Genetic Variation of Ecophysiological Responses to CO_2 in *Picea glauca* Seedlings

Qing-Lai Dang*, Jodi M. Maepea and William H. Parker

Faculty of Forestry and the Forest Environment, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario, Canada P7B 5E1

Abstract: To investigate genetic variation in the response of white spruce (*Picea glauca* (Moench) Voss) to CO_2 elevations, one-year-old seedlings of 11 provenances from Ontario were grown at 360, 530 and 700 µmol mol^{-1} CO_2 concentrations. After 60 days of treatment, CO_2 assimilation rate (A) at growth [CO_2] was 32% and 74% higher in the 530 and 700 µmol mol^{-1} CO_2 treatment, respectively, than in 360 µmol mol^{-1} [CO_2]. After 90 days, A under 530 and 700 µmol mol^{-1} [CO_2] was 141% and 223% greater, respectively, than under 360 µmol mol^{-1} [CO_2]. When measured at a common [CO_2] (360 µmol mol^{-1}), however, *A* under 530 and 700 µmol mol^{-1} [CO_2] treatment was 39% and 53% lower than in the 360 µmol mol^{-1} [CO_2] treatment, respectively, after 60 days of treatment, and 22 and 35% less, respectively, after 90 days. The CO_2 elevations also significantly suppressed V_{camx}, J_{max} and TPU after 60 days and 90 days of treatment. The [CO_2] elevations significantly reduced Stomatal conductance and transpiration after 60 days of treatment, but not after 90 days. g_s was 28% and 35% lower under 530 and 700 µmol mol^{-1} [CO_2], respectively, than under 360 µmol mol^{-1} [CO_2] after 60 days of treatment. *E* was 23 and 33% lower under 530 and 700 µmol mol^{-1}, respectively, than under 360 µmol mol^{-1} [CO_2]. Photosynthetic water use efficiency (WUE) was 80% and 157% higher after 60 days of treatment and 145% and 196% higher after 90 days, respectively, under 530 and 700 µmol mol^{-1} [CO_2] than under 360 µmol mol^{-1} [CO_2]. There were no significant between-provenance differences in any of the above responses; nevertheless, the ecophysiological performance of seedlings was closely correlated with the temperature and precipitation regimes of the seed origin. In addition, provenance significantly affected seedling total biomass, shoot mass, shoot/root ratio and height growth.

Keywords: White spruce, boreal forest, global climate change, photosynthetic down-regulation, photosynthetic acclimation to carbon dioxide concentration, seedling ecophysiology of boreal trees.

INTRODUCTION

Over the past 100 years, the concentration of atmospheric [CO_2] has increased from 270 µmol mol-1 (parts per million) to approximately 360 µmol mol^{-1}. Based on IPCC [1], a doubling of the CO_2 concentration in the atmosphere will occur by 2075. Elevated [CO_2] and its associated changes in temperature and precipitation regimes, are predicted to have a dramatic impact on the structure, distribution, productivity and health of boreal forest tree species. Climate change will most likely modify the composition of plant communities in the boreal forest due to the predicted northward migration of species following latitudinal and altitudinal shifts in climate regimes. If the predicted changes in climate become reality, recently established trees will experience different atmospheric CO_2 concentrations and climatic conditions during their rotation [2]. As a result, existing stands will potentially be less adapted to the future climatic conditions. Therefore, it will be essential that present and future planting stocks represent widely adapted populations and seed sources to ensure the long-term regeneration success [3]. Understanding and recognizing the potential effects of climate change on forests will allow resource managers to modify forest management planning strategies and policies to adequately encompass the possibility of climate variability and change and to minimize negative impacts on the health, sustainability, biodiversity, and socioeconomic benefits of the boreal forest.

It is widely accepted that an increase in elevated atmospheric [CO_2] will affect the physiological processes of boreal forest trees [2]. The atmospheric CO_2 concentration is an environmental factor that can limit the efficiency and chemical yield of photosynthetic activity in C_3 plants, including conifers [4]. Under unlimited supplies of water and nutrients, conifers exposed to short-term elevations of atmospheric CO_2 exhibit increased photosynthetic activity [5-7]. However, evidence suggests that the response of photosynthetic processes to an increase in ambient CO_2 is not constant through time. Multiple studies have concluded that exposure to long-term elevations of atmospheric [CO_2] may result in the photosynthetic down-regulation of many tree species [8-14]. This down-regulation in photosynthetic activity may be attributed to the reduction of the amount and activity of the Rubisco enzyme, which catalyzes the carboxylation of RuBP and subsequent synthesis of carbohydrates [15].

Stomata respond to changes in internal CO_2 concentration (C_i) [16]. An increase in C_i will result in a reduction in g_s and a subsequent reduction in leaf-level transpiration (E) and increased water use efficiency (WUE). Stomatal response to C_i as a mechanism for the reduction in g_s under elevated [CO_2] is well documented over a range of herbaceous plant and tree species [5, 6, 8, 12-14, 17-19]. Increases

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*Address correspondence to this author at the Faculty of Forestry and the Forest Environment, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario, Canada P7B 5E1; Tel: (807) 343-8238; Fax: (807) 343-8116;
E-mail: qinglaidang@hotmail.com

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in WUE have also been reported in many studies for well-watered plants [7, 8, 13, 14] and may be beneficial to plants exposed to drought [20].

Seedlings have also shown enhanced rates of growth due to increased carbon uptake under elevated [CO_2] [21]. Eamus and Jarvis [7] and Curtis and Wang [6] have reported on average a 30 – 40% increase in plant dry mass across a range of herbaceous and tree species under doubled [CO_2]. However, the initial growth enhancement may not be sustained over time, possibly as a result of photosynthetic down regulation or shifts in biomass allocation and ontogeny [22, 23].

White spruce [*Picea glauca* (Moench) Voss] is a commercially important tree species in Canada. The physiological and morphological attributes of white spruce are highly variable over its range of geographic distribution as a result of ecological and climatic differences, population sizes, and mating systems [24]. The results of white spruce provenance studies have revealed geographic variation in morphological and physiological attributes including height growth, seedling phenology, branch, root and needle length, number of stomata, and branch pubescence [25, 26]. Despite the numerous studies that have examined geographic variation in various morphological and phenological attributes of white spruce, few studies have investigated genetic variation in physiological traits [1, 18]. While genetic variations among and within populations in response to [CO_2] elevation have been observed for a wide range of coniferous and hardwood species [27-34], there are no such studies reported on white spruce.

Genotypic variations in the physiology and growth of a tree species can enhance its ability to adapt and acclimate to diverse environmental conditions. Understanding how specific white spruce seed sources respond to elevated [CO_2] in terms of physiology and growth is necessary to predict the projected impact of climate change on the boreal forest.

