CRC SUGAR Industry Innovation through Biotechnology

Research Project Final Report

Understanding the reproductive biology and ecology of sugarcane to manage the safe release of genetically modified cultivars

by

Graham Bonnett, Juan Jose Olivares-Villegas, Nils Berding, Terry Morgan and Peter Collins

CRC Project 1b5

Contact:
Dr Graham Bonnett
Theme Leader
CSIRO Plant Industry
QBP, 306 Carmody Road, St Lucia, Qld 4077
Telephone: 07 3214 2352
Facsimile: 07 3214 2920
Email: graham.bonnett@csiro.au

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1.0 SUMMARY

This project set out to obtain basic and previously unavailable information on the ecology and sexual reproduction of sugarcane primarily so advances in sugarcane biotechnology can be utilised to the benefit of the Australian sugarcane industry and the broader Australian economy. The production and commercialisation of genetically modified (GM) sugarcane has attracted increased international interest in recent years, and this has been exemplified by significant, sustained investment in sugarcane biotechnology by large national and international companies. To commercialise GM sugarcane, the proposed cane has to undergo rigorous regulatory assessment including safety to humans and the environment.

A significant part of this assessment relates to how a given sugarcane clone functions in the environment (s) where it will be grown, and the likelihood and impact of transfer of the modified trait to other commercial sugarcane or other sympatric sexually compatible species. While such assessments are performed for each proposed GM sugarcane cultivar under consideration, general information about the sexual reproduction and ecology of sugarcane is also important to help understand potential hazards. For sugarcane, this basic information is scant, largely because the stalk not the seed is the harvested product (i.e. sugarcane is vegetatively propagated) and so the sexual reproduction process have not previously been studied in commercially grown sugarcane. This project undertook a series of studies to help fill the 'information void' on sugarcane.

The project involved several surveys and experiments using cane in farmers' fields to understand the level of flowering and viable seed production under commercial production. Species that could be sexually compatible with sugarcane were determined through analysis of the breeding literature to see what crosses had been achieved with human intervention. This was followed by comparison with botanical records to determine which of the potential species were present in sugar growing regions.

Saccharum spontaneum L. was the only plant that could easily cross with commercial sugarcane that also was present in some Australian sugarcane growing regions. *S. spontaneum* has been used in inter-specific hybridisation with S. officinarum L. to breed modern sugarcane cultivars. Supplementing botanical records with local industry knowledge and surveys we identified five populations of *S. spontaneum* in northern Australia. We also determined the genetic diversity within the populations. These populations and adjacent fields of commercial sugarcane also were studied at the same time to determine flowering times and production of viable seed.

A central part of the project was the convening and use of a consultative group including State and Federal Government regulators. This group met with us once a year, on three occasions to review research progress and help define the most important areas of study. These meetings also ensured information was discussed directly with people who would be significant end-users.

Once the patterns of flowering were starting to be established, further work was undertaken to determine (i) if the *S. spontaneum* plants in one region were capable of receiving pollen from commercial cultivars and producing viable seed (ii) the level of crossing between commercial cultivasr in farmers' fields.

In an attempt to understand why *S. spontaneum* is a very invasive weed in some places where it has been introduced (e.g. Panama) and not as bad in others (e.g.

Queensland) the biology of *S. spontaneum* was studied in Panama. A combination of surveying viable seed production at different sites, analysis of genetic diversity, and comparison to other introduced grass allowed conclusions to be drawn about the genotype/environment interactions leading to invasion and what traits can be assessed in proposed GM canes to ensure sugarcane has not been made more invasive.

Laboratory experiments were conducted to determine the germination response of commercial cane and *S. spontaneum* to temperature and for commercial cultivars also to water availability.

The major outputs from the project were:

(i) identification of sites where *S. spontaneum* grows in northern Queensland.
(ii) determination of when flowering occurs and if seed is produced.
(iii) comparison of the flowering times relative to commercial cultivars to determine when and where there is overlap and consequently the opportunity of transfer of genetic material.
(iv) understanding of the level of viable seed production and its variability within commercial fields of sugarcane.
(v) information on the germination response of commercial sugarcane seeds to temperature and water and for the response of *S. spontaneum* seed to temperature and,
(vi) identification of the features of *S. spontaneum* that make it an invasive weed in Panama but much less so in northern Queensland.

In order to turn these outputs into outcomes, they have been drafted for publication in peer review journals to make them accessible to researchers and to allow regulators to use the information with the knowledge that it has undergone a review process. Direct engagement with staff of Federal agencies responsible for regulating GM sugarcane has also ensured the information is placed directly into the hands of the targeted end-users.

