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IS CLIMATE SIMULATION IN GROWTH CHAMBERS NECESSARY?

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WANG Z. M., JOHNSEN K. H. and LECHOWICZ M. J. *Is climate simulation in growth chambers necessary?* BIOTRONICS 28, 13-21, 1999. In the expression of their genetic potential as phenotypes, trees respond to environmental cues such as photoperiod, temperature and soil and atmospheric water. However, growth chamber experiments often utilize simple and standard environmental conditions that might not provide these important environmental signals. We conducted a study to compare seedling growth in the field to both a standard growth chamber regime and a growth chamber regime programmed to simulate seasonal and diurnal variation in daylength, light intensity, temperature and relative humidity. Twenty-four open pollinated families of black spruce were grown in a nursery bed for one season while *in situ* temperature and relative humidity were recorded. The same 24 families were then grown under two growth chamber protocols; standard conditions (16 hour day and 8 hour night at temperatures 20℃ and 15℃ and 70% and 90% relative humidity, respectively) and climate simulation (programmed variation in diurnal temperature and relative humidity, and daylength). Seedlings from the climate simulation regime were similar to field-grown seedlings with respect to seedling size and dry matter partitioning. Seedlings from the standard growth chamber regime grew larger and allocated more dry matter to the shoots, compared to both other growth regimes. Results clearly indicate that simulating more realistic natural environments in ecophysiological studies can produce results closer to what are observed in the field.

Key words: biomass allocation; *Picea mariana*; black spruce; seedling.

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INTRODUCTION

Environmentally controlled experiments, often using growth chambers, are an indispensable component of ecophysiology research. However, most growth chamber experiments have adopted very simple and unrealistic growing environments (*8*). A typical experiment might run for 90–120 days and every day the same growth regime would prevail: for example, 16 hour day at 26℃ followed by 8 hour night at 22℃. If relative humidity is controlled it is usually held constant at about 70%. In most cases the lights are either all on or all off. In cases where day and night temperatures and daylength are simulated over a growing season, the diurnal variations are seldom mimicked (*2, 15, 16*).

Trees respond to subtle environment cues, such as photoperiod (*10*) and soil and atmospheric drought (*13*), that will not be present in a standard growth chamber regime. Therefore, experiments conducted in such regimes might not detect important main and/or interaction effects of these environmental factors on tree responses. While natural environments cannot be completely mimicked in a growth chamber, newer models can be programmed to systematically vary environmental conditions, such as light quantity, photoperiod, relative humidity and temperature. As such, some researchers have attempted to improve growth chamber experiments by programming growth chambers to simulate aspects of the natural environment (*3, 17, 19, 20*). However, such environmental simulations require more expensive growth chambers and more time and effort to conduct than standard growth chamber protocols.

We have been of the opinion that the extra cost and effort of environmental simulation is justified and will produce experimental results more realistic and useful than standard protocol experiments. However, we are unaware of any experiments that have systematically examined if there are benefits of using a more complicated environmental simulation versus standard and simple growth chamber environments. Here, we present the results of a study designed to compare seedling growth in the field to both a standard growth chamber regime and a growth chamber regime programmed to simulate seasonal and diurnal variation in daylength, light intensity, temperature and relative humidity.

MATERIALS AND METHODS

The field experiment was run in a nursery of the Petawawa Research Forest (77°30′ W, 46°N), Ont., Canada. Two adjacent rectangular plots were set up in the nursery bed. Each plot was divided in the middle to form two blocks. In each block, 120 one liter pots (4 inch top and 3 inch bottom diameters and 5.25 inch height with 4 holes at the bottom) were buried in the sandy soil with the top edges just above the soil surface of the bed. The pots were covered with a thin layer of coarse gravel at the bottom and then filled with soil mixture (peat: black earth: sand=2:2:1).