This study investigated provenance variations in physiological and growth response of one-year old white spruce seedlings from 11 Ontario provenances to elevated [CO_2] for one growing season. The objectives of this study were:

1) To investigate the effects of elevated [CO_2] on the physiology (photosynthesis, stomatal conductance, transpiration, water-use efficiency, and intercellular to ambient CO_2 concentration ratio), biomass, and biomass allocation and growth of one-year old white spruce seedlings.
2) To examine genetic variation among 11 Ontario seed sources of white spruce in the above responses.
3) To determine if the physiological and growth response of young white spruce from distinct genetic sources represent adaptations to local climate conditions.

The findings of this study will provide fundamental information on the appropriate selection and mechanisms of adaptation of individual seed sources of white spruce that will best be able to maximize the utilization of increasing atmospheric [CO_2].

MATERIALS AND METHODS

Experiment Design

The experiment design was a split plot design. The two treatment factors were CO_2 concentration and provenance. The CO_2 concentrations were ambient (360 μmol mol^{-1}), 530 μmol mol^{-1} (1.5 × ambient), and 700 μmol mol^{-1} (2 × ambient). Due to the limited number of greenhouses equipped with [CO_2] controlling and monitoring equipment, replication of the [CO_2] treatments was not possible. The CO_2 treatments represented the main plots. Two benches were randomly located within each CO_2 treatment. Each bench contained a complete replication of all 11 provenances of white spruce (sub-plot, six trees per provenance). The error term for testing CO_2 effects was benches nested with CO_2 treatment and that for testing provenance was the interaction between CO_2 and provenance. Two rows of border seedlings surrounded the test seedlings were used to minimize any edge effects.

Plant Materials

White spruce seeds from 11 sources of different geographic origins in Ontario were obtained from the Canadian Forest Service. The location and elevation of the 11 seed sources are summarized in Table **1**.

Table 1. Seed Source Origins of 11 Ontario White Spruce Provenances

Seed Source	Source	Source ID	Location	Latitude (°N)	Longitude (°W)	Elevation (m)
78	OFRI	7	Bentinck	44.17	81.00	305
1	OFRI	22	Cornwall	45.07	74.83	80
17	CFS	8032	Antrim	45.32	76.18	121
58	CFS	8166	Sinclair Twp.	45.47	79.08	370
86	OFRI	74	Proctor	46.33	82.50	249
121	CFS	8087	Pigeon River	48.02	89.65	306
124	CFS	8088	Shebandowan	48.62	90.18	459
96	CFS	8067	Strathearn	48.72	85.87	335
112	LU	2001.3	Mountain Bay	48.91	87.77	195
81	CFS	8053	Fraserdale	49.03	81.58	215
113	CFS	8078	Auden	50.15	87.88	335

Note: OFRI – Ontario Forest Research Institute; CFS – Canadian Forest Service; LU – Lakehead University.

The Seeds were stratified at approximately 3°C for two weeks and then were sown in 140 mL Jiffy containers at the Lakehead University Greenhouse. Day/night temperatures were set at 30/20°C for the germination phase, which lasted 3 weeks.

When the germination phase was complete, a thermoperiod of 22/12°C was used throughout the remainder of the experiment. Photoperiod was set at 16 hours. For the first 8 weeks after the germination phase, seedlings were fertilized with a starter fertilizer (11-41-8) progressing from 50 to 100 μmol mol^{-1} nitrogen to promote root growth. The fertilizing regime then switched to alternating 20-20-20 and 20-8-20 at 100 μmol mol^{-1} nitrogen, which was progressively increased to 175 μmol mol^{-1} by the end of the first growing season.

Procedures for hardening off of the seedlings started with an application of an 8-20-30 fertilizer in the fall and the seedlings were then blacked-out (kept in darkness) for 14 hours/day for a two-week period. During the hardening off phase, the seedlings were transferred from the greenhouse to an outdoor shade house where they remained until January. The seedlings were watered naturally by precipitation during that time.

Growth Conditions and CO_2 Treatments

The frozen seedlings were thawed at ambient [CO_2] at a 10/ 5°C thermoperiod for seven days and then a 14/7°C thermoperiod for additional two days. After thawing, the Jiffy seedlings were transplanted into 1.4 dm^3 (10.7 x 10.7 x 12.5cm) pots containing a mixture of peat and vermiculite (50/50, v/v). A starter fertilizer (11-41-8) at 100 μmol mol^{-1} N was applied to all seedlings upon commencement of the treatment period to stimulate root growth.

The seedlings were randomly assigned to the three CO_2 treatments (ambient (approximately 360 μmol mol^{-1}), 530 μmol mol^{-1}, and 700 μmol mol^{-1}). The thermoperiod was set at 22/12°C. A two-hour temperature ramping period in the morning and evening was established to coincide with photoperiod. Photoperiod was set at 16 hours. During overcast conditions and early morning and evening hours, supplementary lighting was provided with high-pressure sodium lamps to maintain the photoperiod. Relative humidity was set at 50%. All seedlings were watered to saturation every three days. All seedlings were fertilized once a week with alternating 20-20-20 and 20-8-20 at 150 μmol mol^{-1} N. The electroconductivity of the fertilizer solution was tested to ensure that a uniform concentration was being applied to all seedlings.

The CO_2 concentration and other environmental conditions in the greenhouses were monitored and controlled continuously using an ARGUS® computerized environment control system (Vancouver, Canada). The actual average CO_2 concentrations for the duration of the experiment were 337.5 +/- 16 μmol mol^{-1}, 545 +/-34 μmol mol^{-1}, and 687+/-34 μmol mol^{-1}, respectively for the three CO_2 treatments.

Gas Exchange Measurements

Three seedlings per provenance, block, and CO_2 treatment combination were randomly selected for gas exchange measurements. A PP-Systems CIRAS-l gas exchange system with a Parkinson's Leaf Cuvette for conifers (PP-Systems, Haverhill, MA, USA) was used for the measurements. The CO_2 concentration within the chamber was set to correspond with the growth concentration. Additionally, to evaluate photosynthetic down-regulation, the gas exchange of seedlings subjected to the elevated [CO_2] treatments was also measured under ambient [CO_2]. PAR flux density and air temperature within the leaf chamber were set at 800 μmol m^{-2} s^{-1} and 22.0°C, respectively. The order of measurements among blocks and CO_2 treatments was randomized. However, for operational efficiency, seedlings within the same block were sampled consecutively. Gas exchange measurements were taken on the current-year needles of the terminal shoot. The following parameters were measured and/or calculated according to von Caemmerer and Farquhar [35]: net CO_2 assimilation (A), stomatal conductance (g_s), leaf transpiration rate (E), and the intercellular to leaf surface CO_2 concentration ratio (C_i/C_a). The projected leaf area for each sample was determined using a Regent WinSeedle® leaf analysis system (Regent Instruments Inc., Quebec City, Quebec, Canada). Photosynthetic water use efficiency (WUE) was calculated as A/E. Six provenances were selected randomly to the measurement of A/C_i curves (one curve per provenance). The maximal carboxylation rate (V_{cmax}, μmol m^{-2}s^{-1}), light saturated electron transport rate contributing to RuBP regeneration (J_{max}, μmol m^{-2}s^{-1}), triose phosphate utilization (TPU, μmol m^{-2}s^{-1}), the rate of dark respiration (R_d) and CO_2 compensation point (Γ) were calculated from the A/C_i curves using the Photosyn Assistant software (Dundee Scientific, Scotland, UK). Measurements were taken after 60 and 90 days of treatments.

