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

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Introduction

The American chestnut (*Castanea dentata* (Marshall) Borkh.) was a foundation species in the eastern USA, driving ecosystem dynamics, nutrient cycling, and benefiting wildlife populations[1]. The introduction of chestnut blight (*Cryphonectria parasitica* (Murrill) Barr) made this species functionally extinct, shifting forest systems of North America[2]. The American Chestnut Foundation (TACF) has led the restoration effort by establishing a backcross breeding program in order to repopulate the native range of the American chestnut with blight resistant trees hybridized with blight-tolerant Chinese (*Castanea mollissima* Blume) chestnuts[3]. Little is known about natural regeneration dynamics from seed, as much of the previous research on hybrid chestnuts has been on out-planted nursery seedlings[4]. Common mycorrhizal networks (CMNs) formed with mature trees are a vital component of seed establishment. Little is known about the role of CMNs on seedling growth in stands with remnant chestnut populations, outside of the role of ectomycorrhizal communities (ECMs) in mixed forest stands[5].

The goal of this study was to assess patterns between growth and ECM colonization among monotypic chestnut stands during seed establishment. We hypothesized that if the different chestnut types harbor different mycorrhizal communities, then seedlings would perform better when planted in stands of conspecific trees because of chestnut-specific ECM and CMNs.

Methods

This study was conducted at TACFs Meadowview Research Farm (MRF) in Meadowview, VA using their existing American, Chinese, and their F_1 hybrid stands. In the absence of a sugar maple stand, a 4.5x9 m area directly under an isolated mature tree was used. Seedlings were randomly planted in a 10X20 grid with 0.33m spacing with different flag colors representing different chestnut types. A reciprocal seedling transplant was used to assess the ECM community composition when American, Chinese, and F_1 hybrid chestnuts seeds were planted in corresponding pure stands of each chestnut type alongside a non-ectomycorrhizal sugar maple control stand. 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.

The light environment was quantified and steady state light response curves (Figure 1) were constructed for three randomly selected seedlings of each seedling-site combination to estimate the photosynthetic parameters: maximum photosynthetic rate (A_{max}), dark respiration rate (R_d), quantum yield (QY), and light compensation point (LCP). Five seedlings from each seedling-site combination were then harvested and excised into leaves, stems, and roots for growth characterization.

Individual one-way **analysis of variance (ANOVA) tests were used to compare light environment among sites and photosynthetic parameters among site and seedling type.** Means were compared using Tukey's post-hoc tests. **A two-way ANOVA was used to test the effects of site, seedling-type, and their interaction on growth parameters** (leaf mass, leaf number, total leaf area, and specific leaf mass, shoot basal diameter, total stem length, stem mass, and root mass). Additional one-way ANOVAs tested **the individual effect of site and seedling type** for growth parameters with no significant interaction. Only **seedling differences by site** were tested under one-way ANOVA for growth parameters with significant interactions to better understand the reason for varying development.

All statistical analyses were conducted using SAS v 9.4.

