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Application of an enthalpy balance model of the relation between growth and respiration to temperature acclimation of *Eucalyptus globulus* seedlings

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The enthalpy balance model of growth uses measurements of the rates of heat and CO_2 production to quantify rates of decarboxylation, oxidative phosphorylation and net anabolism. Enthalpy conversion efficiency (η_H) and the net rate of conservation of enthalpy in reduced biosynthetic products ($R_{SG}\Delta H_B$) can be calculated from metabolic heat rate (q) and CO_2 rate (R_{CO_2}). η_H is closely related to carbon conversion efficiency and the efficiency of conservation of available electrons in biosynthetic products. $R_{SG}\Delta H_B$ and η_H can be used, together with biomass composition, to describe the rate and efficiency of growth of plant tissues. q is directly related to the rate of O_2 consumption and the ratio $q{:}R_{CO_2}$ is inversely related to the respiratory quotient.

We grew seedlings of *Eucalyptus globulus* at 16 and 28 °C for four to six weeks, then measured q and R_{CO_2} using isothermal calorimetry. Respiratory rate at a given temperature was increased by a lower growth temperature but η_H was unaffected. Enthalpy conversion efficiency—and, therefore, carbon conversion efficiency—decreased with increasing temperature from 15 to 35 °C. The ratio of oxidative phosphorylation to oxygen consumption (P/O ratio) was inferred *in vivo* from η_H and by assuming a constant ratio of growth to maintenance respiration with changing temperature. The P/O ratio decreased from 2.1 at 10–15 °C to less than 0.3 at 35 °C, suggesting that decreased efficiency was not only due to activity of the alternative oxidase pathway. In agreement with predictions from non-equilibrium thermodynamics, growth rate was maximal near 25 °C, where the calculated P/O ratio was about half maximum. We propose that less efficient pathways, such as the alternative oxidase pathway, are necessary to satisfy the condition of conductance matching whilst maintaining a near constant phosphorylation potential. These conditions minimize entropy production and maximize the efficiency of mitochondrial energy conversions as growing conditions change, while maintaining adequate finite rates of energy processing.

Keywords: calorespirometry; calorespirometric ratio; reductive biosynthesis; gas exchange quotient; natural selection; Q_{10}

1. INTRODUCTION

Changes in the rate and efficiency of respiration are important responses of plant tissues to changing environmental conditions. The dominant paradigm of the relationship between growth and respiration in plants—the growth and maintenance respiration—paradigm, has been the subject of several recent reviews (Amthor 2000; Cannell & Thornley 2000; Thornley & Cannell 2000). These reviews concluded that, despite 30 years of research based on this paradigm, respiration is still poorly represented in whole plant growth models compared with photosynthesis (Cannell & Thornley 2000), and respiration-based models have been unsuccessful in contributing to major crop improvements (Amthor 2000). Hansen *et al.* (1994, 1997, 1998) have promoted calorespirometry (the combined measurement of metabolic heat rate and respiratory CO_2 rate) and an enthalpy balance model as a means of moving forward research on the relationship between respiration and growth. The apparently complex thermodynamic theory that underlies the enthalpy balance approach has been an impediment to adoption of calorimetry by plant scientists and, as a result, calorimetry has rarely been used in plant biology (Ordentlich *et al.* 1991).

In this article, we outline the theory behind the enthalpy balance approach in the hope of making it more accessible to non-experts. Using the enthalpy balance approach and calorimetry, we obtain estimates of growth rate and growth efficiency in growing shoots of *Eucalyptus globulus* seedlings at different growth and analysis temperatures. We relate changes in enthalpic efficiency with temperature to the efficiency of oxidative phosphorylation and propose that the role of alternative respiratory pathways within plant mitochondria can be explained, using non-equilibrium thermodynamic theory (Kedem & Caplan 1965; Stucki 1980), as a means of maintaining near constant phosphorylation potentials by matching the demand for ATP with the rate of phosphorylation.

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2. ENTHALPY BALANCE MODEL OF RESPIRATION AND PLANT GROWTH

Kemp (1996) provides a clear and concise introduction to thermodynamics and enthalpy balances as applied to living systems, while more detailed treatment is provided by Roels (1983). Wadsö (1996) gives a useful introduction to calorimetry, including heat conduction calorimetry. An extensive account of theory, methods and applications of plant calorimetry is given by Criddle & Hansen (1999).

The enthalpy balance model provides a simple description of a complex process because the change in enthalpy of a system is determined only by its initial and final states, regardless of pathway (Hess's law). The enthalpy balance model is based on the first law of thermodynamics (conservation of energy; equation (2.1))—the change in internal energy of the system (ΔU) is equal to the heat added to the system (Q) minus work done by the system (W).

$$\Delta U = Q - W. \tag{2.1}$$

The enthalpy change (ΔH) is the change in internal energy under conditions where changes in pressure or volume are negligible. Growing plants are an open system in which the work done on the system (i.e. energy derived from catabolism of substrate; $W = R_{CO_2}\Delta H_{CO_2}$) minus the heat lost from the system (i.e. metabolic heat production; $Q = -q$) is equal to the change in internal energy (i.e. net synthesis of anabolic products; $\Delta U = R_{SG}\Delta H_B$). Put simply, energy conserved in the products of anabolism is equal to the energy produced from oxidation of substrate that is not dissipated as heat to the surroundings.

