GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2011) 3, 68–78, doi: 10.1111/j.1757-1707.2010.01077.x

REVIEW

The global potential for *Agave* as a biofuel feedstock

SARAH C. DAVIS*†, FRANK G. DOHLEMAN* and STEPHEN P. LONG*†‡
*Energy Biosciences Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA, †Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, ‡Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Abstract

Large areas of the tropics and subtropics are too arid or degraded to support food crops, but *Agave* species may be suitable for biofuel production in these regions. We review the potential of *Agave* species as biofuel feedstocks in the context of ecophysiology, agronomy, and land availability for this genus globally. Reported dry biomass yields of *Agave* spp., when annualized, range from <1 to 34 Mg ha^{-1} yr^{-1} without irrigation, depending on species and location. Some of the most productive species have not yet been evaluated at a commercial scale. Approximately 0.6 Mha of land previously used to grow *Agave* for coarse fibers have fallen out of production, largely as a result of competition with synthetic fibers. Theoretically, this crop area alone could provide 6.1 billion L of ethanol if *Agave* were re-established as a bioenergy feedstock without causing indirect land use change. Almost one-fifth of the global land surface is semiarid, suggesting there may be large opportunities for expansion of *Agave* crops for feedstock, but more field trials are needed to determine tolerance boundaries for different *Agave* species.

Keywords: bioenergy, biomass, CAM, desert, ethanol, henequen, marginal lands, productivity, semiarid, sisal

Received 20 July 2010 and accepted 18 September 2010

Introduction

Biomass feedstocks that grow on semiarid lands could be a sustainable answer to increasing demands for renewable fuels that do not conflict with food and feed production. If high yielding crops that require minimal inputs of water and nutrients can be grown on lands that are marginal for food crops, land use competition could be reduced. Plants that use the Crassulacean Acid Metabolism (CAM) pathway have low water requirements and are productive in semiarid regions because they assimilate carbon at night thereby decreasing the diffusive gradient of water out of leaves and improving water use efficiency (Nobel, 1994). Water use is decreased because potential evapotranspiration increases exponentially with temperature, and by opening stomata during cooler nighttime conditions, less water is lost per CO_2 assimilated. It follows that the greater the diurnal variation in temperature, the greater the water use efficiency advantage of CAM. Because of this efficient resource use, CAM plants have recently been introduced as potential bioenergy crops (Smith, 2008; Borland *et al*., 2009).

In the context of biofuels, CAM physiology provides benefits beyond nutrient and water use efficiency. Lignin represents one of the major impediments to digestion of lignocelluloses to release sugars, a process that allows for the fermentation of cellulose and hemicelluloses to fuels. Current plant feedstocks can be up to 30% lignin (Somerville, 2007), a cross-linked polymer that gives the xylem adequate strength to withstand the tensions imposed during transpiration. However, in CAM plants, tension in the xylem is low because transpiration is low and thus less lignin is required. As a result, lower amounts of lignin are evident in the composition of CAM tissue. *Agave* leaves, for example, have 3–15% lignin by dry weight and up to 68% cellulose (Iñiguez-Covarrubias *et al*., 2001; Santiago *et al*., 2002, Vieira *et al*., 2002, Garcia-Reyes & Rangel-Mendez 2009). Another characteristic of CAM physiology that is advantageous for biofuel feedstock is the accumulation of soluble nonstructural carbohydrates in plant tissue to support PEPC-mediated carboxylation (Borland *et al*., 2009). The low lignin and high soluble

Correspondence: S. C. Davis, Institute for Genomic Biology, 1206 W. Gregory Dr. Urbana, IL 61801, USA. tel. + 1 217 244 5952, e-mail: davissc@illinois.edu

carbohydrate reserves of CAM plants would require less energy for conversion to fuels and may therefore result in a higher quality feedstock (Smith, 2008; Borland *et al.*, 2009).

The *Agave* genus is composed exclusively of obligate CAM plants and includes species that are grown commercially. Importantly, they thrive in conditions that are unsuited to major food crops and pasture grasses. The most widespread commercial uses of *Agave* spp. are for fibers and beverages, derived usually from the leaves and from the stem, respectively. At the peak of production in 1964, over 1 Mha of *Agave* were cultivated globally for sisal fibers (FAO, 2010), the majority of which were in Africa (Lock, 1969; FAO, 2010). Since then, the widespread production of synthetic fibers caused a decline in sisal production (Nobel, 1994) and there were <0.5 Mha of sisal planted by 2008 (FAO, 2010). In the 1990s, $\sim$70 000 ha of *Agave*, predominantly the species *A. tequilana*, were cultivated in México for the production of alcoholic beverages, along with 200 000 ha of *A. fourcroydes*, or henequen (sometimes called sisal), grown for fiber production (Nobel, 1994). *Agave sisalana* (sisal) is also native to México, but is now primarily grown in Brazil and Eastern Africa for fiber.

The potential of CAM plants for biofuel feedstocks has been recently reviewed (Borland *et al.*,. 2009), yet there is very little information about biofuel production from CAM plants at a commercial scale. There is also no systematic record of field-scale dry matter productivity, which is crucial information for establishing the economic and logistic viability of a biofuel enterprise. *Agave* species cultivated for fiber have quite different production systems from those cultivated for beverages, but both are examples of commercially scaled agriculture that would be relevant to a bioenergy production chain. This paper will synthesize information from existing literature to evaluate the potential of *Agave* production systems for near-term sustainable biofuel feedstocks. We review the form, diversity, and ecology of *Agave*, the agronomy of the major crop species, and finally the land availability for biofuel production from this group of plants.

Review of *Agave* form, diversity and ecology

Agave is a genus of some 200–300 species, within the family Agavaceae, although it is sometimes placed in the Liliaceae or Amarylidaceae (Purseglove, 1972; Colunga-García Marín *et al.*, 2007). Gentry (1982) recognizes two subgenera, *Agave* and *Littaea*, in North America. The natural distribution of the *Agave* genus is limited to the Americas, with the greatest diversity of species in México, although many species have been distributed across mediterranean and dry tropical regions for agricultural, hedgerow and horticultural uses (Purseglove, 1972). All species of the genus that have been analyzed use CAM and it is assumed that the genus as a whole uses CAM physiology (Szarek & Ting, 1977; Szarek, 1979).

