Seasonal influences on carbohydrate metabolism in the CAM bromeliad *Aechmea* ‘Maya’: consequences for carbohydrate partitioning and growth

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INTRODUCTION

In plants with crassulacean acid metabolism (CAM), temporal regulation of carboxylation processes mediated via ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPC; nocturnal CO₂ uptake) is required to avoid futile cycling of carbon ([CO₂], water availability, light intensity and temperature, is ubiquitous among plants with crassulacean acid metabolism (CAM). The present study examined how seasonal changes in light availability, as experienced by greenhouse CAM crops in northern latitude regions, influence diel carboxylation patterns and impact on carbon gain and seasonal accumulation of biomass.

**Methods** In the CAM bromeliad *Aechmea* ‘Maya’ integrated measurements of leaf gas exchange, diel metabolite dynamics (e.g. malate, soluble sugars and starch) and biomass accumulation were made four times a year, i.e. in winter, spring, summer and autumn.

**Key Results** During the brighter seasons (spring and summer) daytime Phases II and IV were dominated by C₄ carboxylation, whilst the higher diurnal uptake in the autumn and winter was characterized by equal contributions of both Rubisco and PEPC. As a consequence, net CO₂ uptake showed a significant depression at the end of the day in the darker months when supplementary illumination was turned off. Remarkable seasonal consistency was found in the amount of storage reserves available for nocturnal carboxylation, a consequence of predominantly daytime export of carbohydrate in spring and summer whilst nocturnal export was the major sink for carbohydrate in autumn and winter.

**Conclusions** Throughout the different seasons *Aechmea* ‘Maya’ showed considerable plasticity in the timing and magnitude of C₃ and C₄ carboxylation processes over the day/night cycle. Under low PPFD (i.e. winter and autumn) it appears that there was a constraint on the amount of carbohydrate exported during the day in order to maintain a consistent pool of transient carbohydrate reserves. This gave remarkable seasonal consistency in the amount of storage reserves available at night, thereby optimizing biomass gain throughout the year. The data have important practical consequences for horticultural productivity of CAM plants and suggest a scenario for reconciling carbohydrate partitioning between competing sinks of nocturnal acidification and export for growth.
Plasticity in the deployment of C₃ and C₄ carboxylation processes in CAM plants can be analysed by monitoring the shifts in net CO₂ uptake and metabolites that define the four phases of carbon supply and demand over a 24-h cycle (Osmond, 1978). PEPC is activated at night (Phase I) with the resultant uptake of CO₂ leading to nocturnal accumulation of malic acid. During the day, PEPC is deactivated, and malate decarboxylation releases CO₂ which results in stomatal closure (Phase III) and CO₂ is fixed via Rubisco. These major CAM phases are punctuated by Phase II at the start of the day and Phase IV at the end of the day. The deactivation of PEPC in Phase II and reactivation of PEPC in Phase IV may be extremely protracted, depending on species and environmental conditions (Borland and Griffiths, 1997). Thus, circadian control of PEPC activation status is subject to modulation by metabolites (i.e. cytosolic malate) which provides flexibility for optimizing carbon gain under changing environmental conditions (Borland et al., 1999; Borland and Taybi, 2004). There is also evidence thatRubisco activation status is modulated reciprocally with that of PEPC to curtail competition for CO₂ such thatRubisco activation status is low in Phase II and peaks only a few hours before the end of Phase IV (Griffiths et al., 2002), implicating the clock in some measure of control over C₃ carboxylation. The day/night turnover of carbohydrate is a further key component in determining the magnitude of C₄ carboxylation by providing substrate for PEPC-mediated CO₂ uptake. Temporal regulation of carbohydrate partitioning is required to apportion carbohydrates between the provision of substrates for growth and export versus the retention of carbohydrate to provide PEP for C₄ carboxylation (Borland and Dodd, 2002; Ceusters et al., 2008a).