In the enthalpy balance model (equation (2.2); after Hansen *et al.* (1994)), R_{SG} is the net specific rate of conversion of substrate carbon into anabolic products or specific growth rate (Cmol s^{-1} mg^{-1}), R_{CO_2} is the specific rate of total CO_2 production ('CO_2 rate', Cmol s^{-1} mg^{-1}), q is the specific metabolic heat rate ('heat rate', W mg^{-1}), ΔH_{CO_2} is the enthalpy change for combustion of substrate to CO_2 per mole of CO_2 released (kJ $Cmol^{-1}$) and ΔH_B is the difference between the enthalpy change for combustion to CO_2 and water of anabolic products and that of substrate (kJ $Cmol^{-1}$).

$$R_{SG}\Delta H_B = -R_{CO_2}\Delta H_{CO_2} - q. \tag{2.2}$$

By convention, ΔH is negative for exothermic reactions. Note that ΔH_B is not the heat of combustion of anabolic products. ΔH_B is, taking account of ground states for nitrogen and sulphur, the difference between the heats of combustion of anabolic products and that of the substrate. More reduced anabolic products have a larger ΔH_B. ΔH_B can be derived from the chemical composition, carbon content, degree of reduction or heat of combustion of biomass similarly to glucose values (McDermitt & Loomis 1981; Gary *et al.* 1995) and is negatively correlated with glucose values. Calculated from the degree of reduction, typical values of ΔH_B range from 20 to 60 kJ $Cmol^{-1}$ for most plant tissues, but may be as low as 10 for high carbohydrate tissues such as rice or wheat seed, and greater than 100 for tissues with large oil contents such as peanut, rape and sesame seed. $R_{SG}\Delta H_B$ is the net rate of conservation of enthalpy in anabolic products.

The substrate for respiration varies but, in most situations, can be assumed to be simple sugars (e.g. sucrose) in aqueous solution for which $\Delta H_{CO_2} = -469$ kJ $Cmol^{-1}$ (Gnaiger 1990; Kemp 1996; Battley 1999). This value will change for different substrates or if CO_2 and water are not the only catabolic products. For carbohydrates with a degree of reduction of four, $\Delta H_{CO_2} = \Delta H_{O_2}$ (enthalpy change for combustion of substrate to CO_2 and water per mol of O_2 consumed, kJ mol^{-1} O_2). ΔH_{O_2} is known as the 'oxycaloric equivalent' and changes relatively little with substrate (-430 to -480 kJ mol^{-1} O_2 (Gnaiger 1990; Kemp 2000)). ΔH_{CO_2} is much less negative than this for some highly oxidized compounds (e.g. oxalate) and may exceed -700 kJ $Cmol^{-1}$ for some lipids.

From equation (2.2), enthalpy conversion efficiency (η_H) and carbon conversion efficiency (the fraction of substrate carbon conserved in anabolic products, ε_C) can be defined as in equations (2.3) and (2.4). η_H is the fraction of enthalpy conserved in anabolic products from oxidation of substrate (Roels 1983). Both η_H and ε_C decrease as the ratio q/R_{CO_2} increases. In the absence of growth, $q = -R_{CO_2}\Delta H_{CO_2}$ and η_H and ε_C are both zero. Combining equations (2.2) and (2.3) gives R_{SG} as a function of R_{CO_2}, η_H, ΔH_{CO_2} and ΔH_B (equation (2.5)).

$$\eta_H = -R_{SG}\Delta H_B/R_{CO_2}\Delta H_{CO_2} = (\Delta H_{CO_2} + q/R_{CO_2})/\Delta H_{CO_2}, \tag{2.3}$$

$$\varepsilon_C = R_{SG}/(R_{SG} + R_{CO_2}) = \eta_H/(\eta_H - \Delta H_B/\Delta H_{CO_2}), \tag{2.4}$$

$$R_{SG} = -R_{CO_2}\Delta H_{CO_2}\eta_H/\Delta H_B. \tag{2.5}$$

The enthalpy change for the conversion of simple sugars to anabolic products, via the reaction $C_{sugar} \rightarrow C_{product} + CO_2$, is essentially zero (McDermitt & Loomis 1981). Little or none of the energy from ATP hydrolysis is conserved in chemical bonds in anabolic products (Battley 1987, p. 409). Hence, heat produced from cells during aerobic metabolism is the result of oxidation and q is a linear function of the rate of oxygen consumption ($q = -R_{O_2}\Delta H_{O_2}$ (Patel & Erickson 1981; Kemp 2000)). This relationship forms the basis of 'indirect calorimetry' in which oxygen consumption is measured and used to calculate metabolic heat rate. Ordentlich *et al.* (1991) found good agreement between rates of oxygen consumption measured with an oxygen electrode and heat production measured in potato tubers and cucumber leaves with a heat conduction calorimeter. It follows that q/R_{CO_2} is inversely correlated with the respiratory quotient (R_{CO_2}/R_{O_2}), while η_H and ε_C are positively correlated with the respiratory quotient. $R_{SG}\Delta H_B$ is proportional to the difference between the net rates of decarboxylation ($R_{CO_2}\Delta H_{CO_2}$) and oxidation ($R_{O_2}\Delta H_{O_2}$), which will vary with relative demand for ATP and reductant. In effect, calorespirometry measures both the respiratory quotient and respiratory rate. From the oxycaloric equivalent and Thornton's rule ($\Delta H_{CO_2} = \gamma_i/4\Delta H_{O_2}$ where γ_i is the degree of reduction (Kemp 2000)), the enthalpy balance model can be expressed (equation (2.6)) in terms of carbon and oxygen rates and the degree of reduction of substrate (γ_S) and anabolic products (γ_B).

$$(\gamma_B - \gamma_S)R_{SG} = \gamma_S R_{CO_2} - 4R_{O_2}. \tag{2.6}$$

Willms *et al.* (1999) derived an equivalent expression to equation (2.6) and defined $(\gamma_B - \gamma_S)R_{SG}$ as the 'diverted reductant utilization rate'. From equation (2.6) it can be seen that, in growing tissues, q/R_{CO_2} and the respiratory quotient are determined not only by the degree of reduction of substrate, but also by the degree of reduction of anabolic products and the net rate of anabolism R_{SG} (see also Cen *et al.* 2001). It is also evident from equations (2.5) and (2.6) that the growth rate can only be calculated if the degree of reduction of anabolic products differs from that of the substrate. For example, for a tissue only synthesizing cellulose from glucose, $\Delta H_B = 0$, $(\gamma_B - \gamma_S) = 0$, $\eta_H = 0$, and the growth rate is undefined.

