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FINAL REPORT – SRDC PROJECT BSS325

SMUTBUSTER: ACCELERATED BREEDING OF SMUT-RESISTANT SUGARCANE
VARIETIES

by

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EXECUTIVE SUMMARY

Sugarcane smut is caused by the fungus, *Ustilago scitaminea* Syd., and is one of the most serious diseases of sugarcane. At the end of 1983, only the sugar industries of Australia and Fiji remained free from smut. Sugarcane smut was reported for the first time in Australia in July 1998 in the Ord River Irrigation Area. Eight years later, smut was identified on the east coast of Australia at Childers. By December 2007 sugarcane smut was widespread and established in the Bundaberg–Isis, Central Queensland and Herbert River districts, and by 2010 the Mulgrave, Tully and Burdekin districts were also infested.

The average yield loss reported in papers at the time of the east coast smut incursion was 6% yield loss for each 10% increase in per cent-infected plants. To minimise losses susceptible varieties will need to be completely replaced with equivalent/higher yielding smut-resistant varieties, as fast as possible. This would necessitate changing ~80% of the 2006 sugarcane crop. Replacement of susceptible varieties will be achieved, not only by rapid scale-up of smut-resistant varieties, but also accelerated development of high yielding, smut-resistant varieties. The parental pool of high breeding value, smut-resistant germplasm was however severely limited, adversely impacting the core crossing program.

Without a significant plant breeding response, the rate of genetic gain for productivity would decrease and fewer productive, smut-resistant varieties would be released from the BSES-CSIRO Sugarcane Variety Improvement Program. The SmutBuster project was a key component of the RD&E response to sugarcane smut with the specific objective of developing high yielding smut-resistant varieties through the utilisation of high breeding value parental germplasm with susceptible reaction to smut.

The SmutBuster selection program runs parallel to the core selection program, effectively doubling the size of the selection population. SmutBuster will rapidly screen populations of ~30,000 seedlings derived from RxS, IxS and SxS crosses for smut resistance in Bundaberg each year for five years. Clonal screening of a subset of each seedling population, comprising 10,000 clones, will be conducted using traditional inoculation. Clones with no visible smut symptoms and acceptable appearance emanating from stage 1 screening trials will be planted into SmutBuster stage 2 trials in each of the four regions of the industry. Top selections from these trials will join the core program and enter Final Assessment Trials (FAT). Molecular markers and Near Infra-Red (NIR) indirect selection methods will be further researched for possible implementation. Research to develop an effective seedling screening method will also be conducted.

The major industry outcome from SmutBuster will be a wider choice of more productive smut-resistant varieties. As more productive varieties do not involve additional resource inputs and smut-resistant varieties necessitate less frequent plough-out, positive environmental and social benefits are expected to be large.

SmutBuster is also expected to have a positive impact on the rate of genetic gain for tonnes sugar/ha/year in the near future. From a breeding perspective, the shorter parent generation interval practised in SmutBuster will dramatically increase the frequency of smut resistant genes in the parent populations.

Presently marker strategies do not explain enough of the trait variation to warrant their implementation, whilst NIR methods are technically not feasible due to unreliability of the instrument and low predication accuracy. Smut screening research proved that dip inoculation of true seedlings four weeks after germination followed by inoculation of one 3-eye sett from selected clones was most effective at eliminating the majority of smut susceptible clones and was the method employed in the SmutBuster program from 2008–2010.

The SmutBuster selection program is presently only mid-way to completion and no commercial sugarcane varieties have been released within the time-frame of this project to date. The establishment of the first FATs that include material from the SmutBuster program (2006 seedlings) is scheduled for 2012. The first commercial varieties from SmutBuster are expected to be released to the industry in 2016. Commercial varieties emanating from this project in the future will be protected under the Plant Breeder's Rights Act 1994.

1.0   BACKGROUND

Sugarcane smut is caused by the fungus, *Ustilago scitaminea* Syd., and is one of the most serious diseases of sugarcane (Comstock, 2000). The disease was originally reported from Natal, South Africa in 1877 and has since become widespread in most sugarcane producing regions. By the end of 1983, only the sugar industries of Australia and Fiji remained free from smut (Ferreira & Comstock, 1989). Increasing concern in Australia about the potential for smut to enter prompted preparation of a contingency plan in 1997 (Croft and Magarey, 1997). BSES recognised the threat from smut and commenced screening of Australian varieties for resistance to smut in Indonesia in 1998 (Croft *et al.,* 2000).

Smut was reported for the first time in Australia in July 1998, identified in a field of NCo310 in the Ord River Irrigation Area. Wind borne spores from Indonesia have been suggested as the most likely path of entry (Riley *et al.,* 1999). Sugarcane smut was first identified on the east coast of Australia at Childers in June 2006 (Wilcox *et al.,* 2008). By December 2007, sugarcane smut was widespread and established in the Bundaberg–Isis, Central Queensland and Herbert River districts (Croft *et al.,* 2008a). Further smut spread was reported by Magarey *et al.* (2010a) for the more recently affected Mulgrave, Tully and Burdekin districts, based on industry reporting of new farms infested.

When sugarcane smut was found at Childers in June 2006, the sugarcane crop in the Bundaberg/Isis sugarcane Pest Quarantine Area (PQA5) comprised 76% smut-susceptible varieties, mostly Q188[b] and Q205[b] (Croft *et al.,* 2008a). The arrival of smut has necessitated replacing the majority of the sugarcane crop from smut-susceptible to smut-resistant varieties. In 2010, over 30% of the Herbert crop was still supplied by highly susceptible varieties (Magarey *et al.,* 2010b).

Although difficult to estimate, the yield loss due to smut can be severe in susceptible varieties and suitable conditions. Lee-Lovick (1978) reported losses of up to 73%. Bailey (1979) reported losses of 17 and 22% in the varieties NCo376 and NCo310 (respectively) in South Africa. When the Australian east coast smut incursion occurred, the average yield loss reported in papers that compared per cent yield loss with per cent infected plants was 6% yield loss for each 10% increase in per cent-infected plants (Croft *et al.,* 2008a). Based on this, together with the predicted spread of smut, Watson (2007) estimated total industry losses for the four regions to be $357-442M. Maximum yield losses of around 60% are consistent with later findings of Magarey *et al.* (2010b) in variety Q157 at Abergowrie.

To minimise losses susceptible varieties will need to be completely replaced with equivalent/higher yielding smut-resistant varieties, as fast as possible. Replacement of susceptible varieties will be achieved by rapid scale-up of current smut-resistant varieties and accelerated development of high yielding, smut-resistant varieties. Initial smut screening trials conducted in Indonesia from 1998 characterised 69% of Australian clones (parents and varieties for release) as susceptible (rating 7–9) to smut (Croft *et al.,* 2008b). Initially, not only was the portfolio of smut-resistant varieties for release limited, but also the parental pool of high breeding value, smut-resistant germplasm. Without a significant plant breeding response, the rate of genetic gain for productivity would decrease and fewer productive, smut-resistant varieties would be released from the BSES-CSIRO Sugarcane Variety Improvement Program.

The SmutBuster project (BSS325) is a key component of the RD&E response to sugarcane smut with the specific objective of developing high yielding smut-resistant varieties through the utilisation of high breeding value parental germplasm with susceptible reaction to smut.

2.0   OBJECTIVES

The overarching objective of SmutBuster is to:

- Undertake a sugarcane R&D program focused on the utilisation of the parental pool of high breeding value germplasm with susceptible reactions to smut to breed new, smut-resistant, varieties with high tonnes of sugar per hectare.

Specific objectives include:

- Accelerated development of high yielding smut resistance varieties for use by the Australian sugarcane industry.

- Increasing the frequency of smut resistance genes in parent and selection populations, gradually reducing the current need to double the size of the variety improvement program.

- Implementation of new, innovative and efficient selection methods in sugarcane breeding populations segregating for smut resistance, including the use of:

- Marker assisted selection;

- NIR prediction of smut resistance;

- Seedling screening methods.

- Continuously monitor and improve research progress towards developing smut resistant varieties.

Good progress has been made towards the accelerated development of high yielding smut resistant varieties for use by the Australian sugarcane industry. This includes:

- 2006 seedlings – plant crop harvest data from the 2010 harvest of the first Clonal Assessment Trials (CAT) with about 2,000 clones, planted in Bundaberg, Mackay, Burdekin and Meringa, were analysed to identify tentative selections for final stage trials. These tentative selections will be propagated along with the core program tentative selections in 2011. First ratoon crop data will be collected from the tentative selections in the CATs in 2011. Final selections for planting in the 2012 Final Assessment Trials (FAT) will be made on combined plant and first ratoon crop data.

- 2007 seedlings – about 1,650 clones were planted into CATs at Bundaberg and Mackay in 2010. Unfortunately, one part of the Bundaberg CAT that included 806 clones was abandoned because of poor germination. No trials were planted in the Burdekin, or at Meringa, due to the failure of the Charters Towers propagation plots in 2009. However, 100 of the best clones identified from the southern and central region were resent to Charters Towers in 2010 and these will be included in northern and Burdekin CATs in 2011.

- 2008 seedlings – based on visual appearance and lack of smut symptoms, about 2,000 clones were propagated in Bundaberg, Mackay and Charters Towers in 2010. Germination in these propagation plots has been good, despite initial concerns. No diseases to restrict seedcane movement from Charters Towers quarantine plot have been recorded. Seedcane collected from these propagation plots will be used to establish the 2011 SmutBuster CATs in all four regions.

- 2009 seedlings – based on visual appearance and lack of smut symptoms, about 8,950 clones were selected and planted as 3-eye setts on the Bundaberg Smut farm during October 2010. At the first survey in March 2011, 75% crosses had some smut, with an average smut infection rate of 9.03%. After the second smut survey in June 2011, about 2,500 clones will be selected to plant in propagation plots at Bundaberg, Mackay and Charters Towers.

▪ 2010 seedlings – about 29,500 dip inoculated seedlings were transplanted to the field in February 2010. Following slashing of the seedlings in November 2010, an average smut infection of 6.2 % was recorded in March 2011 in the first ratoon. The percentage smut recorded was similar to the plant crop survey (5.7%) completed in November 2010. This is substantially lower than recorded during the same period last year in the 2009 seedling population (21.2%). Further smut stools will be tagged in June 2011 prior to selecting and planting ~10,000 3-eye setts.

Marker and NIR methods have been tested. Presently marker strategies do not explain enough of the trait variation to warrant their implementation (although this may change with analysis of new data), whilst NIR methods are technically not feasible due to unreliability of the instrument and low predication accuracy of the model.

The dip method for smut inoculating seedlings is the most effective and has been adopted since 2008.

3.0 METHODOLOGY

3.1 Accelerated development of high yielding smut resistant varieties for use by the Australian sugarcane industry

The SmutBuster selection program runs parallel to the core selection program, effectively doubling the size of the selection population. Five crossing-selection series have been initiated as part of the SmutBuster program, commencing in 2006. Each series progresses for six years before merging with the core selection program at the FAT stage. The SmutBuster project culminates when the final SmutBuster series merges with the core program in 2016.

The focus of SmutBuster is to identify smut-resistant clones for use as parents and/or commercial varieties from crosses involving high breeding value, smut-susceptible parents. Seed from crosses between RxS, IxS and SxS parents was the starting point for each of the selection series. In total, 1861 crosses involving 872 parents were sown for inclusion in the five series. Of these parents, 363 (42%) were susceptible to smut. Seed was generally sown in November each year. After two days in the germination chamber at 36°C, the germinating trays were placed in polytunnel screenhouses. When seedlings were about 5 weeks old, they were inoculated by dipping seedlings in a smut spore suspension (10^6 spores/mL water) prior to potting into jiffy pots. The pots were incubated at 31°C overnight to facilitate smut infection and were then placed on outside benches.

