RESEARCH PAPER

Nocturnal versus diurnal CO₂ uptake: how flexible is *Agave angustifolia*?

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Abstract

Agaves exhibit the water-conserving crassulacean acid metabolism (CAM) photosynthetic pathway. Some species are potential biofuel feedstocks because they are highly productive in seasonally dry landscapes. In plants with CAM, high growth rates are often believed to be associated with a significant contribution of C₃ photosynthesis to total carbon gain when conditions are favourable. There has even been a report of a shift from CAM to C₃ in response to overwatering a species of *Agave*. We investigated whether C₃ photosynthesis can contribute substantially to carbon uptake and growth in young and mature *Agave angustifolia* collected from its natural habitat in Panama. In well-watered plants, CO₂ uptake in the dark contributed about 75% of daily carbon gain. This day/night pattern of CO₂ exchange was highly conserved under a range of environmental conditions and was insensitive to intensive watering. Elevated CO₂ (800 ppm) stimulated CO₂ fixation predominantly in the light. Exposure to CO₂-free air at night markedly enhanced CO₂ uptake during the following light period, but CO₂ exchange rapidly reverted to its standard pattern when CO₂ was supplied during the subsequent 24 h. Although *A. angustifolia* consistently engages in CAM as its principal photosynthetic pathway, its relatively limited photosynthetic plasticity does not preclude it from occupying a range of habitats, from relatively mesic tropical environments in Panama to drier habitats in Mexico.

Key words: *Agave*, biofuel, climate change, crassulacean acid metabolism, C₃ photosynthesis, CO₂ response, drought stress, temperature response.

Introduction

Most, if not all, of the approximately 200 species in the New World genus *Agave* (family Asparagaceae, subfamily Agavoideae; Chase et al., 2009; Govaerts et al., 2013) exhibit the nocturnal uptake of CO₂ and accumulation of malic acid characteristic of crassulacean acid metabolism (CAM) (Nobel, 2003, 1996a). In addition to a water-use-efficient carbon metabolism, these archetypal dry-land CAM plants sport a xerophytic, water-conserving morphology that includes succulent, angled, persistent leaves with thick cuticles, sunken stomata, and a rosette leaf configuration around a central stem that channels rain water and condensate to the base of the roots (Gentry, 1982).

As biomass becomes an increasingly valuable commodity for energy generation, there is realization that the seasonally dry or semi-arid landscapes inhabited by *Agave*, whilst not optimal for growing traditional water-demanding food crops, may nonetheless be suitable for biomass generation (Borland et al., 2009; Chambers and Holtum, 2010; Davis et al., 2011; Holtum et al., 2011; Yan et al., 2011). In such landscapes, water-use-efficient *Agave* can accumulate biomass at annual rates that approach those produced by C₄ plants like sugar cane and *Miscanthus* in higher rainfall regions (Nobel, 1991, 1996a; Somerville et al., 2010). For example, productivities...
of 25, 35, and 47–50 Mg dry weight ha\(^{-1}\) year\(^{-1}\) have been reported for the CAM species *Agave tequilana*, *Ananas comosus* (pineapple) and *Opuntia ficus-indica*, respectively (Nobel, 1996a). *A. tequilana* is now being trialed in the seasonally dry Australian tropics as a biofuel feedstock (Chambers and Holtum, 2010; Holtum et al., 2011).

The extent and capacity for day-time CO\(_2\) fixation in well-watered agaves tends to be limited, but its expression differs among species (see Nobel, 2003, for a review). Interestingly, well-watered plants of mature *Agave deserti* did not exhibit afternoon CO\(_2\) fixation, but when ‘overwatered’ (watered daily for 10 weeks) they switched to overwhelmingly C\(_3\) photosynthesis (Hartsock and Nobel, 1976).