3. GROWTH AND MAINTENANCE RESPIRATION

The growth and maintenance respiration paradigm (equation (3.1)) divides total respiration (R_{CO_2}) into two components: respiration used to drive biosynthesis of new biomass (growth respiration, R_G), and respiration used to drive anabolic reactions that maintain or replace existing structures and conditions for cell viability (maintenance respiration, R_M). Following observations by McCree (1970), Thornley (1970) applied Pirt's microbiological model to plants, equating the slope of a plot of respiration rate versus growth rate to a growth coefficient $((1 - Y_G)/Y_G)$, where Y_G is the growth yield, and the intercept to a maintenance rate (equation (3.2)). Maintenance respiration corresponds to the production and hydrolysis of ATP (Amthor 2000) to drive reactions that are not directly related to producing new biomass. Y_G is similar to carbon conversion efficiency except that Y_G is specific to the growth component of respiration (compare equations (3.2) and (3.3)). For some applications, the maintenance component of respiration has been further subdivided into as many as nine components, including protein turnover, phloem loading, ion transport, nitrogen fixation, etc., and 'residual' or 'residual maintenance' respiration (see Amthor 1989, 2000; Thornley & Cannell 2000, pp. 56–64).

$$R_{CO_2} = R_G + R_M, \tag{3.1}$$

$$R_{CO_2} = [(1 - Y_G)/Y_G]R_{SG} + R_M, \tag{3.2}$$

$$R_{CO_2} = [(1 - \varepsilon_C)/\varepsilon_C]R_{SG}. \tag{3.3}$$

The obstacles to measuring R_M and Y_G are considerable and well documented (Breeze & Elston 1983; Amthor 1989, 2000; Chiarello *et al.* 1989; Thornley & Johnson 1990; Shinano *et al.* 1996; Hansen *et al.* 1997, 1998) and partly result from the well-recognized lack of rigorous division between growth and maintenance processes (Cannell & Thornley 2000). Further subdivision of the maintenance component compounds the problem. Assuming a value for the ratio of oxidative phosphorylation to oxygen consumption (P/O ratio), maximum Y_G can be calculated from biochemical pathways using an approach pioneered by Penning de Vries *et al.* (1974), and on this basis many researchers have calculated 'construction cost' from the chemical composition or heat of combustion of biomass (McDermitt & Loomis 1981; Williams *et al.* 1987; Gary *et al.* 1995). However, in the past decade it has been demonstrated that less efficient respiratory pathways that reduce Y_G and ε_C, especially the alternative oxidase pathway, are active continuously and at high and variable rates within plants (Guy *et al.* 1989; Ribas-Carbo *et al.* 1995; Gonzàlez-Meler *et al.* 1999). Although Y_G varies almost continuously with changing environmental conditions, analyses of least-cost pathways can only provide an estimate of the, rarely attained, maximum potential growth yield. A variable P/O ratio will also affect the cost of maintenance calculated from pathway analyses (Amthor 2000).

4. ENTHALPY CONVERSION EFFICIENCY AND THE P/O RATIO

The P/O ratio in mitochondria of plant tissues is variable and depends on the relative proportions of reductant oxidized by the cytochrome pathway and by the rotenone-insensitive bypass and external dehydrogenase, as well as by the activity of the alternative oxidase pathway and proton 'leakage' (Amthor 2000). Recent isolation of mitochondrial uncoupling proteins in plants lacking thermogenic tissues (Maia *et al.* 1998) raises the possibility that these proteins may also cause reductions of the P/O ratio. The P/O ratio may be as large as 2.1 for oxidation of all $FADH_2$ and NADH within the mitochondrial matrix via complex I, II and the cytochrome-*c* pathway, and oxidation of cytosolic NADH via the external dehydrogenase (Amthor 2000). The P/O ratio decreases with increasing activity of the rotenone-insensitive bypass and the alternative oxidase pathway. Variable activity of these pathways results in P/O ratios ranging from 0 to *ca.* 2.1.