The aim of the present work was to examine how seasonal changes in integrated photosynthetic photon flux density (PPFD), as experienced by greenhouse CAM crops in northern latitude regions, influence the diel carboxylation pattern, and to establish the implications for carbon gain and seasonal accumulation of biomass in the constitutive CAM bromeliad *Aechmea* ‘Maya’. Cultivars of *Aechmea* are grown as ornamentals in temperate northern hemisphere regions such as Europe and North America (Ceusters, 2008). Integrated measurements of leaf gas exchange, diel metabolite dynamics (e.g. malate, glucose, fructose, sucrose and starch) and biomass accumulation were made four times a year, i.e. in winter, spring, summer and autumn. Specific questions that were addressed included: (a) How do seasonal changes in PPFD affect the magnitude and duration of C₃ and C₄ carboxylation processes over the day/night cycle of CAM? (b) Is there metabolic flexibility for adjusting the provision of carbohydrates for export over the day/night cycle in a tropical CAM species subjected to seasonal changes in PPFD? (c) What are the implications for carbon gain and biomass accumulation throughout the year?

**MATERIALS AND METHODS**

**Plant material**

*Aechmea* ‘Maya’ is a spineless cultivar resulting from a cross between *A. tessmannii* and *A. fasciata*. These species are CAM bromeliads and belong to the subfamily of Bromeliioideae (Benzing, 2000; Londers et al., 2005). Previous studies have indicated that *Aechmea* ‘Maya’ is obligate CAM (Ceusters et al., 2008b, 2009c). In the greenhouse (Leuven, Belgium) natural daylight was supplemented by additional artificial light (PPFD = 30 μmol m⁻² s⁻¹) between 0600 and 2200 h. From May until September the greenhouse was partially shaded (approx. 25 %). During daytime a minimum temperature of 21 °C was maintained while at night the minimum was 19.5 °C. Relative humidity was controlled, fluctuating around 60 % during daytime and increasing to approx. 75 % at night. CO₂ concentrations in the greenhouse ranged from 300 to 400 μmol mol⁻¹. Plants were watered once weekly with a conventional nutrient solution of 1 mS cm⁻¹ (Londers, 2006).

**Experimental sampling**

Plants, 12 months old (*n* = 10) were sampled every 2 h during a day/night cycle of 24 h in January (winter), April (spring), July (summer) and October (autumn) 2005. Additionally, net CO₂ exchange was measured on three consecutive days from three replicate plants from the same group. The average integrated daily PPFD (QS, Agopie Instruments Inc., Logan, UT, USA) was approx. 2-4 mol photons m⁻² d⁻¹ in winter; 6-8 mol photons m⁻² d⁻¹ in spring; 4-8 mol photons m⁻² d⁻¹ in summer and about 3-2 mol photons m⁻² d⁻¹ in autumn. Typical diurnal variations in PPFD incident on leaves for the different seasons are shown in Fig. 1. The average day/night temperatures were 21/19.5, 24/19.5, 23/19.5 and 23/19.5 for winter, spring, summer and autumn, respectively.

**Gas exchange measurements**

Net CO₂ exchange was measured on the youngest fully expanded leaves, using an LCi portable photosynthesis system (ADC BioScientific Ltd, Great Amwell, Herts, UK). The top part of the leaf was enclosed in a broad leaf chamber (6.25 cm²) and the incoming air was passed through a 25-L bottle to buffer short-term fluctuations in the CO₂ concentration. Gas exchange data were collected over a 24-h period using a 15-min interval (*n* = 3 plants). Net CO₂ uptake was quantified for specific periods during the 24-h time course by integrating defined areas under the CO₂ exchange curves (Griffiths et al., 1986).

**Metabolite analyses**

Starting from 0900 h, leaf samples were taken from the upper one-third of young fully expanded leaves (*n* = 10 plants) every 2 h during a complete 24-h day/night cycle. Leaf samples were immediately frozen in liquid nitrogen.

