The photosynthetic response of American chestnut seedlings to differing light conditions

Heather M. Joesting, Brian C. McCarthy, and Kim J. Brown

Abstract: Restoration attempts to reintroduce American chestnut trees to the eastern deciduous forest by means of a disease-resistant Chinese–American hybrid seed are in progress. Knowing the light conditions required for optimum seedling performance is necessary to maximize the success of reintroduction. American chestnut (*Castanea dentata* (Marsh.) Borkh.) seedlings were planted in two replicate forests in Vinton County, Ohio, in areas that had been thinned (more available light) and in control areas (intact canopy, less available light). The photosynthetic capacity of 12 seedlings per treatment was assessed using an infrared gas-exchange analyzer. Seedlings in the thinned treatment reached light-saturating rates of photosynthesis at an irradiance level approximately 33% higher than did the seedlings in the control treatment. Seedlings grown in the thinned treatment had a significantly greater maximum rate of photosynthesis (A_{max}), dark respiration rate (R_d), and daily carbon gain per seedling than seedlings grown in the control treatment. The light compensation point (LCP), quantum efficiency (ϕ), leaf mass per area (LMA), and leaf nitrogen concentration per unit leaf area (N_{area}) were not significantly different between treatments. American chestnut seedlings in the thinned treatment clearly maximize leaf-level photosynthetic capacity. These results will aid land managers in planning reintroduction trials by providing information on the light conditions required for maximum seedling success.

Résumé : Des essais de restauration sont en cours pour réintroduire le châtaignier d'Amérique (*Castanea dentata* (Marsh.) Borkh.) dans la forêt décidue de l'est en utilisant les semences d'un hybride entre une espèce chinoise et l'espèce américaine résistant à la maladie. Pour maximiser le succès de la réintroduction, il est nécessaire de connaître les conditions lumineuses requises pour optimiser la performance des semis. Dans ce but, des semis de châtaignier d'Amérique ont été plantés dans deux forêts du comté de Vinton, en Ohio, à des endroits qui avaient été éclaircis (plus de lumière disponible) et à des endroits qui ne l'avaient pas été (canopée intacte, moins de lumière disponible). La capacité photosynthétique de 12 semis par traitement a été estimée à l'aide d'un analyseur d'échanges gazeux à infrarouge. Les semis plantés aux endroits éclaircis ont atteint le taux de photosynthèse à saturation lumineuse à un degré d'irradiation environ 33 % plus élevé que les semis plantés aux endroits non éclaircis. Le taux maximal de photosynthèse (A_{max}), le taux de respiration nocturne (R_d) et le gain journalier en carbone par semis étaient significativement plus élevés chez les semis croissant aux endroits éclaircis comparativement aux endroits non éclaircis. Le point de compensation lumineux (LCP), l'efficacité quantique, la masse foliaire par unité de surface (LMA) et la teneur en azote par unité de surface foliaire (N_{area}) n'étaient pas significativement différents entre les traitements. Les semis de châtaignier plantés aux endroits éclaircis maximisent nettement la capacité photosynthétique de la feuille. Ces résultats aideront les aménagistes du territoire à planifier les essais de réintroduction en utilisant l'information sur les conditions de lumière requises pour que les semis aient le maximum de succès.

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Introduction

American chestnut (*Castanea dentata* (Marsh.) Borkh.) was once an important component of the eastern deciduous forest in North America. A century ago, the geographic distribution of this species ranged from Alabama north to New England and Ontario and west through Tennessee and the Midwest (Russell 1987). The range covered 24 eastern states and contained an estimated 3.5 billion trees (Brewer 1995). Around 1900, chestnut blight caused by the fungal pathogen *Cryphonectria parasitica* (Murr.) Barr. was accidentally introduced by infected wood from Asia (Paillet 1984). The fungal disease spread at a rate of 40–55 km·year^{-1}, and by the early 1950s, virtually every American chestnut stand within the native distribution of the species had been destroyed (Brewer 1995). In that region, American chestnut presently exists in the forest as a component of the understory, limited in size by the still-present chestnut blight. These understory stump sprouts develop until they reach roughly 10 cm DBH, at which point they become infected by the blight (McEwan et al. 2006). The chestnut blight effectively prevents the trees from reaching reproductive maturity; hence the few American chestnut individuals that remain are populations of stump sprouts experiencing periodic dieback (Paillet 1988, 2002).

Restoration efforts are currently under development for American chestnut. One of these involves the use of disease-resistant hybrid seed. A traditional plant-breeding program between American chestnut and disease-resistant Chinese chestnut (*Castanea mollissima* Blume) was started in 1983

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(Burnham 1988). The envisioned end-product is a sixth-generation seed that is 94% American chestnut and fully resistant to the chestnut blight (Burnham 1988). To achieve this goal, a series of backcrosses with the blight-resistant Chinese chestnut have been done that will reduce the Chinese chestnut characteristics by one-half in each generation (Diskin et al. 2006). In a study that compared the leaf, twig, and bud characteristics of the hybrid and parental populations, Diskin et al. (2006) found that the hybrids were generally intermediates of the parental populations. The morphological characteristics of the third backcross generation most closely resemble those of American chestnut (Diskin et al. 2006).

Information regarding optimal microsite planting conditions is needed for maximum success in establishing hybrid chestnut seeds; these data will be useful to restoration ecologists and forest managers. Little research has been published on American chestnut physiology, such as how seedlings respond to light, soil moisture, and other environmental conditions (16 citations for American chestnut, compared with 1184 for *Quercus* spp. and 584 for *Acer* spp., according to the ISI Web of Science search engine, December 2006). In addition to establishing seedling growth responses (Jacobs and Severeid 2004; McCament and McCarthy 2005), assessing both leaf acclimation and photosynthetic response of American chestnut seedlings to different light conditions will provide some mechanistic understanding of how these seedlings operate across a range of forest light availabilities.