The first two seedling populations were transplanted to the field in May/June. The latter seedling populations were transplanted to the field two months earlier in February or March. Logistically, this fitted the program better by freeing up the benches for routine smut screening, allowing more time for smut to develop and allowed the crop to be ratooned at an ideal stage for further smut transmission and better for crop growth. Over the five series, in excess of 134,000 seedlings have been established in stage 1 trials, the last four series located on the smut farm 10 km south of Bundaberg. Seedlings were planted in family replicated plots, the number of replications dependant on the number of seedlings in a family. A count of the number of seedlings with smut was completed in November/December of the same year as planting prior to the seedling population being ratooned (slashed). Inspections for smut (counts and tagging) in the ratoon crop were carried out in March and again in July the year following planting. Selection for second stage screening trials was done during October/November. Because of relatively small

numbers of clones showing smut symptoms (tagged), from the second series the number of clones selected for second stage screening was increased from the planned 5,000 to about 10,000 seedlings. Based on visual appearance about 40% of seedlings were selected within the seedlings showing no smut symptoms in each family plot. A 3-eye sett was cut from each selection, dip-inoculated in family groups in a smut spore suspension, incubated at 31°C overnight and then planted directly to the field in what was termed the 3-eye trial/stage.

To date only the first four series have reached the 3-eye stage. The fifth, and final series, will be planted into this stage in October/November 2011. The 3-eye trial design follows on from the seedling trial design; family replicated plots. Two smut inspections (counts and tagging) were carried out in the 3-eye trials, in March and again in June/July. During October, when the trial is about 11 months of age, about 2,000-2,500 clones were selected, based on good visual appearance and lack of smut symptoms. Stalks from the selections were harvested, sorted and distributed to Bundaberg (2), Mackay (2) and Charters Towers (4) for propagation.

The propagation plots are required to bulk-up sufficient material for planting CATs in all four regions. Clones are un-replicated in the propagation plots, and are propagated for one year. To date, three series have been planted in propagation plots, the last two series still to follow in 2011 and 2012. The Charters Towers propagation site has been strategically selected to also serve as a quarantine station for moving sugarcane from the south to the north. The Charters Towers propagation plots supply seedcane material to both the Burdekin and Northern programs and the plot size is double that of Bundaberg and Mackay. For a number of reasons the 2009 Charters Towers propagation plots failed and no CATs were planted in the Burdekin and northern regions in 2010. Given the failure, the project leaders (Cox and Croft) visited Charters Towers in early July 2010 to assess the situation and to ascertain what remedial measures needed to be implemented to ensure this does not happen again. On the trip, a possible alternate site was visited and a decision was made to move to this site. A new lease agreement was signed and Dr Cox visited the site again in September along with local Burdekin staff. The new site has a number of advantages over the old site, including clearly superior farm management capabilities and an excellent irrigation system with access to significant water storage as well as the Burdekin River. A manager was also appointed on a part-time basis to oversee the Charters Towers site. The plots were closely inspected and sampled for RT-PCR assay to ensure no Fiji leaf gall or mosaic was present. All clones with sufficient number of stalks in propagation plots, excluding those with smut, were selected and planted into CATs.

Between May and August 2009, about 1,956 clones from the 2006 series were planted into CATs at Bingera, Mackay, Burdekin and Meringa. Only two of the planned four 2007 series SmutBuster CATs were planted in spring 2010 (~1630 clones). No plantings occurred at Meringa or the Burdekin due to the failure of the 2009 propagation plot at Charters Towers. Unfortunately, due to excessive wet weather conditions and poor drainage at one of the Bundaberg sites, half of the southern 2010 CAT had sub-optimal germination and has been abandoned. Plans to establish the 2008 series CATs are well underway with seedcane from the Charters Towers propagation plots harvested the last week of May 2011 and the Burdekin and Northern CATs planted in early June.

The six SmutBuster CATs planted in 2009 were CCS sampled, harvested and weighed in spring 2010. Individual fibre measurements were not recorded, except in trial RAC09-28. However, due to problems with SpectraCane[TM] over half the samples had no fibre value and the standard fibre value of 13% for the central region was used for these missing samples. Standard fibre values of 12, 12.5 and 13 per cent were used for the southern, northern and Burdekin trials, respectively. Fibre percentage is used in the calculation of EGV. The analysis was done using ASReml on the combined data of all the trials. In trials where

competition among plots was significant, plot cane yields were adjusted using the competition model developed by Stringer *et al,* 2011.

The SmutBuster crossing and selection program is mid-way to completion. During 2010 and 2011 tasks in all five series of seedlings will be undertaken. The workload in the SmutBuster program will start diminishing from 2012 when the oldest series merges with the core program at the FAT stage. The progress of each series is summarised in Figure 1. During 2010/11, for the first time, SmutBuster clones could be compared with those arising from the core program as they progress through CATs (results presented in outputs).

Year	2006 series	2007 series	2008 series	2009 series	2010 series
2006	Plant seedlings (ex Meringa)				
2007	Select & plant 10K inoculated 3ES	Plant seedlings (inoculated)			
2008	Select & prop 2200 clones S, C, CT	Select & plant 10K inoculated 3ES	Plant seedlings (inoculated)		
2009	Plant CAT S, C, A, N	Select & prop 2500 clones S, C, CT	Select & plant 10K inoculated 3ES	Plant seedlings (inoculated)	
2010	Harvest P crop	Plant CAT S, C, A, N	Select & prop 2500 clones S, C, CT	Select & plant 10K inoculated 3ES	Plant seedlings (inoculated)
2011	Harvest 1R crop	Harvest P crop	Plant CAT S, C, A, N	Select & prop 2500 clones S, C, CT	Select & plant 10K inoculated 3ES
2012	Plant FAT (core)	Harvest 1R crop	Harvest P crop	Plant CAT S, C, A, N	Select & prop 2500 clones S, C, CT
2013		Plant FAT (core)	Harvest 1R crop	Harvest P crop	Plant CAT S, C, A, N
2014			Plant FAT (core)	Harvest 1R crop	Harvest P crop
2015				Plant FAT (core)	Harvest 1R crop
2016					Plant FAT (core)

Completed	Partially completed	To be completed

Figure 1 - SmutBuster series progress chart

3.2 Increasing the frequency of smut resistance genes in parent and selection populations, gradually reducing the current need to double the size of the variety improvement program

The SmutBuster crossing program of making crosses between RxS, IxS and SxS parents was completed in 2010. During this period, the core BSES-CSIRO Sugarcane Improvement Joint Venture concentrated on producing RxR, RxI and IxI crosses. Progeny from these crosses are now well advanced in the core selection program and will be having a positive effect on increasing the frequency of smut resistant genes in the selection population. There is, however, significant value inherent in crossing to and among varieties classified as susceptible, and the further use of smut-susceptible parents has continued in the core crossing program in 2011, although cautiously and conservatively, as recommended by the Review of the Smut Response (Hoy *et al.,* 2009) (Appendix 7).

Accelerated smut-resistant parents from the SmutBuster program are planned for inclusion in the core crossing program. From the 2006 series, 31 parents have already been selected and planted in the photoperiod facilities at Meringa and Bundaberg in 2010 for crossing in 2011. This generation interval is much shorter than practised in the core program. The aim is to continue to do this for the remaining four series to dramatically increase the frequency of smut resistant genes in the breeding and selection populations. These parents were selected based on appearance grade and CCS data from the central and southern propagation plots plus smut and Fiji leaf gall resistance ratings.

3.3 Implementation of new, innovative and efficient selection methods in sugarcane breeding populations segregating for smut resistance, including the use of:

3.3.1 Marker assisted selection

This project made use of a sugarcane DArT array that had been recently developed (Heller-Uszynska *et al.*, 2010). The array provided 15,360 DNA markers which were used to screen three different sugarcane populations to identify those markers that were significantly associated with smut resistance. These populations included two genetically broad populations (1) the association mapping (AM) population and the (2) parent population. Both of these populations consisted of important parents, varieties and untested clones (AM only). The other population was genetically narrow and is referred to as the (3) narrow population. It included clones from a group of bi-parental crosses with related parents. These three populations were chosen to increase the likelihood of finding robust marker-smut associations independent of population.

Identifying markers potentially associated with smut resistance was a complicated process involving different statistical models and marker types and is detailed in Appendix 1. From these analyses the best subset of markers was selected for arraying on a smaller array referred to as a DArTPlate. This array allowed the optimal markers to be screened across a greater number of clones more cheaply. Details of how the markers were selected are included in Appendix 1.

To test the validity of markers for smut resistance two tests were carried out. Initially models were developed from one of the populations to predict smut ratings on the other two. The second test used the best model developed from the first test to predict smut ratings on a fourth population. Importantly, this population had also been screened using NIR allowing a direct comparison between the marker and NIR techniques. Data from the first approach are presented below. Data from the second approach will be forwarded in a supplementary report to be submitted after data have been analysed.

The first approach used a method similar to Crossa *et al.* (2010) to predict smut ratings in clones. Essentially, all markers for smut resistance that had been selected for re-arraying on the DArTPlate were included in the analysis for each of the three populations. Here these populations could be seen as 'training populations', to build regression models for prediction on other populations. Using these developed models, marker data from untested individuals can be inputted to produce estimates of a clone's phenotypic value. To deal with the large number of predictors inherent in DNA marker analysis, two methods were compared in developing these models including ridge regression and Bayesian regression (reviewed in Jannik *et al.*, 2010). A summary of the results in given below in Outputs 4.3.1 and Appendix 1 has more details.

3.3.2   NIR prediction of smut resistance

The proof of concept activities to develop NIR methods for predicting the smut ratings of sugarcane clones were performed within the BSS307 project, with future activities regarding smut ratings to be completed within the SmutBuster BSS325 project.

This report describes recent work activities to validate the developed NIR methods on a set of 300 clones from a Bundaberg CAT trial.   This trial was also rated using traditional methods and DNA marker methods and serves as an exercise to evaluate both of these predictive methods against current procedures.   The trial was sampled twice by the NIR method, in both 2009 and 2010.   The results described below are for the 2009 data.   The 2010 data may be flawed as we began experiencing difficulties with the NIR instrument at this stage.   We will perform a similar analysis to this one for the 2010 data and will report that at a later date.

For the NIR procedures, two staff members were each required for a total of 2 weeks in order to complete the spectral collection.   Usually, three different stalks were sampled for each clone, and three replicate NIR spectra were obtained from one of the "mid buds".   This results in 9 spectra being obtained.   The spectra were processed in the usual way and were put through two different partial least squares (PLS) regression models.   This led to the production of 9 different rating predictions for each for the two models.   These data were examined manually and clear outliers were removed (predictions that were either negative or above 10.6, and data that was clearly different to the majority of other results).   The developmental work behind these models and descriptions of how we do some of this work can be found in previous BSS307 milestone reports.

3.3.3   Seedling screening methods

The success of the SmutBuster program hinged on successfully identifying infrequent smut-resistant clones from RxS, IxS & SxS crosses.   This would entail inoculating approximately 30,000 true seedlings and 10,000 3-eye sets annually.   There was, however, no accepted method for inoculating true seedlings with smut.   Two experiments, in 2007 and 2009, were established at the Bundaberg smut research farm to determine an inoculation method for true seedlings which would produce a reliable indication of field resistance.

The 2007 experiment was established to examine three methods for inoculating true seedlings.   Fifty seedlings from 12 families with three resistance categories, susceptible, intermediate and resistant, were included in each method.   The treatments in this experiment were:

1. Dip inoculation of four week old seedlings.   The seedlings were teased from the germination medium, washed in water and dipped in a smut spore suspension (1×10^6 spores/mL) for 10 min.   The seedlings were then planted into potting mix in 90 mm peat pots and placed in an incubation chamber at 31°C overnight.   The pots were planted to the field 4-6 weeks later.

2. Trimming and spray inoculation of seedlings at the 3-6 leaf stage, six weeks after transplanting from the germination trays. Plants were trimmed to approximately 20-30 mm above the growing point and then sprayed with a smut spore suspension (1×10^6 spores/mL) onto seedlings and incubated overnight in a chamber at 31°C before planting to the field.

3. Natural spread by planting the seedlings between smut infected spreader rows.

4. Control – un-inoculated seedlings grown to maturity in a low smut risk area and then screened for smut resistance using the standard dip-inoculation method to estimate the resistance in each of the families.