Four ATP are produced per six CO_2 produced in glycolysis, pyruvate decarboxylation and the citric acid cycle, and up to 25 ATP are produced per six O_2 consumed during oxidative phosphorylation (Amthor 2000). The overall rate of ATP production (R_{ATP}) can be expressed as in equation (4.1).

$$R_{ATP} = (4R_{CO_2} + 12R_{O_2}\mathrm{P/O})/6. \tag{4.1}$$

From Thornton's rule (Patel & Erickson 1981; Kemp 2000) and equations (2.2), (2.3) and (4.1), one obtains equation (4.2) from which it is evident that decreased efficiency, which is equivalent to an increase in the proportion of reducing equivalents used for regeneration of ATP, can result from either a decreased P/O ratio or from an increase in the demand for ATP per unit CO_2 respired (R_{ATP}/R_{CO_2}).

$$\eta_H = 1 - (6R_{ATP}/R_{CO_2} - 4)/12\mathrm{P/O}. \tag{4.2}$$

This analysis is not greatly affected by whether the flux of hexose is via glycolysis or the pentose phosphate pathway. The number of ATP produced from substrate-level reactions per six CO_2 only decreases from 4 to 3.7 as the proportion of hexose oxidized via the pentose phosphate pathway increases from 0 to 33%. The contribution of substrate-level reactions to overall ATP production is small at high-to-moderate P/O ratios. A failure to consider the pentose phosphate pathway could result in R_{ATP} being underestimated at small P/O ratios. However, as the P/O ratio decreases, the relative flux of hexose via the pentose phosphate pathway is likely to decrease owing to a relative

increase of demand for NADH compared with NADPH. The assumption that substrate-level reactions produce four ATP per six CO_2 would also be false if there were significant activity of enzymes that bypass phosphorylation steps in glycolysis (Buchanan *et al.* 2000, p. 669).

Anapleurotic dark fixation of CO_2 catalysed by phospho*enol*pyruvate carboxylase (PEPC) could affect the measured CO_2 rates in growing tissues if the flux through PEPC is comparable with that through pyruvate kinase. Although malate can be converted back to pyruvate by the malic enzyme with the release of CO_2, the contributions of this pathway to pyruvate synthesis appear small (Dieuaide-Noubhani *et al.* 1995; Edwards *et al.* 1998). However, it also appears that the end product of much of the flux through PEPC is glutamate (Dieuaide-Noubhani *et al.* 1995; Edwards *et al.* 1998). This involves the decarboxylation of isocitrate to produce α-ketoglutarate, such that there is no effect of PEPC on CO_2 production. CO_2 production may be affected if biosynthesis of aspartate is a major output of the citric acid cycle in the tissues being studied (Edwards *et al.* 1998).

η_H (or ε_C or q/R_{CO_2}) in plants has been observed to either increase or decrease with increasing temperature, reflecting genetic adaptation to growth conditions (e.g. Taylor *et al.* 1998). However, to infer changes of the P/O ratio from changes of η_H with temperature, the ratio R_{ATP}/R_{CO_2} must be known as temperature changes. If the ratio of maintenance to growth remains constant with temperature then changes in R_{ATP}/R_{CO_2} can be inferred from changes in the ratio $R_{SG}\Delta H_B/R_{CO_2}$. It is generally accepted that the rate of maintenance respiration increases with temperature similarly to total respiration and total metabolic activity (Amthor 1989, pp. 76–80). The pattern of change of growth respiration with temperature is less certain, although McCree (1982) concluded that maintenance respiration and growth rates were positively correlated. Given that maintenance is essential to support growth and that many biochemical reactions are the same for growth and maintenance, it seems reasonable to assume that the ratio of growth to maintenance respiration and, therefore, $R_{ATP}/R_{SG}\Delta H_B$ will remain approximately constant in the 'normal' growth temperature range. R_{ATP} can be determined at maximum η_H with equation (4.1) by assuming a maximum P/O ratio of 2.1 at maximum η_H.

5. METHODS

Eucalyptus globulus seedlings were grown at two temperatures (16 and 28 °C) for four to six weeks prior to measurements. Plants were grown in 15 cm diameter pots filled with coarse sand and irrigated twice daily with a commercially available plant nutrient solution (Miracid) according to the manufacturer's instructions. R_{CO_2} and q were measured on excised shoots at 5 °C intervals from 10 to 40 °C with Hart Scientific model 7707 and CSC model 4100 differential scanning calorimeters operated in isothermal mode (Criddle *et al.* 1990). q was obtained as the average of measurements before and after measuring the heat rate in the presence of a CO_2 trap. Each rate measurement takes between 25 and 40 min to complete, with more rapid thermal equilibration at higher temperatures. R_{CO_2} was calculated from the increased heat rate resulting from the reaction of respired CO_2 with NaOH when a 50 µl vial of 0.4 N NaOH was placed in the ampoule with the sample (Criddle *et al.* 1990). The increased heat rate in the presence of the CO_2 trap is converted to R_{CO_2} by dividing by 108.5 kJ $Cmol^{-1}$. The heat rate typically increases by 20–30% in the presence of the heat trap. These heat conduction calorimeters directly measure heat rate to within ±3 µW. Hence, for a typical measured heat rate of 300 µW, the error of the heat rate measurement is about 1%, while the error of the CO_2 rate measurement ranges from 5 to 10%. The error of the calculated growth rate is slightly greater again.

Separate tissue samples were used for measurements at low temperatures and high temperatures. Sample ampoules have a volume of 1 ml, and the fresh weight of sample tissues was typically 50–150 mg depending on whether samples were taken for measurements at low temperature (10 and 15 °C; larger samples) or high temperature (20–40 °C; smaller samples). After measurements, tissue samples were dried at 70 °C in a vacuum oven overnight and reweighed. All results are expressed on a dry weight basis. η_H and $R_{SG}\Delta H_B$ were calculated from q and R_{CO_2} assuming glucose in aqueous solution as the substrate ($\Delta H_{CO_2} = \Delta H_{O_2} = -469$ kJ mol^{-1} CO_2 or O_2). The respiratory quotient was calculated assuming $R_{O_2} = q/\Delta H_{O_2}$. Q_{10} (the proportional increase of respiration rate for a 10 °C rise in temperature) was calculated based on the rate of CO_2 production.

