

# The Biology of the Saccharum spp.

(Sugarcane)



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This document provides an overview of baseline biological information relevant to risk assessment of genetically modified (GM) forms of the species that may be released into the Australian environment.

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#### **PREAMBLE**

This document addresses the biology of the *Saccharum* spp. hybrid which is grown as commercial sugarcane, with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of *Saccharum* spp. hybrid, general descriptions of its morphology, reproductive biology and biochemistry, biotic and abiotic interactions. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to provide baseline information about the non-genetically modified (GM) parent organism for use in risk assessments of GM *Saccharum* spp. that may be released into the Australian environment.

Sugarcane is a tall growing monocotyledonous crop that is cultivated in the tropical and subtropical regions of the world, primarily for its ability to store high concentrations of sucrose, or sugar, in the stem. Modern sugarcane cultivars that are cultivated for sugar production are founded on interspecific hybrids between *S. spontaneum* and *S. officinarum* (*Saccharum* spp.). Sugarcane is an ancient crop. Its use as a garden crop dates back to around 2500BC. At present it is grown as a commercial crop primarily in South America (Brazil), North/Central America (USA, Mexico), Asia (India, China, Thailand) and Australia. Sugarcane in this document refers to the *Saccharum* spp. hybrids as described above.

#### SECTION 1 TAXONOMY

Sugarcane belongs to the genus Saccharum L., of the tribe Andropogoneae in the grass family (Poaceae). This tribe includes tropical and subtropical grasses and the cereal genera *Sorghum* and Zea (corn). The tribe is further divided into groups, with sugarcane in the Saccharinae Benth. It then may be divided into two subtribes, with sugarcane in the Saccharastra, sometimes called Saccharininae, although this level of group is not an official ICBN designation (Daniels & Roach 1987). The taxonomy and phylogeny of sugarcane is complicated as plants from five genera share common characteristics and form a closely related interbreeding group known as the 'Saccharum complex'. The Saccharum complex comprises Saccharum, Erianthus section Ripidium, Miscanthus section Diandra, Narenga and Sclerostachya (Daniels & Roach 1987). These genera are characterised by high levels of polyploidy (polyploids have more than two sets of chromosomes) and frequently unbalanced numbers of chromosomes (aneuploidy) making it difficult to determine taxonomy and resulting in many revisions of the taxonomic relationships (Daniels & Roach 1987; Sreenivasan et al. 1987). Recent molecular analysis of the genera in the Saccharum complex has led to suggestions that the taxonomy should be rearranged as many of the divisions appear to be polyphyletic (Hodkinson et al. 2002).

The Saccharum genus traditionally comprises six species: S. spontaneum, S. officinarum, S. robustum, S. edule, S. barberi, and S. sinense (D'Hont et al. 1998). However, Irvine (1999) has suggested that the genus should be reduced to just two species, grouping together S. robustum, S. edule, S. barberi, S. sinense and S. officinarum as the species S. officinarum and leaving S. spontaneum as a species. His proposal was based on the interfertility of the grouped species and the lack of distinct characteristics to separate them into individual species. Other authors have suggested that Erianthus is a synonym of Saccharum and the Erianthus spp. should be included in the Saccharum genus (Burner & Webster 1994).

S. officinarum was named by Linnaeus in 1752 in Species Plantarum (Daniels & Roach 1987). The word Saccharum is thought to have been derived from the Sanskrit 'sharkara' (Ritter 1841 as cited in Daniels & Roach 1987). It is also known by the common name of noble cane. Sugarcane is thought to have resulted from complex introgression between S. spontaneum, Erianthus arundinaceus and Miscanthus sinensis (Daniels & Roach 1987), although some data

supports it originating from *S. robustum* (as discussed in Amalraj & Balasundaram 2006). *S. officinarum* has a chromosome number of 2n = 80, with a basic chromosome number of ten, making this species octoploid (having eight copies of each chromosome). However, *S. officinarum* is not a simple polyploid, as it is both an autopolyploid (more than two sets of homologous chromosomes derived from a single species) and also an allopolyploid (possessing two or more unlike sets of chromosomes) (Sreenivasan et al. 1987). *S. officinarum* has chromosomes in common with both of the genera *Miscanthus* and *Erianthus* section *Ripidium* (Daniels & Roach 1987; Besse et al. 1997a), although molecular data has suggested that this is due to common ancestry, rather than any direct involvement of these genera in more recent evolution (Besse et al. 1997a; Grivet et al. 2004).

S. spontaneum is a highly polymorphic, disease resistant, vigorous species with high fibre content. It has 2n = 40 to 128 chromosomes and is a complex polyploid with a probable basic chromosome number of eight or ten (Panje & Babu 1960; Sreenivasan et al. 1987; D'Hont et al. 1996). It can be distinguished from the cultivated Saccharum by thinner canes and a narrow inflorescence (Purseglove 1972). Characteristics of the spikelets at the end of the tertiary branches of the inflorescence are also used by taxonomists to help distinguish this species from other Saccharum spp.

S. barberi and S. sinense have been in cultivation since prehistoric times in northern India and China respectively. This has lead to considerable interbreeding with other genera and species, consequently these species are thought to be ancient intergeneric hybrids (Daniels & Roach 1987). S. barberi is thought to be the product of S. officinarum x Erianthus (sect. Ripidium) introgression, while S. sinense is thought to be derived from S. officinarum x Miscanthus introgression. Each contains chromosomes homologous to S. officinarum and S. spontaneum as well as to those from members of the Erianthus and Miscanthus genera, again indicating the complex origins and inter-relationships within the Saccharum genus (Daniels & Roach 1987).

S. robustum is a wild species. It is thought to be the ancestral species from which S. officinarum is derived (D'Hont et al. 1998; Brown et al. 2007) and there is some speculation that it may be an intermediate step in the evolutionary pathway between S. spontaneum and S. officinarum (as discussed in Daniels & Roach 1987). It is a diverse riparian species that grows in the wet tropics as a vigorous perennial up to 10 m tall (Purseglove 1972). It is often used for house and fence posts (Bakker 1999). Two major groups within the species are known, those that have 2n=60 and 2n=80 chromosomes (Daniels & Roach 1987).

S. edule is morphologically similar to S. robustum except that the flower spike or inflorescence is compacted. It is cultivated as a vegetable in the islands of the Pacific and Papua New Guinea, where it is known as 'navisco' in Vanuatu or 'pitpit' in New Guinea (Grivet et al. 2004). S. edule is thought to be derived from introgression of S. officinarum or S. robustum with other genera (Daniels & Roach 1987).

A summary of the members of the Saccharum genus is shown in Table 1.

Table 1. Members of genus Saccharum (Buzacott 1965; Daniels & Roach 1987)

Species	Classification	Sugar content	Chromosome number
S. spontaneum L.	Wild species	Very low	2n= 40–128
S. robustum Brandes and Jeswiet ex Grassl	Wild species	Very low	2n= 60–200
S. officinarum L.	Noble canes	High	2n= 80
S. barberi Jeswiet	Ancient hybrid	Low	2n= 111–120
S. sinense Roxb.Amend. Jeswiet	Ancient hybrid	Low	2n= 80–124
S. edule Hassk.	Cultivated species	Low- Compacted inflorescence eaten as a vegetable	2n= 60–80 with aneuploid forms

#### SECTION 2 ORIGIN AND CULTIVATION

# 2.1 Centre of diversity and domestication

Commercial sugarcane hybrid cultivars have arisen through intensive selective breeding of species within the *Saccharum* genus, primarily involving crosses between *S. officinarum* and *S. spontaneum* (Cox et al. 2000; Lakshmanan et al. 2005).

S. officinarum accumulates very high levels of sucrose in the stem but has poor disease resistance. The origins of S. officinarum are intimately associated with the activities of humans, as S. officinarum is a purely cultivated or garden species which is not found in the wild (Sreenivasan et al. 1987). The centre of origin of S. officinarum is thought to be in the Indonesia/New Guinea area (Daniels & Roach 1987) where it has been grown as a garden crop since 8000 B.C. (Fauconnier 1993). It has been proposed that S. officinarum evolved from the selection of sweet forms of *S. robustum*. The canes may have previously been used for house building, fencing and archery (Daniels & Roach 1987) and may have been selected, possibly with the aid of animals such as pigs or rats that were attracted to sweeter individual plants (Daniels & Roach 1987). Its cultivation spread along the human migration routes to Southeast Asia, India and the Pacific, hybridising with wild canes. It reached the Mediterranean around 500 B.C. (Fauconnier 1993). From there it spread to Morocco, Egypt, Syria, Crete, Greece and Sicily, the main producers until the 15<sup>th</sup> Century, followed by introduction to West Africa and subsequently Central and South America and the West Indies (Fauconnier 1993). It is thought to have reached Australia on the First Fleet, but did not establish until reintroduced in 1817 from Tahiti (Bull & Glasziou 1979).

The centre of diversity of *S. officinarum* is thought to be in New Guinea (Daniels & Roach 1987), a view supported by amplified fragment length polymorphism (AFLP) marker analysis (Aitken et al. 2006b).

S. spontaneum is believed to have evolved in southern Asia (Daniels & Roach 1987). It accumulates little sucrose and has thinner stalks and higher fibre content than S. officinarum (Jackson 2005). It is a highly polymorphic species with resistance or tolerance to many pests and diseases (Bull & Glasziou 1979). S. spontaneum is an adaptable species and grows in a wide range of habitats and at various altitudes in the tropics through to temperate regions from latitude 8°S to 40°N extending across three geographical zones. These are: a) the East zone which is South Pacific islands, Philippines, Taiwan, Japan, China, Vietnam, Thailand, Malaysia and Burma; b) the Central zone, which includes India, Nepal, Bangladesh, Sri Lanka, Pakistan, Afghanistan, Iran and the Middle East and c) the West zone which includes Egypt, Sudan, Kenya, Uganda, Tanzania and other countries in the Mediterranean (Panje & Babu 1960; Tai & Miller 2001).

Currently, sugarcane is grown in over 60 countries worldwide between latitudes 30°N and 30°S (Bull & Glasziou 1979).

# 2.1.1 Commercial hybrid cultivars

Until the end of the 19<sup>th</sup> century most of the cultivars commonly grown were derived from *S. officinarum*, *S. sinense* and *S. barberi* (D'Hont et al. 1996).

Commercial hybrid cultivars of sugarcane are mainly descended from interspecific hybridisation between *S. officinarum* and *S. spontaneum* (Bull & Glasziou 1979). However other Saccharum species have also been used as parents. An analysis of parents used in breeding programs determined that two *S. sinese*, *S. barberi* and *S. robustum*, 19 *S. officinarum* and "a few" *S. spontaneum* clones had been involved in the breeding of the commercial cultivars available at that time (Roach 1989). Other authors have suggested that the modern

cultivars are founded on only 20 S. officinarum and less than ten S. spontaneum derivatives (Patade & Suprasanna 2008). The basic breeding concept involved the combination of vigorous growth, ratooning ability and tolerance to abiotic stresses and disease resistance from S. spontaneum and high sucrose content from S. officinarum (Berding et al. 2004b). This (Reffay et al. 2005)interspecific hybridisation has increased the geographic range of economic sugarcane production (Berville et al. 2005). The process of backcrossing was termed 'nobilisation' by Dutch breeders and is characterised by asymmetric chromosome transmission (Sreenivasan et al. 1987). Consequently, the genetic component from S. spontaneum is reduced in commercial hybrid cultivars. Estimates of the origin of chromosomes in these commercial hybrid cultivars using both genomic *in-situ* hybridisation (GISH) and AFLP markers have suggested that approximately 80% are derived from S. officinarum and 10% are from S. spontaneum, with the remainder being recombinant chromosomes from the two species produced by the natural process of synapsis during meiosis (D'Hont et al. 1996; Hoarau et al. 2001). However, a later study on different cultivars, using GISH and other methods, estimated their genetic complement as mainly S. officinarum, with approximately 15–20% S. spontaneum chromosomes and less than 5% translocated or recombinant chromosomes (Cuadrado et al. 2004). An AFLP study of offspring related to Mandalay, an important Australian cultivar, suggested that 11% were recombinant chromosomes (Reffay et al. 2005).

Interspecific hybridisation between *S. officinarum* as the female parent and *S. spontaneum* as the male parent produces progeny that have a triploid chromosome number (2n + n = 100 to 130) (Sreenivasan et al. 1987). This arises as the female parent transmits 2n chromosomes, whereas the male *S. spontaneum* parent transmits the normal n chromosomes. Asymmetric transmission also occurs the first time that the hybrid is backcrossed to *S. officinarum* (Lu et al. 1994) and is thought to be either through endoduplication or fusion of two nuclei during meiosis. This phenomenon facilitated breeding of modern sugarcane cultivars as the 'officinarum' qualities recovered more quickly in the hybrids, thus requiring fewer rounds of backcrossing to produce high sucrose cultivars (Sreenivasan et al. 1987).

Hybridisation between *S. officinarum* and *S. spontaneum* culminated with the release of a cultivar called POJ2878 ('Java Wondercane') in 1921 in Java (Indonesia), which became an important cultivar, allowing for a 35% increase in sugar production over the previous best cultivars (Jeswiet 1929; Cox et al. 2000). Most commercial cultivars used in Australia today can be traced to this cultivar (Cox et al. 2000). A study of 40 commercial cultivars grown around the world showed 61% average genetic similarity (Lu et al. 1994).

A list of cultivars approved for commercial cultivation in Australia can be found on the Queensland (QLD) government website <sup>1</sup>(Anon. 2010). These are cultivars which have been selected by taking into account productivity, pest and disease resistance and milling characteristics (Croft et al. 2000).

#### 2.2 Commercial uses

Sugarcane is grown for its sucrose content and is mostly consumed as sugar, a processed product. Sugarcane can also be used for other processed products (see Section 2.2.2) or may be consumed raw. Cane can be squeezed or chewed to extract the juice, which is known as 'caldo de cana' or 'garapa' in Brazil, 'chediraz' in northern India and 'aseer asab' in Egypt. It is generally a popular drink in countries in which sugarcane is grown, and is available on a small scale in Australia.

In 2009, world production of sugar was estimated to be approximately 1,683 million tonnes (t) which was grown on 23.7 million ha (FAO data at

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<sup>&</sup>lt;sup>1</sup> http://www.legislation.qld.gov.au/Acts SLs/Acts SL P.htm

http://faostat.fao.org/site/567/default.aspx#ancor). Brazil was the largest producer at 645 million t, with Australia producing 34 million t. The world production of sugar from sugarcane is approximately six times that from sugarbeet, the other source of sugar.

Australia is one of the major exporters of sugar. In 2005 it was Australia's second biggest export crop (Plant Health Australia 2009), with export earnings of \$1.52 billion per year (Croft et al. 2008).

# 2.2.1 Sugar production

Sugarcane is an established agricultural field crop with a long history of safe use. In Australia, sugarcane has been cultivated for over 150 years, with the majority being grown in QLD with some smaller areas in northern New South Wales (NSW) (Canegrowers 2009).

Sugar is initially extracted from the raw cane at sugarcane mills distributed throughout the growing regions. The cane is shredded and the juice extracted by crushing. The juice is then clarified by heating in the presence of lime (Ca(OH)<sub>2</sub>). The lime complexes with phosphorus in the juice to produce a precipitate of calcium phosphate, which is allowed to settle out taking other impurities with it. Flocculants are added to speed up this precipitation process (Mackintosh 2000).

Clarified sugar juice is concentrated by evaporation to produce 'syrup'. The syrup then goes through multiple rounds of crystallisation to extract the sucrose. The syrup is boiled and the sucrose crystallises from the remaining molasses fraction as it cools. This mixture is known as massecuite, and the sugar crystals are separated from the molasses by centrifugation. This process is repeated three times in Australian sugar mills. Thus clarified sugar juice is boiled and centrifuged the first time to produce 'A' sugar and 'A' molasses. 'A' molasses is then boiled again to produce 'B' sugar and 'B' molasses. The 'B' molasses is boiled a third time to produce 'C' sugar which is mixed with water and is used to seed the next round of crystallisation (Mackintosh 2000). The 'C' molasses is referred to as 'final' or 'blackstrap' molasses (Preston 1988). The 'A' and 'B' sugar are dried to produce raw sugar, which is shipped in bulk to sugar refineries worldwide for further refining, resulting in a highly purified product.