Agave plants are perennial evergreen xerophytes, ranging in size from several centimeters to 4 m and producing large flowering stalks 2–12 m tall after 5–15 years (Gentry, 1982; Valenzuela-Zapata & Nablan, 2004). They have a basal rosette of large stiff, lanceolate, succulent and persistent leaves, often with a terminal spine, and sometimes with spiny margins. In transverse cross-section, the leaves are crescent shaped. The epidermis is highly cuticularized and the stomata are deeply sunken at the base of hypostomatal cavities. The mesophyll includes elongated water storage cells as well as idioblasts, which contain calcium oxalate crystals (Blunden *et al.*, 1973). Stems are short and thick, or basal with leaves formed around the terminal meristem that they encircle. Shoots are typically monocarpic, i.e. they die after flowering, however side shoots may allow the plant to persist. Flowers are usually formed on a massive spike, sometimes termed a pole, and have a paniculate inflorescence (Gentry, 1982). Many *Agave* cultivars do not flower or are sterile, and propagation is either by suckers or from bulbils produced on the flowering stem (Purseglove, 1972).

Agave plants typically form a spreading fibrous root system that arises adventitiously from the stem base and, although shallow, may spread some distance. For example, in the case of *A. sisalana*, anchor roots of 2–4 mm in diameter may spread up to 5 m from the stem base, but vertically remain within the top 40 cm of soil. These roots become suberized, but produce smaller feeder roots along their length, that can bare numerous root hairs (Purseglove, 1972).

Agave spp. often grow on rocky soils of poor quality in regions with extreme drought and elevated temperatures. Optimum growth can be achieved with high annual rainfall amounts of 102–127 cm, but high production has also been observed in *Agave* spp. that grow in regions with only 25–38 cm of annual rainfall (Kirby, 1963). When considering drought, it is important to note that total precipitation is not the only determinant. The pattern of precipitation, i.e. a few high intensity events vs. an even distribution of smaller events will have a profound effect, the former being most typical of the semiarid tropics and subtropics. Water vapor pressure deficit, radiation and windspeed are the determinants of potential evapotranspiration, and all three can be high in the semiarid tropics and subtropics. Soil porosity and topography determine drainage and can also cause frequent droughts in otherwise high precipitation zones. For example, the major growing region of henequen (*A. fourcroydes*) in the Yucatán, México, is characterized by shallow calcareous gravel soils overlaying porous limestone. Even though precipitation reaches 760 mm yr^{-1}, the land is unsuitable for other crops (Purseglove 1972; Colunga-García Marín *et al.*, 2007).

Because droughts are frequent in semi-arid regions, viable perennial crops must not only be efficient in their use of water, but also capable of surviving without any accessible water between rainfall events. *Agave* spp. achieve this not by tolerance to low water potential, but by hydraulic isolation. During a dry-down event the roots shrink, leaving an air gap between the soil and root surface. This prevents dehydration due to water moving from the plant to soil as the soil water potential declines. In parallel, the thick cuticle and closed sunken stomata prevent water loss to the atmosphere and maintain high plant water potential. Even though roots of *Agave* are susceptible to cavitation, the high stem water potential limits the occurrence of cavitation during prolonged droughts (Linton & Nobel, 1999).

From 14 different studies that report annual *Agave* productivities, it is clear that eco-physiological responses of species to different environments will affect the success of new commercial plantations. There are substantial differences in the reported productivities of *Agave* spp. (Fig. 1), but these differences are confounded by regional variation (Fig. 2). Climate is likely to be a major determinant of yields within and among species. For example, *A. lechuguilla* produces only 3.8 t ha^{-1} yr^{-1} but occurs in areas with moderate annual rainfall (427 mm) while *A. mapisaga* produces 32 t ha^{-1} yr^{-1} in regions with 848 mm of annual rainfall (Fig. 3). Consider as another example that the yield of *A. salmiana* ranges from a mean of 10 t ha^{-1} in an arid but relatively cool region to 34 t ha^{-1} in a semiarid region with moderately warm daytime temperatures (Fig. 3). Much of the data on dry matter yield is based on extrapolation from measurements on single plants, rather than yields measured over large areas, which adds uncertainty to the true yields that might be obtained in large-scale plantings.

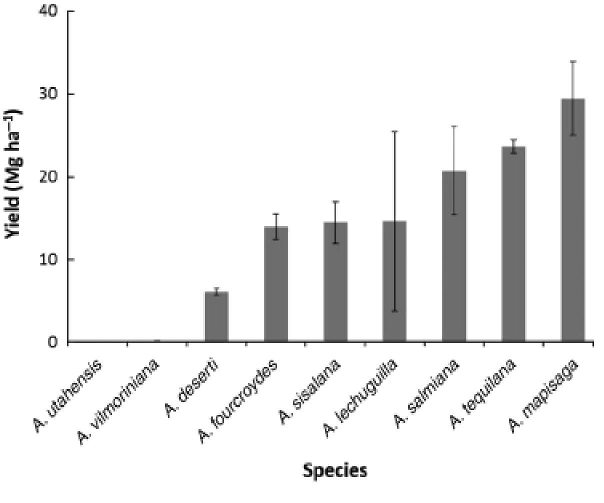


Fig. 1 Productivity of nine cultivated *Agave* species under nonirrigated conditions (Clary & Jameson 1981; Nobel 1984, 1985, 1990, 1991, Nobel & Meyer 1985; Nobel & Hartsock, 1986; Nobel & Quero, 1986; Nobel & Valenzuela-Zapata, 1987; Nobel *et al.*, 1992; Idso & Kimball, 1995). Bars represent means ± SE.