R_{ATP} at the temperature at which η_H was maximum was calculated from η_H at that temperature with equation (4.2) and assuming that P/O = 2.1 at that temperature. Assuming that the ratio of R_{ATP} to $R_{SG}\Delta H_B$ was the same at all temperatures, P/O at other temperatures was then calculated with equation (4.2) and η_H at each temperature.

All results are mean ± standard error of three measurements (one shoot from each of three plants) per treatment. Stated errors include both experimental error and plant variability.

6. RESULTS

$-R_{CO_2}\Delta H_{CO_2}$ always exceeded q, indicating that the rates of growth were positive under all measurement conditions (figure 1). Rates of respiration measured in seedlings grown at 16 °C were greater than those of seedlings grown at 28 °C, showing acclimatization of respiratory rate to growth temperature ($p < 0.05$; paired t-test). R_{CO_2} initially increased quickly ($Q_{10} > 2$) but Q_{10} fell to less than 2 at 25 °C and was close to 1 between 25 and 35 °C. Q_{10} increased slightly from 35 to 40 °C.

η_H generally decreased with increasing temperature (figure 2). The ratio q/R_{CO_2} increased from 284 kJ $Cmol^{-1}$ at 10–15 °C to 408 kJ $Cmol^{-1}$ at 35 °C, which corresponded to a decrease of the respiratory quotient from 1.65 to 1.15. η_H did not change with growth temperature (analysis of covariance between 15 and 35 °C with temperature as covariate). Maximum η_H (0.39) was recorded at 15 °C for the plants grown at 16 °C, and at 10 °C for the plants grown at 28 °C. Minimum η_H (0.13) was observed at 35 °C in both cases. Above 35 °C, η_H appeared to increase, especially in plants grown at 28 °C, opposite to the trend in the data up to 35 °C. The data at 40 °C also produced changes in the trend of the P/O ratio (figure 3) and $R_{SG}\Delta H_B$ (figure 4). The abrupt changes in trends and relatively small change in q between 35 and 40 °C, compared with the change in R_{CO_2}, suggest that metabolic intermediates from glycolysis and the citric acid cycle might be accumulating at 40 °C, increasing the apparent efficiency of enthalpy conservation. This would

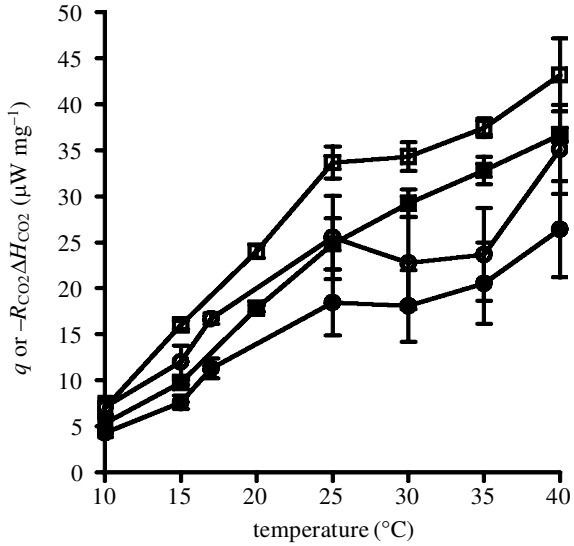

Figure 1. Respiratory rate ($-R_{CO_2}\Delta H_{CO_2}$) and heat rate (q) obtained from calorespirometric measurements on small, rapidly growing shoot tissue of *Eucalyptus globulus*. The respiratory CO_2 rate (R_{CO_2}) is multiplied by $-\Delta H_{CO_2}$ (469 kJ $Cmol^{-1}$) for direct comparison with the metabolic heat rate (q). 50 μW mg^{-1} is equivalent to 106 pmol CO_2 mg^{-1} s^{-1}. Plants were grown at 16 and 28 °C and measurements of q and R_{CO_2} were made at 5 °C intervals from 10 to 40 °C. Results are mean ± standard error of three measurements (one shoot from each of three plants) per treatment. (open squares, 16 °C $-R_{CO_2}\Delta H_{CO_2}$; filled squares, 16 °C q; open circles, 28 °C $-R_{CO_2}\Delta H_{CO_2}$; filled circles, 28 °C q.)

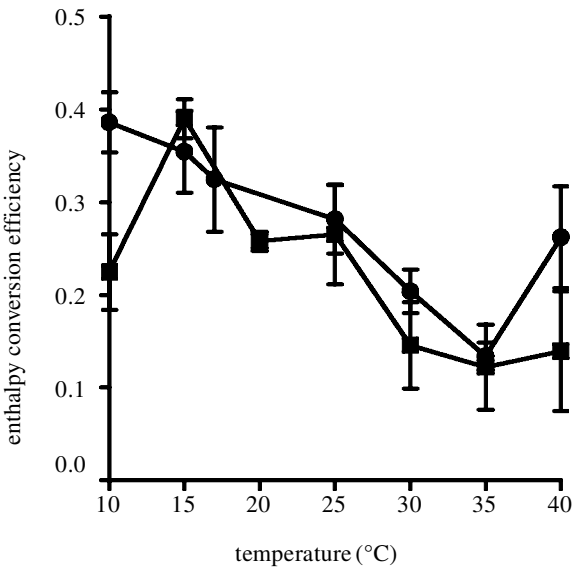

Figure 2. Enthalpy conversion efficiency (η_H) at different measurement temperatures of *Eucalyptus globulus* shoots grown at either 16 °C (squares) or 28 °C (circles). Results are mean ± standard error of three measurements (one shoot from each of three plants) per treatment.

violate the assumption in equation (2.2) that CO_2 is the only product of catabolism and would also result in the rate of growth ($R_{SG}\Delta H_B$) at 40 °C appearing artificially high. Hence, the P/O ratio was not calculated at 40 °C.