In Australia, growers are paid under a variable revenue sharing arrangement (Todd et al. 2004). Sugarcane quality is measured at the mill and partly determines the actual return the grower receives. The formula to determine payment to the grower is complex however, there are three measures of cane quality that are important, brix, pol and commercial cane sugar (CCS). Brix is the percentage of dissolved solids on a weight per weight basis and is measured by refractometer or density meter. Pol is a measure of the degree of rotation of polarised light through a known quantity of clarified juice, which estimates sucrose content. These two measures of juice quality (corrected for fibre content of the stem) allow determination of the level of impurities in the cane (ie. Brix minus Pol equals total impurities in the cane). This allows estimation of the extractable sugar content or CCS of a grower's cane (Mackintosh 2000). The average CCS in Australia is around 13%, but can be as high as 18% (Jackson 2005).

# 2.2.2 Byproducts of sugar production

Several by-products are produced from crushing sugarcane at the sugar mill. In Cuba, it has been estimated that up to 31 products are produced from sugarcane. These include refined sugar, raw sugar, molasses, alcohol, rum, yeast, bagasse, syrups, dextran, confectionary, crude wax, and glucose (as reviewed by Allen et al. 1997). One hundred tons of sugarcane is estimated to produce 14.3 t raw sugar, 27.2 t bagasse, 5.2 t filter cake, 2.6 t molasses and 50.7 t waste water (Allen et al. 1997).

#### Ethanol

A pilot plant is being constructed in QLD to explore the production of ethanol from bagasse (O'Hara et al. 2009), however, in some countries, sucrose is being fermented to produce ethanol (Schubert 2006). In 2006 in Brazil, 47% of the sugarcane crop was used for ethanol production, yielding 17.8 million litres (summarised in Goldemberg & Guardabassi 2009). In 2006, around 40% of fuel used in cars in Brazil was ethanol (Orellana & Neto 2006).

#### Bagasse

Bagasse is the fibrous portion of sugarcane that remains after the juice has been removed. It consists of two types of fibre: the long fibres in the rind, and the shorter, softer fibres in the pith of the cane stem, which constitute 55% of bagasse dry weight. Bagasse cellulose fibres are longer (1–1.5 mm) than hardwood fibres (0.7–1 mm), but shorter than softwood fibres (2.5–5 mm) and are suitable for papermaking. Bagasse is used to make paper in many countries, although not in Australia (Allen et al. 1997). The pith material of the stem is considered a contaminant for papermaking and it must be removed for high quality paper making. Internationally, bagasse has also been used to make particleboard, a construction panel that can be used for cabinets and laminate flooring (Nelson 1998). More recently panels have been prepared using bagasse as the basis for both the resin and the fibres in the board (Hoareau et al. 2006).

Bagasse is used as an animal feed but its use is limited by low digestibility, even for ruminants. Steam treatment of the bagasse improves its digestibility so that it can be used in the fattening of cattle (Pate 1982; Playne 1984; de la Cruz 1990; de Medeiros & Machado 1993; Allen et al. 1997; UN Industrial Development Organisation 2002). Bagasse has also been used as food for shrimp (Freeman et al. 1992).

Bagasse is burnt for heat to produce steam as a source of power to run the sugar mills, with excess energy directed to the electricity grid (Sreenivasan et al. 1987; Mackintosh 2000).

Bagasse is also an effective bio-sorbent and may be used in waste water management. For example, chromium, cadmium, nickel and dyes, common pollutants found in synthetic waste water, are effectively adsorbed by bagasse (Khattri & Singh 1999; Krishnani et al. 2004; Khan & Amin 2005).

#### Molasses

Molasses is the thick syrupy residue left after the sucrose has been removed from the clarified sugar juice (syrup). The 'C' molasses (final or blackstrap molasses) is used for alcohol fermentation, as a stock feed supplement and as a fertiliser for cane fields (Sreenivasan et al. 1987; Sansoucy et al. 1988; Mackintosh 2000). Rum is produced by fermentation of molasses using yeast (*Saccharomyces cerevisiae*) (Purseglove 1972).

# Other products

Trash is the plant material left after harvesting of the sugarcane stalks. In northern QLD it is generally retained in the field as mulch. Baled trash is used as garden mulch and as a low-grade cattle feed in the south-east QLD growing region (Dawson 2002).

Sugarcane wax comprises both the waxy coating on the outside of the stalk, concentrated mainly at the nodes, and the lipids found throughout the cells (Allen et al. 1997). Sugarcane wax is used in cosmetics and pharmaceutical products and it has also been used to lower cholesterol (see Section 5.3 for more information).

Sugarcane ash, the residue produced when the sugarcane bagasse is burnt as fuel in the boilers, and filter mud, the solids left after filtering the cane juice, are often used as fertilisers on

sugarcane farms (Qureshi et al. 2000). It is estimated that 1 t of sugarcane crushed in QLD produces 0.01 t of sugarcane ash and 0.05 t of mill mud (Qureshi et al. 2000). These provide a good supply of many plant nutrients, although nitrogen may need to be added (Calcino 1994). In Australian banana plantations, sugarcane ash has been shown to enhance the growth of bananas by suppressing nematodes, possibly due to improved soil health (Broadley et al. 2004).

# 2.3 Cultivation in Australia

It is believed that *S. officinarum* was brought to Australia in 1788 on the First Fleet but cultivation was not immediately successful (Bull & Glasziou 1979). The first official record of sugarcane in Australia was in Port Macquarie in 1821, and the first record of sugar manufacture was in 1823 (Buzacott 1965). The first Australian commercial sugar mill began operation in 1864. Sugarcane cultivation spread along the QLD-NSW coastline and a system of large central sugar mills, supplied with cane by independent farmers, was introduced by the Colonial Sugar Refining Company (now Sucragen) (Queensland Sugar Corporation 2002)(Figure 1).



Figure 1. Sugarcane growing in Bundaberg, QLD. Photo taken by OGTR, August 2007.

# 2.3.1 Commercial propagation

Propagation of sugarcane is different to the majority of other field crops since commercial sugarcane is propagated vegetatively. A variety or cultivar refers to the specific clone or genotype that has been vegetatively propagated through setts (whole stalks or shorter stem segments), also known as billets or seed canes. The term 'seed cane' is used to distinguish them from true, sexually produced, seed. The planting material is usually grown on-farm as transport is often not practical due to the large volume of material required and the short viability of the harvested cane (3–4 weeks). Primary seed cane are raised in areas approved by the Cane Protection and Productivity Board as being free of disease and this cane is then distributed to the growers who multiply enough cane for their own crop planting (Croft et al. 2000). In Australia, it is estimated that 880 million setts are produced annually for planting (Mordocco et al. 2009).

Commercial sugarcane is also propagated by allowing the regrowth of the stems of the stools that remain in the soil after harvest of the previous crop (ratooning).

There have been some trials with sugarcane plants generated through *in vitro* micropropagation, although this method is not yet used commercially in Australia (Shannon et al. 2008). Micro-propagation of sugarcane provides a reliable and fast method for mass propagation of clonal material. Micro-propagation of meristem tissue has also been used to obtain disease-free planting material (Lakshmanan et al. 2005) and this is commonly used in Brazil for generating nursery material (Irvine 2004). Plants can be regenerated directly from meristem tissue or indirectly (*de novo*) from callus derived from meristem or non-meristematic cells. Thin cell layer culture of immature leaf or inflorescence tissue can also be used for the direct regeneration of plants (Lakshmanan et al. 2005), and can be combined with an automated culture system to reduce labour costs (Mordocco et al. 2009).

# 2.3.2 Scale of cultivation

In Australia, sugarcane is commercially cultivated over a 2100 km stretch from northern NSW (approximately 30°S) to northern QLD (approximately 17°S), with the actual planting area distributed unevenly across this range (see figure 2). About 95% of Australia's raw sugar production originates in the QLD coastal region. There was a small industry in the Ord region of Western Australia (WA) but this ceased production in 2008 (Canegrowers 2009).

In the 2008/09 season, Australia's sugarcane crop harvest was 31.5 million t, from 391,000 ha (Australian Bureau of Statistics 2010). A world comparison of productivity of sugarcane in 1999 indicated that Australia had the highest productivity at 88.97 t cane ha<sup>-1</sup> (Baldani et al. 2002). In the period 1990–1995, the highest average sucrose yield for the QLD sugarcane industry was 12 t sucrose ha<sup>-1</sup> with the highest maximum sucrose yield of the Burdekin region at 17.4 t sucrose ha<sup>-1</sup> (Berding et al. 2004b).

Most sugarcane farms are family-owned and the size of the farms varies from around 40 ha to 250 ha (Canegrowers 2009). Sugarcane is also grown in home gardens in coastal regions (Plant Health Australia 2009).

# 2.3.3 *Cultivation practices*

Sugarcane will grow on a wide variety of soil types, providing adequate fertility is present, although heavy soils are preferred (Purseglove 1972). In Australia, it is generally grown in fine textured sandy loam, clay loam and clay soils (Blair & Stirling 2007). It requires high temperatures, high rainfall (1525 mm year<sup>-1</sup>) or irrigation and good soil fertility (Purseglove 1972).

Setts (cuttings from mature cane stalks) are generally planted within a few days of harvest of the cane, in order to achieve a high frequency of germination. Row spacing is about 150 cm. Buds on planted setts, or on the plant bases remaining after harvest, germinate within two weeks. Sugarcane cultivars differ in their degree of temperature sensitivity, but in general germination is slow at soil temperatures below 18°C and increases rapidly up to about 35°C (Bull 2000). Because sugarcane originated in the wet tropics, yields are much higher when the crop is supplied with adequate water. In Australia, nearly 40% of the land growing sugarcane uses either full or partial irrigation (Ham 1994 as cited in Meyer 1997).



**Figure 2.** Map of sugarcane industry in Australia, October 2010 (used with permission from Australian Sugar Milling Council).

The cultivation of sugarcane relies on the extensive use of fertilizers and pesticides. Nitrogen especially is widely used. Nitrogen is lost to surface runoff, groundwater, soil storage and the atmosphere (Freney et al. 1994; Weier et al. 1996; Bohl et al. 2000; Macdonald et al. 2009). In Australia, there is a declining nitrogen usage from an average of 206 kg N ha<sup>-1</sup> for the 1997 crop to 164 kg N ha<sup>-1</sup> for the 2008 crop (Wood et al. 2010). Phosphorus is usually applied at 22–80 kg ha<sup>-1</sup> and potassium at 10–140 kg ha<sup>-1</sup> (Bull & Glasziou 1979). Insecticides such as chlorpyrifos may be used to control insect pests, and herbicides, including atrazine, diuron, and paraquat can be used for weed control (Hargreaves et al. 1999). It is estimated that herbicides comprise 90% of the pesticides used on sugarcane farms (Christiansen 2000). These are used both within the crop and in other areas on the farm to reduce nesting areas and food sources for rats (Christiansen 2000). In addition, rodenticides such as coumatetrally and zinc phosphide, and fungicides such as triadimifon and propiconazole, are used to control rodents and diseases (BSES Ltd 2006; Dyer 2010). Chemicals may also be used to help ripen the sugarcane, and increase the accumulation of sugar in the stalk. Etherel ((2-chloro-ethyl) phosphonic acid), a growth regulator, is registered for use in Australia, but is not widely used due to variable yield

responses between cultivars and the shorter harvest season (McDonald et al. 2000). Studies have shown inconsistent effects of ripeners due to the sugarcane variety, water deficit stress and the combination of chemicals used (Donaldson & Inman-Bamber 1982; Donaldson 1994; McDonald et al. 2000; McDonald et al. 2001).

Sugarcane is planted in NSW in September-October and in the spring or autumn in QLD. In NSW sugarcane is harvested from mid June to late November, after either one or two years, depending on the region (McGuire et al. 2003). In OLD harvesting occurs between June and December (Queensland Sugar Corporation 1997). Sugarcane is routinely harvested mechanically by cutting stems close to the ground. Cane can be harvested green or after burning. Burnt cane harvesting was introduced during the 1940's due to labour shortages (Christiansen 2000) and to reduce the incidence of rat-borne diseases amongst cane cutters (Wood 1991). This remained the main harvesting method until the 1980's (Ridge & Norris 2000). The majority of sugarcane in QLD is now harvested using the green cane method. It allows the leafy tops of the cane to be left on the ground as a protective trash blanket layer. This in turn protects the soil from erosion and also acts as organic mulch, thereby retaining the nutrients in the soil (Wood 1991; Queensland Sugar Corporation 1997). Green cane harvesting and trash blanketing is known to dramatically reduce soil erosion (Prove et al. 1995) and subsequent herbicide runoff (Kealley 2009). However, in Southern areas trash blanketing may increase susceptibility to frosts and slow down the growth of ration crops due to decreased soil warming (Kingston 2000). In 2003 over 90% of the cane in NSW was burnt prior to harvesting (McGuire et al. 2003). However, this practice is changing and the cane is being harvested green in these areas, with the trash servicing a renewable energy power plant (NSW Sugar 2009).

Sugarcane grows perennially and the root system or stool that remains in the ground will resprout. Ratoon crops grow faster than the original plant crop. Although several ratoon crops are possible, cumulative stool damage from harvesting and weed control operations and the impact of pests and diseases eventually lead to declining yield. The number of ratoon crops steadily increased between 1955, when an average of 1.8 ratoon crops were grown, and 1986 with an average of 4.3. There is variation between areas though, with a greater number of ratoon crops in Mackay (average of 5.5 in 1986) and fewer in Burdekin (average of 3.3 in 1986) (Chapman 1988). More recently a maximum of four ratoon crops are typically grown before ploughing out the crop and replanting (Bull 2000). Ratoons may also be removed by ploughing and treating with herbicide (glyphosate) (Willcox et al. 2000).

After ploughing out the previous ratoons, another sugarcane crop may be planted immediately or the ground left fallow. Alternatively sugarcane may be grown in rotation with legumes, with sugarcane again planted the following winter (Willcox et al. 2000). Until the mid-1970's sugarcane farmers were required to leave part (initially 25% then reduced to 15% in 1964) of their land free of sugarcane and a legume, generally cowpea (*Vigna unguiculate*), was often planted. This helped to reduce the build-up of disease, provide nitrogen for the next sugarcane crop and provide ground cover to prevent soil erosion (Garside et al. 2001). Since the mid-1970's when this restriction was lifted many farmers plough out ratoons and re-plant with sugarcane within 4–8 weeks.

Recently there has been a move back towards legume breaks, and also incorporating controlled traffic planting practices and minimum till in the sugarcane industry. A single pass of heavy machinery over the planting area has been shown to cause soil compaction (Braunack & Peatey 1999) and multiple passes reduce crop yields (Garside et al. 2009). The adoption of controlled traffic planting practices, where GPS guidance is used to direct machinery to the same path in

the field enables the planting beds to be kept separate from the vehicular traffic zones and thus avoids soil compaction and stool damage in the growing areas. This results in reduced cultivation of the beds, which lowers costs and may also reduce weed problems (Garside et al. 2004).

If rotation crops are used, the crop varies depending on the growing region (Queensland Department of Primary Industries and Fisheries 2007a). Soybean is considered highly suitable as a rotation crop, either grown for green manure or for grain production (Queensland Department of Primary Industries and Fisheries 2005). It has been shown to fix more nitrogen, produce more dry matter and withstand dry conditions better than cowpea (Garside et al. 2000). Other promising legume crops include peanuts, mung beans and navy beans. Forage crops such as sorghum, fibre crops such as Kenaf and hemp, and fruit such as melons or bananas also show potential in a rotation system with sugarcane (Queensland Department of Primary Industries and Fisheries 2007a). Experiments have indicated that including a legume crop to break the sugarcane monoculture enhances the yield of both the following sugarcane plant crop and the subsequent ratoon crops (Garside et al. 2001; Garside & Bell 2007).

# 2.4 Crop improvement

New varieties are generated through conventional breeding programs, which rely on the maintenance of germplasm stocks for breeding material. Lines with desirable genotypes are used for hybridisations to produce new lines. Sugarcane breeding for improved cultivars is a time-consuming process, taking upwards of ten years from initial crosses to final agronomic assessment of elite cultivars (Cox et al. 2000). Genetic modification techniques have been developed which may permit more economical and efficient development of novel GM sugarcane lines (see Section 2.4.2) (Lakshmanan et al. 2005).

Garside et al. (1997) reported that the Australian sugar industry had reached a productivity plateau in the period 1970–1990. In that period, 50 new cultivars were released and plant breeders estimated productivity gains of 1% per year (ie. 0.01 t ha<sup>-1</sup> year<sup>-1</sup>). However, the CCS decreased by ~1 unit. More recently, breeding has explored a number of traits including biomass production, stress tolerance, drought tolerance, low temperature stress tolerance, disease tolerance (Ming et al. 2006) and there has been little increase in sugar content in modern cultivars (Jackson 2005).