Restoring American chestnut to the eastern deciduous forest could have two major impacts. Economically, the tree is an excellent source of timber, the tannins are useful for tanning leathers, and the nuts are an edible crop (Buttrick 1999). The timber is valuable; it is fairly rot-resistant and lightweight. American chestnut was the dominant timber species in its range in preblight logging (Brewer 1995). Environmentally, the restoration of American chestnut could have an impact on the carbon sequestration of eastern deciduous forests. American chestnut could also have important food-web impacts, such as providing nesting sites for birds and a food source for deer, wild turkeys, and bears.

Few studies have documented the response of American chestnut seedlings to varying light environments. Griffin found that some degree of canopy openness increased the survival of American chestnut sprout clusters, and a relatively closed canopy resulted in an increase in sprout mortality (Griffin 1989). A study in which American chestnut seedlings were grown in pots at four levels of irradiance (4, 12, 32, and 100% of full sunlight) showed that net photosynthesis rates of American chestnut seedlings increased with light availability (Wang et al. 2006). Total biomass and root:shoot ratios also increased with light availability (Wang et al. 2006). Although this study provides evidence that increasing light availability results in an increase in tree performance, very few studies have been conducted to assess the responses of seedlings to varying light environments under field conditions (Jacobs and Severeid 2004; McCament and McCarthy 2005).

In contrast to the lack of American chestnut seedling physiology data, many studies have been conducted on the effects of different light conditions on the photosynthesis rates of hardwood seedlings such as *Acer* spp. (Ellsworth and Reich 1992; Kubiske and Pregitzer 1996; Lei and Lechowicz 1997; Naidu and DeLucia 1998; Beaudet et al. 2000), *Quercus* spp. (Kubiske and Pregitzer 1996; Naidu and DeLucia 1998), and *Betula* spp. (Wayne and Bazzaz 1993; Kubiske and Pregitzer 1996; Beaudet et al. 2000). The collective results indicated that the photosynthetic capacity of seedlings was greater when seedlings were grown in environments with more available light rather than in those with less available light. Nitrogen concentration per unit leaf area (N_{area}), which is a driving variable for photosynthesis, has also been found to be greater in seedlings grown with more available light (Ellsworth and Reich 1992; Wayne and Bazzaz 1993; Abrams and Mostoller 1995; Kubiske and Pregitzer 1996; Naidu and DeLucia 1998). Nitrogen is required by rubisco and other photosynthetic enzymes involved in light harvesting; therefore, the greater light level a seedling experiences, the more nitrogen that seedling would require (Grassi et al. 2002). A similar result was found for leaf mass per area (LMA), with greater LMA in the seedlings grown in environments with more available light (Abrams and Mostoller 1995). A study of *Betula alleghaniensis* Britton, *Acer saccharum* Marshall, and *Fagus sylvatica* L. seedlings showed a significantly greater mean daily carbon gain per unit leaf area in seedlings grown with more available light, but no significant difference in mean whole plant daily carbon gain between light treatments (Beaudet et al. 2000). Whole-plant carbon gain is closely associated with the survival and growth of a seedling because it includes the effects of biomass allocation and other plant-level characteristics (Beaudet et al. 2000). The biomass and photosynthesis rates of seedlings have been found to increase with more available light (Ellsworth and Reich 1992; Kubiske and Pregitzer 1996; Naidu and DeLucia 1998; Beaudet et al. 2000; McCament and McCarthy 2005), which would result in greater whole-plant daily carbon gain.

The objective of our research was to characterize the photosynthetic acclimation by the leaves of American chestnut seedlings to two different light environments, namely more available light (open understory, mean percent canopy openness of 22.59 ± 1.11%, calculated using hemispherical photography and Gap Light Analyzer software (McCament and McCarthy 2005)), and less available light (dense understory, mean percent canopy openness of 13.65 ± 0.94%, calculated as above) under field conditions. In addition, we wanted to evaluate the relationship between N_{area} and LMA under each light condition and relate these to photosynthetic capacity. A final objective was to estimate the daily carbon-gain potential of seedlings grown in each light environment.

Materials and methods

Study site

The study sites, Zaleski State Forest (39°18′N, 82°21′W) and Raccoon Ecological Management Area (39°14′N, 82°22′W), are located in Vinton County, Ohio. In 2003, the maximum temperatures of the region ranged from –0.8 °C in January to 26.8 °C in July and the minimum temperatures ranged from –8.8 °C in January to 16.5 °C in July. Total rainfall in 2003 was 2131.6 mm, with a mean of 177.55 mm per month (cdo.ncdc.noaa.gov/). The two sites are part of the USDA Forest Service Fire and Fire Surrogate study, which