At a final meeting in April 2010 a summary of project results was presented to staff of the Office of the Gene Technology Regulator, Food Standards Australia and New Zealand, Australian Pesticide and Veterinary Medicines Authority and the Department of Environment, Water, Heritage and the Arts.

The impacts of this project will become apparent as our regulatory bodies use science-based decisions about the experimental and commercial release of GM sugarcane and the conditions under which such releases should occur to ensure they are managed to minimise risks to surrounding environments.

2.0 BACKGROUND

This project has been the first in the world to investigate the sexual reproduction of sugarcane in farmers' fields. This has been necessary because of the research and development of GM sugarcane. Whilst the development of the tools and techniques to produce stably transformed sugarcane has been on-going for some time (Lakshmanan et al., 2005), no GM sugarcane cultivars are grown commercially. However, technical progress has continued towards producing elite GM events in sugarcane suitable for commercial production but research to support the next step of the commercialization phase, regulatory approval was not being addressed.

Regulatory approval in Australia for growing GM sugarcane and using sucrose derived from it for food needs to be given by the Office of the Gene Technology Regulator (OGTR) and Food Standards Australia and New Zealand (FSANZ). Depending on the introduced trait, the Australian Pesticides and Veterinary Medicine Authority (APVMA) and Therapeutic Goods Administration (TGA) also may have to give approvals. The OGTR is responsible for the safety of humans coming into contact with the GMO and safety to the environment. FSANZ ensures food stuffs made from the GMO are safe to eat. In this project an understanding of the sexual reproduction of sugarcane was studied to provide basic information about the aspects of safety to the environment that would needed to be considered for sugarcane. The generic issues assessed for any GM plant are the likelihood of transfer of the introduced gene to other crops of the same species or other sexually compatible species; and the transgene's ability to establish in the environment and make the crop or a sexually compatible species weedier.

There had been multiple limited, experimental releases of GM sugarcane (Lakshmanan et al., 2005), including several in Australia. To assist in making their decisions about these releases, the OGTR collated the available information about the biology and ecology of sugarcane into a single manual (Annonymous 2004). This document showed that there was a paucity of experimental data and the data that were available had been drawn from breeding programs and not studies in commercial fields. Breeders have historically been the only scientists interested in sugarcane flowering and seed production as the stalk, not seed is both the plant part from which crops are propagated and is the harvested portion of the crop. Further, overseas experiences of sugarcane being genetically isolated (i.e. not flowering) in Hawaii (Wang et al., 2005) and mainland China (Chen, 2010) did not match with observations of the Australian sugar industry or incidentally with more widespread observations in Hawaii (Humbert, 1968) or China (Bonnett, personal observations). Consequently there was a need to understand the level of sugarcane sexual reproduction, presence of compatible species and the ability of seed to germinate and establish into new plants. In addition the issue of what would make sugarcane weedier was addressed through a comparative study of the weedy relative of sugarcane, *S. spontaneum* in Panama and Australia.

3.0 OBJECTIVES

The objectives as stated in the proposal were:

This project will provide data (i) to inform decisions made by regulators about the release of genetically modified (GM) sugarcane and (ii) so the industry can design rational monitoring protocols for experimental releases and strategies for the environmentally safe commercial release of GM sugarcane.

Specifically the project will:

- Develop a framework to analyse the risks of transgene(s) establishing (i) in plants in the environment outside of cultivated sugarcane and (ii) in plants within non- GM sugarcane surrounding GM sugarcane.
- Use the information to suggest strategies to reduce any significant risks.
- Publish the results contributing to the framework in peer-reviewed, scientific journals.

As the project developed and through interactions with regulators it became apparent that it was the regulators' role, in particular the OGTR to develop frameworks to analyse risks and not ours. Consequently we have concentrated on obtaining the data that would allow them to work out what the likely hazards relating to sugarcane may be with respect to the environment and consequently the risks associated with any proposed transgene.

The objectives to provide baseline data and identify potential hazards have been met and this will be complete when the papers (presented in the Appendices) that are currently in advanced draft are submitted to scientific journals and published.

In Bonnett et al., (2010) discussed the potential to manage risks through eradication of *S. spontaneum* should this be desirable is discussed. As no significant risks were identified for the introduction of traits having a neutral effect on survival outside of cultivation, no strategies to reduce risk have been proposed. However, the comparative analysis of weediness of *S. spontaneum* in Panama and northern Australia has identified traits that if altered could lead to increased germination and survival of seedlings and possibly weediness. Methods to assess changes to these traits have been developed as part of the study and would be easy to use. Deliberate attempts to alter abiotic stress resistance (to temperature or water availability) are traits that could pleiotrophically increase germination and survival of sugarcane seeds.

