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CAM: not so much a curiosity, more a lesson in integrated management

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Introduction

Why do we continue to be fascinated by CAM? In defence, we often invoke the widespread distribution (it being found in perhaps 7% all plant species) from semi-arid regions to tropical forest epiphytes, with productivities potentially matching those of C_3 crops, whilst the studies of molecular phylogeny increasingly refine our understanding of diversity and evolution (Winter and Smith,1996, Gehrig et al 2001). Alternatively, we may quote the fundamental advances made from studies of CAM plants, be it in terms of molecular characterisation of CAM induction, analysis of circadian rhythmicity, or control of membrane transport, and the interplay between mesophyll conductance, PEPc and Rubisco activity (Borland et al 1999b, Maxwell et al 1999, Luettge 2000). Then again, the basic biochemical framework of CAM might be said to be relatively restricted (compared to the C_4 pathway), with some variation in the mechanisms of decarboxylation and carbohydrate storage. But there are always exceptions- whether in terms of the remarkable phenotypic plasticity found in the hemi-epiphytic stranglers in the genus *Clusia*, or adjustments in the expression of CAM to match changing environmental conditions. Thus, the real reason that CAM continues to engender interest is because of the possibility for integrating the complexities of the CAM cycle across molecular, biochemical and ecological scales, all within a 24 hour cycle.

The fascination starts with the array of CAM lifeforms, whereby succulent leaves, stems or cladodes display remarkable convergence in evolutionary form and function, but coincidentally succulence at the cellular level provides the major physical limitation for CO_2 diffusion (Maxwell et al 1997). Using the traditional framework provided by Barry Osmond, we can map the biochemical and molecular interactions which conspire to overcome these limitations across the Phases of CAM (Osmond 1978). Thus, the fixation of CO_2 at night, catalysed initially by PEPc, leads to the accumulation of malic acid during Phase I of CAM (apart from a few genera which accumulate significant quantities of citric acid); these acids are decarboxylated primarily during Phase III, when stomata are normally closed by the high internal concentrations of CO_2 which can saturate Rubisco. However, the transition between these Phases, seen as the early morning Phase II and late afternoon Phase IV, yet again demonstrate the need for integration of biochemical regulation if the concomitant operation of PEPc and Rubisco is to be avoided. Additional limitations occur if stomata re-open for the plant to undertake conventional C3 photosynthesis during Phase IV, when low mesophyll conductances can lead to C_c, the CO2 the concentration at Rubisco, drawing down to 100 μmol mol^{-1} (Maxwell et al 1997). The

metabolic and molecular regulatory mechanisms which underpin processes from enzyme activation, metabolite storage, light harvesting and carbohydrate partitioning all operate under a range of CO_2 and O_2 concentrations which are equivalent to those encountered across palaeohistorical timescales. How these processes are controlled when CAM activity is induced or repressed by changing environmental conditions, even without widespread availability of transformed plant material, leaves us still much to investigate.

