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Feasibility of Agave as a Feedstock for Biofuel Production in Australia

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Feasibility of Agave as a Feedstock for Biofuel Production in Australia

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Foreword

In the 21st century, water efficiency and diversification of income streams are emerging as key drivers in Australian agriculture.

This report examines the feasibility of growing an extremely water-use efficient desert plant, *Agave tequilana*, in conjunction with sugar-cane or sorghum, to provide feedstock for the production of ethanol. For reasons associated with climate change and a projected increased demand for imported oil, Australian states have mandated ethanol-petrol blends that will require about 748 million litres ethanol *per annum* by 2011. Australian production is currently only 231 million litres.

Agave tequilana is a species that has been used to produce alcohol (ethanol) for two centuries. Varieties have already been generated, cropping systems trialled and fermentation technologies developed. Although the crop is new to Australia, trials can be undertaken immediately in order to fine-tune the crop for production under Australian environmental and market conditions. Most other new potential ethanol-generating crops are still 5 to 10 years away from testing in the field.

It is planned to grow *A. tequilana* as a rain-fed crop. The plant has important attributes including an ability to store sugars throughout the year, a characteristic that may enable it to be processed in sugar-processing plants to prolong crushing periods; low-lignin cellulose fibres in the leaves that should be conducive to Generation 2 ethanol production; and the production of oligofructan carbohydrates that have widespread use in the food, pharmaceutical and nutraceutical industries by virtue of their texture, solubility, sweetness, low digestibility (low glycemic index) and their ability to enhance the growth of beneficial *Bifidobacteria*.

This report demonstrates the feasibility of growing *A. tequilana* in Australia and pinpoints uncertainties in growing a crop that is new to Australian farmers and processors. Both agronomic and financial predictions need to be tested in well-planned field trials.

The research for this report has been supported by Ausagave, James Cook University and RIRDC.

This report is an addition to RIRDC's diverse range of over 2000 research publications. It is part of our Bioenergy, Bioproducts and Energy R&D program which aims to meet Australia's research and development needs for the development of sustainable and profitable bioenergy and bioproducts industries and to develop an energy cross-sectoral R&D plan.

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Craig Burns
Managing Director
Rural Industries Research and Development Corporation

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Executive Summary

What the report is about

The report is an exploration of the biology of *Agave tequilana* and the economic feasibility of growing it as a new crop plant in Australia.

The report introduces the crop to those unfamiliar with it. It examines the demand in Australia for the principal end-use of the crop, ethanol, and the capacity of the crop to provide the necessary feedstock.

Topics explored include a description of the species and the history of the use of *Agave* by *Homo sapiens*, the physiology and ecophysiology of the plant, the chemistry of the carbohydrates it produces and stores, how *Agave* is cultivated in Mexico, where and how it might be be grown in Australia, and what the costs, returns and gross margins of *Agave* production might be. Potential other uses of *Agave* products are also described.

Who is the report targeted at?

As a blueprint for a crop, the report targets those who may produce, provide extension, process, market, finance, research or regulate *A. tequilana* and its products. The collation and assessment of extensive disparate literature provides to those unfamiliar with *Agave* rapid access to the biological, agronomic and production information from Mexico and elsewhere.

Background

Demand is increasing for alternative sources of energy that are secure and produce less greenhouse gas and generate fewer pollutants than fossil fuels. One such energy source is ethanol. Current state legislation mandating the blending of ethanol in petrol will require about 748 million litres of ethanol *per annum* by 2011. Australian production is currently only 231 million litres. Clearly a demand for ethanol-producing feedstock exists.

Of the new crops or cellulosic processes proposed for ethanol production, *A. tequilana* is the only crop that is ready to go into the ground now – cultivars, agronomic systems and fermentation technologies have been developed during two hundred years of cultivation for tequila production.

Aims/objectives

The ultimate objective of this study is to assess the feasibility of growing *Agave tequilana* Weber in Australia as a feedstock for the sustainable production of ethanol. The report introduces *A. tequilana*, detailing why the crop may be of interest in Australia. Information on the biological and agronomic attributes of the crop is collated and potential sites of cultivation identified. An agronomic system is then proposed and costed.

Methods used

In the absence of any cultivation of *A. tequilana* in Australia, agronomic information has been gleaned from Mexican and internationally peer-reviewed literature, discussions with agronomists, industry representatives, researchers in Mexico and the International Society of Crassulacean Acid Metabolism. We have consulted widely for technological, production and financial advice. In Queensland, major biofuels processor and most sugar processors have been consulted as have research organizations such CSIRO, QDPI, BSES, industry bodies such as the Biofuels Association of Australia, and pre-eminent biofuels industry consultants such as BioIndustry Partners Pty Ltd.

Results/key findings

- Comparisons of climate and soils in Mexico and Queensland suggest that *A. tequilana* will grow in certain areas of Queensland at rates that should be commercially viable.

- *A. tequilana* has potential to be grown as a low-input rain-fed crop.

- A number of the potential cultivation sites are also regions where sugar-cane or sorghum is grown and where infrustructure that can process *Agave* is already present.

- By virtue of storing sugars over long periods, *Agave* production may enable sugar mills to extend crushing seasons.

- The predicted gross margins of growing *Agave* should be equivalent at least to those of growing sugar-cane.

- *Agave* low-lignin leaf fibre is a candidate for Generation 2 sugar production from cellulose.

- *A. tequilana* is unlikely to be aggressively weedy.

- Processors and growers have expressed support for *Agave*, but will not invest in the crop in the absence of product from field trials in Australia.

- Potential exists for provision of *Agave* fructans to the food and health industries.

Recommendations

The following recommendations are designed to strengthen the biological and technological bases of growing *Agave:*

- The predicted feasibility of *A. tequilana* as a crop needs to be demonstrated in the field. Trials should be undertaken at a range of sites with differing rainfall and night temperatures.

- Australian-grown product needs to be assessed by processors and the food industry.

- The development of prototypes of mechanized pruners and harvesters needs to be supported.

- A research programme is required to inform extension and vice-versa. The rates of *Agave* growth and carbohydrate production, and the responses to light, water-logging and pests and diseases need to be quantified throughout the life-cycle of plants grown under Australian conditions.

- Market research is required to explore the magnitude and nature of the demand for *Agave* carbohydrates in the food and health industries.

- Information transfer between Mexican and Australian agronomists, processors and researchers needs to be fostered and fast-tracked.

- If *Agave* is successfully integrated into Australian agriculture, a biofuels-oriented plant breeding program will be required. This would best be undertaken in collaboration with Mexican researchers and should include investigation of other *Agave* species.

- The potential of leaf fibre cellulose for ethanol generation should be tested using current and emerging Generation 2 technologies.

1 Introduction

The following report initially addresses the state of the international and Australian ethanol industry, documenting current use and production of ethanol in Australia, assessing future demand and identifying production shortfalls (Chapter 3). It is proposed that *A. tequilana* may be a new crop that can address part of the ethanol shortfall. The species, its history of use and its performance in natural and cultivated habitats in Mexico is then detailed (Chapter 4).

A summary of its physiological characteristics and ecophysiological responses is followed by descriptions of the chemistry of carbohydrates it forms and how similar plants respond to growth at elevated concentrations of CO_2 (Chapter 5).

Potential growing and production areas in Australia are identified on the basis of climatic and edaphic similarities with Mexico and a cultivation system are proposed (Chapter 6). Following a listing of alternate potential uses of *Agave* carbohydrates, a financial case for the production of *A. tequilana* in Australia is compiled on the basis of estimated costs, returns and gross margins (Chapter 7).

The report finishes with an analysis of the potential of *Agave* to be weedy in Australia (Chapter 8), a list of recommendations of actions that would promote the success of *Agave* as a biofuels crop (Chapter 9), and supporting information in the form of appendices (Chapter 10) and references (Chapter 11).

2 Objectives

The ultimate objective of this study is to assess the feasibility of growing *Agave*, in particular *Agave tequilana* Weber in Australia as a feedstock for the sustainable production of biofuels, particularly ethanol.

The report aims to:

- Document current and anticipated production and demand for ethanol in Australia.
- Collate and assess agronomic and production information for *A. tequilana* grown in Mexico, focussing upon the responses of the species to environmental and edaphic variables.
- Describe the biology and physiology of *A. tequilana* and the nature of carbohydrates that it produces.
- Identify climates and soils in Australia that are similar to those in which *A. tequilana* grows in Mexico, and establish whether there are economic opportunities for cultivating *A. tequilana* in these Australian regions. As *A.tequilana* is effectively unknown as a plant and untried as a crop in Australia, the assessment of potential yields and production economics will necessarily be extrapolated from overseas performance.
- Assess potential pest, disease and weed issues.
- Identify potential production issues in Australia.
- Formulate and assess a business case for developing *A. tequilana* as a biofuel feedstock in Australia.
- Examine the potential of *A. tequilana* as a bio-refinery crop (a crop in which all parts of the biomass are utilised thus adding value to feedstock produced for bioenergy).

3 Ethanol: an emerging commodity

3.1 Ethanol as a biofuel

Rising energy demand and oil prices, coupled with acknowledgement that climate is changing and greenhouse gas emissions must be reduced, have stimulated interest internationally in alternative sources of energy that are secure and that produce less greenhouse gas and generate fewer pollutants than fossil fuels. As such, the biofuels bioethanol and biodiesel are liquid-carbon energy sources that are increasing in global economic importance (Figure 3.1). Bioethanol (hereafter referred to as ethanol) is currently produced principally by fermentation of starch or sugars from photosynthetic organisms whereas biodiesel is the product of trans-esterification of fats and oils sourced from photosynthetic or non-photosynthetic organisms. Biofuels constitute around 2.8 % of the global transport fuel supply (International Energy Agency 2008).

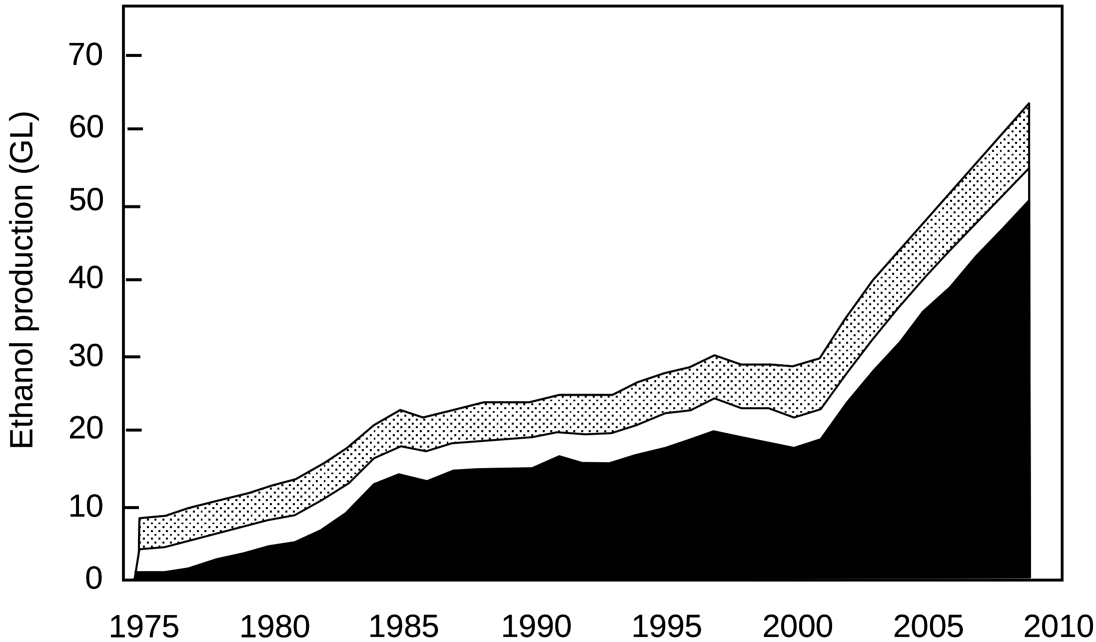

Figure 3.1 Global ethanol production – 1975 to 2009. (Japanese Ministry of Trade and Industry 2009).

Black = fuel, white = beverages, stipple = industrial

Ethanol can be used by current automobile technologies. Ethanol burns with around 30 % less energy than petrol thus a 10 % ethanol-to-petrol mix (v/v, E10) delivers about 97% of the power of standard unleaded petrol. By adding oxygen to the fuel combustion process ethanol produces a cleaner, cooler, faster burn.

In 2008, the USA and Brazil generated 89% of the 49.6 GL (giga litre = billion litres = 10^9 litres) ethanol produced worldwide. The rapid rise in ethanol production in the USA (Figure 3.2), which surpassed Brazil as the largest producer in 2005, has been primed by government policy, in particular the US Energy Independence and Security Act of 2007 that requires American fuel producers to use at least 136 GL of biofuel in 2022. The 2009 Renewable Fuel Standard will reportedly require most refiners, importers and non-oxygenate blenders of gasoline to displace 10.21% of their gasoline with renewable fuels such as ethanol (EPA 2009). Currently, US ethanol production is dependent upon feedstock derived from maize, an energy intensive crop that results in US maize-derived ethanol costing more than twice that of Brazil's cane-based ethanol (Berg & Licht 2004). It is therefore not surprising that the US is a large importer of ethanol, importing about 2.8 GL ethanol in 2008, of which about 0.7 GL was from Brazil. The USA, Japan and the EU are the largest importers of ethanol and Brazil the largest exporter. The USA is also an important exporter of ethanol, mainly to Canada and Mexico and other North American Free Trade Association countries.

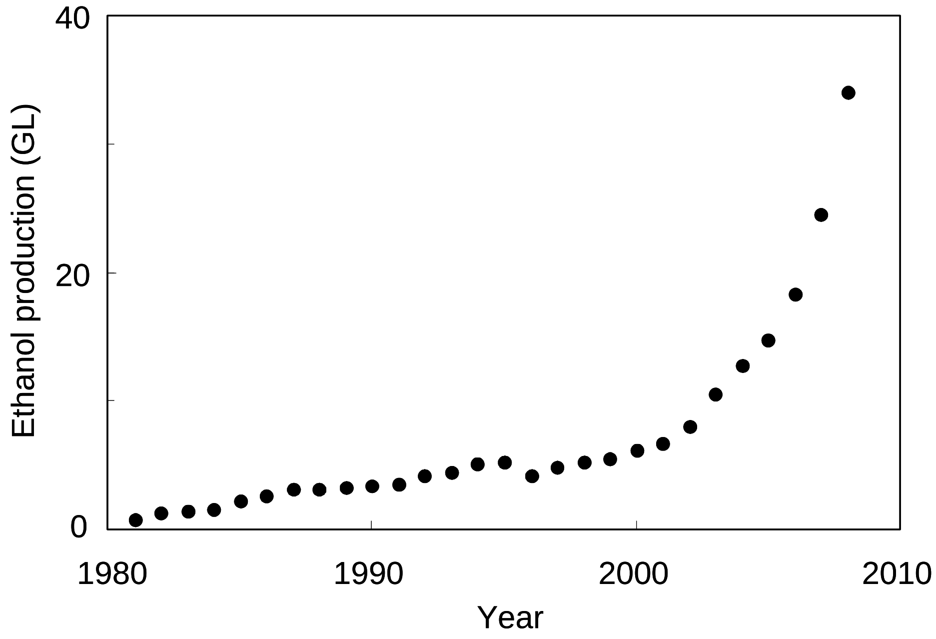

Figure 3.2 US ethanol production - 1980 to 2008 (Renewable Fuels Association 2008).

In 2008, Brazil produced 24.5 GL or 37 % of global ethanol used as fuel (Byerlee et al 2008). Ethanol exports were 5.16 GL, an amount about twice that of petrol exports. The Brazilian industry is based upon an efficient feedstock, sugarcane, and high national demand for ethanol as a fuel. In 2007, the year that an E25 blend (petrol that is 25 % anhydrous ethanol by volume) was legislated as mandatory, ethanol constituted 17 % of the automotive sector energy consumption. In 2008, flexible-fuel vehicles that can run on any proportion of gasoline and anhydrous ethanol constituted 23 % of Brazil's light motor vehicle fleet (DENATRAN 2008). It is expected that the mandated use of ethanol in fuel will spur further development of engines that burn higher concentrations of ethanol.

In 2001, the then government of Australia (GOA) published a 'Biofuels for Cleaner Transport' document that proposed a modest production of 350 ML (mega litre = million litres = 10^6 litres) of biofuels per year by 2010 (for comparison: in 2008, petrol and diesel constituted about 70 % of the liquid fuels sold in Australia - petrol sales were 19.3 GL and diesel sales 17.0 GL, ABARE 2008). Although not mandated in legislative form and equivalent to only about 1 % of transport fuel usage, the 350 ML biofuel value has formed the target, and provided a basis, for subsequent research and industry development (CSIRO, BTRE, ABARE 2003, Australian Government 2005; ABARE 2008).

3.2 Demand for Ethanol in Australia

It has been predicted that in 2009/10 Australian ethanol production capacity will increase while biodiesel is likely to remain unchanged, bringing total biofuel production to around 365 ML (ABARE 2008; Darby 2009), a level exceeding the GOA target of 350 ML. Demand for ethanol reflects an integration of the purchasing patterns of feedstock producers, intermediate producers such as oil companies, service stations, farming co-operatives etc who process, blend and distribute fuels for eventual sale to customers, and the demand by consumers, both national and overseas.

At present, the ethanol excise tax in Australia is 38.143 cents per litre (cL-1). Biofuel producers are eligible for grants that offset the fuel excise tax for the biofuel component, and for grants that support expenditure on capital, distribution and R&D such that the effective excise rates (excise rate minus offsetting grants) are 0 cL-1 for domestic ethanol, and 38 cL-1 for imported ethanol.

Table 3.1 Current and projected use of ethanol in fuel on the basis of legislated or targeted ethanol-in-fuel mandates by Australian states (Government of Australia 2009).

. Levels calculated on the basis of petrol consumption in April 2009

State	Amount of ethanol in petrol		Mandated or targeted ethanol levels and date
	Current	Projected	
	ML	ML	
NSW	94	228	4 % by Jan 2011
VIC	10	219	5 % by 2010
QLD	74	132	5% of regular petrol by 2011
SA	0	0	
WA	0	95	5% by 2010
TAS	0	0	
NT	0	0	
Australia	178	674	
National production	231	350*	

* Previous GOA biofuels 'target' for 2010 – i.e. neither legislated nor policy *per se*

Excise on ethanol will gradually change between 1 July 2011 and July 2015 when the domestic and imported ethanol effective excise tax is anticipated to be similar, 12.5 cL-1 relative to petrol i.e. 1.25 cL-1 E10 fuel. In addition to the GOA, Australian states also provide incentives for ethanol use and production (Table 3.1). New South Wales, which in 2007 mandated at least 2 % volume of ethanol in the total volume of petrol, has increased the level to 10 % by July 2011 (equates to roughly 120 ML ethanol per annum) but only if it is 'economically viable'. Queensland has committed to 5 % of regular unleaded fuel by 2011. Victoria and Western Australia have a biofuel "target" of 5 % by 2010. Tasmania, South Australia and the Northern Territory remain uncommitted.

The major impediments to demand for ethanol include lack of consistent feedstock and fuel supply, uncertain commercial risks for producers and processors who want to act as investors not venture capitalists, limited service station outlets and unattractive relative price. These impediments can be addressed by well tried commercial practices such as increasing the diversity of feedstock supply, fuel supply monitoring, demand incentives, rollout incentives and discounted prices. Consumer confidence has also been a barrier. Although unfounded for modern cars running on E10, many motorists are concerned that ethanol will damage their engines. Consumer education initiatives have reduced such concerns in Queensland and New South Wales, although motorists expect E10 to be cheaper because of its lower energy density.

The demand for ethanol internationally is distorted by tariffs. For example, Jamaica is a major re-exporter to the USA of Brazilian ethanol after its conversion from hydrous to anhydrous form because the USA provides market access concessions that favour ethanol imports from developing countries (ABARE 2008). Australia imposes a tariff of 5 % on imported ethanol (0 % on ethanol from the USA) whereas the USA imposes a US$0.14 L-1 tariff on ethanol imports plus 2.5 % according-to-value tariff. The EU imposes tariffs 6 of €10.2 for every 100 L of denatured alcohol and €19.2 per 100 L of non-denatured alcohol. ABARE (2008) opines that it is possible that the US import duty on ethanol could be lowered to enable the USA to meet its ambitious biofuels supply targets.

Darby (2009) anticipates that Australian domestic production of biofuel as an import replacement for petroleum will grow in significance as the local production shortfall begins to accelerate post-2012. The demand in Australia for petroleum products is projected to increase at around 1.5 % y-1 until 2014 (ABARE 2008) but production of liquid energy (crude oil and condensate) is forecast to peak by the end of 2012 and subsequent increases in consumption will need to be met by increased imports or import replacements. It is stressed that Generation 1 ethanol, the production of ethanol from sugars, is only a part of the solution to Australia's future transport and energy needs. Even if the export fractions of Australian crop production in an average year were used to produce ethanol, an implausible scenario, the following national ethanol fuel blends could be supported: sugar E11, C-molasses < E1, wheat E27 and all other coarse grains E10 (O'Connell et al 2007). Biofuels could move beyond these limits if industries develop around second generation technologies. Nonetheless, for the rural industries concerned, the ability to supply ethanol feedstocks to an Australian industry provides opportunities for diversification and reduces over-exposure to global market fluctuations.

3.3 Ethanol Production in Australia

By January 2008 Australian ethanol production capacity was about 152 ML (Biofuels Association of Australia 2009), 43 % of the 2010 GOA biofuel target. In 2009 ethanol production is expected to be 232 ML (Table 3.2). Together, biofuel production capacity for 2008/09 is estimated to be equivalent to about 0.4 % of total liquid fuel consumption in Australia (Darby 2009).

In 2003, of the 135 ML ethanol produced in Australia 50 ML was blended in fuel, 35 ML was exported (principally to SE Asia) and 42 ML was used locally in pharmaceuticals, foods and beverages, chemical manufactures, paints and thinners, aerosols and cosmetics (Table 3.3; APEC Biofuels 2009). The use of ethanol in fuel has subsequently increased markedly (Table 3.4).

The sugar industry has planned an ethanol production capacity of 186 ML by 2010. Currently two refineries have the capacity to produce ethanol from C grade molasses, CSR at Sarina with an annual capacity of 60 ML and Heck Group at Rocky Point with an annual capacity of 1.5 ML. The latter apparently did not produce ethanol production in 2009.

Ethanol production feedstocks currently used in Australia are (i) molasses using bagasse to generate some of the electricity used in the ethanol production process, (ii) molasses using non-renewable electricity, (iii) grain sorghum, (iv) wheat and (v) waste wheat starch that is a residue from flour production (O'Connell et al 2007). The production of fuel ethanol from molasses and waste wheat starch represents significant value-adding to products that provide low value return to millers.

