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Hoddinott, J.
Department of Botany University of Alberta

Scott, R.
Department of Botany University of Alberta

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THE INFLUENCE OF LIGHT QUALITY AND CARBON DIOXIDE ENRICHMENT ON THE COLD HARDINESS OF THREE CONIFER SPECIES SEEDLINGS

J. HODDINOTT* and R. SCOTT**

Department of Botany, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada.

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HODDINOTT J. and SCOTT R. *The influence of light quality and carbon dioxide enrichment on the cold hardiness of three conifer species seedlings.* BIOTRONICS **25**, 33–44, 1996. *Pinus banksiana, Picea mariana and Picea glauca* were grown in high and low red : far–red quantum flux ratios and CO_2 levels of 350, 700 or 1050 μmol mol^{-1}. Following growth in long days then short days, the seedlings were hardened in a 6/−5℃ thermoperiod in ambient CO_2 levels and red rich light and placed in dark storage at −2℃. After storage, seedlings were cold stressed at various temperatures down to −25℃ and then rewarmed. *Picea glauca* was more cold hardy than *Pinus banksiana* which was more cold hardy than *Picea mariana. Cold stressed Pinus banksiana* grown in far–red rich light had greater triphenyl tetrazolium chloride reduction values than red grown plants but the red grown plants were evaluated as being of higher quality after regrowth following stress. The (P–O)/O chlorophyll fluorescence value for all three species was a good predictor of seedling quality following regrowth after cold storage and cold stress. *Pinus banksiana* and *Picea mariana* grown in red light at ambient CO_2 level had the greatest survival potential following cold storage and cold stress. *Picea glauca* survival was enhanced by doubling the CO_2 level in either light quality.

Key Words : light quality ; CO_2 enrichment ; cold hardiness.

INTRODUCTION

It has been demonstrated that light quality, expressed as the quantum flux ratio, and CO_2 partial pressure in growth rooms influence the growth and physiology of *Pinus banksiana, Picea mariana* and *Picea glauca seedlings* (*9, 10*). Those results are of relevance to plants growing in the natural environment as trees are being subjected to increasing CO_2 levels as a result of human activity (*8*) and because seedlings germinating in areas without overtopping vegetation are exposed to red rich light while plants growing in sub–canopy shade are exposed to far–red rich light. The ratio of red to far–red light, the quantum flux

*Present address, Professional Development Centre, University of New South Wales, Sydney 2052, NSW, Australia.

**Present address, Grand Prairie College, Grand Prairie, Alberta.

or zeta ratio, influences plant growth through the photoequilibrium of phytochrome (*12*).

Trees have been classified according to their response to shade (*2*), *Pinus banksiana* as being very shade intolerant and *Picea mariana* and *P. glauca* as shade tolerant. Natural shade involves a reduction in irradiance by photosynthetically active radiation but, where the shade is due to a vegetation canopy, selective absorption of red light by leaf chlorophyll also causes qualitative changes in natural light and a change in the photomorphogenically significant quantum flux ratio. The present study investigates the influence of the quantum flux ratio on seedlings receiving similar levels of photosynthetically active radiation to allow the photomorphogenic effects of shade to be separated from those due to reduced irradiance.

It has been shown that in *Pinus banksiana* and *Picea mariana* low quantum flux ratios, particularly at high CO_2 levels, were most effective in altering growth, while low ratios and ambient CO_2 levels were most effective in *Picea glauca* (*9*). *Pinus banksiana* chlorophyll levels, chlorophyll O fluorescence and total nonstructural carbohydrate levels were most influenced by quantum flux ratio, while CO_2 level was most effective in influencing those levels in *Picea mariana*. *Picea glauca* levels were unchanged by those treatments (*10*).

Dormancy and frost hardiness is induced in this study by the application of short photoperiods and cool themoperiods (*1, 3*). High CO_2 concentrations have been shown to influence plant responses to cold stress (*11*) and drought stress (*4*) while light quality during the photoperiod or as a night break has been implicated in the development of frost hardiness (*6, 15*).

