Effects of light acclimation on the photosynthesis, growth, and biomass allocation in American chestnut (*Castanea dentata*) seedlings

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Abstract

American chestnut (*Castanea dentata*) is currently regarded as functionally extinct because of chestnut blight. To reintroduce blight-resistant American chestnut back to its historic range, it is imperative to understand the silvics and silviculture of the species. In an outdoor rainout shelter, we grew American chestnut seedlings at four levels of irradiance (4, 12, 32 and 100% of full sunlight) to examine how light intensity affects photosynthesis, growth, and biomass allocation. Net photosynthetic rate increased linearly with increasing irradiance while instantaneous water use efficiency peaked at 32% full sunlight, when seedlings were measured at their acclimated irradiance level. Height and diameter increased with increasing irradiance. However, seedlings only grew laterally under 4% full sunlight. Total biomass increased linearly with increasing irradiance and root to shoot ratio was lowest under 4 and 12% full sunlight. Regardless of irradiance level, >70% of total biomass was allocated to shoot growth. With increasing shade, specific leaf area significantly increased. These observed physiological and morphological light acclimation characteristics indicate that American chestnut is shade tolerant, which partially explains why the species has persisted in the understory for almost a century. The shade-tolerance and fast growing characteristics suggest that an underplanting-and-release or gap-phase regeneration approach would be a suitable silvicultural alternative to a clearcut-and-planting approach for the reintroduction of blight-resistant American chestnut.
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Keywords: Shade tolerance; Biomass allocation; Photosynthesis

1. Introduction

Before the introduction of chestnut blight (caused by the fungus *Cryphonectria parasitica*), American chestnut (*Castanea dentata*) was one of the most widely distributed tree species in eastern North America (approximately from 30°40′ to 44°18′N and 69°46′ to 90°02′W). It extended from central Alabama, north into New Hampshire, Vermont and Maine, west through Tennessee, Kentucky, Indiana and Ohio, and north into southern Ontario (Russell, 1987). Within its native range, American chestnut was often the dominant tree species, comprising an estimated 25% of native eastern hardwood forests (Burnham, 1988). In the southern Appalachian Mountain range, American chestnut comprised 40–45% of the canopy (Reed, 1905; Keever, 1953) and 50% of timber by volume on non-calcareous, well-drained slopes (Zon, 1904;

Buttrick and Holmes, 1913; Brooks, 1937). American chestnut has been found on a wide variety of soil and topographic types, but was most commonly associated with well-drained soils developed on non-calcareous substrates (Frothingham and Earl, 1912; Hawes and Hawley, 1918; Braun, 1950; Russell, 1987; Paillet, 2002; Tindall et al., 2004). It reached 2000 m (6562 ft.) in the southern Appalachians, but only 130 m (427 ft.) in New Hampshire (Russell, 1987). Historically, American chestnut was highly prized for its high quality wood, dependable nut production, and high tannin content (Youngs, 2000; Smith, 2000; Hepting, 1974). Because American chestnut is no longer a major component of the eastern deciduous forest (Paillet, 1988), its silvics (i.e., autecological characteristics) and silviculture have rarely been studied.

Cryphonectria parasitica, an introduced phloem pathogen responsible for predisposing American chestnut to an aggressive canker disease (i.e., chestnut blight), was first detected in the Bronx Zoo of New York City in 1904. By the 1950s, the disease almost completely eradicated American chestnut from the forest canopy within the entire inhabited

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range. Currently, American chestnut is regarded as functionally extinct because it can only persist as understory sprouts in its original distribution range (Paillet, 2002). In fact, American chestnut is listed as an endangered species in Canada (Tindall et al., 2004). To circumvent the canker disease, a backcrossing breeding program has been established by the American Chestnut Foundation (Bennington, VT). Hybrids that exhibit the blight-resistant traits of Chinese chestnut (*C. mollissima*) are scheduled to be available for planting within 3–4 years (Paul Sisco, American Chestnut Foundation, pers. commun.). In an attempt to retain desirable traits of the American chestnut, these hybrids will only possess approximately 6% genetic inheritance from Chinese chestnut. Given the economic and ecological importance of the species prior to its decline (e.g., Russell, 1987; Smith, 2000; Paillet, 2002), blight-resistant American chestnut is expected to be reintroduced back to their native range. Currently, however, we know little about the silvics and silviculture of the species, which could play a fundamental role in the success of the future reintroduction effort.