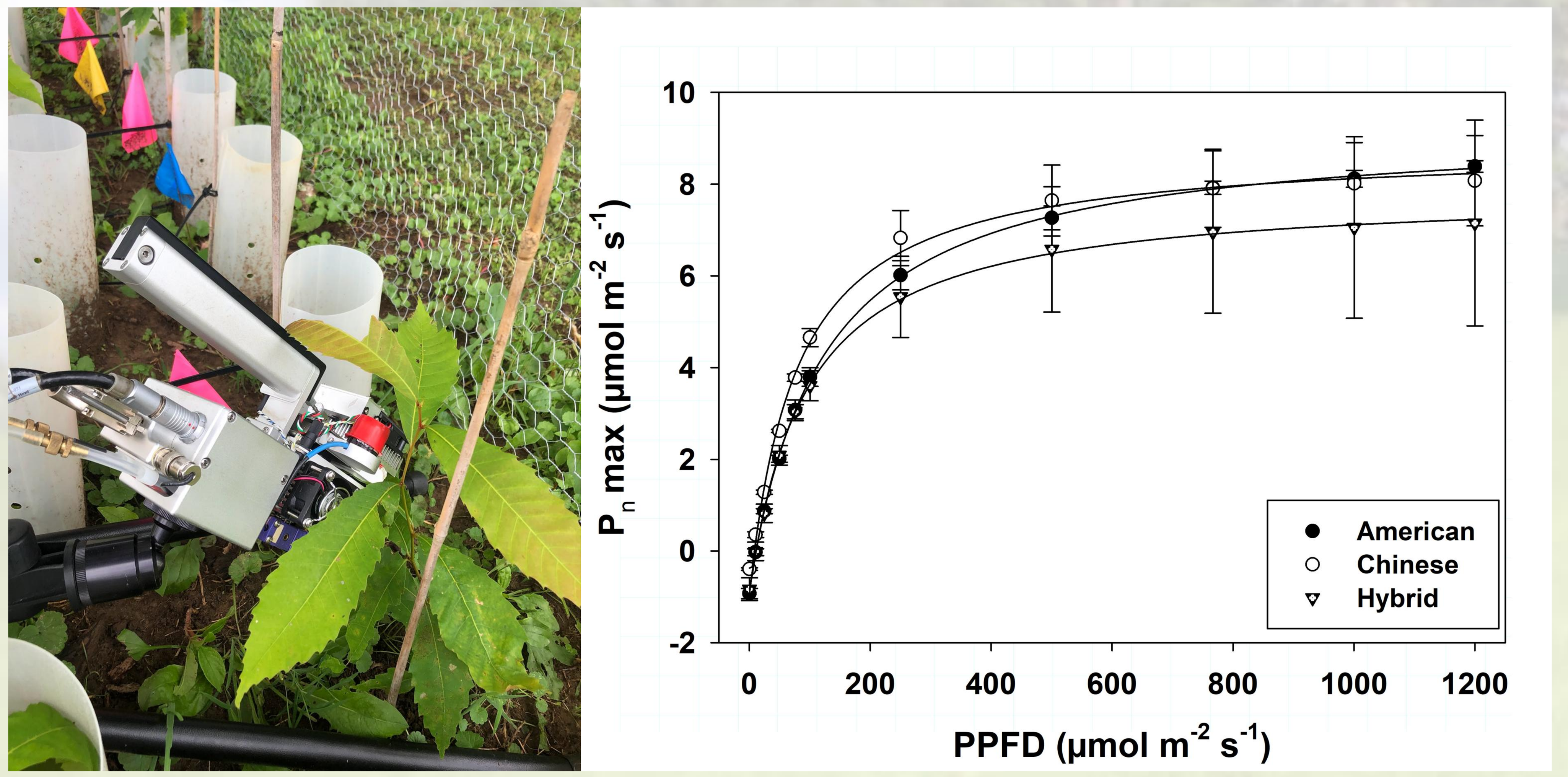

Figure 1. Portable photosynthesis system (Li-Cor 6400, LiCor Bio Sciences) attached to chestnut seedling (left). Net photosynthesis was measured at descending light levels (1200, 1000, 750, 500, 250, 100, 75, 50, 25, 10, 0 µmol m-2 s-1) to create light response curves for American site representing F1 hybrid, American, and Chinese seedlings. Points represent mean (± 1 SE) of 3 trees. American and Chinese (right).