Seeds of 24 open-pollinated black spruce families were cold-stratified at 4℃ for two weeks before planting. On 4 May 1994, three seeds per pot per family

were planted, and five pots (non-contiguous plots) were chosen at random in each block for every family. The pot soil surface was covered with a thin layer of white silicon sand after sowing. Seedlings of the germinated seeds were thinned to one per pot on 22 July. When the soil surface was dry, seedlings were watered with tap water. Seedlings were fertilized on 20 Aug. and 30 Sept. 1994, with 50 ml of 50 ppm nitrogen obtained by mixing 20-8-20 (NPK) fertilizer with water. They were harvested on 20 and 21 Oct. 1994. At harvest, seedling height was measured, root and top were separated by cutting at the soil surface and branches were counted. Roots were washed out, and all harvested materials were dried at 70℃ for two days and weighed to the nearest 0.0001 g.

In the phytotron part of this study, two growth chambers (Conviron Products Company, Winnipeg, Man., Canada), were used to simulate the field conditions, and two were used to create standard conditions. Each chamber was divided into two blocks at the middle to mimic the field blocks, and set at 350 μmol mol^{-1} atmospheric CO_2. All the treatments and schedules followed those in the field. The standard condition was set as 16 hour day and 8 hour night at temperatures 20℃ and 15℃, relative humidities 70% and 90% respectively and daytime light intensity at 600 μmole m^{-2} s^{-1} photosynthetic photon flux density (PPFD). For the simulated field conditions in the phytotron, the two chambers were programmed according to the air temperature and relative humidity that were recorded during the field experiment using an OMNIDATA logger (OMNIDATA, Model 217, OMNIDATA International, Inc., Logan, Utah) set at 30 minute recording intervals. The photoperiod and light intensity were simulated as in (*19*).

The experiment was a split-plot design with the three growth regimes as the main plots, and seedling total weight, root weight, shoot weight, height and branch number were analyzed by ANOVA using SAS. Data were transformed, when necessary, to conform with assumptions required for ANOVA. Dry matter partitioning between root and shoot was analyzed using allometric principles (*12*). The effects of growth regime on dry matter partitioning were analyzed by comparing the allometric parameters from analysis of covariance of natural log transformed dry weights analogous to the analysis of family effects in (*9*). In addition, we analyzed family effects on allometric parameters separately for each growth regime. To assess the importance of family×environment interactions on dry matter partitioning, we compared the correlations of family coefficients of the allometric parameters estimated from the simulated field and the standard growth regimes with the estimates from the field. It was observed that extremely small seedlings (root dry weight$<=$0.007 g) had very large shoot dry weights relative to their root dry weights. We ascribed this to our inability to harvest a large proportion of roots rather than to biological reasons. Thus, 30 of the 1036 seedlings with root dry weights$<=$0.007 g were not included in the allometric analyses.

RESULTS AND DISCUSSION

In general, the simulated field conditions, especially temperature, relative humidity and daylength were very similar to the field conditions but very different from standard conditions. For the chamber set for the standard conditions, 95.2% of the hourly mean temperatures were within the range of ±0.1℃ of the set values, and 88.4% of the hourly mean relative humidities (RH) were within the range of ±2% of the set values. As expected, field conditions varied a lot more than the standard growth chamber regime (Fig. 1). A total of 95% of the temperature readings varied from 4℃ to 26.8℃ with a mean 16.1℃ and 95% of the RH readings varied from 35.5% to 93% with a mean 75.2%. The simulated field condition chamber had 86.8% of the hourly mean temperatures within ±2.5℃ of the set values and 63.3% of the hourly mean RH within ±5% of the set values. In the standard chambers, light was set at 300 μmole $m^{-2} s^{-1}$ one hour after sunrise and one hour before sunset, and the rest (about 90%) of the time it was set at 600 μmole $m^{-2} s^{-1}$ each day. Light intensities were not recorded in the field. However, the 600 μmole $m^{-2} s^{-1}$, which is close to the saturated light condition for black spruce photosynthesis (*22, 23*), is lower than the light intensity in the field when the sky is clear. In the field, black spruce seedlings would be in light below saturation when it was cloudy or in the early morning or in the late afternoon and would be in light above saturation for photosynthesis when it was clear. In the simulated chambers, light was divided into 8 levels (*19, 20*), and only 51.5% of the time light was set at 600 μmole $m^{-2} s^{-1}$ and the rest of the time it was below this level in the day time over the season.