Light is a critical factor affecting the early survival and growth of tree seedlings under a forest canopy, but our knowledge of how American chestnut responds to light is lacking. A review of the literature reveals a paucity of data regarding the growth and photosynthetic performance of American chestnut under light limitation. Several early observations disagreed on the shade-tolerance of American chestnut. For example, Hawley and Hawes regarded American chestnut as a relatively shade-intolerant species that could be excluded by competition with more shade-tolerant species in old growth woodlands. Baker (1950) ranked American chestnut a 3 on a 1 (very intolerant) to 4 (very tolerant) scale, similar in rank to oaks. Paillet (1988) found that chestnut sprouts were present under dense eastern hemlock (*Tsuga canadensis*) canopy, demonstrating a surprising degree of shade-tolerance. When released from the canopy, American chestnut sprouts grow very rapidly, with a height growth rate comparable to black locust (*Robinia pseudoacacia*) (personal observation in the southern Appalachians). In addition, these released sprouts or saplings quickly regained good stem form (Paillet, 2002). The strong ability to persist under high shade and a fast response to release suggest that American chestnut behaves much like a shade-tolerant species. Given the expected reintroduction in the not too distant future, it is imperative to develop an appropriate silvicultural system.

The objective of this study was to investigate light acclimation in American chestnut seedlings growing under a wide range of irradiance, from high shade to full sunlight, in a common garden environment. Specifically, we measured gas exchange and examined changes in photosynthesis, growth, and biomass allocation at four levels of irradiance.

2. Materials and methods

2.1. Plant material

American chestnut seeds were collected near Reedsburg, Wisconsin (latitude 43°32′; longitude 90°1′). In May 2004,

seeds were sown by direct seeding into 3.7 L plastic pots containing standard glasshouse potting substrate (consisting of 45% peat moss, 15% perlite, 15% vermiculite, and 25% bark). Among the 200 seeds sown, 186 were successfully germinated within 10 days. These germinants were allowed to grow under well-watered conditions in a rainout shelter at the Clemson Biosystems Research Complex (Clemson, SC, USA; latitude 34°40′8″; longitude 82°50′40″) for one week before subjecting them to different irradiance treatments. Our rainout shelter was essentially an outdoor environment with a glass roof to exclude precipitation.

2.2. Study design

We grew American chestnut in the rainout shelter under four levels of irradiance: full sunlight (FL), light shade (LS), medium shade (MS), and high shade (HS). The target light intensities for LS, MS, and HS were 35, 15, and 5% of full sunlight. To achieve these light intensities, solar reflecting shade cloth (model XLS Revolux, AB Ludvig Svensson Inc., Kinna, Sweden) was applied onto a 1.5 m × 1.25 m rectangular frame constructed with polyvinyl chloride (PVC) tubes. Forty seedlings were randomly selected from the 186 established seedlings, among which 10 seedlings were randomly assigned to each irradiance treatment. All seedlings were watered on an as need basis to maintain their water supply at or close to field capacity.

Although we used only one shade structure for each light level, the 10 seedlings under each shade structure (light level) were grown individually in pots. More importantly, these seedlings were randomly assigned to each shade structure (light level). Therefore, the 10 seedlings were treated as replicates in our study. However, we acknowledge that the 10 seedlings, grown under the same shade structure, were not completely independent.

2.3. Light measurement

Under each light regime, photosynthetic photon flux density (PPFD) was measured every minute using a line quantum sensor and the average logged every 15 min (LiCor Inc., Lincoln, NE). The line sensor was suspended above the canopy via a fixed PVC support rack that did not exceed the width of the sensor. These measurements were used to verify the shading screen light level.

2.4. Growth measurement

The 40 seedlings (10 per treatment) were measured every 15 days starting 7 June 2004 when the seedlings were placed under different light treatments. Seedling mortality was observed and recorded weekly. Only two seedlings died in the middle of the experiment, one under FL and another under HS. These two seedlings were thus excluded from the study. Height (HT), root collar diameter (RCD), and number of fully expanded leaves (NLVS) were measured five times during the experimental period. Height was measured to the nearest 0.1 cm using a

measuring tape. RCD was measured to the nearest 0.01 mm using a digital caliper. The position and direction of RCD measurements were marked on the stem using a permanent marker in order to maintain consistency. At the end of the experimental period (2 August 2004), all seedlings were destructively sampled. Each seedling was carefully excavated and its roots were carefully washed. Root (RW), stem (SW) and leaf (LW) biomass were determined by drying to a constant mass at 80 °C. Small circular leaf punches with known areas were cut from each leaf to determine specific leaf area (SLA) on a seedling basis.

2.5. Gas exchange measurement

After 2, 4 and 6 weeks of acclimation under each light treatment, net photosynthesis (A_{net}), leaf stomatal conductance (g_s), and transpiration rate were measured using a portable steady state gas-exchange system (CIRAS-I, PP Systems, Amesbury, MA) equipped with a light and temperature controlled cuvette (model PLC5 (B); PP Systems). From the terminal tip, measurements were taken on the youngest fully expanded non-damaged leaf from 0900 to 1230 h. These leaves were tagged and on any given day, measurements were taken in random order to compensate for any effects caused by time of sampling. Measurements were recorded for each seedling after reaching steady state, and PPFD during the measurements was maintained at a level close to the light treatment of each seedling: FL = 1200 μmol m^{-2} s^{-1}, LS = 360 μmol m^{-2} s^{-1}, MS = 180 μmol m^{-2} s^{-1}, and HS = 60 μmol m^{-2} s^{-1}.

