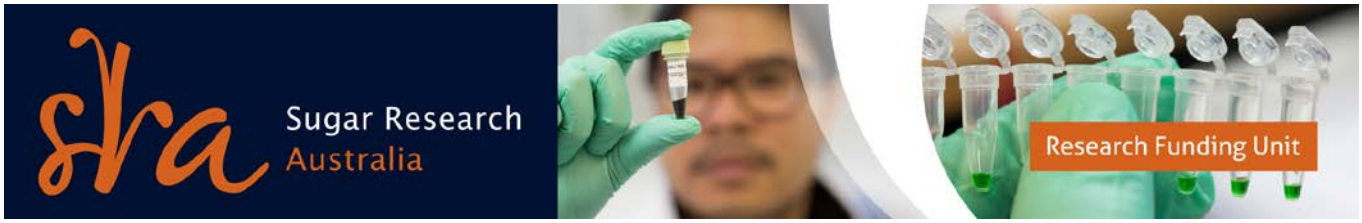

SRA Research Project Final Report

SRA Project Code	**CPI022**		
Project Title	Seed dormancy and establishment: a critical gap in knowledge to support safe deployment of GM		
Key Focus Area in SRA Strategic Plan	Optimally-adapted varieties, plant breeding and release		
Research Organisation(s)	CSIRO		
Chief Investigator(s)	Graham Bonnett		
Project Objectives	To complete the development of the baseline understanding of reproduction of sugarcane in commercial fields that will support regulation of GM cultivars. Specifically to: • Determine if sugarcane seed exhibits dormancy and if so which type. • Determine how long sugarcane seed can remain viable under field conditions. • Determine the abiotic limitations to sugarcane germination and establishment. • Design assays that can be used in the assessment of future proposed GM cultivars to determine if the limits of germination and establishment have been altered.		
Milestone Number	Final report		
Milestone Due Date	01/05/2015	**Date submitted**	
Reason for delay (if relevant)			
Milestone Payment	$40,000		
Milestone Title	Final Report		
Success in achieving the objectives	☒ Completely Achieved ☐ Partially Achieved ☐ Not Achieved		

SRA measures of success for Key Focus Area (from SRA Strategic Plan)	3 varieties which meet the above expectations released per 5 –year period for each region. Percent production from new varieties (<7 years since release) Rate of genetic gain (tonnes of cane per hectare (TCH), commercial cane (CCS), tonnes of sugar per hectare (TSH)). Weighted average disease ratings for varieties in each region.

Section 1: Executive Summary

The development and adoption of new varieties is a major investment in the Australian sugar industry. There are several modern techniques now being introduced into sugarcane breeding, such as introgression of *Erianthus* and the use of molecular markers. As these techniques contribute to better performing varieties these benefits can be readily captured through normal paths to market that have been established for improved varieties. Genetic modification (GM) is providing another method to introduce traits into crop plants. There is an increasing number of countries deploying a large number of GM varieties. This however, requires a different path to market involving regulatory systems that determine if there are potential harms that could result and what the likelihood of this may be. In Australia, environmental harm is assessed by the Office of the Gene Technology Regulator (OGTR). Some of the key considerations in determining the path to harms, in general, are an understanding of the reproductive biology (both sexual and asexual) of the crop. For sugarcane, a crop grown from pieces of stalk to harvest more stalks, the asexual propagation in farmer's fields is relatively well understood. Sexual reproduction, however, has only been the concern of breeders and is not part of the productive cycle in farmer's fields.

In a previous project we have documented that flowering of sugarcane is widespread, though the Australian industry with a greater concentration from the Burdekin to Mossman. Within this area, viable pollen and seed production was found to be most significant from the Herbert to Mossman regions. Additional experiments demonstrated the potential for pollination and hence gene flow between fields and the presence of populations of a compatible wild relative, *S. spontaneum*. It was apparent that seed production occurred at a time not optional for seed germination. Consequently the current project has conducted studies to determine the likely fate in the environment of the sugarcane seeds and relate this to potential paths to harm, so that all future GM sugarcanes can be evaluated against this baseline.

In this project we have used a combination of field and laboratory based studies. A third part of the methodology has been the use of a consultative group to assess both results obtained and plans for the coming year. These annual meetings involved representatives of the end users of the research. Both regulators and sugar industry representatives were active participants in this and have ensured the most relevant studies were conducted and end user engagement throughout the project.

