Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

The Biology and Ecology of Sugarcane (*Saccharum* spp. hybrids) in Australia

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PREAMBLE

This document is designed to be a regularly revised resource document for dealings involving the intentional release (DIR) into the Australian environment of genetically modified (GM) sugarcane either for the purpose of field trials or commercial release. This document addresses the biology and ecology of conventional (non-genetically modified) sugarcane. Included is the origin of sugarcane, general descriptions of its growth and agronomy, its reproductive biology, toxicity and allergenicity and its general ecology. This document also addresses the potential for sugarcane to transfer genes via pollen and seed movement and for weediness. This document will support the detailed risk assessment and risk management plan (RARMP) prepared for each DIR application involving GM sugarcane. However the Regulator will assesses the risks associated with each particular genetic modification on a case by case basis which will be then detailed in the RARMP prepared for each application.

1.   BIOLOGY OF SUGARCANE

SECTION 1.1   ORIGINS AND DISTRIBUTION

Sugarcane is a tall growing monocotyledonous crop plant 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 internodes of the stem. Modern sugarcane varieties that are cultivated for sugar production are complex interspecific hybrids (*Saccharum* spp.) that have arisen through intensive selective breeding of species within the *Saccharum* genus primarily involving crosses between the species *Saccharum officinarum* L. and *S. spontaneum* L. (Cox et al. 2000) (see more details in Section 1.3.3).

S. officinarum or the 'noble canes' accumulate very high levels of sucrose in the stem but have poor disease resistance. *S. officinarum* itself is thought to be the product of complex introgression between *S. spontaneum*, *Eriathus arundinaceus* and *Miscanthus sinensis* (Daniels & Roach 1987). A possible intermediate form in the development of *S. officinarum* is *S. robustum*, a diverse riparian species that grows in the wet tropics with many distinct populations. The origins of *S. officinarum* are intimately associated with the activities of humans as *S. officinarum* is a purely cultivated or garden species with no members found in the wild (Sreenivasan et al. 1987). The centre of origin of *S. officinarum* is thought to be in Polynesia. The species was probably transported throughout south east Asia by humans, leading to a modern centre of diversity in Papua New Guinea and Irian Jaya (Indonesia) where the majority of specimens were collected in the late 1800s (Daniels & Roach 1987).

Hypotheses for the origin of *S. officinarum* involve selection of sweet forms of *S. robustum* for use as food, possibly with the aid of animals such as pigs or rats that were attracted to sweeter individual plants (Daniels & Roach 1987).

S. spontaneum is believed to have evolved in southern Asia. *S. spontaneum* is a much more 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: 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, Afganistan, Iran and Middle east and c) the West zone

which includes Egypt, Sudan, Kenya, Uganda, Tanzania and other countries in the Mediterranean (Tai & Miller 2001; Daniels & Roach 1987; Pursglove 1972).

SECTION 1.2   USES OF SUGARCANE

1.2.1   Sugar production

Sugarcane is an established agricultural field crop with a long history of safe use. It is believed to have become established as a domestic garden crop possibly as early as 2500 BC (Daniels & Roach 1987). Sugarcane has been cultivated in Australia for over 100 years (Canegrowers 2004).

Sugarcane is primarily grown as a source of sugar. Sugar is initially extracted from the raw cane at sugarcane mills distributed throughout the growing region. The cane is shredded and the juice extracted by crushing. The juice is then clarified by a combination of heating in the presence of lime ($Ca(OH)_2$) which 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 (substances added to solutions to produce woolly looking masses of particles which assists settling out suspensions) are added to speed up this process (Mackintosh 2000).

Clarified sugar juice is then concentrated by evaporation to produce 'syrup'. The syrup then goes through multiple rounds of crystallisation to extract the sucrose. It is boiled and the sucrose crystallises from the remaining molasses fraction. The product of this step is known as massecuite. The massecuite is then centrifuged to separate the sucrose from the molasses. 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 purification resulting in a high quality, purified product.

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 and outside the scope of this document however there are three measures of cane quality that are important, which will be briefly mentioned here. 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 passage of polarised light through the clarified juice. 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). Furthermore this allows estimation of the sugar content or commercial cane sugar (CCS) of a grower's cane (Mackintosh 2000).

To calculate CCS it is assumed that three quarters of the impurities remain after the juice is clarified. These impurities end up in the final molasses, which in turn consists of ~40% non-recoverable sugar and 60% impurities. Therefore:

CCS = Pol of juice (corrected for fibre content of stem) – ¾ (impurities in cane x 40/60)

   = Pol in cane - ½ (impurities in cane)

CCS is a measure of how much pure sucrose can be extracted from the cane. The final return that the grower receives is determined by additional factors (see Mackintosh 2000).

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 0.01tonne/ha/year). However, in the same period CCS decreased by ~1 unit.

1.2.2 By-products

Several by-products are produced from crushing sugarcane at the sugar mill. These primarily include bagasse (fibre) and molasses.

Bagasse is the fibrous portion of sugarcane that remains after the juice has been removed. It has several applications, including generation of power for the mill, papermaking and livestock feed. It consists of two types of fibre, which constitute 55% of bagasse dry weight. These are the cellulose fibre of rind, vascular tissue and the pith of the cane stem. Bagasse cellulose fibres are longer (1-1.5mm) than hardwood fibres (0.7-1mm), but shorter than softwood fibres (2.5-5mm) and are suitable for papermaking. The pith material of the stem is considered a contaminant for papermaking and production of high quality paper consequently requires it to be removed. Bagasse is used to make paper in many countries although not in Australia (Allen et al. 1997).

Bagasse is also used as an animal feed but is limited by the low digestibility, even for ruminants (~25%). Chemical, biological or thermo-mechanical treatment improves the digestibility to approximately 65% (Allen et al. 1997; Playne 1984; UN Industrial Development Organisation 2002; de Medeiros & Machado 1993; de la Cruz 1990; Pate 1982).

Further chemical treatment of bagasse can be used to produce other by-products, which may be useful sources of income if further developed in Australia. These include production of various fermented and chemical derivatives of cellulose and fermentation of bagasse to produce fuel ethanol (Allen et al. 1997).

Molasses is the thick syrupy residue left over 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, for fermentation processes and as a fertiliser for cane fields (Mackintosh 2000; Sansoucy et al. 1988).