Fig. 1. Two experimental forests were sampled. Two silvicultural treatments per forest were used, highlighted in thick black box, and two seedlings per plot (six plots in total per treatment) were sampled. (A) Maps of the two experimental forests, Raccoon Ecological Management Area (REMA) and Zaleski State Forest. Maps courtesy of the USDA Forest Service Fire and fires surrogate study Ohio Hills study site (www.fs.fed.us/ne/delaware/4153/ffs/maps.htm). (B) Experimental design of the present study.
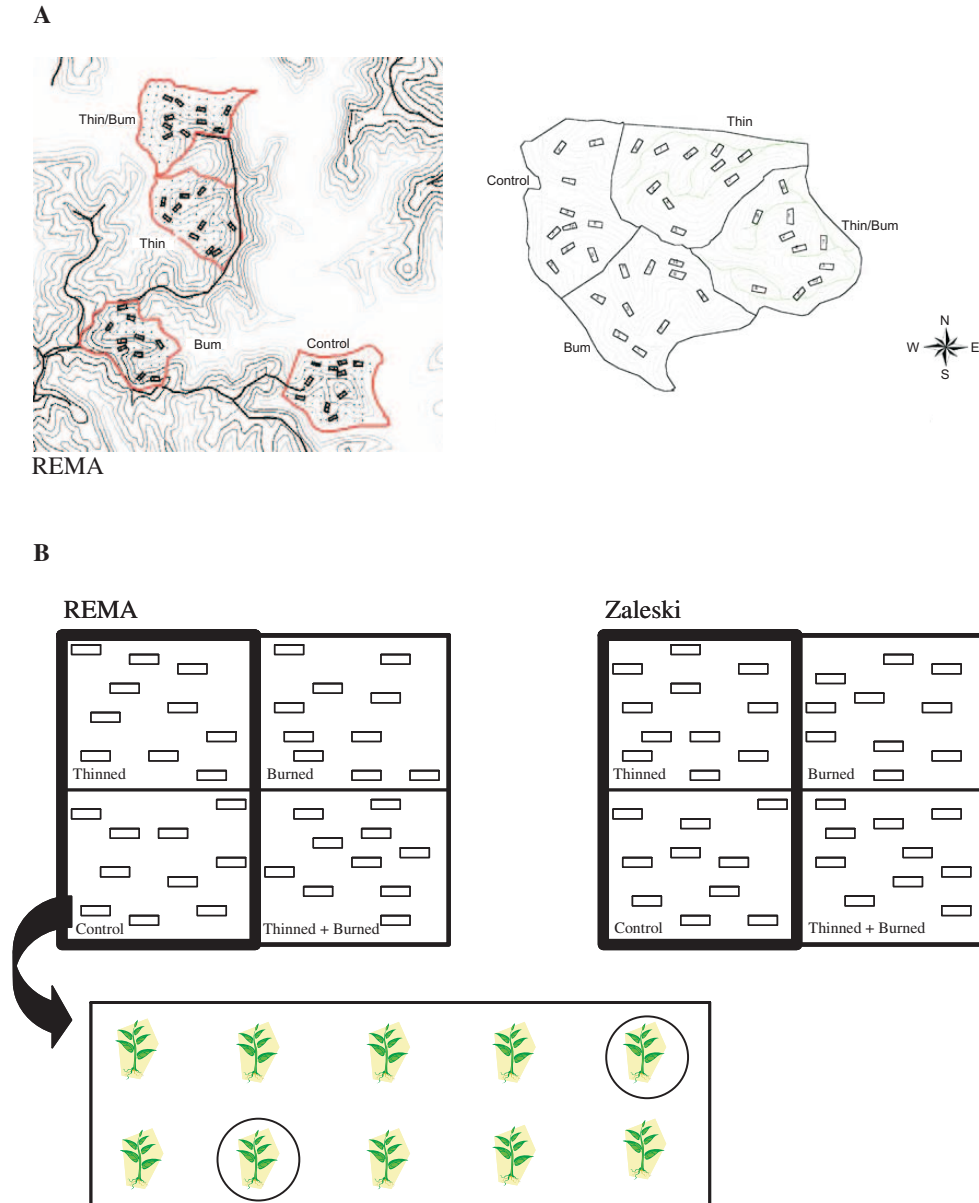
assesses the impact of silvicultural treatments on forest regeneration (www.fs.fed.us/ffs/). Each forest has been divided into four silvicultural treatments: a control treatment, a thinned treatment (30% basal area removed), a burn treatment, and a thinned + burned treatment (Fig. 1A). There were no fully open clearcut conditions available in these experimental forests.

For the present study, only the thinned treatment and the control treatment were investigated, to represent an open understory (more available light environment) and a dense understory (less available light environment), respectively. Within each treatment, 10 permanent 0.1 ha vegetation plots have been established (Fig. 1B). Hemispherical photographs were taken at each plot 1 m from the ground and analyzed for percent canopy openness using Gap Light Analyzer soft-

ware (McCament and McCarthy 2005). The control treatment had a mean canopy openness of 13.65 ± 0.94% and the thinned treatment had a mean canopy openness of 22.59 ± 1.11% (McCament and McCarthy 2005). Fifteen metres from each of these plots, 10 American chestnut seeds were planted in spring 2002 in 2 m × 5 m plots (McCament and McCarthy 2005). The source, treatment, and planting of the seeds is detailed in McCament and McCarthy (2005). In the present study, six plots were sampled per experimental treatment to represent a gradient of percent canopy openness (2 forests × 2 treatments × 6 plots = 24 study plots) (Fig. 1B).

Field methods

In each plot, the photosynthetic performance of 2-year-old American chestnut seedlings was assessed using a LI-6400

infrared gas-exchange analyzer (LI-COR, Lincoln, Nebraska). The internal CO_2 control in the cuvette was used to reach the desired CO_2 level (370 μmol), and different photosynthetic photon flux density (PPFD) levels were simulated using the LI-6400 LED light source. Relative humidity (40 ± 10%), leaf temperature (25 ± 5 °C), and CO_2 concentration (370 μmol) were held constant in the leaf chamber (or cuvette). In contrast, light conditions were changed to measure photosynthetic activity at eight different levels (1600, 1200, 800, 500, 200, 100, 50, and 0 μmol·m^{-2}·s^{-1}) using the LI-6400 LED light source. These measurements were taken in July, August, and September 2003 between 1000 and 1500 on the first mature leaves of two 1-year-old seedlings per plot, for a total sample size (*n*) of 48. The mean daily temperature was 25.4 °C, and the mean monthly precipitation was 149.86 mm. For each leaf measured, a leaf disc 12 mm in diameter was removed using a leaf corer. The disc was immediately stored in an ice container until returned to the laboratory for N_{area} and LMA analyses.