We have conducted extensive field work (i) to collect sugarcane seeds from farmer fields (ii) to assess the longevity of sugarcane seeds under field conditions and (iii) assess the likely competition between sugarcane seeds and weeds in the soil seed bank in and around sugarcane field. These studies were complemented by laboratory experiments that determined the abiotic limits to germination, the level of dormancy and the composition of sugarcane seeds.

We demonstrated that in farmer's fields in the most northerly areas, when sugarcane flowers, it has a low fertility and, the seeds that are produced are short-lived and rare in the soil seed bank in and around sugarcane fields. We established that sugarcane seeds do not display dormancy and germination could occur at relatively low temperatures (Tb=11°C - 16°C) with a base water potential of germination ranging from -1.1 MPa to -1.5 MPa. This means that, at least in Australia the major impediment to seed germination during the flowering period is the water availability. Significantly we showed that the number of weed seeds compared to sugarcane seeds in the seedbank was orders of magnitude higher, hence competition from weeds greatly limits

sugarcane seedling establishment. Interestingly sugarcane seeds contain relatively high levels of lipid material compared to many other grasses. A link between composition and seed longevity was not established.

In conclusion, we demonstrated that, in the Australian environment, the likelihood of sugarcane seeds establishing in the Australian environment is very low. Additionally as a result of the studies we have now developed several techniques that can be used to compare any changes in the response of the processes that comprise sugarcane sexual reproduction as a result of GM changes. When appropriate (for example when deliberate changes to abiotic stress tolerance have been engineered) these can be used to determine any biologically meaningful alterations. The results and implications of the project have been published in International Journals, discussed at ASSCT, discussed directly with regulators and have and will contribute to reference publications (biology documents) used by regulators world-wide.

Section 2: Background

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During the 2000s there was an increase in the interest for developing GM sugarcane for commercial use, including in Australia (Bonnett et al., 2007). Whilst there was significant investment into the development of sugarcane with various GM traits, there was not the same interest in the basic understanding of the biology of sugarcane that would be needed in order for assessment of these proposed cultivars by regulatory authorities. There were significant gaps in the available information, particularly relating to sugarcane sexual reproduction in fields where it was grown. This was important because the paths to harm that would be assessed by regulators included weediness potential in either sugarcane itself or any sexually compatible species growing in the environment. The reason that this information was not available was that unlike seed crops, sugarcane is grown from vegetative pieces to grow and harvest more vegetative pieces (stalks). Consequently there was no role for sexual reproduction in the field and therefore no need to understand the magnitude and its success or otherwise.

The available data was assembled into a biology document by the OGTR initially in 2004. This contained many assumptions and interpretation of data collected primarily from research conducted within breeding programs where flowering and seed production are key processes. In a precursor project we made significant progress in understanding some of the key issues. For example the level of flowering, pollen and seed production in the different regions was assessed (Bonnett et al 2010). The range of species sugarcane has been shown to be sexually compatible with was reviewed and the presence of them in Australia documented (Bonnett et al., 2008). *Saccharum spontaneum* was identified as the most likely species to cross with commercially grown sugarcane in Australia. We therefore conducted studies of *S. spontaneum* in Panama, where it has become a very significant weed. We demonstrated that it was spreading by seed (Bonnett et al., 2014) and that it was not controlled by fire, which in fact probably assists it to remain dominant (Saltonstall and Bonnett 2012).

As information has been generated it has been discussed with the Office of the Gene technology regulator (OGTR). This has led to changes in *The biology of the Saccharum spp (sugarcane)* produced as a reference work by the OGTR and an involvement in the preparation of the equivalent international document produced by the OECD. Some of the work has been recognised in attempts by other nations to draw together the relevant biology of sugarcane in their environments (Cheavgatti-Gianotto et al., 2011).

Both sugarcane and at least one significant population of *S. spontaneum* in Australia produced seed but did appear to be spreading and becoming invasive unlike Panama. Our hypothesis was that the conditions in Panama at the time of seed production were more conducive for seed germination and establishment than

in Australia. When discussing these results it was apparent that an unresolved question was what was the fate of the seeds produced in the Australian environment and could they persist until more favourable conditions during the wet season arrived. Consequently the need for this project was to explore the nature of sugarcane dormancy and longevity, understand the abiotic limits to germination and evaluate the presence in the soil seed bank. Once this information is documented the additional task was to determine if there were relatively simple assays that could be used to determine if those parameters were altered as a result of a GM change. This will become more important as traits that deliberately change the abiotic stress response are introduced as has been the case for a recently approved sugarcane cultivar in Indonesia (Waltz, 2014).