Extraction of organic acids was as described by Ceusters et al. (2008b). Malic and citric acids were measured using high performance liquid chromatography (Waters 510; Waters, Milford, MA, USA) with detection at 210 nm (Waters 484) using an aminex HPX87-H (300 mm x 7.8 mm) resin-based column (Bio-Rad, Hercules, CA, USA). Soluble sugars (glucose, fructose and sucrose) were extracted, subjected to enzymatic treatment (Enzytec, Scil Diagnostics GmbH, Germany) and analysed at 340 nm using
a spectrophotometer (DU-65, Beckman, Fullerton, CA, USA). Starch content was determined as glucose equivalents following digestion with amyloglucosidase (Enzytec, Scil Diagnostics GmbH, Germany) as described by Farrar (1993).

**Carbon budgets**

Carbon budgets followed Ceusters et al. (2008a) and were adapted from a model (Borland, 1996; Borland and Dodd, 2002), which quantitatively describes: (a) the source of carbon in the leaf, i.e. C₃ (Rubisco-mediated CO₂ uptake) or C₄ (from breakdown of malate where 1 mol malate = 4 mol C); (b) the partitioning of this carbon between starch or soluble sugars (sucrose, glucose and fructose) with the excess going to daytime export where 1 mol glucose eq = 6 mol C; (c) the partitioning of carbohydrates between the generation of substrates for CAM, respiratory CO₂ or dark export. The budgets presented are net carbon fluxes, thus taking into account any respiratory losses of carbon during the light period. All numbers presented on budgets are mmol C m⁻² d⁻¹.

**Figure 1** Net 24 h CO₂ uptake (µmol m⁻² s⁻¹; black line) and photosynthetic photon flux density (µmol m⁻² s⁻¹; grey line) incident on the leaf surface (left panel) and diel malic acid (mmol m⁻²; right panel) patterns for young fully expanded leaves of Aechmea ‘Maya’ at each season, i.e. winter (A, E), spring (B, F), summer (C, G) and autumn (D, H). PPFD curves represent typical radiation for each period supplemented with artificial light between 0600 and 2200 h (PPFD = 30 µmol m⁻² s⁻¹). Gas exchange curves are representative of three replicate runs with s.e. <10%. Malic acid data are means ± s.e. (n = 10 plants). The four different phases of CAM are indicated.
Biomass accumulation

To calculate seasonal biomass accumulation, 12-month-old plants (n = 10) were harvested at the beginning of each season and after 3 months of growth. Dry weight was determined after 1 week of drying at 70 °C. Both the absolute growth rate (g dry matter d⁻¹) and the relative increase in dry matter (%) over the 3-month period were calculated.

Data analysis

Where appropriate, the data were analysed using the statistical software package SAS Enterprise Guide 4.0. Before carrying out statistical tests, normality of the data was checked by means of the Kolmogorov–Smirnoff statistic (P > 0.05). Means are compared by Tukey’s studentized range test (α = 0.05).

RESULTS

Leaf gas exchange

Diel gas exchange patterns were monitored in January (winter), April (spring), July (summer) and October (autumn) and integrated net CO₂ uptake was calculated for each of the different CAM phases (Fig. 1 and Table 1). There was a marked depression in net CO₂ uptake around 2200 h (when supplementary lighting was turned off) in winter and autumn, but not for plants measured in spring and summer (Fig. 1). Seasonal changes in integrated PPFD were also reflected in the rate of nocturnal net CO₂ uptake as well as total uptake during the night, which showed marked differences between plants sampled in the spring and summer from those sampled in autumn and winter. More specifically, approx. 50 % more CO₂ was fixed during spring and summer nights compared with autumn and winter nights (Table 1). In contrast, net CO₂ uptake during Phase IV commenced 2 h earlier in plants sampled in autumn and winter and accounted for almost twice as much net CO₂ uptake in comparison to Phase IV for plants sampled in spring and summer. In the latter seasons Phase IV mainly occurred under natural illumination while the same phase was expressed through extension of the photoperiod by low supplementary lighting in autumn and winter. The summer period differed from the other seasons by a higher rate of net CO₂ uptake and total uptake during an extended Phase IV which lasted for 3 h. During the other periods of the year Phase IV only lasted around 1 h. Overall, total diel net CO₂ assimilation was approx. 20 % higher in spring in comparison with the other three seasons, which showed comparable amounts of total net CO₂ uptake over 24 h (Fig. 1 and Table 1).