After 2 weeks from experiment initiation, seedlings growing under FL were also used to construct light and CO_2 response curves. Our purpose is to document some basic physiological parameters for seedlings growing under full sunlight. Prior to light or CO_2 response measurement, plant leaves were illuminated at approximately 750–900 μmol m^{-2} s^{-1} for 20 min in a growth chamber and then measured in random order. Photosynthetic photon flux density was monitored with a quantum sensor (LiCor-189, LiCor, Inc., Lincoln, NE). The PPFD sequence was implemented in the following order: 1200, 900, 600, 425, 300, 200, 100, 50, and 0 μmol m^{-2} s^{-1}. We use the sequence of high to low light level to avoid stomatal conductance oscillation. In a preliminary experiment, we found that the high light level of 1200 μmol m^{-2} s^{-1} did not cause photoinhibition. Carbon uptake was measured and data recorded after exchange rates stabilized. An atmospheric CO_2 concentration of 370 ppm, leaf temperature of 25 °C, and VPD of 1.2 ± 0.2 kPa were maintained within the cuvette during light response gas exchange measurements.

The relationship between photosynthesis and internal CO_2 concentration (A–Q curves, where A is net photosynthetic rate in μmol m^{-2} s^{-1} and C_i is internal CO_2 concentration expressed as the mol fraction of CO_2) was determined on replicates of well-watered seedlings to reduce potential artifacts of stomatal patchiness. Other than CO_2 manipulation, the cuvette conditions for A–d curves were the same as above. Measurements began at a cuvette CO_2 concentration of 370 μmol mol^{-1} and were completed in the following

sequence: 370, 1200, 1000, 800, 600, 370, 175, 150, 100, and 50 μmol mol^{-1}. Non-linear regression techniques for estimating the maximum rate of carboxylation ($V_{c\ max}$), the maximum rate of ribulose 1,5-bisphosphate regeneration (assumed to equal the maximum rate of coupled photosynthetic electron transport) (J_{max}), and triose phosphate utilization (TPU) followed Wullschleger (1993). For each plant, the non-linear regression curve explained > 92% of the variation in A–C_i data.

2.6. Leaf optical property measurement

Leaf absorption, reflectance, and transmittance were estimated with a Minolta SPAD 502 chlorophyll meter (Minolta Camera Co., Ramsey, NJ). The SPAD reading, which is non-linearly correlated with leaf absorption, reflectance, and transmittance (Bauerle et al., 2004), uses a silicon photodiode to derive the ratio of transmittance through the leaf tissue for spectral bands at 650 and 940 nm wavelengths. Five SPAD readings were measured and averaged for each of 10 replicate leaves per treatment on 7 and 21 June and 5 and 19 July 2004.

2.7. Data analysis

We followed methodology described in Parsons et al. (1997), where apparent dark respiration (R_a), quantum efficiency (α), corrected for light absorption following Bauerle et al. (2004), and light compensation point (I_c) were calculated from the linear portion of the initial part of the light response curve and axis intercepts. Model parameters of convexity (ϕ) and light saturation (I_{sat}) were obtained from least squares curve fitting. The non-linear regression coefficients of determination for each curve explained >95% of the variation in the A versus PPFD. Percent leaf light absorptance was calculated on an individual leaf basis by inserting SPAD measurements into the exponential equation (absorptance = $89.2 - 56.8e^{-0.0723(SPAD)}$) developed by Bauerle et al. (2004), which was used to correct for net radiation absorbed when calculating quantum yield. The water use efficiency (WUE) of each seedling was calculated based on net photosynthesis and transpiration rates. Specifically, WUE was calculated on a molar mass basis by dividing the molar masses of CO_2 by that of H_2O. Net photosynthesis, transpiration, g_s, WUE and percent leaf light absorption were averaged over the three measurement times.

Based on biomass measurements, total biomass (TB), aboveground biomass (AB), root to shoot ratio (RSR), leaf weight to total biomass ratio (LWR) and leaf weight to root weight ratio (LWRr) were calculated for each seedling. Based on specific leaf area (SLA, cm^2 g^{-1}) measured from leaf samples and leaf biomass, total leaf area (LA, cm^2) was calculated for each seedling. Leaf area to total biomass ratio (LAR, cm^2 g^{-1}) and leaf area to root weight ratio (LARr, cm^2 g^{-1}) were also calculated.