The influence of the simultaneous application of CO_2 and light quality on cold stress resistance and plant survival following cold storage has not been reported. The present study used two methods to assess treatment effects on plant resistance to freezing stress. The triphenyl tetrazolium chloride (TTC) reduction test detected freezing stress as a lower amount of TTC reduction in tissues due to the inactivation of dehydrogenase enzymes (*13*), while the fluorescence test detected stress as a reduced level of chlorophyll variable fluorescence caused by the inactivation of the water splitting enzyme system in chloroplasts (*16*). The tests demonstrated that *Picea glauca* appeared more cold hardy than *Pinus banksiana* which was more cold hardy than *Picea mariana*. *Pinus banksiana* grown in far-red rich light had greater TTC reduction values than red grown plants but the red grown plants were evaluated as being of higher quality after regrowth. The variable chlorophyll fluorescence values were shown to be good predictors of seedling quality following regrowth after cold storage and cold stress.

MATERIALS AND METHODS

Pinus banksiana Lamb. [jack pine], *Picea mariana* (Mill) B. S. P. [black spruce] and *Picea glauca* (Moench) Voss [white spruce] seedlings were grown for 16 weeks in rooms providing a 18/6 h light/dark photoperiod at 168 ± 20 μmol $m^{-2}s^{-1}$

photosynthetically active radiation (PAR) and a quantum flux ratio of 5.5±0.1 from fluorescent tubes (designated R for red rich light) or 0.7±0.1 from fluorescent tubes supplemented by filtered light from quartz halogen lamps (designated FR for far-red rich). A themoperiod of 18/6 h of 18/16℃ was provided. Rooms were maintained at 350, 700 or 1050 μmol mol^{-1} CO_2 by injection of CO_2 through a system controlled by an infra-red gas analyzer (for additional details on growth conditions see (*9*)).

Plants were then exposed to a 8/16 h day/night photoperiod at a constant temperature of 15℃ with the same light qualities and quantities as in the long photoperiod. After four weeks of short day treatment the *Picea mariana* seedlings were moved to another chamber which provided an eight hour photoperiod from sodium and metal halide lamps at a PAR of 179±15 μmol $m^{-2}s^{-1}$ and a zeta ratio of 2.75±0.04. Over three days the thermoperiod was adjusted from 10/5 to 6/0 then to 6/−5℃ and then maintained at that setting for a further 11 days. Plants were then placed in a dark growth chamber for storage at −2℃. *Picea glauca* and *Pinus banksiana* seedlings were subjected to a similar treatment regime after six and eight weeks of the SD treatment respectively.

After cold dark storage for six weeks a control sample of six seedlings per treatment was placed in a regrowth room which provided 18 h of 350 μmol $m^{-2}s^{-1}$ PAR and a costant temperature of 18℃. Other seedlings were transferred to a dark programmable freezing chamber at −5℃ for 2 h after which time samples of six plants per treatment were removed and placed in the −2℃ dark chamber for two hours then in the regrowth room. The remaining plants were then quickly cooled to −10℃ and held for two hours and samples were removed to the cold room then the regrowth room. Remaining plants were then quickly cooled to −15℃ and the procedure was repeated extending to temperatures of −20 and −25℃.

Seven days after cold stress, seedlings that had been cold stored and stressed had their needles subjected to a modified (*5*) triphenyl tetrazolium chloride (TTC) assay for viability (*13*). At the same sampling time, needle chlorophyll fluorescence in control, −5, −15 and −25℃ treatments was determined with a Branker SF30 Fluorescence Meter. Fluorescence data was calculated as the difference between the maximum (P) and minimum (O) levels of fluorescence normalized to the O level *i. e* (P–O)/O (*16*).

