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Gas exchange and water relations of 3 sizes of containerized *Picea mariana* seedlings subjected to atmospheric and edaphic water stress under controlled conditions

JD Stewart [1]*, PY Bernier [2]**

[1] *Centre de Recherche en Biologie Forestière, Faculté de Foresterie et de Géomatique, Université Laval, Sainte-Foy, Quebec G1K 7P4;*
[2] *Natural Resources Canada, Canadian Forest Service–Quebec Region, PO Box 3800, Sainte-Foy, Quebec G1V 4C7, Canada*

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Summary — Containerized black spruce (*Picea mariana* [Mill] BSP) seedlings of 3 sizes (heights of 18, 35 and 45 cm) were placed in growth chambers and subjected to conditions of low evaporative (20°C, 60% RH) or high evaporative (30°C, 40% RH) demand, with 3 levels of soil water availability in each environment. The large seedlings had the highest rate of net photosynthesis in the cooler environment, but showed the greatest reduction in net photosynthetic rate in the warmer and drier environment, under conditions of limited water supply. The small seedlings were least affected by the warmer and drier environment in which they maintained photosynthetic rates higher than those of the larger seedlings. The decrease in net photosynthesis experienced by the large seedlings in the warmer and drier environment under conditions of limited water availability was associated with a large decrease in stomatal conductance. However, the maintenance of a high level of intercellular CO_2 concentration suggests that most of the limitations to net photosynthesis were of non-stomatal origin. Water content of the root plug was also reduced by increased seedling size, but the differences were least evident under conditions that produced the largest differences in net photosynthetic rates. These results, obtained under controlled conditions, suggest that after outplanting, large seedlings would experience greater reduction in growth than smaller ones only under conditions of high evaporative demand and low water availability.

***Picea mariana* / polyethylene glycol / net photosynthesis / shoot water potential / stomatal conductance**

* Current address: Department of Renewable Resources, University of Alberta, Edmonton, Alberta T6G 2H1, Canada
** Correspondence and reprints

Résumé — Échanges gazeux et relations hydriques chez des semis de *Picea mariana* de 3 tailles différentes cultivés en conteneurs et soumis à différentes conditions de sécheresse atmosphérique et édaphique. *Nous avons soumis en chambre de croissance des semis d'épinette noire (*Picea mariana *[Mill] BSP) de 3 tailles différentes (18, 35 et 45 cm de hauteur) et cultivés en conteneurs, à des conditions de demande évaporative faible (20°C, 60% HR) et élevée (30°C, 40% HR) conjointement à 3 niveaux de disponibilité en eau du sol. Les semis de plus forte taille avaient les taux de photosynthèse nette les plus élevés dans l'environnement frais, mais la plus forte réduction de ce paramètre dans l'environnement plus chaud et sec, sous des conditions de faible disponibilité en eau. Cette forte réduction de photosynthèse nette était associée à une fermeture des stomates. Cependant, le taux élevé de concentration intercellulaire en CO_2 indique que des facteurs non stomatiques étaient principalement à l'origine de cette réduction. Les semis de plus faible taille ont maintenu en conditions chaudes et sèches des taux de photosynthèse nette supérieurs à ceux des semis de plus forte taille. L'accroissement de la taille des semis a réduit la teneur en eau de la motte racinaire, mais principalement sous des conditions n'engendrant pas de différences dans les taux de photosynthèse nette entre les tailles de semis. Les résultats obtenus en conditions contrôlées indiquent que la croissance des semis d'épinette noire de plus forte taille serait plus affectée à la suite de la plantation que celle des semis de plus faible taille à condition seulement que la demande évaporative soit forte et la disponibilité en eau faible.*

Picea mariana */ polyéthylène glycol / photosynthèse nette / potentiel hydrique du xylème / conductance stomatique*

INTRODUCTION

One of the problems faced by outplanted tree seedlings is competition from other vegetation. This problem has often been addressed by attempting to decrease the establishment and growth of the unwanted species through practices such as burning, cultivation, or herbicide application (*eg*, Stewart, 1987; Wood and Dominy, 1988; Campbell, 1990). Another approach is to increase the competitive ability of the planted stock by using larger seedlings than is currently the practice. Large planting stock can overtop competing herbaceous or shrubby vegetation faster than small planting stock (Overton and Ching, 1978; Newton *et al*, 1993), because of its enhanced ability to capture light, and in some cases, to overcome browsing damage (Hartwell, 1973, in Newton *et al*, 1993). In climates with substantial snow accumulation, seedlings with a greater stem diameter are also more resistant to the flattening effect of snow and dead vegetation (Burdett, 1990).