Results and Discussion

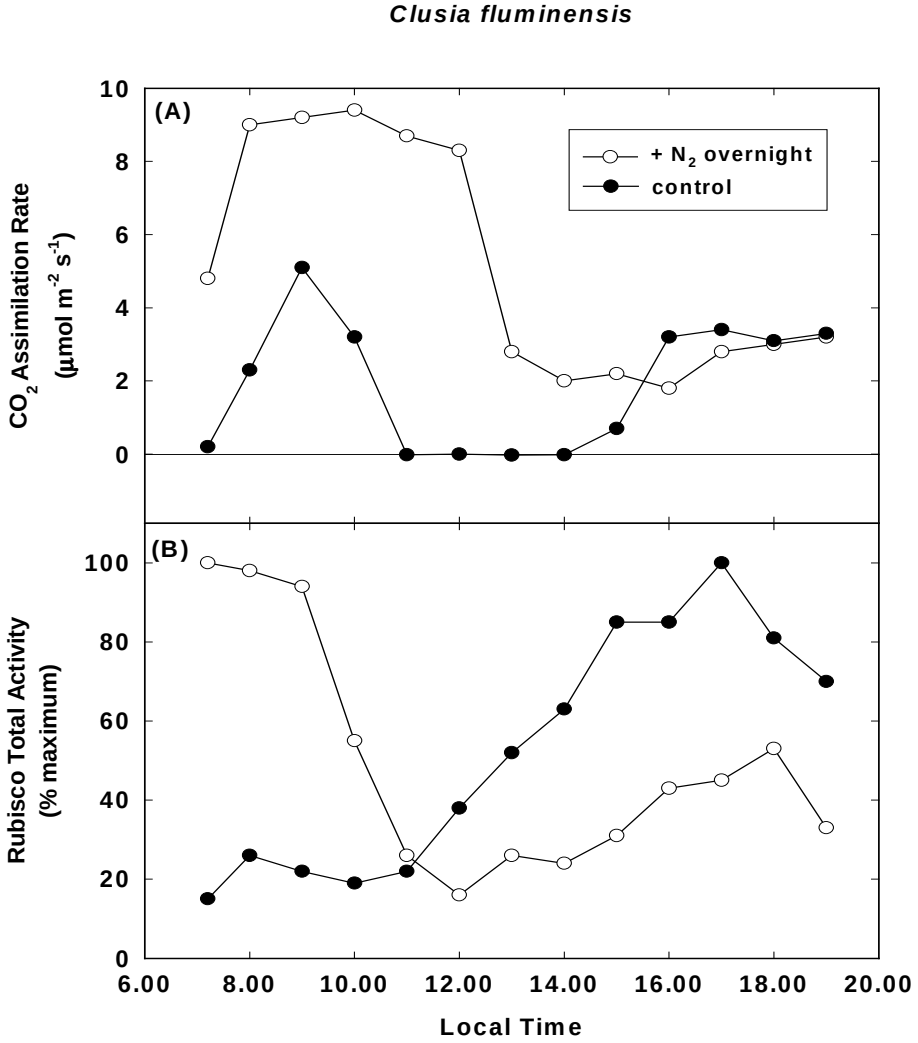

Figure 1 Relationship between CAM activity and Rubisco activation in *Clusia fluminensis*
Gas exchange (CIRAS-1, PP Systems) and Rubisco activity (Maxwell et al 1999) were determined on three replicate pairs of *C fluminensis* leaves, with one leaf having been flushed with N_2 overnight to sustain a O_2-free atmosphere (open circles) whilst the opposite leaf undertook the normal CAM cycle in air (closed circles). Unpublished data of A. Roberts, K Maxwell and H. Griffiths).

During Phase I of CAM, the high substrate affinity of PEPC will help to overcome low stomatal and mesophyll conductances and lead to an effective drawdown of CO_2 into the mesophyll. Activation of PEPC is achieved by phosphorylation via the circadian expression of PEPc kinase (Nimmo 2000, Nimmo et al 2001), with the activity of the kinase moderated by metabolites (Borland et al 1999a; A.N. Dodd, unpublished data) and an inhibitor (Nimmo et al 2001). The activity of PEPc often extends into the light period for several hours after dawn (Phase II) or before dusk (Phase IV), when direct competition with Rubisco could occur (Roberts et al 1997, Borland et al 1999b). Accordingly, we have shown that the regulation of Rubisco in CAM plants is directly related to the extent of CAM activity. For *Kalanchoe daigremontiana*, the carbamylation state progressively increased during Phase II, to reach a maximum just prior to midday, in a pattern mirrored by initial and total extractable activities of Rubisco (Maxwell et al 1999). The slow Rubisco activation and gradual increase in activity which occurred prior to elevated CO_2 during decarboxylation, when PEPc is normally being dephosphorylated and deactivated (Borland et al 1999a,b).

This pattern of regulation is controlled by the activity of the CAM cycle, since by suppressing the uptake of CO_2 by *Clusia fluminensis* overnight by placing leaves in an O_2-free atmosphere, (Figure 1A), there was a large burst of CO_2 uptake to compensate for the limitations imposed overnight as Rubisco was immediately activated at dawn (Figure 1B). For the control plant undertaking the more usual CAM cycle, there was a characteristic burst of CO_2 fixation during Phase II when Rubisco was virtually inactive (Figure 1), this being mediated primarily by PEPc since titratable acidity continued to increase (data not shown). Rubisco activity then increased during Phase III so that the maximum activation occurred at the point when stomata re-opened to allow direct CO_2 fixation during Phase IV(Figure 1). We will show that this diurnal regulation of Rubisco is brought about by changing expression of Rubisco activase (J Girnus and K Maxwell, unpublished data), and so the next question needing to be determined is how Rubisco activase is regulated and which inhibitors are involved.