There are still field trials of genetically modified sugarcane underway in Australia. For the assessment of these trials and future releases this body of work is and will be used and has helped inform the regulators about the industry and provided a greater understanding of the relevant biology of sugarcane.

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.

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.

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.

Bonnett GD, Kushner JNS, Saltonstall K. 2014. The reproductive biology of *Saccharum spontaneum* L.: implications for management of this invasive weed in Panama. *Neobiota* 20 61-79.

Saltonstall K, Bonnett GD. 2012. Fire promotes growth and reproduction of *Saccharum spontaneum* (L.) in Panama. *Biological Invasions,* 14 2479-2488.

Cheavegatti-Gianotto A, De Abreu H M C, Arruda P, Bespalhok Filho J C, Burnquist W L, Creste S, Di Ciero L, Ferro J A, De Oliveira Figueira A V, De Sousa Filgueiras T, Grossi-De-Sá M D F, Guzzo E C, Hoffmann H P, De Andrade Landell M G, Macedo N, Matsuoka S, De Castro Reinach F, Romano E, Da Silva W J, De Castro Silva Filho M, César Ulian E (2011) Sugarcane *(Saccharum x Officinarum*): a reference study for the regulation of genetically modified cultivars in Brazil. *Tropical Plant Biology* **4**, 62-89.

Waltz E (2014) Beating the heat. *Nature Biotechnology* **32**, 610-613.

Section 3: Outputs and Achievement of Project Objectives

Project objectives, methodology, results and discussion

All objectives from the project have been met. In addition the involvement of three students has allowed us to explore related issues and helped provide a broader picture of the biology of sugarcane as it relates to the regulation of GM cultivars.

The students were:

(i) Gabriela Siqueira from Brazil who spent 12 months studying the composition of sugarcane seed. This has resulted in a publication currently under revision in response to referees comments. This is the first report of the make-up of sugarcane seeds.

(ii) Pauline Berger from France who spent three months working on understanding the diversity and level of cross pollination of the population of *S. spontaneum* by the Herbert River. This work was part of the paper written for the 2015 ASSCT proceedings.

(iii) Farheen Bhatti from Pakistan. Farheen has been conducting a PhD on various assays to characterize a GM sugarcane variety that would be needed to support a regulatory application. In Australia Farheen

has been investigating the limits to sugarcane sprouting from buds to determine how altered weediness potential could be characterized.

In this section each of the objectives (and additional findings) are summarized as full details of the methods and results can be found in the papers in Appendix 1.

Section 3 – Objective 1

Determine if sugarcane seeds exhibit dormancy and if so which type.

The production of viable sugarcane seeds from Ingham to Mossman occurs at a time of the year that is rarely conducive to germination. Seeds are produced at a time of the year (winter) when rainfall is low, leading to low soil moisture content, and temperatures are below the optimum for germination.

What was unknown was whether seeds could either remain dormant and germinate during the wetter (summer) months or survive in a soil seed bank and remain viable until better conditions for germination arise.

With respect to investigating dormancy a detailed description can be found in Pierre et al (2015) appended. Briefly seed was collected from numerous commercial fields over two years. Individual seeds were separated from their glumes. Replicate samples of each seed source were either germinated or cut in half and stained with 1% tetrazolium solution as a measure of viability. The number of seeds that germinated was compared to the number assessed as being viable. If more seeds were deemed viable than germinated, the difference could be due to viable seeds not being able to germinate because they were dormant. In none of the samples was the number of viable seeds significantly higher than the number of germinated seeds, disproving the hypothesis that primary dormancy is present in sugarcane seeds

Section 3 – Objective 2

Determine how long sugarcane seed can remain viable under field conditions.

Whilst we demonstrated that sugarcane seed showed no dormancy we still needed to know how long seed could remain viable in the environment. To this end we have conducted a series of experiments with an artificial seed bank and with the soil seed bank in sugarcane fields. The results of these experiments are presented in full in Pierre et al. (2015) included in Appendix 1.

In summary we buried seed in nylon bags at several sites and depths in two seasons and recovered them at monthly time intervals. The figure below summarizes the results of all the experiments and shows that half-life of the seeds was 1.5 – 2.5 months. When looking specifically at the influence of depth on seed decay, we demonstrated that no seeds lived longer than six months or nine months when buried at 5-10 cm or 30 cm, respectively.