Table 3.2 Ethanol production capacity in Australia: current and planned (O'Connell et al 2007, APEC Biofuels 2009, Biofuels Association of Australia 2009).

Company	Location	Feedstock(s)	Capacity	
			2009	Planned
Queensland			ML	ML
CSR Ethanol	Sarina	C-molasses	60	
Heck Group	Rocky Point	C-molasses	1.5[a]	
Bundaberg Sugar	Bundaberg	C-molasses		10
Lemon Tree	Milmerran	sorghum, wheat		67
Downs Fuel Farmers	Dalby	sorghum, wheat	50[b]	80
Austcane	Burdekin	cane juice, molasses		100
Agri Energy	Lake Grace	all grains		90
New South Wales				
Manildra Group	Nowra	waste starch	125	300
Primary Energy	Gunnedah	sorghum		120
Agri Energy	Colleambally	all grains		90
Symgrain	Quirindi	wheat		100
South Australia				
Tarac Technology	Nuriootpa	grape	0.8	
Victoria				
Agri Energy	Swan Hill	all grains		90
Symgrain	West Victoria	wheat		100
Western Australia				
Primary Energy	Kwinana	wheat		160
TOTAL			232	1383

[a] not producing in 2009; [b] 50, ramping up to 80

Table 3.3 Australian ethanol industry, 2003 and 2009 (APEC Biofuels 2009, Government of Australia 2009).

Use of ethanol	2003	2009
	ML	ML
blended in petrol	50	178
used by industry: pharmaceuticals, foods and beverages, chemical manufacture, paints and thinners, aerosols and cosmetics	42	na[1]
exported	35	na
Total production	135	231

[1]na = unable to locate information

Table 3.4 Volume of ethanol-blended automobile gasoline sold in Australia between July 2005 and April 2009 inclusive (Government of Australia 2009)

Fiscal year (July until June)	Ethanol-blended gasoline
	ML
2005-06	56
2006-07	289
2007-08	835
2008-09 (to April inclusive)	1,370

3.4 Ethanol shortfalls in Australia

Although Australia has a national biofuels target of 350 ML by 2010 (source: Department of Infrastructure, Transport and Regional Economics, online: http://www.btre.gov.au/info.aspx?NodeId=16&ResourceId=133), current legislation requires 674 ML ethanol by 2010-2011 for fuel blending alone (Table 3.1). In 2009 the national ethanol processing capacity is only 231 ML. The recent global financial crisis, drought, uncertainties about obtaining sufficient feedstock and a lack of strategic policy direction from the GOA have inhibited investment in the biofuel industry such that many of the proposed biofuel projects listed in Table 3.2 have been shelved during the last 12 months.

With a current production capacity of only 110 ML (Table 3.1), Queensland would have to increase its production 88 % by 2011 just to achieve the anticipated 207 ML required to supply the mandated 5 % ethanol content of regular petrol in Queensland, let alone supply ethanol to other states in Australia. The sugar industry is well placed to supply ethanol, with molasses produced using co-generated energy from bagasse the most energy efficient, and least polluting, source of ethanol (Appendix 1; Cuevas-Cubria 2009). However, ethanol production from sugar is currently only about 60 ML, a shortfall of around 126 ML from the 186 ML planned by 2010. Queensland could theoretically produce 272 ML ethanol (based on 2004 production figures) from fermenting all of the sorghum and molasses exported (Table 3.5), but at the cost of losing those exports. There appears to be commercial space for additional sources of ethanol biofeedstock supply.

Table 3.5 Potential supply of fuel grade ethanol from sugar, molasses and sorghum in Queensland – based on 2004 production figures (recalculated from Urbanchuk et al 2005).

Carbohydrate source	Ethanol yield	2004 production	2004 exports	Potential ethanol production	
				Entire crop	Exported crop
	L ton^{-1}	tons	tons	ML	ML
Molasses	270	1,200,000	400,000	324	108
Sorghum	450	1,400,000	364,000	378	164
Sugar	600	5,500,000	4,019,000	3,300	2,411
Total				4,002	2,683

4 *Agave tequilana*: the plant and the crop

In Australia, *A.tequilana* is effectively unknown as a plant and untried as a crop. In this section we introduce the plant and we compile about its history of use by humans, and the places and conditions under which it is grown in its native and agronomic habitats in Mexico.

4.1 The Plant

4.1.1 Systematic description

The accepted name is *Agave tequilana* F.A.C.Weber, Mus. Nat. D'Hist. Nat. Bull. 8: 220, 1902. The type is a lectotype that was designated as a holotype by Gentry in 1982 (Figure 4.1). The systematic ranking of the species, based upon the Angiosperm Group II system (Angiosperm Phylogeny Group 2003) is: Kingdom: Plantae; unranked: Angiosperms; unranked: Monocots; order: Asaparagales; family: Asparagaceae or Agavaceae[1]; genus: *Agave*; species: *tequilana*. The species has been given other, now discarded, names: homotypic synonym - *Agave angustifolia* subsp. *tequilana* (F.A.C.Weber) Valenz.-Zap. & Nabhan, Kaktus Klub 2004(1): 44, 50 (2004); heterotypic synonyms - *Agave palmaris* Trel., Contr. U.S. Nat. Herb. 23: 116, 1920; *Agave pedrosana* Trel., ibid. p. 116; *Agave pes-mulae* Trel., ibid. p. 117; *Agave pseudotequilana* Trel., ibid. p. 119; *Agave subtilis* Trel., ibid. p. 116.

4.1.2 Species description

Weber (1902) described the species as:

"Plants surculose, radiately spreading, 1.2-1.8 m tall with short thick stems 30-50 cm tall at maturity; leaves 90-120 x 8-12 cm, lanceolate, acuminate, firm fibrous, mostly rigidly outstretched, concave, ascending to horizontal, widest through the middle, narrowed and thickened toward base, generally glaucous bluish to gray green, sometimes cross-zoned, the margin straight to undulate or repand; teeth generally regular in size and spacing or rarely irregular, mostly 3-6 mm long through mid-blade, the slender cusps curved or flexed from low pyramidal bases, light brown to dark brown, 1-2 cm apart, rarely remote and longer; spine

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[1]The Angiosperm Phylogeny Group APG II places the Agavaceae (the traditional family of about 550-600 species and ~18 genera in which the genus *Agave* is placed) within an expanded family Asparagaceae (order: Asparagales, APG 2003). APG II permits the alternative of a separate Agavaceae but expanding the family to include the genera currently in Anemarrhenaceae, Anthericaceae (with *Anthericum* and *Paradisea*), Behniaceae and Herreriaceae. Many treatments have retained Agavaceae as a distinct family.

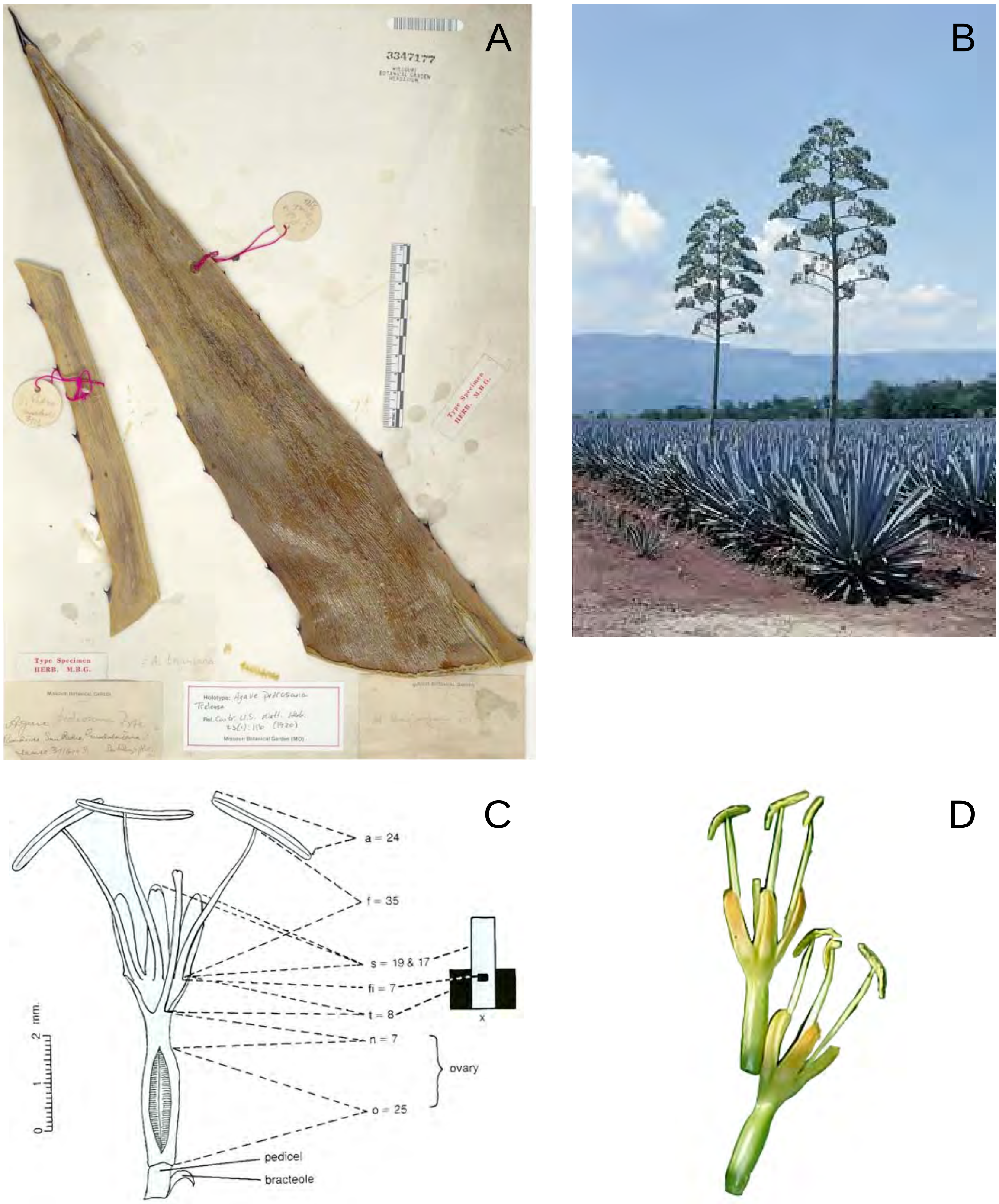

Figure 4.1 (A) The type of *Agave tequilana* F.A.C. Weber in the Missouri Gardens herbarium (MO).

A lectotype designated as holotype by Gentry (1982), the specimen was collected by Trelease in March 1903 from Guadalajara, Jalisco, Mexico. Originally determined as *Agave pedrosana* Trel., it was redesignated January 12 2001 (Tropicos 2009). (B) *A. tequilana* with flower spike (Valenzuela-Zapata 2008). Sugars stored in the stem ultimately provide carbon for the spike. (C) Cross-section of a stylized *Agave* flower with parts measured and a tube/tepal ideogram, x. The white column represents the tepal, the black the tube, and the black square the insertion of the tube. O, ovary body length; n, neck of ovary length; t, tubelength; fi, filament insertion (measured to bottom of tube); s, sepal lengths; f, filament length; a, anther length (from Gentry 1982). (D) *A. tequilana* flowers (modified photo from Valenzuela-Zapata 2008).

generally short, 1-2 cm long, rarely longer, flattened or openly grooved above, the base broad, dark brown, decurrent or not decurrent; panicle 5-6 m tall, large densely branched with 20-25 large diffusive decompound umbels of green flowers with roseate stamens; flowers 68-75 mm long on small bracteolate pedicels 3-8 mm long; ovary 32-38 mm long, cylindric, 6-ridged, with unconstricted short neck, slightly tapered at base; tube 10 mm deep, 12 mm wide, funnelform, grooved; tepals subequal, 25-28 mm long, 4 mm wide, linear, erect but withering quickly in anthesis, turning brownish and dry; filaments 45-50 mm long, bent inward against pistil, inserted *at 1* and 5 mm above base of tube; anthers 25 mm long; ' 'capsula ovata breviter cuspidata; seminibussemi-orbicularibus maximis; hilo sub-ventrali" (Figure 4.1).

4.1.3 Notes on *Agave* and *A. tequilana*

The Monocot Checklist (Govearts et al 2008) contains names of 346 species of *Agave* that are either accepted or unplaced (appendix 2). In contrast, Good-Avila et al (2006) suggests that only 166 species are valid. Either way, the genus *Agave* has undergone early adaptive radiation to become the largest genus in the family Agavaceae, despite a relatively recent origin (8 My ± 2 My). In all probability other *Agave* species may share traits of commercial interest with *A. tequilana*.

Gentry (1982) distinguished *A. tequilana* from its close relatives in *A. angustifolia* by its larger leaves, thicker stems and heavier more-diffusive panicles of relatively large flowers with tepals long in proportion to the relatively short tube (Figure 4.1). The differences are of degree rather than of distinct contrast. Their separation as a species is nominal but is tenable for the group Rigidae, in which species are hard to define. On the basis of morphology, Gentry (1982) provided a key for distinguishing between members of the Rigidae. A subsequent molecular study of retrotransposon sequences shows high levels of retrotransposon polymorphism in *Agave* varieties and species and identified the tequila agaves as a distinct phylogenetic group (Bousios et al 2007).

4.2 History of *A. tequilana*

Aztec legend has it that animals showed *Homo sapiens* how to eat *Agave*. If so, then evidence from coprolites indicates that it first took place at least 9,000 years ago (Callen 1965). Indeed, it has been suggested that *Agave* transplantation was one of the original agricultural pursuits of the Amerindians (Sauer 1965). From 7000 B.C., the use of *Agave* by indigenous people for food, fibre, drink, shelter and various natural products is well documented by preserved quids (chewed fibre rejects), archaeological specimens, fibre artifacts and the tools used in artifact manufacture (Gentry 1982 and references therein). Man moved *Agave* and fostered diversification by creating new genetic combinations.

Agave was eaten after the carbohydrates in the soft starchy white meristem near the short stem and the non-green leaf bases were converted to sugars by direct fire, by baking in stone-lined pits or with hot water. Species with high sapogenin content and other toxic compounds were not domesticated (Gentry 1982).

The first historical records of agaves are Mexican pictographs on ruins and in the codices. Gentry (1982) recommends Goncalves de Lima, in his "El Maguey y el pulque en los codices Mexicanos" (1956), for an excellent resume of history pertaining to agave. The ascendant god seems to have been *Mayahuel*, the Aztec goddess of agaves (Figure 4.2). Before the European invasion, *Agave* was used to produce two types of beverage, aguamiel, the sap from living plants, and pulque, fermented sap. The distillation of the spirits mescal and tequila originated following the Spanish conquest, when the technology of distillation was imported (Gentry 1982). After the conquest of the Mesoamerican highlands, *Agave* cultivation spread rapidly with the Spanish (Gentry 1982). Agaves were transported overseas by both Spaniards and Portuguese for ornamental and fibre use: *A. americana* to the Azores and Canary Islands; *A angustifolia, A. cantala*, and others to Asia and Africa. By the 1700s *A. americana, A. lurida* and others were established along the Mediterranean coasts. In the 1800s agaves became popular throughout Europe as ornamental succulents, though in the north their culture was generally limited to pots and greenhouses, and as fibre industries in colonies in Indonesia and the Philippines. The *A. sisalana* fibre industry was developed in East Africa in the 1900s. *A. tequilana* has not been grown extensively outside Mexico, it is not a major fibre producing species and there was no great demand in Europe for a competitor for the wine and brandy industries.

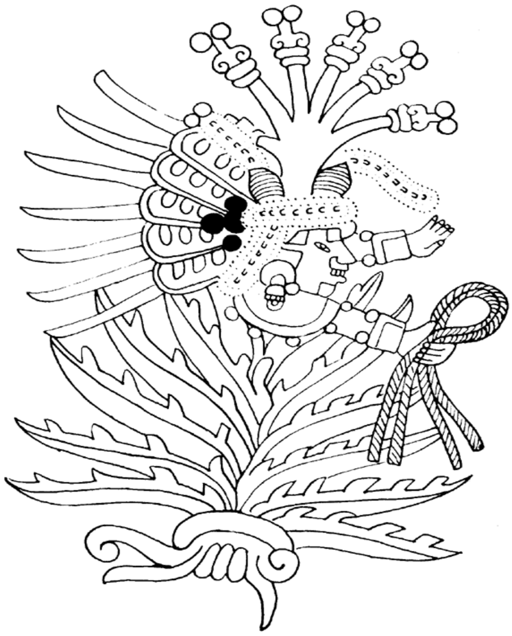

Figure 4.2 Codex reproduction of the goddess of Agave, Mayahuel (from Goncalves de Lima, 1956). Mayahuel is identifiable by the leaves, the stylized *Agave* inflorescence, foaming pulque in her hair and the fibre in her hand.

All tequila is now derived from cultivated varieties of *A. tequilana* Weber, a few populations of which still exist wild in western Jalisco. Under Mexican law, *A. tequilana* is the only *Agave* permitted to be used to produce beverage labelled as tequila. Production is limited to five regions, with most in the state of Jalisco. By 1982, as an industry protection measure, Mexico had embargoed the export of propagation stocks of *A. tequilana* (Gentry 1982). The Mexican Tequila Regulatory Council certifies two types of tequila: ‘traditional’, which is labelled‘100%, de *Agave*’, and 'tequila' which must be made with at least 51% blue agave spirit.

Both tequila and mescal are fabricated from the short broad stem, meristem and leaf bases. The globose, pineapple-like ‘cabezas’ or heads weigh from 25-50+ kg. In the distilleries the heads are traditionally cooked for 30 to 48 h in steam-producing ovens which convert the carbohydrates to sugars. The heads are next macerated and fermented until the sugars are transformed into alcohol, usually by the yeast *Saccharomyces cerevisiae*. Bacterial contaminants such as *Lactobacillus, Streptococcus, Leuconostoc, Pediococcus* and *Acetobacter* may also be present (Cedeño Cruz & Alvarez-Jacobs 1998, Cedeño Cruz 2003). The alcoholic juice is then distilled. Each *A. tequilana* var. azul head contains sufficient carbohydrate to produce about 5 L of ‘100 % *Agave*’ tequila.

4.3 Agave tequilana, the crop

4.3.1 Main production areas in Mexico

A. tequilana is grown in Mexico along a east-west axis from east of Arandas, southwest through Guadalajara, to Magdalena in the west (Figure 4.3). The main areas of production lie in the valley around the town of Tequila in the west, and in the highlands (Los Altos, near Atotonilco and Arandas) to the east. Tequila has been manufactured in the Jalisco area for more than 150 years (Gentry 1982). The oldest region, in the vicinity of Amatitán, developed at the end of the 1600s. Commercial production was established in the city of Tequila in the late 1700s to supply the mining zones in Jalisco. In the 1890s production began in the Jalisco Highlands (Luna, 1991).

Figure 4.3 Map of the Guadalajara region in the state of Jalisco, Mexico, showing important tequila-growing villages/towns mentioned in the text (●).

The tequila growing areas and major mescal growing areas partially overlap (Figure 4.4). According to Gentry (1982), *A. potatorum* is the primary species used to produce mezcal and pulque but others include *A. angustifolia*, *A. asperrmia*, *A. durangensis*, *A. palmeri*, *A. rhodocantha*, *A. salmiana*, *A. shrevei*, *A. weberei*, *A. wocomahi* and *A. zebra.*

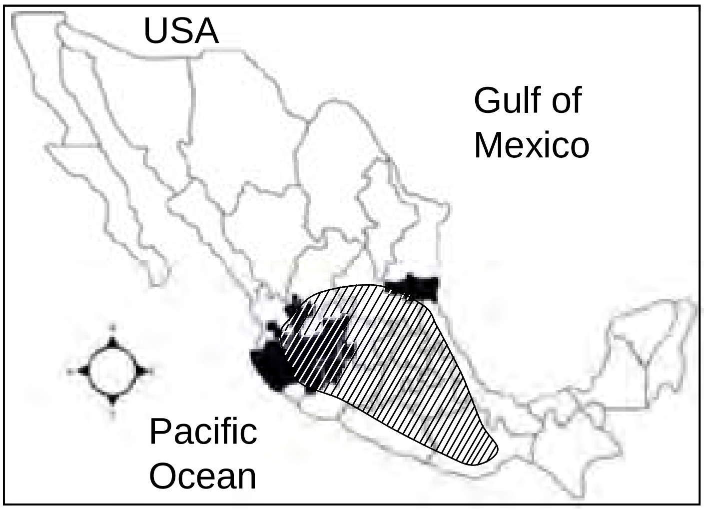

Figure 4.4 Map of Mexico showing region of traditional *Agave* cultivation that includes mezcal (hatched) and the area within which tequila may be produced (solid) (redrawn from Gentry 1982).

4.3.2 Climate

In Jalisco, *A. tequilana* grows in warm sub-tropical and temperate sub-tropical climates corresponding to the USDA climate zones 9A and 10 (Ruiz-Corral *et al.* 2002). The species grows wild in, and is cultivated in, regions with pronounced seasonal rainfall. The annual rainfall of about 800 to 900 mm falls principally during summer. Winters are dry. The temperature conditions for good *Agave* yields in terms of tequila production are a minimum of 10°C and an optimum of 26°C (Figure 4.5). Plants will tolerate maxima of

50°C for limited periods. Because it is mostly found at 800 to 1700 m above sea level the plants grow in regions where day night temperatures are usually separated by about 10°C. When cultivated, planting time is usually immediately prior to the onset of the wet season, from June to September, so that the plants do not suffer from water stress during the first year of growth.

