

Full Length Research Paper

Homogeneous light in shade-house experiment overestimates carbon gains in Norway maple seedlings

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We set up a shade-house experiment with Norway maple growing at two light intensities typical of a disturbed and undisturbed forest canopy from either a homogeneous or a dynamic regime that mimics sunflecks. We show that although horticultural-grade shade-cloths can be finely tuned to provide daily averages of any light intensity, they do not appropriately mimic natural forest understories which are characterized by dynamic light environments composed of intense but short direct light events interspaced with prolonged period of low diffuse light. The results suggest that replicating the dynamic light environment of forests could be very important in experiments aimed at identifying the traits responsible for invasiveness in exotic species in forest understories, such as Norway maple in North America.

Key words: Norway maple (*Acer platanoides*), shade-house, tree seedling growth, photosynthetic response, forest understory, sunfleck, invasive tree species, pot experiment.

INTRODUCTION

Natural forest under stories receive mostly diffuse radiation while the sun is obscured by the canopy, interspaced with periods of intense radiation (sunflecks) when direct light is received through a gap. Both the duration and number of sunflecks, and the daily and seasonal mean percent available light, are functions of the spatial arrangement of the canopy and site latitude and slope that determine the daily and seasonal course of the Sun. Although short in duration, sunflecks may contribute the bulk of the total energy received by under story plants (Chazdon, 1988). Many pot experiments studying the physiological and morphological response of seedlings to available light are conducted using shade-houses covered with horticultural-grade shade-cloths providing daily average light conditions similar in quantity to forest under stories. However, the artificial light provided by these is uniform, and does not account for

the daily variations in light intensity that is experienced by natural seedlings and known to be important for growth, biomass allocation, physiology and morphology (Wayne and Bazzaz, 1993; Robison and McCarthy, 1999; Dalling et al., 2004). Species-specific responses to variations in daily time courses of light could indeed be due to their ability to increase light-use efficiency through rapid induction of photosynthetic capacity following direct light events and minimize the loss of induction between sunflecks (Weber et al., 1985; Chazdon, 1988; Pearcy, 1990), or to their tolerance of high irradiance (Veeranjaneyulu and Leblanc, 1998), and could be important parameters explaining the success of tree species to establish in forests (Lei and Lechowicz, 1997).

A classic explanation for the coexistence of ecologically similar species is the fine-scale differentiation of light (and other resources) requirements for the successful establishment of seedlings (Dalling et al., 2004). This could be particularly important for invasive species in forest under stories. Indeed recent evidence shows that forests may not, as was previously thought, be resistant to invasion of exotic species (Martin et al., 2009). In

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eastern and central North America, Norway maple (*Acer platanoides*) is one such invasive that is competing with native species, especially the ecologically similar sugar maple (*Acer saccharum*), in deciduous forest understories (Reinhart et al., 2005; Webster et al., 2005; Martin and Marks, 2006). In Québec, Canada, the species is threatening natural areas in urban settings (Midy et al., 2007; Lapointe, 2009). Research on the traits that would confer Norway maple some advantage over the similar native sugar maple is ongoing (Kloppel and Abrams, 1995). However, attempts to evaluate seedling differential performance in forest under stories are hampered by the spatial and temporal heterogeneity caused by gaps in the canopy (reviewed in Dalling et al., 1999).

In an effort to calibrate shade-houses to mimic natural forest understories to test hypotheses regarding traits that would confer advantages to the invasive Norway maple, we carried out an initial pot experiment under two types of shade-houses (dynamic vs. homogeneous). Because Norway maple invasion is said to be increased following disturbance, whereas intact understories would provide some resistance (Martin and Marks, 2006), the experiment included two light intensities mimicking a closed canopy (3% available light), and a large gap (23%). Our hypothesis was that homogeneous conditions are not an ideal proxy for forest under stories and over estimate seedling growth and physiological determinants of carbon gain, perhaps impairing our capacity to detect specific adaptations that would confer invasiveness advantages.