Using continuous whole-plant gas exchange, we explored here the potential for photosynthetic plasticity in *Agave angustifolia*, a putative wild ancestor of the agronomically significant species *Agave fourcroydes* and *A. tequilana* (Gentry, 1982; Colunga-Garcia Marin et al., 1999). *A. angustifolia* was chosen because, across its range from Mexico to Panama, it is found in habitats as diverse as coastal dunes at sea level to oak-pine forests at 2200 m (Garcia-Mendoza and Chiang, 2003), and it grows in Panama (the site of this study) in relatively mesic environments where CO\(_2\) fixation in the light is expected to be more favoured than in drier habitats.

In order to determine photosynthetic pathway plasticity of *A. angustifolia*, we examined 24 plants. Our goal was to explore conditions under which plants would markedly upregulate C\(_3\) photosynthetic CO\(_2\) uptake in the light. In two young plants, net CO\(_2\) exchange was continuously monitored for 234 and 281 day/night cycles, during which the plants were exposed to a range of perturbations (light, temperature, CO\(_2\), and watering regime) that have been reported to affect CO\(_2\) uptake in the light in other CAM plants. Furthermore, net CO\(_2\) exchange was monitored in two extremely well-watered mature plants of mature *Agave* (Nobel, 2003, for a review). Interestingly, well-watered plants of mature *Agave deserti* did not exhibit afternoon CO\(_2\) fixation, but when ‘overwatered’ (watered daily for 10 weeks) they switched to overwhelmingly C\(_3\) photosynthesis (Hartsock and Nobel, 1976).

Measurement of whole-plant CO\(_2\) exchange under natural light

A mature plant, established in forest topsoil containing 50 g of Osmocote Plus fertilizer (Scotts-Sierra Horticultural Products), in a 1901 pot was placed inside a ventilated, naturally illuminated chamber constructed of glass panels and an aluminium framework (internal volume 8.8 m\(^3\)). A blower (model 4C054; Grainger Industrial Supply, OH, USA) supplied external air to the chamber at 10.5 m\(^3\) min\(^{-1}\). Within the chamber, air was circulated by four fans and temperature was regulated by a split air-conditioning system (model V1124C2H; Innovair, FL, USA). Whole-plant gas exchange was quantified at 30 min intervals from the rate at which the CO\(_2\) concentration inside the chamber changed when air flow into the chamber was blocked for 5 min, thereby converting the chamber into a closed system. Changes in the CO\(_2\) concentration inside the chamber were measured using a LI-7500 open-path CO\(_2\) analyser (Li-Cor, Lincoln, NE, USA). Calculations of net CO\(_2\) exchange were based on chamber volume that had been corrected for changes of temperature and humidity. Measurements of PFD were taken outside the chamber. PFD inside the chamber was approximately 15% below that outdoors. For further details of methods, see Winter et al. (2009). The experiment was repeated for a second mature plant (data not shown).

Materials and methods

**Plant material**

*A. angustifolia* Haw. was collected from Playa Majagual, Panamá (8°43′ N, 79°45′ E), and grown outdoors in forest topsoil at the Smithsonian Tropical Research Institute, Santa Cruz Experimental Research Facility, Gamboa, Republic of Panama (9°07′ N, 79°42′ W). Opinions differ as to whether *A. angustifolia* is a synonym of *Agave vivipara* L. or whether the two are distinct species (Wijnands, 1983; García-Mendoza and Chiang, 2003; Govaerts et al., 2013). Vouchers of the *Agave* examined in this study were deposited in the herbarium of the University of Panama (JARANDA 4484A and 4484B).

**Measurement of CO\(_2\) exchange in the laboratory**

Bulbs of between 5 and 10 cm in height, comprising two to three leaves, were enclosed in a Perspex cuvette (internal dimensions 11 × 11 × 10 or 20 × 20 × 15 cm) by passing the base of a plantlet through a hole in the cuvette base and sealing the plantlet–cuvette interface with a non-porous synthetic rubber sealant (T erosat VII; Henkel-Teronso, Heidelberg, Germany). The root-containing base of the plantlet outside the cuvette was planted in a 1 litre pot containing potting mix (Cactus, Palm and Citrus Soil; Miracle-Gro Lawn Products, Marysville, OH, USA) and 2 g of Osmocote Plus fertilizer (Scotts-Sierra Horticultural Products, OH, USA).