4.0 METHODOLOGY

This project used field-based and laboratory-based experimentation and surveys to understand the reproductive biology of sugarcane and its wild relative *S. spontaneum*. These approaches were aided by local knowledge obtained from many people active in the sugar industry and by regular meetings of a consultative group comprising mainly of regulators, a main group of targeted end users of the research. Local industry knowledge enabled sourcing of specific experimental sites and populations, whilst the consultative group meetings ensured that we were addressing the highest priority issues and enabled us to calibrate our interpretations of the data with those of experts who would use the data.

The detailed methodology used for particular experiments is fully detailed in the completed and drafted publications in the Appendix. A brief summary of the overall approach is given below.

During the course of the project a series of questions were asked about the sexual reproduction of sugarcane (Figure 1) and about factors that would affect germination and weediness.

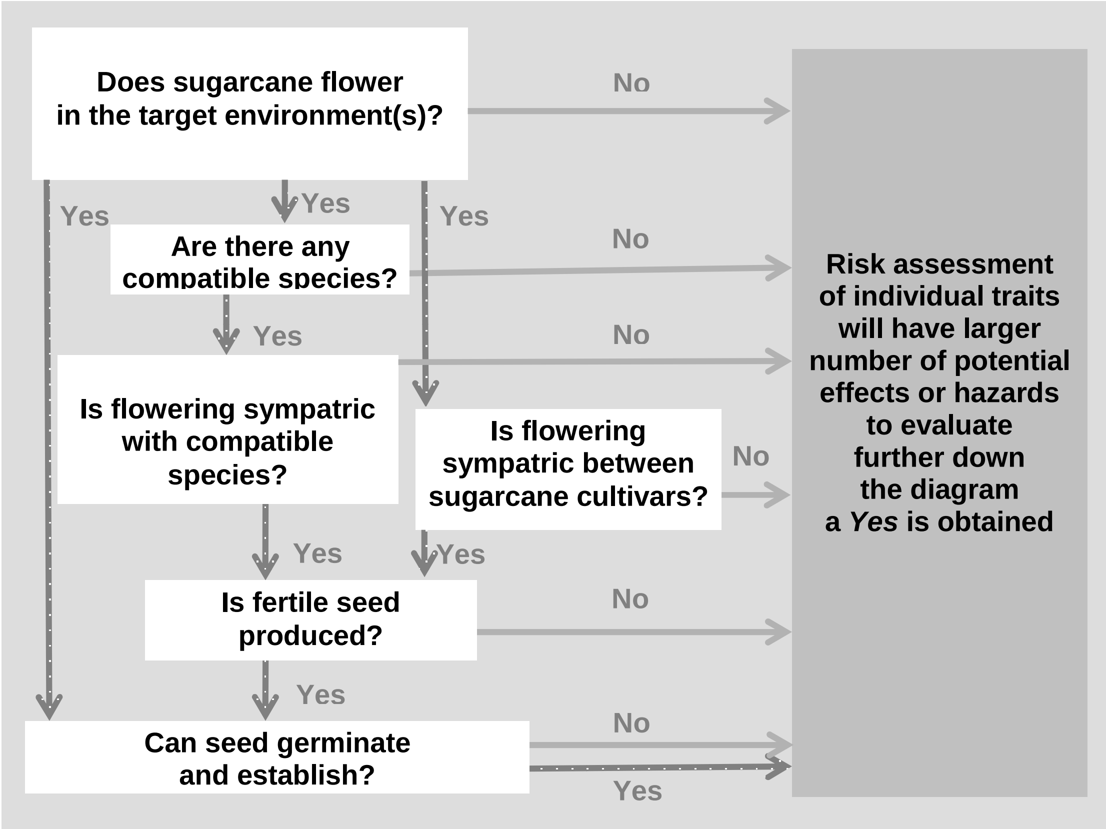

Figure 1 **A generic framework for the identification of potential environmental effects or hazards within a risk assessment, resulting from the sexual reproduction of sugarcane in commercial fields From (Olivares-Villegas et al., 2010).**

Given that sugarcane is grown over a large range of latitudes the study took a regional approach, answering the questions posed in Figure 1 for each of the major sugar growing regions in Australia. The most effort has been devoted to the more northerly regions as these were thought to have the highest level of flowering and pollen fertility. In the final year of the project surveys were also conducted in more southerly regions of the industry, Bundaberg and NSW.