Diurnal patterns of PPFD were measured for each treatment by placing a line quantum sensor in a randomly chosen plot in the control and the thinned treatments (AccuPAR model LP-80, Decagon Devices Inc., Pullman, Washington). Each light bar is a composite of 80 mini photosynthetically active radiation sensors that capture the light environment over a 1 cm × 1 m area. Each sensor was factory-calibrated and they were intercalibrated to time of day and PPFD values. Quantum sensors were positioned approximately 0.3 m from the ground and recorded PPFD every 10 min from 0800 to 1800. A quantum sensor was also placed in an open field approximately 1 mi. (1 mi. = 1.609 km) from the field site at a height of 0.3 m to obtain a reference PPFD. PPFD measurements were taken in August on a mostly sunny day.

Laboratory analysis

Light-response curves were constructed in Sigma Plot 8.0 software (SPSS Inc. 2002) by plotting light availability against the corresponding photosynthesis measurements from the infrared gas-exchange analyzer. Curves were fit using the following equation (Parsons and Ogston 1999):

$$[1] \quad A = \frac{\phi Q + A_{max} - \sqrt{[(\phi Q + A_{max})^2 - 4\phi Q k A_{max}]}}{2k} - R_d$$

where A is net CO_2 assimilation on a leaf-area basis, ϕ is the quantum efficiency, Q is the photosynthetically active radiation, A_{max} is the maximum rate of photosynthesis, k is the curvature factor, and R_d is the dark respiration rate. From the light-response curves, three photosynthetic parameters were determined: A_{max} (μmol·m^{-2}·s^{-1}), R_d (μmol·m^{-2}·s^{-1}), and ϕ (μmol·m^{-2}·s^{-1}). The light compensation point (LCP; μmol·m^{-2}·s^{-1}) was calculated using the following equation (Parsons and Ogston 1999):

$$[2] \quad LCP = \frac{R_d(R_d k - A_{max})}{\phi(R_d - A_{max})}$$

The leaf discs were dried at approximately 65 °C and then weighed to determine LMA. The area of each leaf disc was already determined based on the diameter of the leaf corer

(12 mm). N_{area} was measured using a C:N combustion analyzer (Elementar Vario EL, Hanau, Germany). Acetanilide standards were run approximately every eight samples to maintain confidence in the accuracy of nitrogen measurements for leaf samples.

To determine the effect of photosynthetic acclimation on carbon gain, daily carbon gain was estimated from the photosynthetic parameters and diurnal PPFD patterns. The PPFD measurements were averaged to represent one value for every 30 min. To determine daily carbon gain per leaf (μmol·m^{-2}·s^{-1}), the parameters for each individual leaf calculated from the light curves and the mean PPFD value for each 30 min interval were substituted into eq. 1 (Parsons and Ogston 1999). By multiplying this value by the total leaf area (m^2), the daily carbon gain per seedling (μmol·m^{-2}·s^{-1}) was calculated. To estimate the daily whole-seedling carbon flux (mol CO_2·m^{-2}·30 min^{-1}), the daily carbon gain per seedling was increased by 1800 s and converted to moles. Curves illustrating daily carbon gain per leaf and daily whole seedling carbon flux were constructed using Sigma-Plot 8.0 (SPSS Inc. 2002).

Statistical analysis

A multivariate analysis of variance (MANOVA) was used to determine whether there was a difference in site (two replicate forests, random effect) and (or) treatment (control and thinned treatments, fixed effect). A one-way analysis of variance (ANOVA) was used to confirm which of the variables were responsible for any difference detected in the MANOVA analysis. The variables used in both tests were A_{max}, R_d, LCP, ϕ, LMA, N_{area}, and daily carbon gain per seedling. Variables met all assumptions for the two tests; all data, with the exception of R_d, were log-transformed to fit normality. R_d was winsorized to fit normality. Both analyses were performed in SAS software (SAS Institute Inc. 2000). The PROC MIXED command was used, with treatment (TRT) as a fixed effect and SITE as a random effect. The effect of SITE, TRT, and SITE × TRT was tested, and values were significant if $P \leq 0.05$.
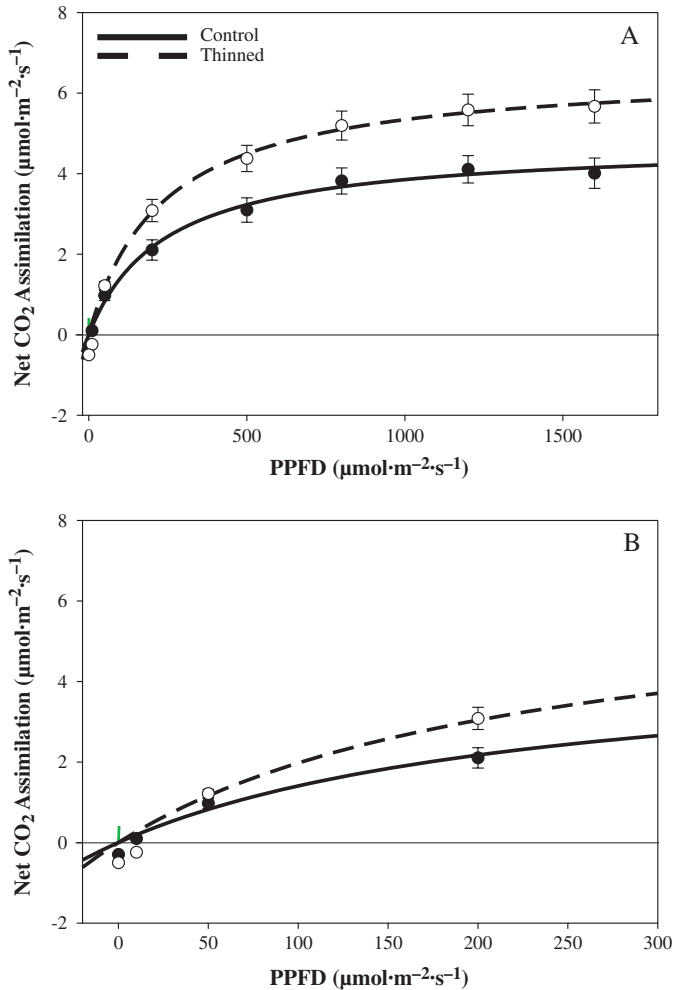
To determine whether any correlations existed between variables, a correlation hemimatrix was constructed. For this analysis, percent canopy openness was added to the variable list. Variables met all assumptions for the correlation procedure; data were transformed to fit normality in the same manner as for MANOVA. The correlation analysis was run using SAS software (SAS Institute Inc. 2000). The PROC CORR command was used to calculate Pearson's correlation coefficients. Correlations existed if $P < 0.05$.