# 2.4.1 Breeding

Sugarcane breeding programs rely on crossing of elite cultivars and usually involve cross-pollination. In the case of self pollination, the arrows (inflorescence) containing the flowers are covered with bags, or the arrows are kept separate from other clones (Sleper & Poehlman 2006).

Lines used in breeding programs are designated as male or female depending on the relative amounts of viable pollen produced, as determined by aceto-carmine or iodine staining (Cox et al. 2000). Cultivars with <10% pollen viability are designated female, cultivars with >20% viable pollen are designated male. Cultivars with intermediate levels of viable pollen (10–20%) are classified as bisexual and may be used as either male or female parents (McIntyre & Jackson 2001). Emasculation using hot water or the reduction in pollen viability in plants grown at low temperatures has been exploited to produce male sterile plants to use as female parents in breeding programs (as discussed in Heinz & Tew 1987).

Sugarcane breeding programs are severely limited by the nature of flowering of each sugarcane cultivar, particularly by a decrease in flowering and pollen viability at high latitudes (Moore & Nuss 1987). Crosses can be made only between cultivars which have overlapping flowering periods. Various techniques have been developed to induce flowering including alteration of

photoperiod so that flowers can be available for crossing when required (Bull & Glasziou 1979).



a) Sugarcane cultivars in the glasshouse ready for crossing



b) Cut male and female inflorescence bagged for crosses (2 weeks)



c) Fuzz developing for future seed harvest



d) Sugarcane seedlings



e) Sugarcane seedlings in growing trays



f) Sugarcane seedlings in a field trial

**Figure 3.** Photographic illustration of the steps involved in artificial crosses performed in *Saccharum* breeding programs. Photo taken by OGTR at BSES station (Aug 2007)

Commercial breeding programs produce assisted crosses between *Saccharum* spp. hybrids under highly favourable conditions. Flowering stalks are cut off and maintained in buckets of

crossing solution. The crossing solution consists of a dilute mixture of acids which help preserve the stalks and provide some nutrients (Cox et al. 2000). Male and female arrows are set up inside canvas lanterns (pollen impervious canvas bags) with the male set above the female to allow pollen to be shed downwards onto the female flowers (Cox et al. 2000). Once pollinated, the stalks are kept in the bucket of crossing solution and allowed to mature, a process taking twelve to fourteen days (Buzacott 1965). In more temperate climates, crossing houses with controlled temperature, light and humidity are used to perform specific crosses.

Figure 3 illustrates some of the steps involved in this process.

To improve the efficiency of breeding and to reliably identify cultivars, modern molecular techniques are being used. Molecular markers can be used to tag genes which are associated with traits of interest, or used to better understand the diversity in the parents used for breeding (Alwala et al. 2006; reviewed in Hotta et al. 2010). Molecular markers have been identified for sugarcane (Selvi et al. 2003; Lakshmanan et al. 2005; Alwala et al. 2008).

ESTs (expressed sequence tags) have been isolated in South Africa from sugarcane meristematic tissue (Carson & Botha 2000) and in Australia and South Africa from sugarcane stem tissue (Carson & Botha 2002; Casu et al. 2003; Casu et al. 2004). A Brazilian consortium has developed a sugarcane EST (expressed sequence tag) program (SUCEST) which produced 238,000 ESTs from 26 cDNA libraries, covering different developmental stages and different organs and tissues (Arruda 2001). ESTs have also been generated in the USA and compared with sorghum and Arabidopsis EST libraries to look for common genes (Ma et al. 2004). These EST projects aim to help expand the knowledge of sugarcane biology and genomics by providing the sequences and possible functions of large numbers of genes that could be related to economically important traits.

As sugarcane is a polyploid, many traits are quantitatively inherited, so QTL (quantitative trait loci) markers are being developed for use in breeding programs. QTLs have been obtained which are associated with stalk number and suckering (Jordan et al. 2004), sugar content (Hoarau et al. 2002; Ming et al. 2002; Aitken et al. 2006a), and yield related stalk traits (such as stalk weight, stalk number and stalk diameter) (Aitken et al. 2008).

An international sugarcane genome sequencing collaboration is also underway to generate sequence data for *S. officinarum*, *S. spontaneum* and a commercial hybrid (Bonnett & Henry 2011).

Mutation breeding has been used in sugarcane to add to the natural genetic variation (Patade & Suprasanna 2008). This includes experiments using tissue culture to induce somaclonal variation. Somaclonal variants for resistance to eyespot disease (*Helminthosporium sacchari*) have been generated through the screening of plants after tissue culture (Larkin & Scowcroft 1983). In some instances selection for the desired trait has been used, for example using eyespot toxin (Larkin & Scowcroft 1983) or for smut resistance (Rodríguez et al 2001 as cited in Patade & Suprasanna 2008). Mutagenesis has also been induced in tissue culture using radiation to produce plants with red rot resistance, tolerance to waterlogging, delayed flowering and altered timing of maturity (reviewed in Patade & Suprasanna 2008) and resistance to downy mildew and improved cane and sugar yield (reviewed in Larkin & Scowcroft 1981).

# 2.4.2 Genetic modifications

Sugarcane has a highly complex genome. This has limited opportunities for crop improvement through conventional breeding of sugarcane (Lakshmanan et al. 2005). Genetic engineering is seen as an important alternative approach for the introduction of new traits into sugarcane. For

an overview of methods and target traits for genetic modification of sugarcane see review by Brumbley et al (2008).

Sugarcane can be genetically modified by microprojectile bombardment (Bower & Birch 1992), electroporation (Arencibia et al. 1995) or *Agrobacterium*-mediated transformation (Arencibia et al. 1998). Positive selection, using the phosphomannose isomerase/mannose-selection system, has been used to produce GM sugarcane plants that do not contain an antibiotic resistance selectable marker (Jain et al. 2007).

Data show that introduced genes are stable in sugarcane and continue to be expressed after asexual and sexual propagation (Hansom et al. 1999; Harrison et al. 2001). However, there is some evidence from field grown GM sugarcane that yield and CCS is reduced, possibly due to the effects of biolistic introduction of DNA into callus. Controls, which had been through the tissue-culture process but were not subjected to biolistic bombardment (ie. not genetically modified), performed better than the GM plants, but still showed reduced agronomic performance. The reduced performance persisted after rationing (Vickers et al. 2005b; Gilbert et al. 2009). Similar results, with a smaller data set were reported from a Cuban field trial (Arencibia et al. 1999). This reduced growth has also been seen from micropropagated plants (Braga et al. 2003). A study of DNA polymorphism after *Agrobacterium* transformation indicated that polymorphism increased four-fold in sugarcane plants which had been through a tissue culture process. Selection by antibiotics or herbicides added to this increased polymorphism (Carmona et al. 2005). Similarly, simple sequence repeat (SSR) marker analysis showed many changes between different GM sugarcane lines produced by microprojectile bombardment and the parent line, thought to be caused during regeneration from callus (Gilbert et al. 2009). The authors suggested that this may be due to replication slippage caused by pausing of the DNA polymerase, or problems with the mis-match repair system.

Transposable elements, natural DNA sequences which cause mutations by moving within the genome, have recently been identified in sugarcane (de Araujo et al. 2005). These are expressed mainly in callus and may be the cause of the observed high somaclonal variation in this tissue (de Araujo et al. 2005). Epigenetic effects may also account for observed unusual growth patterns, however these are often temporary and are usually resolved within a few generations of vegetative reproduction (Birch 1997).

Sugarcane has been genetically modified through the introduction of genes altering a number of traits. Published papers on GM sugarcane describe the genetic modifications for: herbicide resistance (Enríquez-Obregón et al. 1998; Leibbrandt & Snyman 2003); disease resistance by expression of viral coat protein genes from sugarcane mosaic virus (SCMV) or sorghum mosaic virus (SrMV) (Joyce et al. 1998; Ingelbrecht et al. 1999); resistance to leaf scald disease (*Xanthomonas albilineans*) by introduction of a gene for albicidin detoxification (*albD*) (Hansom et al. 1999); resistance to stem borer (*Diatraea saccharalis* F.) (Arencibia et al. 1997; Arencibia et al. 1999) or shoot borers (Kalunke et al. 2009; Arvinth et al. 2010) by introducing *cryIA(b)* or *cry1Aa3*; reduce browning of sugarcane juice by reducing polyphenol oxidase (PPO) enzyme levels through co-suppression or antisense inhibition (Vickers et al. 2005a; Vickers et al. 2005b).

Sugarcane has also been genetically modified to create biofactories for the production of novel compounds. C4 grasses such as sugarcane have a high growth rate and efficient carbon fixation. Sugarcane is seen as particularly advantageous because in addition to the C4 qualities it has a substantial carbon flux through metabolic pathways, and the associated bagasse could be used to generate electricity needed for processing (Twine 2005). For example, GM sugarcane has been modified to produce altered sugars such as trehalose (Zhang et al. 2006; Hamerli & Birch 2011), isomaltose (Wu & Birch 2007) and sorbitol (Fong Chong et al. 2007)

or industrial compounds such as poly-3-hydroxybutyrate (PHB) (Brumbley et al. 2002; Purnell et al. 2007) and p-hydroxybenzoic acid (pHBA) (McQualter et al. 2005).. The first field trial in the US to produce a human pharmaceutical product was conducted with sugarcane genetically modified to produce human granulocyte macrophage colony stimulating factor (GM-CSF) (Wang et al. 2005).

In Australia, field trials of GM plants with altered sugar production, herbicide tolerance, altered plant architecture, enhanced drought tolerance and nitrogen use efficiency, altered sucrose accumulation, and improved cellulosic ethanol production from sugarcane biomass are underway (<a href="http://www.ogtr.gov.au/">http://www.ogtr.gov.au/</a>). In Brazil, there have been a number of field trials for traits such as herbicide tolerance, viral resistance, insect resistance, drought tolerance, sucrose yield and inhibition of flowering (Matsuoka et al. 2009).

# **SECTION 3** MORPHOLOGY

# 3.1 Plant morphology

The morphology and anatomy of sugarcane has been extensively reviewed and so will not be explored in great detail here. See Moore (1987), Bakker (1999) and Cheavegatti-Gianotto (2011) for a comprehensive treatment of the morphology and anatomy of sugarcane.

Sugarcane is a large tropical grass that produces multiple stems or culms each of which consist of a series of nodes separated by internodes. Following germination, the terminal vegetative bud of each shoot lays down a series of nodes. Each node consists of a growth ring or intercalary meristem, the root band (containing root primordia) and a bud above the leaf scar where the leaf sheath attaches, which delimits the node from the internode below. The internodes consist of sucrose storing parenchyma cells and vascular tissue (Moore 1987).

The stem of sugarcane is similar to maize and sorghum in that it is filled with parenchyma cells and is not hollow like many grasses (Griffee 2000). The stem is the major storage area for photosynthate (sucrose) within the sugarcane plant, rather than fruit or seed structures. Transverse sections through an internode reveal vascular bundles surrounded by parenchyma cells with a thick outer epidermis covered in an external layer of wax. Leaves and internodes develop in a basipetal direction in that the leaf blade expands at the base then the internode elongates. As the stem develops, the leaves emerge, one leaf per node, attached at the base of the node, forming two alternate ranks on either side of the stem. At the top of the stem is an apical meristem set on top of a number of very short internodes. Mature stems consist of a number of leaves still enclosed in the leaf spindle, a dozen or so green leaves and a number of senescent leaves, increasing in number with increasing age of the plant. New leaves emerge and expand over a period of between one and three weeks. Internode length can reach over 30 cm, depending on growth conditions, and stems normally reach two to three metres in the normal growing season (Bull & Glasziou 1979; Bull 2000).

The leaf blade is pubescent (hairy) on the abaxial (under) side of the leaf and glabrous (without hairs) on the adaxial (top) side and terminates in a pointed tip. The leaf blade is 2–10 cm across and 60–150 cm long (Fauconnier 1993). The base of the leaf attaches to the stem at the node but then wraps the stem to form a sheath that loosely encloses the internode to which the node subtends.

Sugarcane uses a C4 mechanism of photosynthesis similar to other tropical grasses where the carbon dioxide for photosynthesis is initially fixed by PEP (phosphoenolpyruvate) carboxylase to form a four carbon compound (Hatch & Slack 1966), and consequently the anatomy of the leaves reflects this underlying physiology. The vascular bundles are surrounded by a ring of bundle sheath cells and a ring of mesophyll cells, an arrangement known as Kranz anatomy (Arms & Camp 1987).

Like most grasses, the sugarcane root system is fibrous and shallow. It has been estimated that the top 25 cm of soil contains 50% of the plant roots, with the next 35 cm containing a further 40% of the roots (Fauconnier 1993). However, the effective root zone (ie. the area of roots which are actively extracting water) varies depending on the soil type, from just the topsoil in sodic duplex soils, to 0.9–1.2 m in irrigated clay loam, to 1.8 m in rain-fed conditions (Ham et al. 2000). The root system is dynamic and the areas of active root growth vary depending on the irrigation pattern (Inman-Bamber et al. 2008). The plant also develops buttress roots, that serve to anchor the plant, and some deeply penetrating roots, that grow downwards for up to four metres allowing for water absorption under water stress (Bull & Glasziou 1979). Roots partially die-back after ratooning, although some of the roots can persist for at least four months after harvest and some of the new roots emerge from the old pre-harvest roots (Smith et al. 2005).

# 3.2 Reproductive morphology

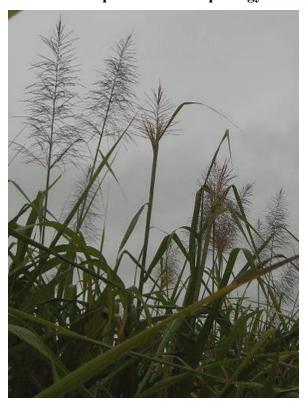
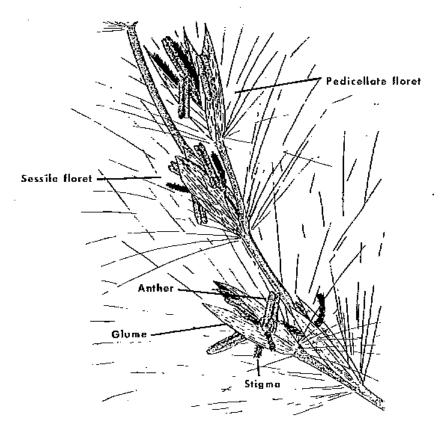


Figure 4. Saccharum spp. hybrid inflorescences. Photo taken by Graham Bonnett, CSIRO.

The sugarcane inflorescence is an open branched panicle, also known as an arrow, whose shape, degree of branching and size are highly cultivar specific (Figure 4). The arrow can bear thousands of flowers (Sleper & Poehlman 2006), estimated to average 24,600 florets (Rao 1980). The arrow consists of a main axis and first, second and third order branches. Attached to the branches are spikelets arranged in pairs, one of which is sessile and one pedicellate, that bear individual flowers (Figure 5). At the base of each spikelet is a row of silky white hairs. Sugarcane flowers consist of three stamens (male) and a single carpel with a feathery stigma (female) typical of wind pollinated flowers. Frequently, the male stamens may be abortive resulting in reduced or absent pollen production (Moore 1987; James 2004; Sleper & Poehlman 2006). Anther colour varies from bright yellow to purple (Moore 1987).



**Figure 5.** Diagram of a portion of a mature raceme of a sugarcane inflorescence showing the arrangement of sessile and pedicellate spikelets and callus hairs (Moore 1987). Reprinted with permission from *Sugarcane Improvement through Breeding*, edited by Don J Heinz, Chapter 3 *Anatomy and Morphology* by Paul H. Moore, copyright (1987). Original figure from Engard and Larsen (1948).

#### SECTION 4 DEVELOPMENT

# 4.1 Reproduction

Sugarcane can reproduce both sexually and asexually. Sexual reproduction is via true seed, often called fluff due to the presence of soft hairs. As discussed in Section 2.3.1 the ability of sugarcane to reproduce asexually is exploited for the production of planting material.

