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# Fixation of $^{14}CO_2$ in tissue cultured red raspberry prior to and after transfer to soil

DANIELLE J. DONNELLY, WILLIAM E. VIDAVER and KONRAD COLBOW

Department of Biological Sciences, Simon Fraser University Burnaby, British Columbia, V5A 1S6 Canada

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**Abstract.** The $^{14}CO_2$ uptake of an aseptically cultured red raspberry clone (*Rubus ideaus* L.) was examined prior to and after transfer to soil. Individual leaves of transplants, both persistent from culture and new ones, were tested 5 weeks after transplant for $^{14}CO_2$ uptake capability. Transplant leaves of successive weekly age classes took up $^{14}CO_2$ at increasing rates per unit area, displaying a spectrum of photosynthetic competence from low levels close to that of leaves from culture, to that of control plants. This is illustrative of acclimatization to the soil environment and was related to transplant light intensity.

## Introduction

Little is known about how tissue cultured plants [8, 9] are re-established in vivo or how transplants acclimatize to soil environments. Evaluation of physiological changes taking place during acclimatization is prerequisite to comprehension of this process and necessary to the development of more efficient transplant protocols. This is important because... the ultimate success of plant tissue culture as a commercial means of plant propagation depends on the ability to transfer plants out of culture on a large scale, at low cost, and with a high survival rate... [1].

Both leaf anatomy and carbon fixation ability of cultured cauliflower [*Brassica* sp.; 5, 6 and 7] and red raspberry plantlets [*Rubus idaeus* L.; 3, 4] are affected by the in vitro environment. Cauliflower transplants do not demonstrate net $CO_2$ uptake until 2 weeks after transfer to soil. New leaves formed the second week after transplantation exhibit greenhouse control levels of $CO_2$ uptake [6]. After transplanting, red raspberry is capable of net $CO_2$ uptake, and developing new leaves are transitional in the sense that both anatomy and photosynthetic $CO_2$ uptake (of new leaves tested in a group) are intermediate between those of plantlets in vitro and greenhouse control plants [3, 4]. To further investigate acclimatization in red raspberry, the photosynthetic capabilities of the persistent leaves retained from culture (unlike cauliflower, red raspberry transplants retain these leaves for many weeks) and successively formed new (transitional)

leaves were examined; $^{14}CO_2$ was used to quantify uptake ability in individual leaves 5 weeks after transplant.

## Materials and methods

The red raspberry plants utilized in these experiments were cloned from a new Haida x Canby selection (BC72-1-7), developed by the British Columbia red raspberry breeding program. Red raspberry plantlets were cultured in 500 ml jars containing a 3 cm wide filter paper support strip around the circumference of the jar, and 30 ml of modified red raspberry rooting medium [2] in that 25 g/L glucose replaced 30 g/L sucrose. Plantlets incubated at $25\,\mu Es^{-1}\,m^{-2}$ were tested at the end of a 4 week culture cycle, the time at which these rooted plantlets were transferred to soil.

Eight plantlets with all leaves tagged were transferred to soil at each of three light intensities, 40, 80 and $120\,\mu Es^{-1}\,m^{-2}$. At weekly intervals all newly emerged leaves of transplants were tagged with petiole markers of an identifying colour. After 5 weeks in soil transplants from each light treatment were used for labelled $CO_2$ fixation experiments. The transplants were held under conditions of elevated relative humidity by closing the flats with transparent covers and misting them regularly. Cultures and transplants were incubated under controlled temperature ($27 \pm 2\,^{\circ}C$) and lighting (16 h photoperiod with 3:1 cool white : warm white fluorescent lighting).

All raspberry specimens; plantlets, transplants and control plants; were subjected to the same $^{14}CO_2$ fixation assay. The experimental apparatus consisted of a closed gas circuit with a plant chamber [10]. This circuit incorporated a gas generation vessel, gas mixing chamber, Geiger counter and pump. The counter was connected to a chart recorder. Thirty $\mu$l of $^{14}$carbonate solution of $12\,\mu Ci$ total activity was placed in a gas generation receptacle, and 2.5 ml of $2N\,H_2SO_4$ added to release the $^{14}CO_2$ into a gas circuit excluding the plant chamber. The pump circulated the gases through the mixing chamber, for 5–10 min, until an equilibrium of $^{14}CO_2$ indicated by the Geiger counter trace, was reached. The specimen was inserted into the plant chamber and exposed to cool white fluorescent light of saturating ($250\,\mu Es^{-1}\,m^{-2}$) intensity. After 1 minute of light exposure the circuit was made to include the plant chamber for a $^{14}CO_2$ exposure period of 5 min (± 10 s). The specimen was then removed from the chamber and a paper punch used to take tissue samples of known area ($0.385\,cm^2$) from each of the many tiny leaves of cultured plantlets and transplants and the large control leaves. When leaves or buds were smaller than the paper punch disc size their outlines were traced onto graph paper for an area estimation. Samples were placed in calibrated test tubes containing approximately 5 ml of hot 80% ethanol. The tubes were capped with aluminum foil and boiled for 2 min in a water bath; this was sufficient to extract all labeled compounds. The extracts were rapidly cooled by placing

the tubes on ice, then made up to 5 ml volume with 80% ethanol. The tubes were agitated and three 100 $\mu$l samples withdrawn from each for $^{14}C$ counting in a Beckmann liquid scintillation counter. Total activity counts were corrected for background activity, then adjusted for volume of the alcohol extract and sample leaf areas [10].