The calculated P/O ratio decreased from its assumed maximum of 2.08 at 10 or 15 °C, to 1.15 at 25 °C and to 0.25 at 35 °C (figure 3). Calculated rates of ATP turnover ranged from 50 to 160 pmol ATP mg^{-1} s^{-1} at 10 and 25 °C, respectively. $R_{SG}\Delta H_B$ values initially increased with temperature, reached a maximum at around 25 °C, and then decreased (figure 4). Note that, although respiration rates depended on the growth temperature, growth rates ($R_{SG}\Delta H_B$) and efficiencies did not because the latter are related to differences between, and ratios of, respiration rates.

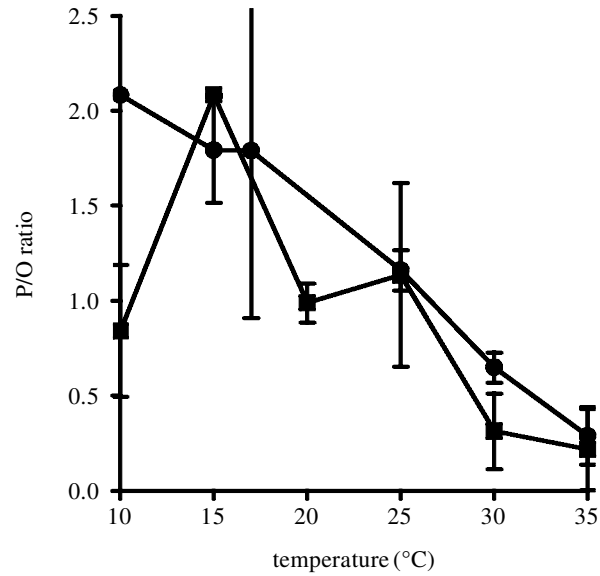

Figure 3. P/O ratio of oxidative phosphorylation at different measurement temperatures of *Eucalyptus globulus* shoots grown at either 16 °C (squares) or 28 °C (circles). Results are mean ± standard error of three measurements (one shoot from each of three plants) per treatment.

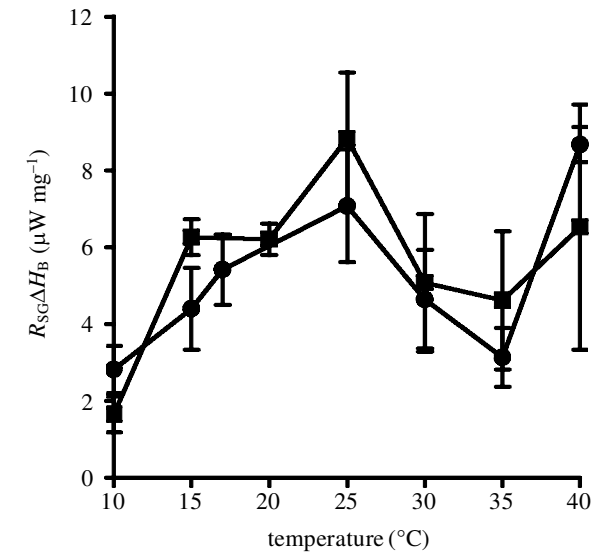

Figure 4. The rate of conservation of enthalpy in anabolic products ($R_{SG}\Delta H_B$) at different measurement temperatures of shoots of *Eucalyptus globulus* grown at either 16 °C (squares) or 28 °C (circles). Results are mean ± standard error of three measurements (one shoot from each of three plants) per treatment.

7. DISCUSSION

Growth rate is a product of respiratory rate and efficiency (see equations (2.5) and (3.3)). Below 25 °C, growth rates of *E. globulus* shoots increased with temperature despite decreasing efficiency because of the increase of respiratory rate. Above 25 °C, the rate of increase of respiration rate decreases and growth rate decreases. Shoot growth of *E. globulus* was most rapid between 20 and 30 °C but most efficient at 10–15 °C. In a natural environment, diurnal temperature variation would result in η_H and ε_C of *E. globulus* being much less than the maximum calculated from least-cost-pathway analyses or related methods. Loomis & Amthor (1999, p. 1594) argued that the alternative oxidase pathway in plants would have little effect (less than a 10% reduction) on carbon conversion efficiency of growing tissues. However, the contribution of other inefficient pathways can further reduce efficiency. From equation (2.4), and assuming a typical value of ΔH_B of 40 kJ $Cmol^{-1}$, we calculate from the data in figure 2 that ε_C would be reduced by up to 30% at 35 °C compared with ε_C at 10–15 °C.

Engagement of the alternative pathway has been observed in response to increased anabolic demand resulting from salinity and drought (Wagner & Krab 1995), temperature (Gonzàlez-Meler *et al.* 1999), illumination (Ribas-Carbo *et al.* 2000*b*), wounding (Kinraide & Marek 1980) and during the de-acidification phase of Crassulacean acid metabolism (Robinson *et al.* 1992), as well as in response to decreased phosphorylation via the cytochrome-*c* pathway resulting from cold stress (Ordentlich *et al.* 1991; Gonzàlez-Meler *et al.* 1999; Ribas-Carbo *et al.* 2000*a*) and phosphorus deficiency (Parsons *et al.* 1999). Other than in thermogenic tissues the function of the alternative oxidase is uncertain. The 'energy-overflow' hypothesis of Lambers (1982) has been largely rejected because it was shown that the cytochrome-*c* and alternative oxidase pathways compete for electrons (Hoefnagel *et al.* 1995). Vanlerberge & McIntosh (1997) suggested that the alternative oxidase might function to balance carbon metabolism and electron transport as the supply or demand for carbon skeletons, reducing power and ATP changes. Criddle *et al.* (2002) have proposed that the alternative pathway might serve to match the rate of phosphorylation with the anabolic demand for ATP, thus maintaining an adequately large phosphorylation potential. As growing conditions change and the rate of ATP consumption increases (or conductance for phosphorylation via the cytochrome-*c* pathway decreases), plant mitochondria must adjust the coupling between electron transport and phosphorylation to maintain both adequate rates and optimal efficiency of mitochondrial energy conversions.