The appendices do not show an experiment to test whether the *S. spontaneum* present in the Herbert River can act as a pollen receptor for pollen from commercial cultivars. This experiment has not been included in the drafts appended.

4.1 *Saccharum spontaneum* x cultivar crosses

Using standard breeding techniques inflorescences of several *S. spontaneum* clones were taken from the Herbert River were used as females and crossed to commercial cultivars concurrently in flower. The crosses were made as breeders would in a cloth lantern and are shown in Table 1.

Seed was matured and then germinated under optimal conditions (paper 2, Appendix). DNA was extracted from germinated seedlings (using a new method adapted to sugarcane for detailed in paper 6 in the Appendix).

Using techniques similar to those described in detail in paper 4 progeny of the crosses that germinated were tested for the presence of a molecular marker absent in the *S. spontaneum* clones used in the cross but present in the commercial cultivar from which the seeds derived. The individuals that had these markers present are hybrids.

5.0 RESULTS

The results are shown in the papers attached in the Appendix. The results of the *Saccharum spontaneum* x cultivar hybridisation experiment are presented below.

5.1 *Saccharum spontaneum* x cultivar crosses

The list of crosses is shown in Table 1. There were very low levels of germination of the seed. All seeds that germinated had the DNA extracted and were tested for hybridisation. The results from one marker is summarised below. Additional markers are in the process of being tested. Several markers are required to determine hybridisation.

Table 1 Crosses between *S. spontaneum* L. growing in the Herbert River (HRS) and commercial sugarcane clones.

Cross	Cross #	Mass of fuzz (g)	Number of seedlings	Hybrids assessed: Marker 2039	Percent hybrids
HRS1[1] x Mida[Φ]	1	10.55	0	0	
HRS2[2] x Mida[Φ]	2	8.18	12	2	16.7
HRS3[3] x Mida[Φ]	3	20.07	5	1	20.0
HRS4[4] x Q162[Φ]	4	8.47	4	0	0
HRS5[5] x Q162[Φ]	5	12.77	168	9	5.36
HRS6[6] x Q162[Φ]	6	12.91	13	2	15.4

[1]18° 35.858′ 146° 16.417′, [2]18° 35.857′ 146° 16.457′, [3]18° 35.862′ 146° 16.452′, [4]18° 35.805′ 146° 16.024′, [5]18° 35.803′ 146° 16.008′, [6]18° 35.896′ 146° 16.610′.

Several controls were performed in this experiment. The first was to demonstrate that the male parents used were fertile and this was tested by using them as the male parent in a cross with Q208[Φ]. For both of these crosses the level of germination of seed also was low, five seedlings per gram of fuzz for both crosses. The second test was to determine the level of seed production of the same genotypes of HRS by sampling mature inflorescences from where they were growing. These genotypes were sampled on two dates and the germination data is presented in Table 2. Clones HRS 4 and 5 produced large numbers of viable seeds. The commercial cultivars also were sampled and they produced less seeds than HRS 4 and 5 but did produce more than when used as males in the crosses with HRS. Combined these data suggest either sub-optimal conditions or timing of the crosses between the HRS and commercial cultivars or some form of incompatibility. The results do indicate however that at least some of the HRS can act as a pollen acceptor from commercial cane.

Table 2 Amount of seed harvested and number of seeds germinating per g of fuzz at 36°C. Inflorescences were collected from *S. spontaneum* plants growing in the river (HRS) or in parental plots for the commercial cultivars.

Genotype	Harvest (30 May 2008)		Harvest (02/03 July 2008)	
	Seed harvested (g)	Germination per g (SE, n=3)	Seed harvested (g)	Germination per g (SE, n=2)
HRS 1	4.10	4.8 (8.25)	9.84	0
HRS 2	4.20	22.2 (9.62)	2.51	78.8 (45.7)
HRS 3	5.20	0	1.80	141.4 (17.5)
HRS 4	2.00	866.7 (354.7)	0.51	212.1 (171.4)
HRS 5	13.7	221.1 (51.7)	7.9	65.6 (28.5)
HRS 6	14.0	33.2 (14.4)	12.2	0
Q162	25.3	26.7 (5.8)	37.1	7.1 (0)
Mida[ɸ]	15.1	10.0 (17.3)	76.6	20.5 (14.8)
Q208[ɸ]	25.0	56.3 (36.9)	*	*

*Not available for sampling as the plants had been harvested

The implication of this experiment is that it is biologically possible for the S. spontaneum growing in the Herbert River to accept the pollen of commercial crops and produce fertile hybrids. Consequently if GM sugarcane was growing in fields close to the River, the GM trait could be unintentionally transferred to *S. spontaneum*. The likelihood of this happening under field conditions has not been assessed.