## Results and discussion

Mature control leaves ($n = 21$) tested through the course of the summer gave activity counts that averaged $34006 \pm 1585$ dpm $cm^{-2}$. Young leaves and leaf buds ($n = 24$) gave activity counts averaging $9415 \pm 711$ dpm $cm^{-2}$. Leaves of plantlets, tested at midday directly from their culture containers, had activity levels of $21149 \pm 1006$ dpm $cm^{-2}$. This was higher than expected, suggesting a $CO_2$ uptake rate greater than observed with gas exchange experiments. Carbon dioxide uptake measured over a longer interval in an infra-red gas analyzer showed that cultured plantlets had $CO_2$ uptake rates of only 2–3 mg $dm^{-2}$ $h^{-1}$ compared to control plant rates of 10–15 mg $dm^{-2}$ $h^{-1}$ [4]. This elevated level of $CO_2$ uptake is most probably brief. Possibly plantlets photorespire non-labeled $CO_2$ originating from glucose in the medium, while control plants and transplants photorespire $^{14}CO_2$ of photosynthetic origin, accumulating proportionately less label.

Measured 5 weeks after transplantation, leaves formed during the first week after transplant to soil had activity levels that were much higher than those of persistent leaves (Figure 1). The most dramatic increase occurred at 120 $\mu Es^{-1}$ $m^{-2}$ where there was a 160% increase. There was an increase of 135% in 40 and 80 $\mu Es^{-1}$ $m^{-2}$ transplants. Carbon dioxide fixation levels at all light intensities were higher in leaves developed during each successive week in the soil environment. The newest growth, developed during the fifth week, achieved activity levels ranging from 165% of culture-formed leaves in the 40 $\mu Es^{-1}$ $m^{-2}$ transplants to almost 200% in those at 120 $\mu Es^{-1}$ $m^{-2}$. Maximum activity levels of new leaves were 53–66% of that of mature greenhouse control leaves, presumably much closer to plants grown at comparable light intensities. There appears to be an advantage to transplanting to the highest light intensity (120 $\mu Es^{-1}$ $m^{-2}$): these transplants had the greatest activity levels per unit and new leaves formed during the first week after transplant to soil at 120 $\mu Es^{-1}$ $m^{-2}$ had the greatest increase in $CO_2$ uptake ability over the persistent leaves. Furthermore, it has been demonstrated that leaf area and dry matter content are significantly higher in new leaves formed at 120 $\mu Es^{-1}$ $m^{-2}$ [4]. The graded increase in $CO_2$ uptake seen in successive week's new leaf growth in all light intensities is apparent despite the fact that these are of increasingly younger age classes. It is clear that during acclimatization photosynthetic carbon fixation ability improves as leaf anatomy undergoes transition away from the effects of culture and this is accelerated by higher transplant light intensities.

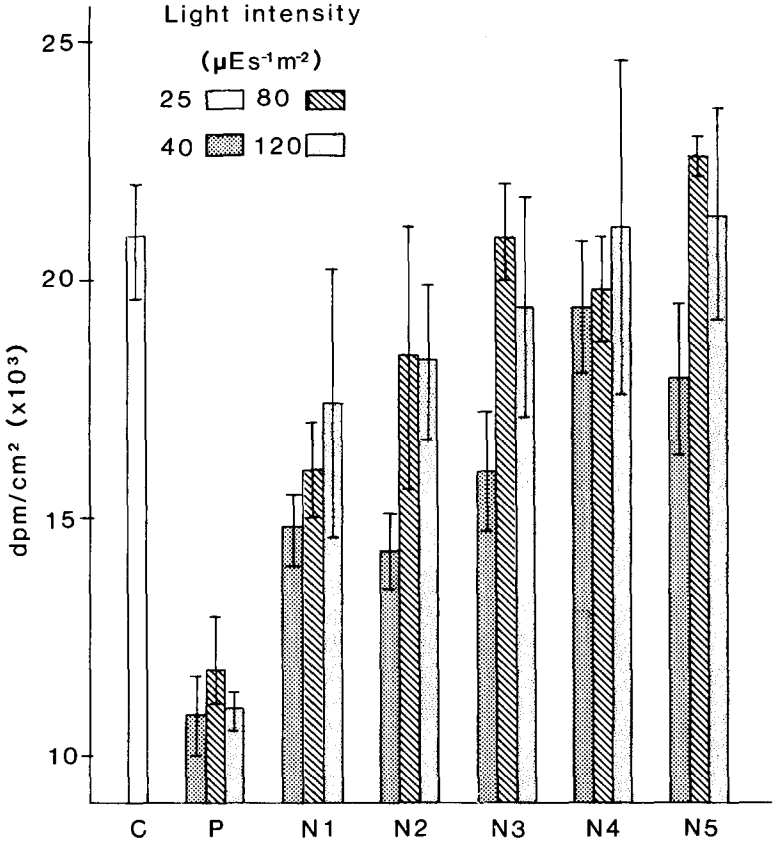


Figure 1. Fixation of $^{14}CO_2$ per unit area (mean and standard error) in leaves of cultured red raspberry plantlets prior to (C) and 5 weeks after transfer to soil. Leaves of transplants were persistent from culture (P) or new (N) ones developed during successive weeks (1–5) after transplantation.

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