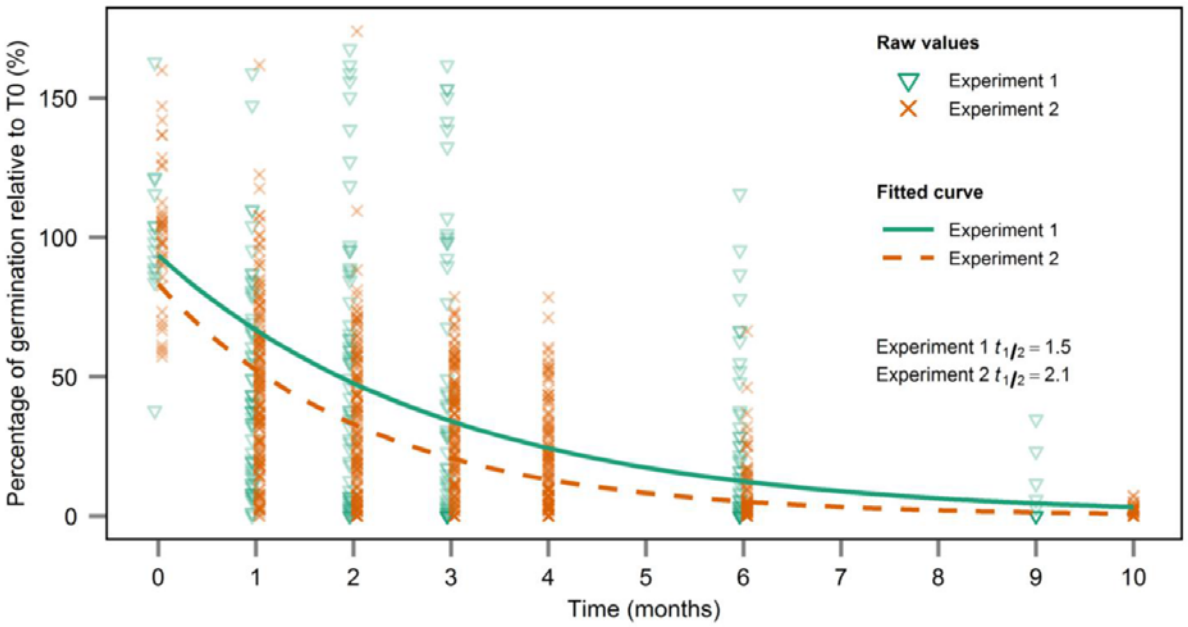

Fig 4. Estimation of seed longevity half-life based on the exponential decay of seed viability. The decay constant was estimated by fitting a nonlinear model and seed longevity half-life was derived by dividing ln(2) by the decay constant.

An analysis of the natural seed bank at two sites over five months was conducted by germinating the seeds in soil samples taken from the field. Germination under ideal conditions in a glasshouse resulted in 12067 seedlings that were classified into 13 families and 40 species. Only eight sugarcane seedlings were germinated, and these were confined to the samples taken in the two months after seed set. Sugarcane seed contributes to a very small proportion of the soil seed bank in sugarcane fields. Furthermore, the lack of viable sugarcane seeds in the samples harvested later corroborates the findings of the artificial seed bank studies that sugarcane seed is short-lived.

Section 3 – Objective 3

Determine the abiotic limitations to sugarcane germination and establishment.

The base temperature for sugarcane seed germination and the base water potential for germination has been established and published in Pierre et al (2014). The interpretation and context setting for these results was further elaborated in Pierre et al (2015a). Both of these publications are presented in full in the appendix.

Briefly, the base temperature for germination was estimated for seed from eight crosses by germinating seed at a range of temperatures. The base temperature was calculated in the range 11.2- 16.4˚C. An additional finding was that the optimum base temperature for seed germination was lower than previously reported and for most sources of seed was around 30˚C. This finding has been communicated to the Plant Breeder at Meringa, who successfully conducted experiments where they demonstrated a statistically significant higher rate of seed germination at 30°C compare to 36°C.]. The base water potential for germination was calculated in the range -1.1 -1.5 MPa. The combination of base temperature and optimal temperature was plotted on top of a range of values for other species (Pierre *et al.*, 2015).We showed that

while sugarcane has a higher base temperature than the other species, it is broadly consistent with the relationship stating that species with a higher base temperature for germination are more sensitive to lower water potential during germination.