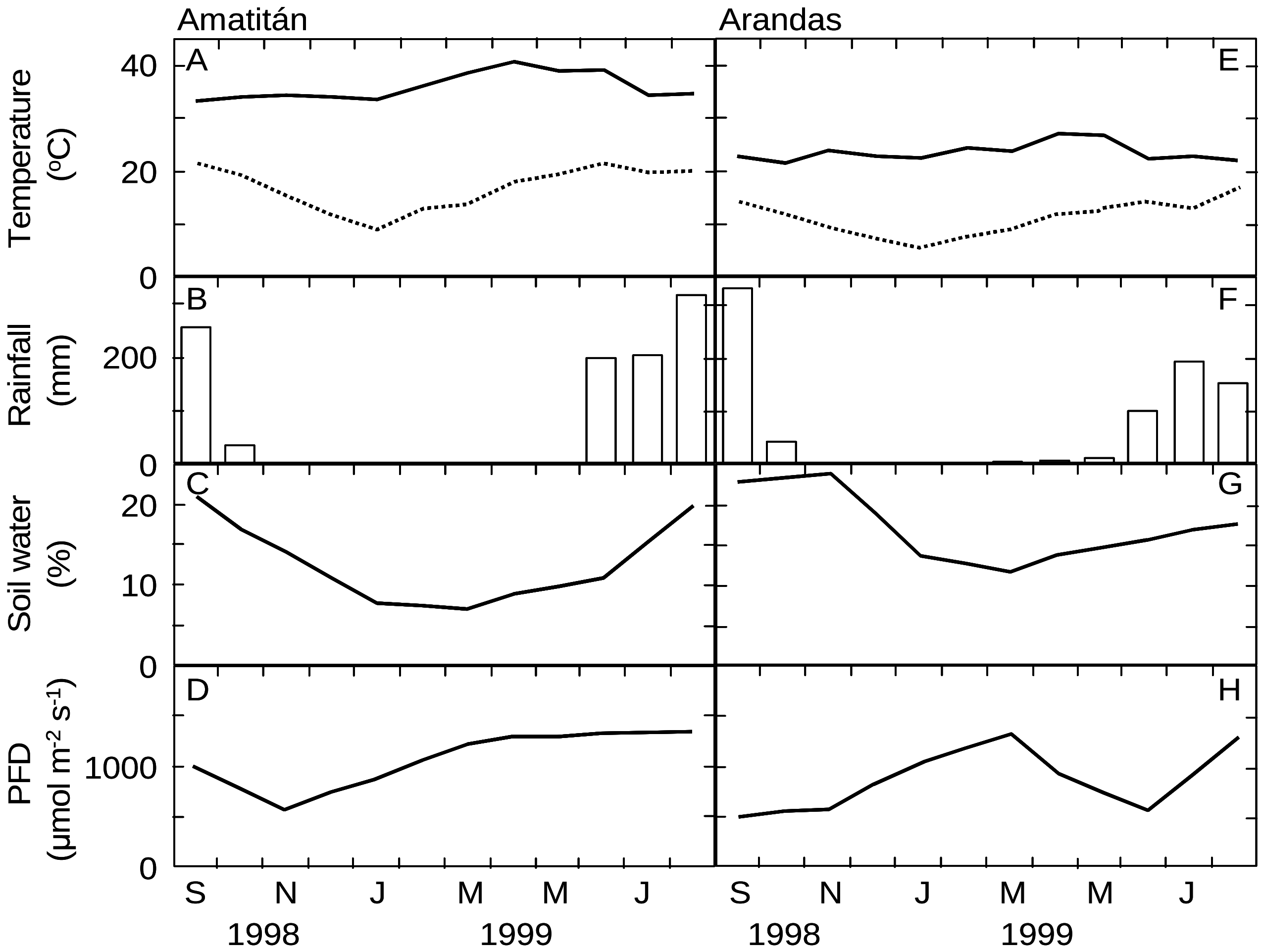

Figure 4.5 Climate during 1998 and 1999 at (left panels) Amatitán in the west of Jalisco at about 1000 m a.s.l., and (right panels) Arandes in the east of Jalisco at about 2000 m a.s.l (see Figure 2.3 for map).

Variables shown are mean monthly maximum air temperature and mean monthly minimum temperature (A, E), monthly rainfall (B, F), mean soil water content in top 10 cm of the soil (C, G), and mean daily PFD (D, H) (redrawn from Pimientia-Barrios et al 2001 & Ruiz-Corral et al 2001).

4.3.3 Soils

In Jalisco, *A. tequilana* grows well in iron-rich, fertile basaltic soils associated with local dormant volcanoes and in black soils in the valleys near Tequila. The non-gravel component of the soil contains roughly 60 % sand, with the remainder silt and clays (Nobel & Valenzuela 1987). A typical cultivation procedure is to add 30-70 g per plant nitrogen as urea. In some areas, phosphorus and potassium is also provided. The elemental composition of *A. tequilana* in comparison to other crop and non-crop plants is shown in Table 4.1.

Table 4.1 Element levels in the chlorenchyma of field-grown agaves and cacti (from Nobel 1988, table 6.1)

Species	Site	N	P	K	Na	Ca	Mg	Mn	Cu	Zn	Fe	B	Acid accumn
		%	ppm	%	ppm	%	%	ppm	ppm	ppm	ppm	ppm	(mol m^{-2})
Agave americana	Mexico	1.53	1,280	1.78	46	3.94	0.59	50	4	49	53	34	0.80
Agave deserti	Palm Desert, CA	1.08	2,760	1.65	10	3.57	0.62	8	2	34	43	20	0.52
Agave fourcroydes	Merida, Mexico	1.69	2,800	1.46	29	4.64	0.61	10	2	33	38	39	0.78
Agave lechuguilla	Saltillo, Mexico	1.14	1,220	1.27	45	6.11	0.40	14	7	36	77	18	0.66
Agave salmiana	San Luis Potosi, MX	1.10	1,790	2.26	46	4.37	0.59	8	4	7	118	26	0.66
Agave sisalana	Nairobi, Kenya	0.6	2,600	1.5	—	1.2	0.32	41	7	15	148	13	—
Agave tequilana	Tequila, Mexico	1.47	3,300	2.97	62	5.33	1.32	53	3	30	155	22	0.70
Agave utahensis	Clark Mtn, CA	0.89	1,450	1.31	66	2.30	0.51	18	1	14	34	19	0.24
Carnegiea gigantea	Buckeye, Arizona	2.48	1,180	1.18	332	1.69	0.60	26	4	21	117	23	0.46
Ferocactus acanthodes	Palm Desert, CA	1.62	1,700	1.95	315	4.62	0.62	122	9	22	161	62	0.38
O. bigelovii	Palm Desert, CA	1.00	1,220	1.52	282	4.98	1.34	46	6	14	219	35	0.19
O. echios	Santa Cruz, Ecuador	1.58	1,720	1.58	484	3.14	1.68	209	3	25	102	18	0.32
O. ficus-indica	Fillmore, California	2.61	3,320	1.18	31	6.33	1.43	54	15	52	88	109	0.81
O. phaeacantha	Kingsville, Texas	2.11	1,970	3.69	179	3.81	1.84	92	4	31	73	23	—
A range of agronomic plants (mean)		2	3,000	2	1,000	2	0.7	70	8	40	150	30	—

Note: Means are presented in % or ppm on a dry-weight basis for six to nine samples from mature field plants.

Nobel (1989) developed an edaphic factor-based *Agave* nutrient index (ANI) that accounted for over 95% of the variation in growth of *A. deserti* between two sites. When applied to *A. tequilana* growing at ten sites at Jalisco in which the N, P and K levels varied 3-fold, the B level varied over 2-fold, and the Na level varied nearly 6-fold, the index accurately predicted the variation in leaf unfolding rate at each site. Although it was stressed that the index was exploratory in its formulation, it shows predictive promise.

4.3.4 Cultivation

Almost all of the available information on cropping of *A. tequilana* relates to the production of tequila (i.e. not biofuels) in a small area in the vicinity of the state of Jalisco (but see Nobel 1988, 1991a, 1991b; Nobel et al 1987, 1998, 2002; Pimentia-Barros 2001). The nature of the mainly small farms (although farm sizes change during boom/bust cycles and some major tequila manufacturers are beginning to invest in larger properties) and the culture surrounding this crop has meant most information and growing skills are passed from one generation to another, and not published.

In Mexico, *A. tequilana* is planted about 15 cm deep, 2 - 4 m apart generally in well-drained raised beds (Cedeño Cruz & Alvarez-Jacobs 1998, Cedeño Cruz 2003). Occasionally *Agave* is sown intercalated with nitrogen-fixing crops such as peanuts, beans, chickpeas or soybeans. Plants are generally not irrigated and fertilization regime depends upon the soil composition (basaltic derived in the highlands and black soils in the valleys), plant age and the financial resources of the grower.

Recently, researchers from eleven Mexican universities have pooled resources to better understand the potential of *Agave* spp. as candidates for biofuel feedstock (Velez Jimenez 2008, see also USDOE 2008). One of the group, Professor R Madrigal Lugo of the Autonomous University of Chapingo, created and maintains the oldest *Agave* germplasm centre in Mexico and has reportedly developed high-yield *Agave* varieties from several species. No literature was uncovered that pertains to these improved varieties, evidently because the researchers are in the process of applying for patents.

4.3.5 Susceptibility to disease and pathogens

In the late 1980s, *A. tequilana* crops in Mexico began to exhibit soft-rot damage. The situation became commercially serious following warmer temperatures and increased rainfall during the 1996 and 1997 seasons. In 2002, 23% or more of the plants produced in Jalisco were affected. The rot-related problems, collectively referred to as TMA (tristeza y muerte de agave, "wilting and death of agave"), have highlighted the low genetic variability in the tequila-producing varieties that have been propagated from asexual suckers for many generations.

The principal diseases seem to be due to bacteria, *Erwinia cacticida* rather than the more common pathogen *E. carotovor*, and a fungus, *Fusarium oxysporum*, the spread of which may be assisted by herbivores, such as the larvae of the weevil *Scyphophorus acupunctatus* Gyll. (Coleoptera: Curculinidae). *Enterobacter agglomerans*, *Pantoea agglomeran*, *Pseudomonas mendocina*, and *Serratia* sp. have also been associated with soft-rot (Jimenez-Hidalgo et al 2004).

Other pathogens of *Agave* in Mexico include the fungus, *Thielaviopsis paradoxa*, that prevents younger plants from forming roots, and nematodes such as *Pratylenchus* sp., *Dorylaimus* sp. and *Helicotylenchus* sp.

No information is available on the susceptibility of *Agave* spp. in Australia to pathogens and herbivores. It should be noted that the health of *A. sisalana, A.Americana, A. fourcroydes* and *A. vivipara* have been naturalised in Australia for around 100 years with little sign of disease.

5 Physiology and ecophysiology

5.1 Photosynthetic pathway

All *Agave* studied to date, including *A. tequilana*, have a capacity to assimilate CO_2 during the light using C_3 photosynthesis and a capacity to assimilate CO_2 in the dark using Crassulacean acid metabolism (CAM; Szarek and Ting 1977, Nobel 1994), a photosynthetic pathway that is present in roughly 6 % of vascular plants (Smith and Winter 1996, Holtum et al 2005). CAM has also been reported in the closely related genera *Hesperaloë* (Ravetta and McLaughlin 1993), *Polianthes* (Reddy and Das 1978) and *Yucca* (Eickmeier 1978).

In CAM photosynthesis, green cells assimilate CO_2 in the dark using the enzyme phosphoenolpyruvate carboxylase (PEPCase) (Figure 5.1). A four-carbon compound, malate, is formed which is stored in large vacuoles as malic acid. During the light, the stomata close and the acid is decarboxylated to produce CO_2 and a three-carbon byproduct. The CO_2 is reassimilated using 1,5-ribulosebisphosphate carboxylase/ oxygenase (Rubisco) and is converted to three-carbon sugars that, with the three-carbon by-product of decarboxylation, are converted to soluble or insoluble carbohydrates (Winter & Smith 1996b, Holtum et al 2005).

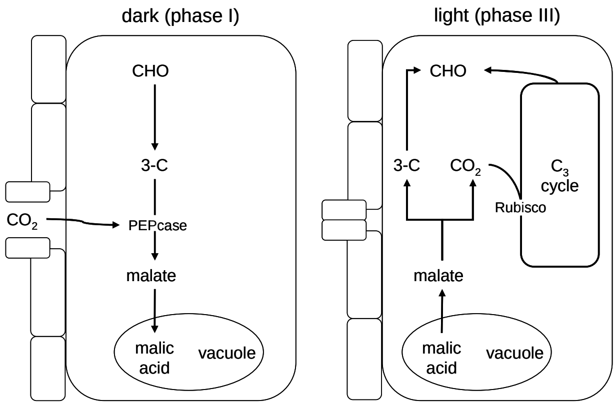

Figure 5.1 The CAM pathway. Phase I: the diffusion of CO_2 through open stomata into green cells, carboxylation by PEPcase, and malic acid storage during the dark (left) and phase III: the conversion of carbon to carbohydrates behind closed stomata during the light (right).

The gas-exchange patterns of CAM plants therefore differ from other plants because they assimilate CO_2 in the dark (Figure 5.2). Internally they are characterized by large reciprocal day-night fluctuations of malic acid and photosynthetic carbohydrates. Although some constitutive CAM species fix CO_2 almost exclusively at night (phase I, Figure 5.2; Osmond 1978), in many, such as *A. tequilana*, CO_2 uptake extends into the early morning (phase II), and may occur in the late afternoon if environmental conditions are favourable (phase IV). The high intercellular [CO_2] associated with decarboxylation suppresses stomatal opening during the middle of the day (phase III).

In the *Agave* species tested to date, *Agave americana*, *A. bracteosa* and *A. desertii*, the decarboxylation of malate during the light is considered to be catalysed by malic enzymes, predominately NADP malic enzyme, not PEP carboxykinase (Dittrich et al 1973, Dittrich 1976).

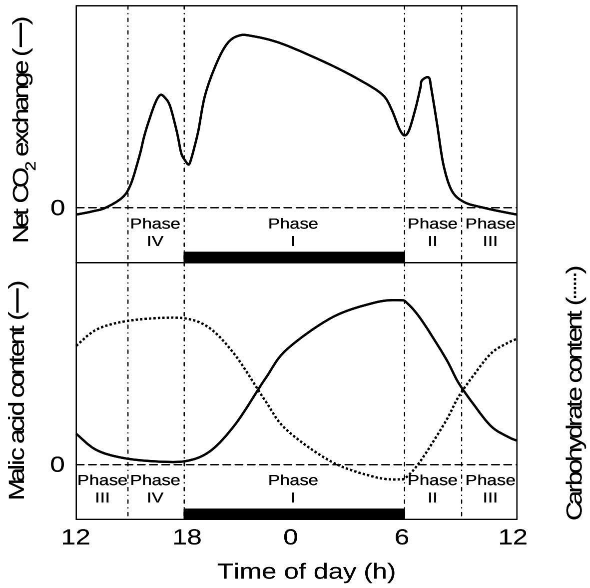

Figure 5.2 The daily cycle of net CO_2 exchange (top panel, solid line), and the reciprocating fluctuations of malic acid (bottom panel, solid line) and storage carbohydrates (bottom panel, dotted line) for a typical CAM plant, illustrating the four phases of CAM (Osmond 1978, Holtum et al 2005). Solid bar represents darkness.

A. tequilana exhibits day-night patterns of gas exchange, both CO_2 and water vapour, characteristic of strong CAM plants that, under well-watered conditions, can assimilate CO_2 during the light and during the dark (Figure 5.3). Indeed, roughly 87 % of carbon gain by *A. tequilana* occurs during the dark (Nobel & Valenzuela 1987).

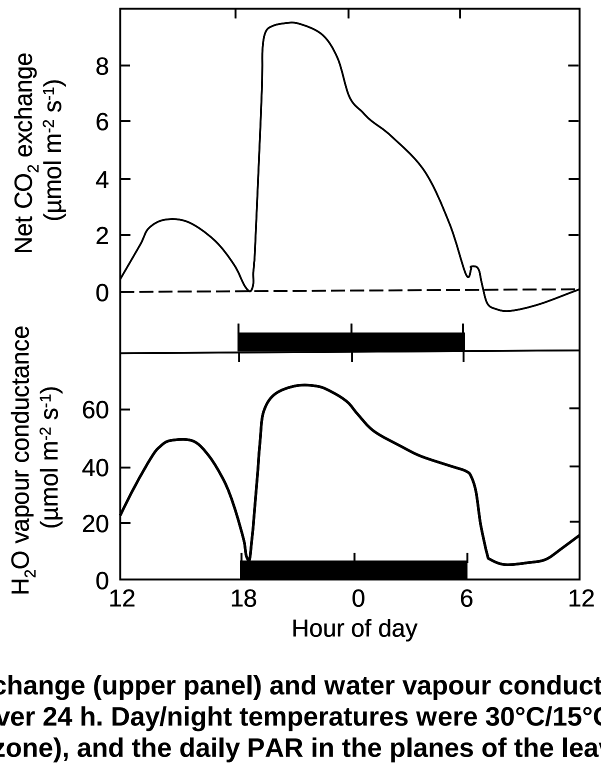

Figure 5.3 Net CO_2 exchange (upper panel) and water vapour conductance (lower panel) for *A. tequilana* over 24 h. Day/night temperatures were 30°C/15°C, the soil was wet (> -0.5 MPa in the root zone), and the daily PAR in the planes of the leaves averaged 20 mol m^{-2}. (redrawn from Nobel & Valenzuela 1987).

The solid bars indicate darkness

CAM plants may exhibit a three- to five-fold higher water-use efficiency (WUE) than C_3 or C_4 plants under comparable conditions because stomata open at night when tissue temperatures average 10–12 °C lower than during the light, and close during midday when temperatures are high (Table 5.1). By concentrating CO_2 at the site of Rubisco, CAM increases the efficiency and optimal temperature for photosynthesis.

Table 5.1 Comparison of agronomic traits for cultivated crops that express different photosynthetic pathways (from Nobel 1991a & Borland et al 2009, Table 2).

Agronomic traits	Photosynthetic pathway		
	CAM	C_3	C_4
Average above-ground productivity (Mg ha^{-1} $year^{-1}$)	43	35	49
Water use efficiency (over 24 h) (mmol CO_2 per mol H_2O)	4 – 10	0.5 – 1.5	1 – 2
Crop water demand (Mg H_2O ha^{-1} $year^{-1}$)	2,580 – 6,450	14,000 – 42,000	14,000 - 28,000

5.2 Productivity

CAM is often considered a mode of photosynthesis that, by virtue of its high WUE, assists plants to survive in environments subject to intermittent water-stress i.e. it is a mechanism adapted for survival, not speedy growth. Indeed, many CAM species exhibit low above-ground dry weight productivity. However, productivity varies with vegetation type and environment. Many species may exhibit low growth rates even when environmental conditions are favourable but some have the capacity to exhibit high productivities when a modicum of water and nutrients are available (Tables 5.1, 5.2).

The flexibility of CAM photosynthesis as expressed by *A. tequilana* was demonstrated by Pimiento-Barrios et al (2001, 2006) who demonstrated that appreciable daily net CO_2 uptake occurred throughout the year for plants grown in a warm subtropical environment (Amatitán, Jalisco) and in a temperate subtropical environment (Arandas, Jalisco) (Figure 5.4). At both localities the unirrigated plants even sequestered carbon during prolonged dry periods, presumably because plant water potential was maintained by leaf succulence. The highest values of daily CO_2 gain at both localities reflected prolonged daily periods of both day and night assimilation. High temperatures in the summer reduced daily net CO_2 uptake.

The capacity of some CAM *Agave* and *Opuntia* to exhibit high productivities appears to be associated with an ability to adjust photosynthetic biochemistry such that an optimal compromise between day- and high night-time uptake is obtained. High rates of carbon gain generally require appropriate night-time temperatures for optimal PEPCase activity (phase I), sufficient light to power rapid malic acid deacidification (phase III), and adequate water to enable afternoon CO_2 uptake (phase IV). *Agave* and *Opuntia* have been exploited agronomically in seasonally water-limited habitats where their above-ground productivities are not only comparable with those of the most water-use efficient C_3 or C_4 crops but they use only 20% of the water required by other plants. Such attributes have been used to highlight the potential of CAM plants for carbon sequestration and as feedstocks for bioenergy production on marginal and degraded lands (Tables 5.3 and 5.4, e.g. Nobel 1988, 1991a, 1991b, 1994, 1996, 2000, Nobel et al 2002, Borland et al 2009). However, their potential as feedstocks for bioenergy production on ‘conventional’ agronomic lands, such as those in the wet-dry tropics and sub-tropics, has been overlooked.

Table 5.2 Annual above-ground dry weight productivity of the most productive C_3, C_4 and CAM species (from Nobel 1996, tables 15.1 and 15.2)

Type and species	Location	Maximal productivity
C_3 crops		(Mg ha^{-1} y^{-1})
Beta vulgaris	California, USA	34
Elaeis guineensis	Malaysia, Sierra Leone	40
Manihot esculenta	Java, Madagascar	45
C_3 trees		
Crypromeria japonica	Japan	44
Eucalyptus globulus	Portugal	40
Eucalyptus grandis	South Africa	41
C_4 crops		
Pennisetum purpureum	El Salvador, Puerto Rico	70 - 88
Saccharum officianarum	Guyana, Hawaii, Queensland	50 - 67
Sorghum bicolor	California, USA	47
C_4 floodplain		
Cyperus papyrus	Kenya	51
Echinochloa polystachya	Brazil	94
CAM crops		
Agave mapisaga	Tequexquinahuqac, Mexico	38 ±2
Agave salmiana	Tequexquinahuqac, Mexico	42 ±3
Agave tequilana	Jalisco, Mexico	25
Ananas comosus		35
Opuntia amyclea	Saltillo, Mexico	45 ±2
Opuntia ficus-indica	Santiago, Chile	47 - 50
Opuntia ficus-indica	Saltillo, Mexico	47 ±3

[1] Under optimal spacing and water supply. Annual rainfall at Tequexquinahuqac was 770 mm; other plants were irrigated.

Table 5.3 Annual aboveground productivities of agaves, cacti and other plants (from Nobel 1988)

Species	Location	Rainfall ($mm\ y^{-1}$)	Plant part	Productivity (Mg dry wt $ha^{-1}\ y^{-1}$)
Agave deserti	California	430	leaves + stem	7.1
Agave fourcroydes	Yucatan, Mexico	1,000	leaves + stem	15.3
Agave lechuguilla	Coahuila, Mexico	430	leaves + stem	3.2
Agave salmiana	San Luis Potosi, Mexico	320	leaves + stem	10.1
Agave sisalana	Tanzania	1,300	leaf fibre	5.1
Agave tequilana	Jalisco, Mexico	1,080	leaves + stem	24.9
Opuntia ficus-indica	Pernambuco, Brazil	1,000	stem	*20*
Ananas comosus (pineapple)	Hawaii	1100	fruit + leaves	~20
Glycine max (soybean)	Illinois	800	leaves + stem	7
Medicago sativa	Arizona, California	200-800	leaves + stem	21-34
Oryza sativa (rice)	California; Japan	500	leaves + stem	10-16
Saccharum officinarum	Guayana, Hawaii, Queensland	2,000	leaves + stem	40-60
Sorghum vulgare	California	600	leaves + stem	8
Triticum aestivum	Australia, UK, Mexico, USA	600-1,000	leaves + stem	4-10
Zea mays	Illinois, Ohio	700-1,000	leaves + stem	11-19
Seven broad- leaved trees	—	—	stem	27
Eleven coniferous trees	—	—	stem	23

Table 5.4 Growth characteristics of CAM plants favourable for cultivation as bioenergy feedstocks, particularly in water-limited regions (from Borland et al 2009, Table 1, who extracted examples from Nobel (1988, 1994), Day (1993), and Winter and Smith (1996c)).