Height, Root Collar Diameter (RCD), and Biomass Measurements

After 90 days of treatment, seedling height was measured from the root collar to the base of the terminal bud. RCD was measured using a pair of digital calipers. The seedlings were harvested and dried for 48 hours at a temperature of 70°C. Oven-dry weights (ODW) of the shoot and root for each seedling were measured. Total seedling ODW and the shoot to root ratio (S:R) were then calculated.

Statistical Analysis

Statistical analysis was performed using SAS/STAT® 8.2 [36]. The GLM procedure combined with the RANDOM/test statement was used to analyze the gas exchange, growth and biomass parameters as a mixed model ANOVA. TEST statements that tested [CO_2] against block and provenance against the block*provenance interaction were incorporated into the model to account for the nonstandard error structure of the split-plot design. When ANOVA showed a significant effect ($p<0.05$), multiple comparisons were performed using Bonferroni t-tests. Since only one A/C_i curve was measured for each provenance, provenance effect was not analyzed for parameters derived from the A/C_i curves.

Multiple linear regressions were performed to investigate the relationships between the response of growth and ecophysiological parameters and monthly and seasonal temperature and precipitation associated with each of the 11 provenances ($p<0.05$). The temperature and precipitation data were obtained from McKenny [37].

RESULTS

Gas Exchange

After 60 days of treatment, CO_2 elevations resulted in a significant increase in CO_2 assimilation rate (A) (Table **2**). Relative to the value in the ambient [CO_2], the increase was 32% and 74%, respectively, for the 530 and 700 μmol mol^{-1} CO_2 treatment. The *A* in the 700 μmol mol^{-1} CO_2 treatment was 32% higher than that at 530 μmol mol^{-1} [CO_2]. No provenance effects or CO_2*P interaction effects were found to have significantly influenced *A* after 60 days of treatment. Despite the lack of statistically significant interactions between provenance and [CO_2], P96 (Strathearn) and P112 (Mountain Bay) appeared to be less responsive to CO_2 elevations than other provenances (Fig. **1a**).

After 90 days of treatment, the CO_2 assimilation rate of seedlings exposed to the 530 and 700 μmol mol^{-1} [CO_2] treatments was 141 and 223% greater than those grown at the ambient [CO_2] (Table **2**, Fig. **2a**). Furthermore, there was a 34 % increase in *A* in seedlings grown in 700 μmol mol-1 compared to the 530 μmol mol^{-1} [CO_2]. Again, there was no significant provenance or CO_2*P interaction effect (Table **2**).

When measured at a common ambient CO_2 concentration (360 μmol mol^{-1}), growth CO_2 concentration was still a significant source of variation in the rate of CO_2 assimilation (A_{360}) after 60 and 90 days of treatment (Table **3**). A_{360} of seedlings grown at 530 and 700 μmol mol^{-1} [CO_2] was 39% and 53% lower than that of seedlings grown at ambient [CO_2], respectively, after 60 days of treatment, and 22 and 35% less than that of seedlings grown at ambient [CO_2], respectively, after 90 days of treatment (Fig. **3**, Table **3**). The CO_2-P interaction or provenance did not significantly influence A_{360} (Table **3**).

The CO_2 elevations significantly suppressed V_{camx}, J_{max} and TPU ($P<0.05$) both after 60 days and 90 days of treatment, but there were no significant differences in these parameters between the two elevated CO_2 treatments (Fig. **3**). The CO_2 elevations did not significantly influence Γ or R_d in either measurement ($P>0.05$, Fig. **3**).

The [CO_2] elevations significantly reduced Stomatal conductance and transpiration after 60 days of treatment, but the effects became statistically insignificant after 90 days of treatment (Table **2**, Figs. **1** and **2**). Mean g_s was 28 % and 35% lower in seedlings grown at 530 and 700 μmol mol^{-1} [CO_2] than at ambient [CO_2] respectively, after 60 days of treatment (Fig. **2b**). E was 23 and 33% lower, respectively, for seedlings grown at 530 and 700 μmol mol^{-1} than those grown at ambient [CO_2] (Fig. **1c**). However, there were no significant differences between the two elevated CO_2 treatments in g_s or *E* ($p>0.05$, Fig. **2b**).

The intercellular to leaf surface CO_2 concentration ratios (C_i/C_a) generally decreased with increasing [CO_2] (Figs. **1** and **2**), but the effect was statistically not significant ($p>0.05$, Table **2**). No provenance or CO_2*P effects were found to significantly influence C_i/C_a after 60 days or 90 days of treatment (Table **2**).

In contrast to *E* and g_s, WUE increased with increasing [CO_2] both after 60 and 90 days of treatment (Table **2**, Figs. **1d** and **2d**). In comparison to the value in the ambient CO_2 treatment, the increase in the 530 and 700 μmol mol^{-1} [CO_2] treatments was 80% and 157%, respectively, after 60 days and 145% and 196%, respectively after 90 days of treatment. There interaction effect between provenance and [CO_2] became significant after 90 days of treatment. Although the provenance effect became more significant as well after 90 days (P = 0.07 versus P = 0.67 after 60 days), it was still statistically insignificant at $\alpha = 0.05$ (Table **2**).

Table 2. P-Values from ANOVA for Gas Exchange Measurements from 11 Ontario Provenances of One-Year Old White Spruce Seedlings Exposed to Ambient, 530, and 700 μmol mol^{-1} [CO_2]. The Measurements were Taken After 60 Days and 90 Days of Treatment

After 60 Days of Treatment

		A		g_s		C_i/C_a		*E*		WUE	
Source	**df**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**
C	2	74.48	0.003*	3955	0.02*	0.120	4.60	1.35	0.04*	481.88	0.007*
P	10	4.14	0.13	97	0.73	0.004	0.55	0.03	0.77	4.24	0.67
CP	20	3.13	0.27	256	0.07	0.014	0.07	0.09	0.07	3.77	0.82
Error	132	3.91		256		0.011		0.08		5.31	

After 90 Days of Treatment

		A		g_s		C_i/C_a		*E*		WUE	
Source	**df**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**	**MS**	**P**
C	2	345.43	0.009*	180.5	0.56	0.109	0.08	0.32	0.28	255.54	0.014*
P	10	2.60	0.70	538.8	0.21	0.019	0.07	0.21	0.16	3.89	0.07
CP	20	5.78	0.12	504.6	0.22	0.011	0.40	0.21	0.12	5.72	0.004*
Error	132	4.89		287.8		0.008		0.10		2.95	

Note: 1) *A* = Assimilation rate, g_s = stomatal conductance, *E* = leaf transpiration rate, WUE = water use efficiency, C_i/C_a = intercellular to leaf surface CO_2 concentration ratio, C = CO_2, P = provenance.
2) * Indicates significant difference at $p \leq 0.05$.