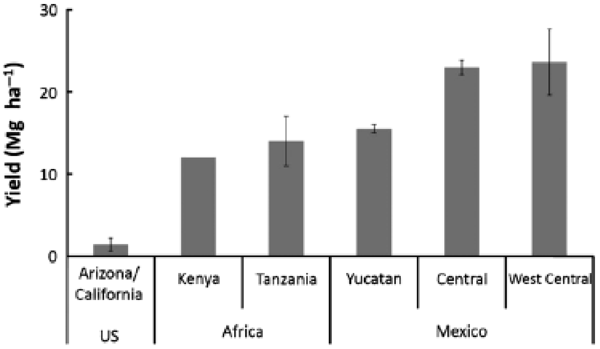
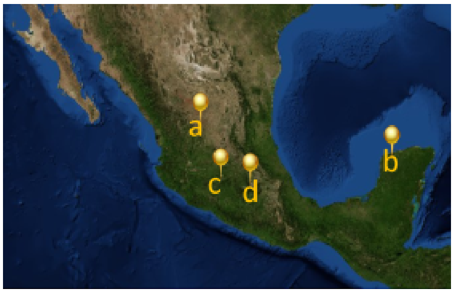


Fig. 2 Productivity of various cultivated *Agave* species under nonirrigated conditions from six locations. Bars represent means ± SE. (Clary & Jameson, 1981; Nobel 1984, 1985, 1990, 1991, Nobel & Meyer 1985; Nobel & Hartsock, 1986; Nobel & Quero, 1986; Nobel & Valenzuela-Zapata, 1987; Nobel *et al.*, 1992; Idso & Kimball, 1995)

The relationship between nighttime temperature and carbon assimilation has been described for *A. tequilana* (Luiz Corral, 2007), but is not necessarily consistent for the majority of other *Agave* species. Using minimum temperature data for the four locations shown in Fig. 3, and assuming 8 h of active CO_2 assimilation daily, the range in estimated CO_2 uptake is less than actual observations. We estimated that the theoretical CO_2 assimilation of *A. tequilana* only ranges from 39 to 42 Mg CO_2 yr^{-1} (or about 21–23 t biomass ha^{-1} yr^{-1}) at sites with mean nighttime temperatures that range from 9.6 to 20.6 °C. The productivity of other *Agave* species are far outside of this range, suggesting that the physiological assumptions for *A. tequilana*, at least in response to nighttime temperatures, may not be directly transferable to other *Agave* spp.

Biodiversity of *Agave*, at the species and genetic levels, has been affected by land management choices in the last century. There has been steady selection pressure on the genetic diversity of *Agave* species that are grown for tequila and fiber (Vargas-Ponce *et al.*, 2009). There has also been a decline in the husbandry practices that historically promoted populations of many species for food, fiber, and forage (Valenzuela-Zapata & Nablan, 2004). *A. fourcroydes* and *A. sisalana* were selected for leaf length to provide long fibers and



Site	Total annual rainfall (mm)	*Agave* species	Annual productivity (Mg ha^{-1})
a	427	*A. lechugilla*	3.8
		A. salmiana	10
b	981	*A. fourcroydes*	16
c	764	*A. tequilana*	24
d	848	*A. mapisaga*	32
		A. salmiana	34

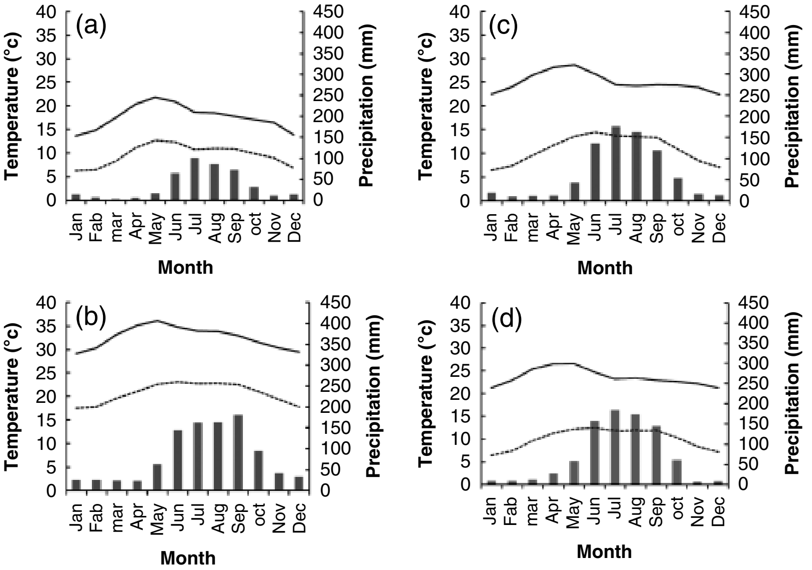


Fig. 3 Climatograms of four locations in Mexico identified in the map legend (upper left) where the biomass of five species (shown in table at lower left) has been measured: (a) Mazatlan, Sin. (b) Merida, Yuc. (c) Morelia, Mich. (d) Tacubaya, D.F. (Nobel 1985; 1990; 1991; Nobel & Hartsock, 1986; Nobel & Valenzuela-Zapata, 1987; Nobel *et al.*, 1992)

Fig. 4 Commercial scale *Agave* production: *A. tequilana* photo courtesy of Alejandro Velázquez Loera (a), piñas from *A. tequilana* (b), *A. fourcroydes* photo courtesy of Abdo Magdub-Méndez (b), fiber from the leaves of *A. fourcroydes* photo courtesy of Abdo Magdub-Méndez (d).

A. tequilana was selected for the high sugar content of the piñas (Fig. 4). Piñas are the swollen and nonstructural carbohydrate rich stem bases that are harvested as the fermentation feedstock for the production of tequila (Fig. 4). There are other varieties of *A. tequilana* that may produce greater biomass, but less sugar, that could be a better choice of feedstock for cellulosic fuels. Published productivities of species vary spatially, and there is no one species that appears to have the greatest productivity across all regions of México or elsewhere. The *Agave* species with the greatest reported annual yields (e.g. *A. mapisaga*; Fig. 1) have not been thoroughly evaluated for commercial-scale plantation agriculture.

An *Agave* plant creates a microhabitat that hosts unique bacteria, fungi and invertebrates. A genetically diverse bacterium, *Zymomonas mobilis*, originally discovered on *Agave* leaves, has been the subject of biotechnological research because of its potential as a fermentative organism with high ethanol tolerance, but the role of micro-organisms in the nutrient budget of *Agave* is unknown. Better understood are a number of parasitic organisms that benefit from *Agave* (Virgen Calleros, 2010), most notably the weevil *Schyphohorus acupunctatus* which burrows into *Agave* and the fungus *Fusarium* spp. which causes severe necrosis in the xylem tissue (González Hernández *et al.*, 2007). Another damaging parasite is the rhinoceros beetle, *Strategus* spp. that can kill a young *Agave* plant in 24 h by eating the root system (González Hernández *et al.*, 2007). Resistance to these pests will most likely be maintained by increasing genetic diversity in crops (Valenzuela-Zapata & Nablan, 2004) or selecting for new resistant clones.