Repeated measures analysis of variance was used to quantify the effect of irradiance level on RCD and HT and changes in RCD and HT over the experimental period. Due to significant interactions between irradiance treatments and measuring

dates, the changes in RCD and HT over the experimental period were analyzed separately for each irradiance level using one-way analysis of variance followed by Bonferroni's multiple comparison. Similarly, differences in RCD and HT among light levels were analyzed separately for each measuring date using one-way analysis of variance followed by Bonferroni's multiple comparison. One-way analysis of variance followed by Bonferroni's multiple comparison was also used to test the difference in physiological variables (A_{net}, transpiration, g_s, WUE, and percent leaf light absorption) and biomass measurements (TB, RW, LW, SW, SLA, RSR, LAR, LARr, LWR and LWRr). Previous studies have raised caution about comparing allometric relationships of plants of different size (e.g., Hunt and Lloyd, 1987; Rice and Bazzaz, 1989), but introducing seedling size (biomass) as a covariate in comparing RSR, LAR, LARr, LWR, and LWRr did not alter our results. All statistical analyses and graphics were conducted using SYSTAT (SYSTAT Software Inc., Richmond, CA). SYSTAT has two build-in tests for compound symmetry or sphericity: the Greenhouse–Geisser statistics and Huynh–Feldt statistics. All repeated measures ANOVA performed in the study passed these tests.

3. Results

3.1. Irradiance treatments

On cloudless days, the maximum PPFDs at solar noon averaged 1750, 560, 209, and 72 μmol m^{-2} s^{-1} or 100, 32, 12, and 4% in the FL, LS, MS, and HS treatments, respectively. Under the same conditions, however, the average PPFD from sunrise to sunset was 827, 264, 99, and 34 μmol m^{-2} s^{-1} in the FL, LS, MS, and HS treatments, respectively. The LS, MS, and HS treatments resulted in the gradient of light environments that are characteristic of understory conditions in gap, moderate, and dense vegetation cover in mature eastern deciduous forests.

3.2. Leaf gas exchange and light absorption

Based on measurements taken from the 10 seedlings acclimated to full sunlight, means of light response parameters were derived (Table 1A). It should be noted that the SPAD measurements taken on all gas exchange leaves on 7 June (as a pre-treatment assessment for any potential differences in leaf optical properties or chlorophyll content) resulted in the following initial mean leaf absorptance values (%): FL = 77.32 ± 2.06, LS = 77.24 ± 1.21, MS = 77.51 ± 1.31, and HS = 77.85 ± 1.14. Pre-treatment SPAD values were not statistically different and they did not differ in post treatment observations. In addition, the use of SPAD values as a surrogate for leaf chlorophyll content did not result in significant variation of mean values among treatments, thus diminishing the possibility of pre-treatment variation in chlorophyll content. It was determined that the I_c and I_{sat} points were 29.48 and 203.50 μmol m^{-2} s^{-1}, respectively. The maximum photosynthetic rate at ambient CO_2 (370 μmol mol^{-1}) was

Table 1
(A) Means of photosynthetic light response curve parameters determined for 1-year-old American chestnut and (B) CO_2 response gas exchange parameters of well-watered American chestnut

Parameter	Mean ± S.E.
(A) Means of photosynthetic light response curve parameters[a]	
R_a (μmol m^{-2} s^{-1})	−1.97 ± 0.19
α (μmol CO_2 μmol^{-1} photon)	0.056 ± 0.01
I_c (μmol m^{-2} s^{-1})	29.48 ± 0.27
ϕ	0.67 ± 0.06
I_{sat} (μmol m^{-2} s^{-1})	203.50 ± 0.65
(B) CO_2 response gas exchange parameters[b]	
A_{max} (μmol m^{-2} s^{-1})	13.40 ± 0.17
$V_{c\,max}$ (μmol m^{-2} s^{-1})	41.51 ± 0.45
J_{max} (μmol m^{-2} s^{-1})	101.72 ± 0.62
Γ (μmol mol^{-1})	12.08 ± 0.17
TPU (μmol m^{-2} s^{-1})	6.36 ± 0.14
CE (initial slope of A_{net} vs. C_i)	0.96 ± 0.08

[a] R_a = apparent dark respiration, α = quantum efficiency, I_c = light compensation point, ϕ = convexity, and I_{sat} = light saturation.
[b] A_{max} = maximum net photosynthesis rate at maximum [CO_2] and saturating light, $V_{c\,max}$ = maximum carboxylation, J_{max} = estimate of the maximum rate of ribulose 1,5-bisphosphate regeneration, TPU = triose phosphate utilization, Γ = CO_2 compensation point, and CE = carboxylation efficiency.