After four weeks in the regrowth chamber, seedlings were evaluated by subjective scoring criteria for *Pinus* and *Picea* regrowth (*7*). Briefly, seedlings showing no damage would be scored at 0 while dead seedlings would be scored at 5. Following regrowth, trays of seedlings were transferred on May 12, 1989 to a shade frame at the University of Alberta Devonian Botanic Garden at Devon, Alberta. After two weeks acclimation, 50 seedlings per light and CO_2 treatment were planted in the field in unshaded conditions. Evaluations of seedling quality were made on August 18, 1989 and again on May 27, 1990 and August 16, 1991.

RESULTS AND DISCUSSION

Triphenyl Tetrazolium Chloride Reduction.

Each CO_2 and light quality treatment batch of seedlings was analyzed with freshly made reagents so direct statistical comparisons between treatments would not be valid. However, by normalizing the data and expressing them as a percentage of the TTC reduction by the control tissues, more general comparisons were possible. Two way analysis of variance was performed on the arc sin transformed percentile data, with values in excess of 100% being taken as equal to 100%. Far-red *Pinus banksiana* seedlings grown at all CO_2 levels tended to have higher TTC reduction values than R ones at all stress temperatures (Table. 1). For FR grown *Pinus banksiana* there was a significant drop in TTC reduction after higher CO_2 concentrations with lower values at lower stress temperatures, with a significant interaction term that may be due to the low −5°C high CO_2 value. For R grown plants there was no effect of CO_2 concentration but there was a significant temperature effect as lower temperatures gave lower TTC values.

No analysis of percentile data was undertaken for *Picea mariana* and *P. glauca* because of the lack of significant treatment effects observed in one-way ANOVA's

Table. 1. Triphenyl tetrazolium chloride reduction by *Pinus banksiana*. Values are percentages of control values expressed as the average value ± the standard deviation for six plants per light or CO_2 treatment.

Light	CO_2	Stress temperature (as % of Control ± S. D.).			
		Control	−5	−15	−25
FR	350	100	143±50	118±27	79±25
	700	100	137±42	125±37	54±23
	1050	100	54±43	101±101	38±23
R	350	100	128±63	92±29	40±21
	700	100	116±51	66±32	45±18
	1050	100	135±5	69±7	74±26

Significance in two way ANOVAs

	CO_2 (μmol mol^{-1})				Light Quality	
	350	700	1050		FR	R
Light	*	**	*	CO_2	***	NS
Temp	***	***	***	Temp	***	***
LxT	*	NS	*	CO_2xT	*	*

FR=Far-Red rich light, R=Red rich light. Entries marked with an asterisk indicate significant effects at the *0.05 **0.01 or ***0.001% levels. NS=not significant.

Table 2. Variable chlorophyll fluorescence (P–O)/O ± the standard deviation for four seedlings of *Pinus banksiana*. *Picea mariana* and *Picea glauca* per light and CO_2 treatment following cold storage (cotrol) and low temperature stress.

Light	CO_2	Stress temperature			
		Control	−5	−15	−25
Pinus banksiana					
FR	350	0.67±0.09	0.70±0.10	0.59±0.13	0.25±0.15
	700	0.36±0.08	0.38±0.07	0.39±0.12	0.20±0.18
	1050	0.42±0.07	0.32±0.26	0.35±0.22	0.17±0.19
R	350	0.67±0.07	0.56±0.15	0.56±0.26	0.37±0.07
	700	0.38±0.10	0.45±0.13	0.35±0.23	0.45±0.08
	1050	0.34±0.15	0.40±0.17	0.51±0.21	0.44±0.17
Picea mariana					
FR	350	0.50±0.06	0.17±0.20	0.20±0.15	0.36±0.16
	700	0.31±0.11	0.34±0.19	0.33±0.17	0.32±0.07
	1050	0.28±0.24	0.30±0.20	0.15	0.18±0.20
R	350	0.54±0.13	0.62±0.14	0.27±0.20	0.25±0.21
	700	0.54±0.06	0.55±0.31	0.23±0.12	0.36±0.20
	1050	0.47±0.11	0.52±0.06	0.29±0.26	0.20±0.16
Picea glauca					
FR	350	0.25±0.12	0.47±0.30	0.47±0.29	0.59±0.19
	700	0.38±0.25	0.48±0.19	0.62±0.19	0.64±0.04
	1050	0.38±0.15	0.59±0.15	0.25±0.17	0.42±0.14
R	350	0.70±0.25	0.32±0.25	0.65±0.22	0.17±0.19
	700	0.39±0.23	0.43±0.12	0.61±0.15	0.50±0.10
	1050	0.49±0.11	0.41±0.17	0.53±0.27	0.50±0.11

