Responses of Plant Growth and Metabolism to Environmental Variables Predicted From Laboratory Measurements

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Abstract—The Arrhenius activation energies, and therefore temperature coefficients, for rates of catabolic production of ATP and for anabolic use of ATP differ. Because the intracellular concentration of ATP and the phosphorylation potential must be controlled within a narrow range for cell survival, a mechanism must exist to balance these rates during temperature variation in ectotherms. We hypothesize that much of this control is accomplished via engagement of temperature-dependent reactions that waste ATP or the potential to make ATP in "futile" cycles and that energy-wasting metabolic cycles are essential for maintaining acceptable phosphorylation potentials across a temperature range. We further postulate that the mitochondrial alternative oxidase (AOX) activity is one important mechanism for "wasting" potential to make ATP and thus for controlling the phosphorylation potential in plants as temperature or other reaction conditions vary. Because of differences in temperature coefficients, the ratio of AOX to COX activities varies with temperature, resulting in a temperature-dependent change in coupling oxidation to phosphorylation. Matching the changes in substrate carbon conversion efficiency to environmental temperature patterns allows plants to maintain constant phosphorylation potentials. Thus, an apparent paradox exists that survival of all organisms in changing conditions depends on an energy loss via "futile cycles."

The Need for Futile Cycles

The biological function of apparently "futile" reactions has long been a subject of speculation. What is the purpose of the ubiquitous reactions that lose energy by cyclic production and breakdown of reaction intermediates or via reactions that result in energy loss by short-circuiting the formation of ATP? The answer to this question is known only for futile reactions employed in specialized thermogenic tissues. "Futile" reactions must contribute positively to fitness in non-thermogenic tissues or they would have been eliminated by natural selection. This study examines the hypothesis that an uncoupled energy loss is required to maintain [ATP] and phosphorylation potential nearly constant in varying cellular conditions and is therefore particularly important for survival of ectotherms in variable temperature environments.

The overall reaction for aerobic growth of plants may be written as in equation 1.

$$C_{substrate} + x(\text{compounds and ions of N, P, K, etc.}) + yO_2 \rightarrow \varepsilon\ C_{structural\ biomass} + (1-\varepsilon)CO_2 \quad (1)$$

Reaction 1 is the sum of two reactions, the catabolic reaction (2)

$$C_{substrate} + yO_2 + nADP + nPi + aNAD^+ \rightarrow CO_2 + nATP + aNADH + aH^+ \quad (2)$$

And the anabolic reaction 3.

$$C_{substrate} + x(\text{compounds and ions of N, P, K, etc.}) + mATP + bNADH + bH^+ \rightarrow C_{structural\ biomass} + mADP + mPi + bNAD^+ \quad (3)$$

Reactions 2 and 3 occur in the condition-dependent ratio $(1-\varepsilon)/\varepsilon$ where ε is the substrate carbon conversion efficiency. Reactions 2 and 3 are energy-coupled through cyclic production and hydrolysis of ATP and redox cycling of NADH. Because the rates of reactions 2 and 3 have different dependencies on temperature and other conditions, the coefficients n and m, and a and b, are generally not equal for the two reactions. Principles of nonequilibrium thermodynamics applied to the energy-coupled system of reactions 1–3 lead to the conclusion that reaction 2 must always produce ATP and NADH at rates equal to or in excess of their rates of use in the biosynthetic reactions of reaction 3 (in other words, $n \geq m$ and $a \geq b$). If ATP is synthesized faster than it is used for biosynthesis, the excess can be disposed of by hydrolytic reactions, but if ATP is synthesized slower than it is used, the phosphorylation potential, or free energy change for hydrolysis of ATP (ΔG_P, Equation 4), falls and cell death ensues. Therefore, the excess ATP and NADH is cycled through condition-dependent uncoupled hydrolysis and oxidation reactions in order to maintain the phosphorylation potential and [ATP] approximately constant.

$$\Delta G_p = -RT\ln [ATP]/[ADP][Pi] \approx \text{constant} \quad (4)$$

The rate of synthesis of ATP is also controlled by condition-dependent reactions that cause a loss of the potential to form ATP, in other words, reactions mediated by uncouplers and through such pathways as the alternative oxidase. The engagement of these less efficient alternate pathways of oxidation to control [ATP] and phosphorylation potential

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varies with reaction conditions. This variation is independent of the rate of reaction 3 because reactions 2 and 3 are not closely coupled. The overall result is a condition-dependent energy loss and a variable efficiency of energy coupling between catabolic and anabolic processes (Wrigglesworth 1997).