Stucki (1980) coined the term 'conductance matching' to describe the condition that maximizes energy efficiency of mitochondrial energy conversions. From the theory of linear, non-equilibrium thermodynamics, Stucki (1980) determined that, to maximize the efficiency of mitochondrial energy conversion, the ratio of the conductance of the load (ATP-utilizing reactions) to that of phosphorylation must equal $\sqrt{(1 - q^2)}$ where the q in Stucki's paper is the degree of coupling between electron transport and oxidative phosphorylation. He concluded from this that, for a given degree of coupling, there is one finite load conductance that permits oxidative phosphorylation to operate efficiently. However, immobile ectotherms such as plants and fungi are faced with frequent, large changes of the demand for ATP (equivalent to large changes in the load conductance). From the principle of conductance matching it is evident that there is also only one degree of coupling that allows oxidative phosphorylation to operate efficiently for a given load. Failing to adjust the coupling of oxidative phosphorylation as the demand for ATP changes results in excessive entropy production and reduced energy efficiency of oxidative phosphorylation.

This results in a trade-off between the rate and efficiency of ATP production: a maximal rate of ATP production is incompatible with maximal efficiency, which could only be maintained at the cost of infinitesimally slow growth (Stucki 1982). Mitochondria rarely maximize the P/O ratio because of the need to maintain adequate rates of phosphorylation. Stucki (1980) likened this to the familiar experience that 'one should not drive a car as fast as possible in order to obtain maximal mileage from a given amount of fuel'. As alternative pathway activity increases, the degree of coupling shifts away from one that maximizes the P/O ratio towards one that maximizes the rate of ATP production with minimal entropy production. Stucki (1980) also concluded that, when the condition of conductance matching is satisfied, the rate of phosphorylation is maximum when the normalized flow ratio (equal to the actual P/O ratio divided by the maximum P/O ratio) is 0.49. Assuming a maximum P/O ratio of 2.08, the rate of phosphorylation should be largest when the P/O ratio is *ca.* 1. From figure 3, this corresponds to a temperature of 26 °C, essentially the same as the temperature of maximum growth rate ($R_{SG}\Delta H_B$) and maximum R_{ATP}. The consistency of the results of this study with predictions from non-equilibrium thermodynamics supports the hypothesis that inefficient pathways might serve to match the supply and demand of ATP within cells. In aerobically grown batch cultures of *Bacillus licheniformis*, Bulthuis *et al.* (1993) also found that the P/O ratio was lower when anabolic demand was greater (specific growth rate was larger) and concluded that lower efficiency was a means to maintain the phosphorylation potential near constant, although they were not able to identify the factor responsible for a smaller P/O ratio.

We assumed a maximum P/O ratio of 2.08 after Amthor (2000). If cytosolic NADH were imported into the mitochondrion via malate then the P/O ratio could be as large as 2.3. Changing the maximum P/O ratio has little effect on the minimum P/O ratio but slightly changes the *y*-intercept of the lines in figure 3. The point at which P/O is half maximal is still at 26 °C. The assumption of maximal P/O at maximal η_H is reasonable. If the P/O ratio were actually 10% less than maximal at maximal η_H then P/O would be half maximal at 25 °C instead of 26 °C but this would not affect our argument.

This explanation is consistent with engagement of the alternative pathway in conditions that increase the demand for ATP for either growth or maintenance, as well as with its role in heat production in thermogenic tissues in which the rate, but not the efficiency, of substrate oxidation is important. It is also consistent with engagement of the alternative pathway in conditions that reduce

conductance of the cytochrome-*c* pathway. This explanation is not inconsistent with the hypothesis that the alternative oxidase prevents over-reduction of the electron transport chain and generation of harmful superoxides (Millar & Day 1997). The production of superoxides also decouples electron transport from phosphorylation. The alternative oxidase may be a 'safe' mechanism for decoupling. Given the important role of the redox poise of the ubiquinone pool in regulating alternative oxidase activity (Day *et al.* 1995), it seems likely that this is the means by which plant mitochondria 'smell' the state of optimal efficiency.

The idea that rates of processes are more important than their efficiency is not new. Cohen (1970) and others (Passioura 1982; DeLucia & Schlesinger 1991; Donovan & Ehleringer 1992; Midgley & Moll 1993) have observed that efficient water use by plants is not necessarily advantageous in water-limited conditions because high efficiency is correlated with slower growth. In competitive situations, plants with larger rates of resource use can have a competitive advantage over slower growing, if more efficient, plants. The existence of the alternative oxidase pathway, and other inefficient pathways within mitochondria, can be explained by Lotka's (1922) principle of maximum power, which argues that natural selection conserves mechanisms that increase the rate of energy flow through a system. Maintaining high rates of energy conversion is a more important evolutionary criterion than maximizing efficiency.