# 4.1.1 Asexual reproduction

Asexual reproduction can be via nodal buds which are found on setts, via rhizomes or via stools (Amalraj & Balasundaram 2006). The parent species of *Saccharum* spp. hybrid differ in their ability to form rhizomes and tillers, with *S. spontaneum* forming dense mats of rhizomes and many tillers, whereas *S. officinarum* forms fewer tillers and rhizomes (Moore 1987; Amalraj & Balasundaram 2006).

# 4.1.2 Sexual reproduction

The ability of sugarcane to reproduce sexually was not recognised until the mid to late 1800's due to its lack of importance as an economic product (Buzacott 1965). Sugarcane flowering is a complex process consisting of a number of steps which are regulated by different photoperiods (Moore & Nuss 1987). Flowering is dependent on interaction of genotypes and environmental factors such as daylength and temperature. Some cultivars can flower profusely in their natural environment but flower sparingly when introduced to other regions (Bull & Glasziou 1979). Cultivars which evolved at high latitudes usually flower earlier than those which originated at lower latitudes, suggesting that they require longer daylengths for floral

initiation (Moore & Nuss 1987). Experiments have also indicated that early flowering cultivars often flower more profusely than later flowering cultivars, possibly due to environmental effects (Moore & Nuss 1987). At Meringa field station, south of Cairns in QLD, an average of 40% of the parental clones used for crossing flowered each year between 1978 – 2003. However, this varied from 13% in 2003 to 75% in 1994 (Berding et al. 2004a). A study of three cultivars in commercial cane fields in the Burdekin region (QLD) in 2006, indicated that one cultivar did not flower, one flowered in some locations and one flowered in all five locations (Bonnett et al. 2007). Observations from the Mulgrave Mill area and the Herbert River region (both in QLD) indicated that commercial sugarcane does flower in these areas (Bonnett et al. 2007).

In Australia, floral development is initiated by shortening day length and occurs from late February to early March (Kingston 2000). Flowering is mostly reliable between latitudes 7° and 12° and non-existent above 30° latitude (Fauconnier 1993). Floral development is induced by photoperiods of approximately 11.5 hours, which often coincides with a natural day length of 12.5 hours. As a result, the period of floral initiation is more defined further from the equator (Bakker 1999). Annual variations in flowering times in a given location are mostly attributable to the differences in night time temperature (Bakker 1999). Cool night temperatures, high day temperatures and lack of moisture interfere with flower initiation. The older and more vigorous stems in a stool are the most likely to initiate flowering (Moore & Nuss 1987). Flower initiation causes the apical meristem to switch from vegetative to floral development. Consequently, flowering of the crop can adversely affect yields (Bakker 1999).

Flowering is not desirable in commercial cane as it uses both energy and sucrose and may lead to pithy islands in the stems (Purseglove 1972). The loss of apical dominance and consequent formation of side shoots leads to reduction in the sucrose content in the stalk. However, if harvesting occurs within 2–3 months of flowering this effect is negligible (Bakker 1999). Measures that have been trialled to prevent flowering include altering planting dates, cutting back the stems, lighting the field for 30 mins at night, applying chemical sprays, applying nitrogen and withholding water (Purseglove 1972; El Manhaly et al. 1984). However, there is some conflicting data on the impact that flowering has on reducing sucrose content in sugarcane stems. As discussed in Moore (1987), some of the conflicting data is due to inappropriate comparisons. Different sugarcane cultivars are affected differently by flowering, and plants that flower may have altered physiology that led to flowering rather than being caused by flowering itself. For example, a series of 35 field trials using epheron, a plant growth regulator, showed reduced flowering and an overall increase in cane weight and sugar yield. However, there was little correlation between reduced flowering and increased yield due to variability between fields (Moore & Osgood 1989). More recent Australian data from experimental plots has shown that cane yield, CCS and sugar yield were all decreased following flowering (Berding & Hurney 2005).

# 4.2 Pollination and pollen dispersal

Sugarcane spikelets open from the top of the panicle, with the outermost spikelets opening first. It takes 5–15 days for all the spikelets on the panicle to open. Spikelets open at sunrise, with anther dehiscence occurring about three hours later, although this is delayed by high humidity (Purseglove 1972).

Sugarcane pollen grains are very small, hairy and wind dispersed. The round-ellipsoidal grains vary in size from 38.25  $\mu$ m x 42.75  $\mu$ m to 67.5  $\mu$ m x 72.0  $\mu$ m (Dutt 1929) and are yellow in colour

Pollen viability from commercial sugarcane fields is low and varies between cultivars and locations in the Burdekin region, showing a range from 1.2–4.4% viability (Bonnett et al. 2007). Studies showed that sugarcane pollen began to lose viability rapidly in less than 30 min (Venkatraman 1922). *S. spontaneum* pollen is rapidly desiccated after dehiscence, having a half-life of only 12 minutes, and is no longer viable beyond 35 minutes, under unmodified environmental conditions (26.5° C and 67% relative humidity) (Moore 1976). At higher humidity the pollen longevity was increased (Moore 1976). Tests with another cane cultivar (Saratha Desi, which is thought to be derived from *S. barberi*) indicated that pollen viability was maintained for two hours in the lab, or one hour when exposed to sunlight (Dutt & Ayyar 1928). Sugarcane pollen stored at 4°C under 90–100% relative humidity retains some viability for up to fourteen days (Moore & Nuss 1987).

Little data is available on sugarcane pollen dispersal. Information from breeding work in which plants were isolated by 20 m in open forest has shown that viable pollen is dispersed over this distance (Skinner 1959). From this work, it was suggested that to prevent contamination of controlled crosses, plants should be isolated by 100 m in open forest, or 300 m in open ground (Skinner 1959).

Sugarcane is a cross-pollinating species although selfing occurs at low levels (Moore & Nuss 1987; McIntyre & Jackson 2001; Tew & Pan 2010). In seven experimental polycrosses the selfing frequencies ranged from from 0–45%. Although the sample size was small, it indicated that the progeny resulting from crosses with a high degree of self-pollination had reduced ability to survive the winter, suggesting reduced vigour (Tew & Pan 2010). The reduction in vigour following self-pollination has been observed previously (Skinner 1959). Sugarcane produces protogynous flowers, where the pistil matures before the anthers. Thus, an individual flower may be cross-pollinated prior to pollen shed from its own anthers (James 2004).

Sugarcane flowers often have reduced male fertility or are male sterile and some are self-sterile (Skinner 1959).

# 4.3 Fruit/Seed development and dispersal

After fertilisation it takes approximately three weeks for the fruit to mature and to be shed (Purseglove 1972). The seed at the top of the panicle which was fertilised first is also the first to mature (Breaux & Miller 1987). These seed are shed as the inflorescence starts to disintegrate, before the seeds at the base reach maturity (James 1980). The mature fruit contain whorls of silky hairs at the base and are adapted for wind dispersal (Purseglove 1972) (Figure 6). No further information has been found in the literature on seed dispersal.

Data from crosses has suggested that a low percentage of florets set fertile seed. One estimate of seed germination showed a maximum of 17.2% in a "very heavy" germinator (Price 1961). Another study showed germination rates of between 3.1 and 22.7% (Rao 1980). Seed collected from commercial fields in the Mulgrave Mill area had variable germination, ranging from 0.9 to 23.6 viable seed g<sup>-1</sup>, depending on the cultivar (Bonnett et al. 2007). Similarly in the Herbert region, even in October, which is four months after the normal flowering time, inflorescences were found containing between 0 and 53.3 viable seed g<sup>-1</sup>. A very small proportion of seed collected further south in the Burdekin region was viable (three seedlings germinated from 30 arrows) (Bonnett et al. 2007).

The naked seed has been measured as  $1.5\pm0.03 \times 0.64 \pm 0.005$  mm and weighing  $0.54\pm0.05$  mg, which is approximately 1850 seeds g<sup>-1</sup> (Rao 1980). One of the sugarcane parent species *S. spontaneum* has seed which weighed 0.39 mg with fuzz, or 0.25 defuzzed (Ellis & Hong 2007).

Mature fuzz consists of the mature dry fruit (caryopsis), glumes, callus hairs, anthers and stigma (Breaux & Miller 1987). The additional parts of the inflorescence are generally handled, stored and sown with the seed because it is not practical to separate them. Although many commercial cultivars of sugarcane can produce seed, it is only used in breeding programs, because the proportion of sugarcane seedlings with agronomic qualities near to those of the parental commercial cultivars is extremely low.



**Figure 6.** *S. spontaneum* seed. Photo by Kristin Saltonstall, Smithsonian Tropical Research Institute, Panama.

# 4.4 Seed germination

Some wild species of sugarcane such as *S. aegyptiacum* (now classified as a subspecies of *S. spontaneum*) have significant seed dormancy, whereas modern cultivars have little seed dormancy (Ellis, Hong and Roberts 1985 as cited in Simpson 1990).

Sugarcane seed has short viability. Preliminary data from on-going experiments has shown that germination of seeds after shedding varied between sugarcane cultivars, but for more than half of the 13 cultivars tested it stayed high for 10–12 weeks when stored under lab conditions at 22°C. Other samples showed a decline in germination from eight weeks (Powell et al. 2008). If stored in polythene at room temperature, fuzz remained viable for 90 to 120 days (Verma et al. 2002). Artificially dried sugarcane seed lost 90% of its viability in 70 days at 28°C if not desiccated (Rao 1980). Modelling of seed longevity using data on germination at different temperatures and moisture contents has predicted that under hermetic storage at -20°C, seed from the parent species *S. spontaneum* will not last as long as ten other crop species, with only potato (*Solanum tuberosum*) showing shorter viability (Ellis & Hong 2007).

Generally in breeding programs the fuzz is sown. However, the fuzz can encourage growth of microorganisms and a large mass of fuzz can prevent seed contact with the soil (Breaux & Miller 1987). Improved germination has been seen when the non-seed parts of the fuzz are removed (Breaux 1981 as cited in Breaux & Miller 1987).

Germination of sugarcane seed occurs better in the light, requires heat and humidity, and takes 25 days for small seedlings to appear (Buzacott 1965; Purseglove 1972). Preliminary data from on-going experiments has shown that seed germinated at 15–42°C under lab conditions, with an optimum germination at 30–36°C (Powell et al. 2008).

As the seed germinates, the primary root emerges first followed by elongation of the plumule. The leaves of the plumule then emerge rapidly. Tiller branches emerge from a bud which forms in the axil of each leaf. Adventitious roots form near the leaf bases (Moore 1987).

The young seedlings are delicate and require optimum temperature, moisture, nutrients and protection from fungal diseases (Buzacott 1965; Breaux & Miller 1987). Information in Breaux and Miller (1987), in part obtained from a survey of sugarcane breeders, suggests that the conditions required to germinate and grow sugarcane seedlings are exacting. Constant care and attention is needed to give seeds and seedlings the conditions required for survival, especially in the first 3–4 weeks post-germination.

Vivipary, when the seed germinates before it detaches from the parent plant, has been observed under experimental conditions in both the parent species *S. spontaneum* and in hybrid sugarcane (Ragavan 1960). It is feasible that moist conditions, similar to the experimentally induced ones, could occur naturally.

# 4.5 Vegetative growth

As discussed previously, sugarcane is propagated from stem cuttings which are referred to as setts, seed, seed-cane or seed-pieces (Purseglove 1972). During the initial stages of germination, root primordia around the nodes of the sett produce a flush of roots, known as sett roots (Bakker 1999). These roots are not connected directly to the primary shoot but are important in maintaining the moisture in the sett. Following formation of the shoot roots, the sett roots blacken and die (Bakker 1999). The primary shoot is made up of a number of closely spaced internodes and nodes below ground. Each node develops new bud and root primordia that are the basis of stool establishment. These root primordia germinate to produce the shoot roots that support further plant growth. The shoot is then independent of the original sett (Bull 2000).

While the shoot roots are developing, some of the new buds below ground also germinate to produce secondary shoots or tillers. These, in turn, develop their own root systems and give rise to shoots (Bull 2000). Shoots usually appear above the soil approximately twelve days after planting, with the first leaf unfurling approximately eight days later (Bakker 1999).

Stem elongation is initially rapid and during this phase the fibre content of the stem is relatively high, whereas the CCS levels are still quite low. Breeding for high above ground biomass in modern sugarcane cultivars means the plant is very top heavy and consequently sugarcane is prone to lodging. Plants recover from lodging by curving of the stem to again grow upright. In Australia, studies have shown that lodging is associated with yield losses in both the wet and dry tropics (Singh et al. 2002).

Growth rate slows and sucrose content increases approximately 120 days after planting (Bull 2000). Maturation and ripening are reversible processes and are associated with the lower rainfall and cooler temperatures of the winter months. During stem growth, each internode operates as an independent unit. While it has a green leaf attached, the internode completes cell elongation and cell wall thickening, and fills with sucrose. Hence internodes generally complete their cycle by the time the attached leaf dies, and the lower internodes are essentially ripe while the upper part of the stem is still growing. The stored sugar is, however, available for translocation to support further tillering and/or growth when conditions are not favourable for photosynthesis (Bull 2000).

As the stem matures, more internodes reach the same condition and sucrose content rises. During this period, the most recently expanded internodes near the top of the stem stop elongating and photosynthates are channelled into storage as sucrose. Factors that affect the maturation of the sugarcane stem include age, nitrogen status and moisture. Environmental

factors that can influence sucrose accumulation include water stress, nutrient status and temperature (Bull 2000).

#### **SECTION 5 BIOCHEMISTRY**

#### 5.1 Toxins

Sugarcane is a well-established agricultural crop with a long history of safe use; it has been cultivated in Australia for over 100 years. Commercial sugarcane is grown as a source of sugar (sucrose) for human food. By-products from processing sugarcane into sugar such as molasses and bagasse have been mainly used as additives in stockfeed.

Sucrose is the primary product of plant photosynthesis and, therefore, common in food crops consumed regularly by humans and animals. Sucrose has an exceedingly long history of human dietary exposure. It has been classified as a non-toxic substance to humans (MSDS 2004). The oral LD<sub>50</sub> of sucrose for rats is 30–35 g kg<sup>-1</sup> body weight (Boyd et al. 1965). Consuming sucrose in extremely large oral dosages may produce gastrointestinal disturbances. Although there is no direct evidence that links sucrose consumption with toxicity, there are several studies indicating that sucrose intake should be limited because it may be associated with health problems (Howard & Wylie-Rosett 2002). Studies found that a high intake of sucrose was associated with cardiovascular diseases, development of type II diabetes, obesity and hypertension (Howard & Wylie-Rosett 2002). In addition, it is well established that sucrose consumption is a risk factor for dental caries (Sreebny 1982; Rugg-Gunn & Murray 1983).

Sugarcane also contains cyanogenic glucosides. These can be cleaved to produce hydrocyanic acid, a poison that acts by inhibiting cytochrome oxidase, thus preventing transfer of oxygen from the blood to the tissues. It is present in many plants, including sugarcane, where it is not at a dangerous level for humans (Rossoff 2002).

A mixture of bagasse and molasses can be used as a food source for cattle. Molasses can be added to cereals at up to 15% in the final mix to improve palatability (Perez 2004). In Cuba, molasses is often used as a much higher proportion of the diet, mixed with urea and fed as 70% of the total diet (Perez 2004). Experiments on cattle in Australia have shown that feeding a diet containing up to 50% molasses may be useful for the beef feedlot industry, especially in northern Australia in areas close to sugar mills (Tomkins et al. 2004). However, feeding of molasses has to be carefully managed as it may be toxic when fed incorrectly or in large quantities. The symptoms of molasses toxicity include reduced body temperature, weakness and rapid breathing and the animal may have difficulty standing (Perez 2004). Molasses toxicity often affects eye-sight and the animal may become blind due to brain damage thought to be cerebro-cortical necrosis (CCN). Studies of an affected ox suggested that the encephalopathy was indistinguishable from CCN (Edwin et al. 1979). The necrosis is likely to be caused by a decrease in energy supply to the brain because of either thiamine or glucose deficiencies (Preston 1988). The glucose deficiency is thought to result from a reduction in propionate, required for gluconeogenesis, due to a complete digestion of molasses in the rumen and therefore a reduction of glucose in tissues and ultimately the brain (Edwin et al 1979 and references therein).

Bagasse, like many other agricultural by-products such as cereal straws, is high in ligno-cellulose and may have a depressing effect on feed intake. The digestibility of bagasse is very poor because of the presence of lignin which protects carbohydrates from being digested by the rumen microbes (de la Cruz 1990; Leng 1991). To improve the nutritive value of lignocellulose materials for livestock, physical or chemical pre-treatments are required (Playne 1984; de la Cruz 1990).