Large seedlings may also have some disadvantages compared with smaller ones. The greater transpiring surface of the larger seedlings may or may not be matched by an increase in the soil water absorption capacity of the root system. A reduction in the soil water absorption capacity per unit leaf area in larger seedlings may result in lower stomatal conductances and lower net assimilation. Negative effects of increased seedling size on survival and growth have been observed with Douglas-fir (*Pseudotsuga menziesii* [Mirb] Franco) on harsh planting sites (Hahn and Smith, 1983).

In order to anticipate problems with respect to water flux, and the resulting negative effects on seedling water relations and photosynthesis, we undertook a controlled environment study using containerized black spruce (*Picea mariana* [Mill] BSP). The seedlings, grown to different sizes in different types of containers, were subjected to 2 sets of atmospheric environmental conditions and 3 levels of soil water availability. Our objectives were 1) to determine if increased canopy size led to an increase in the susceptibility of the seedlings to water stress; and 2) to determine the relative importance of soil and atmospheric drought in the generation of drought stress in the seedlings.

MATERIALS AND METHODS

Containerized black spruce seedlings from a single provenance (EPN-N1-5A-J23-1288) were obtained in 3 sizes from local nurseries in the fall of 1992. Differences in size were achieved through differences in length of culture, container size and fertilization regime. The smallest (size 1) seedlings were grown in 67–50 (67 cavities per tray, 50 cm^3 per cavity) Rigipot containers (IPL Industries, Saint-Damien, QC, Canada) over an 8-month production schedule, with sowing carried out in a heated glasshouse in February, and plants moved outdoors in May. The medium-size (size 2) seedlings were grown in 25–200 Rigipot containers. The large (size 3) seedlings were grown in 45–340 Vent-Block containers (Beaver Plastics, Edmonton, AB, Canada). Both size 2 and 3 seedlings were produced over a 16-month production schedule, with sowing in June in unheated polyethylene tunnels, and seedlings moved outdoors in August for the remainder of the period. Size 1 seedlings received a total of about 15 mg N per cavity. Size 2 and 3 seedlings received about 110 and 170 mg N per cavity, respectively. In all cases, the potting medium was a 3:1 peat/vermiculite mix.

Upon reception from the nursery in November 1992, the seedlings were sorted for uniformity in shoot volume within each size class using displaced water volume. Initial morphological characteristics of a subsample of the seedlings retained for the experiment are presented in table I. After sorting, the seedlings were moved to a 2°C cold room for temporary storage.

The experiment was started in January 1993 and involved the exposure of the seedlings to 2 different atmospheric environments in different growth chambers, with 3 levels of soil water availability. Replications of the atmospheric environment treatments were performed over time because of the limited availability of growth chambers. The seedlings were removed from cold storage and treated in an identical manner for each of the 4 replicates needed to achieve statistical validity of the results. The length of cold storage therefore varied from 8 to 14 weeks, with no significant effect on any of the measured variables (non-significance of replicate effect, table II).

For each of the replicates, a set of 40 seedlings from each size class was removed from cold storage and allowed to recover their metabolic functions for 2 weeks in a pretreatment controlled environment chamber. Conditions in the chamber were set at 20/15°C, 50/100% day/night temperature and relative humidity, respectively, with a 12-h photoperiod. Seedlings were kept well watered.

After the pretreatment, the seedlings were prepared for the experiment. Root plugs were inserted into Spectro-Por 1 dialysis tubes (molecular cut-off weight of 8 000, Spectrum Industries, Los Angeles, CA, USA) that were folded and clamped closed at the bottom end. A sandy loam was used to backfill between the root plugs and the membrane to ensure the continuity of water films between the root plug and the membrane.

Solutions of polyethylene glycol (PEG) 20 000 (JT Baker Inc, Phillipsburg, NJ, USA) were prepared with concentrations of 0, 40, and 80 g PEG/kg H_2O and were used to fill 45-l basins. The concentrations correspond to water potentials of about 0, –0.04 and –0.12 MPa (Williams and Shaykewich, 1969). Four seedlings from each of the 3 seedling sizes were placed at random into holes precut in each basin cover and suspended by their membrane tubes in the solutions

Table I. Initial morphological characteristics (mean ± SD, n = 20) of 3 sizes of containerized black spruce seedlings.