Trait	Example	Comment
High water-use efficiency	5–16 mmol CO_2 per mol H_2O on an annual basis	Typically 4 – 10 times higher than C_3 plants
High drought tolerance	Can grow in areas with as little as 25 mm $year^{-1}$ precipitation	Tissues can tolerate up to 90% loss of water content
Tolerance of high temperatures	Up to 70^oC, based on 50% loss of cell viability after 1 h	Typically upper limit of 50–55 ^oC in C_3 plants
Tolerance of high PPFD	Can tolerate >1000 µmol m^{-2} s^{-1} (or >40 mol m^{-2} d^{-1}) without photoinhibition	Generally more tolerant of high PPFD than agronomic C_3 plants
Tolerance of UV-B radiation	Only 1% incident UV-B transmitted through epidermis of *Yucca filamentosa* (Agavaceae)	Generally thick epidermis and high concentrations of phenolics in CAM plants
Entire shoot surface typically photosynthetic	Whole shoot photosynthetic in leaf- and stem-succulent species; limited bark formation even on stems of arborescent cacti	Many C_3 species are deciduous (shed photosynthetic organs) or woody (limited stem photosynthesis)
High shoot:root ratio and harvest index	Shoot:root ratio as high as 10:1; above-ground biomass readily harvested	
High resistance to herbivores	Effective physical and chemical defences	
High content of non-structural carbohydrate	Especially monocotyledons (20% dry weight); ready conversion of soluble sugars to bioethanol	
Low lignin content	Weak secondary thickening and lack of true wood formation	

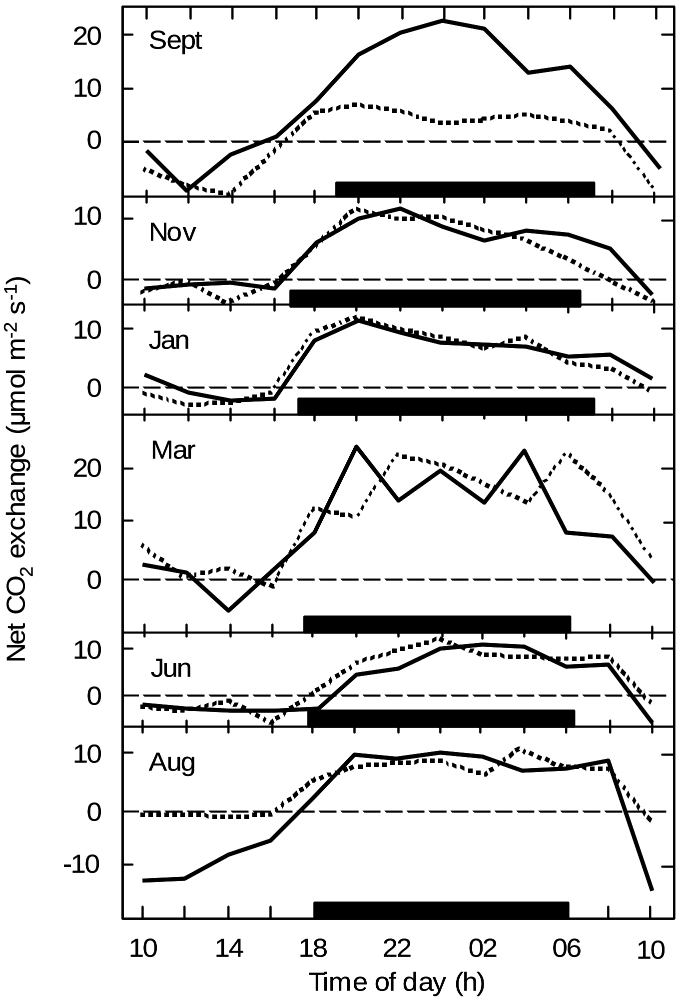

Figure 5.4 Net CO_2 exchange by *Agave tequilana* growing in Jalisco, Mexico, at Amatitán (solid line) 1000 m a.s.l. and at Arandas (dotted line) 2000 m a.s.l. during 1998-1999 (redrawn from Pimientia-Barrios et al 2001).

Environmental conditions throughout the experiment are shown in Figure 5.2. Solid bars represent darkness.

The worldwide cultivation of *Agave* spp. is >500,000 ha (Nobel et al 2002), mostly for fibre and fodder. In 2006, worldwide production of fibre from sisal (*Agave sisalana*) was 246 Gg, with a further 22 Gg being produced from henequen (*Agave fourcroydes*), representing a combined export value of US$200 million (FAO 2008). *Agave* spp. are also used for the production of alcohol, either in the form of tequila of which 284 ML was produced in 2007 from the double distillation of fermented sugars from the stems and attached leaf bases of *A. tequilana* (Ávila-Fernández 2009), or as mescal, a singly distilled beverage.

The potential for *Agave* as an economically viable source of bioethanol with a minimum-waste platform has recently been highlighted in Mexico as well as for the eroded lands of the Great Karoo in SE Africa (Boguslavsky et al 2007, Burger 2008). The high annual productivity of *A. tequilana* (26 Mg dry biomass ha^{-1} $year^{-1}$ on seasonally dry land in refereed literature (Nobel & Valenzuela 1987) or 50 Mg dry biomass ha^{-1} $year^{-1}$ cited in more recent non-refereed literature (Burger 2008) and high total sugar content (27 - 38 %) in leaves/stems/fruits (cf. sugar cane 15 - 22%) have led to reports that distilled ethanol yields of 14,000 L ha^{-1} $year^{-1}$ can be obtained from some cultivars, with further ethanol production possible from cellulose digestion (Burger 2008).

The high productivity of *Agave* is not unique among CAM plants. *Opuntia* are part of natural and agronomic ecosystems in many parts of the world, with commercial cultivation, primarily for fodder and forage, occupying over 1 million hectares. The annual dry biomass productivity for *O. ficus-indica* may attain 47–50 Mg ha^{-1} $year^{-1}$ in cultivation (Table 5.2). In central eastern Australia, a weedy *O. stricta* monoculture occupied >25 million hectares and produced a total biomass of ~1.5 million Gg in ~80 years (Osmond et al 2008).

In a superbly executed field study in Mexico, Nobel & Valenzuela (1987) applied environmental productivity index (EPI) analysis to *A. tequilana* growing under non-irrigated agronomic conditions in commercial plantations (Table 5.5). EPI was developed by Nobel and co-workers (Nobel & Meyer 1985, Nobel 1988, 1991b) to provide a whole-system approach to land use and natural resource management that would inform and improve agronomic practice for CAM cultivation. EPI analyses predicted that the global range of CAM cultivation could be extended for carbon sequestration and biofuel production.

EPI uses monthly effects of PAR (Figure 5.5), water (Figure 5.6) and temperature on nocturnal acidity accumulation to generate indexes of performance (Figure 5.7). The product of the temperature, PAR and water indices provides the EPI (Figure 5.8)

Table 5.5 Dry weight percentages for organs of plants of *A. tequilana* of various ages (n=2) grown on commercial plantations 1 km north of the center of Tequila, Jalisco, at 20°54' N, 103°50' W, 1160 m a.s.l. (redrawn from Nobel & Valenzuela 1987).

Plant dry weight averaged 296 g for 1 year-old plants, 3.51 kg for 3 year-old plants and 28.7 kg for 6 year-old plants

	Age (years)		
	1	3	6
		%	
Unfolded living leaves	66.0	56.7	55.0
Central spike	4.5	7.2	6.8
Dead leaves	9.9	9.4	9.3
Stem	7.4	9.2	14.2
Roots	12.2	11.1	10.3
Offshoots plus their rhizomes	0.0	6.4	4.5

In the study of Nobel and Valenzuena (1987) the main environmental factor that limited growth of *A. tequilana* during the warm wet summer was PAR. During winter, the limiting factor was water. The dry weight gain of the initially 1 year-old unirrigated plant was a creditable 24.9 metric tons ha^{-1} y^{-1}.

Although *A. tequilana* is overwhelmingly grown for the production of beverage-grade alcohol (despite a report of diamond production from tequila (Morales et al 2008), it has recently been recognized that *A. tequilana* has potential as a biofuel feedstock. According to Burger (2008), a small group from the academic and private sectors have a ‘tentative’ agreement with Mexico`s Institut Nacional Ecologia for funding that will enable the cultivation, conservation and patent-protection of selected varieties of *A. tequilana* and *A. angustifolia* for an *Agave*-to-ethanol project. No refereed information is available on these plants to date.

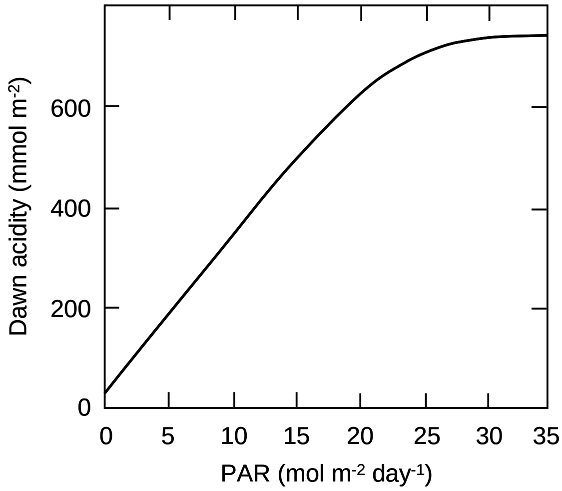

Figure 5.5 Influence of daily PAR on dawn acidity levels for 6 year-old, well-watered *A. tequilana*. Air temperatures ranged from 16 to 32°C (redrawn from Nobel & Valenzuela 1987).

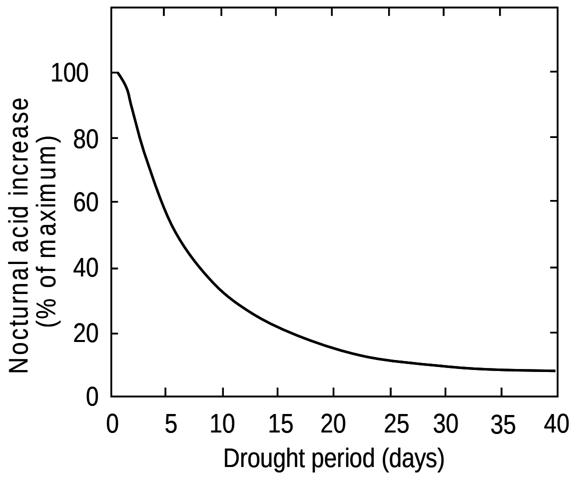

Figure 5.6 Response of nocturnal acidity increases of *A. tequilana* to drought at day/night air temperatures of 30°C/15°C (redrawn from Nobel & Valenzuela 1987).

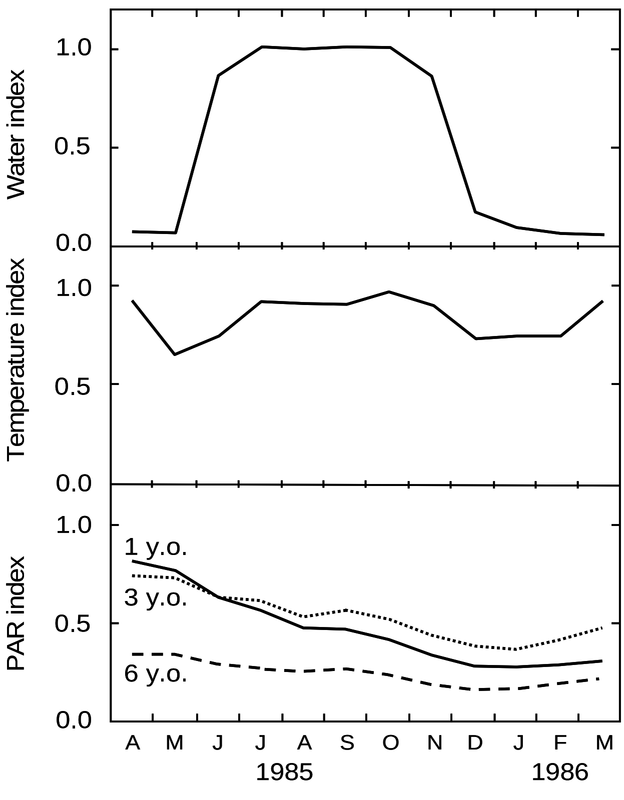

Figure 5.7 Water (top), temperature (middle) and PAR indexes (bottom) calculated for *A. tequilana* of the indicated initial age grown in Jalisco (redrawn from Nobel & Valenzuela 1987).

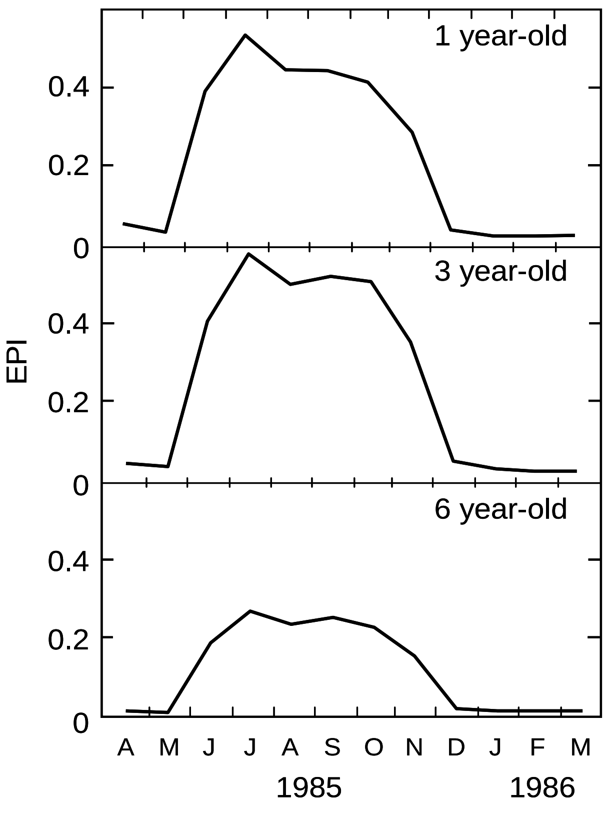

Figure 5.8 Environmental productivity index (EPI) for *A. tequilana* of initial ages of 1 year (top), 3 years (middle) and 6 years (bottom) (redrawn from Nobel & Valenzuela 1987).

5.4 The carbohydrate complement of *A. tequilana*

The carbohydrate content of agavaceous plants is the major attribute that influences their commercial use as fibre, sweeteners and supplements (Ritsema & Smeekens 2003, Urías-Silvas et al 2008). Excess carbohydrates produced in the leaves during photosynthesis are transported to the stem where they are stored as sugars or polymers in vacuoles of succulent parenchyma cells. They subsequently provide a source of carbon and energy for the production of the apical (monocarpic) flowering spike (Figure 4.1B). In leaves carbohydrates are mainly present as low-lignin cellulose and photosynthetic sugar pools, principally fructose and glucose in *A. tequilana*, that are involved in the large day-night fluctuations associated with CAM.

The water-soluble carbohydrate (WSC) content of stems of *A. tequilana* varies depending upon developmental stage. Mature stems examined by Waleckx et al (2007) averaged 283 mg WSC g^{-1} fresh weight of plant, i.e. 28 %, a concentration that will vary with respect to the water content of stems. In comparison, maize contains 16-20 % sugar plus carbohydrate and sugarcane 14-20 %. On a dry weight basis, WSC of between 550 and 900 mg g^{-1} stem dry weight have been reported in genetically identical *A. tequilana* var. azul grown in Jalisco and Guanajuato (Mexico) (Figure 5.9, Mancilla-Margalli & Lopez 2006, Rendon-Salcido et al 2009).

Many *Agave* carbohydrates are water-soluble and are hydrolysed by heat to readily fermentable sugars that are roughly 90% fructose and 8% sucrose. Molasses by comparison may contain, in addition to readily fermentable sugars, some non-fermentable saccharides typically at concentrations of less than 5 % (Bortolussi and O'Neill 2006; Sanchez and Cardona 2008).

In common with about 15% of higher plant species (Cairns 2003, Ritsema & Smeekens 2003, the Agavaceae store carbohydrates mainly as fructans, polymers of β-fructofuranosyl residues (Table 5.6; French 1989). Fructans are commonly water-soluble and synthesized from sucrose accumulated in the vacuole (1). In addition to storage, fructans have been implicated in vegetative development, osmoregulation, cryoprotection and in drought tolerance (Vijn & Smeekens 1999, Ritsema & Smeekens 2003, Vandenende 2004). It should be noted that the oligofructans stored by *Agave* species are often referred to as inulins. This designation appears to be chemically imprecise (Table 5.6).

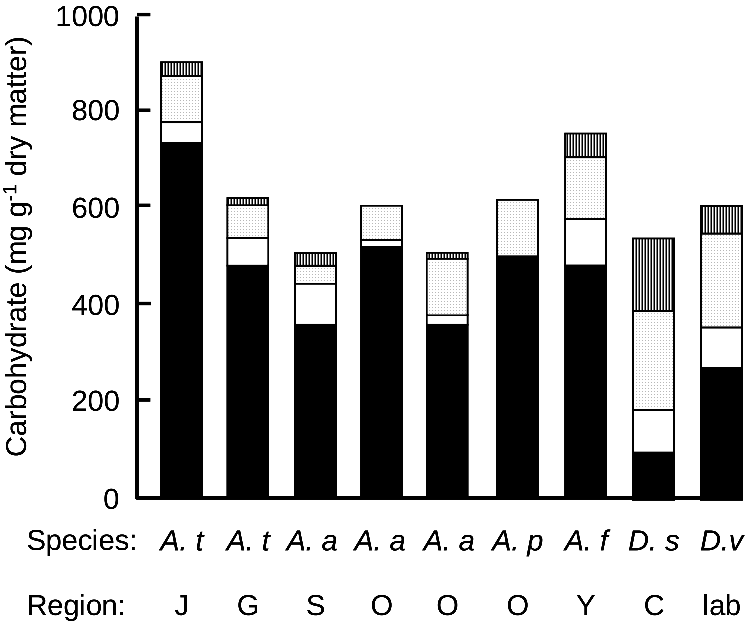

Figure 5.9 Soluble carbohydrate patterns in *Agave tequilana* (*A. t*), *A. angustifolia* (*A. a*), A. *cantala* (*A. c*), *A. potatorum* (*A. p*), *A. fourcroydes* (*A. f*), *Dasylirion* spp. (*D. s*) and *Dahlia variabilis* (*D. v*, a laboratory standard). (redrawn from Mancilla-Margalli & Lopez 2006).

Key: fructans (solid bar), sucrose (open bar), fructose (stippled bar) and glucose (hatched bar). Plants were grown in Mexico in the field at Chihuahua (C, 29^o 30'N, 104^o 30'W, 800m a.s.l., 100-300 mm, 33/2^{o}C), Guanajuato (G, 20^o 26'N, 101^o 43'W, 1780m a.s.l., 700-800 mm, 24/18^{o}C), Jalisco (J, 20^o 32'N, 103^o 40'W, 2000m a.s.l., 705-870 mm, 22/8^{o}C), Oaxaca (O; for *A. a* 16^o 52'N, 96^o 23'W, 1740m a.s.l., 800-2000 mm, 31/8^{o}C; for *A. c* and *A. p* 16^o 30'N, 97^o 59'W, 1440m a.s.l., 600-1500 mm, 24/16^{o}C), Sonoran (S, 29^o 26'N, 110^o 23'W, 380 m a.s.l., <400 mm, 32/15^{o}C), Yucatan (Y, 20^o 58'N, 89^o 37'W, 10 m a.s.l., 700-1110 mm, 40/17^{o}C), or in the laboratory

Table 5.6 The six major types of fructans (Mancilla-Margalli & Lopez 2006)

Fructan type	Characteristic structures	Examples
Type I: linear inulin	β(2-1)-fructofuranosyl linkages	widely described in Asteraceae
Type II: levan (phlein)	with β(2-6) linkages	in grasses eg *Phleum pratense*
Type III: graminans	mixed, usually branched fructans containing type I and II linkages	in wheat and some Asparagales
Type IV: inulin neoseries	contain a glucose between two fructofuranosyl units extended by β(2-1) linkages	in onion and asparagus
Type V: levan neoseries	contain β(2-1)- and β(2-6)-linked fructofuranosyl units on either end of a central sucrose molecule	in oat
Type VI: agavins	contain internal α-D-Glc*p* and β(2-1)- and β(2-6)-linked fructofuranosyl units	*Agave* and *Dasylirion* spp.

Even though usually present as a heterogeneous mixture with varying degrees of polymerization, the type and specific structure of fructans can be species indicative and may have use as taxonomic markers (Bonnett et al 1997, Sims et al 2001, Sims 2003, Peralta-Garcia et al 2007), within the limits of influences by the environmental conditions and developmental stage of the plant. In general, WSC distribution is similar in *Agave* species from the same region (*A. angustifolia*, *A. potatorum*, and *A. cantala* from Oaxaca), whereas it differs in the same species grown in different environments (*A. tequilana* and *A. angustifolia*). It should be noted that the simple sugars extracted in the juice may differ between species, for example, *A. salmiana* contains xylose (Michel-Cuello et al 2008).

The molecular structure of *A. tequilana* fructans was not revealed until Lopez et al (2003) reported that 8 year-old plants contained, in addition to the simple sugars glucose and fructose, a complex mixture of highly branched fructooligosaccharides frequently containing a single glucose moiety. These fructans mainly contained β(2→1) linkages, although β(2→6) branch moieties were present, and had degrees of polymerisation from 3 to 29 units. Subsequently Mancilla-Margalli & Lopez (2006) proposed general structures for three groups of fructans in *Agave* (Figure 5.10). On the basis of the presence of an internal α-D-Glcp in addition to branched linkages these fructans have been termed agavins. Broadly similar carbohydrates have been observed in *A. Americana* (Ravenscroft et al 2009).

