TRANSACTIONSNASTPHL
ISSN 0115-8848 (print)
ISSN 2815-2042 (online)
https://transactions.nast.ph/
Vol. 22 (2000)
doi.org/10.57043/transnastphl.2000.5394

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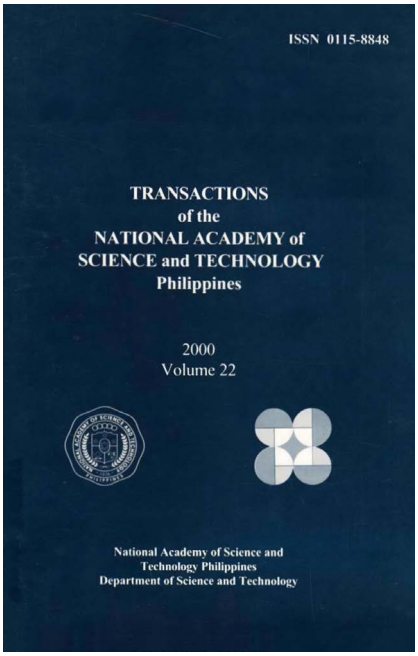


# Influence of Elevated $CO_2$ and Photon Flux Density on Growth, Carbohydrate Content, and Survival in *Pinus radiata* Shoot Cultures Supplied with Varying Sucrose Levels

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**Citation**
Chan MA & Conroy J. 2000. Influence of elevated $CO_2$ and photon flux density on growth, carbohydrate content, and survival in *Pinus radiata* shoot cultures supplied with varying sucrose levels. Transactions NAST PHL 22: 105-118. doi.org/10.57043/transnastphl.2000.5394

*Trans. Nat. Aca. Sci. Tech. Philippines 22: 105-118 (2000).*
*ISSN 0115-8848*

# INFLUENCE OF ELEVATED $CO_2$ AND PHOTON FLUX DENSITY ON GROWTH, CARBOHYDRATE CONTENT AND SURVIVAL OF PINUS RADIATA SHOOT CULTURES SUPPLIED WITH VARYING SUCROSE LEVELS

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## ABSTRACT

The observation that sucrose supply increased growth, despite the presence of $CO_2$ in the headspace during the light period and provision of higher photon flux density (PFD), indicated that carbohydrates were limiting at 350 mL $CO_2$ $L^{-1}$. Hence, in the present study, $CO_2$ in the headspace was enriched to 2,000 µL $L^{-1}$ to investigate whether sucrose could be eliminated from the media and fully autotrophic *Pinus radiata* plants produced *in vitro*. In the first experiment, sucrose was supplied at 0, 3 and 6% at a PFD of 150 µmol $m^{-2}$ $s^{-1}$. Dry matter production and shoot height were greatly enhanced by $CO_2$ enrichment at all sucrose levels indicating that $CO_2$ enhances autotrophy. In addition, vitrified shoots were never observed at 2,000 µL $CO_2$ $L^{-1}$ and the number of senescent shoots were reduced. Increasing the sucrose supply from 0 to 3% stimulated growth even at elevated $CO_2$. On the assumption that more photosynthetic reducing power may have been required to fully develop autotrophy, the PFD was raised to 280 µmol $m^{-2}$ $s^{-1}$ in the second experiment and sucrose was supplied as in the first experiment. Substantial improvement of growth was achieved with a combination of high PFD and elevated $CO_2$ showing that these factors could partially substitute for an external sucrose supply. Maximum growth was achieved at 6% sucrose, 280 µmol photons $m^{-2}$ $s^{-1}$ and 2,000 µL $CO_2$ $L^{-1}$. The requirement for extra sucrose was observed despite a large accumulation of starch at high $CO_2$. Although the reason for this starch accumulation is unknown it may prove beneficial at planting out.

*Key words:* *Pinus radiata*, vitrification, photon flux density, $CO_2$, autotrophy, *in vitro*, sucrose, senescent, shoot culture.

## INTRODUCTION

*P. radiata* shoots grown *in vitro* require $CO_2$ availability during the light period to acquire photosynthetic competence (Antone-Chan, 1993). Achievement of photosynthetic competence enhanced growth and, perhaps most importantly, eliminated the problem of "wet" shoots (Antone-Chan, 1993). However, the addition of sucrose to the media further stimulated growth. Higher PFD was also shown to enhance photosynthetic competence possibly by producing more carbohydrates. These results suggest that carbohydrate supply may still limit growth *in vitro* even when photosynthesis is fully functional at 350 $\mu L\ L^{-1}$.