The gas-exchange cuvette was located inside a controlled-environment chamber (Environmental Growth Chambers, OH, USA) operating under 12 h light (28 °C)/12 h dark cycles (17 or 22 °C as specified). PFD was measured at the top of the cuvette. Air containing 200, 400, or 800 ppm CO\(_2\) was generated by a CO\(_2\)/CO\(_2\)–free-air mixing unit (Walz GmbH, Effeltrich, Germany). Net CO\(_2\) exchange of plantlets was measured in flow-through gas-exchange systems consisting of Walz components and LI-6252 CO\(_2\) analysers (Li-Cor, Lincoln, NE, USA) (Holtum and Winter 2003). Normal watering involved supplying water at least once every second day, and intensive watering involved supplying water twice per day. Drought treatments were imposed by withholding irrigation altogether.

**Growth of plants at 280 and 800 ppm CO\(_2\)**

Twenty plants in 19 l pots were grown with daily watering in forest topsoil for 166 d inside two naturally illuminated glasshouses (internal volume 37.5 m\(^3\) each). Five of the 10 pots in each chamber were supplemented with 5 g of Osmocote Plus fertilizer (Scotts-Sierra Horticultural Products). One glasshouse was maintained at 280 ± 10 ppm (range) CO\(_2\) by passing chamber air through soda lime to lower [CO\(_2\)]. An above-ambient CO\(_2\) concentration of 800 ± 10 (range) ppm was achieved in the second glasshouse by releasing pulses of CO\(_2\) gas into the chamber from a high-pressure cylinder in conjunction with a feedback control system. Within each glasshouse, air was circulated by five fans, and a split air-conditioning system maintained temperatures at close to ambient (Cernusak et al., 2011).
Leaf discs punched from the centre of fully expanded leaves using a cork borer at the end of the light and dark periods were frozen in liquid nitrogen. Organic acids were extracted by sequentially boiling samples in 50% ethanol and water for 5 min. Extracts were cooled to room temperature and titrated with 10 mM KOH to pH 6.5 (Holtum et al., 2004).

Stable isotope analysis

The δ¹³C values of finely ground homogenous powder from the pooled dried leaves of whole plants were measured in an isotope ratio mass spectrometer (Delta V; Thermo Fisher Scientific) in the Stable Isotope Laboratory of the Smithsonian Tropical Research Institute. The abundance of ¹³C in each sample was calculated relative to the abundance of ¹³C in standard CO₂ that had been calibrated against Pee Dee belemnite (*Belemnitella americana*). Relative abundance was determined using the relationship:

\[ \delta^{13}C (\text{‰}) = \left( \frac{^{13}C/^{12}C \text{ of sample}}{^{13}C/^{12}C \text{ of standard}} \right) - 1 \times 1000. \]

The δ¹³C values of two C₄ plant species, *Saccharum spontaneum* and *Portulaca oleracea*, grown in each glasshouse and outside in the open air were used to correct for differences in the δ¹³C value of the source CO₂ (Cernusak et al., 2011). The CO₂ purchased for CO₂ enrichment was from a natural CO₂ spring and a correction of 2‰ was applied.

Results

CO₂ fixation in the dark (CAM phase I; Osmond, 1978) contributed 78% of the carbon gain in a young well-watered *A. angustifolia* for which CO₂ uptake was monitored continuously during 281 consecutive 12 h light/12 h dark cycles (Fig. 1). The predominance of dark fixation was maintained under 12 h PFDs of 400 μmol m⁻² s⁻¹ (17.3 mol m⁻² d⁻¹), 1500 μmol m⁻² s⁻¹ (64.8 mol m⁻² d⁻¹), and 2300 μmol m⁻² s⁻¹ (99.4 mol m⁻² d⁻¹).