	Size 1	*Size 2*	*Size 3*
Seedling height (cm)	18 ± 2	35 ± 4	45 ± 4
Shoot volume (cm^3)	1.22 ± 0.28	13.95 ± 1.17	18.28 ± 1.37
Stem dry mass (g)	0.13 ± 0.03	2.16 ± 0.34	3.05 ± 0.21
Needle dry mass (g)	0.16 ± 0.04	2.28 ± 0.29	3.13 ± 0.32
Root dry mass (g)	0.12 ± 0.04	1.48 ± 0.15	1.54 ± 0.11

Table II. *P*-values and mean square errors from the analysis of variance describing the effects of atmospheric environment, PEG concentration, and seedling size on root plug water content (Θ_{PLUG}), shoot water potential (Ψ_x), net photosynthesis (P_n), shoot conductance to water vapour (g_{sW}) and internal CO_2 concentration (c_i) of black spruce seedlings after 6 and 8 d of treatment.

Effect	df [a]	Θ_{PLUG}	Ψ_x [b]	P_n	g_{sW} [b]	c_i
Replicate (R)	3	0.172	0.193	0.266	0.453	0.069
Environment (E)	1	0.009	0.004	0.0096	0.028	0.192
Mean square error	3	0.225	0.00279	0.000217	0.00226	4 502
PEG (P)	2	< 0.001	0.007	< 0.001	< 0.001	0.052
E x P	2	< 0.001	0.501	0.129	0.296	0.142
Mean square error	12	0.169	0.00564	0.000163	0.00238	9 719
Size (S)	2	< 0.001	0.021	< 0.001	< 0.001	0.420
P x S	4	< 0.001	0.490	0.049	0.634	0.231
E x S	2	0.904	0.006	0.030	0.580	0.019
E x P x S	4	0.089	0.309	0.022	0.018	0.246
Day (days 6 and 8)	3	< 0.001	0.851	< 0.001	0.053	0.433
Mean square error	71	0.142	0.00524	0.00021	0.00334	12 547

[a] *df*: degrees of freedom; [b] analysis performed on log-transformed data.

so that the solution level reached the top of the root plug. Over the course of the experiment, a few membranes developed leaks. Seedlings with leaky membranes were removed from the experiment. The solutions in the basins were stirred with submerged pumps.

Three basins containing solutions with the 3 PEG concentrations were placed in each of 2 controlled environment chambers in which the conditions were set for either a low evaporative demand (E20: 20°C, 60% RH) or a high evaporative demand (E30: 30°C, 40% RH). The corresponding absolute humidity deficits in the chambers were 6.9 and 18.2 g m^{-3} for E20 and E30, respectively. Photoperiod in the chambers was maintained at 12 h. Photosynthetically active radiation at seedling canopy height was about 500 μmol m^{-2} s^{-1}.

On days 2, 4, 6 and 8 after the start of the experiment, 1 seedling of each size was randomly selected from each basin for midday measurements. Gas exchange was first measured *in situ* on a branch tip using a LI-6200 Portable Photosynthesis System (LI-COR Inc, Lincoln, NE, USA). Net photosynthetic rate (P_n), transpiration *(E)*, stomatal conductance to water vapour (g_{sW}) and intercellular CO_2 concentration (c_i) were calculated by the LI-6200. The shoot used for gas-exchange measurements was harvested for dry weight determination in order to standardize gas-exchange measurements by unit needle weight. A second shoot was collected to determine shoot water potential (Ψ_x) using a pressure chamber (PMS Instruments, Corvallis, OR, USA). The remainder of the canopy was retained to measure total foliage dry weight. Finally, the root plugs were weighed fresh, and again after drying at 70°C for 48 h, to determine their volumetric water content (Θ_{PLUG}). While in the growth chamber, care was taken to minimize CO_2 fluctuations (Stewart and Bernier, 1994); CO_2 concentrations were usually about 370 ppm.

As mentioned earlier, the experiment was repeated 4 times, with new sets of 40 seedlings per size placed every second week in the pretreatment chamber. Assignment of E20 or E30 to either of the 2 chambers was done at random for each of the 4 replicates. The experimental design was a split-split-plot. The main plots were the 2 growth chamber conditions. The split-plots were the 3 basins containing the different PEG solutions in each chamber. The split-split-plots were the 12 individual seedlings in each basin arranged in factorial combinations of 3 sizes and 4 sampling dates. The general linear models (GLM) procedure of SAS was used in the statistical analysis. Values of stomatal conductance and shoot water potential were log-transformed in order to homogenize their variance.