Survival of the seedlings was good and varied in a narrow range from 70% to 76% among the three growing conditions, and therefore it was not analyzed as other growth traits were. However, seedling shoot and total dry mass differed significantly among the three growing conditions (Table 1). More interestingly, these differences were not significant between field and the simulated field conditions, but they were significant between these two and the standard growing condition (Fig. 2). In addition, the allocation of dry matter between roots and shoots differed among the environments (Fig. 3). Seedlings from the field and simulated field conditions allocated significantly more dry matter to roots than those from the standard growth chamber regime. Thus, seedlings from the simulated field regime were much closer phenotypically to field-grown seedlings, with respect to size and morphology, than were seedlings grown in the standard growth chamber regime. These results clearly indicate that simulating more realistic natural environments in ecophysiological studies can produce results closer to what are observed in the field.

In standard phytotron conditions, temperature changes suddenly from low to high in the morning and from high to low in the evening. Such a drastic change may disturb plant physiological processes and therefore affect growth (*21*). It has been shown that photosynthetic carbon translocation is active for some time after light-off (*6*) and rapid temperature drop restricts such

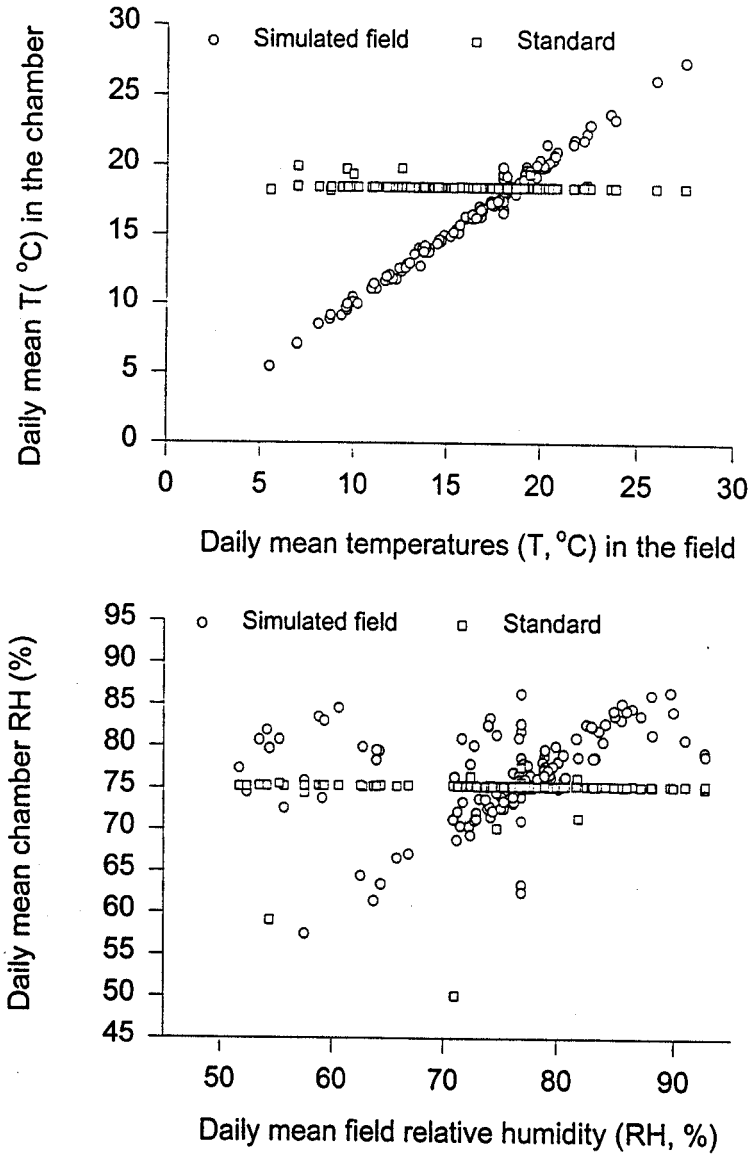

Fig. 1. Hourly mean relative humidity and temperature in the field plotted against the recorded corresponding values in the growth chambers for simulated field and standard conditions over the growth period.

Table 1. ANOVA results for effects tested. Shoot is square root of shoot dry weight; Root is square root of root dry weight; Total is the 4th root of total dry weight; Branch is square root of number of branches plus 1; and Height is the original seedling height. Conditions are the three growing conditions.

Variable	Source	df	MS	F Value	Pr>F
Shoot	Conditions (C)	2	3.174	21.19	0.017
	FAMILY (F)	23	0.039	3.36	0.000
	C*F	46	0.012	1.08	0.385
Root	C	2	0.581	5.65	0.096
	F	23	0.022	3.00	0.000
	C*F	46	0.007	1.03	0.447
Total	C	2	2.416	11.58	0.039
	F	23	0.052	3.24	0.000
	C*F	46	0.015	0.99	0.513
Height	C	2	348.432	3.13	0.184
	F	23	18.725	4.41	0.000
	C*F	46	4.685	1.10	0.350
Branch	C	2	3.934	0.16	0.861
	F	23	0.890	4.05	0.000
	C*F	46	0.319	1.45	0.078

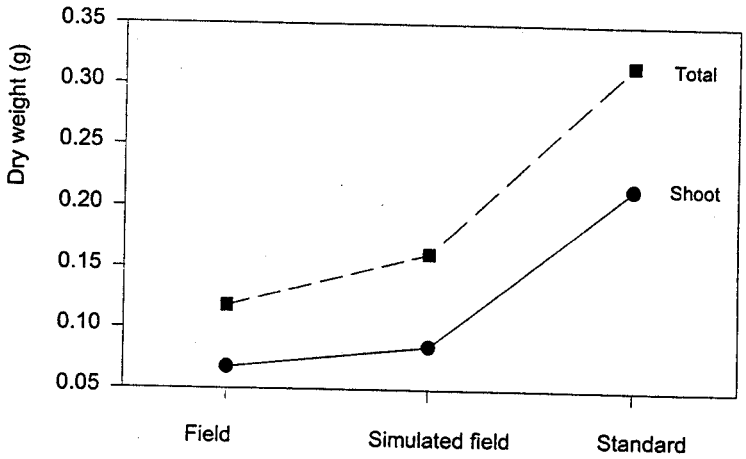

Fig. 2. Total dry weight and shoot dry weight of seedlings in the field, simulated field and standard conditions.