A large number of studies with $C_3$ plants grown in controlled conditions have shown that increasing the $CO_2$ above ambient increases the rate of photosynthesis, carbohydrate production and growth (Conroy, 1992). It is therefore likely that increasing the $CO_2$ concentration in the vial may enhance carbohydrate production and consequently remove the requirement for carbohydrate supply in the media. Removal of soluble sugars from the media would partly solve the problem of contamination, enhance autotrophy in the plantlets (Kozai, 1991) and may enhance survival at planting out. Given that vitrification and contamination are currently the problems which limit the financial success of micropropagation, solving these two problems would be a major achievement with economic consequences. Earlier studies showed $CO_2$ enrichment increased growth of plants *in vitro* (Cournac et al., 1991; Figueira et al., 1991; Infante et al., 1989; Kozai, 1991 and references therein and Mousseau, 1986); however no studies on *P. radiata* have been reported.

In an earlier study on *P. radiata*, it was demonstrated that increasing PFD could not completely substitute for no sucrose in the medium (Antone-Chan, 1993). It is not known whether increasing the $CO_2$ levels can eliminate the need for an external carbohydrate supply or whether higher PFD is needed to support the higher rates of photosynthesis at elevated $CO_2$. An advantage of $CO_2$ enrichment for glasshouse-grown plants aside from increasing growth is that accumulation of carbohydrates facilitates osmotic adjustment during drought (Conroy et al., 1988). If this occurred in *in vitro*-grown plants, death at the planting out stage should be reduced. This would further reduce the cost of micropropagated plants.

This study investigates the influence of $CO_2$ enrichment at different PFD and sucrose supplies on the growth and survival of *P. radiata* shoots *in vitro*. The soluble and structural carbohydrate and starch concentrations are also measured to determine whether extra carbohydrate is accumulated as a result of the treatments.

## Materials and Methods

### *Plant Material*

Shoot tip explants (about 10 mm long) were obtained from the stock plantlets of *P. radiata* D. Don family 20010 which had been maintained and routinely subcultured. Stock plantlets were produced by remultiplication of the adventitious shoots which had been initiated from excised cotyledons. The initiation of *P. radiata* shoots from excised cotyledons was adapted from Aitken-Christie and Thorpe (1984). The stock plantlets were then maintained and routinely subcultured every four weeks on a Modified Le Poivre nutrient medium (Aitken-Christie and Thorpe, 1984) containing 0.6% (w/v) Bacto Davis agar and 3% (w/v) sucrose. There was 30 ml of nutrient-agar medium in each 200 ml sealed jar which was maintained at sterilized culture conditions by autoclaving at 115 psi for 15 to 20 minutes.

The cultures were grown under Philips white, TL 65/80W/33RS fluorescent lights with a photon flux density of 80 to 100 μmol $m^{-2}$ $s^{-1}$ (measured at the level of the lids of culture jars using an Li-188 quantum sensor, LiCor, Inc., USA) over a 14-h photoperiod. The growth room temperature was maintained at 23 ± 2 °C.

## Experimental Design and Culture Conditions

### *Experiment 1*

Shoot explants were randomly chosen from the stock plantlets and transferred to LP/agar media. The media had either 0, 3 or 6% (w/v) sucrose contained in 200 ml unsealed jars. Unsealed jars are culture jars with two holes drilled unto the screwtop lids. One hole was fitted with a rubber seal and the other hole was fitted with a perspex tube 3 cm long and 1.5 cm in diameter. The perspex tube which allowed the atmosphere in the jars to equilibrate with ambient air was loosely covered with a cotton wool, thus preventing any bacterial contamination. The rubber seal facilitated the measurement of gases within the jars.