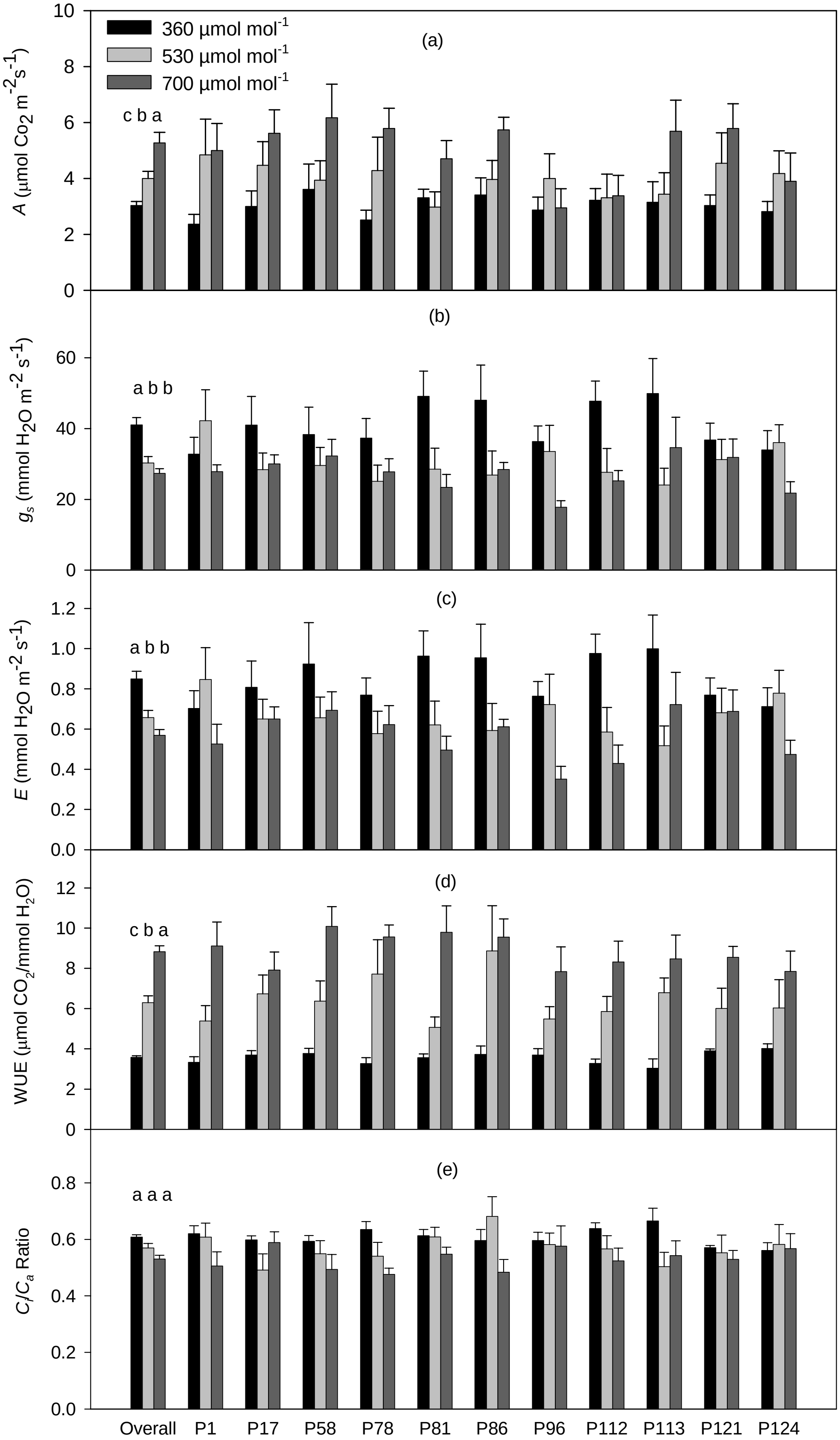

Fig. (1). Net rate of photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), photosynthetic water use efficiency (WUE), and intercellular to leaf surface CO_2 concentration ratio (C_i/C_a) of current foliage in one-year old white spruce seedlings. The gas exchanges were measured at the corresponding growth CO_2 concentrations after 60 days of treatments. Means with different letters indicate significant differences ($P<0.05$). The labels on the x-axis are the seed source codes explained in the text.