Uptake of CO₂ in the dark remained the principal contributor to net carbon gain during 234 day/night cycles of gas exchange when a young *A. angustifolia* was subjected to 300 or 900 μmol m⁻² s⁻¹ light; 200, 400 or 800 ppm CO₂; when well-watered, overwatered, or non-watered; and when night temperatures were 17 or 22 °C (Fig. 2). CO₂ uptake in the afternoon (CAM phase IV) was also always present, whereas the contribution of dawn CO₂ fixation (CAM phase II) to plant carbon gain was minimal. Net CO₂ loss was consistently observed during midday stomatal closure (CAM phase III) except for small CO₂ gains on those days when CO₂ was withheld during the dark while the plant was exposed to 800 ppm CO₂. Over the course of the experiment (Fig. 2), the contributions to net carbon gain by phases I, II, III, and IV were 78.5, 1.7, –4.2, and 24%, respectively.

The contributions of light and dark CO₂ uptake to net carbon gain under 200 or 800 ppm CO₂ differed (Fig. 2). When the CO₂ concentration was reduced from 400 to 200 ppm, CO₂ uptake at a PFD of 300 μmol m⁻² s⁻¹ was reduced by 62% during phase IV and by 16% in the dark. The reductions were 56 and 25%, respectively, at 900 μmol m⁻² s⁻¹. In contrast, when the atmospheric CO₂ concentration was increased from 400 to 800 ppm, CO₂ uptake by the plant at 300 μmol m⁻² s⁻¹ increased by...
64% during phase IV and remained unchanged during phase I. At a PFD of 900 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), the increases in CO2 uptake during phases IV and I were 73 and 10%, respectively.

Withholding water from the plant for 54 d also differentially influenced CO2 fixation in the light and in the dark (Fig. 2). Overall net carbon gain by the plant was initially stimulated but was subsequently reduced as water in the pot became limiting. During the initial 34 d without watering, as CO2 uptake increased by 46% in the dark and decreased by 17% during phase IV in the light, the contribution of nocturnal uptake to net carbon gain rose from 74 to 85%. Subsequently, CO2 exchange fell during the light and the dark. The decrease was proportionally less in the dark such that, at the end of the drought treatment, dark fixation contributed 88% to net carbon gain. In contrast to drought, intensive watering of the plant for 30 d did not affect the relative contributions of light and dark CO2 fixation to carbon gain, nor did decreasing the night temperature from 22 to 17 °C.

CO2 assimilation in the light was upregulated when CO2 was removed from the air supply during the preceding dark period (Fig. 2, d 141 and 142). Fig. 3 details how both the extent and pattern of CO2 exchange in the light differed following exposure to CO2-free air during the night. CO2 was assimilated throughout the light principally via the contribution of an extended phase IV (Fig. 3B). Phase III was transient, and phase II did not increase in duration although the rate of CO2 uptake did increase. During the subsequent dark/light cycle, CO2 exchange returned to the patterns observed prior to the CO2-free treatment (Fig. 3C).

Nocturnal CO2 uptake was the principal contributor to carbon gain in fully mature, well-watered A. angustifolia grown outdoors under natural sunlight (Fig. 4). Day-time CO2 uptake occurred mainly during the late afternoon (phase IV). The contribution of phase II CO2 uptake was variable but generally small, whereas net CO2 loss was consistently observed during phase III midday stomatal closure.

Nocturnal CO2 uptake decreased substantially following overcast days (Fig. 4). For example, the extremely overcast d 4 light period was followed by a night during which there was no net CO2 uptake. Less extreme examples of this trend were evident on d 2, 8, and 12. On days following overcast days, CO2 uptake during the afternoon tended to be more pronounced.

Fig. 5 quantifies the relationships between day-time and night-time CO2 exchange and daily PFD for the mature A. angustifolia illustrated in Fig. 4. Nocturnal CO2 uptake was correlated with the integrated PFD during the preceding light period and contributed predominately to net CO2
uptake at all light intensities that supported positive daily carbon gain. Following sunny days, when the integrated PFD exceeded about 30 mol m\(^{-2}\) d\(^{-1}\), CO\(_2\) uptake at night was saturated, providing 70–85% of the daily carbon gain. Below 30 mol m\(^{-2}\) d\(^{-1}\), total carbon gain fell and the proportional contribution of nocturnal CO\(_2\) uptake to 24h carbon gain rose. Day-time CO\(_2\) exchange became negative at around 21 mol m\(^{-2}\) d\(^{-1}\), whereas night-time CO\(_2\) exchange became negative at about 10 mol m\(^{-2}\) d\(^{-1}\).