6.0 OUTPUTS

The papers published in international journals and conference proceedings are listed in section 10.

In addition there were several additional conference presentations not resulting in a paper and the corresponding abstracts are listed below.

1. Powell R, Olivares-Villegas JJ, Berding N, Schmidt S and Bonnett G 2008 The effect of temperature on germination of *Saccharum* seed. *Proceedings of the Australian Society of Sugar Cane Technologists*, 30, 588.

2. Olivares-Villegas JJ, Powell R, Berding N and Bonnett G Regional differences in the sexual reproductive biology of *Saccharum*. *Proceedings of the Australian Society of Sugar Cane Technologists*, 30, 589.

3. Olivares-Villegas JJ, Berding N, Morgan T, Powell RP and Bonnett GD 2008 Determining the hybridisation potential of sugarcane for the managed deployment of genetically-engineered cultivars. 10th International Society of Biosafety of Genetically Modified Organisms

4. Olivares-Villegas JJ, Powell RP, Berding N, Morgan T, Schmidt S, Bonnett GD 2008. Determining the limits of seed germination of *Saccharum* for the managed deployment of genetically-modified sugarcane. 10th International Society of Biosafety of Genetically Modified Organisms

5. Sexual reproduction of sugarcane in commercial fields: understanding for the development of GM sugarcane. Olivares-Villegas JJ, Berding N, Morgan T, Bonnett GD. Tropical Crop Biotechnology Conference South Africa, July 2009.

6. Olivares-Villegas JJ, Berding N, Morgan T, Bonnett G. Potential sugarcane inter-varietal gene flow: Research for the regulated deployment of genetically modified sugarcane. Genetically Modified Crops Co-existence conference, Melbourne, November 2009.

A summary of the knowledge gained is presented in section 6.

7.0 INTELLECTUAL PROPERTY:

No protected IP has been generated by the project.

From the outset of the project it was agreed that publication of the findings was an important path to impact for the research. This is primarily because to get the findings used by regulators, publication after peer review allows the data to be considered with more weight when being used as part of the decision making process. Consequently, whilst there have been many new findings these have not been considered for protection via patent or trade secret.

1. Defining the need and scope for a project to investigate the sexual reproduction of sugarcane under commercial conditions and its implications for GM sugarcane (paper 1a).

2. Review and collation of the published information providing evidence of crossing possibilities between commercial sugarcane and other species. (paper 1).

3. Identification of locations where *Saccharum spontaneum* is growing in Queensland (paper 1).

4. Knowledge of flowering patterns of commercial sugarcane and *Saccharum spontaneum* (paper 3).

5. Quantification of viable seed production by commercial sugarcane and *Saccharum spontaneum* (papers 3 and 6).

6. Response of germination of commercial sugarcane seeds to temperature and water and *Saccharum spontaneum* to temperature and water (papers 4, 5 and 7) and consequently traits that if altered could increase the weediness potential of *S. spontaneum* in Australia (paper 7).

7. Identification of the features of *Saccharum spontaneum* that make it an invasive weed in Panama (papers 4, 5 and 7) and consequently traits that if altered could increase the weediness potential of sugarcane or *S. spontaneum* in Australia (paper 7).

External sources of money have been contributed to the project.

1. Biotechnology Australia funded the survey of the Mulgrave River by boat in 2006.
2. Queensland Government funded a Queensland\Smithsonian Fellowship to G Bonnett to undertake the study of *Saccharum spontaneum* in Panama in 2009.
3. The Sugar Research and Development Corporation (SRDC) contributed funding for the work in Panama as part of a travel and learning project awarded to G Bonnett.

These have been for specific activities that were approved by the CRC and have impacted positively upon the outcomes of the project and the dissemination of its findings. There has been good co-operation with all parties particularly around generating publicity for the work and its findings as a result of a press release in March 2010 that SRDC and the Queensland Government worked with CRC to produce. SRDC hosted a seminar about the project, in particular the work conducted in Panama on 21 May 2010.