To determine whether N_{area} served as a predictor of photosynthesis (A_{max}), a regression analysis was performed. Because both variables were subject to error, a Type II regression was used. Ranged major axis (RMA) regression was the Type II model utilized because the variables were in different physical units and there were no outliers. RMA regression analysis was performed using the Model II Regression software for Windows (Legendre 2001).

Results

The photosynthetic capacity of American chestnut seedlings was evaluated in thinned and control treatments to

Fig. 2. Mean light-response curves for seedlings grown in control and thinned treatments. (A) Mean light curve for the control and the thinned treatments. (B) Mean light curve at low light levels (0–200 μmol·m^{-2}·s^{-1}). PPFD, photosynthetic photon flux density.

There was a difference in mean ϕ values between treatments ($P = 0.063$), but this difference was not significant (Table 1). There were no significant differences in LCP, LMA, or N_{area} between treatments (Table 1).

A positive correlation existed between percent canopy openness and four variables: A_{max}, R_d, LMA, and daily carbon gain per seedling (Table 2). There was also a positive correlation between A_{max} and the following variables: R_d, LMA, and daily carbon gain per seedling (Table 2). Positive correlations were also present between daily carbon gain per seedling and the following variables: R_d and ϕ (Table 2). A positive correlation existed between LMA and N_{area}, and there was a correlation between R_d and ϕ (Table 2).

The results of the RMA regression analyses indicated a linear regression between A_{max} and N_{area} of $y = 14.378x - 5.460$. The regression was not significant ($P = 0.078$) and had a r^2 value of 0.34. When the data were analyzed separately by treatment using RMA regression techniques, the control treatment had a linear regression of $y = 4.571x + 0.645$ and a r^2 value of 0.34, and the thinned treatment had a linear regression of $y = 28.507x - 14.678$ and a r^2 value of 0.54. Neither treatment had significant regressions ($P = 0.131$ and $P = 0.081$ for the control and thinned treatments, respectively). Model II Regression software (Legendre 2001) does not currently have a function for testing significant differences between slopes and intercepts, so these analyses were not performed.

The mean PPFD values were 162.9 ± 68.05 and 115.4 ± 26.83 μmol·m^{-2}·s^{-1} for the control and thinned treatments, respectively (Fig. 3A). The maximum PPFD values were 1110.8 and 526.5 μmol·m^{-2}·s^{-1} in the control and thinned treatments, respectively (Fig. 3A). The minimum PPFD values were 10.7 and 18.6 μmol·m^{-2}·s^{-1} in the control and thinned treatments, respectively (Fig. 3A). The mean daily carbon gain per leaf in the control treatment was 1.13 μmol·m^{-2}·s^{-1} and total daily carbon gain was 22.51 μmol·m^{-2} (Fig. 3B). The mean daily carbon gain per leaf in the thinned treatment was 1.87 μmol·m^{-2}·s^{-1} and the total daily carbon gain was 37.41 μmol·m^{-2} (Fig. 3B). For the whole plant, the mean daily carbon gain of the seedlings in the control treatment was 15.90 μmol m^{-2}·30 min^{-1} and the total daily carbon gain was 318.06 μmol·m^{-2} (Fig. 3C). The mean whole plant daily carbon gain for seedlings in the thinned treatment was 89.69 μmol·m^{-2}·30 min^{-1} and the total daily carbon gain was 1793.82 μmol·m^{-2} (Fig. 3C).

Discussion

The photosynthetic capacity of American chestnut seedlings was clearly enhanced in the thinned treatment (Table 1, Fig. 2A). The results correspond to those of a study in which the biomass of American chestnut seedlings was measured in four silvicultural treatments (McCament and McCarthy 2005). Seedlings grown in the thinned treatment were larger than those grown in the control treatment in almost all aspects measured (stem height, root length, stem mass, root mass, basal diameter, total biomass, leaf area, number of leaves, and leaf mass) (McCament and McCarthy 2005). Light, measured as percent canopy openness, was the environmental condition correlated most strongly with all parameters measured.

show the extent of photosynthetic acclimation and light response of seedlings. A two-sample t test performed on percent canopy openness between treatments resulted in a significant difference between the control and thinned treatments ($P < 0.001$; SPSS Inc. 2006). The mean light curves indicated that seedlings growing in the thinned treatment reached light-saturating rates of photosynthesis at PPFD levels of approximately 600 μmol·m^{-2}·s^{-1}, which is approximately 33% greater than the PPFD levels for light-saturating rates of photosynthesis for seedlings in the control treatment (Fig. 2A). However, the curve also indicated that at very low light levels (0–25 μmol·m^{-2}·s^{-1}), the seedlings in the control treatment outperformed those in the thinned treatment. At light levels >25 μmol·m^{-2}·s^{-1}, the thinned seedlings outperformed the control seedlings (Fig. 2B). This is an expected result for leaves that are adapted to shade conditions (Lambers et al. 1998).