The biomass of *A. angustifolia* grown in unfertilized soil at 800 ppm CO\(_2\) was double that of plants grown at 280 ppm CO\(_2\), whereas leaf acidities were similar on both mass and leaf-area bases (Table 1). Fertilization increased the biomass 2.5-fold in plants grown at 280 ppm CO\(_2\) and 1.7-fold in plants grown at 800 ppm CO\(_2\). For both unfertilized and fertilized treatments, the \(\delta^{13}C\) values for plants at 800 ppm CO\(_2\) were 1.8‰ more negative than for plants grown at 280 ppm CO\(_2\). In comparison to unfertilized plants, fertilized
plants exhibited greater nocturnal accumulation of H⁺ per unit leaf area, but H⁺ accumulation per unit leaf mass was unchanged.

Discussion

Despite an ability to occupy contrasting habitats, photosynthetic flexibility in *A. angustifolia* does not appear to be exceptional in terms of the proportional contributions to carbon gain of CO₂ uptake in the dark and light. Nocturnal CO₂ uptake was the principal source of carbon in mature *A. angustifolia* and in young plants once they had established. The proportional contribution to daily carbon gain of nocturnal CO₂ uptake remained a remarkably consistent 70–85%, although the amount of CO₂ fixed per day by well-watered plants varied with light intensity and nutrient status. In contrast to *A. deserti* (Hartsock and Nobel, 1976), the day/night pattern of CO₂ exchange in *A. angustifolia* did not shift towards a C₃ pattern when the supply of water was effectively unlimited.

Drought affected plant carbon gain and increased the proportional contribution of nocturnal CO₂ uptake to it (Fig. 2). Two weeks after the cessation of irrigation, drought stress manifested itself as a continuous decline in light CO₂ fixation. Most importantly, the initial 25 d of the decline of CO₂ uptake in the light was accompanied by an increase in the rate of dark CO₂ fixation. This drought-induced upregulation of CAM is a typical feature of facultative CAM. Facultative CAM or facultative components of CAM are not restricted to metabolically flexible annuals such as *Mesembryanthemum crystallinum* (Winter and von Willert, 1972) and *Calandrinia polyandra* (Winter and Holtum, 2011), and perennials such as some species of *Clusia* (Winter et al., 2009), but have also been observed in juveniles of constitutive CAM succulents such as *O. ficus-indica* and *Opuntia elatior* (Winter et al., 2008, 2011).

An attempt to force *A. angustifolia* into a C₃-like photosynthetic pattern by exposing it to 800 ppm CO₂ was partially successful in that daily carbon gain was enhanced and the proportional contribution of CO₂ uptake in the light rose, for example from 29 to 40% during an 11 d treatment (Fig. 2). The ability to maintain this pattern of CO₂ exchange was confirmed following a 166 d exposure to 800 ppm CO₂ after which tissue δ¹³C values were close to those predicted by Winter and Holtum (2002) for a 40% contribution to carbon gain of CO₂ fixation in the light.

In the short-term 800 ppm CO₂ fumigation treatments, CO₂ uptake in the dark was not or was only slightly enhanced (Fig. 2); in the longer-term experiment, there was no enhancement (Table 1), as nocturnal acidification remained unchanged. The contribution of nocturnal CO₂ fixation to carbon gain in CAM tissues is variably responsive to environmental stimuli,
which include night temperature, day-length, light intensity, and atmospheric CO₂ concentration (Neales, 1973; Drennan and Nobel, 2000; Borland et al., 2011). Increased CO₂ assimilation in the light but not the dark has been reported in Ananas grown at 700 ppm CO₂ under a 30/20 °C day/night regime that was optimal for nocturnal CO₂ uptake at ambient CO₂ (Zhu et al., 1999). However, when Ananas was grown at higher night temperatures that were less than optimal for dark CO₂ uptake at ambient CO₂, the growth of plants at 700 ppm CO₂ increased CO₂ uptake in both the light and the dark. Increases in both light and dark CO₂ fixation following exposure to high concentrations of atmospheric CO₂ have been reported for A. deserti (Graham and Nobel, 1996) and Agave salmiana (Nobel, 1996b; Nobel et al., 1996), and for the stem succulents, O. ficus-indica (Nobel and Israel, 1994) and Stenocereus queretaroensis (Nobel, 1996b).