9.08 μmol m^{-2} s^{-1}. The apparent dark respiration rate (R_a) was 1.97 μmol m^{-2} s^{-1}, the quantum yield (α) 0.056 μmol CO_2 μmol^{-1} photon, and the convexity of the light response curve (ϕ) 0.67. Table 1B reports the means of CO_2 response parameters. The A–Q analysis resulted in a maximum net photosynthesis rate at maximum [CO_2] and saturating light (A_{max}) of 13.4 μmol m^{-2} s^{-1}, maximum carboxylation ($V_{c\,max}$) of 41.51 μmol m^{-2} s^{-1}, estimates of the maximum rate of ribulose 1,5-bisphosphate regeneration (J_{max}) of 101.72 μmol m^{-2} s^{-1}, CO_2 compensation point (Γ) of 12.08 μmol mol^{-1}, triose phosphate utilization (TPU) of 6.36 μmol m^{-2} s^{-1}, and carboxylation efficiency (CE) of 0.96.

When measured under their respective irradiance treatments, A_{net} increased with increasing irradiance, with FL > LS > MS > HS (Table 2). Seedlings growing under LS had significantly higher g_s and transpiration rates than all other irradiance treatments. Transpiration rate and g_s under MS were also higher than those under FL. WUE increased with increasing irradiance, with FL > LS = MS > HS (Table 2). Percent leaf light absorption differed among the four irradiance treatments, with HS being higher than either LS or FL and MS and FL being higher than LS (Table 2).

3.3. Growth and biomass allocation

Irradiance treatments affected ($p < 0.001$) RCD, HT, and NLVS. As expected, RCD, HT, and NLVS increased ($p < 0.001$) over the experimental period but their increase depended on ($p < 0.001$) irradiance level (Figs. 1–3). Over the experimental period, height did not increase ($p = 0.203$) under HS, while RCD and NLVS increased ($p < 0.001$) under all irradiance treatments.

At the beginning of the experiment, there were no differences in RCD, HT, and NLVS ($p > 0.542$). Differences in RCD (Fig. 1), HT (Fig. 2), and NLVS (Fig. 3) were detected

Table 2
Physiological variables (means with S.D. in parentheses) of American chestnut seedlings growing under four levels of irradiance

Variables	Light level (%)			
	4	12	32	100
Net photosynthesis[1] (mol m^{-2} s^{-1})	1.82 d (0.32)	2.65 c (0.47)	4.21 b (0.70)	6.09 a (0.96)
Stomatal conductance[1] (mmol m^{-2} s^{-1})	61.17 b,c (16.20)	52.7 c (12.31)	112.10 a (31.29)	74.73 b (21.83)
Transpiration[1] (mol m^{-2} s^{-1})	0.65 b,c (0.14)	0.56 c (0.10)	1.08 a (0.25)	0.79 b (0.20)
Leaf light absorption[1] (%)	84.9 a (1.1)	82.6 a,b (3.0)	77.7 d (3.1)	81.4 b,c (2.9)
WUE[1] (g CO_2 kg^{-1} H_2O)	7.15 c (1.61)	11.78 b (2.30)	10.30 b (4.08)	19.31 a (3.04)

Values in the same row with different letters are significantly different ($p < 0.05$).

[1] Analysis of variance and multiple comparisons were based on log-transformed data. Transformation was made to overcome the problem of unequal variances among groups.

17 days into the experiment and persisted until the study ended. At 60 days (end of the experiment), HS had smaller RCD and lower NLVS than LS and FL; MS had smaller RCD and lower NLVS than FL; HS and MS were shorter than LS and FL (Table 3).
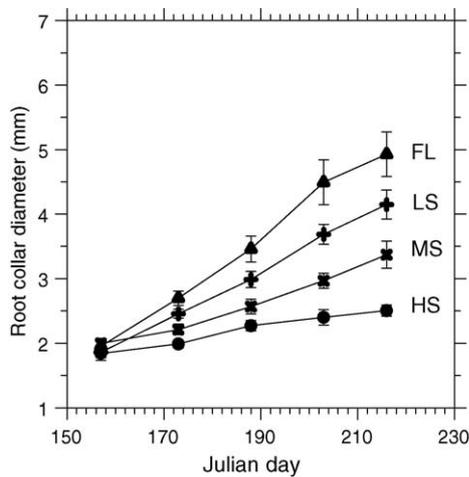

Fig. 1. Changes in root collar diameter (mm) over the experimental period (Julian day). The experiment started on 7 June 2004 and ended on 2 August 2004. The error bar is the standard error of the mean. FL = 100%, LS = 32%, MS = 12%, and HS = 4% full sunlight.