This seedling inoculation experiment was repeated in 2009 with a new treatment where the seedlings were sprayed two days after germination. The 'trim and spray' method was excluded from this experiment due to its poor results in the 2007 experiment. The incidence and severity of smut were recorded every three months. The treatments in the 2009 trial were:

1. Dip inoculation of four week old seedlings. The seedlings were teased from the germination medium, washed in water and dipped in a smut spore suspension (1 x 10^6 spores/mL) for 10 min. The seedlings were then planted into potting mix in 90 mm peat pots and placed in an incubation chamber at 31°C overnight. The pots were planted to the field 4-6 weeks later.

2. Spray germinating seedlings 2 days after germination with a smut spore suspension of (1 x 10^6 spores/mL) and incubation of inoculated seedlings at 31°C for 48 hours. The seedlings were then grown out using normal seedling methods.

3. Natural spread by planting the seedlings between smut infected spreader rows.

4. Control - un-inoculated seedlings grown to maturity in a low smut risk area and then screened for smut resistance using the standard dip-inoculation method to estimate the resistance in each of the families.

4.0   OUTPUTS

4.1   Accelerated development of high yielding smut resistance varieties for use by the Australian sugarcane industry

The six SmutBuster CATs planted in 2009 (2006 series) were all harvested in spring 2010. The analysed data (predicted clone values) has been loaded into the BSES SPIDNet database. Table 1 below gives the trial planting and harvest dates as well as trial averages for TCH, CCS and rEGV.

Table 1 – Average TCH, CCS and rEGV of the SmutBuster CATs harvested in spring 2010

Region	Trial	# Clones	Date		Average		
			planted	harvested	TCH	CCS	rEGV
Northern	MUL09-236	1071	29-Jul-09	9-Aug-10	86.5	15.05	8.51
Northern	MUL09-237	997	3-Aug-09	12-Aug-10	91.3	15.13	8.57
Burdekin	PNR09-28	1738	27-Jul-09	27-Sep-10	77.3	14.03	7.77
Central	RAC09-28	1911	26-May-09	5-Jul-10	87.1	13.87	8.44
Southern	BIN09-25	1064	18-Aug-09	12-Nov-10	94.6	14.81	8.89
Southern	BIN09-26	892	20-Aug-09	17-Nov-10	111.0	13.72	8.50

Encouragingly, 9.1% of clones in the southern 2009 SmutBuster CAT had a rEGV greater than 10, while the northern, central and Burdekin CATs had 5.0%, 4.2% and 0.9%, respectively. Considering the top 250 rEGV clones from each region; there are a total of 510 unique clones, of which 59 clones are common to all four regions and 240 clones are at one

site only. On average, 50% of clones are common between the top 250 rEGV clones from any two regions. Appendix 11 lists the top 30 clones based on average rEGV together with individual trial ranking and rEGV.

The average rEGV of the 2006 series seedlings across all regions was 8.5 for the SmutBuster CATs and 8.1 for the core CATs. The SmutBuster CATs also had a slightly higher average percentage of clones with rEGV greater than 10. It must be noted that the SmutBuster CATs, except in the northern program, are one year behind the core CATs due to a lengthened stage 1 in the SmutBuster program. The northern program, however, is also the only program where the core CATs average rEGV is greater than the SmutBuster CATs average rEGV.

Between 150-250 tentative selections have been made for each region. The 150-250 tentative selections in each of the regions will be included in smut and Fiji leaf gall screening trials in June-July 2011. In August-September 2011, these tentative selections will also be planted into FAT propagation plots. This is the point where the SmutBuster and Core breeding programs merge and 150-250 Core CAT tentative selections will also be planted into FAT propagation plots in each region. The Core and SmutBuster tentative selections will be sampled and harvested in the first ratoon crop after seedcane for the FAT propagation plots and disease screening trials has been collected. Based on the combined analysis of plant and first ratoon crop data, plus disease screening results, the top ~150 tentative selections will be selected for inclusion in FATs. The establishment of the first FATs that include material from the SmutBuster program (2006 seedlings) is scheduled for 2012. The first commercial varieties emanating from SmutBuster will be released to the industry in 2016.

4.2 Increasing the frequency of smut resistance genes in parent and selection populations, gradually reducing the current need to double the size of the variety improvement program

Thirty-one accelerated parents (Appendix 10) were selected from the 2006 SmutBuster series for planting in the photoperiod facilities at Meringa and Bundaberg in 2010 for crossing in 2011. This generation interval is much shorter than practised in the core program. This will dramatically increase the frequency of smut resistant genes in the breeding and selection populations. The best performing clones in the 2006 SmutBuster series CATs are also appearing on the breeding population list eligible for selection as a parent for the 2012 crossing season.

4.3 Implementation of new, innovative and efficient selection methods in sugarcane breeding populations segregating for smut resistance, including the use of:

4.3.1 Marker assisted selection

Predictive models were developed based on marker data to date. These models used DNA markers that showed consistent effects in different populations. The first approach involved using a model developed from one population to predict the smut ratings on the other two populations (see Appendix 1 for details). The best models were significantly correlated with smut rating, however, the amount of variation explained by the model was still small (~14%). The best model appeared to discriminate resistant (R) plants well with 43% of R plants correctly classified (the remaining 57% were classified I and 1% susceptible (Table 2). The drawback of the model is the failure to correctly discriminate S plants from I. A further test on a fourth population, will be carried out using the best developed model which will allow for a direct comparison between markers and NIR and will be submitted in a supplementary report in the coming weeks.

Table 2 - Comparison of the classification of clones by estimated and actual smut rating using markers

Estimated ratings	Actual ratings				% R + I:S
	Resistant	Intermediate	Susceptible	Total	
Resistant	54	30	19	103	82
Intermediate	71	108	97	276	65
Susceptible	1	7	18	26	69
Total	**126**	**145**	**134**	**405**	

A number of models/scenarios have been developed to determine at what point marker-assisted selection would be viable. A summary of this work was extracted from a presentation given by Dr Mike Cox at the 10th ISSCT Breeding and Germplasm workshop in Brazil in May 2011 (Appendix 9). Currently, marker-assisted selection is not cost-effective due to low marker effects and high costs of genotyping. In order to implement marker-assisted selection for smut resistance; 1) the variation explained by markers needs to increase from the current 14% to above 40%, and 2) the genotyping costs need to reduce to below $5/genotype.

4.3.2   NIR prediction of smut resistance

Two regression models have been developed. On their own they have some flaws in the way that they predict smut ratings, one which is biased towards the lower ratings and the other towards the higher ratings. A combined approach with these models is favoured, in that we are happy to accept the results from the first (or low) model when the predictions fall below a rating of 3.5, and will defer to the second model when the first rating is above 4. Because the second model usually over predicts the ratings for the intermediate and susceptible clones, we use a cut off of 4.5 to differentiate between R and I, and 8.0 to differentiate between I and S. This combined approach does a reasonably good job at the resistant end of the rating scale, but still allows too many susceptible clones through the screen.

The results obtained on the blind validation using 300 clones are shown in Table 3. The data in the table needs to be read horizontally to interpret the results from the NIR method. The far right hand column contains the broad classifications of the 300 clones based on the NIR prediction – 29 resistant, 188 intermediate and 83 susceptible. The starting population is defined in the bottom row of the table with the 300 clones consisting of 47 resistant, 53 intermediate and 200 susceptible clones.

Table 3 - Results using the combined model approach for predicting the smut ratings of 300 clones from the Bundaberg CAT

Predicted	Actual Ratings			
	Resistant	Intermediate	Susceptible	Total
R	5	8	16	29
I	34	38	116	188
S	8	7	68	83
Total	**47**	**53**	**200**	**300**

Applying this NIR model as a screening tool would result in 83 clones being discarded, of which 68 are correctly identified as susceptible (shaded cells). The total number of susceptible clones is 200 therefore 132 clones that should have been discarded would be

retained if we used this model. Fifteen clones that are rated intermediate to resistant would be discarded. In order to improve the performance in differentiating intermediate from susceptible clones, we have developed an additional rule based upon the difference in results between the two NIR models. This was based on an examination of the differences between the two models for all clones (Figure 2).
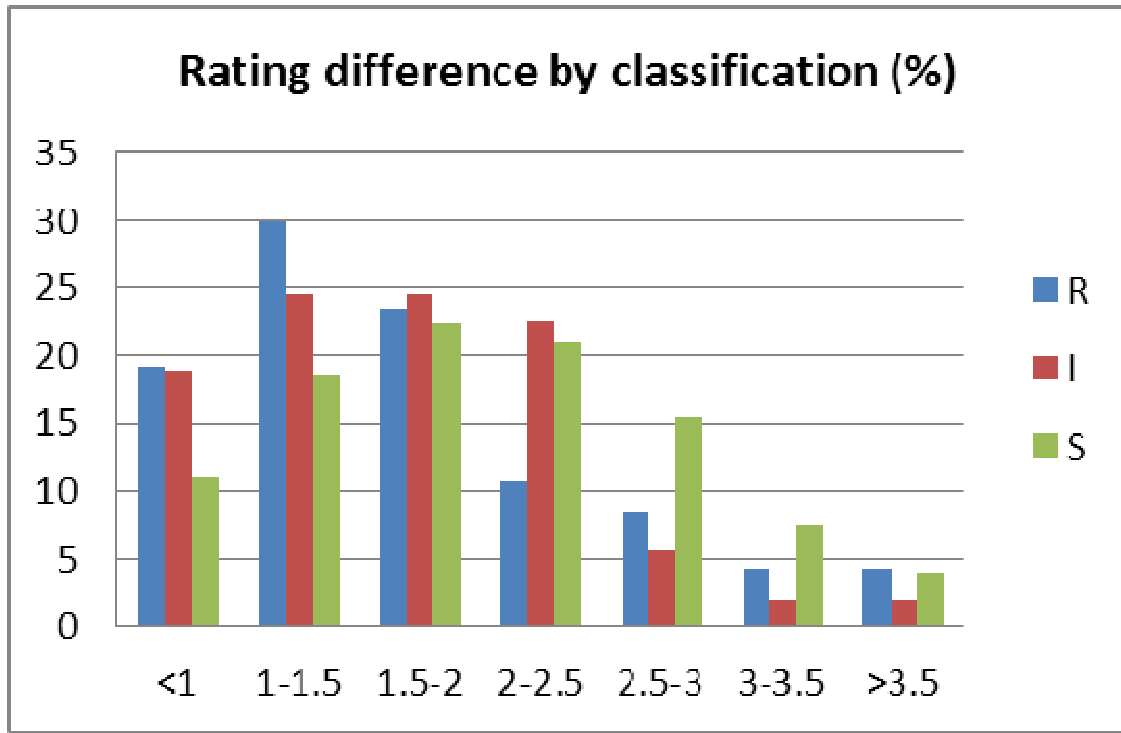

Figure 2 - Plot of the percentage of observation for each class against the differences between the two NIR models

Figure 2 shows that as the difference between the predicted values from the two NIR models increases (moving to the right along the x-axis), so does the likelihood of the unknown clone being susceptible. Similarly, for small differences between the model results, the odds are in favour of that clone being a resistant or intermediate one (for differences below 2 rating units).

An additional rule was added to the interpretation of the models for all clones with a high model value of 7.5 and above, remembering that clones were not rated as susceptible until they reached a value of 8 in this model. For these clones, if the difference between the results of the two models was greater than 2 rating units, it was tagged as being susceptible. This has the net result of helping to identify and recover a number of the susceptible clones which had been incorrectly assessed as intermediate. The results for this approach are shown in Table 4.

Table 4 - Results using the combined model approach with the additional rule for predicting the smut ratings of 300 clones from the Bundaberg CAT

Predicted	Actual Ratings			
	Resistant	Intermediate	Susceptible	Total
R	5	8	15	28
I	30	38	102	170
S	12	7	83	102
Total	**47**	**53**	**200**	**300**

The performance of the model has improved and has identified more susceptible clones. While a number of susceptible clones still pass through this method, it can still perform a reasonable job of culling a number of susceptible clones while not taking out too many resistant and intermediate clones. This screen would remove 102 clones from the population (shaded cells), of which 83 are susceptible.

The interpretation of the data from the NIR models is still incomplete and further approaches need to be considered. These include:

- Automation of crude data treatment using a statistical approach. The crude data were manually sorted but this really needs to be done according to a defined set of rules, for example excluding results which lie +/- one standard deviation from the mean of the set of 9 predictions per model.

- Defining additional rules which can further improve the distribution of clones shown in Table 4.