Each jar contained four explants. There were eight jars (replicates) for each sucrose concentration. Half of the jars containing each sucrose concentration were transferred to a controlled-environment cabinet at the ambient $CO_2$ concentration of 340 μL $L^{-1}$. The other half were transferred to a matched cabinet in which the air was enriched with $CO_2$ to 2,000 μL $L^{-1}$. The PFD was 150 mmol $m^{-2}$ $s^{-1}$ for the 14-h photoperiod. In an earlier study (Antone-Chan, 1993), analysis of the gases in the headspace of the unsealed culture jars have shown that its $CO_2$ concentration increased parallel to that of the chamber atmosphere.

*Experiment 2*

Shoot explants chosen from the stock plantlets were also transferred to LP/ agar media which contained either 0, 3 or 6% (w/v) sucrose as described above. There were also eight jars (replicates) for each sucrose concentration and each jar contained four explants. The $CO_2$ concentration in the growth cabinet was maintained at 2,000 μL $L^{-1}$ for all jars. However, half the jars at each sucrose concentration were exposed to a PFD of 150 μmol $m^{-2}$ $s^{-1}$ and the other half at 280 μmol $m^{-2}$ $s^{-1}$ for 14-h photoperiod. Explants were cultured for 50 days.

**Measurements**

All measurements were made after 50 days in culture. The foliar condition and the number of the plants which survived were assessed. If the number of dead or senescing primary needles per plantlet was less than 25% of the total, the plantlet was counted as surviving. Healthy plants were those which remained green and turgid, with new fascicles and leaf buds formed. The dying or dead plants include those which had stunted growth and had more than 25% yellow or etiolated leaves and dead aerial parts.

The fresh weights and heights of the shoots were measured before these were oven-dried (at 70°C) for 48 hours for dry weight measurements. The length of the three longest fully expanded primary needles of each plant was also measured (in Experiment 1 only).

Approximately 170 mg FW of fully expanded primary needles (pooled samples) from the remaining plants from each jar was used for carbohydrate determination. Tissue extraction and determination of total soluble sugars and starch were done. Total soluble sugars was determined by the anthrone hexose assay, where 0.7 ml sample (tissue extract) was mixed with 1.4 ml of anthrone reagent (2 mg anthrone/ml concentrated sulphuric acid). The mixture was then heated for 8 minutes in a boiling water bath and the concentration of glucose was measured spectrometrically at 620 nm.

Starch was determined by dispersing pellet in 0.2 N KOH at 100°C for 30 minutes. After adjusting the pH to 5.5 by the dropwise addition of 1M acetic acid, glucoamylase reagent was added and then incubated at 30°C overnight. Starch analysis was done on the supernatant using the anthrone hexose assay after boiling the suspension for one minute.

**Statistical Analysis**

The difference between the treatments and the significance of their interactions were assessed by analysis of variance (Student-Newman-Keuls test). The Fisher's exact test for independence in a 2x2 table was used to assess the data on survival. Standard errors were calculated to show the variation about the mean. The analyses were done using the software program CoStat (CoHort Software, Berkeley, CA, 1990).

## Results

### *Experiment 1*

Increasing the $CO_2$ concentration outside the unsealed jars with shoot cultures generally increased dry and fresh weights ($P < 0.01$) (Figure 1). Increasing the sucrose supply also on average increased the dry and fresh weights ($P < 0.01$). However, there was an interaction between the treatments ($P < 0.001$) because of the following: at 350 $\mu LL^{-1}$ $CO_2$, growth continued to increase with each sucrose addition. In contrast, at elevated $CO_2$, dry weight was maximum at 3% and declined at 6% sucrose. Consequently, at 6% sucrose the ambient-grown plants were heavier than those grown at high $CO_2$.

Shoot height was mainly affected by sucrose content in the medium ($P < 0.01$), however there was a significant interaction between $CO_2$ enrichment and increasing sucrose supply ($P < 0.0001$) (Figure 2) in the same manner as the dry weight. Thus, at 0 and 3% sucrose high $CO_2$ increased height, but at 6% sucrose it was decreased by $CO_2$ enrichment.

Needles were generally longer with $CO_2$ enrichment and increasing sucrose supply ($P < 0.05$) (Figure 3).