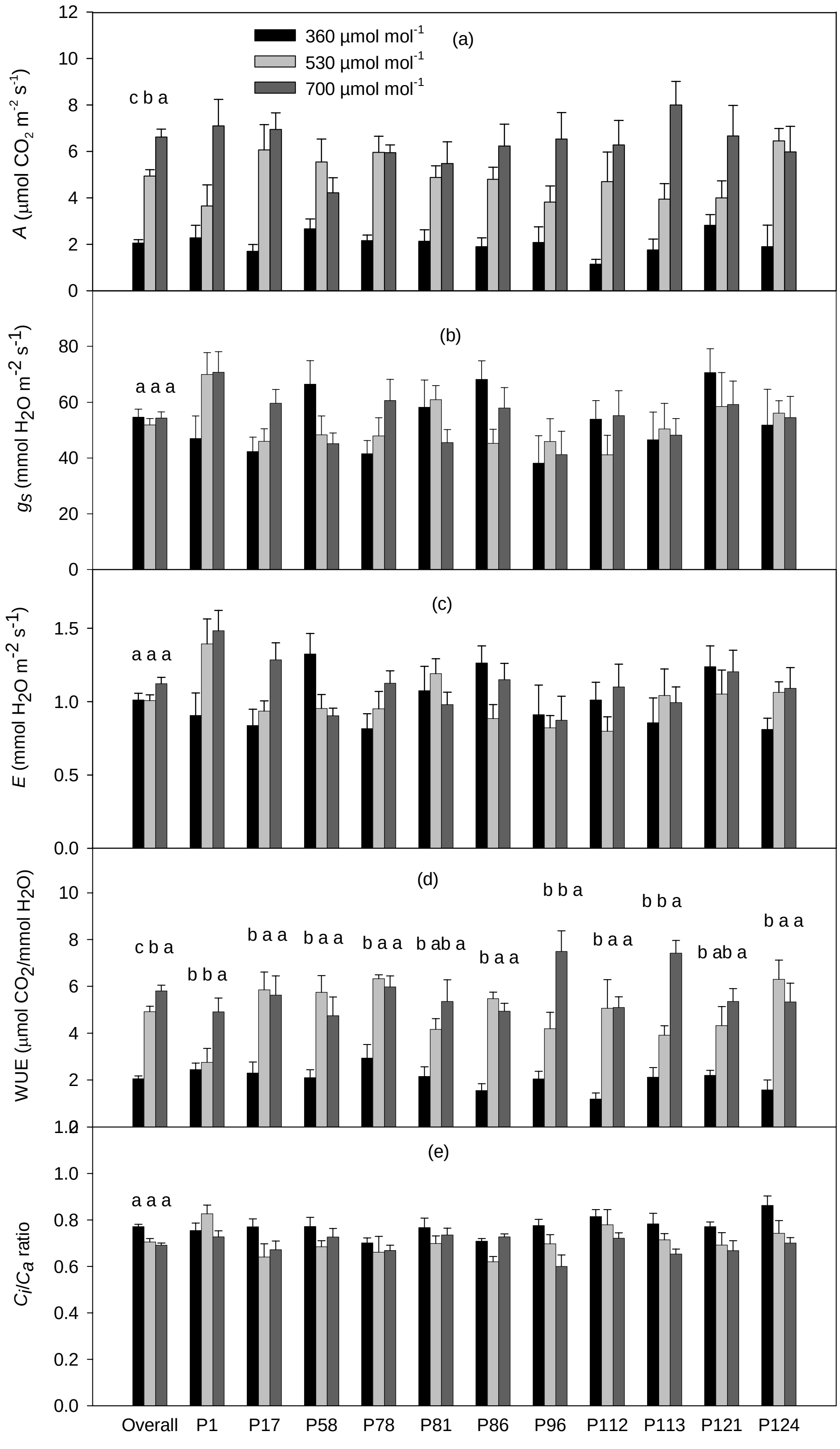

Fig. (2). Net rate of photosynthesis (*E*), stomatal conductance (g_s), transpiration (*E*), photosynthetic water use efficiency (WUE), and intercellular to leaf surface CO_2 concentration ratio (C_i/C_a) of current foliage in one-year old white spruce seedlings. The gas exchanges were measured at the corresponding growth CO_2 concentrations after 90 days of treatments. Means with different letters indicate significant differences ($P<0.05$).

Table 3. **ANOVA Results for CO_2 Assimilation Rate from Seedlings Grown Under Ambient and Elevated CO_2 Concentrations Measured at Ambient [CO_2] After 60 and 90 Days of Treatment**

Source	df	60 Days		90 Days	
		MS	P	MS	P
C	2	46.83	0.01*	25.81	0.03*
P	10	0.23	0.96	1.98	0.28
CP	20	1.12	0.12	1.00	0.84
Error	132	1.71		2.16	

Note: * indicates significant at $p \leq 0.05$.

Biomass and Growth

[CO_2] or [CO_2]-provenance interaction did not significantly affect any of the growth or biomass parameters (Tables **4** and **5**). However, there were significant variations among provenances in seedling total biomass, shoot mass, shoot/root ratio and height (Tables **4** and **5**, Fig. **4**). Seedlings from P86 (Fraserdale), P17 (Antrim), and P1 (Cornwall) exhibited significantly greater shoot biomasses than seedlings from P113 (Auden). In terms of total biomass, significantly higher values were observed in seedlings from P86 and P17 than from P113. Seedlings from P86 and P1 (Cornwall) had significantly greater shoot/root ratios than those from P96 (Strathearn) and P124 (Shebandowan). Seedlings

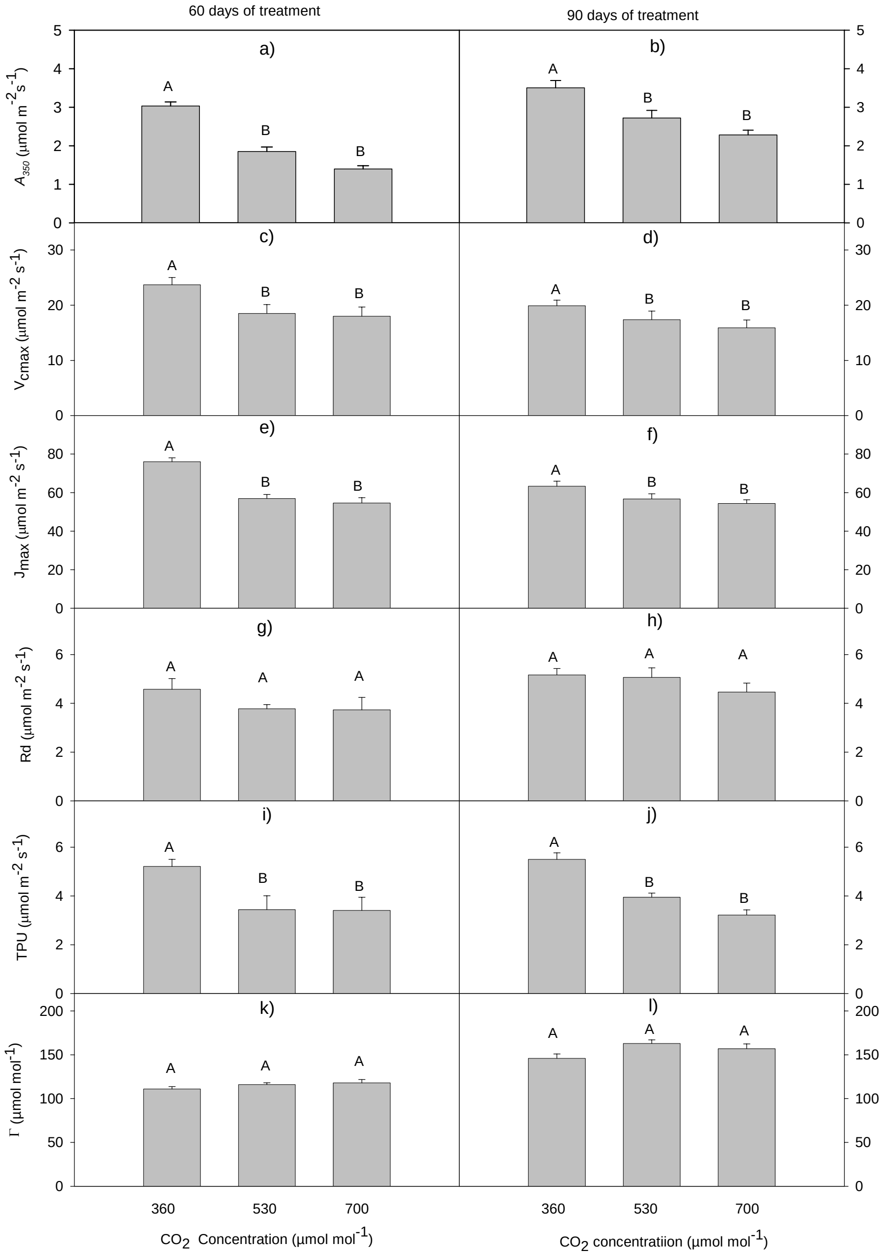

Fig. (3). Net rate of photosynthesis measured at a standard CO_2 concentration for all the seedlings (A_{350}), maximum rate of Rubisco carboxylation ($V_{cmax,}$), maximum rate of photosynthetic electron transport (J_{max}), rate of dark respiration (Rd), rate of triose phosphate utilization (TPU) and the CO_2 compensation point of photosynthesis. Other explanations are as in Fig. (**1**).

of P112 (Mountain Bay) were significantly higher than those of P113 (Auden) and 121 (Pigeon River) (Fig. **4**).