# 5.2 Allergens

Sugarcane pollen is transported by wind and therefore has the potential to act as an airborne allergen. The allergenicity of sugarcane pollen was evaluated in India where 70% of field workers with respiratory disorders showed positive reactions to sugarcane pollen in skin tests (2001). The authors also tested rice and several other plant species and concluded that sugarcane pollen was the most significant allergenic type. However, there are no reports of any major allergic responses to the commercial hybrid cultivars of sugarcane in Australia.

Exposure to organic dusts, such as those present in mouldy sugarcane, can cause bagassosis. Bagassosis is an occupational lung disease of the extrinsic allergic alveolitis type and is caused by breathing dusts containing fungal spores, and/or thermophilic actinomycetes which grow in stored, mouldy bagasse (Lacey & Crook 1988). In Australia, bagasse may be stored covered with tarpaulins at the end of the crushing season to be used to fuel the boilers at the beginning of the next season before fresh bagasse is available (Dawson et al. 1996). The stored sugarcane bagasse contains approximately 50% water and 5% sucrose, so is colonised by bacteria, causing it to heat up and create ideal conditions for fungi and thermophilic bacteria such as Aspergillus fumigatus, Thermoactinomyces vulgaris and Thermoactinomyces sacchari (Lacey & Crook 1988). In India, it is thought that T. sacchari and Saccharopolyspora rectivirgula are the most likely cause of bagassosis (Khan et al. 1995). Prolonged, repeated exposures can lead to permanent lung damage and scarring, and significant disability (Phoolchund 1991; Hur et al. 1994). In Puerto Rico, a study showed a four-fold increase in risk of cancer of the oral cavity amongst sugarcane farmers and farm workers, which may be due to exposure to actinomycetes (Coble et al. 2003). However, a study at two Australian sugar mills did not identify very high levels of airborne bacterial spores and none of the 271 mill workers surveyed showed any symptoms of bagassosis (Dawson et al. 1996).

# 5.3 Beneficial phytochemicals

There have been some reports that very long chain fatty acids/alcohols (policosanols) from sugarcane wax lower cholesterol in humans (reviewed in Hargrove et al. 2004). However, other studies reported no effects on cholesterol (Kassis et al. 2009). Policosanols have also been reported to decrease risk of cardiovascular disease (Janikula 2002) and may have anti-inflammatory effects (Ledón et al. 2007).

Other beneficial phytochemicals from sugarcane include glycolic acid, which can be used in cosmetics, primarily for skin rejuvenation (reviewed in Allen et al. 1997).

#### SECTION 6 ABIOTIC INTERACTIONS

#### 6.1 Abiotic stresses

# 6.1.1 Nutrient stress

The cultivation of sugarcane relies on the extensive use of fertilizers. It has been estimated that a crop of 74 tons of cane ha<sup>-1</sup> removes 107 kg nitrogen, 60 kg phosphorus oxide and 300 kg potassium oxide ha<sup>-1</sup> (Purseglove 1972). The sugarcane plant requires nitrogen for optimum development for yield and sugar content of the canes. Symptoms of nitrogen deficiency are thin, stunted stalks, yellowing leaves with necrosis at the edge and tips and reduced root mass (Calcino et al. 2000). However, excess nitrogen can prolong the crop maturation, resulting in a plant with an excessive leafy canopy, which in turn can make the plant more susceptible to leaf diseases and attack by pests (Bakker 1999). It can also cause excess growth with little storage of sucrose (Irvine 2004).

Phosphorus is required for optimum growth. Deficiencies may manifest in plants with short, thin stalks and stools with a low number of primary stalks, a poorly developed root system and

sometimes leaves that are green-blue in colour. Conversely, an excess of phosphorus can lead to a deficiency of other trace elements such as zinc and iron, thus reducing sugar yields (Bakker 1999).

Potassium is required for many physiological processes. It helps to promote the formation and translocation of sugars, and thus may improve the extraction and purity of the cane juice. Supplementing sugarcane plants that are exposed to excessive nitrogen with potassium can alleviate the symptoms of over-supply of nitrogen. Potassium deficiency results in depressed growth, thin stalks and yellowing of the older leaves with chlorotic spots and ultimately death of the leaf (Bakker 1999). Potassium may also play a role in the ability of sugarcane to withstand dry conditions (Wood & Schroeder 2004). An excess of potassium increases the ash content of sugarcane juice and reduces the recovery of sugar, and, as with phosphorus, it may also lead to a deficiency of other trace elements (Calcino 1994).

Calcium is an important element for plant growth and also a regulator of soil acidity. A deficiency in calcium results in leaf chlorosis and reduced stem diameter. Increasing soil acidity, often due to lime application, can result in an increased fixation of phosphorus, aluminium, iron, manganese and nickel, which may lead to toxicity (Bakker 1999).

Magnesium is important for photosynthesis, being required for chlorophyll function, and is responsible for the green colour in the leaves (it absorbs the blue and red light spectrum). Deficiencies result in leaf chlorosis and stalks of reduced diameter with internal browning (Bakker 1999). Magnesium is usually abundant in Australian soils, although it may become depleted in old canegrowing soils (Calcino 1994).

Other micro element requirements include sulphur, iron, aluminium, zinc, copper, boron, silicon, molybdenum and manganese. Both deficiencies and toxicity to these elements can occur, resulting in symptoms such as reduced growth, reduced root development and a reduction in photosynthesis (Bakker 1999).

# 6.1.2 Temperature stress

#### Low temperatures

Sugarcane cultivars differ in their degree of temperature sensitivity but in general sett germination is slow at soil temperatures below 18°C and the setts may succumb to attack by fungal pathogens before they germinate. Sett germination is increasingly rapid up to about 35°C (Bull 2000).

Experiments have shown that sugarcane plants grow more slowly and have fewer, shorter internodes and fewer leaves at 15°C than when grown at 27°C. The low temperatures also inhibited sucrose export from the leaves to the stalk so the leaves accumulated sugar and starch (Ebrahim et al. 1998).

Flowering is also affected by low temperatures. Cool night temperatures, high day temperatures and lack of moisture interfere with both flower initiation and sucrose accumulation. Temperatures below 18.3°C are non-inductive for flower development (Coleman 1963). In temperate South Africa, pollen fertility has been shown to be limited at temperatures below 21°C (Brett (1952) as cited in Berding 1981). In Meringa, in QLD, artificially increasing the night-time temperature of sugarcane plants to 22–23°C led to increased and earlier flowering (Berding 1981). Experiments have also shown that heated pollen lanterns, used for crossing, can increase seed setting, due to improved fertilisation and embryo development (Berding & Skinner 1980).

Preliminary data from on-going experiments has shown that seed germination is reduced by 60% at temperatures below 30°C (Powell et al. 2008).

Sugarcane is susceptible to frost damage (Griffee 2000). Freezing reduces yields by delaying crop development in spring and by terminating sugar accumulation in autumn (Moore 1987). In northern NSW about a third of the cane is affected by frost, leading to yield losses of 10– 30% annually. Frosts may also affect production in southern QLD and limit the growth of sugarcane in southern regions of Australia (Weaich et al. 1993). The degree of damage varies with the severity of the frost. Leaf browning occurs at temperatures from 0 to -2°C, with temperatures down to - 4°C causing damage to terminal and lateral buds and death of some young internodes. Temperatures as low as -11°C can cause freezing and subsequent cracking of entire stalks. The cracks or damaged buds can allow entry of anaerobic bacteria such as Leuconostoc mesenteroides which can replicate in the damaged tissues and produce dextran. Dextran reduces the sucrose yield at the mill by preventing the crystallisation of sucrose (Irvine 2004). Frost damage varies between sugarcane cultivars, and this is thought to be due to differences in tolerance, rather than differences in morphology which might protect against frosts (avoidance) (Weaich et al. 1993). Management practices, such as retention of a trash blanket increases the susceptibility to frost by preventing radiation of warm air from the soil (Kingston 2000).

#### Hot temperatures

Sugarcane can survive temperatures as high as 45°C, or higher for short periods of time but growth slows at temperatures above 40°C (Moore 1987). However, in Iran sugarcane is grown in the Hapft Tappeh region where the average temperature over the summer months is 45.8 °C (Sund & Clements 1974). Sugarcane grown in the Ord River region of WA, which has a mean November temperatures of 39.4°C², has a lower sucrose content than that grown in cooler regions (Bonnett et al. 2006). Sugarcane exposed to temperatures between 25–38°C had a larger number of shorter internodes which contained lower sucrose levels than similar sugarcane plants grown at 23–33°C (Bonnett et al. 2006). High daytime temperatures (above 31°C) may also inhibit flowering, and very high temperatures at anthesis may reduce seed set. However, it has been suggested that these responses to high temperatures may be due to a water stress effect (as discussed in Moore & Nuss 1987).

# 6.1.3 Water stress

Sugarcane is relatively drought resistant but water stress results in a reduction of sugar production (FAO 2004). It is estimated that irrigation can add 3 t sugar ha<sup>-1</sup>, a figure modelled on an average irrigation of 500 mm (Meyer 1997). Sett germination does not occur in dry soil (Smit 2011). Sugarcane flowering is also reduced by water stress (Moore & Nuss 1987) with watered crops showing a greater number of panicles and a higher percentage of plants flowering (Berding 1995).

#### *6.1.4 Other abiotic stresses*

#### Fire

Fires do not generally kill sugarcane plants, in fact fire may be used to facilitate easier harvesting. It does not destroy the suckers and the plant will subsequently shoot from the nodes or regrow from the stools (FAO 2004).

# Waterlogging

Sugarcane plants can withstand short periods of flooding (FAO 2004). After four days the growing point of the sugarcane plant will die, but it may continue to grow from side shoots once the water has receded (BSES Ltd 2008a). Generally yield loss will be 15–20% after five

<sup>&</sup>lt;sup>2</sup> Bureau of Meterology (http://www.bom.gov.au/climate/data/) accessed 17 Sept 2010

days submergence, 30–60% yield loss after ten days and 37–100% after fifteen days, but this depends on the height of the stalks, with younger cane being more affected than those at 2.5 m tall (BSES Ltd 2008a). Prolonged periods of waterlogging will result in a decline in sugar content (FAO 2004). Waterlogging also results in cooler soil temperatures so germination of setts will be slower and losses from disease may be higher (Ridge & Reghenzani 2000).

#### Altitude

Sugarcane can be grown in a range of altitudes from just above sea level to as high as 3000 m above sea level (FAO 2004).

#### Wind

High winds, especially when combined with heavy rain, can lead to lodging of cane stalks in the field. This leads to problems with harvesting, reduced cane yield and reduced CCS. In northern QLD (an area of high rainfall) a 15–35% decrease in sugar yields have been recorded in a lodged crop compared to an unaffected crop (Singh et al. 2000; Singh et al. 2002). This may be due to rat damage, suckering, and stalk and stool death following lodging (Inman-Bamber et al. 2008).

# Soil pH

Sugarcane prefers a soil pH of 5.0–5.8, although it will tolerate between pH 4–10 (Fauconnier 1993).

#### Salt tolerance

Sugarcane is sensitive to soil salinity. It has been estimated that it will show no reduction of growth in soil with salinity up to 1.1 decisiemens per metre (dS m<sup>-1</sup>) and a 10% growth reduction at 2.2 dS m<sup>-1</sup> (Evans 2006). Sugarcane production is not economic in areas with soil salinity above 4.0 dS m<sup>-1</sup> (Rozeff 1995). It has been further estimated that 10% of the area under sugarcane cultivation in Australia is affected by salinity (Christiansen 2000). Field studies in the Burdekin region showed a negative correlation between cane yield and soil salinity, and showed yield reductions even at salinity levels usually considered too low to be detrimental (Nelson & Ham 1998). It was estimated that for the Burdekin area, there is a 14% decrease in yield for every 1 unit increase in EC<sub>e</sub> (saturation extract electrical conductivity) of the 0–0.5 m depth soil layer (Nelson & Ham 2000). Salinity affects both growth rate and yield of sugarcane, but also the sucrose content of the stalk (Rozeff 1995). Shoot growth has been shown to reduce, although the severity varies between cultivars (Akhtar et al. 2001b), and root growth may be stimulated by increased salinity (Gerard 1978). High salinity has been shown to reduce stalk height and weight, due to reduction in both the number of internodes and the internode length, but not the number of stalks, and may be related to reduced water content (Lingle et al. 2000; Akhtar et al. 2001a). Different life stages may have different sensitivities to salinity, with seed germination showing the least sensitivity (Wahid et al. 1997). In experiments under saline conditions, ratoon crops have shown 2.2–3.7 times greater yield loss compared to plant crops (Bernstein et al. 1966). The addition of potassium and silicon have been shown to help ameliorate the decreases in plant growth and juice quality caused by salinity, and actually have more effect on salt sensitive genotypes compared to salt tolerant genotypes (Ashraf et al. 2009).

#### Aluminium tolerance

High aluminium levels are associated with acid soils, and aluminium toxicity can cause a major reduction in yield in many crops (Delhaize & Ryan 1995). Sugarcane is relatively tolerant of high aluminium levels, although differences in tolerance have been seen between cultivars (Hetherington et al. 1986). Cultivars of the *S. officinarum* parent species generally have higher

levels of tolerance than the *S. spontaneum* parent species (Landell (1989) as cited in Drummond et al. 2001). In an experiment comparing the aluminium tolerances of sugarcane, navybeans, soybeans and maize, which may be grown in rotation with sugarcane, the sugarcane cultivars showed the greatest tolerance. The concentration of aluminium which led to a 10% reduction in root growth were up to ten-fold higher for sugarcane than the other crops tested (Hetherington et al. 1988). Symptoms of toxicity include root stubbing, which leads to susceptibility to water stress and yield loss (Calcino 1994).

#### Other metals

Sugarcane has been shown to tolerate up to  $100 \, \mu M$  copper in laboratory experiments (Sereno et al. 2007). Tolerance to cadmium is higher, with laboratory experiments showing no toxicity at  $500 \, \mu M$  cadmium (the highest concentration tested). Plant damage was seen in other experiments at 2 mM cadmium (Fornazier et al. 2002). The high tolerance to cadmium, and the observation that the sugarcane plants can accumulate cadmium have suggested its use in phytoremediation (Sereno et al. 2007).

# **SECTION 7 BIOTIC INTERACTIONS**

#### 7.1 Weeds

Weeds are a problem in a sugarcane crop due to yield reduction caused by competition or allelopathy and interference with harvesting machinery which reduces product quality (McMahon et al. 2000). In Australia, in 2000, weeds were estimated to cost the sugarcane industry \$70 million each year, in both control costs and lost production (McMahon et al. 2000). As well as controlling weeds within the crop, it is important to control weeds around the farm to reduce any high protein food, such as weed or grass seeds, which rats need to breed (McMahon et al. 2000). See section 7.2.1 for a discussion of rats as a pest of sugarcane.

There are a number of weeds that infest sugarcane plantations including grasses, broadleaf weeds, vines and sedges. Those weeds that are a major problem in Australia are discussed below.

The predominant grasses that occur in sugarcane growing districts include barnyard grass (*Echinochloa crus-galli*), awnless barnyard grass (*Echinochloa colona*), couch grass (*Cynodon dactylon*), wild sorghum (*Sorghum spp.*) and guinea grass (*Panicum maximum*). Pasture grasses in particular can be problematic when the land is subsequently used to grow sugarcane (McMahon et al. 2000). Hymenachne (*Hymenachne amplexicaulis*) is an aquatic grass to 2.5 m tall and has been declared a *Weed of National Significance* (Queensland Department of Primary Industries and Fisheries 2007b). It was planted extensively in northern QLD and the Northern Territory (NT) as a fodder crop and has since escaped from cultivation. It can block irrigation and drainage channels in sugarcane plantations and contaminate sugarcane crops. Spraying with herbicides every three months is used to control hymenachne (CRC for Weed Management 2003). *Imperata cylindrica* is a perennial species that commonly grows on degraded or burnt-off land in most Australian sugarcane-growing districts (Lazarides et al. 1997). It is a common weed in QLD, and although it occurs in all Australian states, it is not listed as a noxious weed in any jurisdiction (National Weeds Strategy Executive Committee 2009).

The most important and prevalent weed of sugarcane is sedge nut grass (*Cyperus rotundus*), although in wetter areas other sedges also occur (McMahon et al. 2000). *C. rotundus* was estimated in 1967 to reduce cane yields by 10 t ha<sup>-1</sup> (Chapman as cited in McMahon et al. 1989). It spreads mainly by tubers, which are produced in very large numbers and are carried in soil and by flood waters. It also reproduces by seed, although apparently only rarely. It

withstands cultivation extremely well, and this process rapidly spreads the tubers around and between fields (DPIW- Tas 2009).