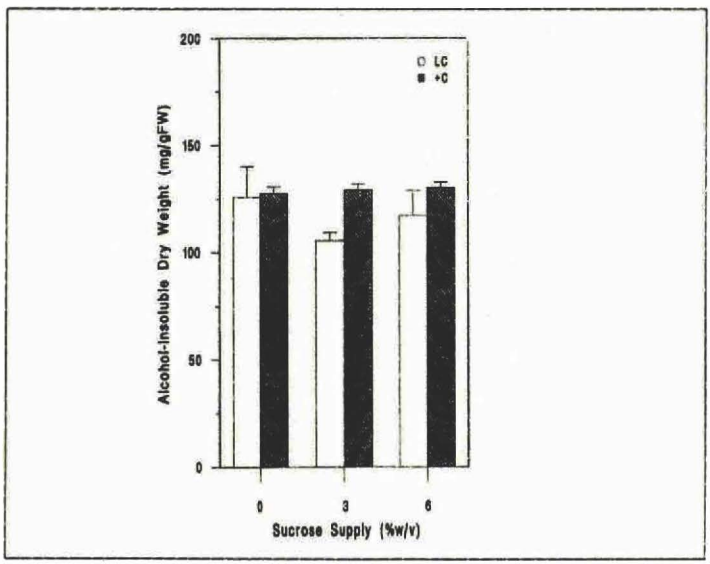


Figure 1. Influence of elevated $CO_2$ n the fresh weight (A) and dry weight (B) of *P. radiata* shoots after 50 days in culture. The plants were grown at 150 µmol photons $m^{-2}$ $s^{-1}$ with varying levels of sucrose in the medium and they were continually exposed to either 350 µL $L^{-1}$ of $CO_2$ (LC) or 2,000 µL $L^{-1}$ of $CO_2$ (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

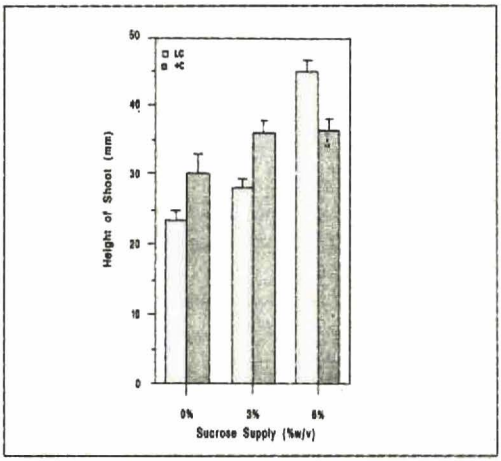


Figure 2. Height of *P. radiata* shoots after 50 days in culture at 150 μmol photons $m^{-2}$ $s^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either 350 μL $L^{-1}$ of $CO_2$ (LC) or 2,000 μL $L^{-1}$ of $CO_2$ (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

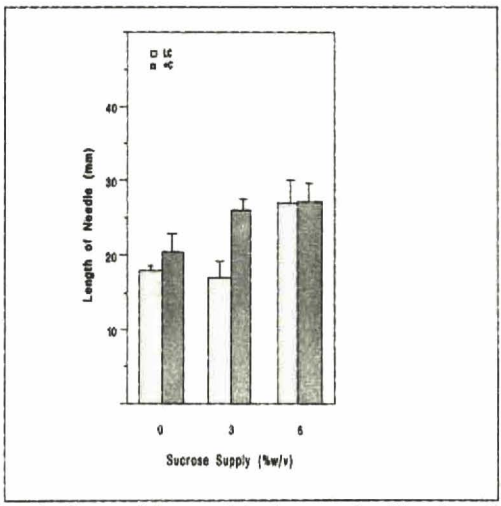


Figure 3. Length of longest fully expanded primary needle of P. radiata after 50 days in culture at 150 μmol photons $m^{-2}$ $s^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either 350 μL $L^{-1}$ of $CO_2$ (LC) or 2,000 μL $L^{-1}$ of $CO_2$ (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

Enriching the air with $CO_2$ also markedly improved survival of plants at each sucrose concentration ($P < 0.01$) (Table 1). Higher sucrose concentrations also enhanced survival and thus a combination of high $CO_2$ and 6% sucrose completely eliminated shoot death.

Elevated $CO_2$ and higher sucrose availability generally increased total soluble sugar concentration ($P < 0.001$) (Figure 4). The significant interaction between the treatments resulted from the large accumulation of sucrose due to $CO_2$ enrichment at 0% sucrose. The increase was negligible at the 3 and 6% sucrose.