The composition of various stages in the production of molasses are shown in Table 1.

Table 1: Sugarcane by-products from the processing of sugarcane (adapted from Preston 1988; Allen et al. 1997). Note that the balance of the fraction in each case is mostly water.

Fraction	Approximate composition (% fresh weight basis)		
	Sucrose	Reducing sugars[*]	Ash[†]
Cane Syrup[1]	45-55	5-10	1-2
High-test molasses[2]	25-30	40-50	2-3
'B' molasses[3]		5-8	80-90
Final molasses[4]	33-37	15-19	10-15

[*] glucose and fructose produced by the action of the enzyme invertase.

[†] mainly inorganic salts.

[1] clarified and concentrated cane juice (syrup) concentrated to the point where the sucrose is almost ready to crystallise.

[2] clarified and concentrated cane juice (syrup) from which no sugar has been crystallised that has been filtered and partially inverted (converted to reducing sugars) to avoid crystallisation.

[3] concentrated soluble residue following the second centrifugation to remove the 'B' sugar.

[4] concentrated soluble residue after the last centrifugation to remove the 'C' sugar, also known as 'C' or blackstrap molasses.

Power is generated from burning bagasse to produce steam to run the mill. Excess energy is directed to the electricity grid. The ash produced is mixed with other impurities (mud) left over after the sugarcane juice is clarified and fine bagasse known as bagacillo to produce **filter cake** which is used as a fertiliser on cane farms (Mackintosh 2000).

Trash refers to the sugarcane plant material left over after harvesting of the sugarcane stalks. It is generally retained in the field as mulch in northern Queensland. Baled trash is gaining in use as garden mulch and as a low-grade cattle feed from the south east Queensland growing region (Dawson 2002).

SECTION 1.3   TAXONOMY AND GENETICS

1.3.1   Tribe Andropogoneae

Sugarcane belongs to the genus *Saccharum* L., of the tribe Andropogoneae in the grass family (Poaceae). This tribe includes tropical and subtropical grasses including the cereal genera *Sorghum* and *Zea* (corn). 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 of *Saccharum*, *Erianthus* section *Ripidium, Miscanthus* section *Diandra*, *Narenga* and *Sclerostachya* (Daniels & Roach 1987). These genera are characterised by high levels of polyploidy and frequently unbalanced numbers of chromosomes (aueuploidy) making it difficult to determine taxonomy and resulting in many previous revisions of the taxonomic relationships (Daniels & Roach 1987; Sreenivasan et al. 1987).

1.3.2   Genus *Saccharum*

The *Saccharum* genus comprises six species *S. spontaneum*, *S. officinarum*, *S. robustum*, *S. edule*, *S. barberi*, and *S. sinense* (D'Hont et al. 1998).

S. officinarum is thought to have resulted from the complex introgression between *S. spontaneum*, *Erianthus arundinaceus* and *Miscanthus sinensis* (Daniels & Roach 1987).

S. officinarum has a chromosome number of 2n = 80 with a basic chromosome number (x) of 10 making this species a polyploid (having more than two chromosome sets eg octaploid, 2n=80 - eight complete sets of chromosomes). However, *S. officinarum* is not a simple polyploid. It is a complex hybrid of different species as it 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). This is indicated by whole chromosomes in *S. officinarum* that are homologous with those in the genera *Miscanthus* and *Erianthus* section *Ripidium* (Daniels & Roach 1987; Besse et al. 1997).

S. spontaneum is a smaller, highly polymorphic, disease resistant, high fibre and highly vigorous species, having 2n = 40 to 128 chromosomes. It is also a complex polyploid with a probable basic chromosome number of eight or 10 (Sreenivasan et al. 1987; D'Hont et al. 1996). It can be distinguished from the cultivated *Saccharum* by thinner canes and a narrow panicle (Pursglove 1972). Characteristics of the spikelets at the end of the tertiary branches of the inflorescence are also used by taxonomists to help distinguish the species from other *Saccharum* spp.

S. barberi and *S. sinense* are thought to be wild species but have been in cultivation since prehistoric times in northern India and China respectively. This had lead to considerable interbreeding with other genera and species and 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 though to be derived from *S. officinarum* x *Miscanthus* introgression, each containing chromosomes homologous to *S. officinarum* and *S. spontaneum* as well as to those from members the genera *Erianthus* and *Miscanthus* again indicating the complex origins and inter-relationships within the *Saccharum* genus (D'Hont et al. 1996; Daniels & Roach 1987).

S. robustum is a wild species thought to be an intermediate step in the evolutionary pathway between *S. spontaneum* and *S. officinarum*. Two major groups with the species are known, those that have 2n=60 and 2n=80 chromosomes respectively.

S. edule is similar morphologically to *S. robustum* except that the flower spike or inflorescence is compacted and it is cultivated as a vegetable in the islands of the Pacific and Papua New Guinea. *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 genetic characteristics of the *Saccharum* species is shown in Table 1.

Table 2: Sugar cane members of genus *Saccharum* (Daniels & Roach 1987; Buzacott 1965).

Species	Classification	Sugar content	Chromosome number
S. spontaneum	Wild species	Nil	2n= 40-128
S. robustum	Wild species	Nil	2n= 60-~200
S. officinarum	Noble canes	High	2n= 80
S. barberi	Ancient hybrid	Low	2n= 111-120
S. sinense	Ancient hybrid	Low	2n= 80-124
S. edule	Wild species	Compacted inflorescence eaten as a vegetable	2n= 60-80 with aneuploid forms

1.3.3   Commercial hybrid cultivars

Commercial hybrid cultivars of sugarcane descended from interspecific hybridisation between *S. officinarum* and *S. spontaneum* (Bull & Glasziou 1979). *S. officinarum* or the 'noble canes' accumulates very high levels of sucrose in the stem but have poor disease resistance. Conversely *S. spontaneum* accumulates no sucrose but is a highly polymorphic species with much higher levels of disease resistance, adaptability and stress tolerance (Sreenivasan et al. 1987). The basic breeding concept involves the combination and improvement of vigorous, disease resistance from *S. spontaneum* and high sucrose content from *S. officinarum*. Increasing sucrose content while maintaining disease resistance of commercial hybrid cultivars has been achieved through a number of back-crossing to several different cultivars of *S. officinarum* (Bull & Glasziou 1979). Consequently, *S. spontaneum* genetic component is reduced in commercial hybrid cultivars. Of the chromosomes in these commercial hybrid cultivars, approximately 80% are derived from *S. officinarum* L. and 10% are from *S. spontaneum* L., with remainder being chromosome from the two species produced by the natural process of synapsis during meiosis (D'Hont et al. 1996).