A combination of the low rainfall during the winter, early spring and temperatures below 30˚C mean that the ability of sugarcane seed to germinate in North Queensland is well below optimal. Combined with short longevity this explains why germinating sugarcane seedlings are rarely seen and don't establish populations outside of cultivation.

More recently with a visiting student from Pakistan (Farheen Bahati) we have looked at what limits sprouting of sugarcane buds. One of the most likely GM changes that can be made to sugarcane that could plausibly increase weediness potential, and therefore a harm, is improved resistance to abiotic stress.

We have designed a system to test the response of sprouting to different water potential (a measure of water stress). The system consists of trays with holes in the base filled with perlite resting on a second tray without holes that acts as a reservoir. The trays are filled with one-eye setts and then subjected to either a water control or solutions with various water potentials that are generated by dissolving various amounts of PEG (polyethylene glycol). A demonstration of this technique is given in the figure below. There were five replicates at each level of water potential and two cultivars (Q242 and Q208) were used in the experiment. Each day, the number of buds that had sprouted was counted and on two occasions the length of the shoots was recorded. The experiment was performed in a glasshouse and the trays were weighed at the start of the experiment and again twice a day and the mass lost from evaporation of water was replaced each day to maintain the differences in water potential between the treatments.

The experiment is now being completed and so we have not yet performed the statistical analysis. However there are clear differences in the response of each cultivar. Cultivar Q242 sprouted within 3 days and there was much less difference between the treatments than for cultivar Q208. So for the initial sprouting of buds, Q242 was relatively insensitive to water potential, whereas for Q208 sprouting was reduced at all levels of PEG and at water potentials of -1.2 and -1.6 MPa. This experiment also gave some insights in the relative sensitivity of sugarcane sprouts to establish. The sprouts of Q242 started dying from day 5 in the treatments containing PEG. However there was only a small loss from the -0.4 and -0.8 MPa treatments compared to the -1.2 and -1.6 MPa treatments, where there was much greater losses. Again Q208 was much more sensitive to PEG treatment than Q242. After six days there was death of sprouts at all treatments, the lower water potentials losing sprouts faster than -0.4 MPa such that after 13 days very few remained alive. We will use this data to calculate base temperature for sprouting.