Signification in two way ANOVAs

	Control	−5	−15	−25
Pinus banksiana				
Light	NS	NS	NS	**
CO_2	***	***	NS	NS
LxC	NS	NS	NS	NS
Picea mariana				
Light	***	***		NS
CO_2	NS	NS		NS
LxC	*	NS		NS
Picea glauca				
Light	**	NS	NS	**
CO_2	NS	NS	NS	**
LxC	*	NS	NS	**

FR=Far-Red rich light, R=Red rich light. Entries marked with an asterisk indicate significant effects at the *0.05, **0.01 or ***0.001% levels. NS=not significant.

on the raw data, and the frequency of values in excess of the control ones.

Chlorophyll Fluorescence After Cold Stress.

Table 2 compares the normalized P level values, i.e. (P–O)/O, after cold stress. For *Pinus banksiana* following control and −5℃ treatments, the values were lower after high CO_2 levels. At −25℃, values were lower after FR. For *Picea mariana* values were lower after FR in the control and −5℃ treatments. *Picea glauca* showed very varied responses but control plants had lower FR values than R ones. No consistant trends of values with temperature were observed for any species.

Plant Survival After Cold Stress.

When plants that had been cold stressed at the end of the dark storage period were allowed to rewarm and grow in long days, R grown *Pinus banksiana* seedling showed lower qualitative scores (better survival) than FR ones at various temperatures, with lower stress temperatures increasing the values of the scores (poorer survival) in both light qualities (Table 3).

Table 3. Qualitative survival of seedlings in a growth chamber after cold stress. Vaues are the averages of qualitative scorings made on six seedlings per light or CO_2 treatment four weeks after being placed in the chamber. ND=not determined. A value of 0.0 indicates undamaged seedlings, 5.0 indicates dead plants.

Species	Light	CO_2	Stress temperature (℃)					
			Control	−5°	−10°	−15°	−20°	−25°
PB	FR	350	0.7	0.3	ND	0.7	ND	3.7
		700	0.2	1.0	ND	3.8	ND	4.7
		1050	2.5	3.0	ND	4.2	ND	4.7
	R	350	0.2	0.2	ND	0.2	ND	2.5
		700	1.2	1.7	ND	2.8	ND	2.8
		1050	0.7	1.0	ND	1.0	ND	1.3
PM	FR	350	3.7	3.7	3.3	2.5	2.2	3.3
		700	0.7	1.8	1.7	4.2	3.0	2.5
		1050	4.0	3.5	3.5	4.7	4.7	4.5
	R	350	0.7	1.0	0.7	2.2	1.2	2.7
		700	1.3	0.5	1.8	1.5	1.8	2.0
		1050	1.8	2.3	3.5	4.0	2.5	3.5
PG	FR	350	1.7	0.3	1.5	1.5	1.3	0.5
		700	0.7	1.7	1.7	0.5	0.3	0.0
		1050	0.5	1.5	1.2	2.8	2.2	2.0
	R	350	0.3	2.7	0.2	0.5	2.5	3.7
		700	1.3	2.2	0.8	1.2	0.8	1.2
		1050	0.7	0.3	1.0	2.0	3.0	2.3

PB=*Pinus banksiana, Picea mariana,* PG=*Picea glauca,* FR=Far–Red rich light, R=Red rich light.