Review of *Agave* agronomic practices

Propagation

Agave species that are cultivated in commercial plantations do not produce seed because the plants are harvested before flowering occurs. The seeds that are produced may not be viable for commercial production because seeded plantlets are more sensitive to the dry periods that are common in semiarid climates, and therefore vegetative propagation has proven necessary for the commercial production of *Agave* spp. (Kirby, 1963; Purseglove, 1972; Nobel, 1994). Because of the high degree of heterozygosity in fertile *Agave*, seed can also be problematic because it produces highly variable stands, which will be challenging for management and harvest. In addition, establishment from seed takes longer than from bulbils or suckers. Bulbils are young plantlets which occur on the inflorescence of mature plants. In sisal production, bulbils are used for vegetative propagation and planting (Nobel, 1994). A single sisal pole may produce 2000 bulbils, which can be harvested from the ground after they have fallen or are shaken from the pole. They are far more numerous than suckers and have been found to give a more uniform crop (Purseglove, 1972).

Although *A. tequilana* plants can also produce bulbils, commercial propagation is most commonly by removal of offset rhizome shoots from mother plants. These are harvested annually. The offsets are grown in nurseries until they reach >750 g of fresh weight, and are then transplanted to commercial fields (Valenzuela-Zapata, 1985). Before new *Agave* individuals are planted, the roots are often sterilized with a 10% formaldehyde solution and left to callus for over a month. Increasingly, micropropagation is being used to clone and propagate plantlets that are then grown in nurseries before planting in the field (Nikam *et al.*, 2003; Ramirez & del Real Laborde, 2007; Ramirez-Malagon *et al.*, 2008). Typically, 2 years of care in a nursery are required before planting micropropagated individuals in the field (del Real Laborde, personal communication).

Nutrient requirements vary throughout the life of an *Agave* individual. Nursery plants are established in peat pots that are supplemented with slow release calcium and magnesium. In the field, a supplemental nutrient solution is applied along with a compost mixture that includes bagasse, a byproduct from tequila processing that often increases soil acidity. Under these conditions, a mixture of nitrogen, phosphorus (P_2O_5), potassium (K_2O), boron, copper, and zinc are typically applied at a ratio of 40 : 120 : 40 : 4 : 2.5 : 16 in the first year and 40 : 80 : 80 : 4 : 2.5: 16 in the second year (Uvalle Bueno & Velez Gutierrez, 2007). Then nutrient amendments are limited to nitrogen, phosphorus, and potassium in the third year at a ratio of 60 : 40 : 80, and only nitrogen is required in subsequent years (Uvalle Bueno & Velez Gutierrez, 2007).

Cultivation

Before the establishment of an *Agave* plantation, the land is usually cleared by burning, bulldozing, or digging to remove existing vegetation and obstructions. Mulching of any vegetation covering the land may be a better method, since it will boost soil organic matter and help preserve any residual soil moisture. In present practice, the land is usually plowed twice to a depth no greater than 15–20 cm and then harrowed (Lock, 1962). However, given the wide spacing, *Agave* would appear a good candidate for no-till planting. In places where *Agave* spp. are planted in exceptionally rocky areas known as 'ceboruco,' the soil is worked by hand and cannot be tilled mechanically (Gobeille *et al.*, 2006).

Young *Agave* plants are transplanted from a nursery into 30 cm deep furrows. Planting density varies with topography, soil quality, and polycultures. *A. sisalana* can be planted with 4900–9900 individuals per ha in optimal conditions (Kirby, 1963). The density of planting for *A. tequilana* ranges up to 6200–7400 plants per ha on steep slopes and 7400–11 000 plants per ha on flat lands (Valenzuela-Zapata, 1985). Row spacing for *A. tequilana* is sometimes as wide as ~3 m with plants within a row ~1.8 m apart (Valenzuela-Zapata, 1985), allowing only 1852 plants ha^{-1}.

Cultivation techniques affect planting densities. Mechanization facilitates planting ~ 1500 individuals per hour (del Real Laborde, personal communication), a process that is far more time efficient than hand planting but full mechanization requires wider row spacing and possibly more land to achieve the same supply of biomass. Mechanization will also affect employment in rural communities. If managed mechanically, rows must be wide enough for tractors to access plants after maturity (3–4 m minimum distance). If manually pruned and harvested, a minimum distance between rows can be reduced to ≤ 2 m (Kirby, 1963). The economic and social trade-offs that result from replacing hand labor with mechanization are important considerations in the development of new *Agave* plantations.

Harvest

The timing of plant maturity can be very specific to individuals, even within a species, and microclimates. At high altitudes and in poor soil conditions, individuals develop more slowly than their lowland relatives. Mature leaves are harvested from *A. sisalana* and *A. fourcroydes* for fiber using a sickle-like knife with a curved end called a 'coa de jima' (Valenzuela-Zapata & Nablan, 2004). Such harvests can continue for 15 years before replanting is necessary. In the case of *A. tequilana* that is used to make tequila, only the piña (stem or head) is harvested after at least 5 years of maturation, and then new plants must be established.

Leaves from *A. tequilana* are typically left on the field to return nutrients to the soil, but very rarely the leaves are used for co-combustion to generate energy at a distillation plant. The use of leaves, for nutrients, co-combustion, or biofuel feedstock, is an important consideration for life-cycle analyses of an *Agave*-based biofuel production system. In the short term, the use of leaves in cellulosic conversion processes or co-combustion could add to the efficiency of *Agave* biofuels, but little is known about the effects of leaf removal on soil organic matter and the long-term sustainability of this agronomic practice.

Mature canopies of crops grown for tequila (piñas) intercept less photosynthetically active radiation than the canopies of crops grown for fiber. Thus, the system used for fiber production has an inherently higher production potential. *Agave tequilana* will barely cover 5% of the ground at the time of planting and covers only about half of the ground surface at harvest. Thus, on average *A. tequilana* will only be able to intercept $<25\%$ of available solar radiation over the 5 years from planting to harvest. Henequen (*A. fourcroydes*) and sisal (*A. sisalana*) by contrast have more full canopies that persists for ~15 years with annual leaf harvests and can therefore intercept more radiation.