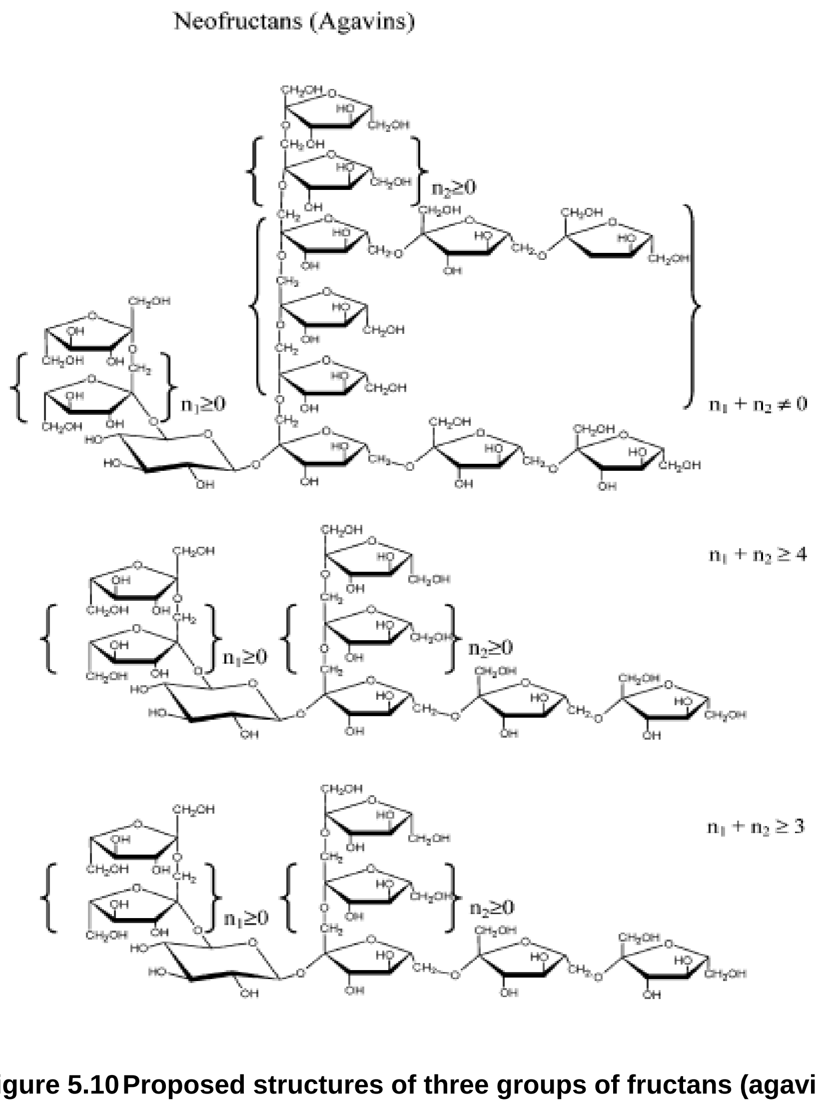

Figure 5.10 Proposed structures of three groups of fructans (agavins) from Agave and Dasylirion. N varies according to species and environmental conditions (from Mancilla-Margalli & Lopez 2006).

Traditionally, deleaved stems are cooked in ovens or in autoclaves to hydrolyze the fructans and soften the stems, which are then cut and shredded to facilitate aqueous extraction of the fructose-rich juice that becomes the fermented broth (Ávila-Fernández et al 2009, Waleckx et al 2008). Although traditional extraction methodologies are still frequently used, the spectacular growth of the tequila industry has resulted in the introduction of modern production technologies (Casas 2006). For example, in “diffusers” a countercurrent contact between shredded uncooked *Agave* and water is optimized, allowing for high extraction efficiency with little loss of fructan associated with the residual fibers. After extraction, chemical (thermal/acid) hydrolysis in autoclaves of fructan in solution is performed.

Enzymatic hydrolysis of polysaccharide is also being explored with the long-term aim of reducing energy requirements, enhancing hydrolysis efficiency (e.g. minimizing the production of phenolics and hydroxymethylfurfural), and simplifying the production process, as hydrolysis and extraction could take place in a single operation (Ávila-Fernández et al 2007, 2009).

An important limitation for tequila producers that is not a constraint for producers of *Agave* as a biofuel feedstock, is that ethanol produced for beverages needs to contain compounds that are important to the final taste of the beverage. As a result the extraction of sugars by tequila producers is modified to allow the co-extraction of more volatile flavour components.

5.5 Climate change and CAM/*Agave* productivity

No reports of responses by *A. tequilana* to elevated [CO_2] (e[CO_2]) were detected by us in our search of the literature. Nonetheless, CAM plants do respond to e[CO_2] (Table 5.6, Figure 5.11) and the formulation of best agronomic practice for production of *A. tequilana* as a biofuel feedstock will require an understanding of how the species and its varieties respond to e[CO_2] (Nobel 1996, Borland et al 2009). Similarly, the responses of *A. tequilana* to changes in the variability and abundance of rainfall, and increases in temperature expected to be associated with climate change during the 21st century will need to be assessed.

The small number of New World CAM plants, that include species of *Agave*, that have been tested at e[CO_2] exhibited average increases in biomass of 35% when grown at double ambient (≈ 700 ppm) [CO_2] (Tables 5.7 and 5.8; Nobel & Hartsock 1986, Nobel 1991b, Nobel & Israel 1994, Drennan & Nobel 2000, Nobel 2000). Such responses of net CO_2 gain to e[CO_2] are similar to those of C_3 species and greater than those of C_4 species.

Acclimatization to e[CO_2] by plants is often associated with build-ups of soluble sugars and polysaccharides that inhibit photosynthesis (feedback inhibition, Stitt 1991). To our knowledge, there are no reports about the effects of e[CO_2] on carbohydrate accumulation in stems of *Agave*. However, for the highly productive desert CAM species, *O. ficus-indica*, subjected to double ambient [CO_2], soluble sugar and polysaccharide contents increases of >60% did not down-regulate photosynthesis (Nobel & Garcia de Cortázar 1991, Cui et al. 1993; Nobel & Israel 1994, Nobel et al 1994), indicating that carbohydrate accumulation was not limiting cladode photosynthesis (Nobel et al 1996, Wang & Nobel 1996). Increased sink strength in daughter cladodes (analogous to the stem and meristem in *Agave*) was associated with increased phloem transport to them (Wang & Nobel 1995, 1996). Greater glucose and malate concentrations in the sink tissues under e[CO_2] may increase the osmolality of sink cells and thus decrease the turgor pressure of the phloem in the sink, resulting in a more rapid movement of photoassimilate into the daughter cladodes (Wang & Nobel 1996, Drennan and Nobel 2000).

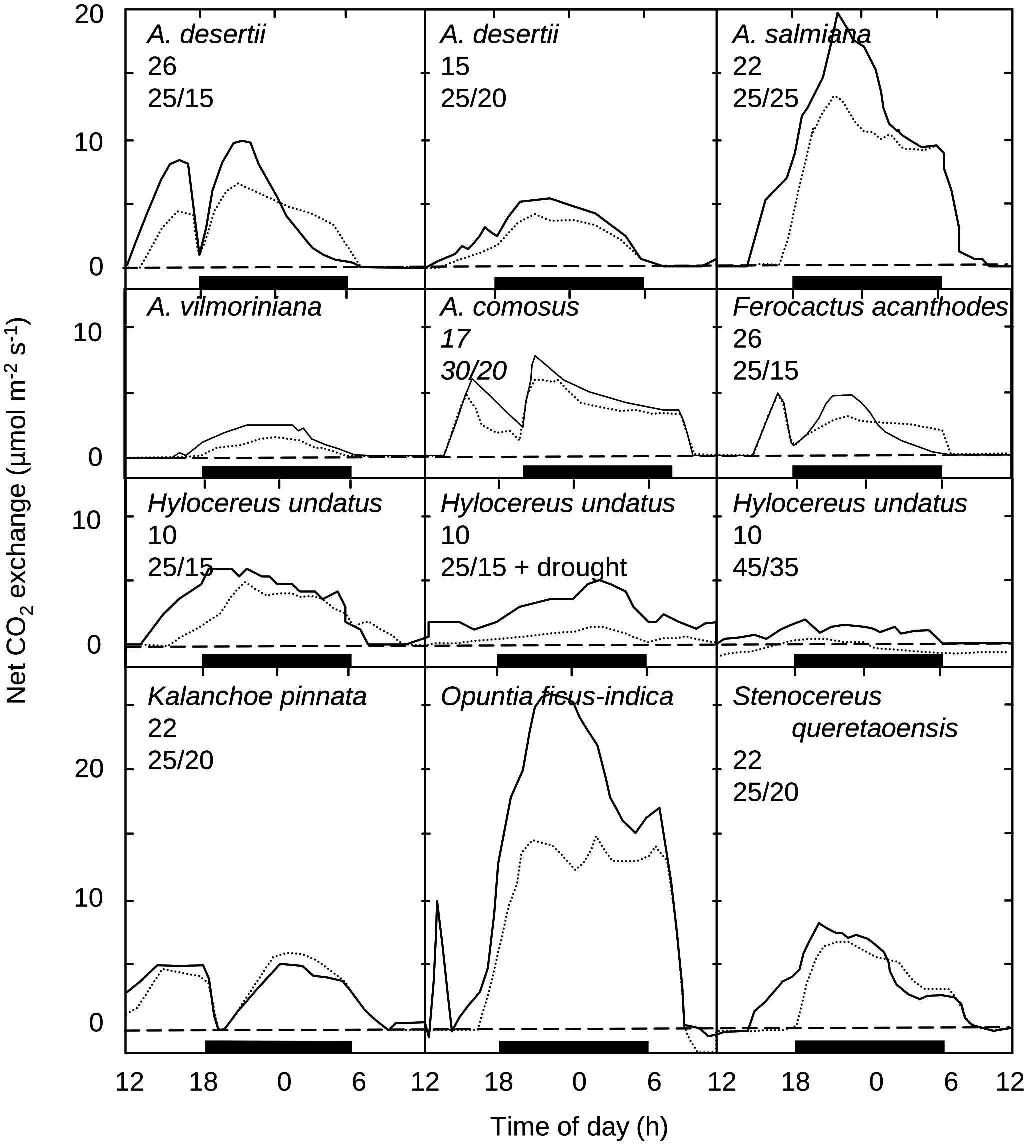

Figure 5.11 Daily time-courses of the CO_2 exchange for a range of CAM species under the current atmospheric [CO_2] (dotted line) and after acclimatization to a doubled atmospheric [CO_2] (solid line) under well-watered conditions except where stated otherwise. In each panel is listed the species and the PPF (mol m^{-2} d^{-1}) and day/night air temperatures ($^oC/^oC$) at which it was grown (see Drennan & Nobel 2000 for references and original figure).

The majority of CAM species subjected to e[CO_2] for extended periods exhibit night-time increases in titratable acidity or malic acid accumulation, indicators of increased dark CO_2 uptake (Figure 4.1). The kinetics vary between species indicating that a range of traits are responsible (Drennan & Nobel 20000). These include interactions between the succulent nature of CAM plant photosynthetic organs and the associated constraints to CO_2 diffusion (Drennan & Nobel 2000, Borland et al 2009).

Table 5.7 Responses of agavaceous species and *Ananas comosus* (pineapple) to elevated atmospheric CO_2 concentrations (from Drennan & Nobel 2000).

Species	Productivity	Morphological changes	CO_2 uptake	Enzymes
Agave deserti	Increased[1a]	Leaves thicker & longer[1], chlorenchyma thicker[1], root cell length increased[2]	Afternoon & night-time uptake increased[1,3], WUE increased[1]	PEPCase decreased[1], Rubisco decreased but activated *in vivo* % increased[1]
Agave salmiana	Increased[4]		Afternoon and night-time uptake increased[4,5]	PEPCase decreased[4], Rubisco decreased[4,5] but activated *in vivo* % increased[4], PEPCase K_M decreased[4]
Agave vilmoriniana	Increased[6]		Night-time uptake increased 8-12%[7]	
*Yucca schidigera*a			Increased[8]	
Ananas comosus	Increased [9-11]	Root : shoot ratio increased[9], leaf thickness increased[9]	Increased morning and night-time uptake[9-12], WUE increased[11]	

[a] References: 1 Graham & Nobel (1996), 2 Drennan & Nobel (1996), 3 Nobel & Hartsock (1986), 4 Nobel et al (1996), 5 Nobel (1996), 6 Idso et al (1986), 7 Szarek et al (1987), (8) Huxman et al (1998), 9 Zhu et al (1997a), 10 Zhu et al (1997b), 11 Zhu et al (1999), 12 Crewes et al (1975)

For *A. deserti*, *A. comosus* and *O. ficus-indica*, despite the greater contribution of CO_2 uptake in the light to the total net daily CO_2 gain, WUE increased at e[CO_2] (Tables 5.8 and 5.9; Cui et al 1993, Graham & Nobel 1996, Zhu et al 1999, Drennan & Nobel 2000). The increase in WUE, and associated higher plant water content, is the result of increased daily net CO_2 uptake and decreases in stomatal conductance.

The majority of CAM species appear to exhibit maximum daily net CO_2 gain at day/night air temperatures of ~25/15 °C irrespective of [CO_2] (Figure 5.11, Drennan & Nobel 2000). By increasing the O_2:CO_2 ratio at the site of CO_2 fixation increased temperatures increase photorespiration but e[CO_2] offsets the increase and may thus contribute to the enhancement of daily net CO_2 gain observed at elevated temperatures for well-watered *A. deserti*, *O. ficus-indica*, *H. undatus and A. comosus* (Nobel & Israel 1994; Raveh et al. 1995; Graham & Nobel 1996; Zhu et al. 1999).

Table 5.8 Response of biomass and net CO_2 gain of CAM species to long-term (> 1 month) exposure to about double ambient [CO_2] (from Drennan & Nobel 2000, Tables 2 and 3).

Species	Biomass increase and (experiment duration)	Change in daily net CO_2 gain	Change in CO_2 gain during the light	
			Ambient [CO_2]	Elevated [CO_2]
	% (months)	(%)	(%)	(%)
Agave deserti	30 (12)	2	22	38
	31 (17)	49	17	24
Agave salmiana	17 (4)	36	15	23
Agave vilmoriniana	28 (6)			
Ananas comosus	23 (4)	15	19	33
Ferrocactus acanthodes	30 (12)			
Opuntia ficus-indica	40 (12)	41–152	-4	17

Table 5.9 Water-use efficiencies (WUE) for CAM plants under ambient and double ambient [CO_2] (from Drennan & Nobel 2000, Table 4)

	WUE (mmol CO_2 mol^{-1} H_2O)	
Species	Ambient [CO_2]	e[CO_2]
Agave deserti	20	42
Ananas comosus	9·5	13
Opuntia ficus-indica	4	7

6 *A. tequilana* as an Australian industry

6.1 Production of *A. tequilana* in Australia: the Ausagave project

In 2003, agronomist Don Chambers of Ausagave identified *A. tequilana* as a crop with potential for areas of seasonally-limited rainfall in Australia. The opportunities for commercial production of *A. tequilana* related to its extremely high sugar and fibre content, and the nature of the sugars. Following travel to Mexico and discussions with Mexican growers and processors in 2004 and 2005, varieties were sourced, export permits obtained, and plants were imported into Australia following submissions to AQIS. Ausagave developed tissue culture propagation techniques and performed basic pot trials under controlled glasshouse conditions.

A desktop study in 2006/07, based on a go/no-go outcome, concluded that *Agave* could be a viable crop in Australia. There is a strong demand for ethanol that is well-documented, considerable demand for fructose-based sugar/sweeteners and oligofructoses, and potential uses for the low-lignin fibre and some secondary metabolites. However, successful implementation of the crop in Australia requires:

1 Production be located correctly: Ausagave identified potential production areas using a model that was developed following inputs from CSIRO (Scion Australasia Limited and CSIRO FFP Pty Limited, participants in an unincorporated joint venture, trading as Ensis), the Bureau of Meteorology (BOM), and Dr JAM Holtum, an academic with expertise in the physiology of succulent plants. The results from the original model inspired Ausagave and BOM to generate a ‘growth day’ model based on optimum minimum and maximum temperatures. Areas near Childers, Rockhampton, Mackay, Ayr and Mareeba have been identified as potential production sites on the basis of the concordance of the modelled climatic and edaphic requirements of *A. tequilana*, the availability of processing infrastructure, and the demand from sugar-cane and sweet sorghum producers for the production of feedstock options. Ausagave is seeking further assistance from RIRDC to establish field trials in these areas.

2 Efficient propagation methods: plants need to be multiplied-up efficiently (vegetative propagation is used in Mexico). Development by Ausagave of a tissue culture protocol has surmounted this problem and produced sufficient plants for the first year of trials. Plants can be deflasked into a plug to enable more efficient mechanisation (Figure 6.1).

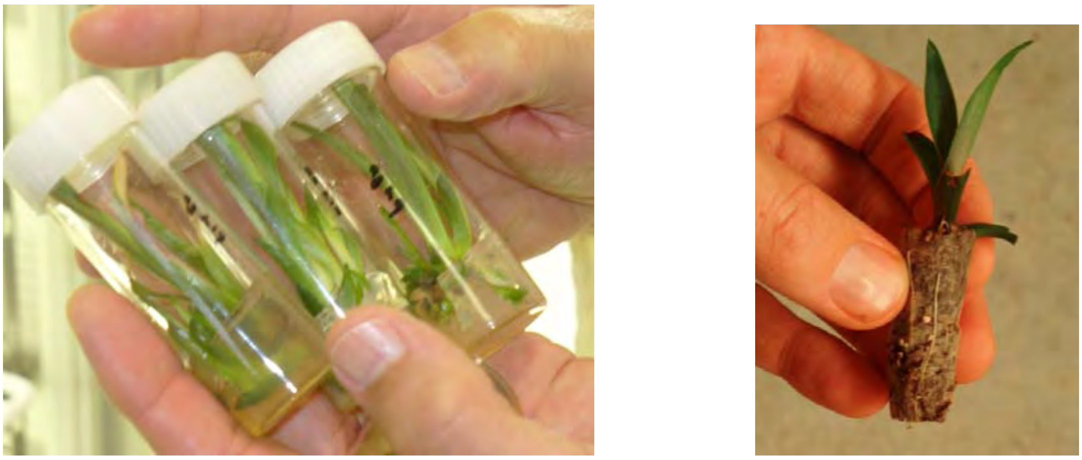

Figure 6.1 Ausagave *A. tequilana* during tissue culture (left panel) and following deflasking into a plug (right panel)

3 Production is mechanised: planting, leaf trimming and harvesting must be mechanised in Australia (hand labour is commonly used in Mexico). In Mexico, some large plantations have adapted plant and equipment developed for the sugar-cane industry for *Agave*. Australian equipment manufacturers are of the opinion that equipment used in Australia can be modified for pruning and harvesting *Agave* (Appendix 3). Field trials are required to provide plants and product that can be used to test and evaluate prototype plant and equipment.

4 Viable business plans: financial models, with independent assessment of the potential income and expenses, need to be constructed and tested for growers, investors and large scale corporate operations. Ausagave and BDO Kendall have developed an interactive model that compares *A. tequilana* with sugar cane. The model has used a 400 ha plantation as an example, showing potential gross margins, costs of production and a returns in chapter 7. Again, field trials are required to establish actual cost of production for crops grown in several locations in Australia.

5 Demonstration of production potential in Australia: growers and processors have expressed considerable interest in the crop, but all agree that *A. tequilana* will not be taken up as a commercial crop until plants are grown, maintained and harvested in Australia under normal agronomic conditions, product is produced and evaluated, and yields and risks are evaluated – these field trials would provide product (juice and fibre) for testing and evaluation.

Whilst no crop of *A. tequilana* has yet been grown in Australia, Ausagave has identified potential co-operators for field trials, planned for the Tablelands, Burdekin, Mackay, Rockhampton and Childers districts in north Queensland. Funding is currently being sought for the trials, which will need to run for five years. During the trials, CO_2 exchange, nitrogen and water dynamics, carbohydrate content and constituents, system inputs and outputs, and sucker-formation will be monitored. Yield will be cross-referenced to Mexican observations and used to fine-tune the farming system (extension protocols and expertise), generate further selection criteria for future demonstration sites (as a stand-alone crop, or in combination with sorghum or sugar cane farming systems), and assess the costs of production.

Processing of plants from the trial sites will test the suitability of harvesting and transport infrastructure and equipment and will provide material to processors in order that they can test extraction methodology, juice yield and composition, and assay for biofuels production. Samples will also be provided to potential producers of value-added *Agave* products. Data will be analysed in terms of expectations from the desk-top study. Predictions from pre-experimental models will be confirmed or modified. Observations will be provided to collaborators to assist development of robust life cycle assessments for *Agave* farmed in Australian agronomic environments.

Because of the importance of the trials, and the potentially steep agronomic learning curve for us, we feel that it is important to reinforce relationships with researchers in Mexico. For us, this will facilitate the exchange of information and the fast tracking of industry knowledge. To this end, we have applied for funds to visit Mexico and for a Mexican researcher/agronomist to visit Queensland.

6.1.1 Comparison of Australian and Mexican climates

On the basis of areas cultivated in Mexico, we developed a model to locate appropriate climates and soils in Australia and then considered local infrastructure and market potential to target initial production areas where field trials should be undertaken. In the selected locations, processing infrastructure is present and many landowners are looking for new crops that will assist them to diversify.

Both Mexico and areas of Queensland experience wet warm summers and cool dry winters (Figure 6.2). The principal differences between the Mexican environments where *A. tequilana* is grown, notably in Jalisco State, and the Queensland areas where we propose growing the crop is that winters are cooler in Mexico as are the hottest months. Presumably these differences are mainly the result of the sites in Jalisco being at higher altitudes than the Queensland sites. We note that many of the Australian sites with potential for growing *Agave* experience more cloud-free days per annum than in Mexico.