Picea mariana also showed lower scores in R grown plants with higher scores occurring in response to lower stress temperatures in both light qualities. *Picea glauca* showed low scores (good survival) over the whole range of temperatures examined, with little obvious difference between R and FR light treatments except at −25 where R grown plants had the poorer survival. The only trend in survival as a result of CO_2 treatments was enchanced quality in *Picea glauca* at intermediate CO_2 levels.

Plant Survival In the Field.

During the first seasons field growth (to Fall 1989) there was a 70% or greater survival of *Pinus banksiana* seedlings (Table 4). Some of the 30% die back must have been restricted to the needles as FR ambient CO_2 grown plants recovered to 94% survival following overwintering (to Spring 1990), although the average quality of the seedlings had declined slightly. Following a second winter and summers growth there had been a decline in the numbers of trees surviving (>64%) but their average quality had improved in most instances (to Summer 1991).

Table 4. Qualitative survival of cold stored seedlings in the field. Values are the average qualitative scores for seedlings ± standard deviation. Values in parenthesis indicate the percentage of surviving seedlings of the original 50 per treatment, outplanted in the spring of 1989, that were scored each year. A value of 0.0 indicates undamaged seedlings, 5.0 indicates dead plants.

Species	Light	CO_2	Fall 1989	Spring 1990	Summer 1991
PB	FR	350	0.3±1.0 (70)	1.0±1.0 (94)	0.02±0.15 (88)
		700	0.1±0.4 (96)	1.3±1.4 (68)	0.13±0.55 (64)
		1050	0.3±1.0 (88)	1.4±1.4 (86)	0.21±0.70 (78)
	R	350	0.3±0.7 (84)	0.3±0.6 (80)	0.14±0.42 (72)
		700	0.1±0.4 (94)	0.8±1.1 (90)	0.08±0.28 (72)
		1050	0.0±0.2 (94)	0.9±1.2 (94)	0.34±0.79 (64)
PM	FR	350	2.7±2.3 (6)	1.0 (2)	5.00 (0)
		700	1.3±1.4 (30)	1.0 (2)	5.00 (0)
		1050	2.3±0.8 (52)	1.4±1.0 (52)	0.0 (52)
	R	350	2.6±1.5 (30)	1.9±1.6 (32)	5.00 (0)
		700	3.0 (2)	2.5±2.1 (4)	5.00 (0)
		1050	4.0±0 (4)	0.8±0.7 (30)	0.0 (50)
PG	FR	350	1.4±1.3 (42)	1.0±1.3 (86)	0.0 (82)
		700	0.9±1.4 (62)	0.6±0.5 (40)	0.0 (40)
		1050	1.1±1.2 (94)	0.4±0.5 (96)	0.0 (92)
	R	350	2.3±1.0 (66)	0.8±0.8 (66)	0.0 (54)
		700	2.1±1.4 (88)	0.3±0.9 (48)	0.0 (44)
		1050	0.8±1.2 (50)	0.5±0.9 (56)	0.0 (48)

PB=*Pinus banksiana*, PM=*Picea mariana*, PG=*Picea glauca*, FR=Far-Red rich light, R=Red rich light.

Picea mariana showed very poor survival after the first summers field growth with the greatest survival being achieved by the FR high CO_2 treatment (52%). Over the next two winters there was no further mortality within that treatment and the quality of the seedlings improved with time. The R high CO_2 treatment showed severe damage during the first summer but there was significant regrowth over the next two seasons with an increase in seedling quality. The 350 and 700 μmol mol^{-1} CO_2 treatments showed such poor quality after the second winter that the Botanic Gardens staff removed them.

Picea glauca seedlings showed 42% or greater survival during the first summers growth and also demonstrated the ability to regrow after the first winter. There was little change in survival over the second winter, with the quality of the surviving seedlings improving. The best survival was shown by FR seedlings after 350 or 1050 μmol mol^{-1} CO_2 treatments.