A. angustifolia could be shifted towards a C₃-like light-only CO₂ uptake pattern by an extreme treatment that required withholding CO₂ during the dark and supplying 800 ppm CO₂ in the light (Fig. 3). In the light, the duration of phase IV increased at the expense of phase III, presumably because small amounts of acid formed at night would be rapidly consumed and the inhibition of stomatal opening by the resulting high internal CO₂ concentration would be transitory. In the 24 h cycle during which CO₂-free air was supplied at night, daily carbon gain fell because the increase in CO₂ gain in the light did not offset the lack of uptake of atmospheric CO₂ during the night.

The stimulation of light fixation following exposure to CO₂-free air at night lasted only during the light period following the treatment. Remarkably, no evidence of a metabolic memory of the CO₂-free treatment was evident in the subsequent night and the day that followed it.

It has been suggested that the feasibility of cultivating strong-CAM plants such as Agave or Opuntia for biofuel feedstock in seasonally dry environments could be improved by developing plants that would fix a greater proportion of CO₂ in the light during the moister parts of the year (Borland et al., 2011). In effect, the proposal is to push the proportional contribution of CO₂ fixation in the light into the vicinity of 50–60%, a proportion that large surveys of CAM plants have revealed as being uncommon in the natural environment (Winter and Holtum, 2002; Crayn et al., 2004; Silvera et al., 2010).

As in A. angustifolia, 24 h CO₂ exchange by the most highly productive CAM species grown in commercial plantations in warm and temperate subtropical dry-land environments, A. tequilana, O. ficus-indica, A. salmiana and Agave mapisaga, is dominated year round by nocturnal CO₂ fixation (Nobel, 1996b; Nobel et al., 1996).

### Table 1. Dry mass, nocturnal increase in leaf tissue acidity, and δ¹³C value (whole shoot) of A. angustifolia grown at 280 and 800 ppm CO₂

| Parameter                          | CO₂ concentration | CO₂ concentration |
|-----------------------------------|-------------------|-------------------|
|                                   | 280 ppm           | 800 ppm           |
|                                   | – fertilizer      | + fertilizer      | – fertilizer | + fertilizer |
| Total dry mass (g)                | 26 ± 4            | 65 ± 12           | 51 ± 5       | 88 ± 11      |
| Nocturnal H⁺ increase:            |                   |                   |             |             |
| µmol g⁻¹ fresh weight            | 226 ± 40          | 210 ± 14          | 184 ± 8     | 207 ± 31   |
| µmol cm⁻²                        | 79 ± 12           | 96 ± 4            | 77 ± 6     | 93 ± 11    |
| δ¹³C (‰)                         | –14.6 ± 0.2       | –14.0 ± 0.2       | –16.4 ± 0.2| –15.8 ± 0.2|

Values are means ± standard deviation (n=5), except for δ¹³C value at 280 ppm + fertilizer, where n=3. Means on the same row with different superscript letters differ significantly (two-way analysis of variance followed by Fisher’s least significant difference test, P<0.05).
1996a; Nobel et al., 1992; Pimienta-Barrios et al., 2001, 2006). In *A. tequilana*, environmental factors that limited growth and productivity in the field were water during the cool dry winter and PAR during the warm wet summer (Nobel and Valenzuela, 1987), not CO₂ fixation in the light. Under these field limitations, a shift towards an increase in the capacity for CO₂ fixation in the light is unlikely to result in significantly increased productivity. Rather, productivity might be expected to be higher if plants were grown at sites that were less cloudy in the summer and more moist in the winter.