Interspecific hybridisation between *S. officinarum* as the female parent and *S. spontaneum* as the male parent produce progeny that have a triploid chromosome number (2n + n = 100 to 130) (Sreenivasan et al. 1987; D'Hont et al. 1996). This phenomenon, thought to arise either through endoduplication or fusion of two nuclei during meiosis (D'Hont et al. 1996), facilitated breeding of modern sugarcane varieties as the '*officinarum*' qualities recovered more quickly in the hybrids, thus requiring fewer rounds of backcrossing to produce high sucrose varieties.

This culminated with the release of a variety called POJ2878 ('Java Wondercane') in 1921 in Java (Indonesia), which became an important variety, allowing for a 35% increase in production over previous best varieties (Cox et al. 2000; Jeswiet 1929). Most commercial cultivars used in Australia today can be traced to this variety (Cox et al. 2000). Sugarcane breeding for improved varieties is a time consuming process taking upwards of ten years from initial crosses to final agronomic assessment of elite varieties (Cox et al. 2000).

SECTION 1.4   CULTIVATION AND GROWTH

It is believed that sugarcane was first brought to Australia in 1788 on the First Fleet but cultivation was not immediately successful. The first official record of sugarcane in Australia was in 1821 at Port Maquarie NSW, but an industry did not develop until a cane plantation was established near Brisbane in 1862. The first Australian commercial sugar mill began operation in 1864. Sugarcane cultivation spread along the Queensland-New South Wales coastline and in 1870 a system of large central sugar mills, supplied with cane by independent farmers, was introduced by the Colonial Sugar Refining Company (now CSR Ltd) (Canegrowers, http://www.canegrowers.com.au/overview.htm).

Currently, in Australia sugarcane is commercially cultivated over a 2100 km stretch from northern New South Wales (approximately 30°S) to northern Queensland (approximately 17°S) with the actual planting area distributed unevenly across this range. A small industry also exists in the Ord district in Western Australia. About 98% of Australia's sugar exports originate in the Queensland coastal region (Canegrowers 2004). Queensland's 2000-01 crop harvest was 28.8 million tonnes of sugarcane, from 424,350 hectares of cultivated land. Each hectare of cane harvested yielded on average 67.8 tonne of cane and 8.94 tonne of sugar. The

gross value of Queensland's 2000-2001 sugar production was $995.6 million (Canegrowers 2004).

1.4.1 Germination and establishment

Commercial sugarcane is propagated vegetatively and germination refers to the initiation of growth from buds present on the planted setts or on the stems of the stools that remain in the soil after harvest of the previous crop. Either whole stalks or stalks cut up into shorter segments called setts are used as planting material (Willcox et al. 2000). Setts 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 150cm. Buds on planted setts, or on the plant bases remaining after harvest, germinate within two weeks of planting (or after harvest of the preceding crop). Setts are prone to fungal attack (due to their high sugar content) and are routinely treated with fungicide to protect them until germination. Sugarcane varieties differ in their degree of temperature sensitivity but in general germination is slow at soil temperatures below 18ºC and will be increasingly rapid up to about 35ºC (Bull 2000). Because sugarcane originated in the wet tropics, yields are much higher when the crop is suppled with adequate water. Thus sugarcane is usually irrigated (Ham et al. 2000).

During the initial stages of germination, root primordia around the nodes of the sett produce a flush of roots. These roots are not connected to the primary shoot but are important in maintaining the moisture in the sett. 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 tertiary shoots (Bull 2000).

1.4.2 Early growth

Stem elongation is initially rapid and during this phase the fibre content of the stem is high whereas the CCS levels are still quite low. Breeding for high above ground biomass in modern sugarcane varieties 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. Only in wetter areas is lodging associated with yield losses (Bull 2000).

1.4.3 Maturation and ripening

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 can influence sucrose accumulation including water stress, nutrient status and temperature.

Sugarcane is usually harvested by cutting stems close to the ground 12 –18 months after planting (Bull 2000). All sugarcane in Australia is harvested mechanically. Sugarcane is routinely harvested before flowering as the process of flowering leads to reduction in stem sugar content.

1.4.4   Ratoon crops

Sugarcane grows perennially and the root system or ratoon that remains in the ground will re-sprout from each stalk. Consequently 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. Thus a maximum of four ratoon crops are typically grown before ploughing out the crop and replanting (Bull 2000). After ploughing the previous ratoons out either another sugarcane crop is planted immediately or the ground is left fallow or planted to legumes with sugarcane again planted the following winter. Planting a legume crop has been shown to improve soil fertility for subsequent sugarcane crops. Removing ratoons with herbicide (glyphosate) rather than by tillage reduces soil compaction and improves soil structure (Willcox et al. 2000).

1.4.5   Eradication of sugarcane

Sugarcane killing practices often result in inadequate eradication of the old crop (Leibrandt 1993). The efficacy of glyphosate on killing sugarcane is affected by various factors such as cane varieties, soil type and stage of cane growth (Turner 1980). Sugarcane growing in light soils was more susceptible to herbicide treatment than that growing on heavy soils (Turner 1980). The growth stage of sugarcane has a pronounced effect on the efficacy of herbicide application. Sugarcane was killed more easily when the height of the leaf canopy was between 0.4-0.75m. Sugarcane appears to be more difficult to kill with herbicide once some stalks have been produced (Turner 1980).

Research showed that slashing of cane suppresses apical dominance and generally enhances chemical cane killing action on the regrowth (Leibrandt 1993). In addition, considerable improvement of eradication was also obtained when a mechanical under-cutter was used to shear the roots following herbicide application.

SECTION 1.5   VEGETATIVE MORPHOLOGY AND ANATOMY

The morphology and anatomy of sugarcane has been extensively reviewed and so will not be explored in detail here. See Moore (1987) 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 with a dormant bud and one or

more rows of root primordia, and a growth ring (intercalary meristem). The internodes consisting of sucrose storing parenchyma cells and vascular tissue.