The MANOVA results indicated there was no significant difference between sites ($P = 0.072$), but there was a significant difference between treatments ($P = 0.004$). The thinned treatment had significantly greater A_{max}, R_d, and daily carbon gain per seedling than the control treatment (Table 1).

Table 1. Mean leaf characteristics for seedlings grown in a control and a thinned treatment.

	Control (13.65%)*	Thinned (22.59%)*	$P^{\dagger}$
A_{max}	4.36 (0.31)	6.73 (0.50)	0.0500
R_d	0.17 (0.03)	0.36 (0.05)	0.0025
LCP	9.10 (1.65)	11.49 (0.82)	0.1115
ϕ	0.026 (0.005)	0.037 (0.005)	0.0625
LMA	37.48 (1.87)	41.86 (2.09)	0.7266
N_{area}	0.703 (0.047)	0.730 (0.031)	0.6214
Carbon gain	3.18×10^{-4} (2.45×10^{-4})	1.79×10^{-3} (1.77×10^{-3})	0.0006

Note: A_{max}, maximum rate of photosynthesis ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$); R_d, dark respiration rate ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$); LCP, light compensation point ($\mu mol \cdot m^{-2} \cdot s^{-1}$); ϕ, quantum efficiency ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$); LMA, leaf mass per area ($g \cdot m^{-2}$); N_{area}, percent nitrogen content per unit leaf area (%); carbon gain, daily carbon gain per seedling ($mol\ CO_2 \cdot m^{-2} \cdot day^{-1}$). Values in parentheses are SEs.
*Canopy openness of the control and thinned treatments was found to differ significantly ($P < 0.001$).
$^{\dagger}P$ values obtained from one-way ANOVA analyses. LCP, LMA, and carbon gain were log_{10}-transformed to fit normality assumption. R_d was transformed through winsorization and A_{max} was $log_{10}(x + 1)$-transformed.

Table 2. Summary of Pearson's correlation coefficients for the relationships among A_{max} ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), R_d ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), lLCP ($\mu mol \cdot m^{-2} \cdot s^{-1}$), ϕ ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), LMA ($g \cdot m^{-2}$), N_{area} (%), percent canopy openness (%), and daily carbon gain per seedling ($mol\ CO_2 \cdot m^{-2} \cdot day^{-1}$).

	A_{max}	R_d	LCP	ϕ	LMA	N_{area}	% Canopy openness	Carbon gain
A_{max}	1.000	0.370*	0.194	0.166	0.339*	0.342	0.607†	—
R_d		1.000	0.231	0.791†	0.212	0.089	0.466*	—
LCP			1.000	−0.284	0.230	−0.217	0.206	—
ϕ				1.000	0.026	0.109	0.295*	—
LMA					1.000	0.573*	0.329*	0.220
N_{area}						1.000	0.351	0.079
% canopy openness							1.000	0.666†
Carbon gain								1.000

Note: For an explanation of abbreviations see Table 1.
*Significant at $P < 0.05$.
†Significant at $P < 0.001$.

American chestnut seedlings grown in the thinned treatment reached light-saturating rates of photosynthesis at an irradiance level greater than those in the control treatment (Fig. 2A). However, at very low light levels (irradiance levels 0–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$), surprisingly few differences existed between the thinned and control treatments (Fig. 2B). The seedlings in the control treatment actually outperformed the seedlings in the thinned treatment in very low light conditions (0–15 $\mu mol \cdot m^{-2} \cdot s^{-1}$; Fig. 2B). This is a common response of leaves that are adapted to shade conditions and of species that are somewhat shade-tolerant (Lambers et al. 1998). The results from the present study and those from McCament and McCarthy (2005) suggest that the general response of American chestnut to light is that of intermediate shade tolerance (King 2003). This means that American chestnut is capable of surviving in a shadier environment, but performs maximally in an environment with more available light. American chestnut seedlings can survive in the understory, but when a canopy gap becomes available, they assume rapid growth to fill the gap.

There have been disagreements regarding the degree of shade tolerance of American chestnut (King 2003; McCament and McCarthy 2005; Wang et al. 2006). Based on the results of a study in which American chestnut seedlings were grown in pots in four different light environments,

Wang et al. (2006) suggested that American chestnut is shade-tolerant rather than intermediate in shade tolerance, as proposed in this paper and other recent papers (King 2003; McCament and McCarthy 2005). In that study, they found that 1-year-old American chestnut seedlings grown in increasing light displayed an increase in net photosynthesis, height, total biomass, root:shoot ratio, and leaf area (Wang et al. 2006). Mean light-response curves taken from the seedlings in full sunlight resulted in a A_{max} of 9.08 $\mu mol \cdot m^{-2} \cdot s^{-1}$, R_d of −1.97 $\mu mol \cdot m^{-2} \cdot s^{-1}$, LCP of 29.48 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and ϕ of 0.056 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and reached light-saturating rates of photosynthesis at PPFD of 203.50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Wang et al. 2006). Some of the physiological characteristics of a shade-tolerant species are low LCPs and points for light-saturating rates of photosynthesis, as well as the ability to survive in the understory (Wang et al. 2006). This definition of shade tolerance is based on physiological characteristics, but the concept of shade tolerance also includes growth analysis and survival, which is more integrative with respect to time. American chestnut seedlings, when grown in approximately 14% and 23% canopy openness, exhibited the physiological traits of shade tolerance (as also seen in Wang et al. 2006). The seedlings in the control and thinned treatments displayed low LCPs and low points for light-saturating rates of photosynthesis (Table 1,

Fig. 3. (A) Daily PPFD values for the control and thinned treatments were measured between 0800 and 1800. Inset figure shows PPFD values in the control (black bars) and thinned (shaded bars) treatments. (B) Mean daily carbon gain per unit leaf area for seedlings in the control and the thinned treatments. Error bars represent SE. (C) Mean daily carbon gain per seedling for seedlings in the thinned and control treatments. Error bars represent SEs.

another species that grows quickly under canopy gaps (Jacobs and Severeid 2004; McEwan et al. 2006). According to both the physiological definition and the "forestry" definition (based on growth and survival), American chestnut is best described as being intermediate in shade tolerance.