Proposals to grow *Agave* as biofuel feedstocks in seasonally dry regions have emphasized their suitability for so-called marginal lands (Borland et al., 2009; Somerville et al., 2010; Davis et al., 2011). Land that is marginal for growing traditional crops may well support high growth rates of water-use efficient, high-temperature-tolerant CAM species, but it remains to be seen whether *Agave* can produce commercially relevant yields on truly marginal lands that are nutrient poor and severely water limited.

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References

Borland AM, Griffiths H, Hartwell J, Smith JAC. 2009. Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands. *Journal of Experimental Botany* 60, 2879–2896.

Borland AM, Zambrano VAB, Ceusters J, Shorrock K. 2011. The global potential for exploitation of plants with crassulacean acid metabolism for bioenergy production. *New Phytologist* 191, 619–633.

Cernusak LA, Winter K, Martínez C, Correa E, Aranda J, García M, Jaramillo C, Turner BL. 2011. Responses of legume versus nonlegume tropical tree seedlings to elevated CO₂ concentration. *Plant Physiology* 157, 372–385.

Chambers D, Hultom JAM. 2010. Feasibility of Agave as a feedstock for biofuel production in Australia. Canberra: Rural Industries Research and Development Corporation.

Chase MW, Reveal JL, Fay MF. 2009. A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society* 161, 132–136.

Colunga-GarcíaMarín P, Coléo-Coléo J, Equiarte LE, Piñero D. 1999. Isozymatic variation and phylogenetic relationships between henequén (*Agave fourcroydes*) and its wild ancestor *A. angustifolia* (*Agavaceae*). *American Journal of Botany* 86, 115–123.

Gray DM, Winter K, Smith JAC. 2004. Multiple origins of crassulacean acid metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. *Proceedings of the National Academy of Sciences*, USA 101, 3703–3708.

Davis SC, Dohleman FG, Long SP. 2011. The global potential for *Agave* as a biofuel feedstock. *GCB Bioenergy* 3, 68–78.

Drennan PM, Nobel PS. 2000. Responses of CAM species to increasing atmospheric CO₂ concentrations. *Plant, Cell & Environment* 23, 767–781.

García-Mendoza A, Chiang F. 2003. The confusion of *Agave viivpara* L. and *A. angustifolia* Haw., two distinct taxa. *Brittonia* 55, 82–87.

Gentry HS. 1982. *Agaves of continental North America*. Tucson: University of Arizona Press.

Govaerts R, Zonneveld BJM, Zona SF. 2013. *World Checklist of Asparagaceae*. Facilitated by the Royal Botanic Gardens, Kew. http://apps.kew.org/wcsp/. Accessed 17 January 2013.

Hartsock TL, Nobel PS. 1976. Watering converts a CAM plant to daytime CO₂ uptake. *Nature* 262, 574–576.

Holtum JAM, Aranda J, Virgo A, Gehrig HH, Winter K. 2004. δ¹³C values and crassulacean acid metabolism in *Clusia* species from Panama. *Trees* 18, 658–668.

Holtum JAM, Chambers D, Morgan T, Tan DKY. 2011. *Agave* as a biofuel feedstock in Australia. *GCB Bioenergy* 3, 58–67.

Holtum JAM, Winter K. 2003. Photosynthetic CO₂ uptake in seedlings of two tropical tree species exposed to oscillating elevated concentrations of CO₂. *Planta* 218, 152–158.

Neales TF. 1973. The effect of night temperature on CO₂ assimilation, transpiration, and water use efficiency in *Agave americana* L. *Australian Journal of Biological Sciences* 26, 705–714.

Nobel PS. 1991. Achievable productivities of certain CAM plants: basis for high values compared with C₃ and C₄ plants. *New Phytologist* 119, 183–205.

Nobel PS. 1996a. High productivity of certain agronomic CAM species. In: Winter K, Smith JAC, eds. *Crassulacean acid metabolism: biochemistry, ecophysiology and evolution*. Berlin: Springer-Verlag, 255–265.