4.3.3   Seedling screening methods

The 2007 trial that investigated different methods of inoculating true seedlings indicated that dip inoculation was most effective (Figure 3). Natural spread was also effective but this method requires more time and considerably larger area. The dip inoculation method allowed 23% of seedlings that develop smut whips to be discarded. The regression results between per cent of smut in the seedling families and their mid-parent smut ratings are given in Appendix 2. The data from the control, however, indicated over 20% of susceptible clones may escape using even the best seedling inoculation method. A second stage of screening using normal dip inoculation of setts is required to adequately screen populations. Inoculation of setts was employed in the SmutBuster program routinely at the 3-eye stage.
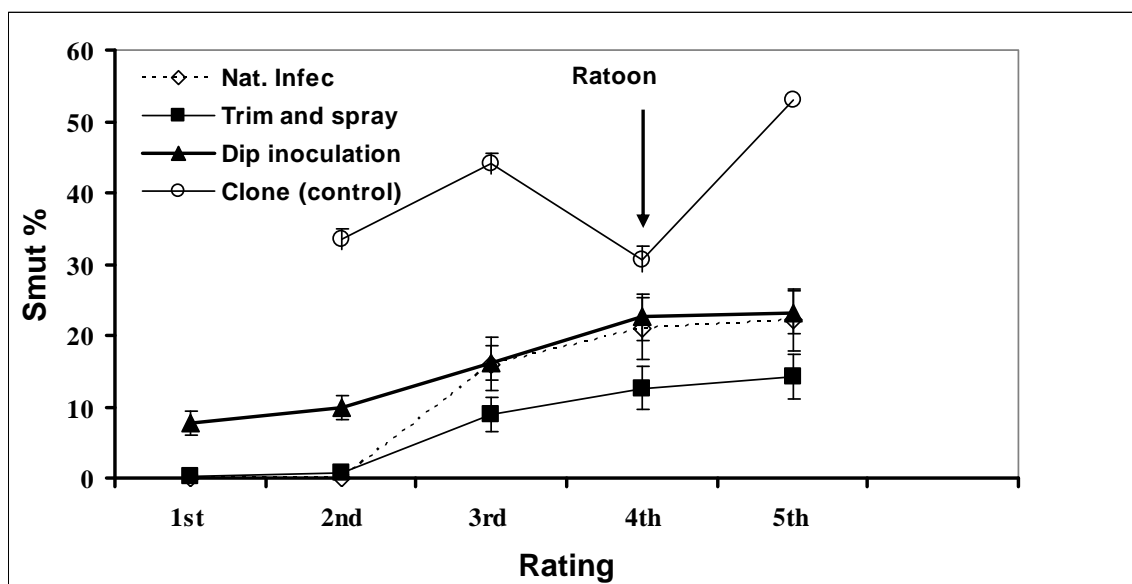

Figure 3 - Smut expression over time on sugarcane true seedlings inoculated using various methods. Clones (control) were planted separately and assessed on plant and ratoon crop. Error bars indicate standard error of means.

The preliminary results (not shown) from the 2009 experiment confirm that the dip method is more effective for the inoculation of true seedlings. The strong relationship between smut incidence in the families and mid-parent rating in the dip inoculation method suggests that this method is giving a good estimate of true resistance in the seedlings. It also confirms previous studies that have shown that smut resistance is a heritable character.

The control seedling clones for the 2009 experiment were inoculated and planted in 2010 using the standard dip inoculation method. This trial was ratooned in late January 2011 and inspections will continue until December 2011. Inspections in the natural spread treatment of the 2009 trial will continue into the ratoon crop and will be completed in June 2012. The final results from this experiment will be available in June 2012 and will be reported in a supplementary report.

Dip inoculation of true seedlings four weeks after germination followed by inoculation of one 3-eye sett from selected clones was the method employed in the SmutBuster program from 2008–2010 to eliminate smut susceptible clones from the SmutBuster populations. We believe that this intensive two stage screening program is highly effective at eliminating the majority of smut susceptible clones.

5.0   INTELLECTUAL PROPERTY AND CONFIDENTIALITY

No commercial sugarcane varieties have been released within the time-frame of this project but commercial varieties emanating from this project in the future will be protected under the Plant Breeder's Rights Act 1994. This IP will be exploited by applying to DEEDI for approval to release the varieties for commercial production in nominated sugarcane growing regions. Varieties will be propagated by BSES and/or agents of BSES for distribution to growers. BSES has agreements in place for the collection of fees for use of varieties covered by Plant Breeder's Rights.

Details of the identity of markers linked to smut resistance will be kept confidential from any party outside the BSES-CSIRO plant breeding program until the information is either protected by patent or published.

6.0   ENVIRONMENTAL AND SOCIAL IMPACTS

Outcomes of this project will be more productive smut-resistant varieties for the Australian sugarcane industry. As more productive varieties do not involve additional resource inputs (e.g. fertiliser) and smut-resistant varieties necessitate less frequent plough-out, positive environmental and social benefits are expected to be large.

7.0   EXPECTED OUTCOMES

The major industry outcome from SmutBuster will be a wider choice of more productive smut-resistant varieties. The economic impact of these varieties can be estimated by comparing the relative economic genetic value (rEGV) of the SmutBuster program and core program clones in FATs. Clones from the 2006 SmutBuster series will be planted into FATs in 2012 and the first results for comparison will be available in 2013.

SmutBuster is expected to have a positive impact on the rate of genetic gain for tonnes sugar/ha/year. Figure 4 shows the actual rate of genetic gain for tonnes sugar/ha/year for the last 20 years together with predicted rate of gains for different scenarios, including: no pre-incursion plan; with SmutBuster; and without SmutBuster. The change in the genetic gain is tracked in the BSES database and should be evident from 2015.

Further quantitative baseline assessments that can be reported in the near future include:

- The relative proportion of smut resistant material in post SmutBuster CATs and FATs relative to a pre-smut incursion baseline.

- Comparison of the plant and first ratoon crop rEGV means of SmutBuster and core CATs.
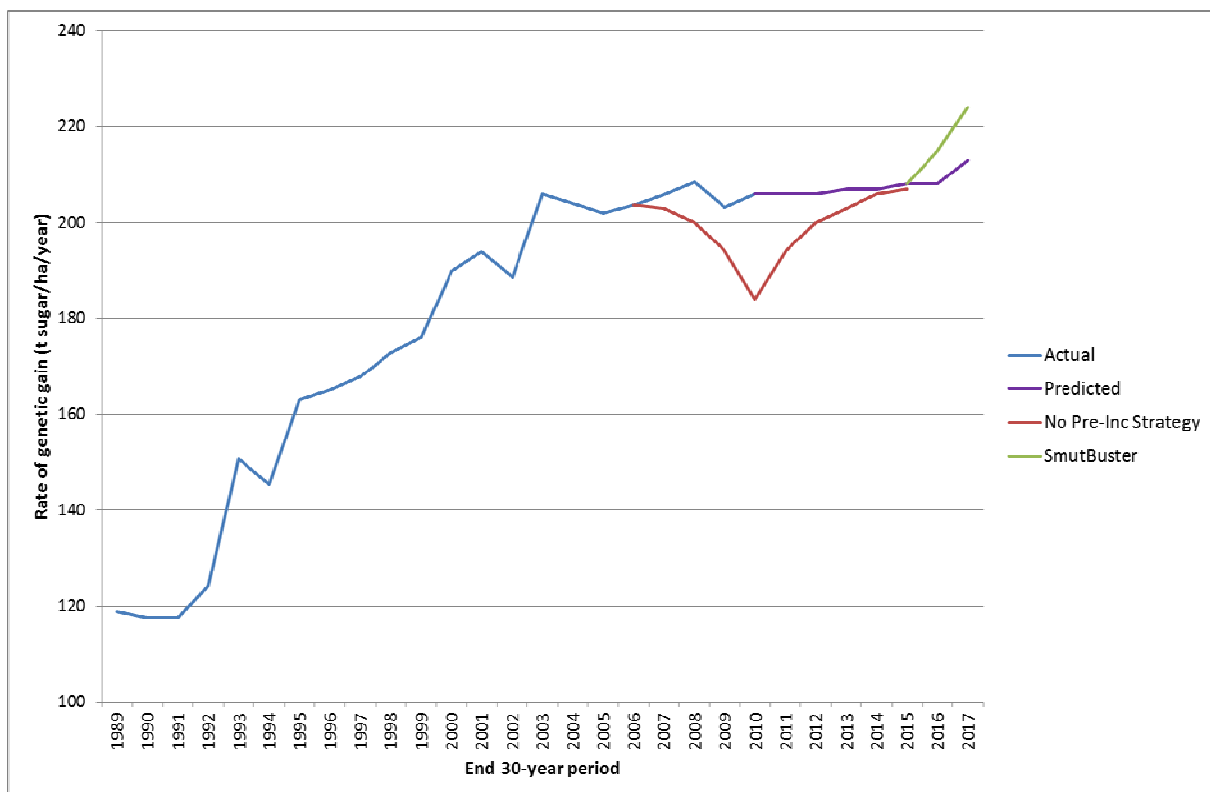

Figure 4 - Rate of genetic gain for tons sugar/ha/year for scenarios with and without i) pre-incursion strategy, and ii) SmutBuster

8.0   FUTURE RESEARCH NEEDS

The dip inoculation method for true seedlings developed within SmutBuster will be researched further in project BSS343 – 'Maximising genetic gain from family and within family selection'. Research project BSS343 will investigate changes required in core field selection schemes to include methods developed in BSS325 and other projects. The aim of this project is to improve the effectiveness and efficiency of selection in core progeny assessment trials (PATs). The inoculation of true seedlings with the methods developed in this project will eliminate many smut susceptible clones at the earliest stage in the selection program. The elimination of these smut susceptible clones will allow more resources to be devoted to testing resistant clones in the latter stages of selection. These improvements will

result in increased rates of genetic gain and the release of more productive varieties for the Australian sugarcane industry.

Research on integrating molecular marker technology in the core breeding and selection programs is continuing in project BSS319. Within BSS319 a broad range of parents will be genotyped with DArT markers. Crosses predicted to produce superior progeny based on marker information have been made and their progeny will be tested in the field. Progeny will also be selected using markers in a further cycle of marker-assisted selection.

9.0   RECOMMENDATIONS

Going back over the recommendations of the 'Review of the R&D Response to Sugarcane Smut with Special Reference to the SmutBuster Research Program' (Appendix 7), as well as the BSES Limited response (Appendix 8), the following points are still relevant:

- Continue epidemiological research related to disease expression, severity, and rates of disease increase in ratoon crops. Recommend research continue.

- The research comparing incidence and severity in inoculated tests should be continued. Recommend research continue.

- The panel recognises the significant value inherent in crossing to and among varieties classified as susceptible, and the further use of smut-susceptible varieties is likely to be justified beyond the life of the SmutBuster program. Continued in 2011 crossing, and recommend continue in future.

- Due to the complex nature of the sugarcane genome, the progress of molecular marker research was expected to be difficult, and this was reflected by progress for selection of markers for smut resistance. Therefore, unless further progress can be made, specific investment for smut resistance selection should be curtailed. Recommend that progress be reviewed in 2011 and decision made whether research is continued or curtailed.

- We recommend that 400 preliminary selections from the CAT stage for one region be tested using the NIR bud scanning method and in inoculated trials on the smut farm. Selections from the two methods should be compared in a further inoculated and NIR trial. This should be continued for several cycles to establish the utility of the NIR method for evaluating smut response. Recommend this research be curtailed until issues with the NIR instrument are resolved.

Considering the cost and effort that have gone into SmutBuster over the last six years it will be a real tragedy if the research were to be curtailed, especially as project progress to date is still largely undetermined. Early indications are that progress will reach, if not exceed, expectations and it is recommended that the project continue through to the end (2016) unless future progress reports prove otherwise. Budgetary constraints are, however, forcing BSES Limited to review their variety improvement program and possibly "cut-down" the Core and/or SmutBuster programs. It is thus recommended that BSES Limited continue to seek a partner, including the SRDC, to continue the funding of the innovative SmutBuster R&D work.