Broadleaf weeds such as blue top/billygoat weed (*Ageratum spp*.) and purslane/pigweed (*Portulaca oleracea*) tend to be less of a problem and can be controlled relatively easily if targeted when the plants are young. Broadleaf weeds tend to be more regional and soil specific (McMahon et al. 2000). The giant sensitive plant, also known as tropical blackberry (*Mimosa diplotricha* or *M. invisa*), is a serious weed in northern QLD and has been identified as a weed that can invade sugarcane crops (Queensland Department of Primary Industries and Fisheries 2007c). It can be controlled using an introduced sap sucking insect (*Heteropsylla spinulosa*) as a biological control agent, or with slashing or herbicide use (McMahon et al. 2000; Queensland Department of Primary Industries and Fisheries 2007c).

Vines have become an increasing problem after the adoption of trash-blanketing, although a thick layer of trash has been shown to inhibit their growth (Fillols & Callow 2010). They have the potential to grow rapidly and if left uncontrolled can impede the harvesters (McMahon et al. 2000). The most problematic vines in sugarcane include bindweed (*Convolvulus spp.*), passionvine (*Passiflora spp.*) and morning glory (*Ipomoea spp.*) (McMahon et al. 2000).

There are a number of herbicides that can be used to control weeds in sugarcane. These include pre-emergent herbicides such as isoxaflutole, imazapic or a diuron/hexazinone mix (Fillols & Callow 2010). Herbicides such as 2,4-D amine can be used on broadleaf weeds. Paraquat, a non-selective herbicide, can be used on broadleaf, grassy and other weeds (McMahon et al. 2000). However, a study in north QLD which evaluated a range of herbicides for their effectiveness against major sugarcane weeds showed unacceptable damage to a number of sugarcane cultivars from post-emergence paraquat application (Makepeace & Williams 1986).

# 7.2 Pests and diseases

The cost of controlling the major pests and diseases of sugarcane to the sugarcane industry in Australia was estimated to be \$111 million in 1996 (McLeod et al. 1999). This included \$14 million in lost production and control costs for pests, and \$97.4 million in loss and control for diseases (McLeod et al. 1999). The major pests and diseases that cause losses in sugarcane production include cane grubs, feral pigs, ratoon stunting disease (RSD), sugarcane rusts, chlorotic streak and soil-borne diseases (McLeod et al. 1999). More recently, sugarcane smut has become a serious threat to the industry. To reduce the impact and prevent new outbreaks of pests and diseases Australia maintains strict control and quarantine guidelines and develops a response strategy in the event of an incursion. To this end the sugarcane industry has worked with government agencies to develop the National Sugar Industry Biosecurity Plan (Plant Health Australia 2009). The major pests and pathogens relevant to the Australian sugarcane industry are discussed below.

# 7.2.1 *Pests*

#### *Invertebrate pests*

There are many insect pests of sugarcane and some insects such as plant hoppers (*Perkinsiella saccharicida*), are also known vectors of diseases (Croft et al. 2000; Allsopp et al. 2002). Appendix 1 gives an overview of these insect pests and the major insect pests are discussed below.

Cane grubs (melolonthine white grubs, larvae of the endemic melolonthine beetle) are major pests affecting the sugarcane industry. In 1996 they were estimated to cost the Australian sugarcane industry over \$11 million a year in production loss and control costs (McLeod et al. 1999). They destroy the roots of the sugarcane plants, preventing water and nutrient uptake and

causing lodging (Allsopp et al. 2000). There are 19 native species of cane grub which cause significant damage in cane fields in different regions, with the greyback canegrub (Dermolepida albohirtum) showing the most widespread damage (Robertson et al. 1995). This was estimated to cause a crop loss of 1 million t of cane in the 2000-2001 season (Chandler & Tucker 2010). The different species occur on different soil types and in different geographic regions and have either a one or two year lifecycle, yet in both cases the damage is caused by the third (final) larval stage (Allsopp et al. 2000). Several methods can be used for the control of these insects (Robertson et al. 1995). The application of the insecticide chlorpyrifos or the biological control agent Metarhizium anisopliae (a fungus that attacks the larvae) soon after planting, control the species for two to three years. Other insecticides such as granular cadusafos and liquid imidacloprid, applied after harvest or applied to the ration stubble, are also of use but require irrigation or rain to make them effective. Insecticide applications are complicated by factors such as stability in different soil types, long term nature and inaccessibility of the crop, difficulties associated with soil dwelling insects, and differences in life-cycle duration of the species (Robertson et al. 1998; Allsopp et al. 2002). Farming practices have also been shown to have an effect on the incidence of Childers canegrubs (Antitrogus parvulus Britton), with ratoon crops, fields that had been ploughed out and immediately replanted, and dryland crops having the largest infestations (Allsopp et al. 2003). A study on the greyback cane grub indicates that it preferentially oviposits on tall sugarcane plants, and that the taller blocks of cane show higher damage levels (Ward 2003).

Other insect pests of sugarcane include sugarcane and yellow soldier flies (*Inopus rubriceps* and *Inopus flavus* respectively), wireworms (*Agrypnus variabilis, Heteroderes spp.* and *Conoderus spp.*), armyworms including day and night feeding species, as well as loopers (Allsopp et al. 2000). Nematodes, including root-knot nematodes and lesion nematodes can be a serious pest. Estimates of yield losses have suggested that they may cause a 10% loss in plant crops and a 7% loss in ratoon crops (Blair & Stirling 2007). This has been estimated to cost the Australian sugarcane industry \$82 million year<sup>-1</sup> (Ogden-Brown et al. 2010).

#### Vertebrate Pests

There are numerous vertebrate pests of sugarcane including ground rats (*Rattus sordidus*), climbing rats (*Melomys burtoni*), wallabies, striped possums (*Dactylopsila trivirgata*), the eastern swamphen (Porphyrio porphyrio), cockatoos (Cacatua galerita), foxes and feral pigs. All these, except for the fox and feral pig, are native to Australia and are consequently protected. Permits for control of native animals in cane fields must be obtained from the relevant Cane Protection and Productivity Board. Rodents are the most serious pest to the sugarcane industry after the cane grub. During the 1999 and 2000 seasons they destroyed 825,000 t of sugarcane valued at \$25 million (Dyer 2005). Ground rats cause more economic damage than climbing rats as they are more numerous, especially south of the Herbert River (Dyer 2005). They cause yield loss directly by gnawing the cane, but the damage also allows the cane to dry out and provides entry-points for bacterial and fungal attack (Dyer 2005). In addition, rats are known to be carriers of the bacterium Leptospira which can result in Leptospirosis disease in humans. The disease can be spread through soil, mud and water that have been contaminated with urine from infected animals (NSW Health 2007). Integrated pest management is now widely employed to discourage and control rats (Smith et al. 2002). Strategies such as controlling crop weeds have been shown to reduce juvenile rat numbers by 50% and reduce crop damage by 60% (Dyer 2005).

# 7.2.2 Pathogens

Various biological agents including bacteria, fungi and viruses cause diseases of sugarcane. Important diseases of sugarcane that have been identified in Australia are listed in Appendix 2 and the major pathogens are discussed below.

Disease control in sugarcane is based on an integration of legislative control, resistant cultivars and other management procedures. Short term spraying options are available, but their economic viability may not be sustained. Hygiene is important to disease management strategies, particularly for diseases transmitted through cuttings such as ratoon stunting disease (RSD) and leaf scald. Cutting one infected stalk may lead to significant infection to the next 100 cuttings which are subsequently cut by the same blade (Croft et al. 2000). Machine harvesters can also transmit disease.

Many sugarcane diseases are also managed through the use of disease-free planting material supplied through Cane Protection and Productivity Boards. Hot-water treatments are used to disinfect planting material. Long hot-water treatment (three hours at 50°C) is used to control RSD. Soaking in ambient temperature running water for ~40 hours followed by three hours at 50°C is used to control leaf scald bacteria. Short hot-water treatment (50°C for 30 minutes) is used to control chlorotic streak and some insect pests (Croft et al. 2000).

#### Bacterial diseases

RSD is probably the most important disease of sugarcane. It is a highly infectious disease caused by *Leifsonia xyli*, (formerly named *Clavibacter xyli subsp xyli*) which infects vascular tissues of sugarcane. It was first reported in QLD in 1944–45 and has been identified in most countries that grow sugarcane (Bailey 2004). It was estimated in the early 1990's that it affected approximately 30% of farms in NSW (Roach et al. 1992; McLeod et al. 1999) and the estimated loss from this disease Australia-wide was \$6.3 million in 1996 (McLeod et al. 1999). The symptoms are poor growth and stunted shoots, which might not be obvious if most plants in the field are infected. The visual symptoms of red-orange dots in the vascular tissues can be seen only when the stalks are cut and sliced (Croft et al. 2000). The disease is transmitted through healthy plants coming in contact with diseased plant material or contaminated cutting implements. Yield loss is higher in dry weather and often becomes more severe in subsequent ratoon crops (Frison & Putter 1993). The incidence of the disease in parts of NSW has been reduced due to an extension campaign promoting clean seed cane (McGuire et al. 2009)

Leaf scald is caused by the bacterium *Xanthomonas albilineans* which infects the vascular tissues of sugarcane. It is found in most sugarcane districts in QLD (Croft et al. 2000), although it is hard to identify and the disease often has a latent period after infection (Bailey 2004). Leaf scald is characterised by a long white to cream streak on the leaves. Severely infected leaves appear scalded and roll inwards, with the top of the shoots becoming chlorotic. Yield loss occurs through the death of infected cane stalks and poor ratooning (BSES Ltd 2005b). Leaf scald can spread by wind-blown rain, plant material and contaminated cutting equipment such as planters and harvesters (Croft et al. 2000). Leaf scald can infect many other grasses which are alternate hosts and act as a reservoir for the disease. Extremes of moisture and temperature favour disease transmission. Resistant cultivars are used to curb the spread of the disease and susceptible plants are not used in breeding programs (BSES Ltd 2005b).

# Fungal and Oomycete diseases

The two major rusts in sugarcane are orange and common sugarcane rusts (Braithwaite et al. 2009). Orange rust is caused by *Puccinia kuehnii* and is not as economically important as the common rust, caused by *P. melanocephala*. These are both obligate parasitic fungi spread by windblown spores. The disease symptoms of the two rusts are distinct. Pustules of the orange

rust are orange and tend to be grouped in clusters, while those of sugarcane rust are reddish brown and are distributed evenly on leaves. Pustules rupture the leaves and allow water to escape from the plant, leading to moisture stress. (Croft et al. 2000). Both diseases are most severe in humid environments with temperatures below 25°C (Walker 1987).

In 1999–2000, sugarcane crops in Australia were affected by an outbreak of orange rust, which severely damaged the most widely grown commercial cultivar, Q124 (Croft et al. 2000). Highly susceptible parents are no longer used in any breeding programs. More recently, cultivars such as Q173 and Q182 have also been found to be affected by the disease (NSW Sugar 2005).

Yield loss from sugarcane rust depends on environmental conditions and was estimated to cause an economic loss of \$3.5 million in 1996 (McLeod et al. 1999).

Sugarcane smut, caused by *Ustilago scitaminea*, is a serious disease of sugarcane that can reduce yields by 30–100% (Watson 2007). Infection occurs through the sugarcane buds from wind-blown spores (Walker 1987). The disease causes severe stunting and multiple thin stalks. It is characterised by black, whip-like structures that form at the growing points of sugarcane plants (Croft et al. 2000) (Figure 7). These whips replace the spindle leaves and are formed in the shoots developing from infected cane cuttings (Frison & Putter 1993). The whips break open to release the mature spores which are spread by wind (BSES Ltd 2006). There was an outbreak of smut in Australia in July 1998 in the Ord River area of WA. This outbreak was controlled and the disease was not detected in eastern Australia until 2006, when it first appeared in Childers, QLD. Sugarcane smut is now seen as being widely spread and established (Croft et al. 2008). Estimated losses in susceptible cultivars are up to 62% in the Herbert region (Magarey et al. 2010). The spread and occurrence of the disease is being controlled through planting of resistant cultivars, using uninfected seed canes and removing infected crops (BSES Ltd 2009b). However, there has not been a complete conversion to resistant cultivars as the resistant cultivars can have a lower yield than the non-resistant cultivars. Yield of resistant cultivars is also dependant on soil type in the region (Watson 2007).

In order to reduce the spread of sugarcane smut, the movement of sugarcane and sugarcane machinery is restricted in QLD by the Plant Protection Regulation 2002<sup>3</sup>. Provisions under the *Plant Protection Act 1989* (QLD) allow for inspectors to order the destruction of diseased cane and practical guidelines have been developed to control the spread of the disease (BSES Ltd 2005a; BSES Ltd 2007b; Queensland Government 2009).

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<sup>&</sup>lt;sup>3</sup> http://www.legislation.gld.gov.au/LEGISLTN/SLS/2002/02SL205.pdf



Figure 7. Smut on Saccharum spp. hybrid in Bundaberg (May 2010). Photo taken by H. Mitchell, OGTR

Pachymetra root rot caused by *Pachymetra chaunorhiza*, an Oomycete, is a disease only found in Australia in the QLD sugarcane districts (Magarey & Bull 2003). It was first identified as northern poor root syndrome in the 1970s, before the disease-causing organism was identified (Magarey 1994). The disease seems to favour high rainfall areas, and spores can survive up to five years in the soil. In northern QLD, surveys indicate that almost every field is infected with the pathogen. The disease is characterised by a soft rot of the primary and some secondary roots, leading to poor root development. Yield loss caused by Pachymetra root rot was estimated to be up to 40% in highly susceptible cultivars (Croft et al. 2000). Fungicides have not been effective at an economical rate and control is based on planting resistant cultivars (Magarey 1996).

Other fungal diseases of sugarcane are minor (see Appendix 2) and cause less impact on yield. *Viral diseases* 

Sugarcane can be affected by a number of viral diseases (see Appendix 2).

Chlorotic streak is thought to be caused by a virus. The disease occurs in all eastern sugarcane districts, especially in wet and poorly drained fields. Lower incidence of the disease is generally found in drier regions (Croft et al. 2000). The symptoms are yellow to white streaks on the leaf, midrib and leaf sheath. Older streaks change to yellow and are more visible than younger streaks. This is followed by the appearance of chlorosis in the middle of the leaves. Internal vascular bundle tissues may be reddish in colour. (Croft et al. 2000). The disease is transmitted by soil water and diseased seed cane. Yield losses may be up to 40%, with waterlogging compounding the losses. Ratooning may also be poor (BSES Ltd 2009a).

Fiji leaf gall (previously called Fiji leaf disease) is caused by *Fiji disease virus* (FDV) and can lead to stunting and death of infected plants (Ridley et al. 2006). The initial symptoms are whitish galls raised on the underside of the leaf blade and midrib. Galls are produced due to the disorder of cell proliferation in the phloem and xylem. Galls can vary from white to green and the surface is usually smooth. When the gall is old, the epidermis may be ruptured and appear brown. At an advanced stage of infection, stem development slows down. Successive leaves become smaller and stiffer with the whole top part of the stem developing a fan-like

appearance (Croft et al. 2000). Fiji disease can be transmitted by infected cuttings and plant hoppers (*Perkinsiella saccharicidae*) are a known vector for the disease. Significant yield loss was recorded in 1970s in QLD (Croft et al. 2000) but due to the intensive management program put in place, there have been no reports of disease incidence since the 1980s. However, FDV is present in southern cane growing areas and plant hoppers are present in all canegrowing areas of QLD and NSW (Ridley et al. 2006).

Worldwide, sugarcane mosaic is caused by a number of potyviruses such as the *Sugarcane mosaic virus* (SCMV). Currently in Australia, only the *Sugarcane mosaic virus* strain A is present, which is a mild form of the virus (BSES Ltd 2008b). The mosaic symptom pattern appears in young growing leaves. Once the leaves are older, infected leaves may appear relatively normal as the mosaic becomes green. Yield loss caused by sugarcane mosaic was 40% in some fields in Australia (Croft et al. 2000). Aphids transmit the disease, as can seed produced by infected cane.

#### 7.2.3 Other biotic interactions

Sugarcane may have symbiotic relationships with a number of bacteria that fix nitrogen.