MATERIALS AND METHODS

Norway maple seeds were collected in fall 2007 from mature trees located in parks and streets within or just outside Mont-Royal Park, Montreal. Those trees are the source of an invasion now taking place inside the Park (Midy et al., 2007; Lapointe, 2009). Seeds were kept dry at 15°C in paper bags until mid-November when they were transferred outdoors to the shade-houses for stratification and germination under experimental conditions. Roofs were removed from the shade-houses in the winter to simulate winter and early spring conditions. The seeds were placed inside in humid sand boxes composed of layers of sand, minced leaf litter; 2.5 cm extruded polystyrene foam, a white plastic sheet and a wire mesh, for insulation and protection from rodents. Germination started on April 1, 2008. 400 seedlings were transferred to 220 mL multi-cell containers in mid-April and placed at random in their respective light regime. The seedlings were transferred to 6.77 L pots on June 15 at which time they had filled their initial containers. All 400 seedlings had survived so a protocol was established to select those to be kept for the rest of the experiment by first eliminating trees with deformities or mechanical damage. The remaining seedlings within each treatment were arranged along a height gradient and extremes at both ends were removed until the sample size for each treatment was obtained, plus extras to be used as buffers at each ends of the shade-houses, for a total of 320 seedlings.

Eight shade-houses were erected using wood frames in fall 2008 at the Montreal Botanical Garden (+45°33.7' - 073°34.3') in a split-plot design comprised of (i) four blocks (ii) two types (homogeneous and dynamic) and two intensities (3 and 23%), with

each treatment assigned at random within its level. Shade-houses were oriented north-south. Each was approximately 6 m long by 5 m wide and 1.5 m high and divided in two at their center. Shade-houses of the same type within a given block shared a common central wall. The east and west walls of all shade-houses (including central walls) were covered with sheets of green Coroplast™ leaving a 30 cm gap covered with black window screen (~50% light transmission) at the bottom for aeration and to keep rodents out. Light treatments were imposed on 14 May 2008 (before that the shade-houses had no roof to simulate the spring conditions of deciduous forests). The roofs of both types and intensity treatments extended to the north and south walls of the shade-houses to simulate a continuous forest cover and minimize border effects. Homogeneous conditions were imposed using different sets of horticultural-grade shade-cloths until the desired intensities were obtained. The same levels were obtained under dynamic conditions using solid roofs of the same material as the walls with movable parts to create two continuous gaps along the length of the roof, the size of which could be precisely adjusted.

The dynamic treatments were successfully calibrated against the homogeneous to obtain the same total daily averages of available photosynthetically active radiation (PAR) (3 and 23%) using data loggers and quantum sensors over several days (see example on Figure 1) as is often done to characterize forest under story light environments (Paquette et al., 2007). Quantum sensors were placed at average seedling height inside the shade-houses between the two rows of seedlings to the east, thus showing slightly longer direct light events in the afternoon than in the morning, while the reverse was experienced by seedlings on the western rows. Intensity calculations are simply the ratios of the total daily inputs measured inside a given shade-house to that measured by another sensor placed on top of one of the shade-house (REF). Light gaps lasted about an hour and 10 min twice a day under the D-H and D-L regimes, respectively. Results from 12 July, 2008 were used for intensity characterisation and daily Carbon gain computations because it showed a good mix of sunny and cloudy conditions at approximately mid-season (Figure 1). Once integrated from sunrise to sunset we obtained the following percent available light for the four different light regimes (other measurements throughout the summer gave similar results): Homogeneous High-light (H-H): 23.04% PAR; Homogeneous Low-light (H-L): 2.66% PAR, Dynamic High-light (D-H): 23.03% PAR, Dynamic Low-light (D-L): 2.65% PAR.

Each shade-house contained 32 maple seedlings on four rows (plus four buffer seedlings at each of the north and south ends), for a total sample size of 256 (not counting the buffers). Statistical analyses were carried out on JMP 8 (SAS Institute Inc., Cary, NC, USA). Blocks and their interactions were assigned as random factors (R) in the following restricted maximum likelihood (REML) split-plot mixed model (Searle et al., 1992; Wolfinger et al., 1994): Block R (4), Type (2), Block x Type R ; Intensity (2), Block x Intensity R , Type x Intensity. Tukey HSD tests were carried out where justified to investigate significant interactions.