The ability of the various physiological parameters measured to predict seedling survival was evaluated by regression analysis of the TTC percentile and fluorescence data against the qualitative scores of seedling health in the growth chamber. The qualitative score is a longer term summative evaluation of the health of the seedlings after growth treatments and cold storage while the physiological parameters are more quantitative and may be obtained earlier giving a formative estimate of the survival potential of the seedlings.

Linear regression analysis of *Pinus banksiana* data showed significal negative correlations between percent TTC reduction and damage scoring for the total population and for R or FR grown plants (Table 5). Significant correlations were seen for R grown and the total population of R and FR grown seedlings of

Table 5. Qualitative survival rating (y) compared to percent TTC reduction (x). Linear regression equations were computed for seedlings from the far-red (FR) or red (R) treatment conditions and for pooled treatments (FR+R) at all CO_2 treatments and all stress temperatures.

Species	Light	Linear regression equation		
PB	FR	$y=-0.0337x+6.048$	$r=-0.7475$*	DF=7
	R	$y=-0.0201x+3.208$	$r=-0.6859$*	DF=7
	FR+R	$y=-0.0244x+4.392$	$r=-0.5659$*	DF=16
PM	FR	$y=-0.0025x+3.652$	$r=-0.2604$NS	DF=13
	R	$y=-0.0210x+4.433$	$r=-0.9132$***	DF=13
	FR+R	$y=-0.0031x+3.085$	$r=-0.2163$NS	DF=28
PG	FR	$y=0.0060x+0.567$	$r=0.1984$NS	DF=13
	R	$y=-0.0316x+4.879$	$r=-0.7701$***	DF=13
	FR+R	$y=-0.0132x+2.958$	$r=-0.3947$*	DF=28

PB=*Pinus banksiana*, PM=*Picea mariana*, PG=*Picea glauca*, DF=degrees of freedom. r=correlation coefficient. NS=not significant. Asterisks indicate significant correlations at the *0.05, **0.01 or ***0.001% levels.

Picea glauca, and R grown *Picea mariana.* When the values based on the abnormally low control value for the FR grown *Picea mariana* seedlings at 350 μmol mol^{-1} CO_2 were removed from the analysis, the regression for the remaining FR plants was still not significant but the total population showed a highly significant correlation ($r=-0.630$, $n=23$).

Solving the regression equations for the total populations of individuals in a species indicated that *Pinus banksiana* showed some recovery (qualitative score of 4 or less) at TTC percentile values above 66% while *Picea glauca* showed recovery above 84%. The TTC test did not allow good prediction of *Picea mariana* seedling survival. For control plants of all three species, there was good agreement between the qualitative scores of plants in the growth chamber and in the field during the first summer.

A TTC reduction value of 50% of the control value was suggested as representing lethal damage in plant tissue (*13*) but it was found that a 60% criterion was a better predictor of survival for cold stressed *Picea glauca* (*5*). However, the present study would suggest that the TTC test has a limited ability to predict survival of cold stressed seedlings, particularly those of the spruces studied, due to the lack of treatment effects on TTC reduction.

When the (P–O)/O chlorophyll fluorescence ratio one week after being placed in the regrowth chamber was compared by linear regression analysis to the qualitative survival scores after one month in the chamber there were very significant negative correlations for all three species (Table 6). Variable chlorophyll fluorescence was a very good predictor of plant survival during the subsequent weeks. A positive correlation between normalized fluorescence values and seedling quality, judged by a root growth capacity test, in *Picea glauca* seedlings has been reported (*16*). A reduction in the F_v/F_o ratio (analogous to the (P–O)/O ratio used in the present study) in *Pinus sylvestris* exposed to temperatures of −32℃ or −34℃ for 3 h has been reported (*14*) and that also correlated with an increase in chlorophyll bleaching.