At the end of the experiment, TB and each biomass component differed ($p < 0.001$) among the four irradiance treatments. Total biomass and SW differed between any irradiance treatment, with FL > LS > MS > HS. FL and LS had higher RW and LW than either MS or HS; MS also had higher RW and LW than HS (Table 3). Root to shoot ratio was lower ($p < 0.001$) under HS and MS compared to LS and FL. Leaf weight to total biomass ratio was lower under FL compared to LS, MS and HS. Leaf weight to root weight ratio was lower under both FL and LS compared to MS and HS (Table 3).

Leaf area supported by each seedling decreased ($p < 0.001$) with decreasing irradiance. Significant differences in LA were detected between all irradiance treatments except between FL and LS (Table 3). Specific leaf area increased ($p < 0.001$) with decreasing irradiance. Significant differences in SLA were detected between any irradiance treatments except between MS and LS (Table 3). LAR differed ($p < 0.001$) among the four irradiance treatments, with HS having larger LAR than either LS or FL and MS having larger LAR than FL (Table 3). Leaf area to root weight ratio increased ($p < 0.001$) with decreasing irradiance, with HS and MS having higher LARr than either LS or FL and LS having higher LARr than FL ($p < 0.001$).
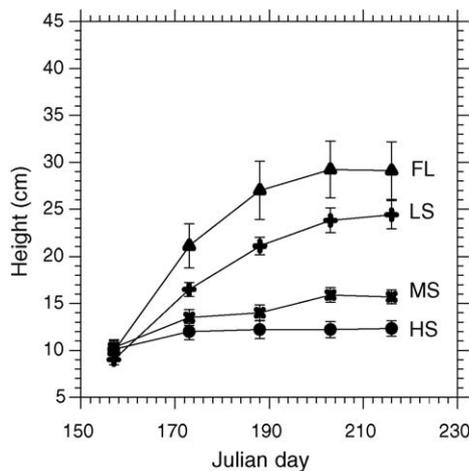

Fig. 2. Changes in height (cm) over the experimental period (Julian day). The experiment started on 7 June 2004 and ended on 2 August 2004. The error bar is S.E.M. FL = 100%, LS = 32%, MS = 12%, and HS = 4% full sunlight.
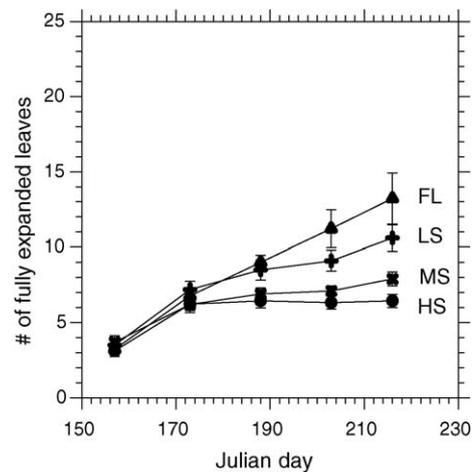

Fig. 3. Changes in the number of fully expanded leaves over the experimental period (Julian day). The experiment started on 7 June 2004 and ended on 2 August 2004. The error bar is the S.E.M. FL = 100%, LS = 32%, MS = 12%, and HS = 4% full sunlight.

Table 3
Growth and morphological variables (means with S.D. in parentheses) of American chestnut seedlings after 2 months under four levels of irradiance

Variables	Light level (%)			
	4	12	32	100
Root collar diameter[1] (mm)	2.51 c (0.25)	3.37 b (0.63)	4.15 a (0.67)	4.93 a (0.97)
Height[1] (cm)	12.3 c (2.3)	15.7 b (2.2)	24.4 a (4.5)	29.1 a (8.6)
Total biomass[1] (g)	1.14 d (0.22)	2.28 c (0.60)	5.08 b (1.56)	7.96 a (3.31)
Rootbiomass[1] (g)	0.26 c (0.08)	0.55 b (0.21)	1.56 a (0.42)	2.33 a (0.72)
Leaf biomass[1] (g)	0.51 c (0.10)	1.02 b (0.29)	2.11 a (0.76)	2.82 (1.07) a
Stem biomass[1] (g)	0.18 d (0.04)	0.35 c (0.09)	0.71 b (0.23)	1.40 a (0.80)
Root to shoot ratio	0.37 b (0.10)	0.40 b (0.13)	0.57 a (0.08)	0.59 a (0.12)
Leaf weight ratio[1]	0.45 a (0.07)	0.45 a (0.04)	0.41 a (0.03)	0.36 b (0.02)
Leaf weight root ratio[1]	2.27 a (0.94)	2.01 a (0.64)	1.34 b (0.22)	1.19 b (0.15)
Leaf area[1] (cm^2)	130.9 c (28.7)	245.5 b (97.8)	483.1 a (191.7)	490.1 a (185.4)
Specific leaf area[1] (cm^2 g^{-1})	256.0 a (19.0)	226.8 b (18.0)	226.4 b (20.1)	175.1 c (12.6)
Leaf area ratio[1] (cm^2 g^{-1})	115.8 a (21.4)	101.8 a,b (12.4)	93.4 b (12.1)	62.8 c (5.3)
Leaf area root ratio[1] (cm^2 g^{-1})	582.9 a (268.0)	455.9 a (144.0)	304.9 b (62.4)	208.0 c (28.3)

Values in the same row with different letters are significantly different ($p < 0.05$).