Yields

A. mapisaga and *A. salmiana* produced the greatest yields observed in 14 studies of *Agave* species. With careful management that includes pruning, fertilization, irrigation, weeding and pest control, *A. mapisaga* yielded 38 Mg ha^{-1} yr^{-1} and *A. salmiana* yielded 42 Mg ha^{-1} yr^{-1} (Nobel *et al*., 1992). These yields far exceed corn, soybean, sorghum, and wheat productivities under intensive management. Even without irrigation, yields of *A. mapisaga, A. salmiana,* and *A. tequilana* can reach 25–26 Mg ha^{-1} yr^{-1} in semiarid conditions of west central México (Nobel, 1991). At the low end of productivity, in arid conditions of the southwestern US (<200 mm yr^{-1}), yields of *A. utahensis* and *A. vilmoriniana* are <1 Mg ha^{-1} yr^{-1} (Clary & Jameson, 1981; Idso & Kimball, 1995). In the Yucatán peninsula of México, where there is greater rainfall (981 mm yr^{-1}) but more porous soil, *A. fourcroydes* has a productivity of ~16 Mg ha^{-1} yr^{-1} without irrigation (Nobel & Valenzuela-Zapata, 1987).

It is uncertain how yields of the more productive species of *Agave* would be reduced in more arid conditions. In fact, side-by-side replicated plot trials that would be necessary for any rigorous comparison of species or cultivars appear to be largely lacking. Most yields have been assessed for individual experimental plants rather than production fields, where yields are likely to be lower.

The composition of harvested *Agave* biomass varies and different methods are required for processing leaves and the piña after harvest. Leaves have greater cellulose and lignin, and the piña has greater amounts of soluble carbohydrates (Table 1). In a regional evaluation of crops in México, it was discovered that the composition of carbohydrates extracted from the same species differed according to the location of source crops that were subject to different climates (Mancilla-Margalli & López, 2006). For several species that were grown in the same region (including *A. angustifolia*, *A. potatorum*, and *A. cantala*), the carbohydrate profiles were similar among species. This underscores the importance of site selection for optimizing biofuel yields.

Biomass to alcohol conversion

The tequila industry provides a model for biofuel production because the plantations and the initial fermentation at a distillery are similar to what would be necessary for fuel ethanol production. Tequila Sauza, one of the largest tequila companies in México,

Table 1 Weight-based percentages of compounds in *Agave* leaves

	*A. tequilana**	*A. fourcroydes*†	*A. sisalana*‡	*A. americana*§	*A. angustifolia*¶	*A. salmiana*||
Leaf						
Cellulose	65	78	43	68	–	–
Hemicellulose	5	5–7	32	16	–	–
Lignin	16	13	15	4.9	–	–
Piña						
Glucose	1–3	4	–	–	0–2	–
Sucrose	4–6	8	–	–	1–10	–
Fructose	7–10	13	–	–	2–7	–
Fructans	43–73	49	–	–	36–51	–
Total soluble carbohydrates	55–90	55	–	–	52–63	–
Bagasse						
Cellulose	43	–	–	–	–	47
Hemicellulose	19	–	–	–	–	13
Lignin	15	–	–	–	–	10
Total soluble carbohydrates	5	–	–	–	–	–

*Iñiguez-Covarrubias *et al.* (2001); Mancilla-Margalli & López (2006); Cedeño-Cruz & Alvares-Jacobs (1999)
†Mancilla-Margalli & López (2006); Vieira *et al.* (2002)
‡McDougall *et al.* (1993)
§Mylsamy & Rajendran (2010)
¶Mancilla-Margalli & López (2006)
||Garcia-Reyes & Rangel-Mendez (2009)

processes approximately 400 Mg of piñas per day into alcohol at a single distillation plant. Approximately 1 L of 40% ethanol is produced for every 5.5 kg of dry biomass. The piña is $\geq$ 52% sugar (Mancilla-Margalli & López, 2006), and can have a greater concentration of soluble carbohydrates than corn grain. If one assumes the current ethanol conversion efficiency for corn grain (Perrin *et al.*, 2009), then an ethanol plant that processes 400 Mg day^{-1} of piñas could theoretically produce 61 million L yr^{-1} of 100% ethanol for fuel (equal to $\sim$417 L t^{-1}). This scale of production (400 t day^{-1}) is similar to that of a tequila distillery, and does not include conversion of the cellulosic parts of *Agave* plants.

The biomass from the leaves left after piña processing could add substantially to the amount of ethanol that can be produced. The leaves of *A. tequilana* that are unused in the process of tequila production equate to $\sim$ 38% of the total plant biomass (Cedeño, 1995; Iñiguez-Covarrubias *et al.*, 2001), and could serve as cellulosic feedstock to produce another 28 million L ethanol yr^{-1}. In total, from piñas and leaves, the production capacity of an ethanol plant that processes *Agave* from the same amount of land used by a single tequila distillery could theoretically produce 89 million L yr^{-1} of ethanol (equal to $\sim$378 L t^{-1}). Assuming crop yields of 24 Mg ha^{-1} yr^{-1} (Nobel & Valenzuela-Zapata, 1987), then 6083 ha would be harvested annually to support this production volume.

There are both solid and liquid byproducts after converting *Agave* to tequila or ethanol. Liquid waste (vinasse) from the initial processing of piñas can be treated (Méndez-Acosta, 2010), but is often recycled to fields without treatment resulting in degradation of water quality. Methane is produced as a byproduct from the water treatment process (Méndez-Acosta, 2010) and could be used for energy. Bagasse is the solid byproduct that is typically composted and reapplied to *Agave* fields, returning nutrients but also contributing to acidification of the soil. Other uses could be for co-combustion to produce heat and power or for additional cellulosic feedstock for liquid fuel (see bagasse composition in Table 1). If the bagasse, which is usually $\sim$40% of piña weight (Cedeño, 1995; Iñiguez-Covarrubias *et al.*, 2001), were also converted to ethanol, an additional 22 million L yr^{-1} of ethanol could be produced. Uses of both vinasse and bagasse would thus be important considerations in a life-cycle analysis of an *Agave*-based bioenergy production system.