Table 4. P-Values from ANOVA for Biomass Measurements from 11 Ontario Provenances of One-Year Old White Spruce Seedlings Exposed to Ambient and Elevated [CO_2] for 90 Days

Source	Df	SM		RM		TM		S:R	
		MS	P	MS	P	MS	P	MS	P
C	2	2.29	0.33	0.3	0.63	3.34	0.50	2.64	0.56
P	10	7.61	0.01*	0.3	0.47	9.48	0.01*	1.96	0.03*
CP	20	2.46	0.17	0.2	0.64	3.71	0.36	0.92	0.36
Error	132	2.14		0.3		3.57		0.74	

Note: 1) SM = shoot dry mass, RM = root dry mass, TM = total seedling dry mass, S:R = shoot to root ratio.
2) * indicates significant difference at $p \leq 0.05$.

Table 5. P-Values from ANOVA for Height and Root Collar Diameter (RCD) from 11 Ontario Provenances of One-Year Old White Spruce Seedlings Grown Under Ambient and Elevated [CO_2] for 90 Days

Source	Df	Height		MS	RCD
		MS	P		P
C	2	132.7	0.25	0.01	0.98
P	10	74.9	0.004*	0.91	0.09
CP	20	23.1	0.16	0.58	0.32
Error	132	29.8		0.52	

Note: * indicates significant difference at $p \leq 0.05$.

Relationships Between Climate Variables and Gas Exchange, Biomass, and Growth of Seedlings

After 90 days of treatment, monthly precipitation, monthly maximum temperature, and mean temperature during the wet and dry quarters of the year accounted for a high percentage of the variation in *A*, *E*, g_s and WUE (up to 93%, Table **6**). The significant climate variables tended to differ for seedlings grown under different [CO_2] (Table **6**). *A*, *E*, and g_s appeared to have become more closely correlated with monthly temperatures but less closed correlated with precipitation as [CO_2] increased, whereas WUE showed an opposite trend (Table **6**). The Ci/Ca ratios were generally poorly correlated with the climate variables, with the exception of June and August precipitation under ambient CO_2 concentration.

Climate variables explained 63 to 91% of the variation in total seedling biomass and shoot to root ratio over the three CO_2 treatments (Table **7**). Monthly precipitation, seasonal mean temperatures, and fall minimum and maximum temperatures were the dominant variables. Compared to the gas exchange and biomass measurements, climate variables were relatively weaker in predicting the growth measurements of height and root collar diameter, which is evident in the absence and/or lower R^2 values (Table **7**).

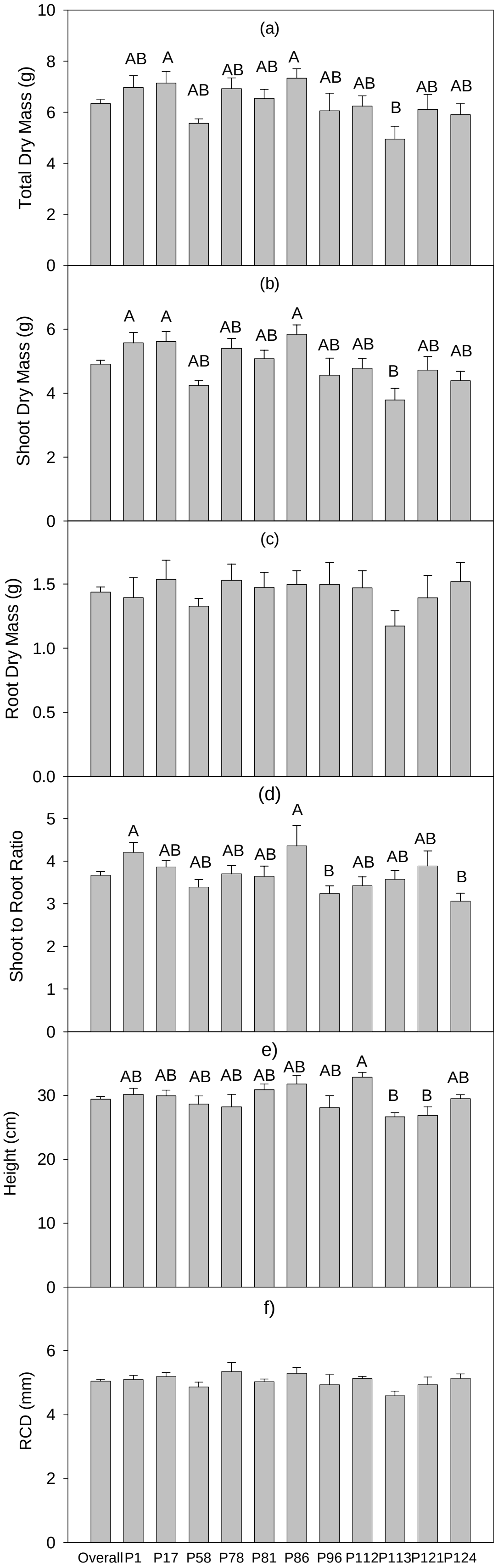

Fig. (4). Seedling total dry mass, shoot dry mass, root dry mass, shoot:root ratio, height and root collar diameter. The measurements were taken at the end of the experiment. Other explanations are as in Fig. (**1**).

Table 6. R^2 Values for Multiple Regressions Between Gas Exchange Parameters and Climate Variables from 11 Ontario Provenances of One-Year Old White Spruce Seedlings After 90 Days of Exposure to 360, 530 and 700 µmol mol^{-1} CO_2

Parameter	360 µmol mol^{-1}		530 µmol mol^{-1}		700 µmol mol^{-1}	
	Climate Variable(s)	R^2	Climate Variable(s)	R^2	Climate Variable(s)	R^2
A	PrecipDP SepPrecip OctPrecip	0.789	MtempWetQ MayPrecip SepPrecip DecPrecip	0.922	TempSeas MTempDryQ MayPrecip	0.931
g_s	None(p<0.05)	0.000	JulyMinTemp JulyPrecip	0.738	DiurnRan PrecipSeas AugMaxTemp	0.885
E	PrecipWetQ SepPrecip	0.641	MTempWarmQ	0.690	JunMaxTemp AugMaxTemp	0.876
WUE	MTempDryQ TempRanP3 MarMaxTemp	0.906	MtempWetQ PrecipWP MayPrecip DecPrecip	0.926	MayPrecip	0.423
C_i/C_a	JunPrecip AugPrecip	0.802	None(p>0.05)	0.000	None(p>0.05)	0.000