The higher CO_2 treatments provided to *Pinus banksiana* during the long day growth and the short day hardening phases were slightly detrimental to the control and −5℃ seedlings as judged by their fluorescence characteristics and qualitative scores following rewarming. However, no differences were found in

Table 6. Qualitative survival rating (y) compared to (P–O) /O values (x). Linear regression equations were computed for seedlings from all light and CO_2 treatments and all stress temperatures.

Species	Linear regression equation		
Pinus banksiana,	$y=-8.102x+5.345$	$r=-0.7527$***	DF=22
Picea mariana	$y=-6.047x+4.586$	$r=-0.6933$***	DF=22
Picea glauca	$y=-4.541x+3.465$	$r=-0.6486$***	DF=22

DF = degrees of freedom. r = correlation coefficient. Asterisks indicate significant correlations at the ***0.001% level.

the fluorescence properties of seedlings of *Pinus radiata* grown at ambient and enriched CO_2 levels in non-stressed control or drought conditions (*4*). Following the lowest temperature stress, R treatments had resulted in a better score in both tests. Field planting gave good results after all treatments but high CO_2 seedlings had a slightly lower quality.

Picea mariana control and −5°C seedlings showed a good agreement between their (P–O)/O chlorophyll fluorescence values and qualitative scores with FR grown seedlings having lower fluorescence values and generally worse qualitative scores. After lower temperature stress no treatment effects were apparent on the fluorescence values or qualitative scores. The FR grown plants showed a less systematic correlation between the two seedling evaluation mathods and were of lower quality than the corresponding R plants. When planted out in the field there was high seedling mortality during the first summer but that mortality was greater for the R treated seedlings. After several years it was only the seedlings grown at the highest CO_2 level that showed significant survival.

These short term observations are at variance with another study (*6*) which found that *Picea mariana* exposed to short day lengths with a FR daylength extension had a higher survival capacity than plants receiving a R daylength extension. The more dramatic changes induced in the status of the plant's phytochrome with the longer duration exposure to the different quantum flux ratios used in the present study may be the basis for the difference between the studies. The present study also used a finer grading system to assess plant damage.

On the basis of electrical conductivity tests on isolated plant apices, it has been demonstrated that *Picea mariana* seedlings exposed to short periods of elevated atmospheric CO_2 had a greater susceptibility to frost damage than control plants (*11*) which contradicts our long term findings in the field. It is possible that the response of isolated plant parts was not the same as in whole seedlings, and the other study did not provide any information on the long term ability of the treated seedlings to regrow.

Picea glauca control seedlings showed good agreement between (P–O)/O chlorophll fluorescence and qualitative scores. High CO_2 level with FR light and low CO_2 with R gave the highest fluorescence and lowest qualitative score in control plants but it was the FR grown plants that did the best in the first field year. However, after −25°C stress it was the mid CO_2 level in both light qualities that gave the best scores.

The qualitative survival scores for the control plants of the three species indicated that *Pinus banksiana* and *Picea glauca* had withstood cold storage better than *Picea mariana.* Ambient CO_2 treatments enhanced the normalized chlorophyll fluorescence in *Pinus banksiana* and R light enhanced it in both spruce species. Qualitative scores for stressed plants in growth chambers indicated that *Picea glauca* was the most cold hardy species following all treatments followed by R treated *Pinus banksiana* and *Picea mariana* with FR grown *Pinus banksiana* and *Picea mariana* being the least cold hardly.

It was more difficult to rank cold hardiness on the basis of the percentile TTC reduction data as the critical value predicting lethal damage was more variable between species.

The improvement of qualitative seedling scores after *Pinus banksiana* and *Picea mariana* growth in R light may be of commercial importance. By cultivating seedlings in R rich light typical of high pressure sodium or metal halide lamps the quality of the seedling stock may be improved. The enrichment of the growth room atmosphere with CO_2 would not be advantageous as, while it would improve seedling growth, it would also result in reduced seedling quality during growth after cold storage and cold stress. *Picea glauca* would improve its hardiness at low temperatures in both light qualities if grown at double the ambient CO_2 level.

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