In Brazil, sugarcane is grown with low nitrogen inputs (50 kg ha<sup>-1</sup>) compared to other countries who use >200 kg ha<sup>-1</sup> (Boddey et al. 1991). The cane is commonly burnt before harvesting in Brazil so little nitrogen is returned to the field. This low nitrogen requirement has led to the suggestion that some cultivars of sugarcane can obtain nitrogen via biological nitrogen fixation (BNF). The occurrence of BNF has been suggested in several pot studies where some cultivars of sugarcane have thrived for several generations without the addition of nitrogen (Boddey et al. 1991; Urquiaga et al. 1992). Differences were seen between plant genotypes, but it was estimated that BNF could account for 25-60% of the nitrogen assimilated in one study (Boddey et al. 2001) and up to 70% in another study (Urquiaga et al. 1992). The organisms responsible for this have not been unequivocally determined. Studies have focussed on endophytic bacteria such as Gluconacetobacter diazotrophicus (previously called Acetobacter diazotrophicus), however these bacteria were not shown to be producing nitrogenase in planta (James et al. 2001). Despite this, a study using G. diazotrophicus - innoculated plants found large increases in nitrogen fixation under nitrogen deficient conditions. This nitrogen fixation did not occur after inoculation with a mutated nitrogenase deficient form of the bacterium (Sevilla et al. 2001).

G. diazotrophicus may also play a role in defence against sugarcane pathogens. It inhibited in vitro growth of Colletotrichum falcatum (red-rot) (Muthukumarasamy et al. 2000) and Xanthomonas albilineans (leaf scald) (Piñón et al. 2002; Blanco et al. 2005). Additionally G. diazotrophicus-inoculated sugarcane stems were resistant to infection by X. albilineans (Arencibia et al. 2006). There is also some evidence that it may promote sugarcane growth by production of a growth promoting factor (Sevilla et al. 2001), such as auxin (IAA; indole-3-acetic acid) or by solubilisation of mineral nutrients (as reviewed in Saravanan et al. 2008).

Other bacterial species have been isolated from sugarcane that may play a role in nitrogen fixation including *Agrobacterium diazotrophicus* (Xing et al. 2006), *Herbaspirillum spp* (Reis et al. 2007) and *Azospirillum* spp. (Baldani et al. 1997). Experiments have shown that coinoculation of *G. diazotrophicus* and *Herbaspirillum spp* gave enhanced sugarcane biomass compared to inoculation with either the single species, or to uninoculated controls (Muthukumarasamy et al. 2006). A field-based experiment and surveys of sugarcane fields in NSW and QLD showed no evidence of biological nitrogen fixation as a source of nitrogen (Biggs et al. 2000).

Vesicular-arbuscular mycorrhizal fungi (VAM) have been found in north QLD sugarcane fields in association with sugarcane roots. These fungi are known to colonise plant roots and may supply the plant with mineral nutrients, especially phosphorous. Pot experiments, using soil and mycorrhial spores from cane fields, showed that the addition of VAM increased the yield of soybean and maize plants. However, no effects have been seen on sugarcane growth from addition of the VAM *Glomus clarum* at various phosphorus levels in pot experiments (Kelly et al. 2001; Kelly et al. 2005). Similar experiments in wheat have shown that although there is no increased yield following root colonisation with VAM, 50% of the phosphorus in the plants had been absorbed via VAM (Li et al. 2006).

### **SECTION 8** WEEDINESS

Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. Weediness in Australia is often correlated with weediness of the plant, or a close relative, elsewhere in the world (Panetta 1993; Pheloung et al. 1999; Groves et al. 2005). The likelihood of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for plants to establish and spread into new environments (eg escapes of commonly used garden plants) (Groves et al. 2005).

Modern *Saccharum* spp. hybrid cultivars do not possess many of the attributes commonly associated with problematic weeds such as seed dormancy, persistence in soil seed banks, germination under adverse environmental conditions and a short life cycle (Baker 1974; Keeler 1989; Keeler et al. 1996).

# 8.1 Weediness status on a global scale

An extensive compilation of the world's weed flora is produced by Randall (2002). Most of the information contained in this book has been sourced from Australia and North America, but also includes numerous naturalised floras from many other countries. Randall (2002) lists twelve species of *Saccharum* which have been identified as having a documented weedy history. However, due to species reclassifications many of these species are now known by alternative names and are no longer in the *Saccharum* genus. The sugarcane parent species, *S. officinarum* is listed as naturalised, introduced, a casual alien, an economic weed and a quarantine weed in some countries. The other sugarcane parent species, *S. spontaneum* is listed as naturalised, introduced, a casual alien, an economic and environmental weed, a noxious weed and a quarantine weed in some countries. *S. spontaneum* is listed as one of the 104 most important world weeds by Holm et al (1997). The hybrid of these two species grown as cultivated sugarcane is listed as an Australian quarantine weed (species prohibited entry under a country's quarantine regulations) (Randall 2002).

S. spontaneum is native to India and recorded as a weed in 33 countries. It has adapted to diverse environments throughout the world, ranging from tropical to subtropical regions, most commonly found in central and south-eastern Asia (Holm et al. 1997). S. spontaneum is a serious agricultural weed in Thailand, the Philippines, India and Indonesia where it competes vigorously on disturbed sites (Holm et al. 1997). It occurs in wastelands, fallow fields, marshes, on banks of streams and ponds, on sand dunes, along railroads and highways and in or around agricultural fields. Pure stands of S. spontaneum can be found in poor agricultural soils, degraded by fire and overuse (Holm et al. 1997; Hammond 1999). It is recorded as a noxious weed in the US (USDA 2010).

S. officinarum and Saccharum spp. hybrids have not been recorded as major weeds (Holm et al. 1997; USDA 2004; Berville et al. 2005).

#### 8.2 Weediness status in Australia

In Australia, sugarcane occurs almost exclusively in managed cultivation. In sugarcane growing districts, transient sugarcane plants may occur around fields, but there is no indication that these form self-perpetuating populations (Bonnett et al. 2007). Thus, sugarcane does not appear to be a problem as a volunteer weed (Berville et al. 2005).

None of six recognised *Saccharum* species (*S. spontaneum*, *S. robustum*, *S. edule*, *S. barberi*, *S. sinensis* and *S. officinarum*) are native to Australia. Only the two parental species of modern cultivars, *S. officinarum* and *S. spontaneum*, are recorded as naturalised in Australia (1990). Naturalised populations of *S. spontaneum* were recorded at several locations in QLD alongside the Mulgrave, South Johnston and Herbert rivers, and in Feluga and the Cardwell Range (Bonnett et al. 2008) as well as alongside the Daly river in NT (Magarey et al. 2007). The populations in Feluga and South Johnston were deliberately planted (Bonnett et al. 2008). Sampling of DNA from the populations has suggested that the plants at the South Johnston, Feluga, Cardwell Range and probably Mulgrave sites are clonally propagated; thus, growth of the populations is by vegetative, not sexual, reproduction (Bonnett et al. 2008).

S. officinarum is listed in the CSIRO Handbook of Australian Weeds as a minor weed found naturalised in some tropical and mediterranean climates in Australia (Lazarides et al. 1997). It has also been listed by Groves (2003) as present in QLD and NSW but not rated as a weed as it was either not a problem, or not present in agricultural areas. Australia's Virtual Herbarium (Australia's Virtual Herbarium 2010) has records of S. officinarum collected in the NT and QLD, however, these may have been collected as representatives of a S. officinarum crop from commercial fields.

Molecular studies have shown *S. officinarum* to have low levels of genetic diversity compared to other *Saccharum* species (Sobral et al. 1994; Janno et al. 1999). Generally this species has less capacity to compete in the natural environment than *S. spontaneum*. However, due to its perennial nature, some populations escape from cultivation and can persist as long as there is sufficient moisture in the root zone. *S. officinarum* has thus become naturalised in some areas in Australia (Hnatiuk 1990).

Saccharum spp. hybrids are not recognised as weeds in Australia. They have lost many of the critical weedy attributes such as profuse tillering, adaptability to biotic stresses and resistance to pests and diseases that were present in the parental species from which the cultivated sugarcane hybrids were derived. As discussed in Section 4, most of the cultivated cultivars exhibit low fertility of both pollen and ovules, so flowers in commercial fields rarely set seed (James 2004). However, data from Bonnett et al (2008) suggests that viable seed production does occur at low levels in some commercial fields. Sugarcane seeds need optimum conditions for germination and survival of the resulting seedlings (see Section 4.4). These conditions may only occur sporadically in natural ecosystems, thus limiting the spread and persistence of sugarcane. There are isolated reports of seeds germinating naturally in the Mulgrave, South Johnston and Herbert sugarcane growing regions (17–18.5°S) but not further south (Bonnett et al. 2010).

## 8.3 Control measures

Sugarcane may be killed by ploughing out the stools and then treating with herbicide (glyphosate) (Willcox et al. 2000). However, minimum tillage practices often result in inadequate eradication of the old crop (Leibbrandt 1993). The efficacy of glyphosate for killing sugarcane is affected by various factors such as cane being in active growth, cane cultivars, soil type and stage of cane growth (Turner 1980). Sugarcane grown in light soils is more susceptible to herbicide treatment than that grown on heavy soils. The plant is killed more

easily when the height of the leaf canopy is between 0.4–0.75 m compared with older cane that has produced stalks (Turner 1980). Glyphosate is ineffective on recently cut ratoons until germination of buds is completed and tillering is advanced (Chedzey & Findlay 1985). Rain may also affect the efficacy of herbicide, so it is best used during the dry season (Owende et al. 1995). Research has shown that slashing of cane suppresses apical dominance and generally enhances chemical cane killing action on the regrowth. In addition, considerable improvement of eradication was also obtained when a mechanical under-cutter was used to shear the roots following herbicide application (Leibbrandt 1993).

### SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

The possibility of genes transferring from *Saccharum* spp. hybrid to other organisms is addressed below. Potentially, genes could be transferred to: (1) cultivated sugarcane populations; (2) other cultivated and naturalised *Saccharum* species; (3) other plant genera and (4) other organisms. For gene transfer beyond the species, potential barriers must be overcome before gene flow can occur successfully. Pre-zygotic barriers include differences in floral phenology, different pollen vectors and different mating systems such as stigmatic or stylar incompatibility systems. Post-zygotic barriers include genetic incompatibility at meiosis, selective abortion, lack of hybrid fitness and sterile or unfit backcross progeny. Even where pre-zygotic and post-zygotic barriers do not exist, physical barriers created by geographic separation can still limit gene transfer to other plants.

Successful gene transfer requires that three criteria are satisfied. The plant populations must: (1) overlap spatially; (2) overlap temporally (including flowering duration within a year and flowering time within a day) and (3) be sufficiently close biologically that the resulting hybrids are fertile, facilitating introgression into a new population (den Nijs et al. 2004).

## 9.1 Intraspecific crossing

The fertility of the commercial sugarcane cultivars is currently poorly understood. This is mainly because seeds are not the primary product of this crop, nor are they used for propagating sugarcane. In addition, asynchronous flowering, both within and between cultivars, makes seed production in the field ineffective (James 1980).

As indicated in Section 4.1.2, sugarcane flowering is variable in the field and the crop is exclusively vegetatively propagated. Different cultivars of sugarcane produce different amounts of pollen.

Self-pollination does occur, which can prevent outcrossing. The frequency of self-pollination can vary widely depending on the parent, with two studies showing 20–100% and 83-100% outcrossing rates in controlled crosses (Hogarth 1980; McIntyre & Jackson 2001).

No insect or animal vectors for sugarcane pollen are known. Pollen viability is low and of short duration under natural environmental conditions (Moore 1976). Even under artificial conditions, storage of sugarcane pollen is difficult and has been the subject of intensive investigations by sugarcane breeders, who would like to store valuable pollen (see Section 4.2).

266. Little data is available on sugarcane pollen dispersal. Information from breeding work in which plants were isolated by 20 m in open forest resulted in 3% and 50% of the offspring respectively being the result of out-crossing (Skinner 1959). From this work, it was suggested that to prevent contamination of controlled crosses, plants should be isolated by 100 m in open forest, or 300 m in the open (Skinner 1959).

Flowering and viable pollen production are both temperature dependent; in Australia they decrease with increasing latitude (Bonnett et al. 2010). This makes intraspecific crossing more likely at higher latitudes than in commercial fields in NSW.

### 9.2 Natural interspecific and intergenic crossing

Sugarcane is closely related to the genera *Erianthus, Narenga, Miscanthus* and *Sclerostachya*. These genera and *Saccharum* are collectively known as the *Saccharum* complex and are expected to be sexually compatible at some levels (Bull & Glasziou 1979; Grassl 1980; Daniels & Roach 1987). There are also reports of sugarcane crossing under controlled conditions with species outside of the *Saccharum* complex (discussed in Section 9.3.2).

# 9.2.1 Natural interspecific crossing

None of the six recognised *Saccharum* species (*S. spontaneum*, *S. officinarum*, *S. robustum*, *S. barberi*, *S. edule* and *S. sinensis*) are native to Australia. Some of these species are maintained within sugarcane research stations as germplasm stocks and have been used in breeding programs to produce new cultivars. There are also records of *S. spontaneum*, *S. officinarum* and *S. edule* in gardens in northern Australia (Magarey et al. 2007). Of these species only *S. officinarum* and *S. spontaneum* are naturalised in Australia (see Section 8.2).

At Meringa research station in QLD, *S. robustum* sheds pollen at the same time of year as sugarcane hybrids and has been shown to freely cross with sugarcane hybrids (Skinner 1959).

Naturalised *S. spontaneum* is considered the most likely species to naturally hybridise with cultivated sugarcane in Australia (Bonnett et al. 2008) as other *Saccharum* species are rare outside of research stations or home gardens. As discussed in Section 8.2, naturalised populations of *S. spontaneum* have been found in areas in QLD. The population of *S. spontaneum* in the Cardwell Ranges is sterile and is isolated from the commercial sugarcane areas by rainforest (Bonnett et al. 2010). The other populations produce viable pollen and are close to sugarcane growing areas. Hybridisation requires synchronicity of flowering between cultivated cane and *S. spontaneum* to enable cross-pollination to occur. *S. spontaneum* has been shown to flower asynchronously with sugarcane in the Mulgrave river region, but synchronously with sugarcane in other more southern areas (Bonnett et al. 2010). There is no data to suggest that hybridisation between *S. spontaneum* and sugarcane occurs.

### 9.2.2 Natural intergenic crossing

As indicated above, the genera *Erianthus*, *Narenga*, *Miscanthus* and *Sclerostachya* are expected to be sexually compatible at some levels with sugarcane (Bull & Glasziou 1979). Some groups of *S. robustum* are thought to be products of a spontaneous hybridisation event between *S. spontaneum x Miscanthus* hybrids, in areas where both species occur naturally (Sreenivasan et al. 1987). However, in order to cross naturally with the *Saccharum* spp. hybrid in Australia, the two species need to be located in close proximity and flower at the same time.

Some accessions of the *Saccharum* complex, such as *Erianthus* and *Miscanthus*, are maintained in many sugarcane research station germplasm collections for sugarcane breeding in Australia. *Erianthus* spp. have not been recorded in other locations in Australia (Australia's Virtual Herbarium 2010).

A number of *Miscanthus* species are sold as garden plants in Australia. *Miscanthus sinensis* has been recorded as a weed in NSW and WA (Lazarides et al. 1997). Specimens have been collected from southern WA, the central coast of NSW, southern South Australia, southern Victoria and in two locations in Tasmania (Australia's Virtual Herbarium 2010). It is considered naturalised in parts of WA and NSW (Hnatiuk 1990). *Miscanthus floridulus* is a noxious weed overseas, however it has not been recorded in Australia (Lazarides et al. 1997; Australia's Virtual Herbarium 2010).

*Narenga spp.* have not been recorded in Australia (Australia's Virtual Herbarium 2010). It has been suggested that the wild cane Hitam Rokan, collected in Sumatra, is a naturally occurring

hybrid of *Saccharum* and *Narenga* (Janaki-Ammal 1942). This suggestion is based on morphological similarity to known synthetic hybrids but has not been confirmed by molecular methods.

Sclerostachya spp. have not been recorded in Australia (Australia's Virtual Herbarium 2010).

Other potentially sexually compatible species outside the *Saccharum* complex are present in Australia.