The requirement demonstrated above for engagement of less efficient pathways in plants to maintain phosphorylation potentials under variable reaction conditions is also apparent from fundamental thermodynamic considerations. The second law of thermodynamics requires that mass and energy flow through the system (described by equation 1) must be accompanied by an entropy increase, in other words, processes such as growth require a transfer of energy from the system to the surroundings. This is measurable as heat loss to the environment (Jou and Llebot 1990). When the reactions occur at steady state, the entropy (heat) loss to the surroundings is minimized (Prigogine 1980). Any displacement from steady state, in other words, varied reaction conditions, results in an increased energy loss to the environment. Various workers (Jou and Llebot 1990, p. 51) have confirmed that these concepts apply to growth of cells under near steady state conditions and have used measurements of heat dissipated as an indication of the increase in entropy during variable growth conditions. We propose that the concepts also apply to rapidly growing plant tissues with adequate substrate supplies. Thus, variation in reaction conditions that perturb steady-state growth (or further displace a growing system from steady state) results in an experimentally measurable increase in heat loss and an overall decrease in energy use efficiency during growth. Several sources infer (but do not rigorously prove for biological systems) that the larger and more frequent the variations perturbing steady-state growth, the greater the energy loss from the system (Zotin 1990; Lewis and Randall 1961, unpublished observations by the authors).

Futile Reactions in Plant Metabolism

Thermodynamic arguments clearly require variable efficiency and increased heat loss with variable growth conditions. However, the mechanisms by which energy is lost and mechanisms by which the energy loss and changing efficiency are matched to environmental conditions to ensure optimal growth and survival are not defined by the thermodynamic arguments. The mechanisms may be derived from analysis of the effects of changing conditions on enzyme activities involved in intracellular ATP energy cycles.

The ATP cycle for aerobic cellular energy metabolism may be presented in the form of two half-cycles, the catabolic formation of ATP and the anabolic hydrolysis and use of ATP (Kemp 1996) (fig. 1).

The concentrations of adenine nucleotides in cells is low (a few mM) and the rate of turnover of ATP is high (up to 1 g ATP per g rapidly expanding plant tissue per day). Thus, rates of the two halves of the cycle in figure 1 must remain carefully balanced to maintain [ATP] constant and the phosphorylation potential in the narrow range required for cell viability (Kemp 1996). However, variation in reaction conditions cause changes in the relative rates of all the reactions involved in ATP synthesis and breakdown. Because all cells experience changing reaction conditions during growth, all must have mechanisms to control the rates of synthesis and breakdown of ATP and the phosphorylation potentials. Such controls have frequently been discussed in terms of energy charge, with emphasis on regulation by adenylate binding to glycolytic enzymes (Atkinson 1977). However, simple feedback inhibition/activation by intermediates in energy metabolism alters rates, but not stoichiometry, and does not facilitate the thermodynamically required changes in stoichiometry and efficiency required during changes in reaction conditions. Multiple, parallel reactions with different effects on energy coupling as shown in figure 1 provide control of phosphorylation potential by altering the energy coupling efficiency.

Homeotherms, which experience relatively small changes in reaction conditions, contain enzymes or enzyme systems such as phosphatases and uncoupling proteins to control phosphorylation potentials during changing reaction conditions (Stucki 1989; Nath 1998). Ectotherms face a major additional problem in maintaining phosphorylation potentials during temperature change and require these enzymes plus enzymes that can adjust metabolic responses to the large temperature change. This becomes particularly evident from observations that the Arrhenius activation energies, and therefore the temperature dependencies of the rates of ATP synthesis and its anabolic use, are not the same (Criddle and others 1997; Taylor and others 1998; Smith and others 1999). Therefore, in the absence of a regulatory mechanism the relative rates of catabolic formation of ATP and its use in anabolic reactions would change continuously with changing temperature and [ATP] would vary with temperature. Ectotherm survival thus requires mechanisms to facilitate varying catabolic/anabolic stoichiometries, control phosphorylation potential and optimize energy use efficiency over a broad temperature range.

