# Effects of Lighting and $CO_2$ Enrichment on Acclimatization of Micropropagated Woody Plants

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**In order to test the effects of $CO_2$ enrichment and light intensity on the acclimatization and ex vitro performance of micropropagated woody plants we have designed and constructed an inexpensive $CO_2$ enrichment/fogging chamber which could be easily adapted for commercial use. $CO_2$ enrichment during acclimatization has been shown to be beneficial with mountain laurel, lilac, grape, apple, and raspberry, but not with serviceberry, blueberry, or sweet cherry.**

## INTRODUCTION

The focus of most technical reports on micropropagation of woody plants is on the optimization of the in vitro chemical and physical environment. However, when it comes to managing the transition from tissue culture to the greenhouse or field environment (stage IV or acclimatization), growers are largely left to fend for themselves. This is not surprising since relatively little research has focused on this final but very critical stage of micropropagation despite the fact that it can be critical in terms of success and the overall profitability of micropropagation. A number of crops such as apple and serviceberry proliferate easily in culture, but high losses in the acclimatization phase can render their micropropagation marginally or wholly unprofitable.

The *acclimatization protocol for woody plants in most commercial* micropropagation operations typically involves the transfer of unrooted micro shoot cuttings from the in vitro tissue culture environment, into an ex vitro modified greenhouse environment characterized by high humidity, and low light intensity. Acclimatization systems usually rely on shaded natural lighting supplemented, in some cases, by supplemental lighting for photoperiod extension. Under these conditions, microcuttings are expected to initiate a new adventitious root system as well as new shoot growth.

A problem inherent in acclimatization systems located within a greenhouse facility is that light intensity varies hourly, daily, and seasonally. Consequently, microcuttings experience not only variable lighting, but also temperature, and to some extent relative humidity as well. Furthermore, in tightly closed systems, such as the polystyrene sandwich-type boxes often used for acclimatization, $CO_2$ concentrations may become limiting during the light period due to photosynthetic utilization of $CO_2$. Although atmospheric $CO_2$ concentration has occasionally been experimentally enriched with beneficial effects during in vitro culture (Kozai, et al., 1988) or during stage IV acclimatization (Desjardins, et al., 1990; Lakso, et al., 1986), there is little commercial application of in vitro or ex vitro $CO_2$ enrichment.

It has been our view that in vitro $CO_2$ enrichment will be technically difficult to implement and economically difficult to justify because of the difficulty of avoiding contamination in a gas-flow-through system. The acclimatization stage would appear to be a particularly appropriate time to intervene with enrichment $CO_2$. It could be accomplished at this stage more easily and less expensively compared to the tissue culture (in vitro) environment, or compared to the post-acclimatization stages of greenhouse production where plants occupy a far larger amount of space which would be much more expensive to treat. Our objectives over the last several years have been to develop a practical, economical ex vitro acclimatization system in which $CO_2$ concentration, light intensity, temperature, and relative humidity can be optimized at an affordable cost to growers.

## MATERIALS AND METHODS

We designed and constructed an acclimatization chamber for experimental optimization of carbon dioxide and lighting which allows us to vary $CO_2$ and lighting in complete 2 × 3 factorial arrangement of treatments, with two levels of $CO_2$ and three levels of light intensity. The overall six compartment chamber was built within an enclosed basement room with no windows. It was 12 ft long × 6 ft wide. The chamber was subdivided into six compartments in a 2 wide × 3 long arrangement. Each compartment measured 4 ft in length and width and sloped from a height of 22 in. at the back, along the central chamber axis, to 12 in. in front. Walls and floor were constructed from 0.5-in. plywood. Interior walls were lined with waterproof bathroom paneling and the bottom with 1/8-in. thick vinyl flooring. Joints were caulked with silicone. Each chamber had a separate lid, consisting of a wooded sash with clear Flex-O-Pane plastic stretched across it. Lids were hinged in the back and sloped from back to front to facilitate cleaning and to maximize light transmission by encouraging beads of condensation to roll off the inside of the lid.