[1] Analysis of variance and multiple comparisons were based on log-transformed data. Transformation was made to overcome the problem of unequal variances among groups.

4. Discussion

The light response of American chestnut is comparable to eastern deciduous trees (e.g., Kubiske and Pregitzer, 1996; Groninger et al., 1996). For example, the light saturation point of American chestnut (203 μmol m^{-2} s^{-1}) is slightly higher than red maple (*Acer rubrum*) (146 μmol m^{-2} s^{-1}; Kubiske and Pregitzer, 1996), but lower than northern red oak (*Quercus rubra*) (252 μmol m^{-2} s^{-1}; Kubiske and Pregitzer, 1996) and white oak (*Q. alba*) (650 μmol m^{-2} s^{-1}; Teskey and Shrestha, 1985). The light compensation point of American chestnut (29.5 μmol m^{-2} s^{-1}) is slightly lower than red maple (35.8 and 32.8 μmol m^{-2} s^{-1}, respectively, Kubiske and Pregitzer, 1996; Groninger et al., 1996), northern red oak (48.9 μmol m^{-2} s^{-1}; Kubiske and Pregitzer, 1996) and tulip poplar (*Liriodendron tulipifera*) (31.2 μmol m^{-2} s^{-1}; Groninger et al., 1996). Because shade-tolerant species generally have lower light compensation and saturation points (Kozlowski et al., 1991), American chestnut is likely more shade tolerant than oaks but similar to red maple.

Since American chestnut leaf biochemical characteristics were not previously reported, we compared our findings to those reported for other tree species. When considering $V_{c\,max}$, J_{max}, and TPU together, American chestnut values are most similar to values reported by Harley et al. (1986) for strawberry tree (*Arbutus unedo*). However, Harley et al. (1986) exposed leaves to ~300 μmol m^{-2} s^{-1} higher PPFD and 2 °C higher temperature. When only considering $V_{c\,max}$ and J_{max}, values reported for bigpod ceanothus (*Ceanothus megacarpus*) (Mahall and Schlesinger, 1982) and poplar (*Populus euramericana*) (Gaudillere and Mousseau, 1989) were similar to our observed American chestnut values. Given the lack of ecophyiological information for American chestnut, the CO_2 response parameters and light response parameters reported in our study should provide basic information necessary for developing process-oriented simulation models.

Both survival and growth are important indicators of shade tolerance (Daniels et al., 1979; Lorimer, 1983). In addition to the full sunlight treatment, we selected the three shade treatments to emulate dense understory (4%), moderate understory (12%), and gap conditions (32%) in eastern deciduous forests. Mortality due to light limitation was not found in our study. Our lowest light treatment (HS) had 4% of full sunlight or averaged approximately 34 μmol m^{-2} s^{-1} PPFD from sunrise to sunset, which is above the I_c of 29.5 μmol m^{-2} s^{-1} determined in our study. This result suggests that American chestnut would survive, at least for the first year, if planted in the understory of a closed canopy forest. The wide spread occurrence of American chestnut sprouts under closed canopies with dense understories is a testament to our finding and the capacity of American chestnut to survive in high shade conditions. However, our study was conducted in a rainout shelter over a relatively short time (one growing season). Caution is advised when applying our results to natural conditions.

Our study indicated that seedling height and diameter growth decreased with decreasing irradiance, with the best growth performance observed under full sunlight. This result is consistent with studies of other eastern deciduous trees (e.g., Groninger et al., 1996). Under HS, seedlings did not significantly grow in height despite significant increases in RCD and NLVS during the experimental period. The result suggests that growing tall is not the best survival strategy for American chestnut under severe light limitation, further supporting the hypothesis that a species allocating more biomass to lateral growth has a greater capacity to capture light in light-limiting environments (Oliver and Larson, 1996). By not growing tall under HS, American chestnut seedlings decreased their height to diameter ratio, thus increased light use efficiency.