Some enzyme reactions that can function to control phosphorylation potential or alter the stoichiometries of oxidative phosphorylation and anabolic use of ATP for production of biomass are shown in figure 1 (reactions indicated with

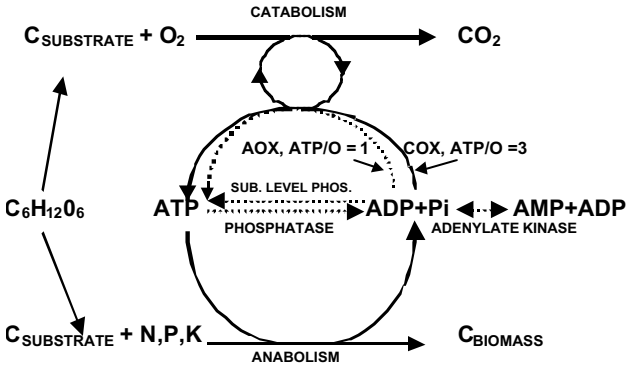

Figure 1—ATP cycle for plant respiration and biomass synthesis.

dashed arrows). For example, variation in relative amounts of substrate level phosphorylation can alter the ratios of substrate level to oxidative phosphorylation and affect overall reaction stoichiometries and energy use efficiency. The adenylate kinase reaction plays a critical role in buffering phosphorylation potentials against small changes in reaction conditions or intermediate concentrations (Stucki 1989). Phosphatases can alter energy-coupling stoichiometries via a classical "futile cycle" hydrolysis of ATP without accomplishing biochemical work. Uncouplers or uncoupling proteins can also cause a "futile" energy loss. However, this group of reactions probably plays a relatively limited role in balancing the overall condition-dependent production and use of ATP during metabolism of healthy, aerobic plant cells.

In contrast, because it is often present with high and variable activity in plant cells, the mitochondrial alternative oxidase (AOX) can have large effects on the efficiency of coupling energy metabolism to biomass production. Substrate oxidation via COX yields a maximum of 3 ATP mol^{-1} O_2. The alternative oxidase enzyme has no phosphorylation site and oxidation via the AOX pathway yields a maximum of 1 ATP mol^{-1} O_2. Oxidative energy that could have been used to synthesize two additional ATP is lost as heat. The AOX catalyzed reaction thus causes a "futile" energy loss. Consequently the number of moles of substrate required to produce one mole of ATP depends on the AOX/COX ratio (Lance and others 1985). Because the temperature coefficients of AOX and COX are not the same (McCaig and Hill 1977; Smakman and others 1982; Purvis 1985; Mc Nultly and Cummins 1987), this ratio changes with temperature (and probably with other reaction conditions). Figure 1 illustrates how changing this ratio changes the substrate carbon conversion efficiency, ε (in other words, the fraction of substrate carbon incorporated into structural biomass).

Thus, we hypothesize that the "futile" waste of the potential to produce ATP and the futile hydrolysis of ATP are the mechanisms by which phosphorylation potentials are controlled in plants growing in a variable temperature environment. We further propose that a primary reaction for adjusting the efficiency of ATP synthesis and maintaining the near constant phosphorylation potential in plants is the mitochondrial alternative oxidase. The relative temperature coefficients of the AOX and COX reactions determine the rate of change in efficiency with temperature and thereby the fitness of a plant for growth in a particular temperature environment. Matching a plant to environmental temperature is achieved by balancing the temperature coefficients of ATP synthesis, ATP use in anabolic reactions and ATP (or the potential to make ATP) wasted so that energy use efficiency is optimized over the time-temperature distribution during the growth season.

A quantitative test of these hypotheses is possible by comparison of data from direct calorespirometric measurements of substrate carbon conversion efficiencies of many different plants (Criddle and others 1997; Taylor and others 1998; Smith and others 1999) with measurements by Gonzalez-Meler and others (1999) of AOX and COX reaction rates of soybean and mung bean at 14 and 28 °C.