10.0 LIST OF PUBLICATIONS

- A Review Committee, comprising Professor Jeff Hoy (Louisiana State University), Dr Bob McIntosh (Honorary Professor, Sydney University Plant Breeding Institute) and Dr Mac Hogarth AM (formerly BSES Plant Breeder and SRDC Director) reviewed the SmutBuster project during the week 16-20 February 2009. Their report was presented to BSES, CSIRO, QDPI&F and SRDC management on 20 February 2009. A copy of the report and the BSES response is attached as Appendix 7 and Appendix 8.

- The following paper was presented at the 27th ISSCT Congress in Mexico in March 2010 (Appendix 6).

  MC Cox, BJ Croft, RC Magarey, N Berding and SA Bhuiyan. (2010) Sugarcane smut in Australia: history, response and breeding strategies. Proceedings of International Society of Sugarcane Technologists 27.

- The following paper presented at the 32nd ASSCT conference held in Bundaberg in May 2010 was reprinted in the International Sugar Journal 2011, Vol. 113, No. 1345 (Appendix 5).

  DE Purcell, JP Oxley, MC Cox, BJ Croft, MG O'Shea (2010). On-site rapid screening for sugarcane smut resistance using Near-Infrared (NIR) spectroscopy.

- The following poster was presented at the joint 4th Asian Conference on Plant Pathology and 18th Biennial Australasian Plant Pathology Society Conference, 26 – 29 April 2011, Darwin, Australia (Appendix 3).

  SA Bhuiyan, RS James, BJ, Croft, MC Cox (2011). Development of inoculation method for screening of true seedlings of sugarcane against smut.

- An Information Sheet (IS11002) titled "SmutBuster program" was printed in March 2011. The Information Sheet briefly describes the what, why and how of the SmutBuster program. A copy is attached as Appendix 4.

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APPENDIX 1

Association mapping

Association mapping refers to finding markers in physical linkage with QTL in populations derived from a random selection of clones with different levels of relatedness. Successful application of this approach relies on some sections of genome derived from certain ancestral clones remaining "intact" among a genetic population of interest. This can arise when the set of genotypes of interest all trace back, over a small number of generations, to some common ancestors. This situation leads to what is called "linkage disequilibrium" where some DNA markers and some nearby alleles affecting traits of interest still haven't undergone recombination, and are in "coupling phase" linkage more often than in "repulsion phase" linkage. This will be the case in genomes of parents in core sugarcane breeding programs, which trace back over a small number (maybe 4 to 8 in most cases) of generations to a relatively small number of key ancestors. Recent research has confirmed that linkage disequilibrium can be readily detected across many modern sugarcane cultivars, suggesting that linkage between markers and traits may be possible to achieve in our sugarcane commercial germplasm. However, several potential complications may also easily arise with this approach for the unwary player, and it is important that it is rigorously evaluated before application. Another complication is that effects of some alleles are associated with complex interactions with other alleles, and hence effects are conditional on genetic backgrounds in which they are found. One way to address this limitation, but also maximise the level of exploitable variation captured in QTL mapping research by including important "interaction" effects is to work on specific genetic populations in which significant improvement would be of high value.

Populations

Three different sugarcane populations were used to identify those markers that were significantly associated with smut resistance. These populations included two genetically broad populations (1) the association mapping (AM) population and the (2) parent population. Both of these populations included important parents, varieties and untested clones (AM only). The other population was genetically narrow and is referred to as the (3) narrow population. It included clones from a group of bi-parental crosses with related parents. Table m1 gives the details.

Table m1 - Trait data available for DArTPlate construction

Population name	Population type	Genotypes with smut ratings	Markers types available discrete (continuous)
Association mapping	Broad	448	1531 (15360)
Parent	Broad	674	1988 (15360)
Narrow	Narrow	405	1400 (15360)

There was overlap in clones between the parent and AM populations. Table m2 shows this along with the shared discrete markers between populations. These were used to quantify the repeatability of the DArT assays, which has good (r > 0.9; results not shown).

Table m2 - Common clones and discrete markers between the three populations

		Clones in common	
	AM	179	0
Discrete markers	1140	Parental	0
in common	743	939	Narrow

Marker types

All clones were genotyped using DArT markers (Jaccoud *et al.*, 2001). For each clone, two types of polymorphic DArT markers were obtained which were termed "discrete" (ie. able to be scored clearly into two classes, corresponding to "present" (1) and "absent" (0)) or continuous (15,360 markers classed as "continuous" (ie. all clones have the marker and the score is potentially correlated to the number of copies of each marker allele per genotype)). Typically only ~10% of continuous markers can be classified as discrete. Discrete markers with a frequency larger than 0.9 and less than 0.1 were excluded. All continuous markers were used in the analyses.

Marker models

To identify marker~trait associations a number of models were used. As it was very difficult to evaluate which model is more accurate we chose an approach to incorporate the best markers from several different models and populations. A list of the models used is shown in Table m3. The simplest model (M1) ignores any population structure and tests single marker trait associations (trait~mk_i associations) and builds up models from this data. Models two and three include either pedigree (M2) or genetic similarity (M3) to account for population structure. Models four, five and six are correlated two marker models where two linked markers (determined by correlation) ($mk_i + mk_j$) are used instead of a single marker to test for trait associations. Again population structure is accounted for by either pedigree (M5) or genetic similarity (M6). Model 7 looks at marker x marker interactions in the absence of population structure. Models 8 and 9 (M8 & M9) analyse all markers simultaneously and use different methods to reduce the number of markers into correlated groups (markers grouped by association tests, ridge regression in M8 and Fischer's pair wise association in M9) and then run analyses with these groups to identify the best subset of markers.

Table m3 - Models used to identify marker-trait associations

Model type	Model	mk_i	$mk_i + mk_j$	$mk_i * mk_j$	all mk	Pedigree	Genetic similarity
Forward selection	M1	Y	-		-	-	-
Forward selection	M2	Y	-		-	Y	-
Forward selection	M3	Y	-		-	-	Y
Forward selection	M4	-	Y		-	-	-
Forward selection	M5	-	Y		-	Y	-
Forward selection	M6	-	Y		-	-	Y
Forward selection	M7	Y		Y		-	-
Backward selection	M8	-	-		Y		
Stepwise selection	M9				Y		

When population structure was included in the model (M2, M3, M5, M6) it was done so using the additive relationship coefficient from the pedigree of all the clones in the population and their ancestors. This approach is similar to the model developed by Yu *et al.* (2006) with some modification. Analysis of each marker was conducted independently and the model could be written as the following:

$$\mathbf{y} = \mathbf{X}_1\mathbf{m} + \mathbf{Z}_1\mathbf{a} + \mathbf{R} \ [\text{Eq.1}]$$

where $\mathbf{y}$ = a vector of measured value for smut

$\quad\quad\mathbf{m}$ = a vector of fixed effects of a marker and its plate number in the laboratory;

$\quad\quad\mathbf{a}$ = a vector of random polygenic effect for clones in trials and their ancestors

with a ~ $N(0,\mathbf{G})$ where $\mathbf{G} = \mathbf{A} \otimes \left(\sigma^2_g\right)$ and **A is the**

relationship matrix derived from pedigree (Henderson 1976) and $\sigma^2_{g_i}$ is genetic variance.

$\mathbf{R}$ = residual effects from errors and modelled as diag($\mathbf{R}_i$)

$\mathbf{X}_1$ and $\mathbf{Z}_1$ are design or incidence matrices.

To examine interactions among markers, all 2-way interactions between discreet markers were examined (M7). For example in the AM population for discrete markers, a total of 1531 markers were examined, giving a total of (1531 x 1531)/2 − 1531 = 1.17 million separate analyses for each trait! Given there were a total of 15360 continuous markers, the number of all possible 2-way interactions was too many to do in a practical timeframe (it was estimated to take several years given our available computing capacity), and a method to reduce the number of markers involved was employed. This method consisted of firstly clustering the markers into groups based on similarity of scores across genotypes, and then examining all 2-way interactions between a random marker from each group.

Model 8 was carried out using proprietary methods of DArT Pty Ltd. Model 9 was undertaken based on the models of Crossa *et al.* (2010). However, this model was found to be too computer intensive for such a large number of markers. Instead it was developed for

predicting smut phenotypes based on a smaller set of markers identified as detailed in the next section.

Marker selection

A significant similarity in the ranking of markers for smut was found in models M1 to M6 in the AM and parent populations, to simplify selection only results from M2 were chosen to go forward with in these two populations. These results were compared with results from M1 on the narrow population and M7 and M8 on all populations.

The ultimate goal of our marker selection was to identify a set of 384 markers which could be re-arrayed to allow more cost effective screening of a large number of genotypes (costs estimated to be 1/5 of the price for full DArT analysis). The array also included markers for TCH, CCS and other diseases. Details of selection steps in shown in Table m4. Essentially, most emphasis was put on markers or marker*marker interactions which were identified in more than one population.

Table m4 - Steps in marker selection for the sugarcane DArTPlate

Model	Step	Alpha. tch	Alpha. ccs	alpha. smut	Effects	Description	Note
M2	1.2	0.1	0.1	0.1	NA	significant over 3 ppns and 2 types of markers	
M2	1.3	0.1	0.1	0.1	consistent over three ppns	significant over 3 ppns for discrete markers	
M2	1.4	0.1	0.1	0.055	consistent over three ppns	significant over 3 ppns for either discrete or continuous markers	
M2	1.5.1	0.05	0.1	0.1	consistent over two ppns	significant narrow ppn discrete marker that also significant at either AM	
M2	1.5.2	0.05	0.1	0.1	consistent over two ppns	significant narrow ppn discrete marker that also significant at parent ppn	
M2	1.6.1	0.05	0.025	0.025	consistent over three ppns	significant narrow ppn continuous marker that also significant at AM ppn	
M2	1.6.2	0.05	0.025	0.025	consistent over two ppns	significant narrow ppn continuous marker that also significant at parent ppn	
M2	1.7	0.005	0.005	0.001	consistent over three ppns	significant in both AM and parent ppns for either discrete or continuous markers	
M2	2	0.1	0.05	0.05	consistent over three ppns	significant markers in both types from narrow ppn	
M2	3				consistent over three ppns	significant discrete markers in narrow ppn	10 for each trait
M2					consistent	significant continuous	10 for

					over three ppns	markers in narrow ppn	each trait
M7	4					common interaction over three ppns	
M7	5					significant interaction in narrow ppn	
M2	6				consistent over three ppns	most significant markers in AM and parent ppns	6 for each ppn x trait x Mktype combination, or 72 in total
M2	7				NA	most significant markers in parent ppns for PA, LS and FJ	
M8	8				consistent over three ppns	Markers identified by DArT Pty Ltd	

Alpha = refers the P-value used for discrimination.
Effects = refers to the difference in the population mean with and without the marker.
Ppns = populations.

Model predictions

To test the validity of markers for smut resistance two tests were carried out. The first used models developed from the populations to predict smut ratings on the others. The second test used the best model developed from the first test in a blind test to predict smut ratings from a group of tentative FAT selections. Data from the first approach is presented below. Data from the second approach has not yet been received and will be forwarded in a supplementary report to be submitted after data has been collected and analysis carried out.

Approach 1:

An approach similar to that used by Crossa *et al.* (2010) was used to predict smut ratings in clones. Essentially, all markers for smut resistance that had been selected for rearraying on the DArTPlate were included in analysis for each of the three populations which were used as 'training populations'. From these analyses, models were developed which allow genotypic data from untested individuals to be used to produce estimates of a clone's phenotypic value. To account for the large number of predictors inherent in DNA marker analysis, two methods were compared in developing models including ridge regression and Bayesian regression (Table m5). Initially, the AM population was used to construct the model with discrete markers only and this was then used to predict the narrow and parent populations. Three models were used including M1: restriction in model to those common markers shared between populations; M2: use of coefficients from models for prediction based on all markers in each population and M3 assume all markers are in common and that discrete markers not shared between populations are either all present or all absent in population. Comparison of the predictions produced by these models is shown in Figure m1.