For some time we have been intrigued by the patterns of light use as a diagnostic for the CAM Phases, rather than simply demonstrating photoinhibition. The measurements of Rubisco activity (see above) were all undertaken under constant light intensity (PFD of 500 µmol m^{-2} s^{-1}), so as to remove any effect of delayed induction. It was striking that for *K. daigremontiana*, non-photochemical quenching (NPQ) was highest at dawn and dusk: there was a sharp decline from 2.5 to 0.5 during Phase II, stabilising during Phase III, only to increase again during Phase IV (Maxwell et al 1999). There are energetic implications for the combined operation of PEPc and Rubisco, with the so-called "futile cycle" potentially avoided by the co-regulation of PEPc and Rubisco, as we have shown above. Our attention was first drawn to this problem when undertaking measurements of Apparent Quantum Yield (AQY) using a leaf disc electrode for the epiphytic bromeliad *Guzmania monostachia* maintained under exposed conditions (Maxwell et al 1992). We initially showed how the overall quantum efficiency and V_{max} adjusted during the diurnal cycle, with both declining at midday. We now include the data at dawn and dusk on these days (Figure 2), whereby both AQY and V_{max} were then lower than at midday. Thus it would appear that the entire photochemical efficiency of these plants is down-regulated overnight, with zeaxanthin content and NPQ poised to resume at dawn each day (see also Maxwell et al 1995). This phenomenon has also frequently been observed during diurnal measurements of various *Clusia* species (eg de Mattos et al 1999), in that the

quantum yield of PSII fluorescence is low at dawn and dusk as drought stress progresses.

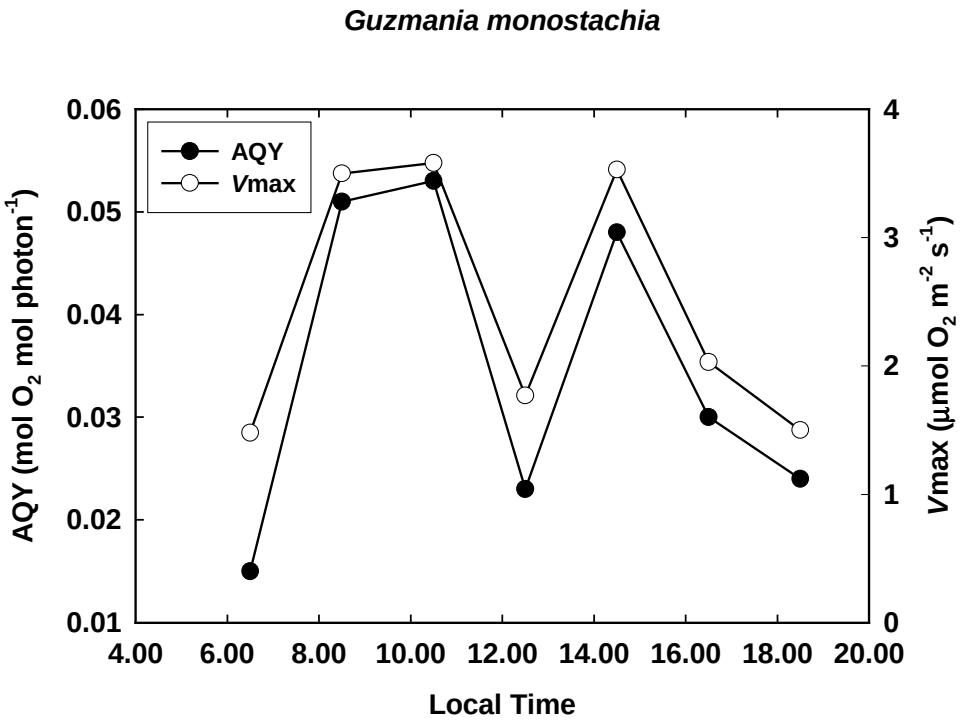

Figure 2 Diurnal changes in Apparent quantum yield and *Vmax* in *Guzmania monostachia*
Measurements were made on five replicate leaves at each time point under saturating CO2 in a Hansatech Leaf Disc Electrode (LD2) system, on plants maintained under fully exposed conditions in Trinidad