Demonstration of Futile Cycle Function From Changes in Substrate Carbon Conversion Efficiency With Temperature

Substrate carbon conversion efficiencies of plants can be calculated from direct calorimetric measurements of rate of heat loss (q) and either the CO_2 production rate (R_{CO2}) or the rate of O_2 consumption (R_{O2}) for rapidly growing plant tissues. The ratio of heat loss per mole CO_2 produced (or O_2 consumed) is a direct, and intuitive, measure of efficiency. The more heat lost per C-mole respired to CO_2, the less efficient the transfer of respiratory energy into anabolic products. The relation between q/R_{CO2} and ε is shown in equation 5 (Hansen and others 1994)

$$q/R_{CO2} = -(1-\gamma_p/4)\,\Delta H_{O2} - [\varepsilon/(1-\varepsilon)]\Delta H_B \qquad (5)$$

where γ_p is the chemical oxidation state of the stored photosynthetic products used as substrate for biomass production, ΔH_{O2} = -455 kJ $mole^{-1}$, and ΔH_B is the enthalpy of incorporation of C into biomass, as kJ $mole^{-1}$ C. As q/R_{CO2} increases, ε decreases (so long as the chemical nature of the photosynthetic substrate and biomass remain constant).

Simultaneous measurement of q and R_{CO2} on rapidly growing seedling tissues of cold climate plants, such as maize cultivars adapted for cultivation in the northeastern U.S. and eastern Canada, show increasing q/R_{CO2} as temperature increases (Criddle and others 1997; Taylor and others 1998) (table 1). Thus, ε for these plants decreases with increasing temperature. For example, an increase in

Table 1—Measured changes in q/R_{CO2} with temperature and calculated substrate carbon conversion efficiency.

	q/R_{CO2}, kJ mol^{-1}		ε, percent[a]	
	14 °C	28 °C	14 °C	28 °C
Maize cultivar[b]				
G17 (cool climate)	250	425	67	56
814 (warm climate)	400	295	44	60
T10 (cool climate)	225	425	70	23
Tom Thumb (warm)	444	338	10	54
Other species	q/R_{CO2}			
Cool climate	14 °C	28 °C		
Eucalyptus globulus	245	>700		
Cabbage	310	>800		
Strawberry	320	500		
Cheat Grass	328	455		
Warm climate				
Eucalyptus grandis	414	347		
Tomato	425	375		
Lily	305	255		
Rice (*Italico livorno*)	404	345		

[a]Calculations of ε with equation 5 used measured values of q and R_{CO2} and the assumption that ΔH_B = 100 kJ mol^{-1} C incorporated into plant biomass, with carbohydrate as the substrate carbon source, in other words, γ_p = 0.
[b]Data from (Taylor and others 1998).

temperature from 14 to 28 °C caused an increase in q/R_{CO2} from about 250 to 425 kJ mol^{-1} in the cold-climate adapted Pioneer Seed maize cultivar G-17. This corresponds to a decrease in ε from 67 to 56 percent. In contrast, there is a decrease in q/R_{CO2} from about 400 to 295 kJ mol^{-1} when temperature is increased from 14 to 28 °C for the warmer climate adapted Pioneer Seed maize cultivar 814. This corresponds to an increase in efficiency of seedlings from about 44 to 60 percent over this range (table 1). Values for additional maize cultivars and other species are included in table 1 to show that changes in q/R_{CO2} and ε for maize represent common trends for warm and cool climate plants (Smith and others 1999; Criddle and Hansen 1999). Thus, simple experimental measurements on plant tissues quantify values of ε and show that ε changes systematically with temperature in a pattern specific to growth temperature conditions.

Warm climate cultivars commonly increased while ε of the cooler climate cultivars decreased with increasing temperatures in the range studied (table 1). Since thermodynamic considerations require that efficiency must change with temperature, it is not unexpected that each species or cultivar is adapted to have higher efficiency in the temperature range to which it is adapted. Note that the designation of warm and cool climates refers to temperatures experienced during the growth season and not necessarily to annual average temperatures at a site. The four maize cultivars of this study and most of the additional examples were grown in common conditions with paired comparison plants, so the observed efficiency differences are genetically defined responses to environmental temperature, not a consequence of differences in acclimation during growth.