Metabolite dynamics

Nocturnal CO₂ fixation via PEPC is inextricably bound up with formation of malic acid and this was reflected in a 50 % higher accumulation of malic acid during Phase I in plants sampled in spring and summer compared with those analysed in winter and autumn (Fig. 1; P < 0.05). Plants sampled in summer also continued to accumulate malic acid for 3 h at the start of the day (Phase II) and consequently net malic acid breakdown was delayed in these plants compared with those sampled at other times of the year. Malic acid decarboxylation was completed some 2 h earlier (i.e. at 1500 h) in plants sampled in autumn and winter, resulting in Phase IV net CO₂ uptake starting earlier (Fig. 1) compared with plants sampled in spring and summer when decarboxylation was completed and Phase IV commenced by 1700 h. Some malic acid accumulated during Phase IV in all plants indicating that PEPC was active at this time, particularly during the spring. In total, almost 40 % more malic acid was shuttled through the diel CAM cycle during spring (P < 0.05), compared with the other three seasons, which showed comparable diel accumulation/degradation of malic acid (P > 0.05). Citric acid content did not fluctuate significantly (P > 0.05) during 24 h (results not shown).

The diel patterns of starch accumulation/depletion were less affected by the different seasons than the 24-h time courses of malic acid content (Fig. 2). Maximal starch contents were similar over the four seasons (mean of 49 ± 5 mmol Glc eq m⁻²) and peaked at 1700 h regardless of season and dipped to a minimum of 16 ± 3 mmol Glc eq m⁻² at 0900 h (Fig. 2). Thus, the magnitude of starch degradation proved to be very constant throughout the year and liberated 32 ± 5 mmol Glc eq m⁻², which could, potentially, be used to provide 64 mmol PEP for nocturnal carboxylation.

The diel patterns for sugar content, i.e. glucose, fructose and sucrose were also similar in the different seasons. Glucose and fructose contents fluctuated, respectively, around 4 ± 1 mmol m⁻² and 3 ± 1 mmol m⁻² and so did not participate in the net diel carbohydrate flux required to sustain CAM (results not shown; P > 0.05). Sucrose showed a diel pattern of accumulation and depletion, analogous to starch, but smaller in magnitude (Fig. 2). Thus, sucrose degradation could potentially deliver 5 ± 1 mmol m⁻² sucrose, equivalent to approx. 10 mmol Glc eq m⁻² or 20 mmol PEP to sustain malate formation.

Table 1. Integrated net CO₂ uptake for the times of each of the four CAM Phases (mmol CO₂ m⁻² phase⁻¹) by young fully expanded leaves of Aechmea ‘Maya’ (n = 3) for each season

<table>
<thead>
<tr>
<th></th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Phase IV</th>
<th>Total 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>January (winter)</td>
<td>42.5 ± 3.0b</td>
<td>0-4 ± 0.1c</td>
<td>0-5 ± 0.4a</td>
<td>28.5 ± 0.8a</td>
<td>74.6 ± 3.2b</td>
</tr>
<tr>
<td>April (spring)</td>
<td>64.2 ± 8.5a</td>
<td>3-9 ± 1.3b</td>
<td>0-8 ± 1.2a</td>
<td>18.9 ± 1.7b</td>
<td>88.5 ± 7.2a</td>
</tr>
<tr>
<td>July (summer)</td>
<td>57.3 ± 3.1a</td>
<td>8-3 ± 1.5a</td>
<td>2-1 ± 2.5a</td>
<td>12.7 ± 3.2a</td>
<td>75.1 ± 10.2b</td>
</tr>
<tr>
<td>October (autumn)</td>
<td>44.8 ± 4.8b</td>
<td>1-3 ± 0.4c</td>
<td>2-0 ± 1.7a</td>
<td>29.9 ± 2.5a</td>
<td>74.0 ± 6.8b</td>
</tr>
</tbody>
</table>