8.0 ENVIRONMENTAL AND SOCIAL IMPACTS:

From the project:

There should be no significant detrimental environmental impacts as a result of conducting the project. Whilst inflorescences and seed were removed from farmers' fields and the wider environment this is not expected to have an impact. Any reduction of *S. spontaneum* in the environment as a result of the project would probably be considered an environmental benefit as the species is not indigenous. Care has been taken not to spread *S. spontaneum* beyond its existing range.

Seed was placed on the ground in some environments. This could however, happen naturally as the seed is wind blown and abundantly produced (even though it may not be viable). However, no experiments were successful in germinating either seed from commercial sugarcane or *S. spontaneum in situ*.

A societal benefit has accrued through the capability built within the project that did not previously exist associated with the Australian Sugar Industry. This has also led to a new level of engagement between the sugar industry and regulatory authorities, adding to the reputation of the industry as a safe custodian of GM cultivars. Both the capability and reputation will be further developed through the execution of new projects. One SRDC project focussing on the information required by FASANZ in relation to food uses of sucrose from GM sugarcane will shortly be underway.

From implementing its findings:

As the projects findings are implemented by regulators in their decision making process about the release of GM sugarcane, the environment should not be harmed as the aim of the project was to highlight potential environmental hazards for assessment. Similarly any environmental benefits and societal benefits from reduced or more benign herbicides or improved productivity that may have been delayed whilst the basic information was collected by the project may now accrue earlier.

9.0 EXPECTED OUTCOMES

A key outcome planned for the project was that the data generated would be discussed with and placed in the hands of those responsible for the regulation of GM sugarcane in Australia. The OGTR, DEHWA, APVMA, DEEDI were consulted before the project started and were made aware of the existence of the proposed project and its aims. The OGTR and DEHWA have been participants in the consultative committee that have met on three occasions in Cairns, Ingham and Murwillumbah.

Towards the end of the project a meeting was held at OGTR (Canberra), where three staff of OGTR, four from DEWHA, two from FSANZ and four from APVMA were in attendance. The project findings were presented and discussed.

Evidence that OGTR in particular is aware of the work and its outputs can be found (i) in Table 3 where the papers published early in the project have been cited in appraisals and risk management plans (ii) in direct interactions with OGTR (Louise Matthews in May 2009, where they were after unpublished information to assist their process) (iii) by request to assist by commenting on The Biology of Sugarcane document being prepared by OGTR (Dr Heidi Mitchell) for the OECD.

Table 3 Analysis of citations via Google Scholar (15 May 2010) for papers published to date in the project.

Paper	Number of citations
Bonnett G.D, Nowak E, Olivares-Villegas J.J, Berding N, Morgan T, Aitken K.S. **2008**. Identifying the risks of transgene escape from sugarcane crops to related species, with particular reference to *Saccharum spontaneum* in Australia. Tropical Plant Biology **1**:58-71	5*
Bonnett GD, Berding N, Morgan T, Fitzgerald P. **2007**. Implementation of genetically modified sugarcane – the need for a better understanding of sexual reproduction. *Proceedings of the Australian Society of Sugar Cane Technologists*. **29**:258-266	3#

* 3 by OGTR
# All 3 by OGTR

Industry has been made aware of the work and its findings through (i) talks at ASSCT, and papers in the proceedings in 2007 and 2010, (ii) SRDC seminar 21 May 2010, (iii) addressing Sugarcane Gene Technology Group on several occasions including a summary of the projects findings on June 2 2010, (iv) CRC Biotechnology workshops to growers and millers and extension staff 2008, 2009.

Technology developers and the broader scientific community will continue to be made aware of the work through presentations (ISSCT, Mexico, March 2010, 11th International Biosafety of Genetically Modified Organisms conference, Argentina, November 2010) and journal publications, more of which will be published shortly (see appendix).

10.0 FUTURE NEEDS AND RECOMMENDATIONS

As a result of the project there has been a significant advance in the production of baseline data describing the sexual reproduction of sugarcane and its relative *S. spontaneum* in Australian environments.

There are several pieces of the biological puzzle however, which have not been fully addressed by the project or have arisen from the results obtained.

- The result that *S. spontaneum* growing in the Herbert River can act as an acceptor of pollen from commercial cultivars in controlled experimentation, begs further questions, does this happen naturally and if so how frequently? This has yet to be addressed and could be tested using similar techniques employed in the project to demonstrate the presence of DNA from the pollen parent in progeny. Further we have not demonstrated that the hybrids produced are themselves fertile and competitive in the region. Pan et al. (2004) did show that crosses between *S. spontaneum* as the female and elite clones produced progeny that were themselves fertile in F1 and both BC1 and BC2 generations. There were a few inflorescences of *S. spontaneum* produced whilst commercial cultivars were in flower in the Mulgrave region. S. spontaneum is clonal and rarely produced seed in this region. An experiment to test the potential of this S. spontaneum clone to accept pollen from commercial cultivars could, depending on the results could eliminate the possibility.