Three compartments along one long side of the chamber were equipped with a $CO_2$ enrichment system while the three compartments along the other side were at the ambient $CO_2$ level of the room (approx. 450 pm). $CO_2$ level in the enriched side was monitored and controlled with a $CO_2$ monitor/controller (Horiba Instruments Inc., Irvine, California) set to open a solenoid connected to a tank of compressed $CO_2$ when the $CO_2$ level in the chambers dropped below 1,200 pm.

Humidification was provided by four inexpensive, ultrasonic cool-fog humidifiers designed for household use. One humidifier was located at each corner of the overall chamber, and fog was distributed to each of the six individual chambers via ½-in. diameter PVC tubing. Humidifiers were connected to a timer set to a 5-min on/5-min-off schedule, which resulted in 98±2% relative humidity.

Illumination (16 h/day) was from four 8-ft long Cool White florescent tubes running over each high/low $CO_2$ pair of chambers, perpendicular to the long axis of the chamber. Experimental variation in light intensity was accomplished in early experiments by varying the number of florescent tubes above each pair of chambers, or, in later experiments, four tubes were placed over each pair of compartments and the medium and low light compartments were shaded with one or two layers, respectively, of Saran shade cloth laid directly on top of the lids.

Temperature control within the room (not the compartments themselves) was achieved by means of an air conditioner. To achieve as much uniformity as possible of temperature, $CO_2$ concentration, and relative humidity between compartments