The $CO_2$ treatments had a dramatic effect on starch accumulation ($P < 0.001$) and there was on average a four-fold increase in starch due to $CO_2$ enrichment (Table 2). Increasing sucrose availability also tended to cause an increase in starch content but not to the same extent as $CO_2$ enrichment. Consequently, the starch to soluble sugar ratio was greatly enhanced at the higher $CO_2$ level ($P < 0.001$) (Table 2). The effects due to sucrose supply on starch production were also significant ($P < 0.05$), showing that a strong interaction existed between sucrose supply and $CO_2$ enrichment.

The alcohol-insoluble dry weight represents the structural carbohydrate component of the plant, the major proportion of which is the cell wall. Neither sucrose supply nor $CO_2$ enrichment had a significant effect on this carbohydrate fraction (Figure 5).

Table 1. Influence of $CO_2$ enrichment at a PFD of 150 μmol photons $m^{-2}$ $s^{-1}$ on the survival of P. radiata shoots after 50 days in culture with varying levels of sucrose in the nutrient medium. The values (%) refer to the number of plants which either survived (healthy) or were dying/dead in relation to the total number of explants used.

| Sucrose supply (%w/v) | $CO_2$ μl $L^{-1}$ | Condition of the plant[a] | |
|---|---|---|---|
| | | Healthy | Drying/dead |
| 0 | | 350 | 44 56 |
| | 2,000 | 94 | 6 |
| 3 | 350 | 75 | 25 |
| | 2,000 | 94 | 6 |
| 6 | 350 | 100 | 0 |
| | 2,000 | 100 | 0 |

[a]The main effects due to CO2 enrichment and sucrose supply were significant (P<0.05).

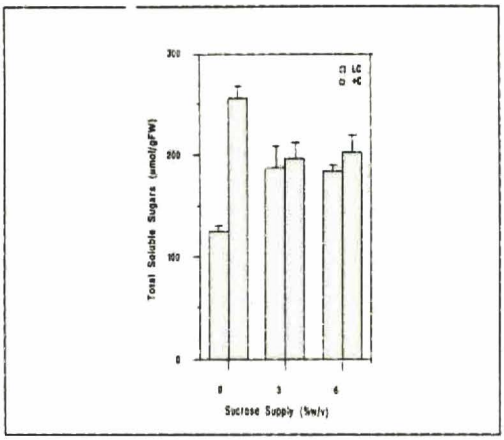


Figure 4. Influenced of elivated $CO_2$ on total soluble sugars (glucose equivalents) of *P. radiata* after 50 days in culture at 150 μmol photons $m^{-2}$ $s^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either 350 μL $L^{-1}$ of $CO_2$ (LC) or 2,000 μL $L^{-1}$ of $CO_2$ (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

Table 2. Influence of $CO_2$ enrichment at a PFD of 150 μmol photons $m^{-2}$ $s^{-1}$ on starch production of P. radiata shoots after 50 days in culture with varying levels of sucrose in the nutrient medium. Each value represents the mean of four replicates + standard error.

| Sucrose supply (%w/v) | $CO_2$ μL $L^{-1}$ | Starch production | |
|---|---|---|---|
| | | Starta (umol/gFW) | Starch soluble sugars ratio[b] |
| 0 | 350 | 41.7 + 6.7 | 0.34 + 0.07 |
| | 2,000 | 146 + 13 | 0.57 + 0.056 |
| 3 | 340 | 27.1 + 1.9 | 0.15 + 0.01 |
| | 2,000 | 188 + 9 | 0.88 + 0.15 |
| 6 | 340 | 72.5 + 8.4 | 0.37 + 0.04 |
| | 2,000 | 195 + 13 | 0.96 + 0.05 |

[a]The main effects due to $CO_2$ enrichment and sucrose supply were significant ($P<0.001$ and $P<0.05$, respectively) and there as also a significant interaction between these factors ($P<0.05$).