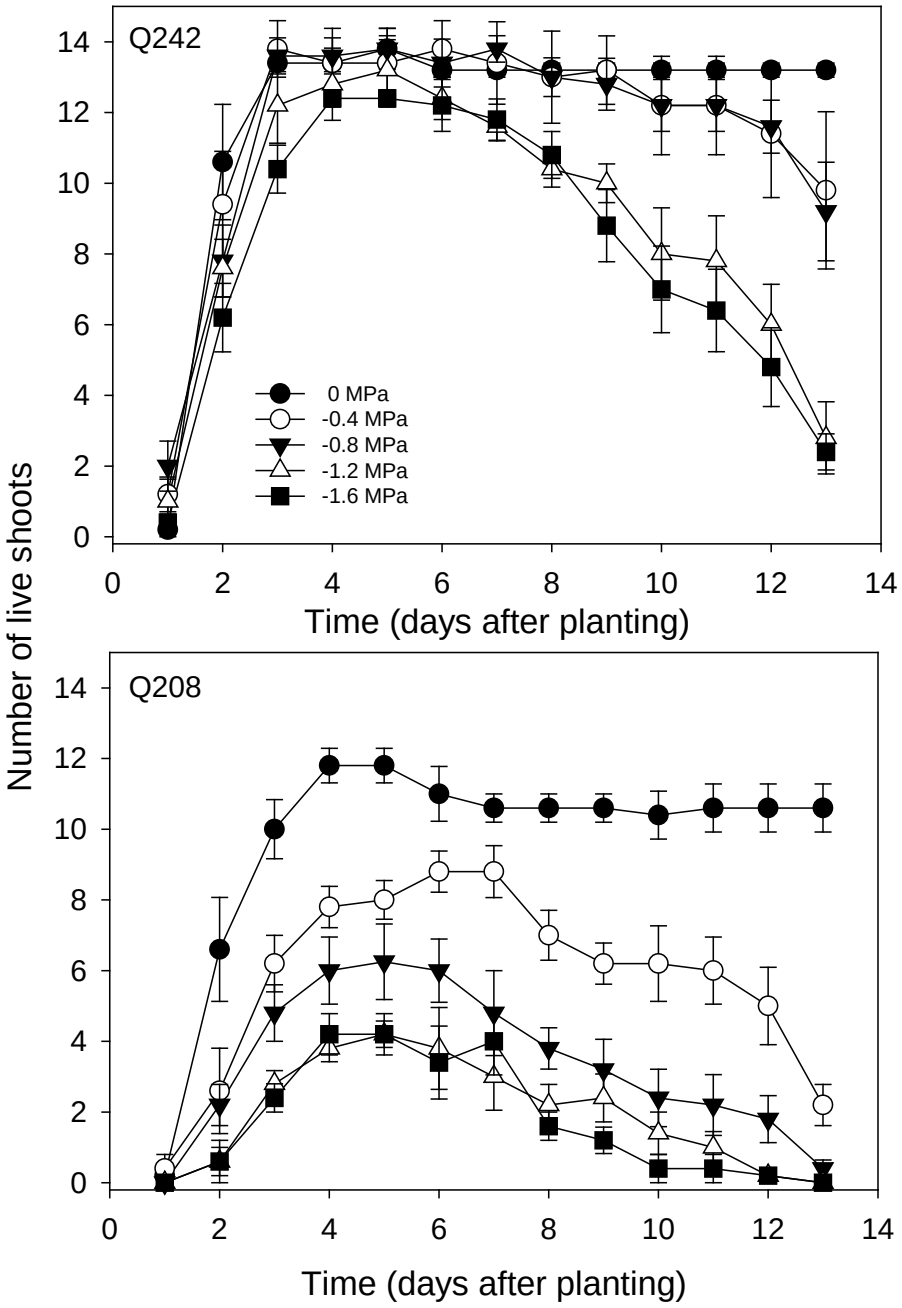

Figure legend. Time course of sprouting of two cultivars of sugarcane at various concentrations of PEG to generate different water potentials.

Section 3 – Objective 4

Design assays that can be used in the assessment of future proposed GM cultivars to determine if the limits of germination and establishment have been altered.

For sugarcane, traits that are designed to have an effect on abiotic tolerance (e.g., cold tolerance or drought tolerance) on crops could also have an impact on other processes that are limited by stress. In order to determine if the introduction of a particular transgenic has caused pleiotropic changes to important processes

then quick and easy assays need to be developed to test this. In particular the processes that might lead to increased potential for weediness should be considered. We have now determined the thermal and water-potential limits to sugarcane seed germination (Pierre et al 2014) and are working out the moisture limited germination of vegetative sets. For seed we have determined the base and optimum temperature for germination of seed from various sources. The methods described in Pierre et al (2014) have demonstrated that they can distinguish between cultivars and therefore could pick up changes, in seeds derived from GM cultivars. Table 1 summarises the assays that could be used to measure the impact of traits that affect potential for weediness.

Table 1, Phenotypes, consequence of change and method of assaying in GM cultivars to determine any change.

Parameter*	Consequence of change	Assay/test
Altered flowering time,	Earlier flowering may reduce chances of viable seed production and establishment. Delayed flowering may reduce chances of cold temp during development and decrease the time between flowering and suitable germination conditions.	Side by side comparison of GM cultivars and source cultivars (or equivalent). May have to use flag leaf initiation in field trials if flowering not allowed.
Increased seed production+	If more seeds are produced there is potentially an increase in weediness potential (particularly if other parameters are also changed)	Assay weighed aliquots of fuzz at 30˚C as per the standard assay in Pierre et al (2014). Compare GM to non-GM comparator. Will need to work out how to get seeds.
Changed limits of seed germination+	Potential to increase spread by increasing ability to germinate and establish in more places.	Assays described in Pierre et al (2014)
Additional findings New entry changed longevity	Potential to increase the proportion of seeds surviving until good conditions for germination arise.	Assay for following longevity in burial experiments described in Pierre et al (2015). Would use the 5-10 cm depth and follow monthly for six months to get quickest answer. Done under the breeding regulations.
Changed limits of bud sprouting+	Potential to increase spread by increasing ability to sprout and establish plants vegetatively in more places.	Assays described above in this section.
Altered competitiveness+	This could be a phenotype resulting from multiple possible changes.	Competition assay currently being developed – see above.

*Changes to these parameters do not necessarily mean that additional risk management is required, but assessment of any altered potential harm given the actual transgene incorporated.
+These assays would need to be conducted either under a field release license that allowed flowing for the purposes of collecting this data OR under management controls that allow crossing and seed production.