Data are means ± s.e. and those in each column followed by a different letter are significantly different by Tukey’s studentized range test (P < 0.05).
Carbon budgets

To integrate measurements of net CO₂ uptake with net malate and carbohydrate turnover, leaf carbon budgets were constructed for plants sampled during each season (Fig. 3). The autumn and winter periods were characterized by a comparable total daily carbon input of approx. 260 mmol m⁻² d⁻¹. The major part of this input was delivered by breakdown of malic acid, which was previously built up during Phases IV and I. However, C₃ carboxylation also made a direct contribution to CO₂ sequestration in Phase IV, which was marked by nearly equal amounts of net CO₂ uptake mediated by Rubisco and PEPC (Table 2 and Fig. 1). The major sink for assimilated carbon was the daytime accumulation of starch. A further 20% of fixed carbon was allocated to the accumulation of soluble sugars and about 3% was used to sustain C₄ carboxylation during Phase IV. These processes allowed a minimal daytime net export of carbon of 5% in autumn whilst daytime net export was completely abolished in winter. In both autumn and winter, starch degradation was the major source of carbon skeletons for the nocturnal synthesis of PEP to sustain C₄ carboxylation. Additionally, limited breakdown of soluble sugars, i.e. sucrose, occurred during the period of malate formation. In total, 70% of carbohydrate degradation was used to sustain PEPC-mediated CO₂ uptake, whilst the remaining 30% of carbon was available for export/respiration during the period of malate formation in autumn and winter.

In spring, the daily net carbon input was increased by 45% to approx. 360 mmol m⁻² d⁻¹ due to elevated synthesis of malic acid during Phases IV and I, and, to a lesser extent, to

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**Fig. 2** Diel pattern of starch (mmol Glc eq m⁻²; left panel) and sucrose (mmol m⁻²; right panel) for young fully expanded leaves of *Aechmea* 'Maya' at each season, i.e. winter (A, E), spring (B, F), summer (C, G) and autumn (D, H). Data are means ± s.e. (n = 10 plants) and the four different phases of CAM are indicated.
the occurrence of significant net CO\textsubscript{2} uptake in Phase II. In contrast to the situation in plants sampled in winter and autumn, in spring the transitional Phases II and IV consisted exclusively of PEPC-mediated CO\textsubscript{2} uptake (Fig. 1 and Table 2). In spring, starch accumulation was still the major daytime sink for carbon but 25\% of carbon was exported during daytime. To sustain the higher rates of net dark CO\textsubscript{2} uptake in plants sampled in the spring, most of the starch
and soluble sugar pools were degraded to form PEP, thereby allowing only 5% of carbon to be used for export and respiration during Phases I and IV.

In summer, the daily input of carbon was diminished to 286 mmol m\(^{-2}\) d\(^{-1}\) but this was still 15% higher than that measured in autumn and winter, mainly due to more malic acid accumulated during Phase I. The net uptake of CO\(_2\) during Phase II, which was largely mediated by PEPC, also contributed to daily carbon gain (Table 2). During Phase IV, uptake of CO\(_2\) was solely attributed to PEPC activity (Fig. 1 and Table 2). Comparable to the diel fluxes of carbon noted during spring, some 25% of acquired carbon was exported during the day in summer. To fulfil the needs of malate formation, the remaining carbohydrate was allocated to the synthesis of PEP for nocturnal carboxylation, thereby abolishing virtually any net export at night.