This leads us to the implications for the way that light use and Rubisco activity is regulated between the more succulent, constitutive CAM plants, as compared to C3-CAM intermediates. The latter group is often characterised by a much wider range of daytime CO_2 fixation patterns, occasionally incorporating continuous CO_2 uptake across 24 hours. This occurs when the CO_2 supply from decarboxylation is insufficient to close stomata, allied to the extremely high potential carboxylation capacities (V_{max}) measureable for many CAM plants (Borland and Griffiths 1996).
Most recently, we have sought to appease the promptings of Barry Osmond, which have chastened us for nearly a decade, when he suggested that the dark doings of CAM plants may furnish an explanation (Osmond et al 1996). As part of a study comparing mesophyll conductance and efficiency of light use across a range of members of the genus *Kalanchoe*, we will now show that highly succulent, constitutive CAM plants are unable to activate light reactions and Rubisco activity at night when reactivated with saturating light intensity for 10 minutes. In contrast, the less succulent species, which display greater plasticity in the range of Phase II and IV gas exchange characteristics, were able to reactivate Rubisco under these conditions, similar to *Nicotiniana* (K Maxwell, unpublished observations). In the less succulent species, electron transport rates at night were similar to those achieved during Phase IV in the light, which contrasted markedly with the succulent *K. daigremontiana*,

which did generate sustained NPQ after the light treatment, even at low PFDs . We conclude that the extent of Rubisco regulation and inhibition is related to the absolute dependence on nocturnal CO_2 fixation, which is particularly acute in more succulent leaves, where the mesophyll conductance is lowest.

Whilst more detailed studies on the molecular and biochemical characterisation of decarboxylation processes are urgently required, one other component of the CAM pathway which also integrates, and perhaps controls, the entire CAM cycle, is that of carbohydrate supply and demand. We will report studies on the C_3-CAM intermediate *Mesembryanthemum crystallinum* which have analysed the way that starch degradation limits the CAM cycle. Circadian control is exerted over the expression of the major starch decarboxylating enzymes (β amylase and starch phosphorylase, STP) and key evidence has come from studies manipulating the CAM cycle under continuous light or CO_2-free conditions, showing that carbohydrate supply, rather than PEPC activation, really regulates the extent of CAM activity (A.N. Dodd, A.M. Borland and H. Griffiths, unpublished data).

Finally, these observations have implications at the ecological scale, where the plasticity in the CAM cycle can optimise CO_2 fixation by day or night, particularly in those less succulent leaves which are perhaps less irreversibly committed to the strict co-regulation of PEPc and Rubisco. Firstly, the magnitude of nocturnal acidification being dependent on light intensity of the previous day (Borland et al 1999b) shows that carbohydrate partitioning is a key component of the CAM cycle. This is now supported by the way that the circadian transcription of key enzymes occurs in the light, in advance of the dark period activation. Secondly, the observation that Rubisco regulation is intimately associated with the CAM pathway has been demonstrated in data presented above, as well as the observation that when *G. monostachia* induces CAM, Rubisco then showed delayed activation (K Maxwell, unpublished observations). This pattern of regulation is closely allied to the induction of photochemistry, electron transport and NPQ, which can be repressed at dawn and dusk in constitutive CAM species. Finally, there are direct ecological advantages for CAM epiphytic bromeliads, a small number of which are exclusively found in the wettest upper montane forest formations. Here, we hypothesized that CAM would make a major contribution to carbon gain because of the plasticity inherent to the CAM pathway, should gas exchange be limited by surface moisture. This has been borne out by observations under field conditions, in that daily carbon gain in both rainy and dry seasons exceeded that for sympatric C3 bromeliads with similar lifeforms (S Pierce, K Winter and H Griffiths, unpublished observations).

In conclusion, research on this pathway is always mainstream for those of us committed to CAM, which to us is not so much a curiosity, more a way of life. We hope that this short review of the latest developments in physiological ecology shows the continued potential for demonstrating the integrated management of metabolism. In context, the interplay between molecular, biochemical and environmental regulation, across dark and light periods, will continue to illuminate processes relevant to all plants.

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