Table m5 - Methods and Models run to test which approach is best

Method	Model	Predictions	Discrete markers selected in model
Ridge	M1	3	31
Ridge	M2	3	23
Ridge	M3a	3	29
Ridge	M3b	3	29
Ridge	M1	9	27
Ridge	M2	9	20
Ridge	M3a	9	26
Ridge	M3b	9	26
Bayesian	M1	3	32
Bayesian	M2	3	25
Bayesian	M3a	3	27
Bayesian	M3b	3	27
Bayesian	M1	9	29
Bayesian	M2	9	20
Bayesian	M3a	9	26
Bayesian	M3b	9	26

Method refers to how the model was constructed. Model refers to how the predictions were made including M1: restriction in model to those common markers shared between populations; M2: use of coefficients from model for prediction based on all markers and M3 use all markers and assume discrete markers not shared between populations are either all present or all absent in population. Prediction show whether ratings were predicted on a 3 point scale (Resistant, Intermediate or Susceptible) or the standard 9 point scale (1 to 9 with 1 being resistant).

Importantly, the method did not appear to make much difference and both ridge (_r) and Bayesian (_l) regressions produced highly correlated predictions (e.g. p_am_r and p_am_l).
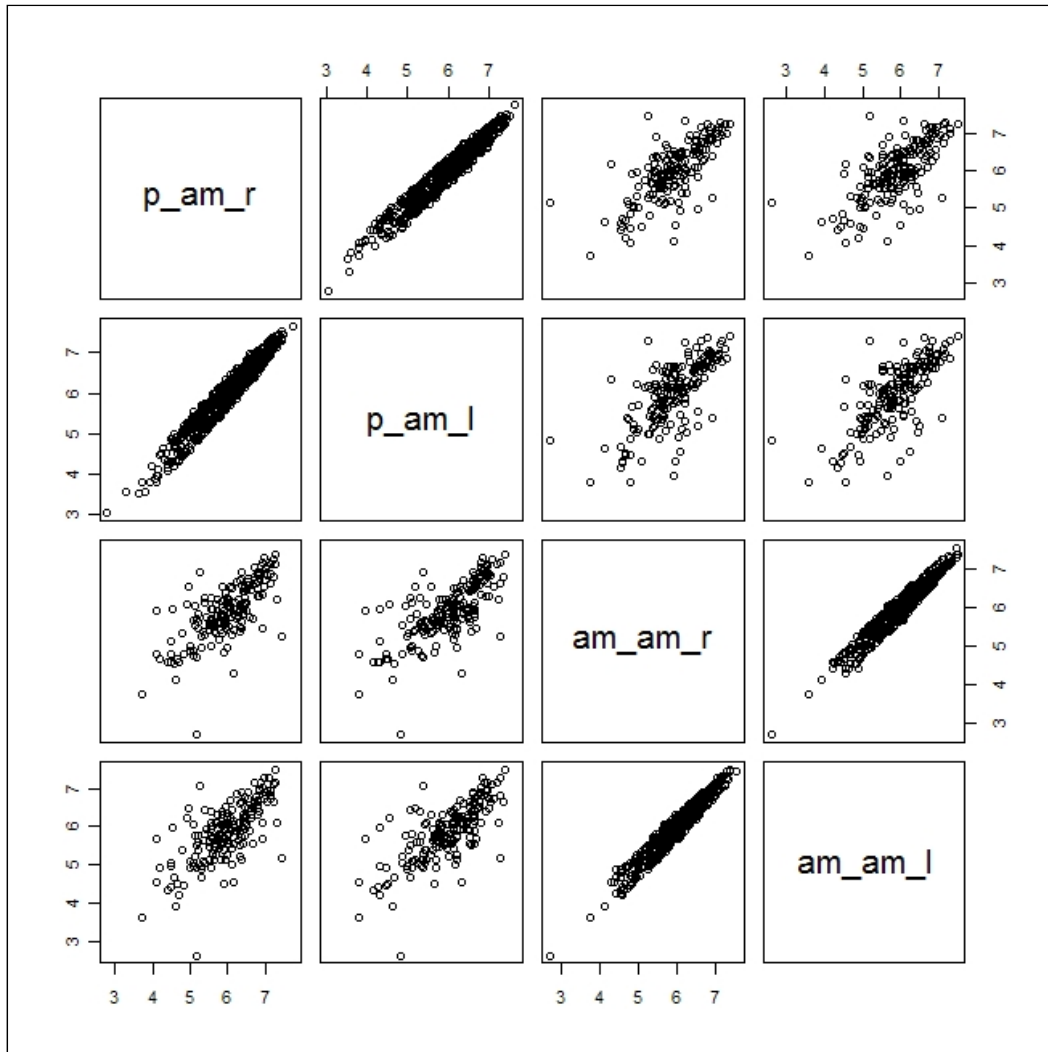
Figure m1 - Method comparison: Correlation of actual smut rating (Smut) of the parent and the AM populations from M1, with predictions for each based on the AM model (parent = p_am-r and p_am_l; AM = am_am_r and am_am_l)

A comparison of the different models was also made. Essentially models M1 and M3a and M3b all performed similarly, with M2 performing poorly. A subset of the results is shown below (Figure m2) with comparisons of the parent and AM populations with ridge regression. This analysis indicates that although the estimated smut ratings were significantly correlated with the actual ratings, the amount of variation explained by the best model was still less than 10%.
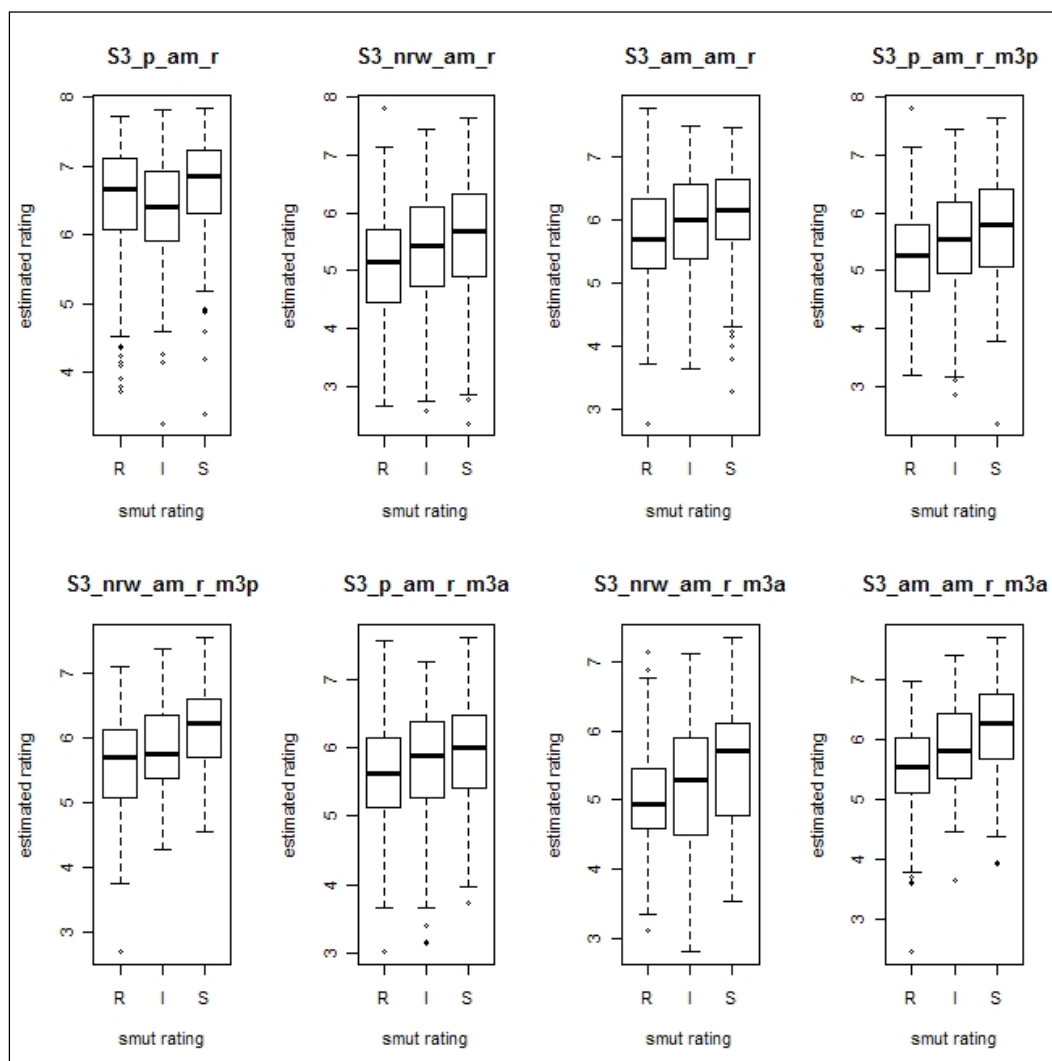
Figure m2 - Model comparison: Boxplots of actual smut ratings vs predicted smut ratings. Results from 8 of the models are shown which include M1 (S3_p_am_r, S3_nrw_am_r, S3_am_am_r), M3a (S3_p_am_r_m3p, S3_nrw_am_r_m3p, S3_am_am_r_m3p) and M3b (S3_p_am_r_m3a, S3_nrw_am_r_m3a, S3_am_am_r_m3a). All analyses shown use ridge regression and a converted 3 point resistance scale

The poor performance of the model can best be seen from a comparison of the actual smut rating and estimated smut rating for a population. Figure m3 shows a density plot of the distribution of actual smut ratings for the narrow population (solid line) over-laid with the estimated smut ratings of the narrow population using based on a model produced from the AM population (dashed line). Differences in distribution are significant with estimated ratings clustered between 4 and 7. This is presumably due to the fact that the markers used in this model explain only a proportion of the variation for smut resistance.
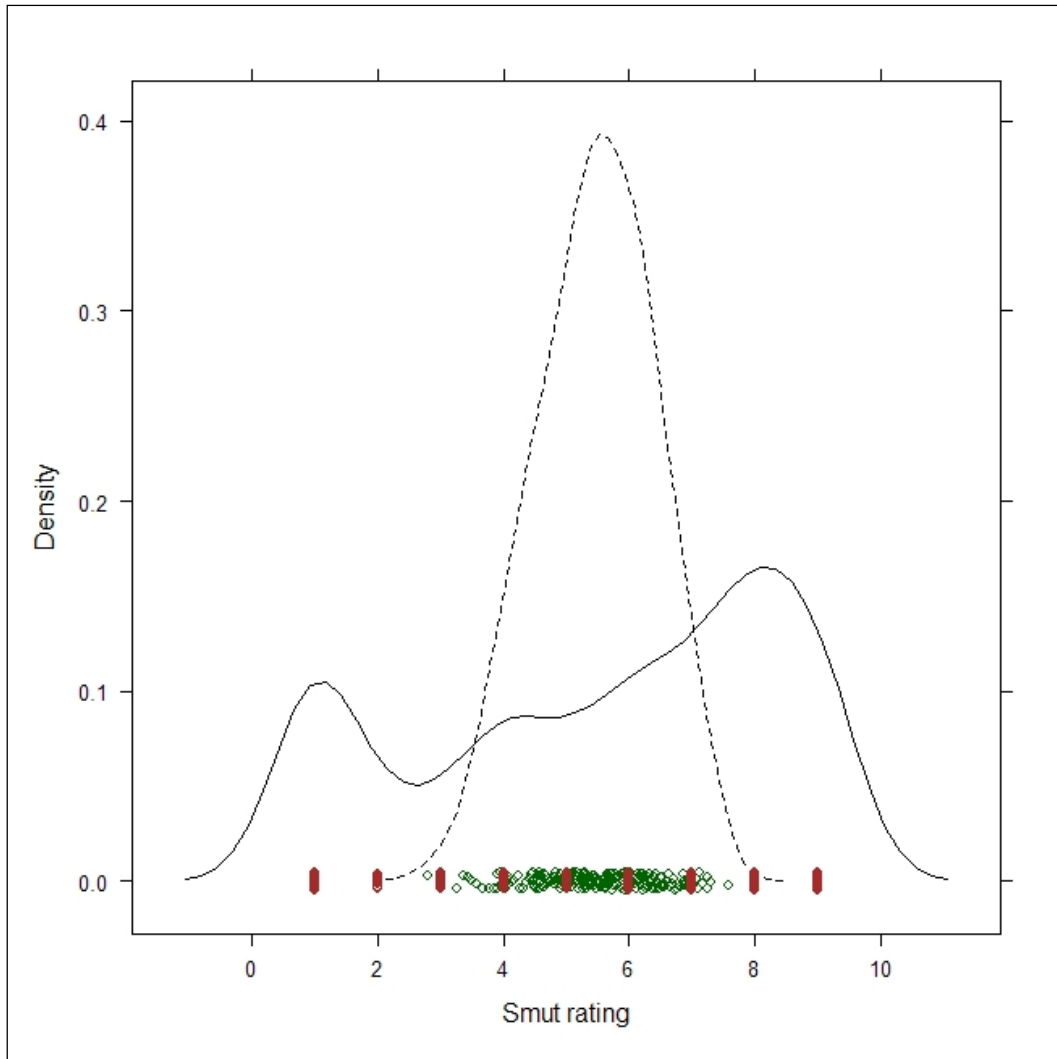
Figure m3 - Comparison of smut rating distributions on the narrow population between actual ratings (solid line) and estimated ratings using an AM model (dashe1d model)