The lack of a significant difference in ϕ values between treatments was apparent in the mean light curves (Fig. 2B). The efficiencies with which the seedlings were converting light to CO_2 were approximately equal, and this may be primarily species-specific ϕ (Table 1). The slopes of the curves were similar; the point of light-saturating rate of photosynthesis was the area of the curve that was significantly different (Fig. 2A). The lack of a relationship between irradiance level and ϕ was in accordance with the results of other hardwood-tree studies (Ellsworth and Reich 1992; Beaudet et al. 2000; Aranda et al. 2004). These data further support the suggestion that American chestnut is intermediate in shade tolerance. Acclimation to light appeared to be the same for both treatments; however, the amount of light fueling photosynthesis was greater for seedlings in the thinned treatment, which resulted in greater photosynthetic capacity.

The relationships between percent canopy openness and the photosynthetic parameters indicated that as light availability increases, A_{max}, R_d, LMA, and daily carbon gain per seedling increase linearly. Sun-loving plants that are grown in environments with more available light typically produce leaves with a larger LMA than sun-loving plants grown in environments with less available light (Abrams and Mostoller 1995; Matsuki et al. 2003). The increase in LMA may be a response to mesophyll expansion and the development of the photosynthetic apparatus (Ellsworth and Reich 1992). The increase in LMA in this study corresponded well to the leaf-biomass increase found for these seedlings by McCament and McCarthy (2005) (Table 2).

No significant difference in N_{area} was found between treatments, and no significant correlation was found between N_{area} and any photosynthetic variable (Table 2). There was also no significant correlation found between N_{area} and percent canopy openness (Table 2). The lack of relationship between N_{area} and percent canopy openness was contrary to those found in other studies, which showed greater N_{area} in leaves from environments with more available light (Ellsworth and Reich 1992; Wayne and Bazzaz 1993; Abrams and Mostoller 1995; Kubiske and Pregitzer 1996; Naidu and DeLucia 1998). The lack of a relationship between N_{area} and A_{max} contradicts the results of in other studies, which showed a positive relationship between N_{area} and photosynthesis (Table 2; Abrams and Mostoller 1995; Kazda et al. 2000; Grassi et al. 2002; Ripullone et al. 2003). Because nitrogen is partitioned among the enzymes required for both the light and dark reactions of photosynthesis, a plant that has a greater rate of photosynthesis would be expected to have leaves with greater N_{area} (Grassi et al. 2002). The relationship between LMA and N_{area} is comparable to what has been found in other studies (Niinemets 1997; Kazda et al. 2000). Further investigations of the relationship between the photosynthesis of American chestnut seedlings and N_{area} are required to reach a conclusion about this relationship.

The diurnal pattern of PPFD showed a large peak in the control treatment at approximately 1350 (Fig. 3A). This ex-
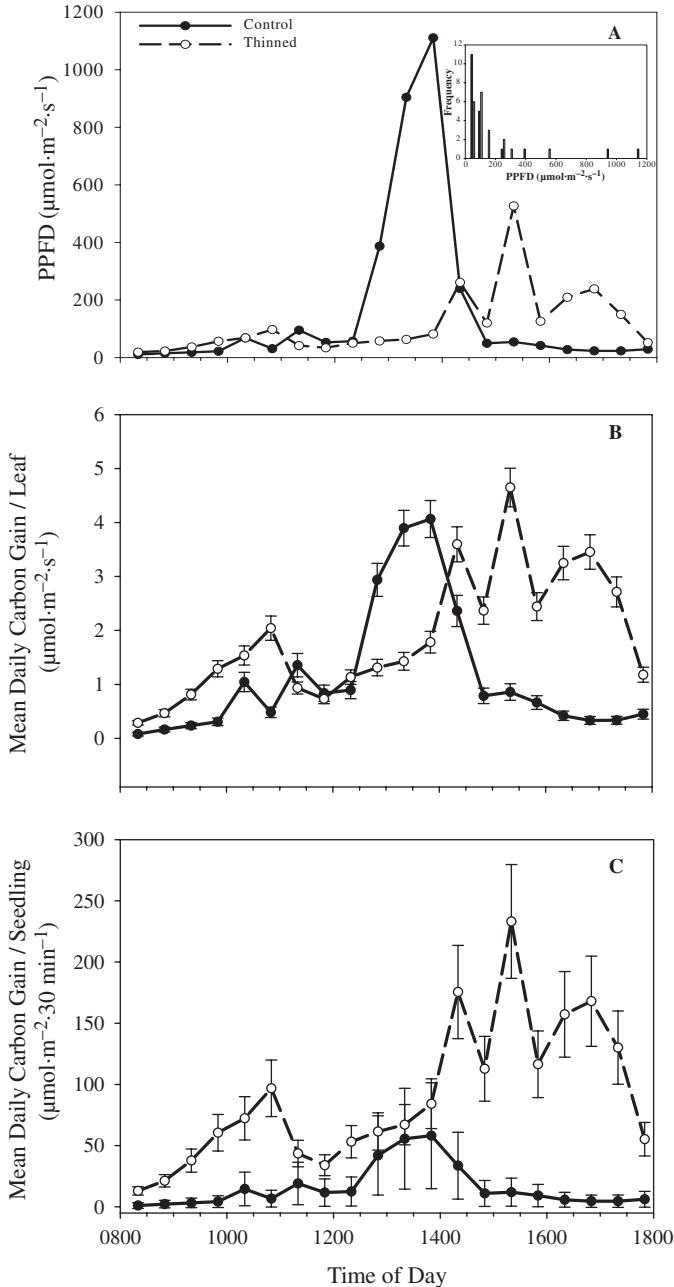
Fig. 2). However, many shade-tolerant species rarely exhibit significant growth under high light conditions (e.g., *Acer rubrum* L.). In a study of the survival and growth of American chestnut seedlings grown under four silivicultural treatments, it was found that total biomass increased with increasing light (McCament and McCarthy 2005). American chestnut has been shown to take advantage of gaps in the canopy quickly to attain canopy stature and its growth has been compared to that of *Liriodendron tulipifera* L.,