Seedling response to the imposed light regimes was evaluated over three classes of variables: growth, biomass allocation and physiology. Growth and biomass were measured at the end of the growing season. Total height from the ground (H; cm), and stem diameter just above the root collar (D_0 ; mm) were evaluated. Then the seedlings were cut at ground level, the soil carefully sifted out to collect the complete root systems, and the above- and below-ground dry weights measured (AGB, BGB; g). In July and August, 2008 we evaluated light response curves for a subsample of 60 trees selected at random within alternating treatments and blocks. Measurements were carried out under constant CO₂ concentration and leaf temperature using a LI6400 portable photosynthesis system fitted with an autonomous light source and CO₂ injector (LiCor, Lincoln, NE, USA). Photosynthesis was measured during rainless mornings on the last mature leaf of each seedling following

Table 1. Mean growth, biomass partitioning, physiological response, daily carbon gains (mmole CO₂ m⁻² day⁻¹) and REML analysis results of Norway maple seedlings to four light regimes.

	H (cm)	D ₀ (mm)	AGB (g)	BGB (g)	A _{max}	R	φ	θ	C gain
H-H	27.1	5.8 A	5.39	2.87 A	10.02 AB	-0.630	0.0691	0.496	192
H-L	5.8	2.2 B	0.31	0.28 B	7.37 AB	-0.229	0.0798	0.494	51.2
D-H	22.5	5.5 A	3.99	2.29 A	11.20 A	-0.740	0.0732	0.495	158
D-L	5.2	1.8 C	0.21	0.14 C	5.97 B	-0.171	0.0662	0.497	24.2
Type	0.0483	0.0235	0.0289	0.0068	0.5741	0.8854	-	-	NA
Intensity	< 0.0001	< 0.0001	< 0.0001	0.0001	0.0238	0.0478	-	-	NA
Type*Int.	0.5273	0.0011	0.2010	< 0.0001	0.0046	0.5503	-	-	NA
R ² (N)	0.83 (256)	0.90(256)	0.90(256)	0.92(256)	0.58 (60)	0.41 (60)	- (60)	- (60)	NA

REML results (log transformed responses) are given below least-square means; H: total height; D₀: stem diameter; AGB and BGB: above and belowground biomass; A_{max}: maximum photosynthetic capacity; R: dark respiration; φ: quantum yield; θ: convexity. REML did not converge for both φ and θ (no significant effect found). Light regime effect on C gain could not be tested as it was computed using means per regime for each photosynthesis parameter. Tukey HSD tests are given where justified to investigate significant interactions; means not sharing a letter are significantly different.

a protocol where stable state gas exchange was averaged at six increasing light intensity: 0, 25, 100, 250, 500 and 1000 μE m⁻² s⁻¹. Curves were fitted in JMP with a four-parameter non-rectangular hyperbola (Prioul and Chartier, 1977).

$$A = \frac{\phi I + A_{\max} - \sqrt{(\phi I + A_{\max})^2 - 4\theta \phi I}}{2\theta} - R \quad (1)$$

where A (μmole CO₂ m⁻² s⁻¹) is the net photosynthesis, A_{max} the maximum photosynthetic capacity at high light, I the light intensity (μE m⁻² s⁻¹), φ the quantum yield and θ a parameter describing the convexity of the curve.

The measured photosynthetic responses to available light of seedlings growing in each of four growth environments were then used in a modelling exercise to account for the differences in light regimes. Daily Carbon gains (mmole CO₂ m⁻² day⁻¹) were resolved and summed up over a whole mid-summer day using Equation 1 (assuming steady state photosynthesis) for a light intensity I computed every minute using real data from July 12 (Beaudet et al., 2000).