Maize (*Zea mays*) may be grown throughout Australia as an irrigated or dryland crop depending on rainfall conditions. It is, however, grown mostly in QLD (Atherton Tableland, Burnett, Darling Downs) and southern NSW (Murrumbidgee, Murray and Lachlan River Valleys) (OGTR 2008). Australia's Virtual Herbarium has records of *Z. mays* collected in QLD, NSW and Tasmania (Australia's Virtual Herbarium 2010). It is listed in the CSIRO Handbook of Australian Weeds (Lazarides et al. 1997). It has also been listed by Groves (2003) as present in NSW but not rated as a weed as it was either not a problem, or not present in agricultural areas.

Wild *Sorghum* species are among the weeds of Australian sugarcane crops and are widespread in Australia (Hnatiuk 1990; McMahon et al. 2000). Thirty-two species of Sorghum have been recorded as present in Australia (Australia's Virtual Herbarium 2010). Six of these are listed in the CSIRO Handbook of Australian Weeds, two of which (*S. x almum* and *S. halpenese*) are listed as noxious weeds in parts of Australia (Lazarides et al. 1997). Seven sorghum species are listed by Groves (2003) as present in a number of states, with some of the species being naturalised and known to be a major problem at four or more locations within a State or Territory.

*Imperata cylindrica* is an Australia native plant. It is sold as a garden plant in Australia as cultivars such as 'rubra', known as Japanese blood grass. It is recorded in WA, NT, SA, QLD, NSW, VIC and TAS (Australia's Virtual Herbarium 2010). It is listed in the CSIRO Handbook of Australian Weeds as a weed of pastures (Lazarides et al. 1997), but is not listed by Groves (2003).

This suggests that many of these species are present in sugarcane growing areas in Australia, so there may be potential for crossing to occur. However, no natural hybrids have been recorded and, as discussed in Section 9.3, viable hybrids with these species have only been produced under experimental conditions using large numbers of plants, often with male sterility to prevent self-pollination.

# 9.3 Crossing under experimental conditions

There is limited data available on crosses between *Saccharum* spp. hybrid and other species. Data is presented on crosses with *Saccharum*, *Erianthus*, *Miscanthus*, *Bambusa*, *Sorghum* and *Imperata*. Information on crosses performed with the parent species, *S. spontaneum* and *S. officinarum* is included in this Section as it may be indicative of the potential for successful crossing with the hybrid.

### 9.3.1 Species in Saccharum complex

Although many attempts to cross between *Saccharum* spp. hybrid and the species in the *Saccharum* complex may have occurred in sugarcane research stations, limited publications are available. Hybrids have been reported under experimental conditions with *Miscanthus*, *Narenga*, *Erianthus* and *Sclerostachya* (Sreenivasan et al. 1987).

#### Saccharum

Successful controlled crosses have been obtained using pollen from commercial *Saccharum spp.* hybrid and *S. spontaneum* as the female parent. This required heat emasculation of *S. spontaneum* to reduce self-pollination (Pan et al. 2004).

#### Erianthus

A number of species of *Erianthus* have been used for crossing with sugarcane. There is an early report of a cross between *S. spontaneum* and *Erianthus ravennae*, which produced fertile hybrids, although these were not confirmed by molecular methods (Janaki-Ammal 1941).

Crosses between *Erianthus arundinaceus* and hybrid *Saccharum* spp. produced putative intergenic hybrids which had characteristics from the male Erianthus parent. However, these were shown to be selfed progeny which did not possess isozyme marker bands characteristic of Erianthus (Lee et al. 1998). A small number of successful crosses were made between Saccharum spp. hybrid and E. arundinaceus in 1983 in Meringa (Lee et al. 1993). Of 96 attempted crosses made at BSES in QLD between E. arundinaceus and S. officinarum or hybrid Saccharum spp., 26 were successful producing over 1000 seedlings. Thirty-seven of the seedlings were identified as genuine hybrids, but only 19 survived, all derived from S. officinarum as a female parent and E. arundinaceus as a male parent. All of these hybrids had poor vigor, were sterile and showed chromosome elimination (Piperidis et al. 2000). Nonetheless, Cai et al. (2005) have successfully identified a fertile intergeneric cross between E. arundinaceus and S. officinarum using microsatellite markers and 5S rDNA. This plant was obtained from crosses performed at a Chinese research institute. Genomic slot blot hybridisation (GSBH) has also been used to confirm hybrids between S. officinarum (as the female parent) and E. arundinaceus (as the male parent) and to determine that 43% of the F1 progeny were selfs (Besse et al. 1997b). Isozyme electrophoresis, sequence-tagged PCR, RFLP and GISH have also been used to confirm intergenic hybrids of S. officinarum x E. arundinaceus (D'Hont et al. 1995).

Crosses have been performed with *E. rockii*. These used *S. officinarum* or *S. officinarum* x *S. spontaneum* as the female parent to produce viable hybrids (Aitken et al. 2007). Seed imported from China into Australia was tested using DNA markers which confirmed the following crosses: *S. officinarum* with *E. arundinaceus*; *Saccharum* spp. hybrids with *E. arundinaceus*; and *Saccharum* spp. with *E. rockii* (Foreman et al. 2007). Similarly crosses between *Saccharum* spp. hybrids and *Erianthus fulvus*, using the *Saccharum* spp. as the female parent, have produced hybrids, confirmed using SCAR markers (sequence-characterised amplified region) (Wang et al. 2009). Hybrids of *S. officinarum* and *Erianthus procerus* have also been generated (Rajeswari et al. 2009).

Crosses have also been performed using elite sugarcane cultivars as the female parent with North American *Erianthus spp. E. alopecuroideum, E. contortus* and *E. giganteus*. Seed was produced, but it is not known if the progeny were true hybrids (Burner & Webster 1994).

### Narenga

Crosses have been made between *Narenga porphyrocoma* and *S. spontaneum* (Kandasami 1961). Analysis of hybrids between *N. porphyrocoma* and *S. officinarum* showed intermediate characteristics and low male and female fertility (Janaki-Ammal 1942). Hybrids from a cross between *S. officinarum* and *N. porphyrocoma* showed viable pollen in tissue culture (Bonnett et al. 2008).

#### Miscanthus

Hybrids have been reported from crosses between *Miscanthidium violaceum* (= *Miscanthus flavescens*) and *Saccharum* spp. hybrids (Brett 1954). Other crosses with *Miscanthus* have been verified by *Alu*-PCR, using short interspersed nuclear elements (SINE) (Alix et al. 1999).

## Sclerostachya

Parthsarathy (1948) reported a cross between *S. officinarum* and *Sclerostachya fusca*. Further crosses between *S. officinarum* and *S. fusca* have also been performed. An F1 hybrid with these two parents has been described as containing 55 chromosomes, and used in tissue culture to regenerate plantlets (Sreenivasan & Sreenivasan 1984). Four crosses between *S. spontaneum* and *S. fusca* have been described, which produced 79 offspring (Kandasami 1961).

# 9.3.2 Species outside Saccharum complex

Hybridisation with *Saccharum* has also been attempted with some members of distantly related genera belonging to tribe Andropogoneae, such as *Imperata cylindrica* (blady grass), *Sorghum* spp. and *Bambusa arundinaceae* (bamboo) (Thomas & Venkatraman 1930; Janaki-Ammal 1938; Rao et al. 1967; Nair 1999) as well as *Zea mays* (maize) from the tribe Maydeae (Janaki-Ammal 1938; Janaki-Ammal 1941). In some of these reports intergenic hybrids were claimed, however some could not be accepted as true hybrids (Grassl 1980; Bonnett et al. 2008). As discussed in Bonnett et al (2008), altered morphological characters and chromosome numbers can occur in self-pollination and are not in themselves proof of hybrid production.

### Maize

A cross was reported between *Z. mays* and *S. officinarum*, using male sterile sugarcane as the female parent (Janaki-Ammal 1938; Janaki-Ammal 1941). This plant was sterile, had 52 chromosomes, was morphologically different from both parents and resolved from both parents based on cluster analysis of RAPD markers (Janaki-Ammal 1938; Janaki-Ammal 1941; Janaki-Ammal et al. 1972; Nair et al. 2005; Nair et al. 2006). Another report suggested that the hybrid embryos of maize and sugarcane aborted during development. This was partially overcome by embryo culture, although all the seedlings died when transferred to soil (Hrishi & Marimuthammal 1968).

#### Bamboo

Early crosses of *Bambusa arundinacea* with two *Saccharum* spp. hybrids produced 29 hybrids (Venkatraman 1937). Histological analysis showed that the hybrids had altered chromosome numbers from the parents, and many of the hybrids were male sterile (Janaki Ammal 1938). A cross of *B. arundinacea* with *S. officinarum* produced two progeny (Raghavan 1952). However, it has been suggested that neither of these were genuine hybrids (Nair & Ratnambal 1970; Grassl 1980). Histological analysis of crosses between *B. arundinacea* and *S. officinarum*, *S. robustum*, *S. spontaneum* or seven *Saccharum* hybrids indicated that with *Saccharum* as a female parent the hybrid embryos aborted during the early embryogenic stage (Rao et al. 1967). Four mature putative hybrid seeds were obtained from 960 crosses using *B. arundinacea* as a female parent, all with either *S. spontaneum* or *S. robustum* as male parents. These either failed to germinate from seed or produced abnormal seedlings which did not survive (Rao et al. 1967).

### Sorghum

Sorghum species have been artificially crossed with Saccharum spp. hybrids and S. officinarum (Thomas & Venkatraman 1930; Gupta et al. 1978; Grassl 1980; Nair 1999). These studies used Saccharum spp. as both the female or male parent and often used large numbers of sterile lines.

Four hybrids were produced using *S. officinarum* as the male parent (Nair 1999). One of the sterile hybrids was induced to flower by gamma irradiation of calli, and appeared to be female fertile, although male sterile (Sobhakumar & Nair 2005).

Generally, the hybrid offspring have been of low vigour and fertility, but back crossing to both parents has been achieved (Grassl 1980; Sreenivasan et al. 1987). However, Grassl (1980) recorded that after the 4<sup>th</sup> to 5<sup>th</sup> generation of backcrossing to sorghum, the sugarcane chromosomes had been eliminated from the intergeneric hybrids. The initial reports used morphological and cytological characteristics to identify hybrids, however, more recent work has used RAPD molecular markers to confirm that the hybrids are genuine (Nair et al. 2005; Nair et al. 2006).

Experiments using different sorghum species have shown that pollen-pistil incompatibility is the major barrier to the production of sorghum hybrids (Hodnett et al. 2005). Consequently, breeding work using a sorghum *iap* (inhibition of alien pollen) mutant in which the incompatibility is removed as the female parent has produced a number of hybrids with *Saccharum* spp. The hybrid seed produced needed careful management to avoid either vivipary or lack of germination due to an impenetrable seed coat. The hybrids had varied phenotypes from very poor growth to very vigorous, though two of the vigorous plants were male sterile (Hodnett et al. 2010).

### *Imperata*

There is one report of an experimental cross between *Imperata cylindrica* and a *Saccharum* spp. hybrid, producing triploid progeny resembling sugarcane which could apparently self-fertilise to produce F2 progeny (Janaki-Ammal 1941). However, other authors have suggested that these may not have been true hybrids (Nair & Ratnambal 1970).

Thus, intergeneric gene transfer involving existing commercial sugarcane hybrids may be possible, by hand-pollination under experimental conditions designed to overcome natural barriers to cross-pollination, but such hybrids have not been observed in the wild.

# **APPENDICES**

Appendix 1. Invertebrate pests of sugarcane and their control summarised from Agnew (1997).

Common Name	Species	Affected Plant Part	Control
Cane grubs	19 native species of beetle larvae	Roots – significant root damage destabilises stool leading to lodging	primarily insecticide sprays
Soldier fly	Inopus rubriceps and I. flavus larvae	Roots – poor germination	no chemical control, plough out and leave bare fallow for a season, replant late
Ground pearls (bugs)	Eumargarodes laingi and Promargarodes australis nymphs	Roots – form cysts in soil (pearl) and feed on sap	no chemical control, tolerant cultivars, plough out and leave bare fallow for a season
Cicadas	3 species, nymphs	Roots – sap feeding	no chemical control, plough out and leave bare fallow for a season
Funnel ants	Aphaenogaster pythia	Roots – weakens stools	no chemical control, plough out
Symphylans	Hanseniella spp.	Roots – poor crop establishment	encourage rapid germination, insecticides
Nematodes	several	Roots – interfere with water and nutrient absorption	nematicides
Wireworms (click beetle larvae)	Agrypnus variabilis and Heteroderes spp.	Shoots – bore into the buds of setts or the growing point	insecticides in plant crops (none for ratoon crops)
Black beetles	Heteronychus arator and Metanastes vulgivagus	Shoots – chew into young shoots causing death of the shoot	no chemical control, plough out and leave bare fallow for a season, insecticides registered for <i>H. arator</i> control in NSW only
Rhyparida beetles	Rhyparida morosa and R. dimidiata	Shoots – chew into young shoots causing death of the shoot	none available
Butt weevil		Shoots – also bore into setts and ratoons (occurs rarely)	none available
Stenocorynus weevil	Stenocorynus spp.	Shoots – also chew roots of germinating setts, weakening growth (occurs rarely)	none available
Whitefringed weevil	Naupactus leucoloma	Shoots – chews leaves (occurs rarely)	none available
Large moth borer	Bathytricha truncata	Shoots – chew into young shoots causing death of the shoot (minor pest)	none available
Ratoon shoot borer	Ephysteris promptella	Shoots – chew into young shoots causing death of the shoot	no chemical control, damage only severe under drought conditions
Bud moth	Opogona glycphaga	Shoots – chew buds preventing germination (occurs rarely)	none available
Field crickets	Teleogryllus oceanicus and T. commodus	Shoots – chew buds preventing germination (occurs rarely)	none available
Mole cricket	Gryllotapla sp.	Shoots – chew buds and young shoots	none available
Wart eye	unidentified mites	Shoots – buds fail to germinate	none available
Sugarcane weevil borer	Rhabdoscelus obscurus	Stem – bore into stems allowing other diseases in	no chemical control, quarantine between growing areas of sugarcane and palms

Common Name	Species	Affected Plant Part	Control
Termites	several species	Stem – hollow out stems	no chemical control, remove dead wood from cane fields
Locusts	several species	Leaf and stem – chewing	cultivation before eggs hatch
Armyworms and loopers	various species	Leaf and stem – chewing	plants usually recover from early damage
Planthopper	Perkinsiella saccharicida	Leaf and stem – sap feeding, vector for Fiji disease	Fiji disease resistant cultivars
Linear bug	Phaenacantha australiae	Leaf and stem – sap feeding, damaged leaves more susceptible to fungal diseases	natural enemies
Mealybug	Saccharicoccus sacchari	Leaf and stem – sap feeding	natural enemies
Aphids	3 species	Leaf and stem – sap feeding	natural enemies
Scale insect	Aulacaspis madiunensis	Leaf and stem – sap feeding	disease free planting material

**Appendix 2.** Diseases of sugarcane that cause yield losses in Australia (Frison & Putter 1993; McLeod *et al.* 1999; Croft *et al.* 2000).

Common name	Causal agent	Control
Bacterial		
Leaf scald	Xanthomonas albilineans	Resistant cultivars
Ratoon stunting disease (RSD)	Clavibacter xyli subsp. xyli	Disease free planting material
Red stripe (Top rot)	Acidovorax avenae subsp. avenae	Resistant cultivars
Fungal		
Rusts	Puccinia melanocephala and P.kuehnii	Resistant cultivars
Yellow spot	Mycovellosiella koepkei	Resistant cultivars
Pachymetra root rot	Pachymetra chaunorhiza	Resistant cultivars
Sugarcane smut	Ustilago scitaminea	Resistant cultivar, hot water treatment
Pineapple disease	Ceratocytis paradoxa	Fungicide applied to setts
Eye spot	Bipolaris sacchari	Resistant cultivars
Red rot	Glomerella tucumanensis	Resistant cultivars
Pokkah boeng ('tangle top')	Fusarium monoliforme (Gibberella fujikuroi) and F. subglutinans (G. subglutinans)	plants usually recover without need for disease control
Viral	,	
Chlorotic streak	Unknown, probably virus	Disease free planting material, good drainage
Fiji disease	Fiji disease phytoreovirus (FDV)	Resistant cultivars
Mosaic diseases	Potyviruses: Sugarcane mosaic virus (SCMV), Sorghum mosaic virus (SmMV), Maize dwarf mosaic virus (MDMV), Johnson grass mosaic virus (TGMV), striate mosaic associated virus.	Disease free planting material and resistant cultivars

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