.076) or root-shoot ratio ($r^2 = 0.0001$, p = 0.949). Inoculation also had no significant effect on total biomass by seed-type (American: $r^2 = 0.101$, p = 0.173; Chinese: $r^2 = 0.095$, p = 0.1855; F_1 hybrid: $r^2 = 0.122$, p = 0.132).

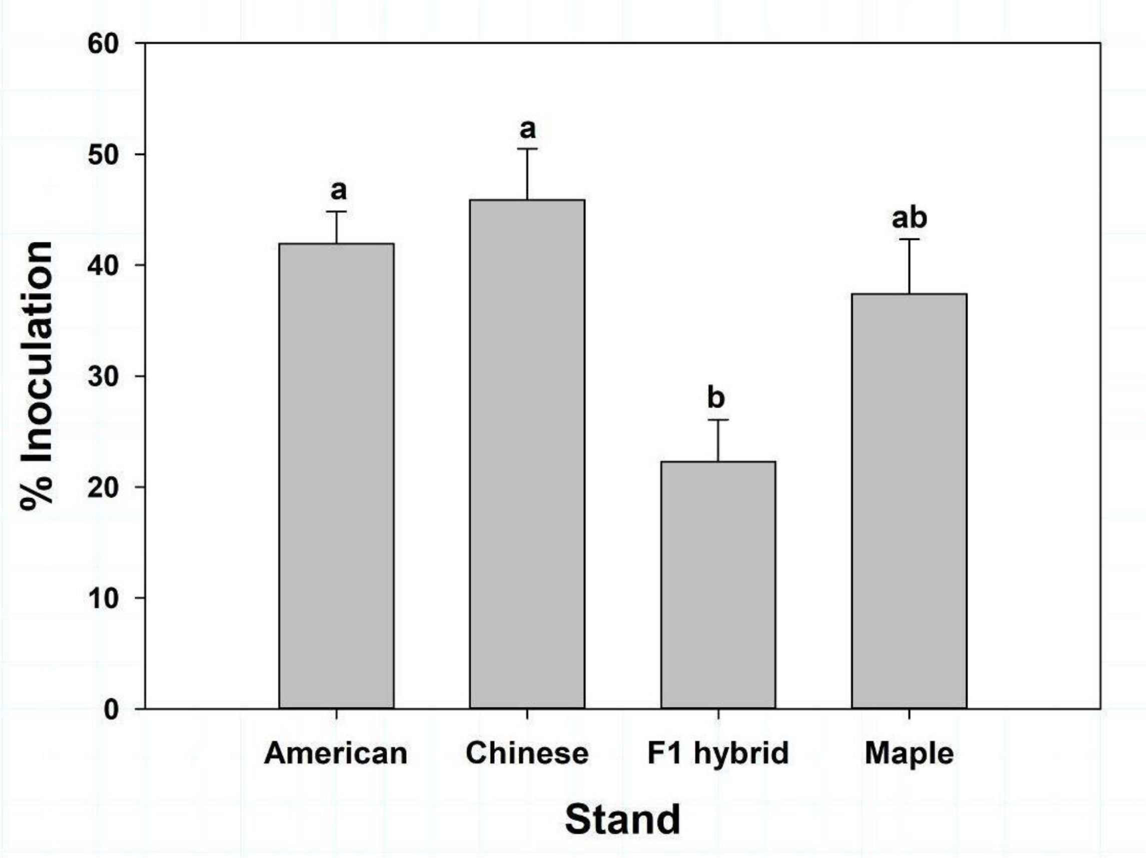

Figure 7. Mean + SE inoculation frequency (% inoculation) for each stand. Bars with different letters represent significant differences between means (p < 0.05).