RESULTS AND DISCUSSION

In general, we found large and significant differences in most response variables between seedlings growing under high and low light intensities, as expected (Table 1). The type of light regime (homogeneous vs dynamic) also had a significant effect on most response variables. In all cases for growth and biomass partitioning the same trend was observed, with larger seedlings under high light, as well as under homogeneous light regimes. Some interactions were noted between type and intensity, revealing effect sizes that differed slightly between regimes (Table 1 - HSD tests). The effect of regime type on stem diameter and belowground biomass (BGB) was large on seedlings growing under low light whereas it was negligible between both high light regimes.

The type of regime had no general effect on physiological

response, but the maximum photosynthetic capacity (A_{max}) and respiration rate (R) were affected by intensity, with an increased A_{max} and a corresponding increase in night time respiration under high light, as expected (Bazzaz, 1996) and reported in Wayne and Bazzaz (1993) for *Betula* species (Table 1). There was also an interesting pattern highlighted by a significant interaction for A_{max}. While A_{max} reached an absolute minimum under a dynamic low light regime (D-L), it was actually increased under dynamic high light (D-H). REML analyses did not converge for the other two photosynthesis parameters evaluated, revealing no significant effect (Table 1).

Daily Carbon gains (C gain) were simulated for a single model seedling from each light regime using parameters and light intensities from July 12. Although, this response was not measured per se and statistical inference could not be made, simulated C gain integrated the effect of differences in photosynthetic capacity and light availability for every minute of a typical mid-summer day under each of the four regimes. Whereas seedlings growing under homogeneous light have access to a constant, uniform supply of light, those growing under dynamic light must adapt to a daily succession of short events of intense light separated by prolonged periods of shade (Figure 1). Furthermore, the light response curve for photosynthesis is not linear, and A_{max} is reached at rather low levels (around 600 - 700 μE m⁻² s⁻¹) with respect to the light available in dynamic shade-houses during sunfleck events (1200 - 1500 μE m⁻² s⁻¹ - Figure 1), thus making these large increases in light intensities not compensating for the low photosynthetic activity that can only be achieved between sunfleck events in terms of daily carbon gain (Figure 2), which Wayne and Bazzaz (1993) referred to as the "saturation hypothesis". Important differences in simulated C gain between light

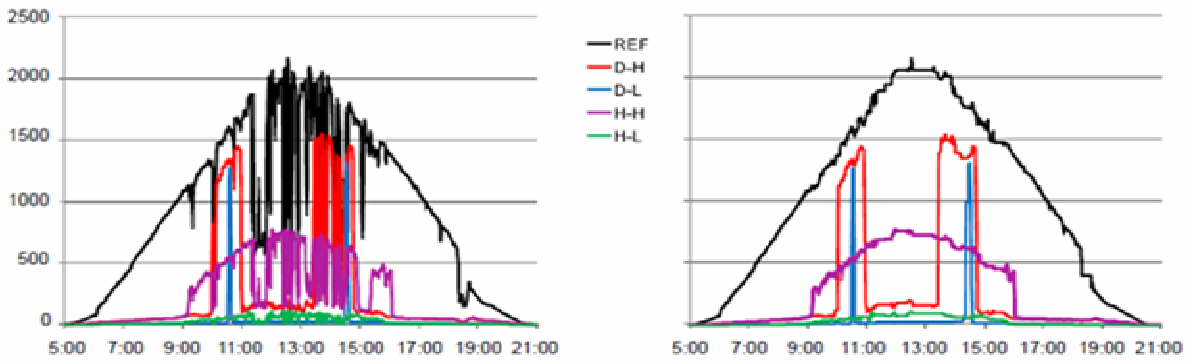


Figure 1. Top: Measured light ($\mu\text{E m}^{-2} \text{s}^{-1}$) plotted against time (minutes) for the two dynamic (D) and homogeneous (H) shade-houses under high (H) and low (L) light regimes, and an outside reference (REF), on a partly cloudy day (12 July, 2008). Bottom: Idealized light availability beneath the same four regimes (same data but cloudy periods removed and smoothed curves between sunny periods).