- A related question arises in relation to the *S. spontaneum* population in the Herbert River. Is it possible to eliminate this population and consequently the hazard? This will be discussed further with the SGTG group in early June.

- The project has clearly shown that the conditions for germination of seed arising from commercial sugarcane and *S. spontaneum* are not favourable at the time of production. There are no observations of germination occurring in the wet season (January-April) following seed production (in the previous May-July) but definitive studies on dormancy and seed survival through the winter into summer would highlight this if carry over of viable seed occurs.

- Along with dormancy, there have been no definitive studies on the role of light in the germination of sugarcane or *S. spontaneum* seed.

- Sugarcane seedlings have been observed to germinate occasionally in the field. However, they have never been followed to determine if they establish mature plants.

- Pollen flow has dominated discussions of transgene dispersal from many GM crops. In sugarcane, wind dispersal of seed may be a more important avenue of dispersal of a transgene. This has not been studied.

- Whilst the project concentrated on several areas in northern Queensland, and there was a smaller effort in other more southerly regions, these latter regions were not covered comprehensively. In addition some regions (e.g. Atherton Tablelands, Mossman) were not investigated at all. The likely responses may be able to be predicted from modelling or data could be gathered when trials were conducted in these areas.

- The data collected to date would appear to show that for sugarcane the release of traits such as those already approved in Australia for cotton and canola (herbicide resistance and insect resistance) would not add any additional issues on top of those addressed for the approved crops. However, there are two types of transgene that closer scrutiny and further research would be required for.

  - Firstly there are several traits under development aimed at improving performance under abiotic stress. It is not obvious how competitive sugarcane would be outside of cultivation with these traits or how their presence in seeds may affect the ability to germinate and produce new plants. Investigating ways of testing this for sugarcane may be required in the near future.

  - For plants that are producing products not designed for food (plastics/enzymes) the presence in the environment is likely to undergo more scrutiny. Strategies for gene containment may have to be more fully developed for these traits.

The knowledge, understanding and contacts made with the regulatory process and the agencies involved in regulating GM technologies as a result of conducting this project has led to a new project post-CRC. The aim of this project is to provide the baseline information required by the Australian regulator responsible for the food uses of GM plants (Food Standards Australia and New Zealand, FSANZ).

11.0 PUBLICATIONS ARISING FROM THE PROJECT

Journal papers

1. Bonnett G.D, Nowak E, Olivares-Villegas J.J, Berding N, Morgan T, Aitken K.S. 2008. Identifying the risks of transgene escape from sugarcane crops to related species, with particular reference to *Saccharum spontaneum* in Australia. *Tropical Plant Biology* 1 58-71.

2. Powell RP, Olivares-Villegas JJ, Berding N, Schmidt S, Bonnett GD. Germination of *Saccharum* seed: Response to temperature and viability over time.

3. Olivares-Villegas JJ, Bonnett GD, Berding N, Morgan T. Sexual reproductive biology of sugarcane (*Saccharum* spp. Hybrids) and of a sexually compatible species, *Saccharum spontaneum* L., in Australia.

4. Bonnett GD, Saltonstall K. Seed production and germination contributes to the invasiveness of *Saccharum spontaneum* in Panama.

5. Bonnett GD, Saltonstall K. Why is *Saccharum spontaneum* the dominant introduced grass in Central Panama?

6. Olivares-Villegas, Bonnett GD, Berding N, Li JC, Perroux J. Sexual hybridisation of commercial sugarcane (*Saccharum* spp. Hybrids) in cultivated settings.

7. Bonnett GD, Olivares-Villegas JJ, Letondor C, Saltonstall K. Comparison of *Saccharum spontaneum* in Panama and in northern Australia.

<u>ASSCT and ISSCT papers</u>

1a Bonnett GD, Berding N, Morgan T, Fitzgerald P. 2007. Implementation of genetically modified sugarcane – the need for a better understanding of sexual reproduction. *Proceedings of the Australian Society of Sugar Cane Technologists*. 29 258-266.

2a Bonnett GD, Olivares-Villegas JJ, Berding N, Morgan T 2010. Sugarcane sexual reproduction in a commercial environment: Research to underpin regulatory decisions for Genetically Modified sugarcane. *Proceedings of the Australian Society of Sugar Cane Technologists* 32 1-9.