As the stem develops the leaves emerge, one leaf per node, attached at the base of the node. The leaves eventually form 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 seven 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 2000).

The leaf blade is pubescent (hairy) on the abaxial (under) side of the leaf and glabrous (without hairs) on the adaxial (top) side (Moore 1987). Sugarcane uses a C4 mechanism of photosynthesis similar to other tropical grasses and consequently the anatomy of the leaves reflects this underlying physiology. 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.

The stem of sugarcane is the major sink for photosynthate (sucrose) within the sugarcane plant, rather than fruit or seed structures. Transverse cross section through an internode reveals vascular bundles surrounded by parenchyma cells with a thick outer epidermis covered in an external layer of wax (Moore 1987). Developing leaves and internodes develop in a basipetal direction in that the leaf blade expands from the tip to the base then the internode elongates.

The 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.

Like most grasses the sugarcane root system is fibrous and shallow. However, the plant develops buttress roots that serve to anchor the plant and some deeply penetrating roots that grow downwards for 5-7 metres allowing for water absorption under water stress (Moore 1987).

SECTION 1.6   REPRODUCTION

1.6.1   Sugarcane flowers

Sugarcane forms an open panicle type of inflorescence, whose shape, degree of branching and size are highly variety specific. The inflorescence or arrow consists of a main axis and first, second and third order branches. Attached to the branches are spikelets arranged in pairs that contain individual flowers. The sugarcane flower consists 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).

1.6.2   Flower initiation and seed propagation

The occurrence of flowering under field conditions is variable, influenced by variety as well as by environmental conditions. Flowering is most reliable and occurs earliest between

latitudes 7º and 12º and later northward and southward in the tropical region (Fauconnier 1993; Moore & Nuss 1987). Flowering in northern Australia (latitude 15º-20º) begins at the start of May. Flower initiation causes the apical meristem to switch from vegetative to floral development, causing stalk elongation to cease, consequently flowering of the crop can affect yields. The older and more vigorous stems in a stool are the most likely to initiate flowering (Moore & Nuss 1987).

The ability of sugarcane to reproduce sexually was not recognised until 1888. Floral development is initiated by shortening day length to an intermediate value, and occurs in Australia from mid February to mid March (Cox et al. 2000). Cool night temperatures, high day temperatures and lack of moisture interferes with flower initiation. Flowers take two to three months to mature after initiation.

Sugarcane is a cross-pollinating species although selfing occurs at low levels (McIntyre & Jackson 1995; Moore & Nuss 1987). Although sugarcane flowers often have reduced male fertility they are rarely male sterile. Sugarcane pollen is very small, hairy and wind dispersed. It 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 (26.5º C and 67% relative humidity) (Moore 1976; Venkatraman 1922). As a result, viable pollen is not expected to disperse far in the field. Sugarcane pollen stored at 4°C under 90 -100 % relative humidity retains some viability for up to 14 days (Moore & Nuss 1987).

As intensity of *Saccharum* flowering is dependent on interaction of cultivars and environmental factors such as daylength and temperature, some varieties can flower profusely in their natural environment but flower sparingly when introduced to other regions (Bull & Glasziou 1979). Sugarcane breeding programs are severely limited by the nature of flowering of each sugarcane variety (Bull & Glasziou 1979), particularly decrease in flowering and pollen viability at high latitudes (Dunckelman & Legendre 1982). However, controlled breeding is also possible when cultivars of low pollen viability are used as a female parent while low seed viability cultivars are used as a male parent (Bull & Glasziou 1979). Crosses can often be made but only between varieties which have overlapping flowering period. Various techniques have been developed including alteration of photoperiod which can induce flowering so that flowers could be available for crossing when required (Bull & Glasziou 1979).

Sugarcane seed or 'fuzz' is the entire flower panicle without the main flower axis and larger lateral axes. Mature fuzz consists of the mature dry fruit (caryopsis), glumes, callus hairs, anthers and stigma. The superfluous parts of the inflorescence are generally handled, stored and sown with the seed because it is not practical to separate them. Although many commercial varieties of sugarcane can produce seed, fuzz is only used in breeding programs, as the proportion of sugarcane seedlings with agronomic qualities near to those of the parental commercial clones is extremely low. Sugarcane fuzz is short lived, loosing 90% of its viability in 80 days at 28ºC if not desiccated (Rao 1980).

SECTION 1.7   DISEASES AND PESTS

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). The major pests and diseases that cause losses in sugarcane production include canegrubs, feral pigs, ratoon stunting disease (RSD), sugarcane rusts, chlorotic streak and soil-borne diseases (McLeod et al. 1999).

1.7.1 Diseases

Various biological agents including bacteria, fungi and viruses cause diseases of sugarcane. Diseases of sugarcane that have been identified in Australia are listed in Table 3.

Disease control in sugarcane is based on an integration of legislative control, resistant varieties, and other management procedures. Long-term disease management in Australia focuses on the development of disease resistant cultivars (McLeod et al. 1999). 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 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 the disease.

Many sugarcane diseases are also managed through the use of disease-free planting material supplied through Cane Protection and Productivity Boards. To obtain such planting material hot-water treatments are used to disinfect planting material. Long hot-water treatment (3 hours at 50ºC) is used to control RSD. Soaking in ambient temperature running water for ~40 hours followed by 3 hours at 50ºC is used to control leaf scald bacteria. Short hot-water treatment (50ºC for 30 minutes) is used to control chlorotic streak and some insect pests (Croft et al. 2000). Minimising cultivation also can encourage healthy microbial communities and reduce disease-causing organisms (Allsopp et al 2000).

Bacterial diseases

Ratoon stunting disease (RSD) (*Clavibacter xyli subsp xyli*)

The RSD is caused by *C. xyli* subsp *xyli* which infects vascular tissues of sugarcane. The ratoon stunting disease of sugarcane is probably the most important disease of sugarcane. In Australia, the estimated lost from this disease was $6.3m/year (McLeod et al. 1999). The symptoms are poor growth and resulting in stunting 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). Yield loss often becomes more severe in subsequent ratoon crops.