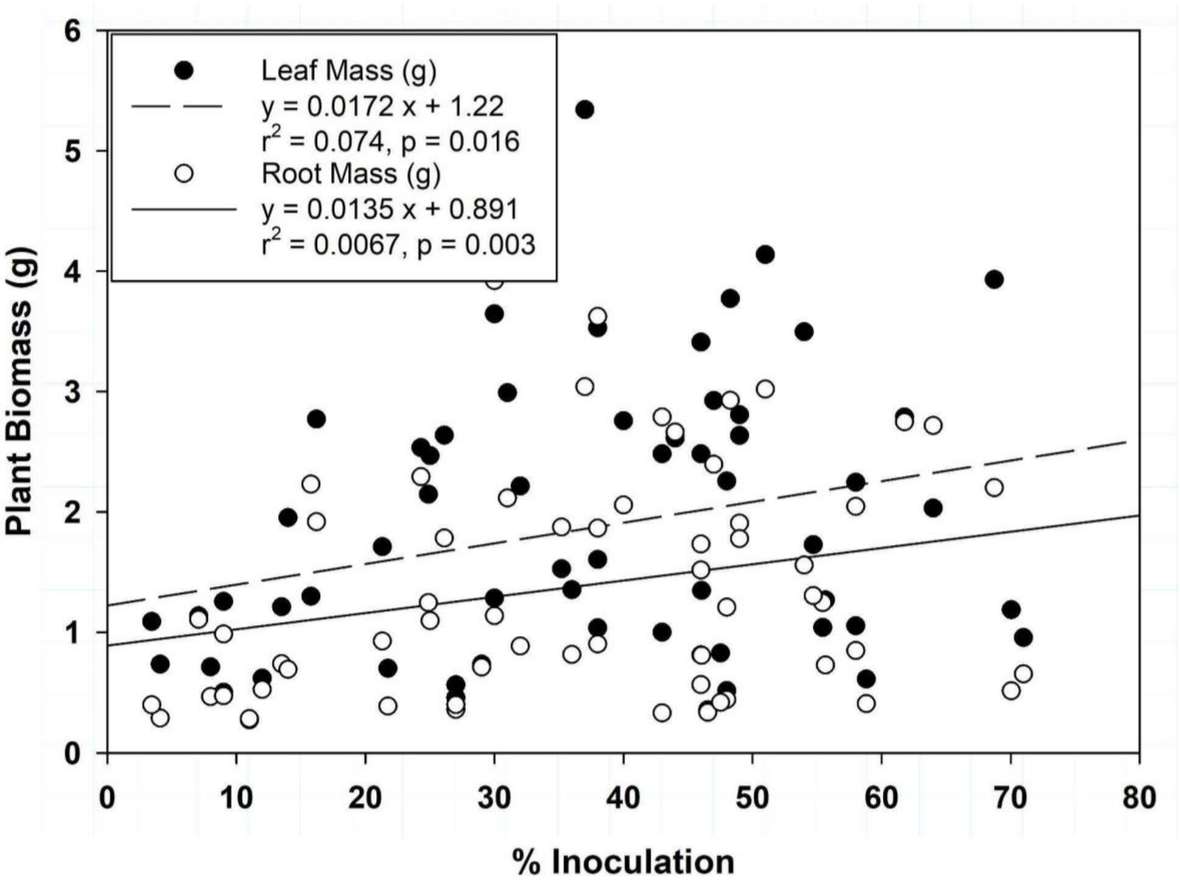

Figure 8. Leaf and root biomass as a function of root mycorrhizal colonization (% inoculation).

Discussion

Steady State Light Response Curves

Previous research has found higher A_{max} in American chestnuts relative to other Chinese and hybrid chestnut varieties (Knapp et al. 2014). This was likely the result of longer growth times (4 years from established seedling) compared to our 4 months from seed, leading to the lack of significant seed-type differences in our results. This is likely due to a comparatively low sample size, as this study measured n = 36 total seedlings with 3 replicates per seed x stand combination while Knapp et. al 2014 measured n = 40 in total seedlings with 8 replicates per seedling-type. It is also possible that variations in chestnut seedling age may have impacted the final growth dynamics in our stands, limiting our ability to accurately characterize trends within a limited growth period. Differences in photosynthetic parameters between stand-types were likely driven by different light environments, as shade intolerant species, like the Chinese chestnut, tend to have higher light-saturated photosynthetic rates when compared to more shade tolerant species like the American chestnut (Boardman 1977). However, other studies have shown American chestnuts to be extremely plastic under variable light conditions, showing high photosynthetic activity species under high light conditions as well as shade-tolerance in low light conditions (Knapp et al. 2014), implying the exact cause is likely multi-faceted. While this study found no significant seed-type trends within the high-light American stand, A_{max} was significantly higher across all seed-types planted at the American stand, showing a likelihood that light availability was a significant factor in variations in A_{max} across stands.

Growth Parameters

Light availability likely influenced the higher performance of seedlings in the American stand across a majority of the growth parameters. However, the maple stand also showed significantly higher means for a majority of the significant stand-type effects with a significantly lower light availability. This may be a result of insufficient light quantification as there was a technical malfunction with the quantum sensor used at the maple stand, leading only n = 10 observations to be averaged for analysis while the other stands received n = 20. This would not be surprising as the control ‘stand’ consisting of an individual tree might lend itself to higher light availability than the denser stands used for analysis, possibly showing some confounding factors in discussing the influence of light availability at the control stand. Additionally, this created a non-uniform canopy, allowing the time-of-day light measurements were taken to skew our light estimates, something that was not accounted for in the mid-day measurements used in this study.