Evidence That the "Futile" AOX Reaction Supplies a Temperature-Dependent Change in Substrate Carbon Conversion Efficiency

Gonzalez-Meler and others (1999) measured the activities of AOX and COX in mung bean and soybean at two temperatures. They showed (a) simultaneous and continuous engagement of AOX and COX pathways in both mung bean and soybean (see also Hoefnagel and others 1995; Guy and others 1989; Ribas-Carbo and others 1995), (b) relatively high activities of both AOX and COX at 14 °C and 28 °C, and (c) large differences in the temperature dependencies of AOX and COX and therefore different substrate carbon conversion efficiencies at the two temperatures. Thus, the thermodynamic requirement for a changing efficiency with changing reaction temperatures can be satisfied in part by the presence of the parallel AOX and COX pathways. The remaining questions are: can the futile reaction catalyzed by AOX (a) provide both a mechanism for the thermodynamically required and experimentally observed changes in efficiency as temperature changes, and (b) provide a means for quantitatively matching the efficiency change to requirements of a specific environment.

The studies of Gonzalez-Meler and others (1999) partially answer these questions. With AOX and COX activity data at only two temperatures, it is not possible to calculate meaningful temperature coefficients, particularly when the high temperature (28 °C) may be above the optimum temperature for growth of one or both species. However, the limited available data do show large, species-dependent differences in ratios of activities at the two temperatures.

To consider changes in substrate carbon conversion efficiencies specifically due to the effects of temperature on activities of AOX and COX, we assume that plants with no AOX activity operate with a substrate carbon conversion efficiency near 0.7 (Stucki 1989), and that short-term, bidirectional, day-to-day changes in temperature affect the AOX/COX ratio predominantly by differences in responses to temperature rather than by other mechanisms (however, see discussion below). Increasing the temperature from 14 to 28 °C increased the AOX/COX ratio for soybean but decreased the ratio for mung bean. Table 2 shows that soybean ε increases from about 0.57 to 0.61 as temperature is increased between these two values, while ε for mung bean decreases from 0.64 at 14 °C to 0.61 at 28 °C. Thus, substrate carbon conversion efficiency changes with temperature in opposite directions for mung bean and soybean. ATP production efficiency of soybean increased at

Table 2—Calculated substrate carbon conversion efficiencies (ε) and growth rates for soybean and mung bean.

	Soybean				Mung bean			
T °C	ε[a]	ε/(1-ε)	R_{O2}[b]	Growth rate as (R_{O2} X ε/(1-ε))	ε	ε/(1-ε)	R_{O2}	Growth rate as (R_{O2} X ε/(1-ε))
1X AOX								
14	0.57	1.35	6.2	8.4	0.64	1.74	10.6	18.5
28	0.61	1.56	25.5	39.8	0.61	1.56	32.9	51.3
2X AOX[c]								
14	0.45	0.82	9.4	7.7	0.60	1.51	13.4	19.9
28	0.50	1.00	42.5	42.5	0.56	1.30	44.5	57.7

[a]Values of ε were calculated from AOX and COX activities at 14 °C and 28 °C reported by Gonzalez-Meler and others (1999). The calculations assume (a) a maximum substrate carbon conversion efficiency of 0.7 for both species when total respiration is via COX, (b) production of 3 ATP per O_2 via the COX pathway and 1 ATP per O_2 via the AOX pathway, and (c) that substrate carbon conversion efficiency is proportional to the total amount of ATP produced per mole O_2 for plants containing both AOX and COX.

[b]R_{O2} is total oxidation rate via AOX plus COX.

[c]Values of ε in the presence of 2 x AOX are based on the reported near doubling of AOX activity with little change in COX activity following cold acclimation of mung bean and soybean (Gonzalez-Meler and others 1999).

the higher temperature while efficiency was higher for mung bean at the lower temperature. The similarities of results in tables 1 and 2 show that a significant change in efficiency with temperature for both warm and cold climate plants could be accounted for by the presence of AOX and the ratios of the temperature dependencies of AOX and COX.