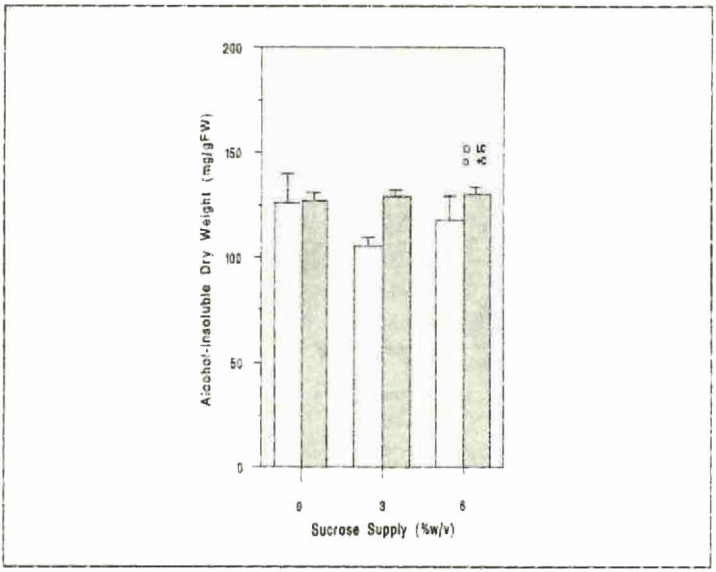


Figure 5. Influenced of elivated $CO_2$ on alcohol-insoluble dry weight of *P. radiata* shoots after 50 days in culture at 150 μmol photons $m^{-2}$ $s^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either 350 μL $L^{-1}$ of $CO_2$ (LC) or 2,000 μL $L^{-1}$ of $CO_2$ (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

## Experiment 2

Increasing the PFD at a $CO_2$ concentration of 2,000 μL $L^{-1}$ increased both the dry and fresh weights at all sucrose concentrations ($P < 0.01$) (Figure 6). The largest relative increase was at 6% sucrose giving a significant interaction between PFD and sucrose supply. Consequently, weights continued to increase with higher sugar availability at the higher PFD. In contrast, there was a decline in plant weight with increasing sucrose concentration at the lower PFD (Figure 6). This confirms the results of experiment 1 (Figure 1).

Increasing PFD increased shoot height at each sucrose level ($P < 0.01$) (Figure 6A). There was an interaction between sucrose supply and PFD because higher sucrose availability only increased height when the PFD was 280 μmol $m^{-2}$ $s^{-1}$. The same general trend was observed in experiment 1, however there was a small increase in height when the sucrose supply was increased from 0 to 3% (Figure 2).

Because of the elevated $CO_2$ used in this experiment there were very few unhealthy shoots even at 0% sucrose (Table 3).