Nobel PS. 1996b. Responses of some North American CAM plants to freezing temperatures and doubled CO₂ concentrations: implications of global climate change for extending cultivation. *Journal of Arid Environments* 34, 187–196.

Nobel PS. 2003. *Environmental biology of agaves and cacti*. Cambridge: Cambridge University Press.

Nobel PS, García-Moya E, Quero E. 1992. High annual productivity of certain agaves and cacti under cultivation. *Plant, Cell & Environment* 15, 329–335.

Nobel PS, Israel AA, Wang N. 1996. Growth, CO₂ uptake, and responses of the carboxylating enzymes to inorganic carbon in two highly productive CAM species at current and doubled CO₂ concentrations. *Plant, Cell & Environment* 19, 585–592.

Nobel PS, Israel AA. 1994. Cladode development, environmental responses of certain CO₂ uptake, and productivity for *Opuntia ficus-indica* under elevated CO₂. *Journal of Experimental Botany* 45, 295–303.

Nobel PS, Valenzuela AG. 1987. Environmental responses and productivity of the CAM plant, *Agave tequilana*. *Agricultural and Forest Meteorology* 39, 319–334.

Osmond CB. 1978. Crassulacean acid metabolism: a curiosity in context. *Annual Review of Plant Physiology* 29, 379–414.

Pimienta-Barrios E, Zaño-ouden-Hernández J, García-Galindo J. 2006. Seasonal photosynthesis in young plants of *Agave tequilana*. *Agrociencia* 40, 699–709.

Pimienta-Barrios E, Robles-Murguía C, Nobel PS. 2001. Net CO₂ uptake for *Agave tequilana* in a warm and a temperate environment. *Biotropica* 33, 312–318.

Silvera K, Santiago LS, Cushman JC, Winter K. 2010. The incidence of crassulacean acid metabolism in Orchidaceae derived from carbon isotope ratios: a checklist of the flora of Panama and Costa Rica. *Botanical Journal of the Linnean Society* 163, 194–222.

Somerville C, Youngs H, Taylor C, Davis SC, Long SP. 2010. Feedstocks for lignocellulosic biofuels. *Science* 329, 790–792.

Wijnands DO. 1983. *The botany of the Cremelins*. Rotterdam: Balkema.

Winter K, García M, Holtum JAM. 2008. On the nature of facultative and constitutive CAM: environmental and developmental control of CAM expression during early growth of *Clusia*, *Kalanche*, and *Opuntia*. *Journal of Experimental Botany* 59, 1829–1840.

Winter K, García M, Holtum JAM. 2009. Canopy CO₂ exchange of two neotropical tree species exhibiting constitutive and facultative CAM photosynthesis, *Clusia rosea* and *Clusia clyndrica*. *Journal of Experimental Botany* 60, 3167–3177.
Winter K, Garcia M, Holtum JAM. 2011. Drought-stress-induced up-regulation of CAM in seedlings of a tropical cactus, Opuntia elatior, operating predominantly in the C3 mode. Journal of Experimental Botany 62, 4037–4042.

Winter K, Holtum JAM. 2002. How closely do the δ13C values of crassulacean acid metabolism plants reflect the proportion of CO2 fixed during day and night? Plant Physiology 129, 1843–1851.

Winter K, Holtum JAM. 2011. Induction and reversal of crassulacean acid metabolism in Calandrinia polyandra: effects of soil moisture and nutrients. Functional Plant Biology 38, 576–582.

Winter K, von Willert DJ. 1972. NaCl-induzierter Crassulaceensäurestoffwechsel bei Mesembryanthemum crystallinum. Zeitschrift für Pflanzenphysiologie 67, 166–170.

Yan X, Tan DKY, Inderwildi OR, Smith JAC, King DA. 2011. Life cycle energy and greenhouse gas analysis for Agave-derived bioethanol. Energy and Environmental Science 4, 3110–3121.

Zhu J, Goldstein G, Bartholomew DP. 1999. Gas exchange and carbon isotope composition of Ananas comosus in response to elevated CO2 concentration and temperature. Plant, Cell & Environment 22, 999–1007.