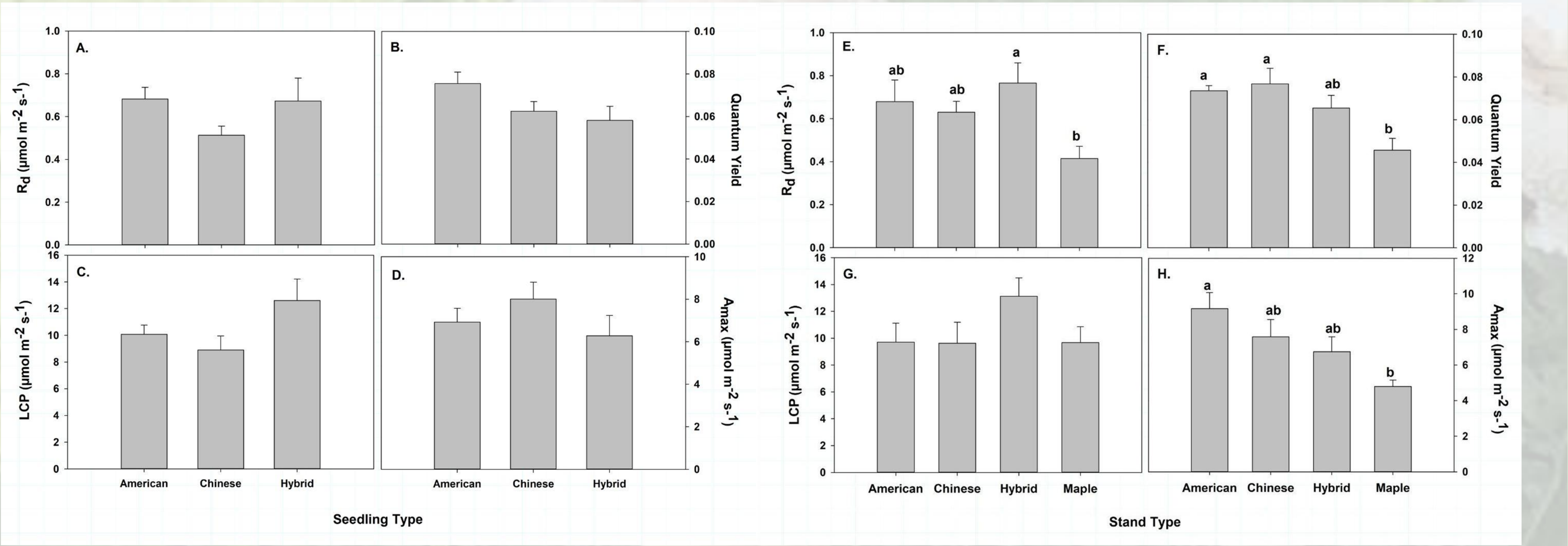

Figure 2. Mean (± 1 SE) dark respiration (R_d), quantum yield (QY), light compensation point (LCP), and maximum photosynthesis (A_{max}). Bars with different letters represent significant differences in seedling (A-D) and stand (E-H) type at $p < 0.05$.