American chestnut has demonstrated a clear light acclimation at both the leaf and plant levels. Similar to previous studies on other eastern deciduous species (e.g., Gottschalk, 1994), our

study found a significant increase in SLA with increasing light limitation. Specific leaf area is an important index of leaf structure that is highly correlated with light environment (Tucker and Emmingham, 1977; Tucker et al., 1987). The significant differences in LAR found between irradiance levels indicated that the same amount of total biomass supported more leaf area with decreasing irradiance, which is consistent with other studies (e.g., Callaway, 1992; Gottschalk, 1994; Valladares et al., 2002). With increasing irradiance, A_{net} increased from HS to MS to LS to FL and thus more biomass was produced by the same amount of leaf area. In addition, greater SLA and LAR indicate a higher efficiency in capturing light resources under shade (Givnish, 1988; Wilson, 1988; Oliver and Larson, 1996).

Compared to the FL and LS, American chestnut seedlings growing under HS and MS allocate less to roots than shoots as indicated by their lower root to shoot ratio. Similarly, with decreasing light intensity, they allocated more to leaves at the expense of roots and stems, indicated by their higher LWR and LWRr. Regardless of light level, American chestnut invests >70% of its total biomass to aboveground growth. This allocation pattern is comparable to tulip poplar, red maple and black gum (*Nyssa sylvatica*) (Latham, 1992; Groninger et al., 1996), but contrasts with white oak (Wang and Bauerle, 2006) and mockernut hickory (*Carya tomentosa*) (Latham, 1992). Both mockernut hickory and white oak allocated <35% to the aboveground, which is believed to allow them to survive better under a frequent top-kill due to surface fire as well as on dry sites or during drought periods (e.g., Johnson et al., 2002). It appears that American chestnut adopts a life history strategy similar to tulip poplar and red maple, despite that fire has been attributed to its pre-blight dominance (Foster et al., 2002). The role of fire in regenerating American chestnut needs to be further investigated.

Based on its physiological and growth responses to irradiance, American chestnut may be best described as a shade-tolerant species. Because of its shade-tolerance, American chestnut has persisted as understory sprouts for many decades since the blight (Paillet, 1984, 1988, 2002). Zon (1904), however, suggested that chestnut sprouts are more shade-tolerant than seedlings. Therefore, the persistence of sprouts may or may not suggest the long-term survival of planted American chestnut seedlings in a low light environment. Further field testing of both existing chestnut sprouts and hybridized blight-resistant seedlings is warranted.

5. Management implication

The pre-blight dominance of American chestnut has been attributed to its prodigious sprouting capacity and rapid growth (Frothingham and Earl, 1912). Prolific sprouting of American chestnut is considered an adaptation for long-term survival in the forest understory (Paillet, 2002). As confirmed by our study, American chestnut is shade-tolerant, a trait that is perhaps exhibited even more in sprouts (Zon, 1904; Paillet, 1988). Therefore, sprouting becomes an effective reproductive strategy for American chestnut because it awaits and captures

crown openings (Paillet, 1984). In two relatively recent studies, American chestnut was reported to be a fast growing species. Latham (1992) found that American chestnut seedlings ranked higher than mockernut hickory, northern red oak, American beech (*Fagus grandifolia*), black gum, and tulip poplar across a broad range of resource combinations that affect competitive ability. After 6 and 7 years of growth in southwest Wisconsin, Jacobs and Severeid (2003) reported that American chestnut grew significantly faster than black walnut (*Juglans nigra*) and northern red oak. These two studies were conducted on seedlings, and sprouts were believed to grow much faster than seedlings during early stages of development (Zon, 1904). In the southern Appalachians, American chestnut sprouts grew as fast as black locust after release from a hurricane event (personal observation).

Because American chestnut is shade-tolerant and responds to release extremely well, clearcutting and planting may not be needed when reintroducing the species into existing forests. Instead, an alternative for American chestnut reintroduction could be an underplanting-and-releasing silvicultural system. Blight-resistant American chestnut seedlings can be planted in the understory of closed-canopy forests or naturally occurring gaps. After establishment, these seedlings would then be released by canopy removal. The shade-tolerance of American chestnut would allow flexibility in the release scheduling. The fast growth of American chestnut would ensure its competitive advantage over co-existing sprouters (e.g., oaks, maples, and tulip poplar) and invaders (e.g., black locust and tulip poplar). By strategically planting American chestnut on suitable target areas with desired spatial configuration, the maximum potential of natural dispersal could be captured, and its pre-blight dominance may be gradually realized.

6. Conclusions

Significant changes in physiology, growth, and biomass allocation were observed in American chestnut seedlings along an experimental light gradient, suggesting high plasticity in morphological and physiological acclimation to light. These results indicate that American chestnut is shade tolerant, which partially explains why American chestnut has persisted as understory sprouts for several decades even under the canopy of very shade-tolerant species. Given the economic and ecological importance of the species prior to blight, it is anticipated that blight-resistant hybrids will be reintroduced as soon as they become available. The shade-tolerance and fast growing characteristics of American chestnut suggest that an underplanting-and-releasing silvicultural system would be a plausible alternative to a clearcutting-and-planting silvicultural system.

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