Leaf scald (*Xanthomonas albilineans*)

Leaf scald is caused by a bacterium *X. albilineans* which infects the vascular tissues of sugarcane. It is found in most sugarcane districts in Queensland (Croft et al. 2000). The symptom of leaf scald is characterised by the long white to cream streak on the leaves. The severe infected leaves appear scald and rolling inwards. The top of the severe infected shoots turn chlorosis. Yield loss occurs through the death of infected cane stalks.

Leaf scald can spread by wind-blown rain, plant material, contaminating cutting equipments such as planters and harvesters (Croft et al. 2000). Leaf scald can infect many other grass weeds which are alternate hosts and act as a reservoir for the disease. Extremes of moisture and temperature conditions favour disease transmission.

Fungal diseases

Orange rust (*Puccinia kuehnii*)

Orange rust is caused by *P. kuehnii*. The symptom is distinct from the common rust caused by *P. melanocephala*. Pustules of the orange rust are orange while those of common rust are

reddish brown. Leaf lesions tend to be grouped in cluster while those of common rust are distributed evenly on leaves. Pustules cause rupture to the leaves and allow water to escape from the plant, leading to moisture stress. Rain favours the development of orange rust but inhibits the development of common rust (Croft et al. 2000).

In 1999-2000, sugarcane crops in Australia were affected by the outbreak of orange rust, which had severely damaged the most widely grown commercial cultivar Q124 (Apan et al. 2003). Later, the new promising cultivar of high yield and resistance to orange rust Q205 was developed (Courtney 2002).

Sugarcane rust (*Puccinia melanocephala*)

Sugarcane rust is caused by *P. melanocephala* an obligate parasitic fungi. Estimated loss from sugarcane rust in Australia was $3.5m (McLeod et al. 1999). The symptoms started from tiny, elongated spots, light green to yellow on leaves. These spots later enlarge and turn to reddish brown. Yield loss depending on environmental conditions was estimated to be 10-20% in Australia (Bernard 1980) and 20-40% in USA (University of Florida 2004).

Pachymetra root rot (*Pachymetra chaunorhiza*)

Pachymetra root rot is a disease only found in Queensland sugarcane districts (Magarey & Bull 2003). The disease seems to favour high rainfall areas. In northern Queensland, surveys indicated almost every field is infected with the disease. 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% (Magarey 1994).

Sugarcane smut (*Ustilago scitaminea*)

Sugarcane smut is a serious disease of sugarcane that can reduce yields by 20-30%. The disease causes severe stunting and is characterised by black, whip-like structures that form at the growing points of sugarcane plants. These whips replace the spindle leaves and are formed in the shoots developing from infected cane cuttings (Frison & Putter 1993). There was an outbreak of smut in Australia in July 1998 in the Ord River area of Western Australia. This outbreak was controlled and the disease has not been detected in eastern Australia (Croft et al. 2000). Short hot-water treatment and use of resistant varieties can control smut.

The movement of sugarcane and sugarcane machinery is restricted in Queensland by the *Plant Protection Act 1989* (Qld) and the *Plant Protection Regulations 2002* (Qld). Provisions under the *Plant Protection Act 1989* (Qld) allow for inspectors to order the destruction of diseased cane.

Other fungal diseases of sugarcane are minor diseases (Table 3) and cause less impact on yield.

Viral diseases

Chlorotic streak

The cause of the disease is unknown but it is thought that the disease may be caused by virus. The disease occurs in sugarcane districts with wet and poorly drained fields. Lower incidence of the disease is generally found in drier regions (Croft et al. 2000). The symptoms are the yellow to white streaks on the leaf and on the midrib and leaf sheath. Older streaks change to yellow and more visible than younger streaks. Finally chlorosis starts to appear in the middle of the leave. Internal vascular bundle tissues may show reddish in colour. Yield can be reduced by 70% (Croft et al. 2000).

The disease is transmitted in soil by water. It is common in areas with poor drainage and flood prone areas. Chlorotic streak can also transmitted through cuttings. Infected cuttings show poor germination, slow and number of stool reduced.

Fiji disease

Fiji disease is caused by *Fiji disease virus* (FDV) genus Figivirus, family Reoviridae. The symptoms is whitish galls raised on the underside of the leaf blade and midrib. Galls produced due to the disorder of cell proliferation in the phloem and xylem. The colour of the galls can vary from white to green and the surface of the gall is usually smooth. When the gall is old the epidermis may be ruptured and appear brown. At advance stage of infection, stem development slows down and successive leaves become smaller and stiffer, the whole top part develops a fan like appearance (Croft et al. 2000) .

Fiji disease can be transmitted by infected cutting, or by a vector: plant hoppers (*Perkinsiella saccharicidae*). Significant yield loss has been recorded in 1970s in Queensland (Croft et al. 2000). Due to the intensive management program put in place, no reports of the disease incidence since the 1980s.

Sugarcane mosaics

Sugarcane mosaic is caused by a number of potyviruses (Table 3) such as the *Sugarcane mosaic virus* (SCMV). The mosaic symptom pattern appears in young growing leaves. Once old, infected leaves may appear more normal as the mosaic become green. The record of yield loss caused by sugarcane mosaic was 40% in some fields (Croft et al. 2000). Aphids are the vector for transmission of the disease. Seed produced by infected cane can also transmit the disease.

Table 3. 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 varieties
Ratoon stunting disease (RSD)	*Clavibacter xyli* subsp. *xyli*	Disease free planting material
Red stripe (Top rot)	*Acidovorax avenae* subsp. *avenae*	Resistant varieties
Fungal		
Rusts	*Puccinia melanocephala* and *P.kuehnii*	Resistant varieties
Yellow spot	*Mycovellosiella koepkei*	Resistant varieties
Pachymetra root rot	*Pachymetra chaunorhiza*	Resistant varieties
Sugarcane smut	*Ustilago scitaminea*	Resistant variety, hot water treatment
Pineapple disease	*Ceratocytis paradoxa*	Fungicide applied to setts
Eye spot	*Bipolaris sacchari*	Resistant varieties
Red rot	*Glomerella tucumanensis*	Resistant varieties
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 varieties
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 varieties

1.7.2 Pests

There are many insects and animals that are pests of sugarcane. In addition some insects such as plant hoppers (*Perkinsiella saccharicida*) are known vectors of other diseases (Croft et al. 2000; Allsopp et al. 2000). Information below is summarised from 'Australian Sugarcane Pests' (Agnew 1997).