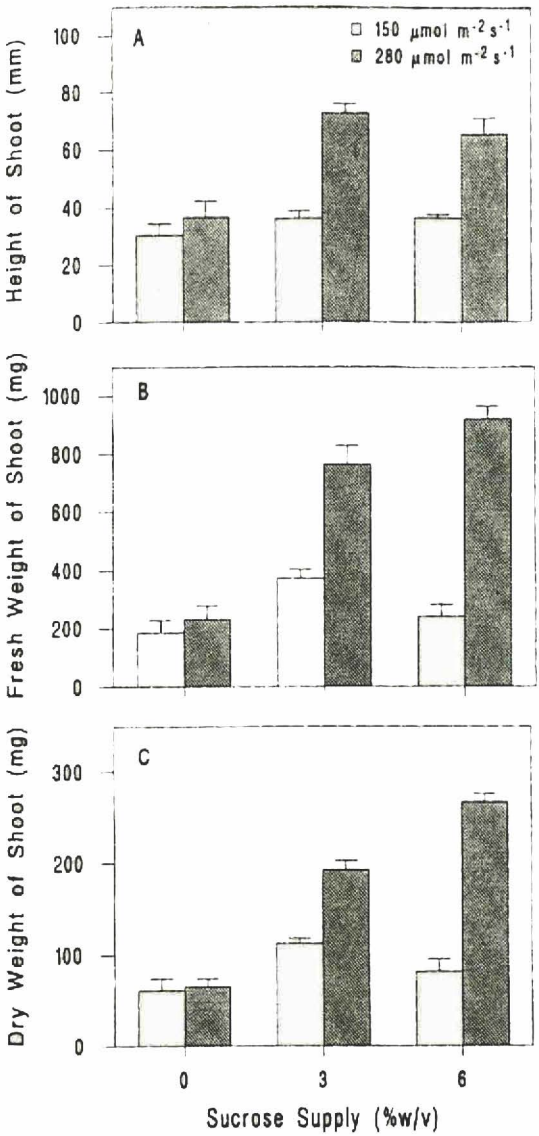


Figure 6. Influenced of elivated $CO_2$ on alcohol-insoluble dry weight of *P. radiata* shoots after 50 days in culture at 150 μmol photons $m^{-2}$ $s^{-1}$ with varying levels of sucrose in the medium. The plants were continually exposed to either 350 μL $L^{-1}$ of $CO_2$ (LC) or 2,000 μL $L^{-1}$ of $CO_2$ (+C). Each bar represents the mean of four replicates. One standard error is indicated at the top of each bar.

Table 3. Influence of photon flux density (PFD) on the survival of *P. radiata* shoots after 50 days in culture at 2,000 µl $L^{-1}$of $CO_2$. The plants were grown in an LP/agar medium with varying levels of sucrose. The values (%) refer to the number of plants which either survived (healthy) or were dying/dead in relation to the total number of explants used.

| Sucrose supply (%w/v) | PFD µmol $m^{-2}$ $s^{-1}$ | Condition of the plant[a] | |
|---|---|---|---|
| | | Healthy | Drying/dead |
| 0 | 150 | 94 | 6 |
| | 280 | 83 | 17 |
| 3 | 150 | 94 | 6 |
| | 280 | 100 | 0 |
| 6 | 150 | 100 | 0 |
| | 280 | 100 | 0 |

[a]The differences due to PFD were not significant.

## Discussion

The highest shoot productivity of *P. radiata* over 50 days in culture was achieved by providing a PFD of 280 µmol $m^{-2}$ $s^{-1}$ at a $CO_2$ concentration of 2,000 µL $L^{-1}$ and a 6% sucrose concentration in the medium (Figure 6). Failure to provide sufficiently high PFD resulted in a depression of growth by high $CO_2$ at 6% sucrose (Figure 1). Shoot height was affected in a similar way (Figures 2 and 6A). These findings indicate that elevated $CO_2$, PFD and sucrose supply all played a role in shoot growth. The effect of these vial factors were probably mediated through effects on photosynthesis and water relations (discussed in Chapter 4 and 5 in Antone-Chan, 1993).

$CO_2$ enrichment has the potential to increase photosynthesis *in vitro* especially in the presence of high light levels (Desjardins et al., 1988; Fujiwara et al., 1988). The enhancing effects of elevated $CO_2$ on the rate of $CO_2$ assimilation has long been known in most $C_3$ species (Kimball, 1983). The reason is that the first enzyme in the $C_3$ photosynthetic pathway, ribulose-1,5-bisphosphate carboxylase (Rubisco) has an affinity for oxygen as well as $CO_2$. Dissipation of energy through photorespiration reduces the rate of photosynthesis by 40-50% (Sharkey, 1985). Raising the $CO_2$ concentration increases the ratio of $CO_2$ to $O_2$ at the site of fixation in the chloroplast thereby, increasing photosynthetic rates. Thus, the response of photosynthesis to increasing $CO_2$ levels from 0 to approximately 1,000 µL $L^{-1}$ is linear at saturating PFD (Farquhar and Von Caemmerer (1982).

At $CO_2$ concentrations above this, RuBP regeneration becomes limiting because insufficient reducing power is produced by the light reactions of photosynthesis. Hence, a family of $CO_2$ response curves, plateauing at increasing $CO_2$ levels can be constructed by measuring photosynthesis at increasing PFD. Consequently, in my experiments, at $CO_2$ concentrations of 2,000 $\mu L\ L^{-1}$, which would have almost completely suppressed photorespiration, higher PFD was required to promote maximum growth (Figure 6). It is therefore, essential to increase the PFD if gains in growth are to be made by increasing the concentration of $CO_2$.