Note: PrecipDP = in driest period of the year, SepPrecip = September precipitation, OctPrecip = October precipitation, MtempWetQ = mean temperature in wettest quarter of the year, MayPrecip = May precipation, DecPrecip = December precipitation, TempSeas = Temperature seasonality (i.e., difference in temperature between coldest and warmest seasons), MTempQ = precipitation in driest quarter of the year, JulyMinTemp = July minimum temperature, JulyPrecip = July precipitation, DiurnRan = mean diurnal range of temperature, AugMaxTemp = August maximum temperature, MTempWarmQ = mean temperature in warmest quarter, JunMaxTemp = June maximum temperature, TempRanP3 = temperature range for period 3 (i.e., the entire growing season starting on date following March 1 after 5 consecutive days with mean daily temperature greater or equal to 5 degrees and ending on date following August 1 when minimum daily temperature falls below -2 degrees. Units are days), MarMaxTemp = March maximum temperature, PrecipWP = precipitation in wettest period.

Table 7. R^2 Values from Multiple Regressions Between Growth and Biomass Measurements and Climate Variables from 11 Ontario Provenances of One-Year Old White Spruce Seedlings After 90 Days of Exposure to Ambient (360), 530 and 700 µmol mol^{-1} CO_2

Parameter	360 µmol mol^{-1}		530 µmol mol^{-1}		700 µmol mol^{-1}	
	Climate Variable(s)	R^2	Climate Variable(s)	R^2	Climate Variable(s)	R^2
Total biomass	MTempDryQ AnnPrecip Marprecip	0.887	TprecipP4 NovMaxTemp NovPrecip	0.887	OctMinTemp	0.629
Shoot:Root	DayStart MTempP3	0.762	MtempWarmQ AprPrecip MayPrecip	0.864	NovMinTemp FebPrecip NovPrecip	0.910
Height	None(p>0.05)	0.000	None(p>0.05)	0.000	None(p>0.05)	0.000
RCD	TempRan	0.478	DiurnRan JulyPrecip	0.677	MarPrecip NovPrecip	0.568

Note: MTempDryQ = mean temperature in driest quarter, AnnPrecip = annual precipitation, MarPrecipP4 = March precipitation, TPrecip = total precipitation for period 4, NovMaxTemp = November maximum temperature, NovPrecip = November precipitation, OctMinTemp = October minimum temperature, Day-Start = Julian day for start of the growing season, MTempP3 = mean temperature for period 3, MTempWarmQ = mean temperature in warm4est quarter, AprPrecip- April precipitation, MayPrecip = May precipitation, FebPrecip = February precipitation, TempRan = annual temperature range, DiurnRan = mean diurnal range of temperature.

DISCUSSION

Net CO_2 assimilation of one-year old white spruce seedlings was enhanced by CO_2 elevations, despite reductions in photosynthetic capacity. Net A was enhanced by 223% after 90 days in seedlings exposed to 700 µmol mol^{-1} as compared to the ambient CO_2 concentration. The increase in *A* is generally greater than those in the literature. In a meta-analysis of [CO_2] responses in trees, Curtis and Wang [6] reported a 54 % increase in *A* in studies that were conducted in greenhouses with pot sizes less than 2.4L. Gunderson and

Wullschleger [10] estimated an overall increase in tree photosynthetic activity of 44% in response to high atmospheric [CO_2] levels.

The increase in net photosynthesis may largely be explained by an increase in carboxylation efficiency of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco). Under ambient atmospheric CO_2 concentrations, Rubisco is CO_2 substrate-limited. As [CO_2] partial pressure increases, the ratio of oxygenation to carboxylation decreases, thereby increasing net photosynthesis over photorespiration [15].

Jach and Ceulemans [38, 39] reported that significant variation existed in the response of *A* to elevated CO_2 concentrations in *Pinus sylvestris* L. as a result of needle age (current year versus one-year old foliage), seedling age, and time of growing season. In this study, photosynthetic measurements were only taken on current year needles. Thus, the results of this study should not be extended to all needle ages, tree ages, or all times of the growing season.

When measured under ambient CO_2 concentration, mean *A* of the current foliage of one-year old white spruce seedlings grown at elevated [CO_2] was lower than that of seedlings grown at ambient [CO_2], which suggests that the seedlings in the present study exhibited photosynthetic down-regulation as a result of exposure to 530 and 700 µmol mol^{-1} CO_2 concentrations after 90 days of treatment. This conclusion is further supported by the declines in V_{cmax} and J_{max}. Photosynthetic down-regulation has been reported in some, but not all studies investigating the effects of elevated concentrations of CO_2 on trees. These results agree with those found by Centritto and Jarvis [28] for *Picea sitchensis* (Bong.) Carr., Tissue *et al.* [40] for *Pinus ponderosa* Dougl. ex P. & C. Laws, Zhang and Dang [14] and Cao *et al.* [8] for *Pinus bankciana* Lamb and *Betula papyrifera* Marsh.

Photosynthetic down-regulation is most prominent in plants under nutrient and rooting volume limitations and/or reduced sink strength [12, 21]. When the seedlings were extracted from the pots after the gas exchange measurements, rooting volume did not appear to be limited by pot size in this study. However, since the fertilization regime was the same for all treatments in the experiment and the seedlings in under elevated CO_2 grew faster, presumably having greater demand for nutrients, the seedlings under elevated CO_2 concentrations may have been limited by nutrients as compared to those under ambient CO_2, thus resulting in photosynthetic down-regulation. Cao *et al.* [8] have reported that the magnitude of photosynthetic down-regulation in *Betula papyrifera* marsh decreases with increasing nutrient supply.

This study suggests that stomata can acclimate to CO_2 concentrations. The CO_2 elevations significantly reduced g_s and E of white spruce seedlings after 60 days of treatment, but the effects became insignificant after 90 days of treatments, suggesting that the stomata became less sensitive or insensitive to CO_2 concentrations following a longer period of exposure to elevated CO_2 and consequently stomatal conductance became less limiting to photosynthesis. This acclimation explains the greater increases in photosynthetic rates in seedlings under elevated CO_2 in comparison to those under ambient CO_2. In a meta-analysis of 48 studies, Curtis and Wang [6] reported an insignificant 11% reduction in g_s in response to elevated [CO_2]. Similarly, Roberntz and Stockfors [41] reported that elevated [CO_2] had no significant effect on g_s in current year growth of 30-year-old *Picea abies* (L.) Karst. However, tree and leaf age has been reported to affect the degree of reduction in g_s, with responses from younger conifer seedlings and foliage being more prominent than those shown by older trees [38, 42]. Sage [12] and Ward and Strain [23] suggest that the lack of a significant response from g_s and E may be indicative of an acclimation response of the photosynthetic components of the white spruce seedlings to increased [CO_2] over time.