3a Olivares-Villegas JJ, Berding N, Morgan T, Bonnett GD 2010. A support framework for deployment of genetically modified sugarcane: identifying potential risks from sexual reproduction of commercial cultivars. *Proceedings of the International Society of Sugar Cane Technologists* 27 CD.

<u>SRDC Special Report</u>

1b Fitzgerald P and Bonnett G. 2007. Research Strategies for future Farming Systems – GM sugarcane. In *Research and Development Strategies to Advance the Australian Sugarcane Industry*. SRDC Technical Report 1/2007. pp 94-115. SRDC, Brisbane.

<u>Book chapter</u>

1c Bonnett GD , Henry RJ. *Saccharum*. In Wealth of Wild Species: Role in Plant Genome Elucidation and Improvement Volume 8: Wild Relatives of Industrial Crops. C. Kole ed. Springer. In press.

Media coverage

As a result of a CRC press release (23 March 2010) detailing the work in Panama, the following media coverage of the story has been detailed in Table 4.

Table 4 Medium and date of publication/broadcasting of stores from CRC Press Release dated 23 March 2010

Medium	Date
Gympie Times	25 March 2010
News Mail, Bundaberg	25 March 2010
ABC Radio Rural Report, Cairns	29 March 2010
ABC Radio Queensland country hour (Far North, Capricornia, North Queensland, North West Queensland, Tropical north, Southern Qld and Wide Bay).	29 March 2010
SRDC news	30 April 2010

North Queensland Register	5 May 2010
Australian Canegrower	17 May 2010
Australian Sugarcane April-May Vol 14 No 2 p4	May 2010
Australasian Science	Drafted May 2010 for July 2010 publication.

12.0 ACKNOWLEDGMENTS

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14.0 APPENDIX 1

Publications, completed and in draft.

A list of publications is displayed in the left hand column. The Cover Sheet button directly above this column will return you to the Annual Report with a single click. A navigation bar containing miniature versions of each page (of the currently selected document) can be turned on by pressing F4 or clicking on a publication in the list.

(01) Bonnett G.D, Nowak E, Olivares-Villegas J.J, Berding N, Morgan T, Aitken K.S. 2008. Identifying the risks of transgene escape from sugarcane crops to related species, with particular reference to *Saccharum spontaneum* in Australia. *Tropical Plant Biology* 1 58-71.

(02) Powell RP, Olivares-Villegas JJ, Berding N, Schmidt S, Bonnett GD. Germination of *Saccharum* seed: Response to temperature and viability over time.

(03) Olivares-Villegas JJ, Bonnett GD, Berding N, Morgan T. Sexual reproductive biology of sugarcane (*Saccharum* spp. Hybrids) and of a sexually compatible species, *Saccharum spontaneum* L., in Australia.

(04) Bonnett GD, Saltonstall K. Seed production and germination contributes to the invasiveness of *Saccharum spontaneum* in Panama.

(05) Bonnett GD, Saltonstall K. Why is *Saccharum spontaneum* the dominant introduced grass in Central Panama?

(06) Olivares-Villegas, Bonnett GD. Berding N, Li JC, Perroux J, Sexual hybridisation of commercial sugarcane (*Saccharum* spp. Hybrids) in cultivated settings.

(07) Bonnett GD, Olivares-Villegas JJ, Letonder C, Saltonstall K. Comparison of *Saccharum spontaneum* in Panama and in northern Australia.

(08) Bonnett GD, Berding N, Morgan T, Fitzgerald P. 2007. Implementation of genetically modified sugarcane – the need for a better understanding of sexual reproduction. *Proceedings of the Australian Society of Sugar Cane Technologists*. 29 258-266.

(09) Bonnett GD, Olivares-Villegas JJ, Berding N, Morgan T 2010. Sugarcane sexual reproduction in a commercial environment: Research to underpin regulatory decisions for Genetically Modified sugarcane. *Proceedings of the Australian Society of Sugar Cane Technologists* 32 1-9.

(10) Olivares-Villegas JJ, Berding N, Morgan T, Bonnett GD 2010. A support framework for deployment of genetically modified sugarcane: identifying potential risks from sexual reproduction of commercial cultivars. *Proceedings of the International Society of Sugar Cane Technologists* 27 CD.

(11) Fitzgerald P and Bonnett G. 2007. Research Strategies for future Farming Systems – GM sugarcane. In *Research and Development Strategies to*

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