Engagement of AOX Benefits Plants Growing in Stress Conditions

An important consequence of temperature adaptation of energy metabolism based on futile cycle mechanisms becomes apparent when the effect of AOX activity on growth rate is considered. Growth rate is determined by the product of the respiration rate (R_{CO2}, or R_{O2} assuming the respiratory quotient = 1) multiplied by [ε/(1-ε)] (19). In the studies of Gonzalez-Meler and others (1999) cold acclimation of mung bean and soybean seedlings increased total oxidase rate of both plants by increasing AOX about two-fold with little change in COX activity. When AOX increases, the overall oxidase rate is higher, substrate carbon conversion efficiency is lower, but R_{CO2} multiplied by ε/(1-ε) is little changed. Doubling AOX activity at 28 °C, soybean changes growth rate only from 39.8 to 42.5 $Cmol^{-1}\ s^{-1}\ mg^{-1}$ and mung bean from 51.3 to 57.7 $Cmol^{-1}\ s^{-1}\ mg^{-1}$ (table 2). Thus, growth rate at a given temperature is little affected by increasing (or decreasing) AOX because the changes in total respiration rate are closely offset by reciprocal changes in the efficiency of production of ATP per mole of O_2 respired. Irrespective of whether ε increases or decreases with temperature, growth rate is essentially self-regulating. AOX synthesis or activation thus provides a mechanism for increasing electron flow, without major effects on growth rate.

Mediation of the thermodynamically required changes in substrate carbon conversion efficiency with changing temperature via introduction of futile reactions such as AOX can have important benefits to cell survival. Engagement of AOX can help maintain rapid electron flow (though at lower efficiency) and plant viability when the COX pathway is blocked or stressed. The continued flow of electrons via AOX under temperature or other stress conditions that may otherwise shut down respiration is consistent with the previously proposed role of AOX in contributing to the protection of cells from harmful oxidative reactions and buildup of fermentation products when plants are stressed (Wagner and Krab 1995; Purvis and Shewfelt 1993).

Stresses, by definition, hinder plant growth. The common denominator linking all stresses is an effect on metabolism that decreases energy use efficiency with increasing stress. Any changes that differentially affect the rates of reactions involving ATP synthesis and use triggers changes in the requirement for futile cycling to maintain d[ATP]/dt = 0 and a near constant phosphorylation potential. Thus, AOX (or another reaction with similar effects) is required to "waste" increasing amounts of energy in the presence of increasing stress. In support of this conclusion, alternative oxidase has been shown to be induced by many stresses including low temperature, drought, some herbicides, hydrogen peroxide and some protein synthesis inhibitors. Increasing AOX in response to stress has correctly been discussed in context of the need to maintain electron flow as a defense mechanism, but the required role in changing energy coupling efficiencies to control phosphorylation potential and maintain viable cells has not been described.

The importance of AOX in providing means for response to stress is emphasized in studies by Parsons and others (1999). They demonstrated that, compared to control cells, tobacco cells lacking alternative oxidase were greatly restricted in their ability to adapt to phosphate-limited growth. The growth limitation was ameliorated by addition of an uncoupling agent that provided (in the context of this communication) the "futile," non-phosphorylating pathway necessary for adjustment of energy coupling stoichiometries and phosphorylation potentials when AOX was absent. We predict that the tobacco cells lacking AOX will also fail to tolerate changing temperature and many other stress conditions. We propose that these cells are similar to homeotherms that have more limited need for futile reactions because of smaller changes in growth conditions.

Summary

As immobile ectotherms, plants must adjust energy metabolism to nearly continuous variation in temperature. Thermodynamic arguments as well as experimental measurements show that this is accomplished via changing stoichiometries of energy-coupled reactions and changing energy use efficiencies. Adaptation of a plant to a particular environment includes selection for the temperature coefficients for ATP synthesis, for anabolic use of ATP, and for reactions wasting ATP or the potential to synthesize ATP that allow phosphorylation potentials to be maintained nearly constant over the required temperature range.

We have demonstrated that AOX activity can fill the essential role of altering both the rate and efficiency of respiratory metabolism in plant cells in response to temperature change. This is the mechanism by which AOX fulfills the additional roles (mostly protective) that have been ascribed to AOX (McCaig and Hill 1977; Parsons and others 1999). Thus, whatever other functions it accomplishes, a primary role of AOX is maintenance of the phosphorylation potential in the appropriate range for cell viability when temperature changes. Variable temperature requires all ectotherms to have enzymes with functions analogous to the plant alternative oxidase. Ectothermic animals that lack an alternative oxidase may satisfy this requirement by a combination of uncoupling factors and phosphatases, or analogous enzymes.