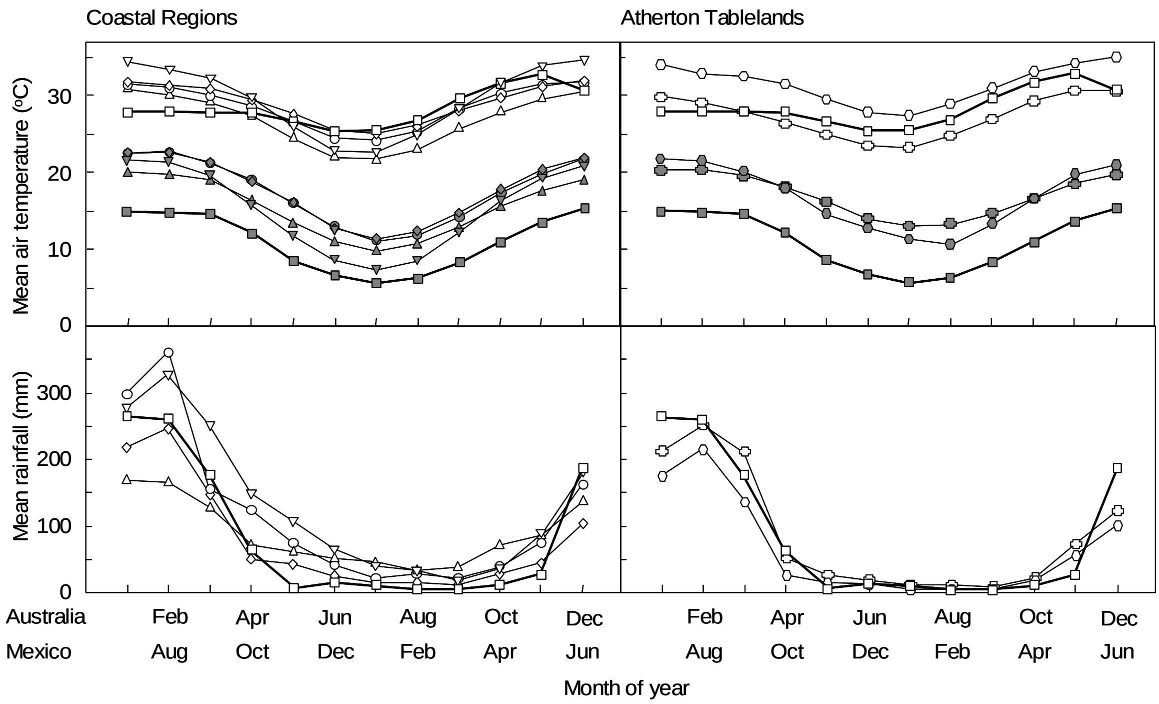

Figure 6.2 Mean monthly maximum (open symbols) and minimum (closed symbols) temperatures (upper panel) and mean monthly rainfall (lower panel) of potential trial sites for *A. tequilana* along east coast regions of Queensland (left panels) and the Atherton Tablelands (right panels), Australia, in comparison to Tequila, Mexico (□, ■; lat. 20.88°N, long. 103.83°W, 600 m a.s.l.). (BOM 2009).

The coastal sites are Ayr (◇, ◆; 52 year mean, lat. 19.62°S, long. 147.38°E, 12 m a.s.l.), Childers (△, ▲; 60 year mean, lat. 25.24°S, long. 152.28°E. 109 m a.s.l.), Mackay (▽, ▼; lat. 21.12°S, long. 149.22°E, 30 m a.s.l.) and Proserpine (○, ●; 20 year mean, lat. 20.49°S, long. 148.56°E, 20 m a.s.l.). The Atherton Tableland sites Dimbulah (, ⬣; 11 year mean, lat. 17.15°S, long. 145.11°E, 407 m a.s.l.) and Walkamin (, ✚; 41 year mean, lat. 17.13°S, long. 145.43°E, 594 m a.s.l.)

6.1.2 Comparison of Australian and Mexican soils

A. tequilana is grown in many soil types in Mexico as described earlier, with the emphasis on mineral soils that impart certain flavours and characteristics to the tequila. In many cases the soil nutrient levels are marginal, as this slows the growth of the *Agave* plant, a factor considered important for the development of secondary compounds that impart flavour to tequila. The higher yielding crops are mostly grown on well-drained volcanic and red Krasnozem soils with a slightly acid pH. The soil composition varies across the proposed Australian growing regions from well-drained volcanic red soils on the Atherton Tablelands to more sandy and clay-containing soils on the coast. For production of *A. tequilana*, the main soils to avoid are those prone to water-logging. Most free-draining agricultural soils should provide an adequate base. The nutrient status will determine any fertilizer requirements.

6.2 Cultivation practices in Australia

A. tequilana has yet to be grown commercially in Australia and has never been grown for biofuel feedstock production. Cultivation conditions thus have to be estimated based on current practices used to grow *Agave* species in Australia at the beginning of the 20th century (cf chapter 9) and on the basis of systems used to grow *A. tequilana* for tequila production in Mexico (Cedeño Cruz & Alvarez-Jacobs 1999, Cedeño Cruz 2003).

Plant Propagation: as discussed earlier, replanting in Mexico is performed with suckers (ramets derived from rhizomes) that are separated at the age of 3-4 years (Sánchez 1991, Valenzuela 1992, Valenzuela-Sánchez et al 2006). Suckers are a cheap source of planting material compared to seed or tissue culture but provide no genetic variability as all plants are clones. Ausagave has developed tissue culture protocols that will enable rapid multiplication of robust disease-free plants. Nevertheless, adequate variation will require a breeding programme, probably best undertaken in conjunction with Mexican researchers. The tissue culture process has been refined to utilize 'plugs' that generate strong young plants amenable to a rapid mechanical planting process.

Varieties: Ausagave selected and imported to Australia 27 robust, phenotypically different commercial varieties from various locations and growers to provide genetic variability that could be tested and trialled in Australia. Most were selections of *A. tequilana* with two of *A. angustifolia*. It is noted, however, A. *tequilana* var. Azul growing in fields over 100 km distant from each other was genetically similar. Indeed, the crops were one of the most genetically uniform populations ever encountered in the history of evaluating plant populations for genetic diversity (Vega et al 2001).

Planting and Plant Density: Historically, A. *tequilana* is planted in row widths that suit both hand labour and harvest, where donkeys still cart the piñas from the field. Planting is done by hand at a density of around 2,000-4,000 plants ha^{-1}. In recent years plant densities have been increased and plants are being harvested earlier. For Australian plantings, Ausagave has decided on a density of 5,000 plants ha^{-1}, which will be mechanically planted in row spacing of 2 metres.

Fertiliser: *Agave* fertilization is based on soil composition, plant age and growth rate. Typically urea is the nitrogen source, with up to 250 kg ha^{-1} added directly into the soil. In some areas, phosphorus and potassium fertilization are added. The initial trials planned by Ausagave will use a pre-plant NPK followed up by side-dressing as the crop grows. The rates and types of fertilizer will be based on soil and leaf tests balanced against the nutrient removal predictions.

Irrigation: In Mexico, nearly all *A. tequila* is rain-fed and this is the intention for production in Australia. However, a pre-plant irrigation may benefit in areas of low soil moisture at planting.

Pest and Weed Control: Stands of Agave naturalised in Australia are generally healthy, consistent with a low threat from local pests and diseases. Ausagave has conducted limited trials and has observed botrytis infestation when plants are grown under continuous extremely high humidity. The major pests observed to date have been garden snails and rabbits. A. *tequilana* grows slowly in the early years and is a poor weed competitor. Ausagave has undertaken some initial screening of herbicides, but field trials will be critical in identifying potential control measures for use within mass production systems.

Pruning and sucker removal: Discussions between Ausagave and the IBS – Centre of Engineering Innovation suggest that pruning can be performed using a modified hedge-row vine trimmer with the leaves transferred to a haul-out vehicle for transport to a processor. The trimming knives will most likely be replaced with cutting discs. A prototype for sucker removal will be based on the 'vine dodger' concept using a rod weeder mechanism to remove and windrow the plants.

Harvesting: Unlike most crops that have specific harvest times, *Agaves* can be harvested all year round, although once the flower spike begins to develop the sugar content of the stem decreases rapidly. Field testing will confirm the best times for harvesting under Australian conditions. The most appropriate harvest time may be influenced by the availability of processing equipment and the final end-use.

As yields can vary between 300 and 500 t ha^{-1}, with the weight of a harvested plant varying from 60 to 120 kg, it will be essential to have a robust mechanical harvester. Ausagave has had discussions with IBS – Centre of Engineering Innovation and the current plan is to use a modified whole-stick cane harvester as the 1st prototype, matched to a suitable haul-out vehicle. Prototypes have been developed in South Africa, but will need to be tested in the field. Trials will allow this.

The sites in coastal Queensland identified as potential *A. tequilana* cropping areas all have access to processing mills and support a large number of potential growers familiar with the sugar industry. However, the coastal climate is subject to a substantial wet season. Despite growing plants in raised beds, in the absence of trials we do not know how the crop might respond to prolonged soil saturation. It may be that regions further from the coast may offer the best long-term production areas. Dimbulah (Atherton Tablelands) has a similar climate to Jalisco (Figure 6.2), supports entrepreneurial growers who have previously cultivated tobacco, and is close to a sugar processing plant.

6.3 An opportunity for Mexico – Australia technology exchange

A. tequilana has long been grown in Mexico but not in Australia. The potentially steep agronomic learning curve for Australian industry and researchers can be made easier by developing relationships between Mexican and Australian industry and researchers, thereby fast-tracking the dissemination of information.

6.4 Potential range of products from *A. tequilana*

Agave has potential as a bio-refinery crop, a crop in which all parts of the biomass are utilized thus adding value to feedstock produced for bioenergy. As such it could provide further diversification of income for producers.

Aside from the production of alcoholic beverages and sugar replacement, *Agave* leaves contain soluble sugars that can be extracted and low-lignin cellulose fibres (roughly 22 % of above-ground dry weight) that can be used for generation 2 ethanol production. Alternatively dried leaves can be used for energy cogeneration. The oligofructans have potential for polymerization and esterification into compounds such as rayon and cellophane, amongst others.

The combined attributes of fructan chemistry in terms of texture, solubility, sweetness and low digestibility have resulted in their widespread use in the food industry in roles that include:

- texture improvement – yoghurts, bakery products, cheese, soft drinks, pet food
- sugar reduction – dental health products, diabetic products, ice-cream, sweets, pet food
- fibre enrichments – baked foods, bread, yoghurt, cereal bars, per food
- fat substitution – meats, yoghurts, sweets, cheese, ice-cream, fat spreads, pet food
- stabilizer – yoghurt, dairy drinks, soft drinks
- speciality - tequila nectar

By enhancing the growth of beneficial *Bifidobacteria* in monogastrics (such as humans, pigs, dogs, cats etc) fructans aid digestion. Associated benefits that have been reported include reductions in allergies and reduced antibiotic use in livestock. Pharmaceutical and nutraceuticals industries have interest in fructan metabolism because the prebiotic control of *Bifidobacteria* has associated potential applications in reducing of risks of diseases such as constipation, infectious diarrhea, some cancers, osteoporosis, atherosclerotic cardiovascular disease, obesity, and non-insulin dependent diabetes. Currently the principal sources of commercial-grade fructans in Australia, chiefly inulin, are chicory roots from Europe and South America (Monti et al 2005) and Jerusalem artichoke from China.

7 A financial case for production of *A. tequilana* in Australia

7.1 Estimates of production

Agave tequilana has yet to be grown under agronomic conditions in Australia and thus the following financial calculations are based on surrogate values and should be regarded as indicative. Production values are based on those reported for plants grown for tequila production in Mexico. The costs of cultivation, maintenance and harvesting are estimated from costs of growing other crops in Australia, and gross income is based upon price estimates provided by processors in Queensland. We have assumed a 5 year cycle similar to that used by some modern tequila producers. However, it has yet to be demonstrated that such a cycle is preferable for growing *Agave* for biofuel production. It may be that a shorter cycle is preferable if the rate of sugar accumulation is greater in younger plants. Such information can only be supplied from field trials. Some of the assumptions used for preparing the financial case for growing *A. tequilana* in Australia are presented in Table 7.1.

We have assumed a harvestable plant mass of 80 kg, with a stem mass of 45 kg (~56 % of whole plant mass), which seems to be the industry norm for plants grown under well-managed conditions in Jalisco (Table 7.2). According to recent reports from Mexico (e.g. Burger 2008 and Velez-Jimenez 2008, not peer reviewed and plants not seen by us) *Agave* varieties with stem fresh weights of over 200 kg have been developed. These plants have reportedly been dry grown in marginal areas; however, we believe the marginal areas were within a summer rainfall region receiving up to 800 mm rainfall *per annum*.

Ausagave has access to many of these new varieties through its association with colleagues in Mexico, and is expecting plants ready for trials later in 2009.

The proposed plant density of 5,000 plants ha^{-1} is higher than that typically used in Mexico. However, in Mexico plants space between rows is required for movement of farm labour. We anticipate that mechanical pruning and harvesting will permit closer spacing of rows in Australian crops and the use of plant and machinery that straddles the rows will again provide the opportunity for higher plant densities.

The 5 year cycle referred to above needs to be tested in Australia against the sugar content and the cellulosic yields of *A. tequilana* in various locations. The 5 year cycle is also convenient in the comparison to sugar cane, as many cane crops are grown on a 5 year rotation. It should be noted that many tequila producers still prefer waiting up to 8 years or more to have the slower growing plants provide enhanced flavours for the production of tequila. Such agronomic procedures are not relevant when plants are grown for biofuel feedstock.

Table 7.1 Assumptions used to calculate indicative financial bases for the production of *Agave tequilana*.

Character	Assumed value	Comments
Total soluble sugars (TSS)	28 %	On a wet weight basis
Total fermentable sugar (TFS)	24 %.	89 % conversion from TSS to TFS
Ethanol per tonne TFS	600 L	
Price for TSS	\$300 t^{-1}	Conservative estimate
Plant density	5,000 ha^{-1}	Greater than unirrigated systems used for tequila production
Typical plant mass at harvest	80 kg	Above-ground mass, all organs
Gross yield	400 t ha^{-1}	Wet harvest weight over 5 years @ 5,000 plants ha^{-1}
Fibre content	32 %.	On a wet weight basis
Ethanol from cellulose	400 L t^{-1}	We assume that the conversion operates at 60% efficiency i.e. 240 L t^{-1}
Price for fibre	\$40 t^{-1}	
Bagasse	40%	43% cellulose, 19% hemicellulose, 15% lignin, 3% total nitrogen, 1% pectin, 10% residual sugars and 9% other compounds

Table 7.2 Assumed composition of an *A. tequila* crop at year 5 after planting.

Plant organ	Plant mass and composition			Crop mass and composition at 5,000 plants ha^{-1}		
	Mass	Sugar	Fibre	Mass	Sugar	Fibre
	kg wet mass	%	%	t ha^{-1}	t ha^{-1}	t ha^{-1}
Stem	44.8	28	26	224	63	58
Leaves	28.8	20	36	144	29	52
Other	6.4	10	50	32	3.2	16
Total	80	24	32	400	95	126

Under the assumptions of Tables 7.1 and 7.2, the annualized production of ethanol from *A. tequilana* is likely to be at least equivalent to that of sugar-cane (Table 7.3). *A. tequilana* contains a higher percentage of sugars than does sugar-cane, whereas it is expected that sugar-cane will yield greater biomass over a 5 year cycle.

Table 7.3 Comparison of ethanol production from sugars extracted from *A. tequilana* and sugar-cane. Assumptions for *Agave* were as per Tables 7.1 and 7.2. Sugar-cane calculations assume 100 t ha^{-1}.

Species	Total soluble sugars	Ethanol
	%	L ha^{-1} year^{-1}
Agave tequilana	24	10,230
Saccharum officianarum	14	7,560

Like many agaves, *A. tequilana* leaves and stems contain appreciable fibre, about 32 % on a wet weight basis, in the form of bundles that are 23–52 cm long and 0.6–13 mm wide (Iñiguez-Covarrubias et al 2001). The fibre is a potential source of ethanol production (Table 7.4). The low-lignin high-cellulose constitution of the fibre suggests that current cellolosic technologies may be sufficiently efficient to convert the cellulose to sugars (or other products). The conversion of fibre to ethanol could significantly increase the gross margins for *A. tequilana*.

Table 7.4 Potential total plant ethanol yield per hectare over 5 years from *A. tequilana*.

Plant part	Ethanol		
	Sugar	Fibre	Total
	L ethanol ha^{-1}		
Head	33,869	13,978	47846
Leaves	15,552	12,442	27994
Other	1,728	3,840	5568
Whole plant	51,149	30,259	81,408

7.2 Gross margins

A comparison of cumulative gross margin for sugar-cane and estimated cumulative gross margin for *A. tequilana* suggests that gross margins are likely to be comparable for *A. tequilana* and sugar-cane (Figure 7.1) in a 2 crop situation. In reality it is more likely that a farmer will plant less area per year but will planting each year to ensure a continuous supply of product with a similar area under crop.

The cost of planting material is the single largest expense. Once many plants are being produced it is likely that the cost per plant will reduce significantly. In addition, the model does not include the value of the suckers that can be used to plant additional areas.

The return for the A. *tequilana* juice (TSS) has been calculated on the basis of a conservative \$300 t^{-1}. Several processors have indicated that the price should more likely be around \$400 t^{-1}, the proce assumed for sugar-cane. Likely price increases for energy, fertilisers and water over the next 5 to 10 years should enhance the profitability of A. *tequilana* as it is a low input crop.

In areas where cane is less productive A. *tequilana* would be extremely competitive based upon the projected returns and costs of production assumed herein. The early establishment costs of *Agave* will be a key decision-making issue, as it may take 3 to 4 years before the grower breaks even. The example in Figure 7.1 has been based on a farmer contracting the planting and harvesting.

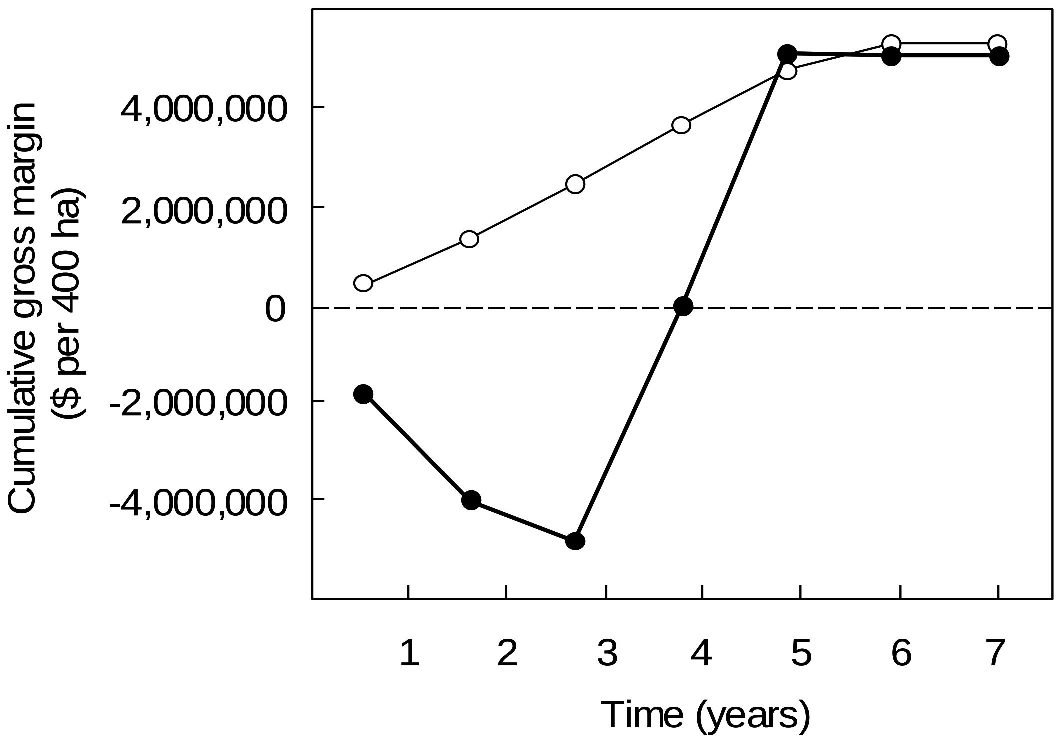

Figure 7.1 Cumulative gross margin for a 400 hectares of sugar-cane (○, ref) and estimated cumulative gross margin for 400 hectares of *A. tequilana* (●).

The calculations assume: for *Agave* – 5,000 plants ha^{-1}, 200 ha sown in year 1 and 200 ha sown in year 2, 200 ha harvested at year 5 and 200 ha harvested in year 6 with a yield of 400 t wet mass ha^{-1} (80 kg $plant^{-1}$) and 24 % fermentable sugars; for sugar-cane – 200 ha planted for 2 years and harvested each year with a yield of 100 t ha^{-1} $year^{-1}$ wet biomass and 14 % CCS. In order to err on the conservative side, sugar-cane sugar was priced at \$400 t^{-1} whereas *Agave* sugars were priced at \$300 t^{-1}.

The break-down of costs and returns in Table 7.5 is indicative only, more rigorous estimates await the outcomes of field trials. The gross returns are based upon the sale of TFS at \$300 t^{-1} and the bagasse at \$40 t^{-1}. Investment inputs i.e. the plants/planting material, will reduce after year 3 as the *A. tequilana* crop can supply replants via suckers.

We have not explored, or incorporated into the *Agave* business plan, non-ethanol markets for fructans or non-ethanol markets for cellulose. Fructans currently sell for prices that are two to three times that of sucrose. Sugarcane bagasse is primarily composed of 25% lignin, 25% hemicellulose and 40–50% cellulose (Pandey et al 2000) whereas bagasse from *A. tequilana* is about 43% cellulose; 19% hemicellulose and 15% lignin (Cedeño Cruz & Alvarez-Jacobs 1999, Cedeño Cruz 2003, Hernández-Salas et al 2009). The increased glucan and decreased lignin content of agave fibre may provide a good source of fermenting sugars produced by chemical and enzymatic hydrolysis, and saccharification.

Table 7.5 Estimated annualized gross returns, production costs and gross margins for sugar-cane (*Saccharum officianarum*) and *Agave tequilana.*

Production costs and returns for *A. tequilana* need to be tested using field trials.

	Saccharum officianarum		*Agave tequilana*	
	$ ha^{-1}	$ t^{-1}	$ ha^{-1}	$ t^{-1}
Gross Return	5,600	56	6,692	84
Investment inputs (cost of plants)	151	2	1,844	23
Operational expenses (soil preparation, planting, growing and harvesting)	2,840	28	2,321	29
Total Production Costs	2,991	30	4,165	52
Gross Margin	2,610	26	2,527	32

8 Agave as weeds

8.1 Legal status

8.1.1 Australian Federal legislation

Neither *A. tequilana* nor any other *Agave* species is on the following key lists that identify weeds in Australia

- Australian Government, Dept. of Agriculture, Fisheries and Forestry (DAFF) Exotic Weeds Watch List (Australian Government 2009)
- Weeds Australia, Australian Weeds Committee National Initiative National Portal weeds register (Weeds Australia 2009a)
- Australian Noxious Weeds Database (Weeds Australia 2009b)
- National Environmental Alert List (Australian Government 2009)
- DAFF/DEE Weeds of National Significance list (Australian Government 2009)
- Sleeper Weed list (Australian Government 2009)
- Species Targeted for Eradication (Australian Government 2009)

A. tequilana is on the Australian Quarantine and Inspection Service permitted entry list for nursey stock - species may be imported subject to the conditions C5012, C7301, C7302, C7300 (Australian Quarantine and Inspection Service 2009)

8.1.2 South Australian legislation

Neither *A. tequilana* nor any other *Agave* species is listed as a proclaimed weed by the South Australian Dept. of Water, Land and Biodiversity Conservation (Government of South Australia 2009)

A. americana and variegated *Agave* are listed as potential sleeper weeds in Australian Grazing Management Zones (GMZs) tropical savannas (GMZ 2), Mitchell grass downs (GMZ 3), southern Australian sheep and cattle grazing (GMZ 8), extensive sheep grazing (GMZ 9), highly modified rangelands (GMZ 10) on the basis of listings by Grice & Martin (2005). Inclusion of *Agave* in the list is precautionary as there is no evidence of weediness of the species in these areas to date (Grice AC 2009 personal communication).