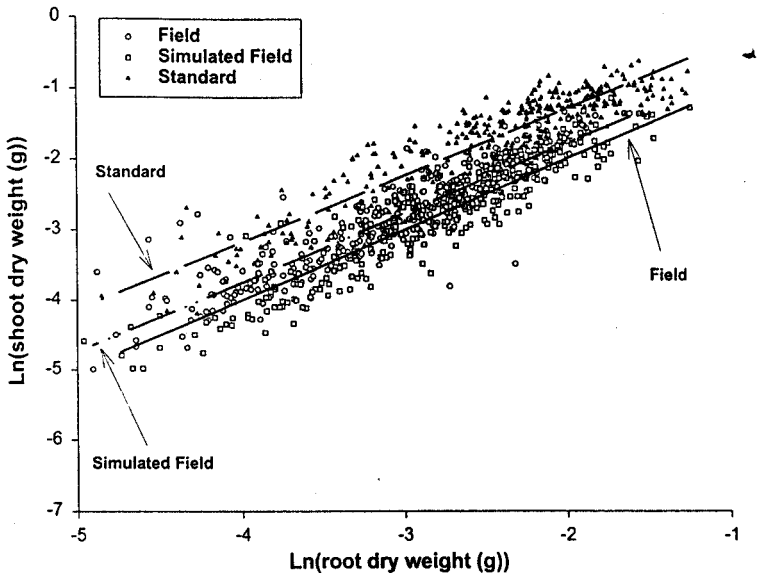

Fig. 3. Allometric relationships between seedling Ln (root dry weight) and Ln (shoot dry weight) for the field, simulated field and standard conditions: original data points with the corresponding regression lines.

translocation from source leaves to sinks (*18*). Such a restriction might have contributed to the greater biomass allocation to shoots in standard regime than in field and simulated field conditions in this study.

In our study, seedlings grew less in the field and simulated field conditions than in the standard conditions (Fig. 2). This indicates that the standard condition was more favorable than the other two conditions for seedling growth probably due to high light intensity (600 μmole $m^{-2}s^{-1}$) integrated over a longer summation of daylength. Also, temperatures in the field and simulated field conditions were on average cooler than in the standard condition and varied in a wide range. Some of the low and high temperatures might have been stressful to the seedlings under field and simulated field conditions, or the greater warmth in the standard conditions may have promoted more seedling shoot growth. Ransom soybean and Blue Lake bean grew less in programmed diurnal temperatures than in the average day and night temperatures obtained from the program (*3*). Therefore, field and simulated field conditions may not be the most favorable for plant growth in some situations, which further indicates the importance of simulating realistic field conditions in controlled environmental studies if results are to be extrapolated to the real world.

The 24 black spruce families varied significantly in their shoot, root and total dry mass (mean family total dry weight ranged from 0.126±0.027 g to 0.266±0.023 g), height and branch number. However, their relative performances in total growth and growth components did not vary significantly among the three growing conditions: no family×growth regime interactions were

detected (Table 1). With respect to dry matter partitioning, the standard regime produced seedlings where family variation in root/shoot partitioning was actually more similar to field results than seedling from the simulated field regime. That is, when analyzed by growth regime, family effects in the analysis of covariance between shoot and root dry weights were statistically significant ($p<0.05$) in the field and standard regimes and family slope estimates were related ($r=0.44$) between these two environments; there was no statistically significant ($p>0.10$) variation in family allometry under the simulated field regime and there was no relationship among family slopes between the field and simulated field regimes.

In nature, climatic conditions, such as temperature, light and humidity, change in a coordinated way both diurnally and seasonally. Plants are adapted to such changes, and many of their physiological and phenological responses, such as germination, photosynthesis, timing of flowering, leaf senescence, winter bud formation, dormancy development, and bud opening, to environments are closely controlled by these changes (*4, 5, 7*). However, our study was conducted for only one season while the seedlings were all in the free-growth stage. In studies over one growing season, black spruce seedlings become increasingly dependent on fixed growth and bud-set and needle primordia initiation will be dependent on daylength responses and autumn climate (*14*). In addition, genetic differences in root/shoot partitioning can be greatly impacted by differential timing of budset (*10, 11*). In a study examining the potential for differential black spruce family responses to climate change, Wang *et al.* (*19, 20*) did not detect G×E interactions until the second simulated growth season. Early selection, sometimes in controlled environments, is now included in operational tree breeding programs (*1*). Although, with the exception of dry matter partitioning, differential family performance was not detected in our study, seedlings from the simulated environment were phenotypically much more similar to the field grown trees. Although not demonstrated in our study, this suggests that climate simulation might also produce more reliable genetic responses, particularly for multi-season studies when photoperiodic and thermoperiodic responses are important. Experiments as we have performed here, but for multiple seasons, are still needed to examine the necessity of environmental simulations in genetic studies.

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