To improve the model it was decided to only use a single model and add in continuous markers (M1 with Bayesian regression used). Different populations (AM, narrow (nrw), parent (p) populations were used to build the prediction model. Comparison of models showed that inclusion of both marker types increased the variation explained to ~14% in the best models (Figure m4).
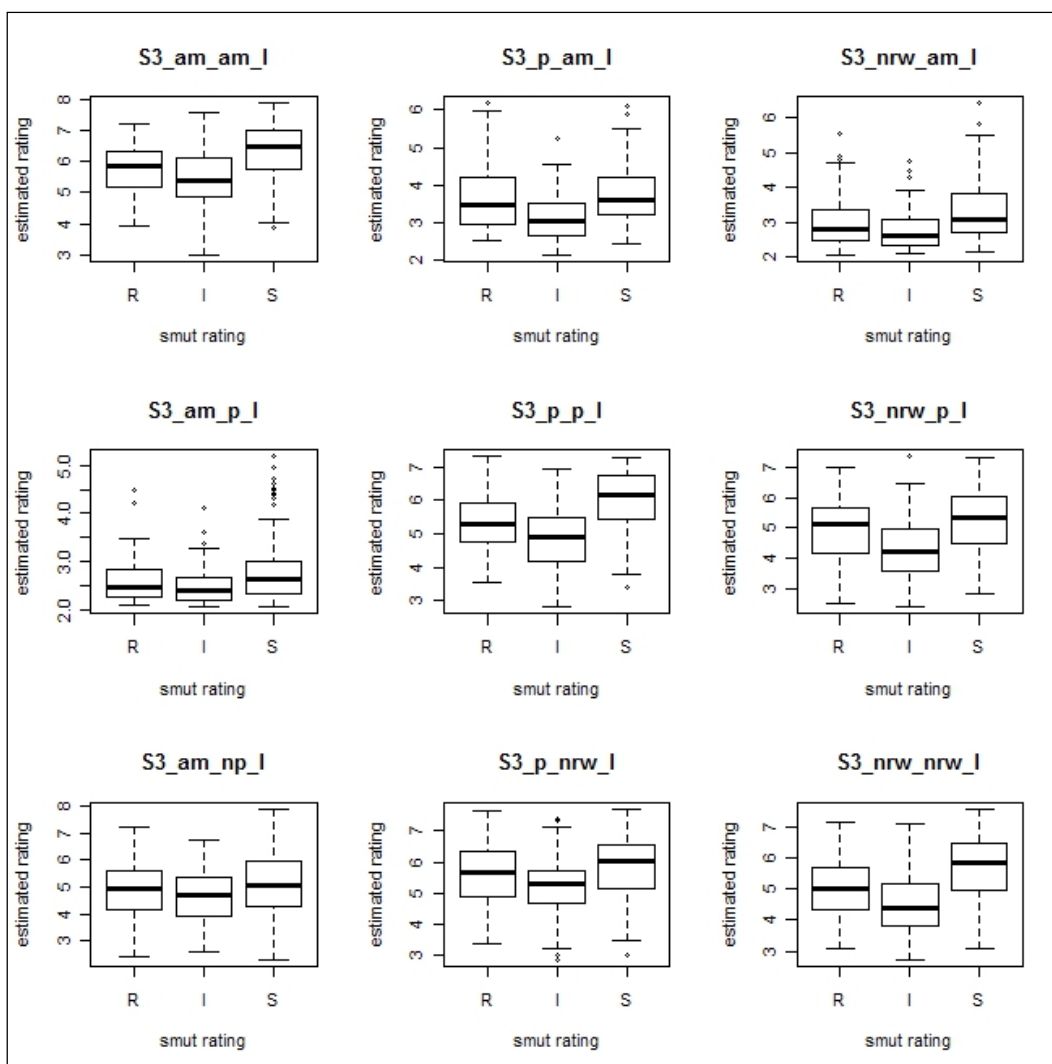
Figure m4 - Model comparison: Box-plots of actual smut ratings vs predicted smut ratings. Results from M1 using all three models to predict smut ratings. S3_p_am_I for instance refers to estimation of the parent populations using the am population. All analyses shown use Bayesian regression and a converted 3 point resistance scale

Density plots also showed that the distribution of the estimated ratings from these models better matched the distribution of actual ratings (Figure m5).
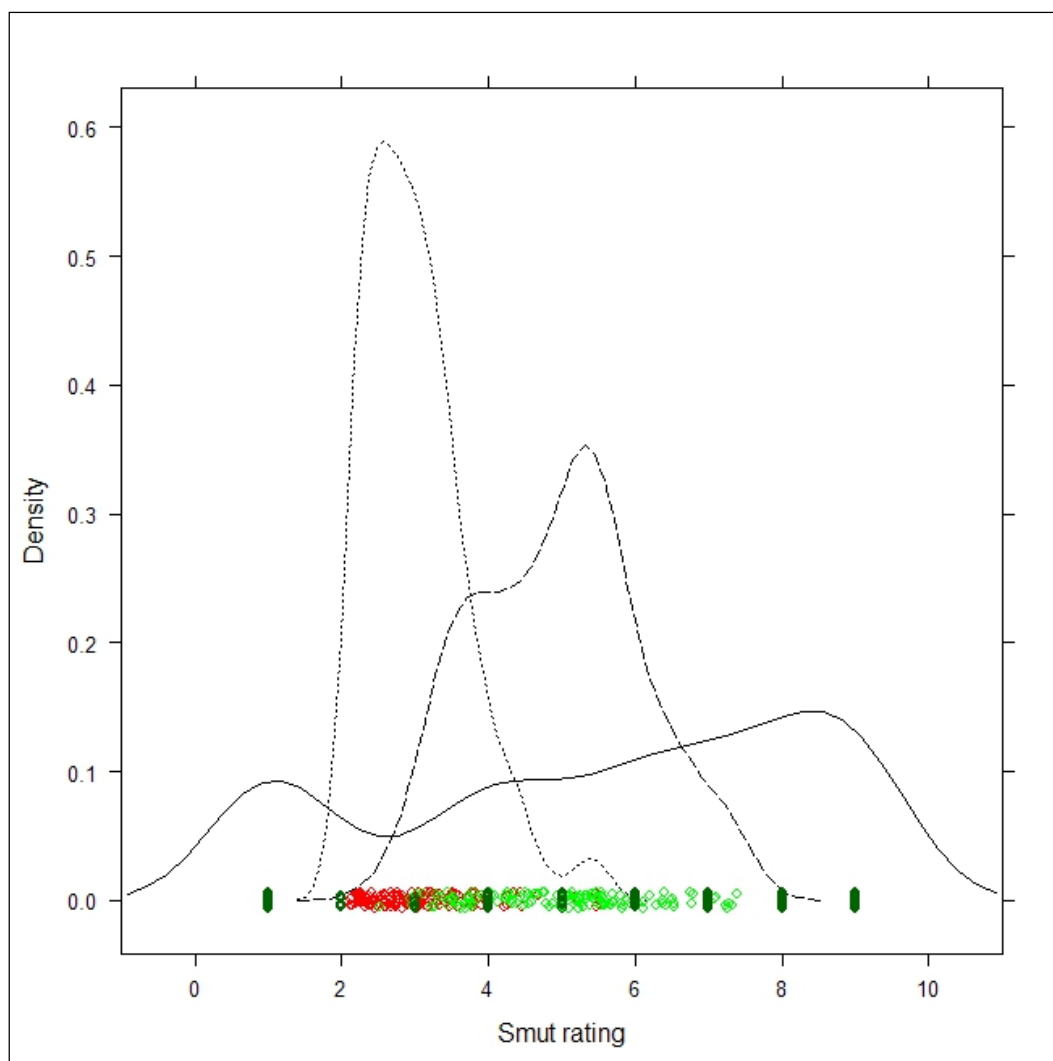
Figure m5 - Comparison of smut rating distributions on the narrow population between actual ratings (solid line) and estimated ratings using an AM model (dotted line) and estimated ratings from the parent population (dashed line)

In summary, Approach 1 suggests that perhaps moderate levels of variation may be explained by markers. The best model appeared to identify resistant plants reasonably well, however, it still classified many resistant and susceptible plants as intermediate. This new model will be tested on the FAT selections in the second approach to examine its efficacy. Failure to predict more than 10% of the variation would suggest that the current marker strategy is probably not cost effective.

Approach 2: Blind test

A population of 282 clones from tentative selections from a Southern and NSW FAT series (trials MQN08-21; MQN08-22; MQN08-23; MQN08-29 were sampled) were selected to test predictions made with markers. DNA from these clones was extracted and genotyped using the DArTPlate. Results from this will be presented in a supplementary report.

APPENDIX 2

The regression results between per cent of smut in the seedling families and their mid-parent smut ratings indicate that the dip inoculation method is superior to the other two methods (Figures p1, p2 and p3). There was a highly significant correlation between smut % and mid-parent rating in the dip inoculation method (Figure p1). Significantly high correlation was also observed between smut % and mid-parent rating in the natural infection method (Figure p3), whereas, a poor correlation was observed in the trim and spray method (Figure p2).

The correlation between the average smut incidence (%) of 20 clones from each family and the mid-parent smut rating of the parents of the families was r = 0.55 (Figure p4). The control treatment where setts of 20 clones selected from each family were dip inoculated using the standard method was used for this analysis. Unfortunately some parents were no longer available and the resistance of the parents could not be confirmed under the same conditions as the progeny.
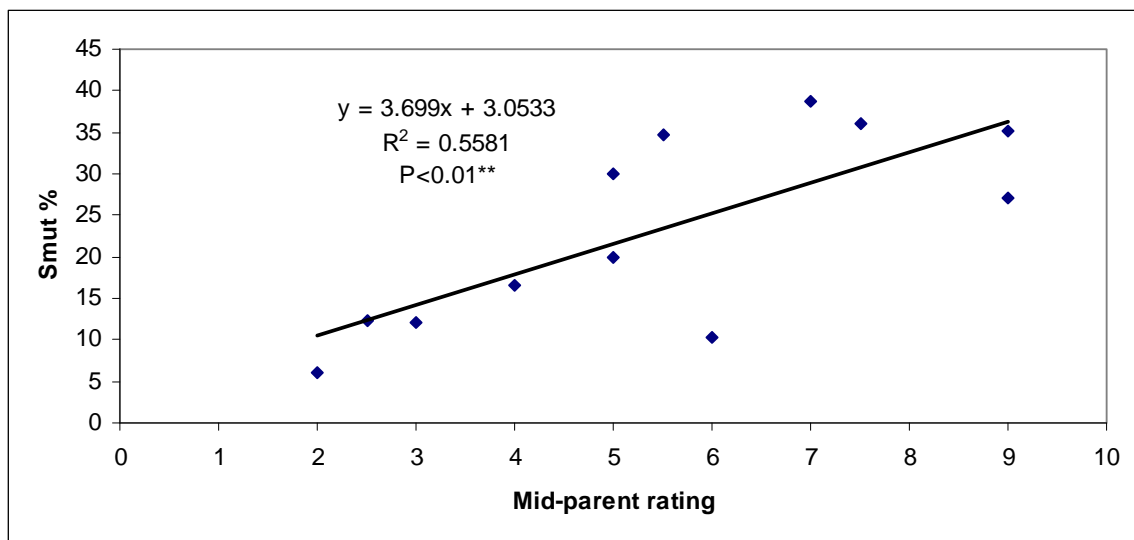