Animal pests of sugarcane are numerous and include ground rats, climbing rats, feral pigs, wallabies, foxes, the striped possum, the eastern swamphen and cockatoos. Ground rats (*Rattus sordidus*), wallabies, striped possums (*Dactylopsila trivirgata*), the eastern swamphen (*Porphyrio porphyrio*) and cockatoos (*Cacatua galerita*) are native to Australia and consequently are protected. Permits for control of native animals in cane fields must be obtained from the relevant Cane Protection and Productivity Board. Integrated pest management is now widely employed to discourage and control economically damaging pests. This includes strategies such as controlling weeds that may harbour rats (Allsopp et al. 2000).

Table 4 Insect pests of sugarcane (Agnew 1997).

Common Name	Species	Affected Plant Part	Control
Canegrubs	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 varieties, 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 *H. arator* 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 – bore into setts and ratoons (occurs rarely)	none available
Stenocorynus weevil	*Stenocorynus* spp.	Shoots – 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
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 varieties
Froghopper	*Eoscarta carnifex*	Leaf and stem – sap feeding, leaves look scorched	tolerant varieties

Common Name	Species	Affected Plant Part	Control
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

2. TOXICITY AND ALLERGENICITY

Section 2.1   Toxicity

Sugarcane is a well-established agricultural crop with a long history of safe use. Sugarcane 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 food 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 LD_{50} of sucrose for rats is 30 g/kg body weight (MSDS 2004). Consuming sucrose in extremely large oral dosages may produce gastrointestinal disturbances. Although there is no direct evidences that link 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 increases the risk factor for dental caries (Rugg-Gunn & Murray 1983; Sreebny 1982).

A mixture of bagasse and molasses is used as cattle feed. When fed in large quantities and incorrectly, molasses may be toxic. The symptoms of molasses toxicity are reduced body temperature, weakness and rapid breathing. The animal may have difficulty standing (Perez R. & de Azucar 2004). Molasses toxicity often affects eye-sight and the animal may become blind. This indicated damage to the brain and the clinical syndrome was similar to that of cerebro-cortical necrosis. Necrosis in the brain readily develops and allows rapid diagnosis (Preston 1988). The necrosis is likely to be caused by either a decrease in energy supply to the brain because of thiamine deficiency or glucose deficiency.

Bagasse is the highly fibrous residue remaining after cane is pressed to remove sucrose. Like many other agricultural by-products such as cereal straws, bagasse 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 ligno-cellulose materials for livestock, physical or chemical pre-treatments are required (de la Cruz 1990; Playne 1984).

Section 2.2   Allergenicity

Sugarcane pollen is transported by wind and therefore has potential to act as an airborne allergen. Allergenicity of sugarcane pollen was evaluated by Chakraborty et al. (2001). In the skin tests that were conducted, 70.58% of field workers with respiratory disorders showed highly reactive skin tests. 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.

3. WEEDINESS

In Australia sugarcane occurs almost exclusively in managed cultivation. In sugarcane districts, transient sugarcane plants may occur along roadsides or railways where it can establish after displacement during transport, but there is no indication that these form self-perpetuating populations.

SECTION 3.1 WEEDINESS OF *SACCHARUM* SPP. HYBRIDS

Commercial cultivars of sugarcane are hybrids of *S. officinarum* and *S. spontaneum*. They are not recognised as weeds in Australia or anywhere in the world. They have lost many of the critical weedy attributes that were present in the parental species from which the cultivated sugarcane hybrids were derived (Holm et al. 1997). Most commercial cultivars of sugarcane are routinely harvested before flowering as the process of flowering leads to reduction in stem sugar content (Moore & Nuss 1987). As discussed above, when allowed to flower, sugarcane flowering is erratic and variable and sugarcane seed is short-lived (Rao 1980). Even if seed is produced, the chance of it germinating, surviving to reproductive maturity and spreading is low.

Volunteer sugarcane seedlings of modern cultivars are non-invasive in natural habitats and are likely to be controlled by natural herbivores during early stages of growth or be out-competed by other weeds. There has been one instance of volunteer sugarcane, of cultivar CP29-116, probably growing for 30-40 years in southern Queensland. These volunteer canes consist of only a few stools and have not spread further.

The potential for *Saccharum* spp. hybrids to become weeds in Australia is low because canes are harvested before they flower as mentioned above. Unaided dispersal of the sugarcane vegetative materials is unlikely due to the size and weight of the stem sections. Animal pests such as feral pigs have the strength to remove plant material and take them elsewhere. But there are no reports of feral pigs or other animal pests of sugarcane indulging in such activities. Establishment and spread of volunteers are unlikely as the seedlings of modern cultivars are highly susceptible to pests, weeds and therefore unlikely to establish and spread without human intervention. Un-germinated stem cuttings tend not to survive for long as they are rapidly degraded by soil micro-organisms due to their high sugar content.

SECTION 3.2 WEEDINESS OF *SACCHARUM* SPECIES

None of five recognised *Saccharum* species (*S. spontaneum, S. robustrum, S. barberi, S. sinensis* and *S. officinarum*) are native to Australia. Some of these species are maintained within Australian sugarcane research stations as germplasm. Only two parental species of modern cultivars are recorded naturalised in Australia ie *S. spontaneum* and *S. officinarum* (Hnatiuk 1990).

3.2.1 *Saccharum spontaneum*

S. spontaneum is native to India and recorded as a weed in 33 countries and is 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). It is classified as a noxious weed in 42 states of the USA (USDA 2004). 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. *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). Pure stands of *S. spontaneum* can be found in poor agricultural soils degraded by fire and overuse (Hammond 1999);(Holm et al. 1997).

Because of higher genetic variability of polyploid species, *S. spontaneum* has more variable morphology and physiology, enabling it to develop higher phenotypic plasticity and effectively compete in disturbance prone and changeable habitats. *S. spontaneum*'s competitive advantage is due to its diverse reproductive abilities. *S. spontaneum* can reproduce by both vegetative propagation (layering, root and stem cutting) and seeding. It invades aggressively both underground and above ground by extending rhizomes, tillers (secondary shoots) and tertiary shoots, which together form thick stools or clumps (Pursglove 1972). The dense root mat of *S. spontaneum* makes it impossible for young seedlings to penetrate and emerge. *S. spontaneum* is highly variable, tolerating a broad range of soil types and moisture contents. It is shade tolerant and produces many small wind dispersed seed (Pursglove 1972). These weedy traits have not been incorporated into commercial hybrids.