RESULTS

Measurements on days 2 and 4 showed that the seedlings were still adjusting to treatment conditions as root plug water contents gradually dropped from near saturation at day 0 to levels in near-equilibrium with the dynamics of water exchange of each treatment. Initial analysis of variance therefore showed a systematic interaction between all main effects and day of measurement (analysis not shown). In order to focus the present report on the effect of treatment conditions at or near equilibrium, the effects of treatments were evaluated by performing an analysis of variance on the data obtained on days 6 and 8 of treatment only. Of the 144 seedlings selected for measurements on those 2 d in the 4 replicates, 18 developed leaks. The results obtained on the 126 remaining seedlings are presented in table II. The effect of the day of measurement (day 6 or 8) was still significant for many variables (table II), but there were no interactions of the 'day' factor with any of the other treatment factors (not shown). This indicates that seedling conditions were still evolving, but that the passage of time would not cause changes in the conclusions reached on the relative effects of the treatments, all treatments being affected equally by the passage of time. Only data from day 6 are presented graphically in order to reduce the complexity in the presentation of our results.

In general, the effects of seedling size, PEG concentration and atmospheric environment were highly significant on all variables but c_i, with many significant interactions among treatment factors (table II).

Root plug water content generally decreased with increased seedling size, increased PEG concentration and increased evaporative demand (fig 1). However, this effect was not uniform across size and PEG concentrations, as shown by the significant size x PEG interaction (table II). The effect of size dominated at low PEG concentrations, but was nearly absent at 80 PEG as root plug water content dropped to near-uniform low values across all seedling sizes.

Shoot conductance also decreased with increased seedling size, increased PEG concentration and increased evaporative demand (fig 2). The significant 3-way interaction (table II) reveals that the pattern was not uniform. In fact, the highest values of g_{sW} were obtained in the E30 environment, in size 1 seedlings. However, the combination of high PEG concentration and large seedling size always yielded low values of g_{sW}.

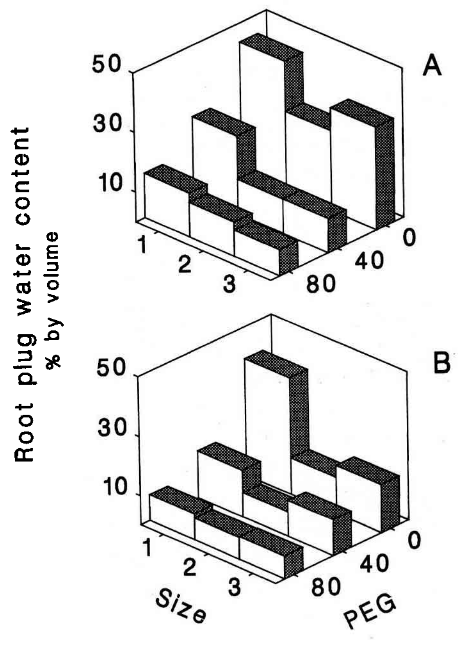

Fig 1. Peat water content of the root plugs by seedling size and PEG concentration of the solutions (g PEG/kg H_2O): **A)** under low evaporative conditions (20°C, 60% RH); and **B)** under high evaporative conditions (30°C, 40% RH)

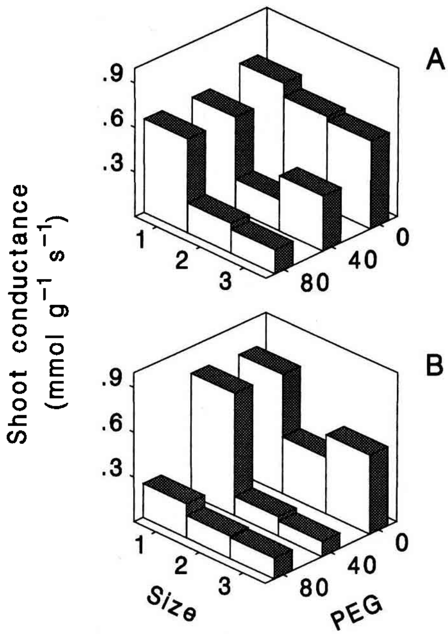

Fig 2. Shoot conductance to water vapour by seedling size and PEG concentration of the solutions (g PEG/kg H_2O): **A)** under low evaporative conditions (20°C, 60% RH); and **B**) under high evaporative conditions (30°C, 40% RH).