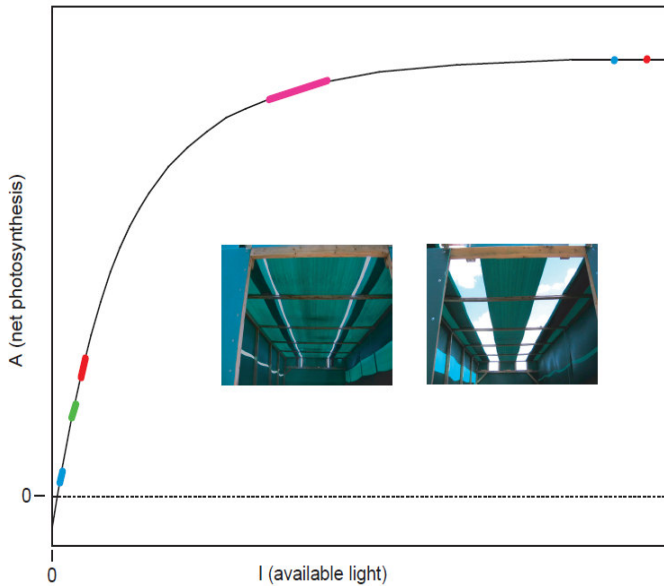


Figure 2. Theoretical light response curve for seedlings growing under the four light regimes. Homogeneous shade-houses (purple and green) provide constant light at levels that are higher than those received by seedling under dynamic regimes (red and blue) for most of the day. The duration of gap events are small and do not compensate for the loss in C gain relative to homogeneous conditions because maximum photosynthesis is reached at much lower levels. Inserts are photographs of the dynamic shade-houses from a seedling perspective through the open door at the north end (left D-L, right D-H). Late-afternoon sunflecks are visible on eastern walls.

intensities were therefore observed, but also between dynamic and homogeneous conditions for the same intensity. Homogeneous conditions are much more favourable to seedling growth than are dynamic ones, especially under the low light regime, showing 112% increase in C gain between dynamic and homogeneous

regimes. Furthermore, the true differences are probably even more important given that (i) shade tolerant species are susceptible to photo-inhibition and may actually have reduced photosynthesis at high light levels following the closure of stomata to prevent water stress damage (Veeranjaneyulu and Leblanc, 1998), and (ii) we assumed steady state photosynthesis. Indeed the time for complete induction of a leaf from the time it is exposed to a sudden sunfleck event following prolonged low light conditions may be important, especially for seedlings growing at low light levels (Percy et al., 1997), thus making these events, especially those lasting only a few minutes as in our low light dynamic regime, less important in terms of C gain than is simulated using steady-state photosynthesis. Although the observed physiological differences between regime types were small and mostly not significant, the simulation of daily Carbon gains allowed us to understand how differences in light regimes translated into large and significant differences in size and biomass by the end of the growing season in favour of trees growing under homogeneous light regimes. It should be noted that during the 2008 summer we observed some significant increases in maximum temperatures inside the dynamic shade-houses on some sunny, windless days, especially under low-light, possibly due to a lack of roof openings for ventilation which could have impacted on the growth of our seedlings. However, these were mostly the results of direct light events which were not only of short duration, but also somewhat realistic as natural sunflecks also increase temperatures locally in forest understories.

Several variables reacted strongly to different shade house types tested, especially at the low level of available light. In general, and as hypothesized, seedlings grew significantly better under traditional horticultural shade-houses providing constant diffuse light. Shade cloths are used extensively in the horticultural industry for good reasons. But our experiment did not attempt to find

optimal artificial growing conditions, but conditions that mimic natural ones. As the natural light environment in forests is not homogenous, but rather composed of heterogeneous events of full light interspaced by prolonged shade conditions, the use of dynamic shade-houses such as those used in the present experiment is recommended to better mimic natural forest under stories. Furthermore, the growth and physiological differences we found between the two regimes were much larger than the expected range that would confer an invasive species an advantage over native species in forest understories. Using the present results as guide, we can now proceed to identify the physiological and morphological traits that would confer Norway maple its advantage over the native sugar maple in a comparative study of controlled pot experiments using dynamic shade-houses to better mimic natural under story light conditions.

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