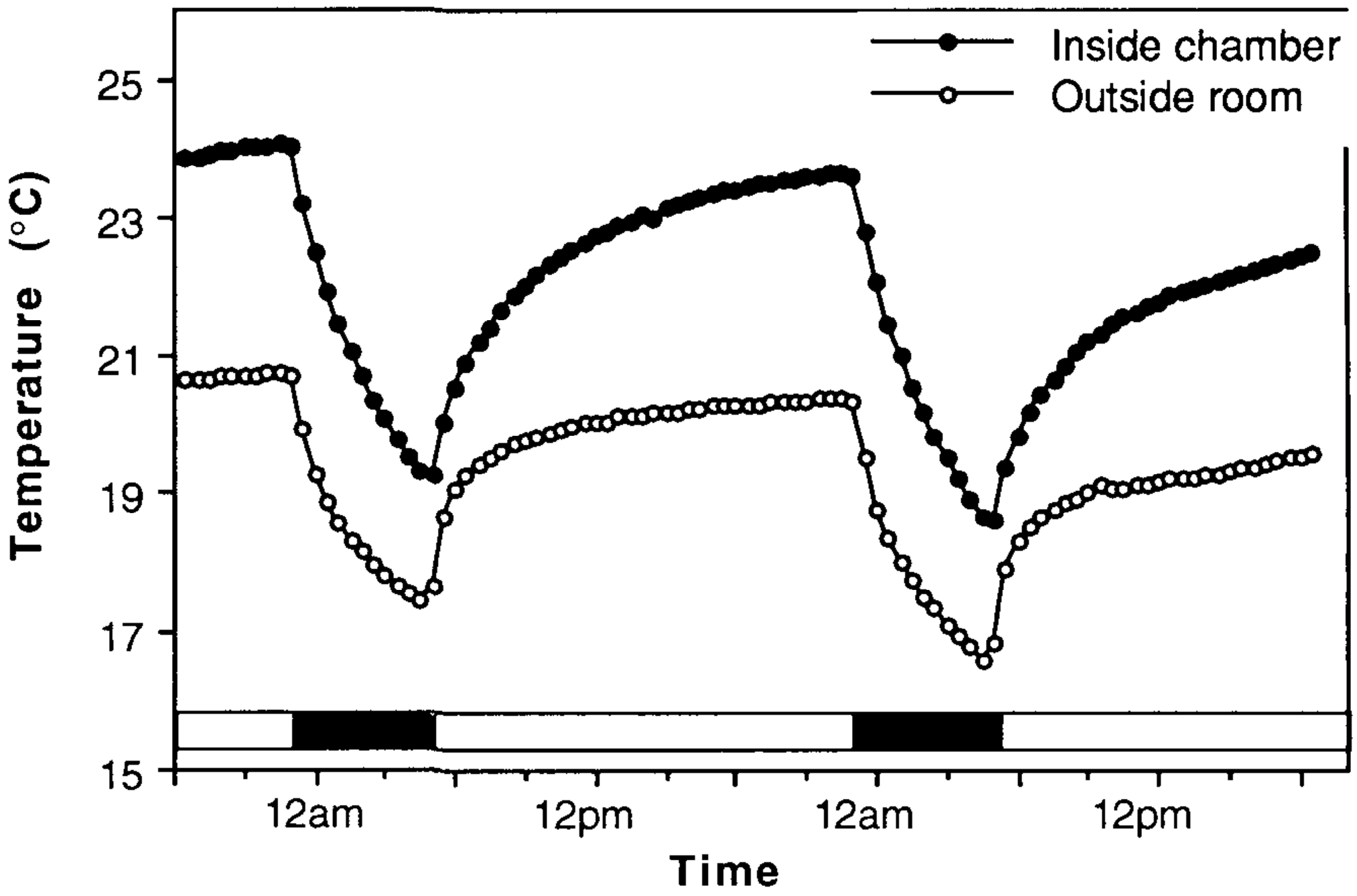


**Figure 1**. Acclimatization chamber and room temperature variation over time.

(within a $CO_2$ treatment), the three compartments on either the high or the low $CO_2$ side of the chamber were interconnected with a pair of ventilation fans in their common interior walls. The two fans in each common wall blew in opposite directions to set up circular air flow with in the three compartments.

Over the past several years, we have conducted the basic 2 × 3 factorial experiment consisting of two $CO_2$ levels (450±50 and 1,200±200 pm) and three light intensities (specified below for each experiment) with eight species of woody ornamental and fruit crops including mountain laurel (*Kalmia latifolia*), lilac (*Syringa vulgaris*), serviceberry (*Amelanchier canadensis*), grape (*Vitus labruscana*), apple (*Malus sylvestris* var. *domestica)*, sweet cherry (*Prunus avium*), blueberry (*Vaccinium corymbosum*), and red raspberry (*Rubus idaeus*). In all cases, unrooted microcuttings from Stage II shoot cultures were transplanted without rooting hormone into flats containing a suitable rooting medium (peat in the case of mountain laurel, and peat vermiculite for other species). Mountain laurel and grape microcuttings were donated by the commercial tissue culture laboratories (Knight Hollow Nursery, Madison, WI and Congdon and Weller Nursery, North Collins, NY, respectively). The other species were cultured in our own micropropagation laboratory at Cornell using standard shoot proliferation media and protocols.

## RESULTS AND DISCUSSION

Figure 1 shows the relationship between photoperiod and temperature in the experimental compartments and in the outer room. Heat generated from the florescent lights increased steadily throughout the light period. Chamber temperature was approximately 3°C higher than room temperature at any given time due