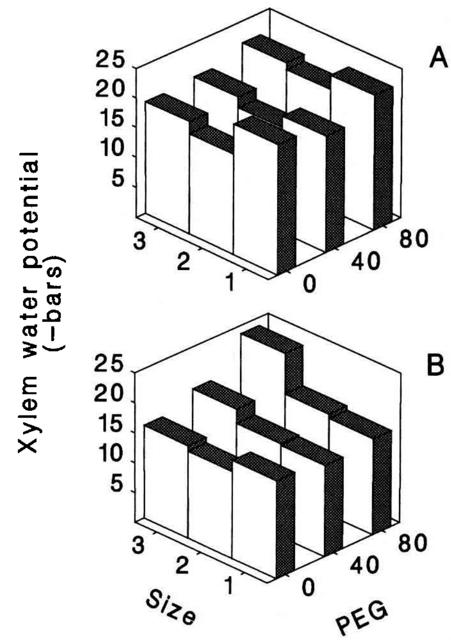

Fig 3. Shoot water potential of seedlings by seedling size and PEG concentration of the solutions (g PEG/kg H_2O): **A)** under low evaporative conditions (20°C, 60% RH); and **B)** under high evaporative conditions (30°C, 40% RH).

Shoot water potential was only moderately responsive to any of the 3 treatment factors. In general, Ψ_x increased (became less negative) with an increase in the evaporative demand (fig 3), a response certainly linked to the concurrent drop in g_{sW}. The exception to this behaviour was the drop in Ψ_x in the E30 environment in size 3 seedlings with increasing PEG concentration, a response that shows up as a significant size x environment interaction in table II.

The response of net photosynthesis was quite complex as all 3 treatment factors interacted significantly (table II). In general, P_n decreased with an increase in evaporative demand and PEG concentration (fig 4) in a pattern that paralleled that of g_{sW} (fig 2). The effect of seedling size varied both with PEG levels and environment. The largest seedlings showed the largest rates of net photosynthesis by unit needle dry weight under conditions of limited stress (E20, low PEG), but the lowest rates under stressful conditions (E30, high PEG) (fig 4).

DISCUSSION

The initial hypothesis of this work was that increased seedling size would lead to increased water stress and decreased net photosynthesis. The results show that increased seedling size did indeed cause

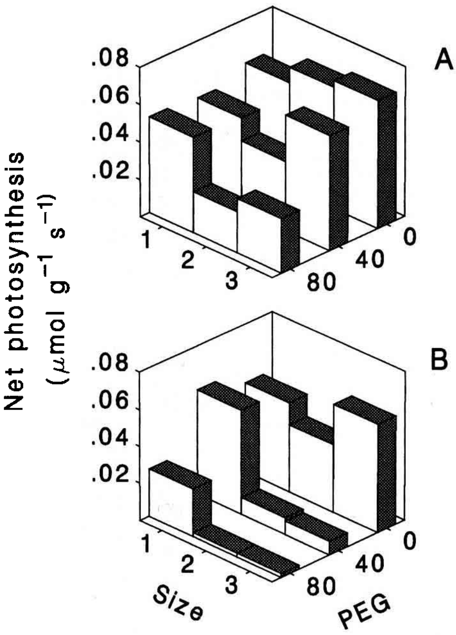

Fig 4. Net photosynthesis per unit foliage mass by seedling size and PEG concentration of the solutions (g PEG/kg H_2O): **A)** under low evaporative conditions (20°C, 60% RH); and **B)** under high evaporative conditions (30°C, 40% RH).

such effects, but only under the harshest conditions imposed, *ie* highest PEG concentrations and highest evaporative demand. Neither of these 2 factors taken individually resulted in a greater depression of net photosynthesis in larger seedlings than in smaller ones, except in the case of the E20, 80 PEG treatment.

Stomatal conductances and rates of net photosynthesis under the 0 PEG treatment in the low evaporative environment are comparable to rates observed on black spruce both under controlled conditions (Wang and Macdonald, 1993; Yue and Margolis, 1993) and in the field (Blake and Sutton, 1988; Macdonald and Lieffers, 1990). The treatments also created a range of water availability conditions in the peat plug that were quite comparable to those in the field. The average peat volumetric water contents ranged from 44% under the mildest conditions to 9% under the harshest. A moisture-release curve obtained on disturbed samples of peat substrate (results not shown) reveals that the corresponding soil water tensions range from about –0.01 to –0.15 MPa. The wet portion of that range is similar to tensions measured in planting areas normally targeted for black spruce (*eg,* Bernier, 1993). The dry portion probably represents extreme conditions for that species.