A third limitation to photosynthesis has been described by Sharkey (1985), i.e., triose phosphate utilization limitation. When there are insufficient sinks for the carbohydrates produced by photosynthesis, feedback inhibition of photosynthesis occurs possibly via altering Rubisco activity. In many species, feedback inhibition can be delayed by diverting triose phosphate from sucrose to starch production in the chloroplast. Starch accumulation was observed in the pine plantlets grown at 2,000 $\mu L\ CO_2\ L^{-1}$. The starch to soluble carbohydrate ratio was on average of 0.8 at elevated $CO_2$ compared with 0.3 at ambient $CO_2$ (Table 2). Hence, the increase in shoot dry weight at elevated $CO_2$ was partly attributable to the accumulation of starch. Although increasing the PFD would have also increased photosynthetic rates (as demonstrated in previous experiments (Chapter 5 in Antone-Chan, 1993), the starch to soluble carbohydrate ratio was not substantially increased. Morin et al. (1992) have suggested that the increase in starch in glasshouse-grown clover at elevated $CO_2$ was caused by altering the ratio between photorespiration and photosynthesis. Changes in Pi flux into the chloroplast and/or nitrogen flux through the photorespiration cycle may be responsible for changing the starch to soluble sugar ratio at elevated $CO_2$ (Conroy, 1992).

The finding that starch accumulation partly accounted for the increase in dry weight is significant for plant survival on planting out because starch reserves could be degraded to soluble sugars and serve as substrate for growth or for osmotic adjustment. An interesting observation was that structural carbohydrate (estimated as alcohol-insoluble dry weight) was unaffected by either $CO_2$ enrichment or higher sucrose supply. It is a common observation that leaves of high $CO_2$-grown plants appear "stiffer". It is therefore likely that changes in cell wall properties on water relations are responsible. This is supported by the findings in an earlier experiment (Antone-Chan, 1993) that the relationship between relative water content, osmotic potential and water potential was altered when $CO_2$ was available in the culture vessels.

Higher $CO_2$ availability eliminated senescent shoots. Earlier (Antone-Chan, 1993), it was demonstrated that vitrified (or "wet") shoots could be eliminated by facilitating gas exchange between the air and the jars. It was concluded that the presence of $CO_2$ as substrate for photosynthesis during the light period may have contributed. Increasing the $CO_2$ even higher, may have further enhanced longevity by providing carbohydrate reserves in the form of starch. The finding that higher sucrose supply also eliminated dead shoots supports this idea (Table 1).

Increasing $CO_2$ from 350 to 2,000 μL $L^{-1}$ resulted in increased needle length as well as starch accumulation, irrespective of sucrose level at a PFD of 150 μmol $m^{-2}$ $s^{-1}$. This increase in expansion may have been due to greater substrate availability, improved water relations or changes in the elastic properties of the cell walls. It was demonstrated that *P* was maintained better when $CO_2$ concentration was increased and sucrose was provided (Chapter 4 in Antone-Chan, 1993). In the present study, the response of elongation at high PFD and $CO_2$ irrespective of sucrose may indicate an effect of $CO_2$ on water relations and/or direct effect on elongation.

## Conclusion

Enrichment of the atmosphere of the unsealed jars with $CO_2$ to a concentration of 2,000 μL $L^{-1}$ enhanced the growth of *P. radiata* shoots in vitro provided that sufficient reducing power could be generated. Consequently, the growth increases were only achieved at a PFD of 280 μmol $m^{-2}$ $s^{-1}$. $CO_2$ enrichment also reduced the number of senescent plants.

Increasing the $CO_2$ concentration also greatly enhanced starch accumulation. It was, therefore, surprising that despite the accumulation of carbohydrate, increasing the concentration of sucrose in the media to 6% further increased growth. At the lower PFD this was not observed.

The results indicate that maximum growth is achieved at high $CO_2$ concentrations and PFD and with 6% sucrose. In practical terms it would be possible to remove the sucrose from the media because the decrease in growth may be more than offset by the reduced likelihood of contamination. High $CO_2$ is also advantageous because it leads to accumulation of starch which on planting out would be a valuable carbohydrate reserve.

The results are also interesting in terms of basic plant physiology. Many questions have been raised about the influence of external sucrose supply, $CO_2$ enrichment and PFD on accumulation of carbohydrates and their influence on shoot elongation. Whether high $CO_2$ has an impact on growth other than via its effect on photosynthesis remains to be investigated.

## ACKNOWLEDGEMENTS

Assoc. Prof. E.W.R. Barlow
Dr. Malcolm L. Reed
Patrick O'Connell (CSIRO Division of Food Research)
Jacob Milczanowski
Dr. Benjamin O. Chan
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