Despite the absence of a significant reduction in g_s and E, WUE was enhanced as a result of elevated [CO_2]. Increased WUE in response to CO_2 elevations has been reported for many species of well-watered plants [7] including young trees, such as *Pinus bankciana and Betula papyrifera* [8, 14], *Picea abies* and *Picea mariana* (Mill.) BSP [31, 33, 43, 44].

In spite of increased CO_2 assimilation rates, exposure to elevated [CO_2] did not significantly increase the biomass production and growth of young white spruce after 90 days of treatment. This result is in disagreement with the average biomass gains of 29% and 40% across a range of tree species reported [6-8, 14, 28, 30, 45]. A possible cause for the lack of biomass gain in the seedlings may have been the relative short length of the treatment period. Elevated CO_2 concentration also did not significantly affect biomass allocation. This finding agrees with the majority of the studies which have shown that elevated [CO_2] rarely alters the allocation of biomass between roots and shoots [6, 23, 46, 47].

The data did not show a significant provenance effect on gas exchange parameters of one-year old white spruce seedlings after 90 days of treatment. The lack of a provenance effect on the gas exchange variables agrees with the results reported by Houpis *et al.* [29] for *Pinus ponderosa* and by Liu *et al.* [27] for *Populus tremuloides* Michx. However, the significant relationships between gas exchange parameters and the local climate variables of seed origins for the provenances tend to suggest that there should have been differences in gas exchanges among the provenances. Furthermore, the probability to support the non-significance conclusion is marginal (0.07 as compared to the threshold of 0.05). Therefore, we conclude that the lack of statistical significance in gas exchange was due to the small sample size [27] that we used in the gas exchange measurements. A larger sample size will increase the degree of freedom for the error term and enhance the sensitivity of ANOVA for detecting significant differences [48].

Despite the absence of a significant provenance effect on the gas exchange of one-year old white spruce seedlings, there were strong correlations between the gas exchange variables [A, g_s, E, and WUE) and monthly climate variables for the 11 Ontario provenances of white spruce at ambient and elevated CO_2 concentrations. These findings agree with those reported by Liu *et al.* [26] for four provenances of *Populus tremuloides* from northwestern Ontario. The results show that local climates associated with the 11 provenances can be used to predict physiological processes of white spruce seedlings under ambient and elevated CO_2 concentrations. Furthermore, these results suggest that the physiological processes occurring within white spruce from genetically distinct sources are sensitive to changes in local precipitation and temperature levels and have adapted to local climates.

Local climate was able to predict physiological processes of white spruce seedlings grown in 530 and 700 μmol mol^{-1} [CO_2] to a noticeably lesser extent than those grown at ambient [CO_2]. Furthermore, the significant climate variables tend to be different between the different CO_2 treatments. These results may suggest that there is strong interactive effect between the environmental conditions of temperature, precipitation and atmospheric CO_2 concentration on white spruce physiology. Future studies that incorporate temperature and precipitation conditions that simulate those predicted in a future climate, in addition to elevated [CO_2], may improve the ability of local climates to predict physiological processes in high atmospheric [CO_2] environments.

In contrast to the lack of significant differences in gas exchange traits, substantial variation in the biomass and the heights and diameters of the white spruce seedlings existed between provenances. Genotypic variation in biomass, height, and diameter in white spruce has also been reported by Nienstaedt and Teich [26], Morgestern [24], and Morgenstern and Copis [49]. The largest seedlings which originated from P112 – Mountain Bay, P86 – Proctor, and P17 – Antrim did not appear to have any geographical relationship in terms of latitude, longitude, or elevation. The consistently smallest seedlings originated from the northernmost provenance in the study; P113 (Auden). Of all the provenances included in the study, the local climate associated with P113 was represented by the lowest mean annual temperature and precipitation levels, the shortest growing season, and the largest annual and diurnal temperature ranges [30]. The overall weak performance of P113 suggests that the extreme climate conditions in the Auden area had a considerable effect on the productivity of seedlings from that provenance in comparison to seedlings from the other provenances when grown in a common environment. The inconsistency between gas exchange and growth/biomass parameters in detecting inter-provenance differences suggests that gas exchanges parameters are not effective for examining differences between different provenances of white spruce. Gas exchange parameters are generally expressed on a per unit leaf area or leaf mass basis, as is the case in this study. However, the growth and biomass accumulation is determined by the total production of carbohydrates by the seedlings, which can be approximated by the product of exchange rate, total amount of leaf per tree (either leaf area or mass) and the length of time when photosynthesis is carried out. In other words, gas exchange rate is only one of the three factors affecting carbohydrate production and thus carbon budget and growth. Therefore, gas exchange data should be interpreted with caution. When the total carbohydrate production is important for answering a research question, we recommend using the total photosynthesis per plant rather than per unit leaf.

The CO_2*P interaction did not appear to significantly influence net assimilation, stomatal conductance, transpiration, or the atmospheric to intercellular CO_2 concentration ratio of one-year old white spruce seedlings. A lack of significance of the CO_2*P interaction on gas exchange of one-year old white spruce seedlings agrees with the findings of Liu *et al*. [26] for young *Populus tremuloides* and Johnsen and Seiler [30] for *Picea mariana*. Results from the present study disagree with those reported by Wang *et al*. [34] and Centritto and Jarvis [28] who found a significant CO_2*genotype interaction in photosynthetic responses of *Populus tremuloides* and *Picea sitchensis* respectively. There was a between-provenance difference in response of WUE to elevated [CO_2] after 90 days of treatment. This finding may suggest that the seedlings originating from provenances demonstrating higher WUE, P113 and P96, may perform better in a future climate in which the incidence of drought is predicted to increase [10].

Wang *et al*. [50] conducted a similar study investigating CO_2*provenance interactions on the physiology and growth of *Picea mariana*. Although they reported no significant CO_2*provenance interactions after one growing season, several traits were significantly affected by the interaction after a second growing season [33]. Thus, a study spanning two or more growing seasons is warranted in order to further understand the CO_2*provenance interaction on the physiology of young white spruce. Additionally, as discussed previously on provenance effect on gas exchange, the small sample size used in this study could have been a contributing factor for the non-statistical significance of the effect.

The ability of the physiological and growth processes of white spruce to acclimate to local climate conditions will be critical as the predicted changes in atmospheric CO_2 concentrations and temperature and precipitation regimes associated with global warming manifest themselves across Ontario. The lack of significant differences between difference provenances in their response to CO_2 elevations suggests that seed sources of white spruce in Ontario selected for superior growth characteristics in the present climate should remain to be superior in the predicted future climate.

The findings of this study pertain to a short-term greenhouse experiment. Further studies that span multiple growing seasons and that integrate the simulation of the future climate and resource availability conditions (temperature, precipitation, nitrogen availability) associated with elevated atmospheric [CO_2] are warranted in order to obtain a better understanding of the response of white spruce provenances in Ontario to climate change.

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