Alternative oxidase activity is adjusted by several factors in addition to temperature. Concentrations of α-ketoacid metabolites, disulfide reduction, oxidation state of quinones, allosteric regulation, gene expression and possibly other factors all fine-tune AOX activity (Buchanan and others 2000). Activity responses to some of these effectors may be slow while others act rapidly and may be difficult to distinguish from simple Arrhenius temperature effects on enzyme activity. Each could be expressed differentially as temperatures change. Thus, the presence of various activating mechanisms of AOX can cause activity changes with temperature to differ from those calculated simply on the basis of temperature coefficients for this discussion. However, all of these moderators of AOX activity must work against a

background of nearly continuous, bi-directional temperature change with instantaneous effects on enzyme activity that change relative rates of AOX and COX activities, irrespective of enzyme level and activation state. No matter what the ultimate cause, AOX has a temperature dependence of activity that differs from the temperature dependence of COX and the pattern of response to temperature differs with species, cultivar, and genotype.

Unique consequences of altering respiration by variation in rates of AOX (or analogous "futile" reactions) are: (a) Changing the level of AOX activity alters electron flow, but the resulting changes in rates of electron flow and changes in efficiency are offsetting so that there is a self regulation of ATP availability for anabolism. Therefore, growth rates at a given temperature are little changed by alteration of AOX levels. (b) Via changes in AOX activity, plant respiration rate may be increased to counter stress effects, with an energy cost, but without altered growth rates or major regulatory changes in other aspects of plant metabolism. (c) Because adjustment of efficiency with temperature follows from the physical consequences of differences in temperature coefficients of AOX and COX, no complex biological regulatory system need be involved to meet the requirements of variable temperature. (d) Evolutionary adaptation of a plant to an environment by natural selection must include selection for the relative values of temperature coefficients of AOX and COX (in other words, efficiency change with temperature) that optimize growth within that environment.

Within photosynthesizing plant cells, chloroplasts also have an ATP cycle with different temperature coefficients for synthesis and use of ATP and must also maintain near constant phosphorylation potentials for proper function. Because there is no direct chemiosmotic link between chloroplasts and mitochondria, the chloroplasts require a separate class of futile cycle enzymes to maintain potentials when reaction conditions change. Differences in temperature coefficients between Calvin cycle and photorespiration activities (Mohr and Schopfer 1995) suggest that the "waste" of ATP by photorespiration may be an important key to maintaining phosphorylation potentials in chloroplasts during variation in temperature. The necessary waste of ATP in both mitochondria and chloroplasts suggests that the many unsuccessful attempts to improve crop productivity by reduction of "wasteful" reactions such as AOX and photorespiration can only achieve success for crops grown in a narrow temperature range.

References

Atkinson, D. E. 1977. Cellular energy metabolism and its regulation in metabolism. New York: J. Wiley and Sons.

Buchanan, B .B.; Gruissem, W.; Jones, R. L. 2000. Biochemistry and molecular biology of plants. Rockville, MD: American Society of Plant Physiologists.

Criddle, R. S.; Smith, B. N.; Hansen, L. D. 1997. A respiration based description of plant growth rate responses to temperature. Planta. 201: 441–445.

Criddle, R. S.; Hansen, L. D. 1999. Calorimetric methods for analysis of plant metabolism. In: Kemp, R. D., ed. Handbook of thermal analysis and calorimetry, Vol. 4: from macromolecules to man. Elsevier Science: 711–763.

Gonzalez-Meler, M. A.; Ribas-Carbo, M.; Giles, L.; Siedow, J. N. 1999. The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. Plant Physiology. 120: 756–772.

Guy, R. D.; Berry, J. A.; Fogel, M. L.; Hoering, T. C. 1989. Differential fractionation of oxygen isotopes by cyanide-resistant and cyanide-sensitive respiration in plants. Planta. 177: 483–491.

Hansen, L. D.; Hopkin, M. S.; Rank, D. R.; Anekonda, T. S.; Breidenbach, R. W.; Criddle, R. S. 1994. The relation between plant growth and respiration: a thermodynamic model. Planta. 194: 77–85.