The effect of seedling size on root plug water content, evident mostly under the mildest conditions, reflects the limits imposed by the different interfaces in the delivery of water from the PEG solution to the roots. Peat is a poor water transport medium at water contents corresponding to even mild tensions (Örlander and Due, 1986; Bernier, 1992). In the field, such interfaces are therefore also present as the relatively coarse peat–vermiculite mix of the root plug must serve as a transmission medium between the mineral soil and the roots. Consequently, differences in root plug water content among seedling sizes should also occur in the field.

The lack of large variations in shoot water potential shows the level of stomatal regulation of water loss by the seedlings. Water potential levels were actually greater (less negative) in the harsher E30 environment than in the E20 environment for most size x PEG combinations, except for size 3 seedlings under the 80 PEG treatment. As treatments progress from the mildest (E20, 0 PEG) to the harshest (E30, 80 PEG), increasing stomatal closure is needed to maintain such a favourable internal water status. There was no clear relationship between shoot water potential and net photosynthesis.

The similarity in the general pattern of response between shoot conductance and net photosynthesis appears to be evidence of g_{sW} controlling the rate of P_n by limiting the supply of CO_2. However, the computation of internal CO_2 concentration reveals values that do not show c_i as limiting P_n (fig 5). For example, the highest c_i values coincide with the lowest P_n measurements (E30, 40 PEG, sizes 2 and 3). These results suggest that changes in P_n were not caused by internal CO_2 depletion following stomatal closure. In fact, in black spruce, stomatal limitation to P_n appears to be important only at relatively low values of stomatal conductance (Stewart *et al*, 1995). Instead, the parallel drop in P_n and g_{sW} suggests a common mechanism of regulation. Possible candidates are the chemical signals sent by root tips as soil water availability decreases. Such signals have been shown to regulate stomatal processes (Davies *et al*, 1990). The drying of roots has also been shown to reduce seedling growth (Coutts, 1981).

The large seedlings maintained high net photosynthetic rates under conditions of mild and moderate water stress. In the field, this high rate multiplied by their foliage biomass, plus the initial greater height, should translate into absolute growth rates exceeding those of the smaller seedlings. Studies using bare-root Sitka spruce (*Picea sitchensis* [Bong] Carr) seedlings (South and Mason, 1993), and bare-root and containerized Douglas-fir seedlings (Newton *et al*, 1993) have shown superior absolute growth of large stock under normal planting conditions. Only when planted on harsh sites did larger Douglas-fir seedlings perform more poorly than smaller ones (Hahn and Smith, 1983). Given these results, we expect the largest black spruce seedlings to grow faster and be better competitors than the smaller seedlings in situations where atmospheric and soil drought stresses are minimal. On drought-prone sites, the smallest seedlings should grow best. In the latter environments, the stress itself will reduce the intensity of competition.

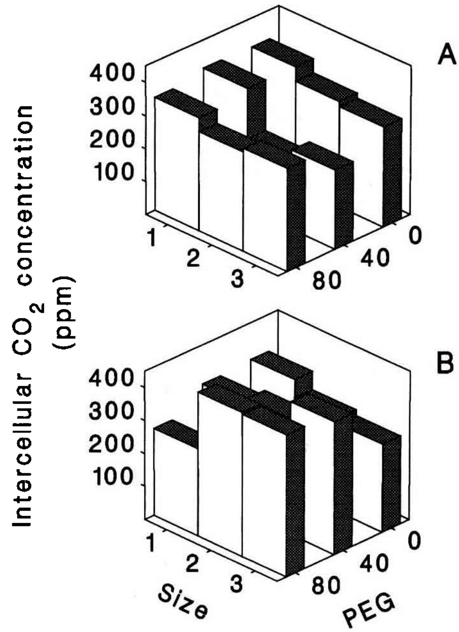

Fig 5. Intercellular CO_2 concentration by seedling size and PEG concentration of the solutions (g PEG/kg H_2O): **A)** under low evaporative conditions (20°C, 60% RH); and **B)** under high evaporative conditions (30°C, 40% RH).

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