**Biomass accumulation**

Plants produced most of the biomass in the period from spring to summer with an increase of dry matter of 74 ± 13%, equivalent to a gain of 0.44 ± 0.08 g dry matter d\(^{-1}\) (Table 3). During the three months from summer to autumn, plant total dry matter increased by 37 ± 7% (0.21 ± 0.04 g dry matter d\(^{-1}\)). Despite receiving considerably less light in the months intervening between autumn to winter and from winter to spring, plant dry matter increased by 48 ± 12% and 59 ± 14%, respectively, which equated to 0.27 ± 0.06 to 0.32 ± 0.07 g d\(^{-1}\).

### TABLE 2. Relative contribution of PEPC- and Rubisco-mediated CO\(_2\) uptake during transitional Phases II and IV for young fully expanded leaves of Aechmea ‘Maya’ for each season

<table>
<thead>
<tr>
<th>Phase</th>
<th>C(_3) carboxylation (%)</th>
<th>C(_4) carboxylation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C(_3)</td>
<td>C(_4)</td>
</tr>
<tr>
<td>January (winter)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>April (spring)</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>July (summer)</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>October (autumn)</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Percentage of C\(_4\) carboxylation is quantified by comparison of malic acid build-up and total net CO\(_2\) uptake in each period whereby 1 mol malic acid corresponds to 3 g CO\(_2\). The remaining CO\(_2\) uptake is attributed to C\(_3\) carboxylation through direct Rubisco activity.

* CO\(_2\) uptake during Phase II was negligible in winter and autumn and consequently no carboxylation percentages are calculated.

### TABLE 3. Seasonal relative increase in total plant dry matter (%) and absolute growth rate (g dry matter d\(^{-1}\)) for 12 month-old Aechmea ‘Maya’ plants (n = 10)

<table>
<thead>
<tr>
<th>Relative increase in dry matter</th>
<th>Absolute growth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>January–April</td>
<td>58 ± 13(^{+})</td>
</tr>
<tr>
<td>April–July</td>
<td>73 ± 13(^{1})</td>
</tr>
<tr>
<td>July–October</td>
<td>37 ± 6(^{+})</td>
</tr>
<tr>
<td>October–January</td>
<td>48 ± 12(^{+})</td>
</tr>
</tbody>
</table>

Data are means ± s.e. and those in each column followed by a different letter are significantly different by Tukey’s studentized range test (P < 0.05).

### DISCUSSION

**Seasonal impact on C\(_3\) and C\(_4\) carboxylation processes**

The total amount of net CO\(_2\) uptake measured over 24 h was remarkably similar over the different seasons, and despite the 3-fold higher daily integrated PPFD experienced in spring compared with winter, plants took up only approx. 20% more CO\(_2\) in spring, implying that light was not the only limiting factor for net CO\(_2\) uptake throughout the changing seasons. The relative contributions that C\(_3\) and C\(_4\) carboxylation made to net carbon balance varied throughout the year and a reciprocal relationship was observed between the relative magnitudes of daytime and nocturnal CO\(_2\) sequestration across the different seasons. Data obtained from other CAM species suggested the existence of mechanisms for compensating any shortfalls in nocturnal CO\(_2\) uptake by modulating both the amplitude and duration of the following daytime phases (Dodd et al., 2002).

Previous studies have indicated seasonal differences in PEPC-specific activity of 8- and 30-fold in obligate and facultative CAM species, respectively, with highest PEPC found in spring/summer months (Pilon-Smits et al., 1991a, b). In Aechmea ‘Maya’, PEPC dominated net CO\(_2\) uptake in the spring and summer months both during the night (Phase I) but also during the day (Phases II and IV), indicating metabolic flexibility in modulating both the magnitude and duration of PEPC activation in response to seasonal changes in PPFD. The length of time that stomata remain open during Phase II appears to be related to the activation status of PEPC which in turn is determined by the onset of malic acid efflux from the vacuole (Winter and Tenhunen, 1982; Borland et al., 1999). Seasonal changes in the timing of malic acid breakdown in leaves of Aechmea ‘Maya’ are consistent with the above view and previous investigations on this species have consistently shown retention of maximal malic acid content for 3 h after dawn in the summer months (Londers et al., 2005; Ceusters et al., 2008a, b). The tight relationship between an extended Phase II and continued PEPC activation in Aechmea ‘Maya’ over the summer remains an enigma since the factors that regulate the timing and rate of malate decarboxylation in CAM plants remain to be established.