Hoefnagel, M. N. H.; Millar, A. H.; Wiskich, J. T.; Day, D. A. 1995. Cytochrome and alternative respiratory pathways compete for electrons in the presence of pyruvate in soybean mitochondria. Archives of Biochemistry and Biophysics. ABB 318: 394–400.

Jou, D.; Llebot, J. E. 1990. Introduction to the thermodynamics of biological processes. NJ: Prentice Hall: 49–51.

Kemp, R. B. 1996. Heat dissipation and metabolism in isolated mammalian cells. In: Principles of medical biology, Vol. 4: cell chemistry and physiology: Part III. London: JAI Press: 303–329.

Lance, C.; Chauvenau, M.; Dizengremel, P. 1985. The cyanide-resistant pathway of plant mitochondria. In: Douce, R.; Day, D. A., eds. Higher plant cell respiration. Berlin: Springer-Verlag.

Lewis, M.; Randall, M. 1961. Thermodynamics 2nd edition; Revised by Pitzer, K. S.; Brewer, L. New York: McGraw-Hill: 100.

McCaig, T. N.; Hill, R. D. 1977. Cyanide insensitive respiration in wheat: cultivar differences and effects of temperature, carbon dioxide, and oxygen. Canadian Journal of Botany. 55: 549–555.

Mc Nultly, A. K.; Cummins, W. R. 1987. The relationship between respiration and temperature in leaves of the arctic plant *Saxifraga-Ceruna*. Plant Cell. 10: 319–325.

Mohr, H.; Schopfer, P. 1995. Plant Phyiol. Berlin: Springer-Verlag: 198–200.

Nath, S. 1998. A thermodynamic principle for the coupled bioenergetic processes of ATP synthesis. Pure and Applied Chemistry. 70: 639–644.

Parsons, H. L.; Yip, J. Y. H.; Vanlerberghe, G. C. 1999. Increased respiratory restriction during phosphate-limited growth in transgenic tobacco cells lacking alternative oxidase. Plant Physiology. 121: 1309–1320.

Prigogine, I. 1980. From being to becoming: time and complexity and the physical sciences. San Francisco: W. H. Freeman and Co.

Purvis, A. C. 1985. Low temperature induced azide-insensitive oxygen uptake in grapefruit flavedo tissue. Journal of American Society of Horticulture. 110: 385–388.

Purvis, A. C.; Shewfelt, R. L. 1993. Does the alternative pathway ameliorate chilling injury in sensitive plant tissues? Physiologia Plantarum. 88: 712–718.

Ribas-Carbo, M.; Berry, J. A.; Yakir, D.; Biles, L.; Robinson, S. A.; Lennon, A. M.; Siedow, J. N. 1995. Electron partitioning between cytochrome and alternative pathways in plant mitochondria. Plant Physiology. 109: 829–837.

Smakman, G.; Hofstra, R. 1982. Energy metabolism of *Plantago-lanceolata* as affected by change in root temperature. Physiologia Plantarum. 56: 33–37.

Smith, B. N.; Jones, A. R.; Hansen, L. D.; Criddle, R. S. 1999. Growth, respiration rate, and efficiency responses to temperature. In: Pessarakli, M., ed. Handbook of plant and crop stress. New York: Marcel Dekker, Inc. 417–440.

Stucki, J. 1989. Thermodynamic optimizing principles in mitochondrial energy conversions. In: Seis, H., ed. Metabolic compartmentation. New York: Academic Press: 39–69.

Taylor, D. K.; Rank, D. R.; Keiser, D. R.; Smith, B. N.; Criddle, R. S.; Hansen, L. D. 1998. Modeling temperature effects on growth respiration relations of maize. Plant, Cell and Environment. 21: 1143–115.

Wagner, A. M.; Krab, K. 1995. The alternative respiration pathway in plants: role and regulation. Physiologia Plantarum. 95: 318–325.

Wrigglesworth, J. 1997. Energy and life. London: Taylor and Francis Ltd.

Zotin, I. 1990.Thermodynamic bases of biological processes: physiological reactions and adaptations. Berlin: De Gruyter: 27–41.