Australia's Virtual Herbarium (http://www.cpbr.gov.au/avh.html) records *S. spontaneum* collected in the Northern Territory, Queensland and northern New South Wales. Evidence suggests that currently *S. spontaneum* is not a serious weed in Australia, because surviving uncultivated populations have established in the Northern Territory for at least 50 years without further spread.

3.2.2   *Saccharum officinarum*

S. officinarum is cultivated as agricultural crop in many countries in Asia and other tropical climate countries. It has been grown as food crop in the Americas and Indies since the 18th century. It is thought that *S. offiicinarum* was originally selected by humans in Papua New Guinea. *S. officinarum* has escaped the agricultural area and naturalised in some areas but has not been recorded as a major weed in Australia (Hnatiuk 1990; USDA 2004) or elsewhere (Holm et al. 1997). Hnatiuk (1990) reported that *S. officinarum* is naturalised in Queensland and New South Wales. Lazarides et al (1997) recorded it as a minor weed found naturalised in some Tropical and Mediterranean climates in Australia. *S. officinarum* has also been recorded as a minor weed and/or a quarantine species in some countries (Randall, 2002) because it may pose a risk of quarantine disease transmission.

As a result of many years of cultivation, *S. officinarum* has essentially lost the capacity to invade in uncultivated habitats. 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. A few populations of *S. officinarum* have established outside agricultural areas in southern Queensland for 30-40 years. These populations consist of only a few stools and do not result in further spread.

3.2.3   Other *Saccharum* weed species

There are a number of species within genus *Saccharum* which are recognised as weeds elsewhere such as *Saccharum arundinaceum, S. bengalense, S. floridulum, S. narenga, S. procerum* and *S. ravennae* (Randall, 2002). There are no records of these species in Australia (Hnatiuk, 1990; http://www.cpbr.gov.au/avh.html; Randall, 2002).

SECTION 3.3   WEEDINESS OF SPECIES IN THE *SACCHARUM* COMPLEX IN AUSTRALIA

Sugarcane is closely related to the genera *Erianthus, Narenga, Miscanthus* and *Sclerostachya*. These genera including *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).

Miscanthus sinensis has been recorded in southern Western Australia, the central coast of New South Wales (Hnatiuk 1990), southern South Australia, southern Victoria and in two locations in Tasmania (Australia's Virtual Herbarium 2004) and locally is considered weedy (Lazarides et al. 1997). *Miscanthus floridulus* is a noxious weed overseas (Randall 2002). It has been found naturalised in Australia but has not been recorded as a weed (Australia's Virtual Herbarium 2004). *Miscanthus nepalensis* is recorded as locally invasive in New Zealand and Brazil (Randall 2002).

Narenga porphyrocoma has been collected from a single site in Queensland within commercial sugarcane growing areas (Australia's Virtual Herbarium 2004). Other species in the *Miscanthus* and *Erianthus* genera are listed in 'A Global Compendium of Weeds' (Randall 2002) as weeds of agriculture in Asia.

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.

4.   POTENTIAL FOR GENE TRANSFER FROM SUGARCANE TO OTHER ORGANISMS

SECTION 4.1   GENE TRANSFER TO CULTIVATED SUGARCANE AND NATURALISED SUGARCANE

As indicated above, sugarcane flowering is variable in the field and the crop is exclusively vegetatively propagated. Sugarcane is a largely cross-pollinated species with a low frequency of selfing and pollen is dispersed by wind (McIntyre & Jackson 2001). No insect or animal vectors for sugarcane pollen are known. Pollen viability is low under natural environmental conditions (Moore 1976). Sugarcane pollen is rapidly desiccated after dehiscence, having a half life of only 12 minutes and no viability after 35 minutes at 26.5°C and 67% relative humidity (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 for desirable crosses.

Different varieties of sugarcane produce different amounts of pollen. Crossing and selfing therefore varied greatly. Analysis of seed derived from crossing studies showing seed set varied between 3.1-22.7% (Grassl 1980; Rao 1980) reflecting the poor viability of sugarcane pollen. In some case seed were uniform in size but up to 30% were smaller or shrivelled, however, most of them germinated (Rao 1980). Molecular methods have indicated that 0-17% of progeny from apparent hybrid crosses are actually the result of selfing (flower being fertilised by its own pollen, McIntyre & Jackson 2001). Selfing frequencies were found to vary dramatically between 0-18% (McIntyre & Jackson 2001) to 0-80% (Hogarth 1980).

Parents used in breeding programs are classified as male or female depending on relative amounts of viable pollen produced. Sex is often determined by aceto-carmine staining to determine the viability of pollen. Clones with <10% pollen viability are designated as female, clones with >25% viable pollen are designated as male. Clones 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).

Commercial breeding programs produce artificial crosses between *Saccharum* spp. hybrids under highly favourable conditions. 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). However, sugarcane is not an ideal candidate for crossing in conventional plant breeding due to its characteristic non-synchronous flowering and low sexual seed viability (Selman-Housein et al. 2000).

Viable pollen of sugarcane would not be expected to disperse very far. Accordingly, cross-pollination would only be expected to occur to flowering sugarcane plants growing in close proximity. In addition, a very low rate of successful crosses would be expected due to the rapid loss of pollen viability.

SECTION 4.2   GENE TRANSFER TO OTHER RELATED *SACCHARUM* SPECIES

It has been reported that species with the genus *Saccharum* can hybridise with other closely related species (Grassl 1980; Daniels & Roach 1987; Grassl 1980). However, such reports of hybridisation do not generally refer to modern sugarcane varieties (complex hybrids).

Gene flow between *Saccharum* spp. hybrids to other *Saccharum* species is likely to be low, because plants are likely to be harvested before flowering. If seed set happens, seedling survival is poor, and field management methods would limit survival of any volunteer plants.