To summarise, we have met all of the objectives set for the work. In addition we have measured the composition of sugarcane seed and this is described in detail in the paper in the appendix (Siquerira et al., under review). The main finding from this work was the relatively high lipid contained in the seed compared to many grass crops. We could not, however, link this composition to the short half-life of sugarcane seed in the environment. A further study we conducted was to determine the diversity and parentage of the seeds of *Saccharum spontaneum* growing along the Herbert River. This work is presented in Pierre et al., (2015). Briefly we found that the individuals in the Herbert River are diverse and that most of the seeds were the product of hybridization i.e. they were not the result of self-fertilisation. This leaves the possibility open that there could be transfer of genes to *S. spontaneum* from a GM sugarcane in this area (see recommendation 3).

Section 4: Outputs and Outcomes

Key knowledge outputs

- Sugarcane seed exhibits no dormancy, is relatively short-lived, has a half-life in the environment of around two months, and is present transiently and at very low levels in the soil seed bank.
- Sugarcane seed has a base temperature for germination of between 12-16˚C, and a base water potential for germination of -1.1 to -1.5 MPa.
- Methods have been developed that could be used to determine if seed of sugarcane containing a transgene has phenotypic traits making it more likely to establish than the non-GM competitor.
- The major components of the sugarcane seed have been quantified.
- *Saccharum spontaneum* in the Herbert River, is diverse and cross pollinating.

Scientific written outputs

The scientific papers produced by the project are listed in section 9 and included in full in Appendix 1.

Written outputs to inform regulators and technology developers

In addition to scientific publications, this project has contributed both information and staff input to the development of reference works produced by the Office of the Gene Technology regulator. These works are used by technology developers in their applications and regulators in their assessments:

OECD (2013) Consensus document on the biology of sugarcane (*Saccharum spp*). http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282013%2922&doclanguage=en. Accessed 02 Sep 2014.

OGTR (2011) The biology of the *Saccharum spp* (sugarcane). http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/sugarcane-3/$FILE/biologysugarcane11.pdf. Accessed 02 Sep 2014.

An additional output from the project is information for the next update of the Biology document and an annotated document will provided to OGTR indicating where we think there is new information and therefore where updates should be made.

Through engagement with the project the skills of the OGTR in relation to sugarcane itself has been greatly increased. The engagement via the consultative group has then led to attendance at ASSCT and a much greater understanding of the context of sugarcane trials and production systems.

Section 5: Intellectual Property (IP) and Confidentiality

The aims of CPI022 were to provide information regarding sugarcane sexual reproduction to regulators and technology developers; therefore all our data are publicly available via peer-review journal publication, conference presentations and meeting with the industry and regulators.

Whilst we have not developed any protectable IP, we have looked to identify any other opportunities to exploit the findings arising from the work, for example we have worked with breeders at Meringa to test the use of lower germination temperatures for seed from crosses.

Section 6: Industry Communication and Adoption of Outputs

The key messages that have come from the project are:

1. Sugarcane seed exhibits no dormancy, is relatively short-lived, has a half-life in the environment of around two months, and is present transiently and at very low levels in the soil seed bank.
2. Sugarcane seed has a base temperature for germination of between 12-16˚C, and a base water potential for germination of -1.1 to -1.5 MPa.
3. Given 1+2, sugarcane seed in unlikely to be able to germinate and establish in North Queensland.
4. We have developed methods that could be used to determine if seed of sugarcane containing a transgene has phenotypic traits making it more likely to establish than the non-GM competitor.
5. The ability of *Saccharum spontaneum* and sugarcane in the Herbert region to cross pollinate needs to be explored if traits that can increase weediness potential as a by-product of increasing agronomic performance are developed.

These messages have been communicated to:

1. The consultative committee comprising SRA, OGTR, Department of Environment, Chair of Sugarcane Gene Technology Group (SGTG) annually at meetings to discuss results and plans for the next year.
2. The full office of the Office of the Gene Technology Regulator (and other regulators from FSLANZ and APVMA) at a seminar December 2014.
3. The chair of SGTG and CANEGROWERS Staff who were given a briefing in May 2014.
4. To groups of growers and consultants, at Advisor workshops arranged by the PEC unit in Mission Beach, Airlie Beach and Bundaberg in May 2014.
5. The audience of Australian Society of Sugar Cane Technologists annual conference via a paper, April 2015, Bundaberg.
6. The audience of regulators and researches of regulatory sciences at the meeting of the International Society Biosafety of Genetically Modified Organisms via a paper. Cape Town, South Africa, 2015.

a) What new information, if any, is available on the adoption of project outputs?