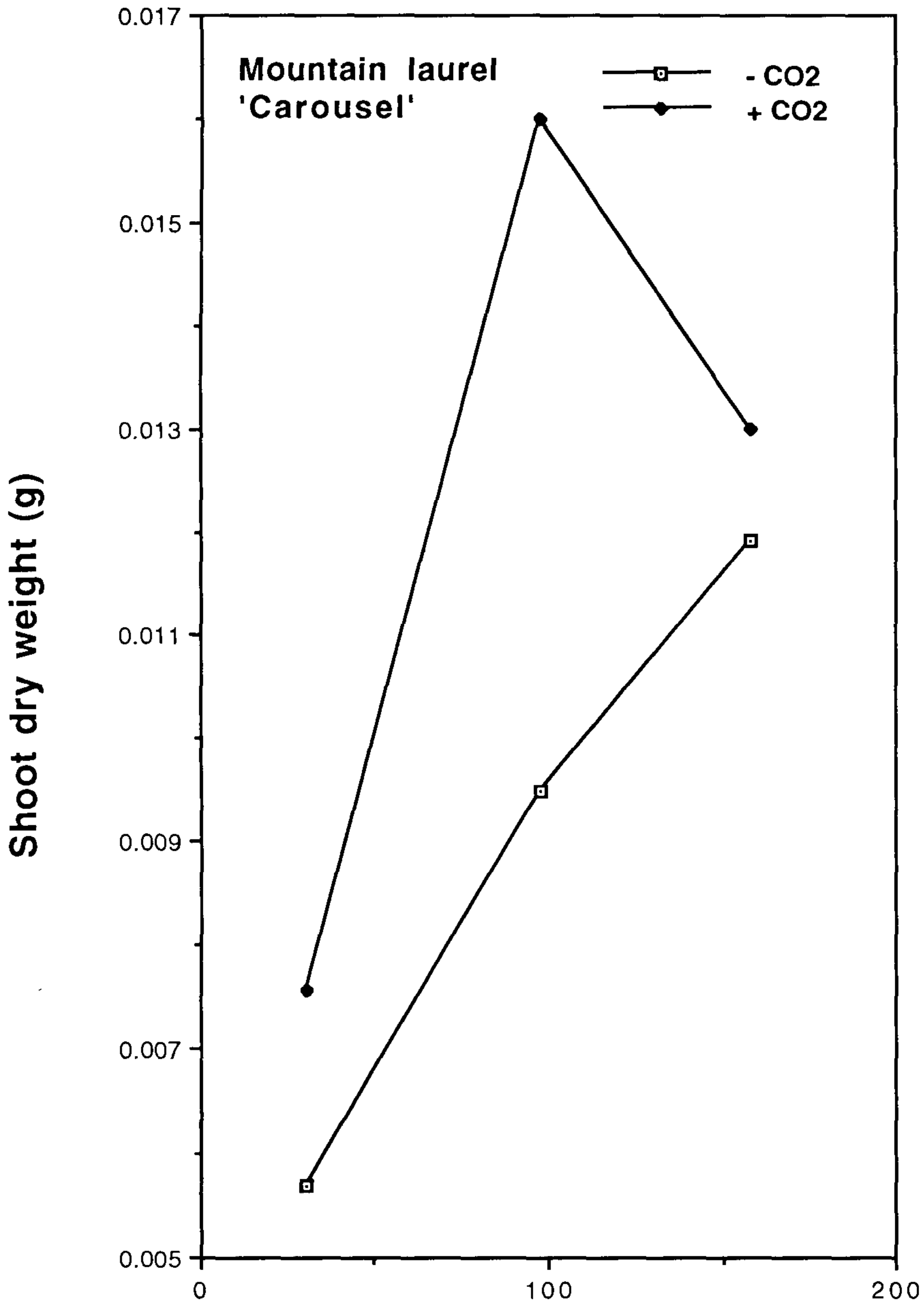


**Figure 2**. Effect of $CO_2$ enrichment and light intensity (irradiance) on shoot growth of mountain laurel.

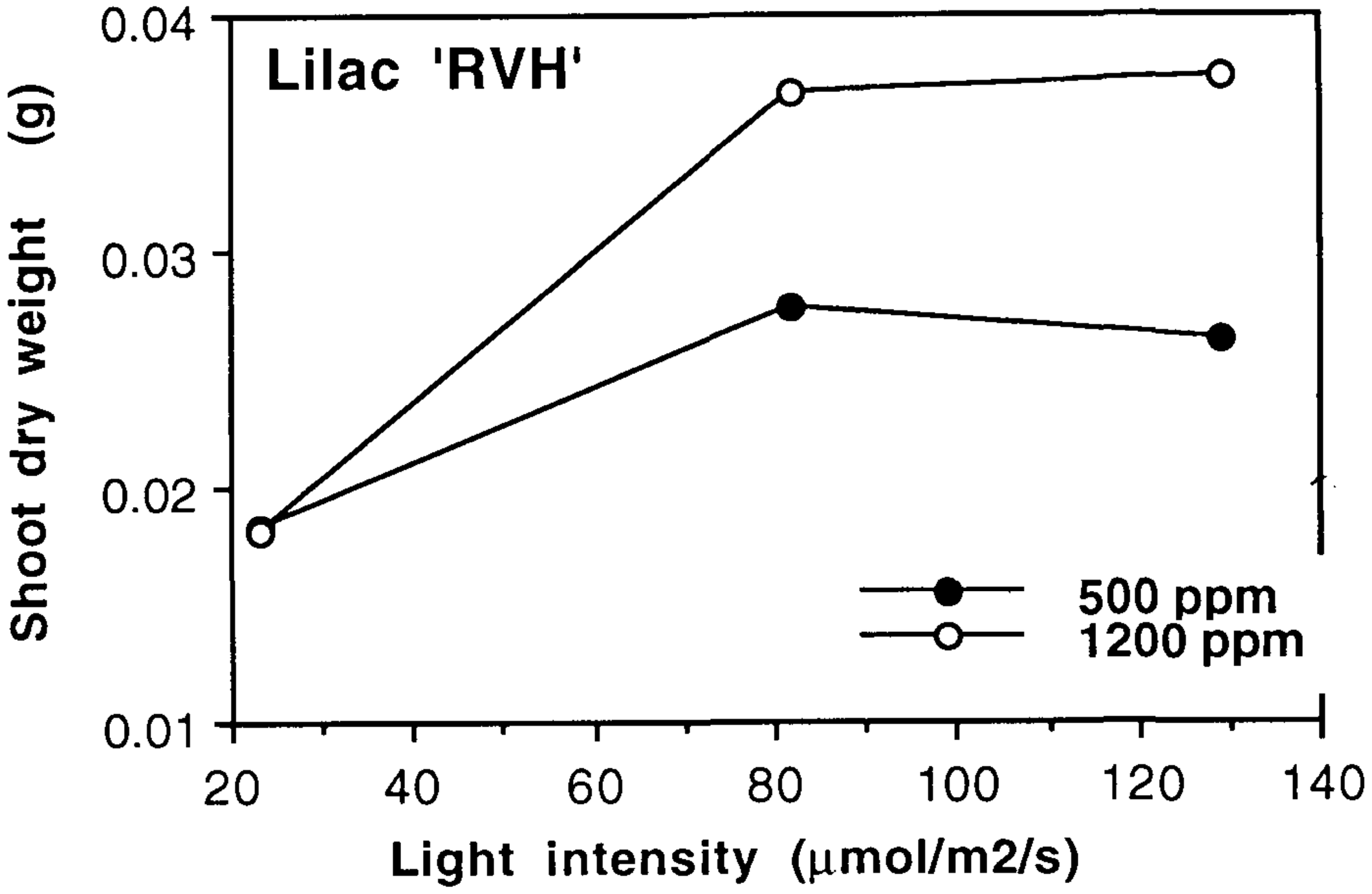


**Figure 3**. Effect of $CO_2$ enrichment and light intensity (irradiance) on shoot growth of lilac.

to a "greenhouse" effect. Between compartment temperature variation was no more than 2°C at any given time due to the intercompartment air circulation fans, despite more than 3-fold difference in illumination level from high to low light treatments. There was, however, seasonal variation in room and chamber temperature ranging from a 23°C (winter) to 29°C (summer) (24-hour average).

Our first experiments were conducted with the mountain laurel cultivars Elf and Carousel. Figure 2 shows that shoot dry weight accumulation after 8 weeks in the fogging chamber increased with increasing light intensity and in response to $CO_2$ enrichment, with the best shoot growth with $CO_2$ enrichment at the medium light level. Similar results were obtained for the effect of $CO_2$ enrichment and light intensity on root dry weight except that the $CO_2$ stimulation was greatest at the high rather than the medium light level.

Figure 3 shows similar results for the lilac cultivar RVH. Both shoot growth and root growth (not shown) were enhanced by $CO_2$ at the medium and high light but not at the low light level.

Red raspberry, apple, and grape are other species which have responded positively to $CO_2$ enrichment. Serviceberry, blueberry, and cherry, on the other hand, have not exhibited a positive response to $CO_2$ enrichment. We believe that the relatively high summer temperatures (29°±2°C) may be a factor in this lack of response to $CO_2$ enrichment, and we are currently modifying the system to achieve better temperature control. Furthermore, we have noted that all species in the Rosaceae family which we have included in these experiments (serviceberry, apple, and cherry), appear to undergo shoot dormancy or "stall out" during acclimatization. In recent experiments with serviceberry we have been able to

overcome this dormancy to some extent by spraying foliage with 100 ppm gibberellic acid ($GA_3$) at the time when microcuttings are beginning to root in the acclimatization fog boxes (about 3 weeks after sticking).

## LITERATURE CITED

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