Land available for *Agave* bioenergy

Based on only the area of abandoned land previously used to grow *Agave* for fiber (FAO, 2010), there is an opportunity to reinstate *Agave* agriculture on 1.87×10^5 ha in Mexico, 3.43×10^5 ha in Africa, and 0.67×10^5 ha in other parts of the world. The *Agave* plantations in Australia pre-date FAO records, but there

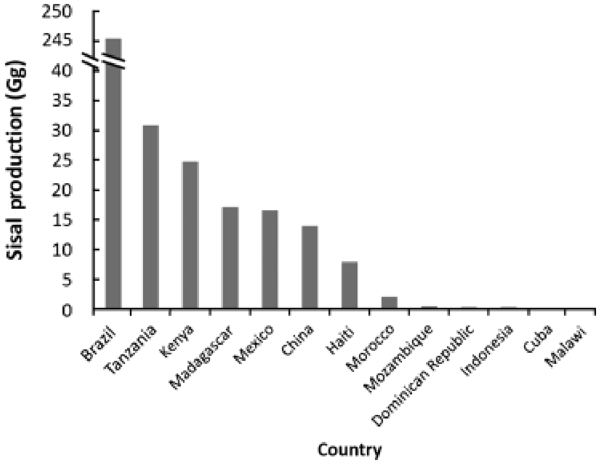


Fig. 5 Sisal, fiber from *Agave sisalana* and *Agave fourcroydes*, production in countries that reported to FAO in 2007 (FAO, 2010).

are regions on the continent that could be allocated to *Agave* feedstock crops (Holtum *et al*., 2011). Conversion of abandoned plantations back to *Agave* would result in minimal indirect land use change because of the low productivity that occurs in these regions. Actual opportunities within and beyond these abandoned lands depend on local politics, economics, and climate change. In total, semiarid regions that could support *Agave* agriculture comprise 18% of the global land surface (Hoekstra & Shachak, 1999).

Land that is marginal, abandoned or of low-productivity represents one of the most appropriate opportunities for biofuel feedstocks because this will minimize indirect land use change (Somerville *et al*., 2010). While the sisal production in Brazil dwarfs the production of *Agave* fiber in other countries (Fig. 5), there are less abandoned land opportunities than there are in other regions of the world where sisal production has declined. The land area devoted to sisal production in Brazil is similar to what it was in 1964, at the peak of global production. In cultivation, *Agave* is not limited to semiarid lands, since it is also productive in areas with high rainfall and good soil water retention. As a result, not all sisal production is on semiarid land. Most of Brazil receives more than 1000 mm of precipitation annually (NOAA, 1991), and is a very large agricultural producer. In considering *Agave* as a biofuel feedstock, it will be important that plans are limited to areas where moisture prevents the viable production of food crops. CAM plants, because of their low stomatal conductivities, will be inherently less efficient than other crops in high moisture environments.

Arid and semiarid regions often intersect with lower economic opportunities because of the inherent limitations on land-based resources. For example, there are large tracts of semiarid to arid environments in Africa that support very few food crops. This may be in part a cause for political and economic instability in some of this region (Blench, 1996; Oketch, 2006). The theoretical minimum amount of water required for CAM plants with an average production of 43 $\mathrm{Mg\,ha^{-1}\,yr^{-1}}$ is 258 $\mathrm{mm\,yr^{-1}}$ (based on Borland *et al*., 2009), less than the precipitation at sites where *Agave* is grown commercially. While the theoretic production of *Agave* represents an opportunity for enhancing land-based resources in arid regions, rigorous replicated field trials that verify such remarkably high yields are lacking. Still, feedstocks that grow where food resources do not could stimulate local economies without displacing existing commodities, in effect minimizing indirect land use change.

Australia has the largest proportion of semiarid land of any continent. The greatest challenge for initiating *Agave* biomass crops in this region may be regulations against the use of exotic species. Although *Agave* spp. are typically not invasive, Australian regulations that govern importation of non-native species are restrictive relative to most other regions of the world. This is because of the unique biogeography of the continent and a history of biodiversity loss due to introductions of well-intended but disastrously aggressive exotic species. For example, *Opuntia stricta*, another CAM species, rapidly expanded onto 25 Mha of farmland after being introduced in central eastern Australia (Osmond *et al*., 2008). While this introduction shows the ability of CAM plants to thrive in this dry environment, it also leaves a historical scar that would likely result in opposition to another CAM plant introduction. Ironically, there was no evidence of invasion from *Agave* plantations that were established, but later abandoned in the early 1900s, in Australia. Policy position and support for *Agave* introductions may be a key factor in determining the success of bioenergy production from this genus in Australia.

The diversity of *Agave* spp. native to México that could serve as bioenergy feedstocks are currently not recognized internationally. Fiber produced from *Agave* is listed as a crop commodity by the Food and Agriculture Organization of the United Nations (FAO) under two categories: 'Sisal' and 'Agave Fibres Nes.' The official FAO definition of 'Sisal' is limited to *A. sisalana*, but the sisal reported for Mexico is actually from *A. fourcroydes* (henequen). According to FAO, the 'Agave Fibres Nes' category includes Haiti hemp (*A. foetida*), henequen (*A. fourcroydes*), ixtle, tampico (*A. lechuguilla*), maguey (*A. cantala*), pita (*A. americana*), and Salvador hemp (*A. letonae*). Clarification of these categories, inclusion of other known species, and bioenergy designations will need to be adopted before a global market for *Agave* bioenergy feedstock can be realized.

Table 2 Global biofuel production (EIA, 2010)

	Region	Gigaliters produced in 2008
Ethanol	United States	27
	Brazil	28
All Biofuels	North America	39
	South America	31
	Europe	13
	Asia	4
	Australia	<1
	Africa	<1
Ethanol potential from Agave with no LUC		6

Bioenergy in a future climate

With respect to future climate change, *Agave* spp. may have an advantage over other crops because CAM physiology is adapted to extreme high temperatures and drought. Interestingly, the areas of the world that we have identified as the most suitable for *Agave* feedstock plantations (Mexico, Australia, and Africa) are also areas where interannual variation in temperature is relatively low (Sun *et al*., 2010). Although there is a general trend of warming in these regions (Sun *et al*., 2010), less sporadic fluctuations in climatic conditions will translate into lower risk for plantations given that they require at least 5 years for establishment. Long-term projections indicate there will be a decline in precipitation in the southwestern US, México, Australia, and northern Africa (IPCC, 2007). The trend toward less plant-available water underscores the importance of establishing crops with high water use efficiency.