The latest draft risk assessment and management plan for DIR135_Limited and Controlled release of sugarcane with enhanced sucrose content, cites the papers published from this project. Evidence that regulators are using the work.
(http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir135/$FILE/Risk%20Assessment%20and%20Risk%20Management%20Plan%20(consultation%20version).pdf)

b) *List any newsletters, fact sheets or any other media coverage.*
We have not sought to publicise this work widely to the public.
However, we have the information that could contribute to fact sheets (frequently asked questions) if the industry was going through the process of releasing GM sugarcane cultivars.

c) *Identify any further opportunities to disseminate and promote project outputs at seminars, field days etc.*
The work has been communicated to several audiences. If the industry moves towards the growing of commercially released GM sugarcane there will be a need to further disseminate the findings to a broader audience.

Section 7: Environmental Impact

The experiments conducted and the recommendations that have arisen from the project are designed to avoid deleterious environmental impact. The results will be used to inform analysis of potential environmental harms that might arise from deployment of GM sugarcane. As well as information to inform this analysis, a series of protocols have been identified that could be used to determine if any changes have been made to a GM sugarcane that could lead to a harm. These tests are more likely to be needed when a trait that is targeting a deliberate change such as abiotic stress is being introduced.

Section 8: Recommendations and Future Industry Needs

We have used the information generated as a result of this project, its predecessor and any other information from around the world to work with the OGTR to determine (i) where the *The biology of the Saccharum spp (sugarcane)* document, should be updated in its next revision in the light of new information and (ii) to identify areas of the biology that are both potentially important from a regulatory perspective and where the data are insufficient, and further under what circumstances this will become important to know.

R1: Work with the OGTR to update *The biology of the Saccharum spp (sugarcane)* document. Identify the areas of most need for any additional information and what might trigger the need to acquire it.

As a result of both industry (through SRDC/SRA) and CSIRO investment we have now developed a significant depth of knowledge in matters related to the scientific support of the regulation of GM sugarcane in Australia (and more broadly). Consequently as new GM traits are developed, this expertise can be called upon to (i) advise on the types of studies that might be required to support regulatory decisions with respect to the environment and food and (ii) where required undertake these studies in an independent manner. This would become particularly valuable when traits beyond input management (herbicide/insect control) are developed as there have been few of these assessed and approved either in Australia or worldwide. In particular we have developed the thinking around trait's conferring an advantage in the face of abiotic stress.

R2: When industry is preparing to release a GM cultivar, the information generated as a result of these projects be used to help develop frequently asked question fact sheets to support the release.

R3: The expertise developed as part of this project be called upon to assist with unanswered or new questions related to the potential harms from GM sugarcane as new traits are evaluated. For example, if a trait was being investigated that as well as conferring an agronomic advantage could also potentially increase weediness

potential was being contemplated, it would then be time to consider further research. It may be necessary to determine whether there was pollen flow to the *Saccharum spontaneum* population in the Herbert River, and if this could increase the weediness potential of that species.

The process of using a consultative group through this and the previous project has proven very beneficial and a very good way of engaging end users. This approach should be encouraged in other projects that are interacting with stakeholders outside of the sugar industry.

Section 9: Publications

A list of Publications arising from the project is given below. The full text of the papers are contained in Appendix 1 and the conference abstracts in Appendix 2

Pierre, J. S., Bonnett, G. D., Berger, P., Aitken, K. S., Saltonstall, K., Rae AL (2015). Developing the baseline understanding of the sexual reproduction of sugarcane in farmer's fields. In R. C. Bruce (Ed.), *Proceedings of the Australian Society of Sugar Cane Technologists* (pp. 227–236). Mackay.

Pierre, J. S., Perroux, J., Whan, A., Rae, A. L., & Bonnett, G. D. (2015). Poor fertility, short longevity and low abundance in the soil seed bank limit volunteer sugarcane from seed. *Frontiers in Bioengineering and Biotechnology*. ***Under review***

Pierre, J. S., Rae, A. L., & Bonnett, G. D. (2014). Abiotic Limits for Germination of Sugarcane Seed in Relation to Environmental Spread. *Tropical Plant Biology*, *7*(3-4), 100–110. doi:10.1007/s12042-014-9141-9

Siqueira, G. F., Pierre, J. S., El Tahchy, A., Glassop, D., Singh, S., Bonnett, G. D., & Rae, A. L. (2015). Sugarcane seed composition and changes during artificial ageing. *Crop and Pasture Science*. ***Under review***

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