Wagner & Krab (1995) reported that the alternative oxidase is generally induced in response to 'stress'. In this study, a reduced P/O ratio was not associated with stress. If stress is defined as a condition that reduces growth rate then, in this study, there was no evidence of stress from 15 to 25 °C, despite decreasing efficiency, because absolute growth rate was increasing. Above 25 °C both the rate and efficiency of growth decreased, but even in this situation the less-efficient pathways might be optimizing efficiency of mitochondrial energy conversion as growing conditions change by minimizing entropy production. Without a reduction in degree of coupling to increase the rate of phosphorylation, the phosphorylation potential would presumably fall and growth rate would decrease even more rapidly than observed. It is more likely that the tissues were stressed at 40 °C when the rate of oxidative phosphorylation might have failed to keep pace with that of decarboxylation. At high temperatures, loss of membrane integrity, inhibition of oxidative phosphorylation and increased glycolysis could explain the sudden, apparent increase of enthalpic efficiency. At least in response to temperature change, there is no evidence that the alternative pathway is engaged specifically in response to stress.

Although we have argued that reduced efficiency of oxidative phosphorylation in plants is largely owing to activity of the alternative oxidase pathway, the reduction in P/O ratio calculated in this study cannot be attributed entirely to alternative oxidase activity because the P/O ratio of the alternative pathway is 38% that of the cytochrome-*c* pathway (i.e. $0.38 \times 2.08 = 0.79$), while the minimum calculated P/O ratio was less than 0.3. This indicates that other inefficient pathways were also active and contributed to reduced efficiency. In addition to the rotenone-insensitive dehydrogenases, it is possible that some uncoupling between mitochondrial electron transport and phosphorylation resulted from proton leakage across the inner-membrane of the mitochondria, especially at high temperatures (Lin & Markhart 1990). Recent isolation of uncoupling mitochondrial proteins in plants lacking thermogenic tissues (Maia *et al.* 1998) also raises the possibility that these proteins may be present in all plants and have roles other than thermogenesis, and that some uncoupled electron transport in plant mitochondria may be attributable to uncoupling proteins. Together, these additional sources of inefficiency make calculation of alternative oxidase activity from calorimetric measurements and P/O ratios problematic. Similarly, measurements of alternative oxidase activity from oxygen isotope discrimination cannot be used to determine P/O ratios or actual growth efficiencies.

We assumed that maintenance respiration was the same proportion of total respiration at all temperatures. This assumption is consistent with maintenance being essential to support growth. Our assumption that changes in enthalpic efficiency with temperature reflect changes in the P/O ratio, and can be partially tested by combining calorimetric measurements with stable oxygen isotope methods that estimate alternative pathway activity, a major cause of reduced respiratory efficiency. Calorimetry cannot be used to estimate changes in the P/O ratio in response to conditions that might change the ratio of growth to maintenance respiration nor can they be used in non-growing tissues, as the enthalpic efficiency of non-growing tissues is always zero regardless of the P/O ratio.

The enthalpy balance model of respiration and plant growth is simple, general and consistent with established concepts. It requires similar assumptions to existing models, such as the nature of the substrate and the chemical composition of biomass, and the information obtained from calorimetry is readily converted into quantities already used in traditional plant growth modelling. Calorimetry is particularly suited to growing shoot material in which the amount of wounding of tissue excised at the petiole is small. There is more wounding of root material owing to the number of root segments that need to be excised a short distance from the root tip to fit in the small ampoule (1 cc), and care is needed handling samples to obtain good results from root material. Calorimetry is also suitable for small branch and twig material. Calorimetry suffers drawbacks in common with closed gas-exchange systems, i.e. constantly changing concentrations of O_2 and CO_2. Another limitation of calorimetry is the fixed and small volume of the ampoules used to contain the samples. Sample sizes must be adjusted or different samples used for measurements at different temperatures. To obtain accurate CO_2 rate measurements, overall heat rates must be large enough that the error of the instrument is small, but not so large that the air inside the ampoule is depleted of oxygen. These competing constraints place tight limits on the range of tissue mass that can be used for analyses.

Similar information could be obtained from simultaneous measurements of CO_2 consumption and O_2 production by growing tissues, using equation (2.6). This approach would require similar assumptions about the degree of reduction of substrate and anabolic products. Note that the respiratory quotient is not solely determined by degree of reduction of the substrate unless growth rate is zero. In growing tissues, anabolism can account for a

significant fraction of CO_2 production. We calculated respiratory quotient values as large as 1.65 for growing shoots of *E. globulus*. In growing tissues, respiratory quotient values will also be determined by the ratio of growth to maintenance and the efficiency of oxidative phosphorylation.

The theory and methods presented in this paper have obvious applications for determining variation of growth rate and efficiency in response to seasonal temperature changes and other environmental conditions such as salinity, waterlogging, nutrition and drought. Calorespirometry has been used to select genotypes for faster growth rate in contrasting environments and to explain variation in growth rate of genotypes between environments. Strong correlations have been found between height and diameter growth of clones of *Sequoia sempervirens* and the temperature dependence and absolute rates of q and R_{CO_2} of their growing tissues (Anekonda *et al.* 1993, 1994). The enthalpy balance model has been able to discriminate clearly between *Zea mays* cultivars adapted to warm or cool growing conditions (Taylor *et al.* 1998). Measurements of q and R_{CO_2} have also been correlated with growth (Criddle *et al.* 1996, 2000) and salt tolerance (Marcar *et al.* 2002) in *Eucalyptus* spp. In non-growing tissues, calorimetry can complement existing methods by providing a measure of maintenance respiration rate and substrate oxidation state in response to widely ranging temperatures.

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