None of five recognised *Saccharum* species (*S. spontaneum, S. officinarum, S. robustrum, S. barberi* and *S. sinensis*) are native to Australia. These species are maintained within sugarcane research stations as germplasm and can hybridise successfully with modern cultivars of sugarcane (*Saccharum* spp. hybrid). These species have been used in breeding programs to produce new varieties. Of these species only *S. officinarum* and *S. spontaneum* are recorded as naturalised in Australia. There is no records of native or endangered species having sexual compatibility with sugarcane in Australia (Daniels & Roach 1987).

There are a number of species within the *Saccharum* genus which are recognised as weed elsewhere, such as *Saccharum arundinaceum, S. bengalense, S. floridulum, S. narenga, S. procerum, S. ravennae* (Randall, 2002). These species have potential to outcross successfully with hybrid sugarcane but as mentioned above there have been no records of these species in Australia (Hnatiuk, 1990; http://www.cpbr.gov.au/avh.html).

SECTION 4.3   GENE TRANSFER TO OTHER GENERA IN *SACCHARUM* COMPLEX

As indicated above, sugarcane is closely related to the genera *Erianthus, Narenga, Miscanthus* and *Sclerostachya*. These five genera (including *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).

Although many attempts to cross between these species may have been attempted in sugarcane research stations, limited publications are available. Of 96 crosses made at BSES in Queensland between *Erianthus arundinaceus* and *S. officinarum* or hybrid *Saccharum* spp., 26 were fertile producing over 1000 seedlings. Only 19 putative hybrids have survived, all were derived from *S. officinarum* as a female parent and *E. arundinaceus* as a male parent (Piperidis et al. 2000). Genuine hybrids were produced at a frequency of 2.8% however all of these hybrids had poor vigour and were sterile (Piperidis et al. 2000). Chromosome elimination has been observed in all putative hybrids. Molecular studies have demonstrated that *E. arundinaceus* is genetically quite distant from *Saccharum* (Nair 1999; Alix et al. 1998).

In conclusion, evidence indicated that even if the commercial hybrid sugarcanes were allowed to flower, the likelihood of gene transfer to other closely related species within the *Saccharum* complex or their hybrids is very low, because of genetic incompatibility.

SECTION 4.4   GENE TRANSFER TO OTHER GENERA IN TRIBE ANDROPOGONEAE

Hybridisation with *Saccharum* has also been attempted with some distantly related genera belonging to tribe Andropogoneae such as *Imperata* (blady grass)*, Sorghum* (sorghum) and *Bambusa* (bamboo) (Nair 1999; Rao et al. 1967; Janakiammal 1938; Thomas & Venkatraman 1930). These claims for intergeneric hybrids are based on anatomical morphological and cytological studies but have never been verified by molecular analysis. A few of these putative intergeneric hybrids could not be accepted as true hybrids (Grassl 1980; Nair 1999; Rao et al. 1967).

Histological analysis of crosses between *S. officinarum, S. robustum, S spontaneum* plus seven *Saccharum* hybrids and *Bambusa* indicated that abortion of hybrid embryos occurred during the early embryogenic stage (Rao et al. 1967). Four mature seeds were obtained from 960 crosses using *Bambusa* as a female parent, all putative hybrid seeds were either from *S. spontaneum* or *S. robustum* as male parents. These putative hybrids either failed to germinate from seed or died at seedling stage (Rao et al. 1967).

Sorghum species have been artificially crossed with *Saccharum* hybrids (Grassl 1980) and *S. officinarum* (Nair 1999). Wild *Sorghum* species are among the weeds of Australian sugarcane crops (McMahon et al. 2000) and are widespread in Australia (Hnatiuk 1990). Generally, the offspring have been of low vigour and fertility, but back crossing to both parents have been achieved (Grassl 1980; Sreenivasan et al. 1987). However, Grassel (1980) recorded that after the 4th to 5th generation of backcrossing to sorghum, the sugarcane chromosomes had been eliminated from the intergeneric hybrids.

Imperata (blady grass) is capable of crossing with *Saccharum* (Sreenivasan et al. 1987). *Imperata cylindrica* is reported in Australia (Clifford & Ludlow 1978; Hartley 1979; Hnatiuk 1990; Clifford & Ludlow 1978; Hartley 1979). *I. cylindrica* is a perennial species that commonly grows on degraded or burnt-off land in most Australian sugarcane-growing districts. It is a common weed in Queensland (Kleinschidmidt & Johnson 1977), and although it occurs in all Australian states (Auld & Medd 1987; Australia's Virtual Herbarium 2004) it is not listed as a noxious weed in any jurisdiction (National Weeds Strategy Executive Committee 2002).

There is one report of experimental cross between *I. cylindrica* and a *Saccharum* hybrid, producing triploid progeny resembling sugarcane, which could apparently self-fertilise to

produce F2 progeny (Daniels & Roach 1987; Sreenivasan et al. 1987). 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 artificial hybrids have not been observed in the wild.

SECTION 4.5   GENE TRANSFER TO OTHER ORGANISMS

The only way by which genes could be transferred from sugarcane to other than plants is by horizontal gene transfer. Such transfers have not been demonstrated under natural conditions (Nielsen et al. 1997; Nielsen et al. 1998; Syvanen 1999) and deliberate attempts to induce them have so far failed (Coghlan 2000; Schlüter et al. 1995).

Transfer of plant DNA to bacteria has been demonstrated under highly artificial laboratory conditions (Gebhard & Smalla 1998; Mercer et al. 1999; Nielsen et al. 1998), but even then only at a very low frequency. Phylogenetic comparison of the sequences of plant and bacterial genes suggests that horizontal gene transfer from plants to bacteria during evolutionary history has been extremely rare, if occurring at all (Doolittle 1999; Nielsen et al. 1998).

Recombination between viral genomes and plant DNA has only been observed at very low levels, and only between homologous sequences under conditions of selective pressure, eg. regeneration of infectious virus by complementation of a defective virus by viral sequences introduced into a genetically modified plant genome (Greene & Allison 1994; Teycheney & Tepfer 1999).

Thus, gene transfer from sugarcane to organisms other than plants is extremely unlikely. A more detailed review of horizontal gene transfer from plants to other organisms is provided in the risk assessment and risk management plans that were prepared in relation to application DIR 051/2004 for the release of GM sugarcane into the Australian environment.

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