Conclusion

There is an immediate land opportunity of almost 600 thousand ha globally for *Agave* bioenergy crops that would not incur indirect land use changes (FAO, 2010). Assuming maximum yields of 26 Mg ha^{-1} yr^{-1} in semi-arid conditions and a conversion efficiency of 380 L ethanol Mg^{-1} of lignocellulosic biomass (Farrell *et al*., 2006), 6.1 billion L of ethanol could be produced from *Agave* with minimal increases in environmental impacts. Although this is a small percentage of the total global biofuel production (Table 2), the assumptions used in this calculation are conservative because they only include *Agave* production on lands that have come out of previous *Agave* cultivation. In reality, if *Agave* production on other semiarid lands were economically and environmentally sustainable, *Agave* could add substantially more value to land-based resources in Africa, Australia, and Mexico. In some places, this new commodity could stimulate local economies, but there are political and legislative obstacles to overcome before *Agave* production can expand globally. For local economies in some regions, it will also be critical not to displace current beverage and fiber production from *Agave*. More research is needed to identify *Agave* species that are optimal for maximizing biomass yields regionally.

Acknowledgements

We would like to thank Rhea Kressman for collecting literature sources and all of the participants of the *Agave* and Bioenergy Workshop held on May 12–13, 2010 in Guadalajara, Mexico for sharing their insights about *Agave* production. This work was supported by the Energy Biosciences Institute.

References

Blench R (1996) *Aspects of Resource Conflict in Semi-arid Africa*. Overseas Development Institute, London.

Blunden G, Yi Y, Jewers K (1973) Comparative leaf anatomy of *Agave, Beschorneria, Doryanthes, and Furcraea* species (Agavaceae-Agaveae). *Botanical Journal of the Linnean Society*, **66**, 157–179.

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

Cedeño MC (1995) Tequila production. *Critical Reviews in Biotechnology*, **15**, 1–11.

Cedeño Cruz M, Alvarez-Jacobs J (1999) Production of tequila from agave: historical influences and contemporary processes. In: *The Alcohol Textbook*, 3rd edn (eds Jacques K, Lyons TP, Kelsall DR), pp. 225–242. Nottingham University Press, Nottingham, UK.

Clary WP, Jameson DA (1981) Herbage production following tree and shrub removal in the Pinyon-Juniper type of Arizona. *Society for Range Management*, **34**, 109–113.

Colunga-García Marín P, Larqué Saavedra A, Eguiarte LE, Zizumbo-Villareal D, (eds) (2007) *En lo ancestral hay futuro: del tequila, los mezcales y otros agaves*, 1st edn. Centro de Investigación Científica de Yucatán, A.C, Mérida, Yucatán, México.

EIA (2010) United States Energy Information Administration: Independent Statistics Analysis. Available at: http://www.eia.doe.gov/emeu/international/ (accessed 22 September 2010).

FAO (2010) Food and Agriculture Organization of the United Nations: Statistics Division. Available at: http://faostat.fao.org (accessed 29 June 2010).

Farrell AE, Plevin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM (2006) Ethanol can contribute to energy and environmental goals. *Science*, **311**, 506–508.

Garcia-Reyes BR, Rangel-Mendez JR (2009) Contribution of agro-waste material main components (hemicelluloses, cellulose, and lignin) to the removal of chromium (III) from aqueous solution. *Journal of Chemical Technology and Biotechnology*, **84**, 1533–1538.

Gentry HS (1982) *Agaves of Continental North America*. University of Arizona Press, Tucson.

Gobeille A, Yavitt J, Stalcup P, Valenzuela-Zapata AG (2006) Effects of soil management practices on soil fertility measurements on *Agave tequilana* plantations in Western Central Mexico. *Soil and Tillage Research*, **87**, 80–88.

González Hernández H, del Real Laborde JI, Solis Aguilar JF (eds) (2007) *Manejo de plagas del agave tequilero Texcoco, Edo*. Colegio de Postgraduados and Tequila Sauza, S.A. de C.V, de México and Zapopan, Jalisco, México.

Hoekstra TW, Shachak M (eds.) (1999) *Arid Lands Management: Toward Ecological Sustainability Urbana*. University of Illinois Press, Illinois.

Holtum JAM, Chambers D, Tan DKY (2011) *Agave* as a biofuel feedstock in Australia. *Global Change Biology Bioenergy*, **3**, 58–67.

Idso SB, Kimball BA (1995) Effects of atmospheric CO_2 enrichment on the growth of a desert succulent: *Agave vilmoriniana* Berger. *Journal of Arid Environments*, **31**, 377–382.

Iñiguez-Covarrubias G, Lange SE, Rowell RM (2001) Utilization of byproducts from the tequila industry: part 1: agave bagasse as a raw material for animal feeding and fiberboard production. *Bioresource Technology*, **77**, 25–32.

IPCC (2007) *Climate Change 2007: Synthesis Report*. Geneva, Switzerland.

Kirby RH (1963) *Vegetable Fibres: Botany, Cultivation, and Utilization*. Hill, Leonard, London.

Linton MJ, Nobel PS (1999) Loss of water transport capacity due to xylem cavitation in roots of two CAM succulents. *American Journal of Botany*, **86**, 1538–1543.

Lock GW (1962) *Sisal*. Green, London.

Lock GW (1969) *Sisal: Thirty Years' Sisal Research in Tanzania*. Longsmans, London.

Luiz Corral JA (2007) Requerimientos agroecologicos y potencial productivo del agave Agave tequilana Weber en Mexico. In: *Conocimiento y Practicas Agronomicas para la Prodduccion de Agave tequilana Weber en la Zona de Denominacion de Origen del Tequila* (eds Rulfo Vilchis FO, Pérez Domínguez JF, del Real Laborde JI, Byerly Murphy KF), pp. 11–36. Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias: Centro de Investigación Regional del Pacífico Centro, Tepatitlán de Morelos, Jalisco.

Mancilla-Margalli NA, López MG (2006) Water-soluble carbohydrates and fructan structure patterns from *Agave* and *Dasylirion* species. *Journal of Agricultural and Food Chemistry*, **54**, 7832–7839.