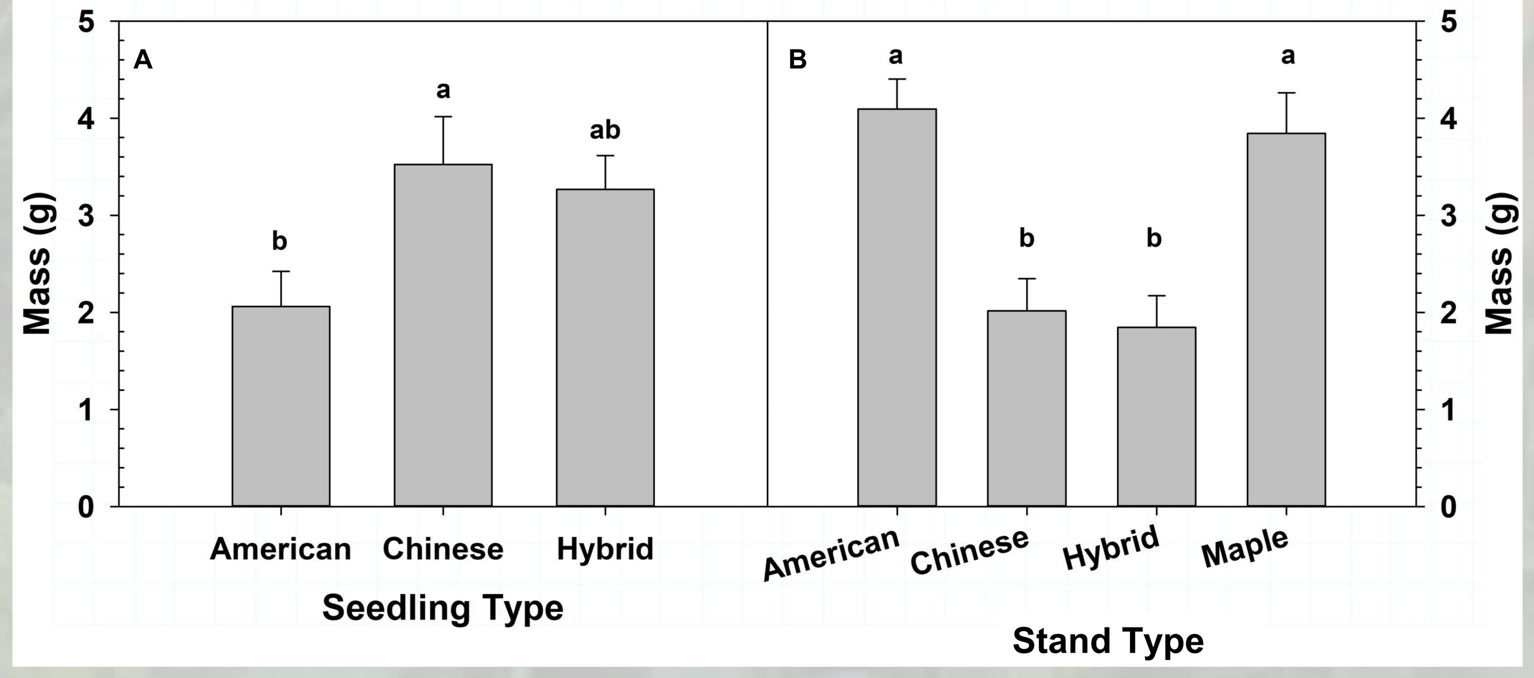

Figure 3. Mean (± 1 SE) Shoot mass (g) for seedling (A) and stand (B) type. Bars with different letters represent significant differences among seedling (A) and stand (B) types at $p < 0.05$.

Results

__Steady-State Light Response Curves:__

Photosynthetic parameters **differed by site but not seed type** (Fig. 2 A-D)

- **R_d was significantly higher in the F_1 hybrid site**, intermediate in the American and Chinese sites and lowest in the Maple site (p=0.0235; Fig. 2E)
- **QY was significantly higher in the Chinese and American sites**, intermediate in the F_1 hybrid site, and lowest in the Maple site (p=0.0019; Fig. 2F)
- **LCP had no significant variation based on site alone** (Fig. 2G)
- **Amax was significantly higher in the American site**, intermediate in the Chinese and F_1 hybrid sites, and lowest in the Maple site (p=0.0055; Fig. 2H)
- **Ambient light was highest in the American stand** and did not differ in remaining stands (p<0.001; not shown)

__Growth Parameters:__

American and Maple sites had significantly higher valus than the Chinese and Hybrid sites for most growth parameters (as represented by shoot mass Fig. 3 A).

Chinese seedlings had consistently higher values for most growth parameters than other seedling types (as represented by shoot mass Fig. 3 B).

Few significant interactions (Leaf area and SLM) with an overall lack of consistent seedling by site interactions.

Discussion

We found that there were differences in photosynthetic parameters between sites (Figure 2) but these were not consistent likely as a result of low sample size (n=3) .

- **The American site had the lowest canopy density** leading to higher light availability and increased seedling growth
- while **Chinese seedlings had higher growth for most parameters despite delayed germination** and replanting in June 2025
- low sample size and high variation likely cause for inconsistent seed by site interactions

ECM colonization data collection is ongoing, with growth parameters waiting to be related to related to those findings.

Previous research has found first year seedling growth to be negatively impacted by conspecifics competition, especially in high density (our seedlings were planted at 0.3m spacing), with this effect diminishing in subsequent years. Because of this and delayed germination in Chinese seeds, we left many seedlings to be harvested in the second year of growth (2026).

Literature Cited

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