8.1.3 Queensland legislation

No *Agave* species is a declared plant in Queensland (under the Land Protection (Pest and Stock Route Management) Act 2002) nor are they listed as class 1, 2 or 3 weeds (Government of Queensland 2009).

8.1.4 New South Wales legislation

No *Agave* is declared in New South Wales under the Noxious Weeds Act 1993 (Government of New South Wales 2009).

8.1.5 Western Australian legislation

No *Agave* species is on the December 2008 updated list of plants that are declared under the Agriculture and Related Resources Protection Act (Government of Western Australia 2009).

8.1.6 Northern Territory legislation

No *Agave* species are declared noxious plants or weeds in the Northern Territory Weeds Management Act 2001 (Government of the Northern Territory 2009).

In the Katherine Region *A. americana* is listed as a potential rangeland sleeper weed (cf. Grice & Martin 2005). There is no evidence of weediness of this species in the region to date (Grice AC 2009 personal communication).

8.2 Agave as an international weed

Agave spp. native to Central and South America have been cultivated in Europe since the 1500s, in Africa and Asia since the 1600s and in Oceania since European colonization (Irish & Irish 2000). In the first half of the twentieth century, sisal supplied about 70 % of the world's long hard plant fibers (Nobel 1994). It is therefore not surprising that agaves have become naturalised in a variety of regions (e.g. Tanzania, Kenya, Mauritius, Philippines; Marais & Coode 1978, Gentry 1982, Nobel 1994, Nobel 2003). It is noteworthy that even when plantations were abandoned *Agave* have been rarely weedy.

A. tequilana is not listed in the Global Compendium of Weeds (Randall 2002). Other Agaves, notably *A. sisaliana*, which have been grown commercially as sources of fibre for at least two centuries, have become naturalised in places and occasionally weedy[1] (Randall 2002). Weediness is best documented in South Africa and Hawaii. In Hawaii, *Agave* was introduced in the 1880s to start a cordage industry. Despite naturalization on all islands except Niÿihau and Kahoÿolawe, no *Agave* is a declared noxious plant in Hawaii. Agaves are easy to see and eradicate. Indeed agaves are still recommended for planting as barrier plants. The Hawaiian Plant- Weed Risk Assessment list (Daehler et al 2004), an assessment of several hundred species for invasive capability in Hawaii and neighbouring Pacific islands, does not include an assessment of *Agave* because it is not considered agressively invasive.

In South Africa, where *A. sisalana* has been planted across the landscape as a soil surface stabilizer in addition to its use as a commercial crop, it is a Category 2 declared plant (a weed with a commercial application and may only be grown in demarcated areas or in biological control reserves). However, even in municipalities with large weed problems, sisal is generally a minor component e.g. in the Umkhanyakude District it constitutes 2 % of weeds (Eichler 2004).

Although Clusius drew an *Agave americana* in Spain in 1546 (Irish & Irish 2000), weediness of *A. americana*, *A. fourcroydes* and *A. sisalana* was not reported until recently in sandy regions in the SE (Badano & Pugnaire 2004, Casimiro-Soriguer & Perez-Latorre 2008). The agaves, which are reproducing clonally and apparently have not been subjected to weed control, exhibit negative, positive or neutral effects on native species, depending upon the size and rooting depth of neighbours.

8.3 Native, naturalised and weedy Agavaceae

8.3.1 Agavaceae native to Australia

In Australia, the Agavaceae (subsumed in the Asaparagaceae in some recent taxonomic treatments) is represented by native genera of *Cordyline* (10 spp.), *Pleomele* (1 sp.) and *Doryanthes* (2 spp., generally placed in the Doryanthaceae) (Anon. 2006). The Australian Agavaceae generally inhabit wet and humid environments in contrast to the Central and South American Agave that are principally plants of arid and semi-arid environments.

8.3.2 Agavaceae naturalised in Australia

In Australia six exotic Agavaceae from the genera *Agave* (*A. americana, A. vivipara, A. sisalana*), *Furcraea* (*F. foetida, F. selloa*) and *Yucca* (*Yucca aloifolia*) are considered naturalised (Figure 8.1; Forster 1987a; Bationoff et al 2002, Anon. 2006). Infestations of Agavaceae are local and in many cases represent escapes from gardens or plantations that have been established for over 100 years (Forster 1987a, 1987b, 1988). *A. attenuata*, which has been widely cultivated in gardens, was not considered by Forster (1988) to be naturalised but is in the NT and Qld by the Australian Plant Census (Anon. 2006).

8.3.3 A history of cultivation of *Agave* in Australia

Agavaceae from Central America, including *Agave americana*, *Furcraea foetida*, *Yucca aloifolia*, *Y. filamentosa* and *Y. gloriosa,* were cultivated horticulturally in NSW and SA as early as 1843 (Swinbourne 1982) and in Queensland by 1864 (Anon. 1908a). In the 1890s *A. fourcroydes*, *A. rigida* and *A. sisalana* were commercially planted in Queensland for sisal production (Turner 1892, McLean 1897, Lock 1962, Gentry 1982). An experimental plantation of *A. sisalana* is also known from Wollongbar, northern NSW (Gorman 1904a, 1904b, Haywood 1907). *A. rigida* from the St. Helena Penal Establishment in Moreton Bay (Anon. 1901) was distributed to as far afield as the Daintree River (Anon. 1901) and Childers (Anon. 1903). *Furcraea foetida* was grown in the districts of Bundaberg, Maryborough and Townsville.

Yields of nearly a ton of fibre a day (Anon. 1904a, 1904b, 1908b, 1910) ensured that interest in agavaceacous fibres remained considerable in Queensland although, for many, the more frequent returns for sugar and dairy farming held more attraction than the 3-4 year wait for returns from fibre (Anon. 1916b). Soaring freight prices associated with the World War I offset high prices associated with revolution in Mexico such that the industry became uneconomic (Anon. 1913, 1915, 1916a) and plants were generally ploughed in or occasionally abandoned.

Some agavaceous species associated with plantations, together with garden escapes, subsequently become naturalised. Occasionally plants have become weedy on a local scale. In such cases they appear to have spread only short distances over decades, even in the absence of efforts to control them. The exhibition of limited invasive capacity by the naturalised species is highlighted by the following Queensland-based observations of Forster (1987b, 1988).

A. americana is probably the most persistent and weed-like of the naturalised Agavaceae most probably due to its widespread planting in suburban and farm gardens, its succulent nature and an ability to reproduce asexually and possibly occasionally sexually (Forster 1987b). Nevertheless, naturalizations are local and easily controlled using herbicides. For example, in 2008 a potentially serious local *A. americana* infestation of 180 m² in northern WA, on an abandoned farm that is now part of the Fitzgerald River National Park, was controlled by herbicides with ease (Figure 8.2, WA Dept of Environment and Conservation 2008).

Naturalized colonies of *A. vivipara* have formed colonies near Biggenden and Rockhampton (Forster 1987b). Although seed has been observed, establishment of bulbils appear to be the main mode of dispersal in these localized populations.

The number and extent of colonies of *A. sisalana* in Queensland is small (especially in SE Queensland, Forster 1987b) compared to what one might expect for a drought-resistant *Agave* that was widely cultivated (Figure 8.1), probably because *A. sisalana*, like *A. fourcroydes,* is a sterile hybrid that does not produce seed (Gentry 1982). Naturalizations between the small townships of Bajool and Sisalana between Rockhampton and Gladstone are possibly descendants from a plantation near Gladstone (Anon. 1912, Forster 1987b).

Reproduction of both *F. foetida* and *F. selloa* appear principally via bulbils, neither capsules nor seed have been observed (Forster 1987b). Considering their long cultivation, easy propagation by bulbils and lack of wide distribution (Figure 8.1), it is unlikely that either poses much of a threat with respect to weediness or invasiveness. On Raoul Island Kermadec (NZ), *F. foetida* was recorded as naturalised in 1944 (West 1996). By 1974 when the first attempt at eradication commenced the largest clump was 30 - 40 m across. Plants, which appeared to have spread clonally, were removed by hand such that in 1994 only one plant was detected (West 1996).

Yucca aloifolia is naturalised at several localities in Qld, NSW and WA (Figure 8.1). In Queensland, small populations are known on foreshores at seaside settlements (see also Bationoff & Franks 1997, 1998; Bationoff & Butler 2002). An inland population at higher altitudes (450m) has been present since at least 1948 and a large population of nearly 50 m across is likely to have been established over a century ago (Forster 1987b).

Agavaceous escapes tend to become weedy on islands where plantations were grown, generally because there has been little or no attempt at control during the naturalization of large populations. For example, on Carlisle and Thomas islands (GBR) uncontrolled descendants of *A. sisalana* plantations persist and have become weeds (Forster 1987b). *A. vivipara* has become weedy on Peel Island in Moreton Bay, where an extensive plantation of sisal was established when it served as a quarantine station (Anon. 1908a cited in Forster 1987b).

On Magnetic Island, near Townsville, *A. sisalana* has become aggressively weedy on rocky promontories in *Auricaria*-containing seasonally dry forests (Jo Peterson personal communication). The main mechanism of dispersal appears to be from bulbils. The plants, probably the progeny of escapees from gardens, are obvious to see because of their size and distinctive shape but awkward to control because they inhabit rocky terrain and have been allowed to grow and spread for many years.

In Mexico, abandoned *Agave* plantations tend to be rapidly recolonized by other species and the new assemblages reach diversity values similar to undisturbed natural habitats (Gonzáles-Iturbe et al 2002). Three factors determine this situation: the low population growth of agaves, their low negative interference with other species, and the ‘nursing’ processes mediated by agaves during early succession (Gonzáles-Iturbe *et al*., 2002). The main reproductive mechanism of agaves in their native habitats is seed production (Nobel, 1988) but the rates of successful establishment are extremely low with many seedlings dying 8–9 days after germination (Jordan & Nobel 1979, Gonzáles-Iturbe et al 2002).

In Australia, the weed potential of *Agave* is often confused by association with the weed potential of prickly pear (*Opuntia* spp.) because both are succulents and drought-tolerant. The two groups of plants are not closely related taxonomically. The eudicot *Opuntia* form new plants (bulbils) from pads (cladodes) that detach easily from the mother plant. Isolated leaves of the monocot *Agave* spp. cannot form bulbils but may form suckers or offsets at the base of stems. The suckers, which are produced from basal stem buds or from rhizomes, do not move very far from the parent, or very fast (a few metres per decade perhaps). Mature escapee plants are extremely visible because of their large size. Many *Agave* do not reproduce sexually in Australia (introduced varieties of *A. sisalana* and *A. fourcroydes* are sterile) and, of those that do, pollinators are uncommon or not present. In horticulture agaves infrequently set seed, which may explain why *Agave* spp. are not appearing on Australian lists of weeds despite the widespread cultivation of agavaceous species in gardens, with over 100 species of *Agave* and 50 of *Yucca* having been imported.

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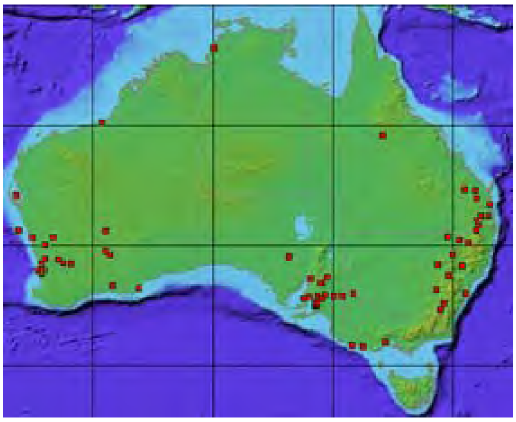
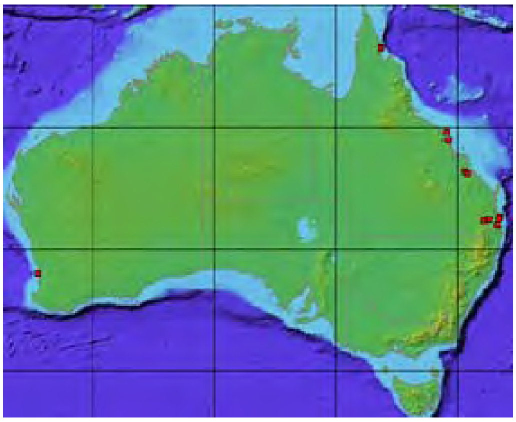
1 The terms naturalised and weeds differ but are not mutually exclusive: a taxon becomes naturalised if it forms colonies or populations that persist and multiply although they are no longer cultivated; a weed is a taxon that grows where humans do not want it to grow. Either may or may not be invasive.

Figure 8.1 Sites from which Australian herbaria have collected Agavaceae that are considered naturalised. *Agave americana* (A), *A. sisalana* (B), ***A. vivipara*** **(C),** ***Furcraea foetida*** **(D),** ***F. selloa*** **(E) and** ***Yucca aloifolia*** **(F) (Australian Virtual Herbarium 2009).**

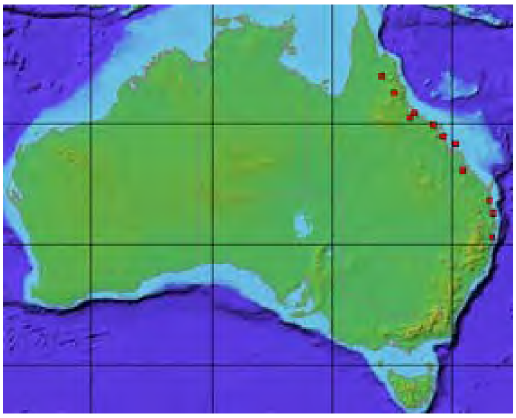
A. *Agave americana* L.
Naturalized (ACT, LHI, NI, NSW, Qld, SA, VIC, WA)



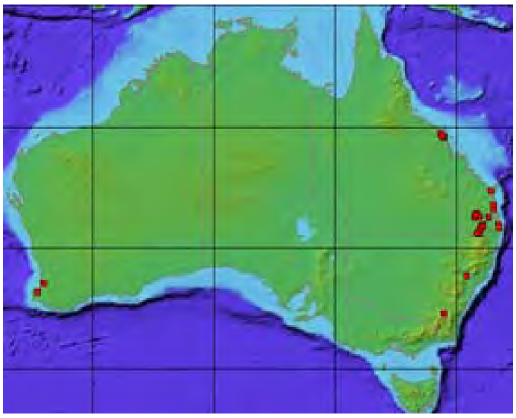
B. *A. sisalana* Perrine
Naturalized (Qld, WA)



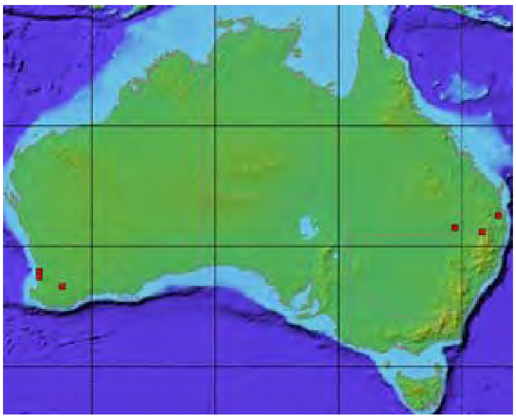
C. *Agave vivipara* L.
Naturalized (NSW, Qld)



D. *Furcraea foetida* (L.) Haw.
Naturalized (LHI, NI, NSW, Qld, WA)



E. *Furcraea selloa* K.Koch
Naturalized (Qld, WA)



F. *Yucca aloifolia* L.
Naturalized (ACT, LHI (sparingly), NSW, Qld, WA)

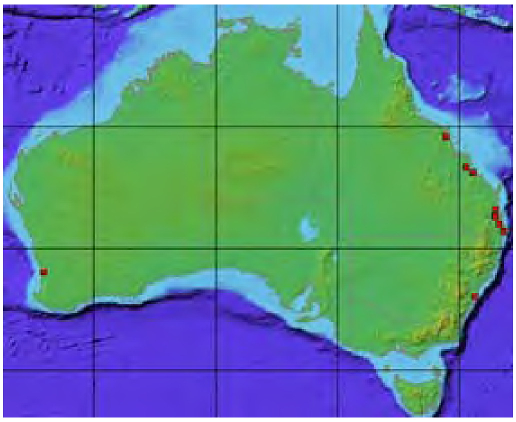


Figure 8.2. Eradication of an infestation of *A. americana* near Ravensthorpe, WA. Plants sprayed in July 2008 (left panel) were revisited in November 2008 (right panel).

Photographs downloaded 13 July 2009 from www.dec.wa.gov.au/news/department-of-environment-and-conservation/succulent-weed-reduction-at-ravensthorpe-a-success.html).

9 Recommendations

We recommend the following activities to further develop and commercially exploit the attributes of *A.tequilana.*

- The predicted feasibility of *A. tequilana* as a crop needs to be demonstrated in the field. Trials should be undertaken at a range of sites with differing rainfall and night temperatures.

- Australian-grown product needs to be assessed by processors and the food industry.

- The development of prototypes of mechanized pruners and harvesters needs to be supported.

- A research programme is required to inform extension. The rates of *Agave* growth and carbohydrate production, responses to light, water-logging and pests and diseases need to be quantified throughout the life-cycle of plants grown under Australian conditions.

- Market research is required to explore the magnitude and nature of the demand for *Agave* carbohydrates in the food and health industries.

- Information transfer between Mexican and Australian agronomists, processors and researchers needs to be fostered and fast-tracked.

- If *Agave* is successfully integrated into Australian agriculture, a biofuels-oriented plant breeding program will be required. This would best be undertaken in collaboration with Mexican researchers and should include investigation of other *Agave* species.

- The potential of leaf fibre cellulose for ethanol generation should be tested using current and emerging Generation 2 technologies.

10 Appendices

Appendix 1 Emissions Associated with Ethanol Production

Under Australian systems of production life-cycle greenhouse gas emissions (expressed as CO_2 equivalents) from a litre of E10 (ULP) are less than from a litre of ULP (Table 1, ABARE 2009) although blending ethanol with petrol requires removal of moisture from the ethanol, a process that is energy intensive (the ethanol portion of ethanol-petrol blends is thus associated with more emissions than pure ethanol but less than petrol).

Table 10.1 Effect of ethanol source on greenhouse gas emissions from E10 (ULP) in comparison to ULP (CSIRO, BTRE, ABARE 2003; Australian Government 2005, Cuevas-Cubria 2009).

unleaded petrol (ULP)	molasses & cogenerated energy	molasses	grain sorghum	wheat	wheat starch waste
	difference in life-cycle greenhouse gas emissions (CO_2 equivalents) of E10 (ULP) with respect to ULP				
g/km	%				
404.98	– 4.2	– 2.7	– 2.0	– 0.7	– 2.7

The level of emissions depends upon the feedstock and production processes used to generate the ethanol as well as the technology used to extract energy from ethanol (e.g. the type of vehicle used and driving patterns). Of the five sources of feedstock used in Australia the production of ethanol from molasses produced using cogenerated energy emits the fewest CO_2 equivalents and the least atmospheric pollutants (Table 2). Ethanol production from waste products will generate fewer emissions and pollutants per km travelled than products grown specifically for fuel production as some of the upstream products are not attributed solely to producing fuel. Similarly, the use of low greenhouse emissions electricity for processing fuel will reduce overall emissions. Technological details associated with the production of ethanol (farming as well as processing) and its use (particularly by vehicles) greatly affect the perceived environmental benefits of ethanol as a fuel (cf Table 2).

Table 10.2 Life-cycle air pollutant production from E10 (ULP) [ULP containing 10 % anhydrous ethanol by volume] and unleaded petrol (ULP). CO is carbon monoxide, NO_x is nitrogen oxide, VOC is volatile organic compounds, PM is particulate matter.

The energy requirements of a passenger car are assumed to be 4.63 MJL^{-1} and the energy density of ethanol is assumed to be 21 MJL^{-1} (recalculated from Cuevas-Cubria 2009, Table 3).

Pollutant & source	E10 (ULP)					ULP
	molasses & cogenerated energy	molasses	grain sorghum	wheat	wheat starch waste	ULP
			g/km			
CO tailpipe	3.547	3.547	3.547	3.547	3.547	4.850
CO upstream	0.287	0.286	0.100	0.361	0.102	0.090
NO_x tailpipe	0.484	0.484	0.484	0.484	0.484	0.461
NO_x upstream	0.473	0.502	0.487	0.543	0.483	0.480
VOC tailpipe	0.144	0.144	0.144	0.144	0.144	0.168
VOC upstream	0.683	0.681	0.680	0.700	0.680	0.669
PM tailpipe	2.008	2.008	2.008	2.008	2.008	3.346
PM upstream - urban	6.692	13.190	13.960	13.930	13.750	7.062
PM upstream - non-urban	7.025	7.007	6.307	7.557	6.757	7.442
Total life-cycle air pollutants						
- urban	14.317	20.842	21.409	21.716	21.197	17.126
- non-urban	14.650	14.659	13.756	15.343	14.204	17.506
Difference in life-cycle pollutants from E10 (ULP) with respect to ULP (%)						
- urban	-16.40	21.70	25.01	26.80	23.77	-
- non-urban	-16.31	-16.26	-21.42	-12.36	-18.86	-

Appendix 2 World Agave species list

The list of names and authorities has been adapted from that available at the Kew Gardens web site. It includes unplaced names.

1 *Agave acicularis* Trel., Mem. Natl. Acad. Sci. 11: 34 (1913).

2 *Agave acklinicola* Trel., Mem. Natl. Acad. Sci. 11: 41 (1913).

3 *Agave* × *ajoensis* W.C.Hodgs., Novon 11: 414 (2001).

4 *Agave aktites* Gentry, U.S.D.A. Agric. Handb. 399: 148 (1972).

5 *Agave albescens* Trel., Mem. Natl. Acad. Sci. 11: 44 (1913).

6 *Agave albomarginata* Gentry, Agaves Cont. N. Amer.: 129 (1982).

7 *Agave albopilosa* I.Cabral, Villarreal & A.E.Estrada, Acta Bot. Mex. 80: 52 (2007).

8 *Agave aloides* Jacobi, Hamburger Garten- Blumenzeitung 22: 265 (1866). Name unplaced.

9 *Agave americana* L., Sp. Pl.: 323 (1753).