tremely high peak was correlated with a PPFD value of 1110.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which was greater than the remaining PPFD values measured for the control treatment. This high PPFD value is most likely the result of a sunfleck. A histogram of the PPFD values for the control and thinned treatments supports this; the greatest frequency of PPFD values for the control treatment is between 0 and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the greatest frequency of PPFD values for the thinned treatment is between 0 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with the remainder being between 200 and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 3A, inset).

The purpose of the diurnal PPFD measurements is to provide a sample data set for modeling purposes, not to characterize the integrated light environment for a growing-season at each of the sites. The PPFD data describe a typical light environment for a growing season day for a seedling growing in a thinned or control treatment. The data were collected to estimate the theoretical daily carbon gain of a seedling growing in a dense understory versus that of a seedling growing in an open understory. The differences in the light environment for each treatment were captured in the hemispherical photographs that were taken at each plot in each treatment and analyzed using GLA software by McCament and McCarthy (2005). To obtain a more accurate measurement for the diurnal pattern of PPFD, measurements should be made with multiple sensors at each plot in which photosynthetic measurements are made. Also, measurements should be taken at several dates throughout summer to coincide with the photosynthetic-measurement dates and to provide a better understanding of the diurnal patterns of PPFD in each treatment. Further study of the potential carbon gain per seedling by American chestnut in varying light environments needs to be done.

The mean daily carbon gain per unit leaf area showed a very similar distribution in instantaneous carbon gain, with means that showed little difference (1.13 and 1.87 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the control and thinned treatments, respectively). However, the mean daily carbon gain per seedling was significantly different in the control and the thinned treatments (Table 1). According to a previous study on the biomass of these seedlings, the seedlings in the thinned treatment had a significantly greater biomass and number of leaves than those in the control treatment; seedlings in the control treatment had a mean of 5 leaves·seedling^{-1}, and those in the thinned treatment had a mean of 12 leaves·seedling^{-1} (McCament and McCarthy 2005). The significant correlation between percent canopy openness and daily carbon gain per seedling supports the greater daily carbon gain per seedling in the thinned treatment. The correlation of daily carbon gain per seedling with A_{max} also supports the greater daily carbon gain per seedling in the thinned treatment, which had a significantly greater A_{max}. These results are consistent with those found in another study on hardwood seedlings in environments with more and less available light (Beaudet et al. 2000).

The photosynthetic rates found in American chestnut are intermediate when compared with other hardwood seedlings, whose photosynthetic rates range from 1.81 (*A. saccharum*) to 10.26 (*Betula populifolia* Marshall) $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the understory and from 1.78 (*A. rubrum*) to 10.53 (*Betula papyrifera* Marshall) $\mu mol \cdot m^{-2} \cdot s^{-1}$ in full sun (Ellsworth and Reich 1992; Wayne and Bazzaz 1993; Kubiske and Pregit-

zer 1996; Naidu and DeLucia 1998). Upon reintroduction into the eastern deciduous forest, American chestnut seedlings will face competition with existing hardwood seedlings, such as oak (*Quercus* spp.) and maple (*Acer* spp.). Oaks have been reported to reach photosynthetic rates of 3.88 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kubiske and Pregitzer 1996) in full sun, and maples have been reported to reach rates of 3.32 (Ellsworth and Reich 1992) and 1.78 (Kubiske and Pregitzer 1996) $\mu mol \cdot m^{-2} \cdot s^{-1}$ in full sun. However, to more appropriately define American chestnut's photosynthetic capacity with regard to other hardwood species, a study should be conducted to determine the photosynthetic rates of American chestnut under clear-cut conditions.

In a study comparing the growth of six co-occurring hardwood species, American chestnut ranked highest in height growth, leaf-area growth, and whole-plant dry mass when compared with *Carya tomentosa* Nutt., *Quercus rubra* L., *Fagus grandifolia* Ehrh., *Nyssa sylvatica* Marsh., and *L. tulipifera* (Latham 1992). Griffin et al. found that American chestnut sprout clusters had low survival rates when grown in areas that had *L. tulipifera*, *Prunus serotina* Ehrh., and *Magnolia acuminate* L. as dominant stems (1991). Plots with *Quercus* spp. and *Kalmia latifolia* L., *Rhododendron periclymenoides* (Michx.) Shinn., and other ericaceous shrubs had high rates of survival of sprout clusters (Griffin et al. 1991). A study on the allelopathic effects of American chestnut on other hardwood species and shrubs showed that extracts from the leaves inhibited germination of eastern hemlock (*Tsuga canadensis* (L.) Carrière) and rosebay rhododendron (*Rhododendron maximum* L.) (Vandermast et al. 2002). These results suggest that a fair amount of competition will occur between American chestnut and other hardwood seedlings. More research needs to be conducted to assess other competition factors between American chestnut and existing hardwood seedlings, such as the release of allelopathic chemicals, competition for resources, and spatial effects.

Land managers should take the results from the present study into account when beginning reintroduction trials. For maximum success, seeds should be planted in environments with more available light, and forests should be managed to retain these light conditions (through silvicultural treatments such as thinning). Further investigations on the response of American chestnut to environmental conditions, particularly water and nutrients, should be conducted.

Acknowledgements

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