Chinese seedlings had a higher biomass across most growth parameters despite a delayed germination requiring re-planting almost one month after the other seeds were planted. This increased growth might have resulted from a larger seed size in the Chinese plants compared to the other chestnuts, providing developing seedlings with additional support from environmental stressors and positively influencing seedling growth (Tumpa et al. 2021). Similarly, the high light affinity of Chinese chestnuts and American chestnut seedlings exposed to high light environments likely influenced the shoot growth of the seedlings in the high-light American stand. Previous research has found a 5.7% increase in stem height across American, Chinese, and B_3F_3 hybrid chestnut species that was significantly pronounced in Chinese trees (Boyd and Craddock 2012). This would explain the significant increase in stem length within Chinese seedlings and across all seedlings at the American stand, but it does not account for the significantly lower length of American seedlings. It is possible the variation in seed size may provide Chinese seeds with alternative establishment strategy, as American chestnuts, like other shade-tolerant species, dedicate a majority of their early growth to their root systems. Chinese seeds may allocate more of their initial energy to above-ground growth, more common among shade-intolerant species, explaining why stem length may have differed during the short establishment period we measured. However, the significantly higher stem length within the low-light maple stand was likely another effect of light timing within our quantification. In future analysis it would be better to assess total daily photon flux rather than mid-day photon flux alone, providing a more accurate estimate of light availability for plant growth.

Increased competitive interactions between seedlings are likely to impact seed-type analysis both across stands generally and within individual stands. The close spacing used when planting seeds for this study (0.33 m) likely increased resource competition. In light of the low survival rates of chestnuts during their first 2 years of growth (Dalgleish et al. 2023), it is possible that this competition offset any notable patterns in establishment relation to biomass analysis. As a result of delayed Chinese seed growth and this short establishment period, this study was a small preliminary harvest, allowing the seedlings to grow for another year so that a secondary harvest could be made in summer 2026 with a higher sample size. Hopefully, the increased sample size and reduced confounding effects from variable growth times and light quantification in our subsequent harvest will elucidate some of these unexpected trends.

ECM Colonization

Despite root and leaf mass showing a significant increase with ECM inoculation, the low explanatory power (r^2) of these models indicates there are likely other factors influencing growth. The American and Chinese stands had a significantly higher inoculation than the F_1 hybrid and maple stands, however there was no significant interaction between seed and stand-type and no significant difference in seed-type alone for inoculation. A previous greenhouse study analyzing the growth of trees (including American, Chinese, and hybrid chestnut seedlings) after one year of growth in rocky, lower shelterwood soil vs. loamy, upper shelterwood soils found that soil type influenced ECM colonization more than seedling type. There was a significant difference in mean colonization between the rocky and loamy soil-types, but no significant seedling-type differences. Additionally, the authors found a lack of interaction in ECM colonization between seedling- and soil-type (D'Amico et al. 2014). This points to a larger trend in soil type influencing ECM colonization in chestnut seedlings more than seedling species alone, disproving our initial hypothesis that conspecific seedlings would benefit from connecting into the ECM communities of monotypic chestnut stands in the first year of growth. However, these CMN's may become more important over time. Soil density, pH, composition, current plant cover, and chemical legacies from past vegetation can lead to an increased presence of allelopathic chemicals that limit both plant and ECM diversity (D'Amico et al. 2014; Sáenz-Hidalgo et al. 2023). Many of our stands had a very tough clay soil that made planting notably difficult. It's likely the tough soil structure might have led to limitations in ECM composition as hyphae struggled to navigate through the compacted terrain. Additionally, the intensive management (tilling, herbicide use, frequent mowing) by TACF across the stands used in this study might have negatively affected the ECM communities present. A complementary study is investigating specific ECM communities on the seedling observed in these stands.

Conclusion

This study examined the growth of American, Chinese, and F_1 hybrid chestnut seeds growing in corresponding chestnut stands in order to further understand the role of ECM communities in facilitating seed establishment. Our goal was to establish the influence of distinct ECM communities present in mature monotypic chestnut stands by determining if there was any beneficial effect of conspecific growth. We found that environmental factors largely influenced the photosynthetic capacity of our seedlings, and the large seed size of Chinese chestnuts might positively affect their establishment in high competition environments. Finally, soil composition paired with influences from light conditions and seed size across the stands underscored many stand-type effects vital to seed establishment, but outside the scope of conspecific seed-type influences. Within a complementary study, we will continue to analyze the specific ECM communities present in our stands and further elucidate the role of specific ECM fungi in perpetuating seed-type trends beyond those observed in this study. Characterization of specific ECM species has found significant differences in colonization between American, Chinese, and hybrid chestnuts with no significant difference in colonization between the soil-types in which the trees were grown (D'Amico et al. 2014). This shows a large potential for our continuing analysis to elucidate additional seed-type effects, expanding our understanding of possible influences of conspecific chestnut growth.

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