10 *Agave americana* subsp. *americana*.

11 *Agave americana* var. *expansa* (Jacobi) Gentry, U.S.D.A. Agric. Handb. 399: 80 (1972).

12 *Agave americana* var. *oaxacensis* Gentry, Agaves Cont. N. Amer.: 285 (1982).

13 *Agave americana* subsp. *protamericana* Gentry, Agaves Cont. N. Amer.: 287 (1982).

14 *Agave angustiarum* Trel., Contr. U. S. Natl. Herb. 23: 139 (1920).

15 *Agave anomala* Trel., Mem. Natl. Acad. Sci. 11: 36 (1913).

16 *Agave antillarum* Descourt., Fl. Méd. Antilles 4: 239 (1827).

17 *Agave antillarum* var. *antillarum*.

18 *Agave antillarum* var. *grammontensis* Trel., Repert. Spec. Nov. Regni Veg. 23: 362 (1927).

19 *Agave applanata* Lem. ex Jacobi, Hamburger Garten- Blumenzeitung 20: 550 (1864).

20 *Agave arcedianoensis* Cházaro, O.M.Valencia & A.Vázquez, *Agave*s Occid. México: 45 (2007).

21 *Agave* × *arizonica* Gentry & J.H.Weber, Cact. Succ. J. (Los Angeles) 42: 223 (1970).

22 *Agave arubensis* Hummelinck, Recueil Trav. Bot. Néerl. 33: 236 (1936).

23 *Agave asperrima* Jacobi, Hamburger Garten- Blumenzeitung 20: 561 (1864).

24 *Agave asperrima* subsp. *asperrima*.

25 *Agave asperrima* subsp. *maderensis* (Gentry) B.Ullrich, Sida 15: 254 (1992).

26 *Agave asperrima* subsp. *potosiensis* (Gentry) B.Ullrich, Sida 15: 254 (1992).

27 *Agave asperrima* subsp. *zarcensis* (Gentry) B.Ullrich, Sida 15: 254 (1992).

28 *Agave atrovirens* Karw. ex Salm-Dyck, Hort. Dyck.: 302 (1834).

29 *Agave attenuata* Salm-Dyck, Hort. Dyck.: 303 (1834).

30 *Agave attenuata* subsp. *attenuata*.

31 *Agave attenuata* subsp. *dentata* (J.Verschaff.) B.Ullrich, Haseltonia 12: 27 (2006).

32 *Agave attenuata* subsp. *dentata* B.Ullrich, Haseltonia 12: 27 (2007). Name unplaced.

33 *Agave aurea* Brandegee, Proc. Calif. Acad. Sci., II, 2: 207 (1889).

34 *Agave avellanidens* Trel., Rep. (Annual) Missouri Bot. Gard. 22: 60 (1911 publ. 1912).

35 *Agave bahamana* Trel., Mem. Natl. Acad. Sci. 11: 40 (1913).

36 *Agave banlan* Perr., Mém. Soc. Linn. Paris 3: 97 (1824). Name unplaced.

37 *Agave baxteri* Baker, Gard. Chron. 1: 392 (1888). Name unplaced.

38 *Agave beauleriana* Jacobi, Abh. Schles. Ges. Vaterl. Cult., Abth. Naturwiss. 1869: 150 (1869).

39 *Agave bernhardii* Jacobi, Nachtr. Ord. Agav. 1: 38 (1868). Name unplaced.

40 *Agave boldinghiana* Trel., Mem. Natl. Acad. Sci. 11: 21 (1913).

41 *Agave boscii* (Hornem.) ined..

42 *Agave bovicornuta* Gentry, Publ. Carnegie Inst. Wash. 527: 92 (1942).

43 *Agave braceana* Trel., Mem. Natl. Acad. Sci. 11: 40 (1913).

44 *Agave bracteosa* S.Watson ex Engelm., Gard. Chron., n.s., 18: 776 (1882).

45 *Agave breedlovei* Gentry, Agaves Cont. N. Amer.: 567 (1982).

46 *Agave brevipetala* Trel., Repert. Spec. Nov. Regni Veg. 23: 362 (1927).

47 *Agave brevispina* Trel., Repert. Spec. Nov. Regni Veg. 23: 363 (1927).

48 *Agave brittoniana* Trel., Mem. Natl. Acad. Sci. 11: 44 (1913).

49 *Agave cacozela* Trel., Mem. Natl. Acad. Sci. 11: 41 (1913).

50 *Agave cajalbanensis* A.Álvarez, Revista Jard. Bot. Nac. Univ. Habana 1: 34 (1980 publ. 1981).

51 *Agave calderonii* Trel., J. Wash. Acad. Sci. 13: 365 (1923). Name unplaced.

52 *Agave calodonta* A.Berger, Hortus Mortolensis: 364 (1912).

53 *Agave cantala* (Haw.) Roxb. ex Salm-Dyck, Index Pl. Succ. Hort. Dyck.: 1 (1829).

54 *Agave cantala* var. *acuispina* (Trel.) Gentry, Agaves Cont. N. Amer.: 569 (1982).

55 *Agave cantala* var. *cantala*.

56 *Agave capensis* Gentry, Occas. Pap. Calif. Acad. Sci. 130: 72 (1978).

57 *Agave caribaeicola* Trel., Mem. Natl. Acad. Sci. 11: 27 (1913).

58 *Agave* × *cavanillesii* D.Guillot & P.Van der Meer, Flora Montiber. 28: 73 (2004). Name unplaced.

59 *Agave cerulata* Trel., Rep. (Annual) Missouri Bot. Gard. 22: 55 (1911 publ. 1912).

60 *Agave cerulata* subsp. *cerulata*.

61 *Agave cerulata* subsp. *dentiens* (Trel.) Gentry, Occas. Pap. Calif. Acad. Sci. 130: 43 (1978).

62 *Agave cerulata* subsp. *nelsonii* (Trel.) Gentry, Occas. Pap. Calif. Acad. Sci. 130: 44 (1978).

63 *Agave cerulata* subsp. *subcerulata* Gentry, Occas. Pap. Calif. Acad. Sci. 130: 44 (1978).

64 *Agave chazaroi* A.Vázquez & O.M.Valencia, Agaves Occid. México: 48 (2007).

65 *Agave chiapensis* Jacobi, Hamburger Garten- Blumenzeitung 22: 213 (1866).

66 *Agave chrysantha* Peebles, Proc. Biol. Soc. Wash. 48: 139 (1935).

67 *Agave chrysoglossa* I.M.Johnst., Proc. Calif. Acad. Sci., IV, 12: 998 (1924).

68 *Agave cocui* Trel., Mem. Natl. Acad. Sci. 11: 19 (1913).

69 *Agave collina* Greenm., Proc. Amer. Acad. Arts 32: 296 (1897).

70 *Agave colorata* Gentry, Publ. Carnegie Inst. Wash. 527: 93 (1942).

71 *Agave concinna* Lem., Hort. Vanhoutt. 1(2): 23 (1846). Name unplaced.

72 *Agave congesta* Gentry, Agaves Cont. N. Amer.: 476 (1982).

73 *Agave cookei* Woodrow, J. Bombay Nat. Hist. Soc. 12: 522 (1899). Name unplaced.

74 *Agave cordillerensis* Lodé & Pino, Int. Cact. Advent. 77: 13 (2008). Name unplaced.

75 *Agave cucullata* Lem. ex Jacobi, Hamburger Garten- Blumenzeitung 21: 124 (1865). Name unplaced.

76 *Agave cundinamarcensis* A.Berger, Agaven: 222 (1915).

77 *Agave cupreata* Trel. & A.Berger, Agaven: 197 (1915).

78 *Agave dasylirioides* Jacobi & Bouch., Hamburger Garten- Blumenzeitung 21: 344 (1865).

79 *Agave datylio* F.A.C.Weber, Bull. Mus. Hist. Nat. (Paris) 8: 224 (1902).

80 *Agave datylio* var. datylio.

81 *Agave datylio* var. *vexans* (Trel.) I.M.Johnst., Proc. Calif. Acad. Sci., IV, 12: 1003 (1924).

82 *Agave davillonii* Baker, Bull. Misc. Inform. Kew 1892: 5 (1892). Name unplaced.

83 *Agave de-meesteriana* Jacobi, Monog.: 218 (1864).

84 *Agave deamiana* Trel., Trans. Acad. Sci. St. Louis 23: 139 (1915). Name unplaced.

85 *Agave decaisneana* Jacobi, Abh. Schles. Ges. Vaterl. Cult., Abth. Naturwiss. 1869: 153 (1869). Name unplaced.

86 *Agave decipiens* Baker, Bull. Misc. Inform. Kew 1892: 184 (1892).

87 *Agave delamateri* W.C.Hodgs. & Slauson, Haseltonia 3: 133 (1995).

88 *Agave deserti* Engelm., Trans. Acad. Sci. St. Louis 3: 310 (1875).

89 *Agave deserti* var. *deserti*.

90 *Agave deserti* var. *pringlei* (Engelm. ex Baker) W.C.Hodgs. & Reveal, Novon 11: 413 (2001).

91 *Agave deserti* var. *simplex* (Gentry) W.C.Hodgs. & Reveal, Novon 11: 413 (2001).

92 *Agave diacantha* Royle, Fibr. Pl. India: 44 (1855). Name unplaced.

93 *Agave difformis* A.Berger, Agaven: 95 (1915).

94 *Agave durangensis* Gentry, *Agave*s Cont. N. Amer.: 433 (1982).

95 *Agave dussiana* Trel., Mem. Natl. Acad. Sci. 11: 26 (1913).

96 *Agave eggersiana* Trel., Mem. Natl. Acad. Sci. 11: 28 (1913).

97 *Agave ehrenbergii* Jacobi, Hamburger Garten- Blumenzeitung 21: 255 (1865).

98 *Agave elizae* A.Berger, *Agave*n: 232 (1915). Name unplaced.

99 *Agave ellemeetiana* Jacobi, Hamburger Garten- Blumenzeitung 21: 457 (1865).

100 *Agave ensifera* Jacobi, Nachtr. Ord. Agav. 1: 14 (1868).

101 *Agave entea* Hartwich, Neu Arzneidrogen: 36 (1897). Name unplaced.

102 *Agave erosa* A.Berger, Agaven: 191 (1915). Name unplaced.

103 *Agave evadens* Trel., Mem. Natl. Acad. Sci. 11: 20 (1913).

104 *Agave felgeri* Gentry, U.S.D.A. Agric. Handb. 399: 60 (1972). view Monocot Checklist

105 *Agave fenzliana* Jacobi, Hamburger Garten- Blumenzeitung 22: 170 (1866). Name unplaced.

106 *Agave filifera* Salm-Dyck, Hort. Dyck.: 309 (1834).

107 *Agave flexispina* Trel., Contr. U. S. Natl. Herb. 23: 133 (1920).

108 *Agave fortiflora* Gentry, U.S.D.A. Agric. Handb. 399: 122 (1972).

109 *Agave fourcroydes* Lem., Ill. Hort. 11(Misc.): 65 (1864).

110 *Agave fragrantissima* Jacq., Enum. Stirp. Vindob., App.: 309 (1762). Name unplaced.

111 *Agave fridericii* A.Berger, Hortus Mortolensis: 12 (1912). Name unplaced.

112 *Agave funkiana* K.Koch & C.D.Bouché, Wochenschr. Vereines Beförd. Gartenbaues Königl. Preuss. Staaten 3: 47 (1860).

113 *Agave galeottii* Baker, Gard. Chron., n.s., 7: 40 (1877). Name unplaced.

114 *Agave garciae-mendozae* Galván & L.Hern., Cact. Succ. J. (Los Angeles) 74: 188 (2002).

115 *Agave gentryi* B.Ullrich, Succulenta (Netherlands) 69: 211 (1990).

116 *Agave ghiesbreghtii* Lem. ex Jacobi, Hamburger Garten- Blumenzeitung 20: 545 (1864).

117 *Agave gigantensis* Gentry, Occas. Pap. Calif. Acad. Sci. 130: 63 (1978).

118 *Agave gilbertii* A.Berger, Monatsschr. Kakteenk. 14: 126 (1904).

119 *Agave glabra* Karw. in M.J.Roemer, Fam. Nat. Syn. Monogr. 4: 292 (1847). Name unplaced.

120 *Agave glaucescens* Otto in M.J.Roemer, Fam. Nat. Syn. Monogr. 4: 292 (1847). Name unplaced.

121 *Agave × glomeruliflora* (Engelm.) A.Berger, Agaven: 95 (1915).

122 *Agave* goeppertiana Jacobi, Hamburger Garten- Blumenzeitung 22: 219 (1866). Name unplaced.

123 *Agave gonzaloi* D.Guillot & P.Van der Meer, in Fl. Montiberica 24: 55 (2004). Name unplaced.

124 *Agave gracilipes* Trel., Rep. (Annual) Missouri Bot. Gard. 22: 95 (1911 publ. 1912).

125 *Agave grandibracteata* H.Ross, Icon. Pl. Hort. Bot. Panorm.: t. 1 (1896). Name unplaced.

126 *Agave granulosa* Scheidw., Wochenschr. Vereines Beförd. Gartenbaues Königl. Preuss. Staaten 4: 286 (1861). This name is unplaced.

127 *Agave grisea* Trel., Mem. Natl. Acad. Sci. 11: 34 (1913).

128 *Agave guadalajarana* Trel., Contr. U. S. Natl. Herb. 23: 123 (1920).

129 *Agave guedeneyrii* Houllet, Rev. Hort. 47: 466 (1875). Name unplaced.

130 *Agave × guemensis* D.Guillot & P.Van der Meer, Stud. Bot. 24: 87 (2005 publ. 2006). Name unplaced.

131 *Agave guiengola* Gentry, Brittonia 12: 98 (1960).

132 *Agave gutierreziana* Trel., Contr. U. S. Natl. Herb. 23: 116 (1920). Name unplaced.

133 *Agave gypsophila* Gentry, Agaves Cont. N. Amer.: 510 (1982).

134 *Agave harrisii* Trel., Mem. Natl. Acad. Sci. 11: 34 (1913).

135 *Agave havardiana* Trel., Rep. (Annual) Missouri Bot. Gard. 22: 91 (1911 publ. 1912).

136 *Agave henriquesii* Baker, Gard. Chron. 1887: 732 (1887). Name unplaced.

137 *Agave hiemiflora* Gentry, Agaves Cont. N. Amer.: 480 (1982).

138 *Agave hookeri* Jacobi, Hamburger Garten- Blumenzeitung 22: 168 (1866). view Monocot

139 *Agave horizontalis* Jacobi, Abh. Schles. Ges. Vaterl. Cult., Abth. Naturwiss. 1869: 148 (1869). Name unplaced.

140 *Agave horrida* Lem. ex Jacobi, Hamburger Garten- Blumenzeitung 20: 546 (1864).

141 *Agave horrida* subsp. horrida.

142 *Agave horrida* subsp. *perotensis* B.Ullrich, Cact. Suc. Mex. 35: 80 (1990).

143 *Agave humboldtiana* Jacobi, Hamburger Garten- Blumenzeitung 22: 264 (1866). Name unplaced.

144 *Agave hurteri* Trel., Trans. Acad. Sci. St. Louis 23: 136 (1915).

145 *Agave impressa* Gentry, Agaves Cont. N. Amer.: 146 (1982).

146 *Agave inaequidens* K.Koch, Wochenschr. Vereines Beförd. Gartenbaues Königl. Preuss. Staaten 3: 28 (1860).

147 *Agave inaequidens* subsp. *barrancensis* Gentry, Agaves Cont. N. Amer.: 342 (1982).

148 *Agave inaequidens* subsp. *inaequidens*.

149 *Agave inaguensis* Trel., Mem. Natl. Acad. Sci. 11: 47 (1913).

150 *Agave indagatorum* Trel., Mem. Natl. Acad. Sci. 11: 42 (1913).

151 *Agave intermixta* Trel., Mem. Natl. Acad. Sci. 11: 32 (1913).

152 *Agave isthmensis* A.García-Mend. & F.Palma, Sida 15: 565 (1993).

153 *Agave jaiboli* Gentry, U.S.D.A. Agric. Handb. 399: 89 (1972).

154 *Agave jarucoensis* A.Álvarez, Revista Jard. Bot. Nac. Univ. Habana 1(1): 6 (1980 publ. 1981).

155 *Agave karatto* Mill., Gard. Dict. ed. 8: 6 (1768).

156 *Agave karwinskii* Zucc., Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur. 16(2): 675 (1833).

157 *Agave* kellermaniana Trel., Trans. Acad. Sci. St. Louis 23: 142 (1915). Name unplaced.

158 *Agave kerchovei* Lem., Ill. Hort. 11(Misc.): 64 (1864).

159 *Agave kewensis* Jacobi, Hamburger Garten- Blumenzeitung 22: 218 (1866).

160 *Agave lagunae* Trel., Trans. Acad. Sci. St. Louis 23: 143 (1915).

161 *Agave laticincta* Verschaff., Cat. 12: 2 (1868). Name unplaced.

162 *Agave lechuguilla* Torr. in W.H.Emory, Rep. U.S. Mex. Bound. 2(1): 213 (1858).

163 *Agave lemairei* Verschaff., Ill. Hort. 11(Misc.): 65 (1864). Name unplaced.

164 *Agave lempana* Trel., J. Wash. Acad. Sci. 15: 395 (1925). Name unplaced.

165 *Agave lindleyi* Jacobi, Abh. Schles. Ges. Vaterl. Cult., Abth. Naturwiss. 1869: 152 (1869). Name unplaced.

166 *Agave littaeaoides* Pamp., Boll. Soc. Bot. Ital. 1909: 119 (1909). Name unplaced.

167 *Agave longipes* Trel., Mem. Natl. Acad. Sci. 11: 36 (1913).

168 *Agave longisepala* Tod., Hort. Bot. Panorm. 2: 34 (1886). Name unplaced.

169 *Agave macroacantha* Zucc., Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur. 16(2): 676 (1833).

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Appendix 3 Letter from IBS

IBS centre of engineering innovation

28 July 2009

Don Chambers
PO Box 32,
Aldgate,
South Australia 5154

Dear Don,

Agave Harvester

As per your discussions with Bill, I am writing this letter on behalf of my division here at IBS, the Centre of Engineering Innovation.

We have been involved with many unique and innovative ideas over the 30 years we have been in business. Some of the ideas began from within the company and have been developed and manufactured in house, like our Ozzy Bulldog Banana Bagging Machines which were designed to stop bagging by ladder, and we are still manufacturing today. As well as other ideas which have come from external sources such as the Banana Picking Head we developed recently.

In most cases, the main reasons for the absence of automation of a process is either due to the fact that the people working in the industry are set in the traditional ways of doing things and don't ever think of different / better ways of doing something, or its simply because the cost of labour is far cheaper than worrying about the need to be efficient.

Our design team are excited about the idea and we have discussed the viability of developing and manufacturing an Agave Harvester and have come up with some ideas already. This is only come about through general chat, and there is no doubt in our minds that we would be able to help you develop and manufacture a harvester prototype in time to test out on your trials.

Yours
sincerely,

A. Seawright

Adam Seawright
Project / Design Engineer

Document3

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Appendix 4 Letter from OSM

OUTSOURCE MANAGEMENT PTY LTD

thinking outside the square!

4th August 2009

To whom it may concern

Outsource Management Pty Ltd and BDO Kendalls of Cairns in Queensland, at the request of Don Chambers of Ausgave, developed a crop comparison model that compares the growing of sugar cane to the growing of the Mexican crop, Agave.

In assessing the average performance of both crops, the following key criteria were used, and assumptions made in this process are listed as follows:

1. The model provided by OSM has been designed in such a way that it is able to recalculate core information by simply changing the information/data in any one or more of the cells and the model will recalculate to bottom line.
2. The model provided to Don Chambers contains sugar cane crop average information from the Bureau of Sugar Experiment Stations (BSES) for sugar cane grown on the Atherton Tablelands of Far North Queensland, cross referenced and supported by sugar cane crop information provided by the Department of Primary Industries, Forestry and Fisheries (Queensland), and the Louisiana State University USA.
3. Information relating to Agave crop average yields and juice yields were provided by Don Chambers. Given that the crop have never been grown in any commercial form in Australia there is currently no information available that could be used to demonstrate the performance of the crop in Australian conditions. Therefore, the information used in the development of the crop comparison model had to come from the country of the Agave plant's origin.
4. According to Don Chambers the information provided to OSM on Agave is up to date information provided by his Mexican Industry contacts and University institutions in Mexico. The average yields used for Agave are considered sensibly conservative, however according to Don Chambers are close to industry averages currently produced by Mexico.
5. Based on the information available, and used in the crop comparison model Agave appears to perform comparably to Sugar cane over the growing cycle of 5 years for an agave crop, and has the potential to produce a return equivalent to sugar cane.

LIMITATION OF LIABILITY

The information contained herein and in Agave/Sugar Cane Crop Comparison was provided from a range of independent sources. Therefore, under no circumstances does OSM or BDO give any guarantee nor provide any warrants on the accuracy of the supplied information, nor does OSM or BDO accept any liability or responsibility for the interpretation or evaluation of this information. Any user or evaluator acknowledges the reliance upon their own skills and judgment in respect of the use of this document and the crop comparison model and the information contained therein.

Under no circumstances shall Outsource Management P/L (OSM) or BDO Kendalls (Cairns) accept or be liable for any charges whatsoever, including but not limited to:

- damages through loss of income,
- loss of profit and or loss of capital,
- interruption to business,
- perceived loss of credibility or impact on reputation
- and / or any other consequential economic or incidental damages resulting from, or out of the use of or inability to use or understand the information contained in the Agave/Sugar Cane crop comparison model regardless of whether or not OSM and BDO have been notified of the possibility of such damages.

Under no circumstances should any business or general decisions be made solely and exclusively on the basis of this or any other project document.

Yours sincerely

Bob Cobavie
Managing Director
Outsource Management Pty Ltd

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Feasibility of Agave as a Feedstock for Biofuel Production in Australia

by Don Chambers and Joseph A. M. Holtum

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Demand is increasing for alternative sources of energy that are secure and produce less greenhouse gas and generate fewer pollutants than fossil fuels. One such energy source is ethanol.

Of the new crops or cellulosic processes proposed for ethanol production, *Agave tequilana* is the only crop that is ready to go into the ground now – cultivars, agronomic systems and fermentation technologies have been developed during two hundred years of cultivation for tequila production.

This study assesses the feasibility of growing *Agave tequilana* Weber in Australia as a feedstock for the sustainable production of ethanol. The report introduces *A. Tequilana*, detailing why the crop may be of interest in Australia. Information on the biological and agronomic attributes of the crop is collated and potential sites of cultivation identified. An agronomic system is then proposed and costed.

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