The net acquisition of external CO\(_2\) by Aechmea ‘Maya’ during Phase IV in the brighter seasons, i.e. spring and summer (representing 20% of 24-h uptake), closely matched the concomitant accumulation of malic acid over this period, indicating exclusive PEPC-mediated net CO\(_2\) uptake over the latter part of the day. Moreover, in spring and summer, the instantaneous rates of net CO\(_2\) uptake measured over the late part of the day continued to rise, even into the dark period whilst, for plants analysed in autumn and winter, a significant depression in net CO\(_2\) uptake occurred when illumination was turned off. It seems likely that this drop in net CO\(_2\) uptake at nightfall noted in the darker months could be attributed to deactivation of Rubisco which was responsible for more net CO\(_2\) during Phase IV in the autumn and winter months as compared with spring and summer. Possibly, extending the
natural photoperiod, from 1800 h to 2200 h in autumn and winter with low photon fluencies (30 \( \mu mo l \) \( m^{-2} \) \( s^{-1} \)), supported substantial CO2 uptake in the C3 photosynthetic mode during the latter part of Phase IV. Previous studies in CAM species indicated that Rubisco activation status is modulated reciprocally with that of PEPC, curtailing competition for CO2 in such a way that Rubisco activation status is low in Phase II and peaks only a few hours before the end of Phase IV (Griffiths et al., 2002). Such regulation might involve transcriptional control of Rubisco activase which gives rise to a peak in protein abundance of the activating enzyme in Phase III that then declines over Phase IV (Maxwell et al., 1999; Griffiths et al., 2002). The results presented for Aechmea ‘Maya’ imply metabolic flexibility in the diurnal regulation of Rubisco activase in response to seasonal changes in light intensity, although it is equally possible that a higher PPDF enables PEPC to out-compete Rubisco in terms of net CO2 uptake in Phase IV in spring and summer.

Seasonal impact on carbohydrate partitioning and biomass accumulation

Stoichiometric considerations indicated that starch was the predominant substrate for nocturnal carboxylation throughout the year, while sucrose was also metabolized to a lesser extent to sustain malic acid synthesis. In contrast to the seasonal plasticity in the timing and magnitude of C3 and C4 carboxylation processes over the day/night cycle as described above, the magnitude of nocturnal starch and sucrose breakdown remained remarkably constant throughout the year. Thus, seasonal differences in the magnitude of nocturnal carboxylation were not dictated by carbohydrate availability. Metabolic flexibility for adjusting the provision of carbohydrates for export over the day/night cycle was observed in the different seasons. Daytime export was the major sink for carbohydrate in spring and summer whilst nocturnal export was the major sink in autumn and winter. These differences in carbohydrate allocation may be attributed to seasonal differences in PEPC activity as described above. Thus, lower PEPC activity in the autumn and winter months meant less demand for nocturnal provision of PEP and more carbohydrate was available for export and maintenance at night compared with plants in the spring and summer where higher PEPC activity claimed more carbohydrates for nocturnal provision of PEP. In line with total CO2 uptake, the carbon budget approach showed higher export of assimilates to sustain maintenance and growth in spring, compared with the other seasons. This was reflected by the higher rates of growth and biomass accumulation in the spring. This observation also calls in to question the dogma that the growth and productivity of CAM species is directly related to the magnitude of Phase IV net CO2 uptake (Winter, 1985), since plants in the autumn and winter showed substantially greater Phase IV activity under an artificially extended photoperiod but lower rates of growth and biomass accumulation compared with those sampled in the spring. However, artificial extension of the photoperiod, even with the low fluence rates used in this experiment (i.e. 30 \( \mu mo l \) \( m^{-2} \) \( s^{-1} \)), permitted a gain of biomass in the darker seasons that resulted in comparable growth during winter, summer and autumn.