McDougall GJ, Morrison IM, Stewart D, Weyers JDB, Hillman JR (1993) Plant fibres: botany, chemistry, and processing for industrial use. *Journal of the Science of Food and Agriculture*, **62**, 1–20.

Méndez-Acosta HO, Snell-Castro R, Alcaraz-González V, González-Álvarez V, Pelayo-Ortiz C (2010) Anaerobic treatment of tequila vinasses in a CSTR-type digester. *Biodegradation*, **21**, 357–363.

Mylsamy K, Rajendran I (2010) Investigation on physio-chemical and mechanical properties of raw and alkali-treated *Agave americana* fibre. *Journal of Reinforced Plastics and Composites*, doi: 10.1177/0731684410362817.

Nikam TD, Bansude GM, Aneesh Kumar KC (2003) Somatic embryogenesis in sisal (*Agave sisalana* Perr. ex. Engelm). *Cell Biology and Morphogenesis*, **22**, 188–194.

NOAA (1991) Climates of the World. Asheville, NC. Historical Climatology. Series 6-4.

Nobel PS (1984) Productivity of *Agave deserti*: measurement by dry weight and monthly prediction using physiological responses to environmental parameters. *Oecologia*, **64**, 1–7.

Nobel PS (1985) PAR, water and temperature limitations on the productivity of cultivated *Agave fourcroydes*. *Journal of Applied Ecology*, **22**, 157–173.

Nobel PS (1990) Environmental influences on CO_2 uptake by Agave, CAM plants with high productivities. *Economic Botany*, **44**, 488–502.

Nobel PS (1991) Achievable productivities of certain CAM plants: basis for high values compared with C_3 and C_4. *New Phytologist*, **119**, 183–205.

Nobel PS (1994) *Remarkable Agaves and Cacti*. Oxford University Press, Oxford.

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

Nobel PS, Hartsock TL (1986) Temperature, water, and PAR influences on predicted and measured productivity of *Agave deserti* at various elevations. *Oecologia*, **68**, 181–185.

Nobel PS, Meyer S (1985) Field productivity of a CAM plant, *Agave salmiana*, estimated using daily acidity changes under various environmental conditions. *Physiologia Plantarum*, **65**, 397–404.

Nobel PS, Quero E (1986) Environmental productivity indices for a Chihuahuan Desert CAM plant, *Agave lechuguilla*. *Ecology*, **67**, 1–11.

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

Oketch MO (2006) Determinants of human capital formation and economic growth of African countries. *Economics of Education Review*, **25**, 554–564.

Osmond B, Neales T, Stange G (2008) Curiosity and context revisited: crassulacean acid metabolism in the Anthropocene. *Journal of Experimental Botany*, **59**, 1489–1502.

Perrin RK, Fretes NF, Sesmero JP (2009) Efficiency of midwest US corn ethanol plants: a plant survey. *Energy Policy*, **37**, 1309–1316.

Purseglove (1972) *Tropical Crops*. Longman, London.

Ramirez IV, del Real Laborde JI (2007) Metodos de propagacion del *Agave tequilana* Weber, var. azul. In: *Conocimiento y practicas agronomicas para la produccion de Agave tequilana Weber en la zona de denominacion de origen del tequila* (ed Rulfo Vilchis FO), pp. 57–67. Instituto Nacional de Invertigaciones Forestales, Agricolas y Pecuarias. Centro de Investigacion Regional del Pacifico Centro, Tepatitlan de Morelos, Jalisco, Mexico.

Ramirez-Malagon R, Borodanenko A, Perez-Moreno L, Salas-Araiza MD, Nuñez-Palenius HG, Ochoa-Alejo N (2008) In vitro propagation of three *Agave* species used for liquor distillation and three for landscape. *Plant, Cell, Tissue, and Organ culture*, **94**, 201–207.

Santiago DI, Rodriguez De Garcia N, Mogollan G (2002) Potential for pulp and paper of agave plant fibers. *Revista Forestal Venezolana*, **46**, 19–27.

Smith AM (2008) Prospects for increasing starch and sucrose yields for bioethanol production. *The Plant Journal*, **54**, 546–558.

Somerville C (2007) Biofuels. *Current Biology*, **17**, 115–119.

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

Sun F, Roderick ML, Farquhar GD, Lim WH, Zhang Y, Bennett N, Roxburgh SH (2010) Partitioning the variance between space and time. *Geophysical Research Letters*, **27**, LI2704, doi: 2710.1029/2010GL043323.

Szarek SR (1979) Occurrence of Crassulacean acid metabolism – a supplementary list during 1976-1979. *Photosynthetica*, **13**, 467–473.

Szarek SR, Ting IP (1977) Occurence of Crassulacean acid metabolism among plants. *Photosynthetica*, **11**, 330–342.

Uvalle Bueno JX, Velez Gutierrez C (2007) Nutricion del *Agave tequilero* (*Agave tequilana* Weber, var. azul). In: *Conocimientos y practicas agronomicas para la produccion de Agave tequilana Weber en la zonade denominacion de origen del tequila* (ed Rulfo Vilchis FO), pp. 71–88. Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias. Centro de Investigacion Regional del pacifico Centro, Tepatitlan de Morelos, Jalisco, Mexico.

Valenzuela-Zapata AG (1985) The tequila industry in Jalisco, Mexico. *Desert Plants*, **7**, 65–70.

Valenzuela-Zapata AG, Nablan GP (2004) *Tequila: A Natural and Cultural History*. University of Arizona Press, Tucson, Arizona.

Vargas-Ponce O, Zizumbo-Villareal D, Martínez-Castillo J, Coello-Coello J, Colunga-García Marín P (2009) Diversity and structure of landraces of *Agave* grown for spirits under traditional agriculture: a comparison with populations of *A. angustifolia* (Agavaceae) and commercial plantations of *A. tequilana*. *American Journal of Botany*, **96**, 448–457.

Vieira MC, Heinze T, Antonio-Cruz R, Mendoza-Martinez AM (2002) Cellulose derivatives from cellulosic material isolated from *Agave lechuguilla* and *Agave fourcroydes*. *Cellulose*, **9**, 203–212.

Virgen Calleros G (2010) Pests and diseases of *Agave tequilana* Weber var. azul. Presentation at *Agave and Bioenergy Workshop*, May 12–13, 2010, Guadalajara, Mexico.