It appears that there was a brake on the amount of carbohydrate exported during the day under limiting PPDF (i.e. autumn and winter), which meant that there was remarkable seasonal consistency in the amount of storage reserves available at night. As such, the leaf carbon budgets suggest a scenario for reconciling carbohydrate partitioning between competing sinks of nocturnal acidification and export for growth, a conundrum that continues to challenge CAM scientists (Borland and Dodd, 2002; Osmond et al., 2008). In the proposed model (Fig. 4), two discrete, but interacting pools exist to apportion carbohydrate derived from the decarboxylation of malate and from Phase IV photosynthesis. The primary pool stores carbohydrate for subsequent nocturnal acidification and export, while a secondary pool furnishes the daytime export of carbohydrate. Only when the primary pool is filled does the secondary pool receive carbon skeletons derived from the gluconeogenic recovery of decarboxylation products as well as from Phase IV photosynthesis. The carbohydrate status of the secondary pool will determine the amount of sugar available for export during the day but may also feed forward to stimulate nocturnal carboxylation, presumably via sugar-signalling for increased PEPC activity. Thus, in the spring and summer months, increased input to the secondary pool resulted in enhanced daytime export and increased nocturnal carboxylation as a sink for carbohydrate. In the autumn and winter months, the reduced carbohydrate status of the secondary pool resulted in little daytime export and an attenuation of nocturnal carboxylation so that more sugars were available for export at night. The model implies that leaf carbohydrate status might serve as a central checkpoint gearing nocturnal carboxylation and export for growth to one another in order to optimize plant growth throughout the year. The precise mechanisms responsible for this are unknown but perhaps are a reflection of pervasive circadian control of carbohydrate partitioning in CAM plants which

![Fig. 4 A model for reconciling carbohydrate partitioning between competing sinks of nocturnal carboxylation and export for growth that comprises two discrete, but interacting pools that can receive carbohydrate (C) derived from the decarboxylation of malate and from Phase IV photosynthesis. The primary pool (1) stores carbohydrate for subsequent nocturnal metabolism (i.e. carboxylation and export) while a secondary pool (2) is responsible for daytime export. Pool 1 is the major sink for carbohydrate and only when pool 1 is filled does pool 2 receive carbon skeletons derived from the gluconeogenic recovery of decarboxylation products as well as from Phase IV photosynthesis. The carbohydrate status (X) of pool 2 determines the amount of sugar available for export during the day but may also feed forward to stimulate nocturnal carboxylation.](attachment:figure4.png)
would deliver comparable amounts of nocturnal reserves to maintain the potential for nocturnal carboxylation irrespective of changes in PPFD.

In conclusion, *Aechmea ‘Maya’* showed considerable seasonal plasticity in both the timing and duration of the employment of either C3 or C4 carboxylases to optimize CO2 sequestration during the diel cycle. However, there was a remarkable seasonal consistency in the amount of storage carbohydrates which was attributed to metabolic flexibility for adjusting the provision of carbohydrates for export over the day/night cycle. These observations have important practical consequences for horticultural productivity of *Aechmea* and potentially other CAM ornamentals such as *Phalaenopsis* and *Kalanchoe*. Extension of the natural photoperiod with low fluence rates maintained substantial photosynthesis in the C3 mode during the latter part of the day when PPFD was limiting in autumn and winter. In combination with an altered pattern of carbohydrate partitioning, the resulting photosynthetic plasticity assures an equal biomass gain with an altered pattern of carbohydrate partitioning, the result of changes in PPFD.

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**LITERATURE CITED**