$y = 3.699x + 3.0533$
$R^2 = 0.5581$
$P<0.01**$

Figure p1 - Relationship between smut incidence of true seedlings and their mid-parent smut rating using dip inoculation method

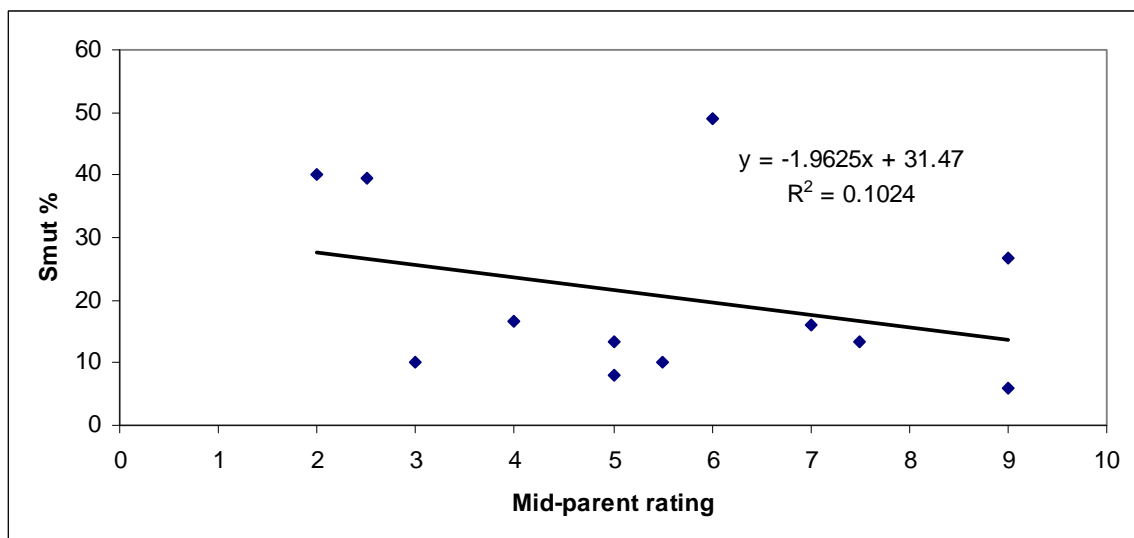
$y = -1.9625x + 31.47$
$R^2 = 0.1024$

Figure p2 - Relationship between smut incidence of true seedlings and their mid-parent smut rating using trim and spray method
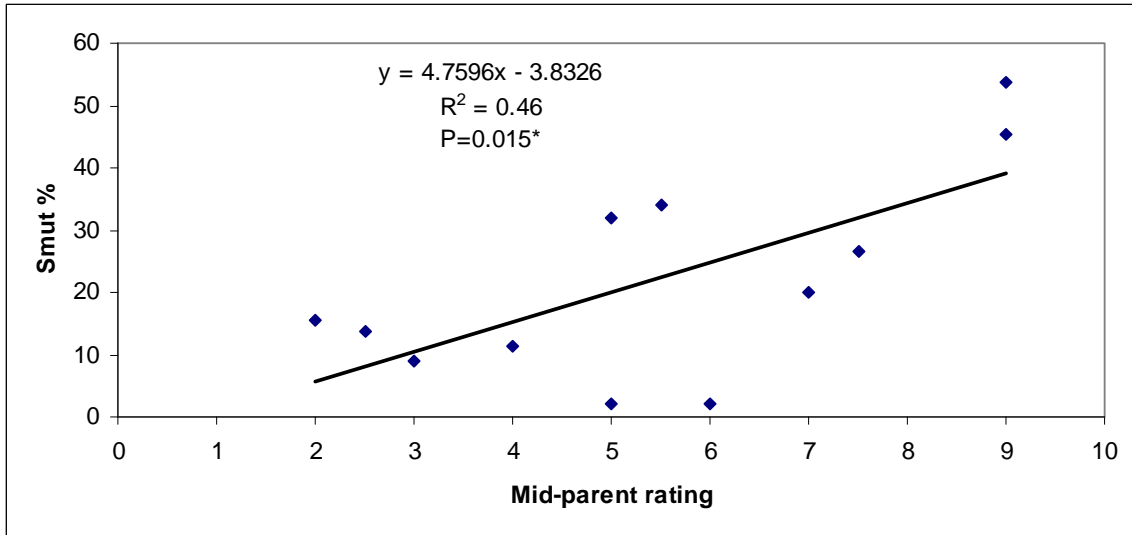
Figure p3 - Relationship between smut incidence of true seedlings and their mid-parent smut rating using natural infection method
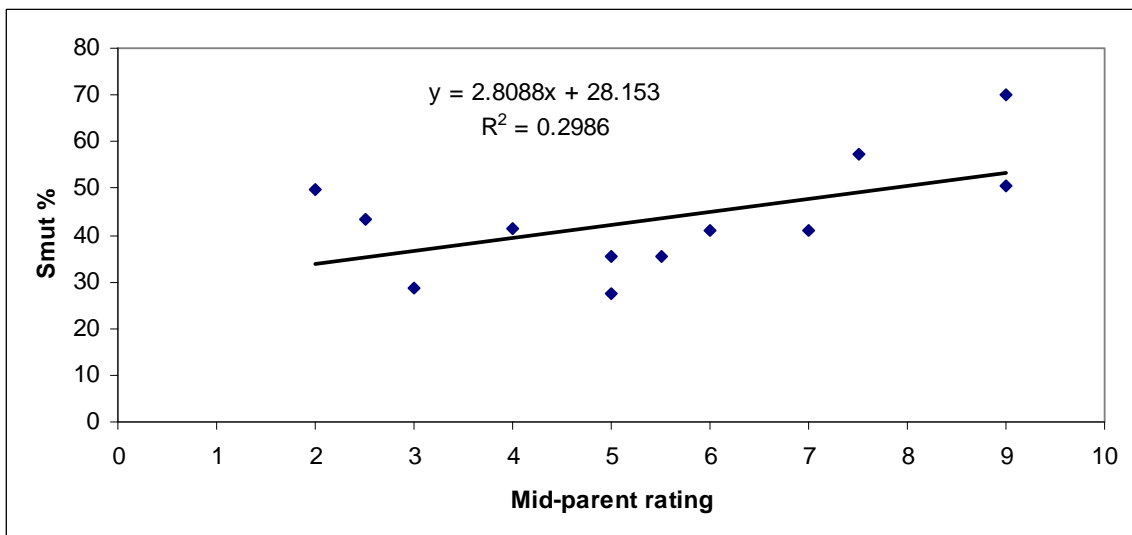

Figure p4 - Relationship between the average smut incidence (%) of the 20 clones from each family and the mid-parent smut rating of the parents of the families. This graph shows the results for the control treatment where clones were inoculated as setts using the standard dip inoculation method

APPENDIX 4

APPENDIX 7

**Review of the R&D Response to Sugarcane Smut
with Special Reference to the SmutBuster Research Program**

**Professor Jeff Hoy
Professor Bob McIntosh
Dr Mac Hogarth, AM**

20 February 2009

EXECUTIVE SUMMARY

The incursion of sugarcane smut in Queensland in 2006 represented a potentially severe crisis for an extremely important Australian agricultural industry. The severity of the incursion was alleviated by the preparedness of the sugarcane industry as a result of the smut incursion plan and earlier collaborative testing of germplasm for smut resistance, firstly in Indonesia and, after 1998, in Western Australia as well.

Even though the industry was well prepared, there was a huge effort to combat the disease following the incursion into Queensland, particularly during the period when eradication was considered a possibility. Much was accomplished by BSES and QDPI&F staff who were under severe pressure to address the many uncertainties created by the presence of the disease.

It was soon realised that the only solution to the problem was for the industry to adopt smut-resistant varieties. As 80% of the commercial crop consisted of smut susceptible varieties, this required a comprehensive review of the sugarcane improvement program with the objective of developing resistant varieties with acceptable agronomic performance in the shortest possible time. An appropriate program was developed, and was funded by BSES, CSIRO, QDPI&F, and SRDC. Some of the funding provided by the Federal Government, through SRDC, included a requirement to review the smut resistance breeding strategy early in 2009.

The outcomes in terms of adoption of new, smut resistant varieties by growers and the adoption of smut resistance breeding strategies by the BSES-CSIRO sugarcane improvement program appear to have been very effective. Resistant varieties are being widely adopted, additional resistant varieties are being released to the industry, and a comprehensive research program was developed to minimise the effect of sugarcane smut on the industry.

In order to ensure the recovery of valuable genes for agronomic traits in germplasm classified as susceptible, the SmutBuster sub-program was initiated. This was to enable accelerated development of smut-resistant clones with high agronomic value. SmutBuster includes a substantial research component addressing a wide range of screening methods.

Having received presentations by participants in the BSES-CSIRO sugarcane improvement program, 16-19 February, 2009, the review panel commissioned to examine the R&D response to sugarcane smut make the following specific recommendations:

Recommendation 1: One more series of SmutBuster crosses should be initiated. An assessment of progress should then be made to decide when to curtail the program.

Recommendation 2: The panel recognises the significant value inherent in crossing to and among varieties classified as susceptible, and the further use of smut-susceptible varieties is likely to be justified beyond the life of the SmutBuster program.

Recommendation 3: The level of resistance in SmutBuster crosses should be assessed by ratooning the inoculated disease trials rather than ploughing those trials out after the plant crop.

Recommendation 4*: An assessment of the relative agronomic performance of SmutBuster crosses should be made by including a random sample of SmutBuster crosses in Stage 1 of the core program.*

Recommendation 5*: Continue epidemiological research related to disease expression, severity, and rates of disease increase in ratoon crops.*

Recommendation 6*: The research comparing incidence and severity in inoculated tests should be continued in all smut resistance screening trials.*

Recommendation 7*: Evaluate the effect of age and bud position on response to inoculation to improve the reliability and consistency of results from smut screening trials.*

Recommendation 8*: We support the continuation of experiments to confirm that the accelerated methods of assessment give a true reflection of smut development under conventional farming practices.*

Recommendation 9*: Preliminary research already under way on pathogen assays using tissue staining and other approaches should be continued under the auspices of SmutBuster, but their potential should be critically evaluated in June 2010.*

Recommendation 10*: Due to the complex nature of the sugarcane genome, the progress of molecular marker research was expected to be difficult, and this was reflected by progress for selection of markers for smut resistance. Therefore, unless further progress can be made, specific investment for smut resistance selection should be curtailed.*

Recommendation 11*: We recommend that 400 preliminary selections from the CAT stage for one region be tested using the NIR bud scanning method and in inoculated trials on the smut farm. Selections from the two methods should be compared in a further inoculated and NIR trial. This should be continued for several cycles to establish the utility of the NIR method for evaluating smut response.*

Table of Contents

1. Introduction

Sugarcane smut was found in the Isis mill area of the Queensland sugar industry in June 2006. BSES Limited (BSES), the principal R, D &E organisation serving the industry, and the Queensland Department of Primary Industries and Fisheries (QDPI&F) immediately responded to the outbreak by quarantining the farm on which the disease was found and other smut infested farms in the vicinity. Initially, there was a massive effort directed towards the eradication of the disease. However, within five months, it became clear that it would be impossible to contain the disease. BSES, and its partner in genetic improvement, CSIRO Plant Industry, then assumed lead responsibility for the R&D response to controlling the disease.

Based on information from other countries that had controlled smut, it was obvious that the only economic method of control was resistant varieties. It was important to reduce productivity losses from the release of unproductive but resistant varieties, and minimise the extended impact on the breeding program. BSES-CSIRO had already recognised the problems they would face if smut were to arrive and had developed new strategies to breed resistant varieties that had acceptable productivity in addition to the pre-emptive work carried out since 1997. Funding, initially, was provided by BSES, CSIRO and QDPI&F. In 2008, the Federal government provided $2m for the production of improved sugarcane varieties, and this funding was provided through SRDC. The BSES-CSIRO sugarcane improvement joint venture was successful in obtaining the funding for work towards the development of productive, smut resistant varieties in a project called SmutBuster.

The SRDC-BSES research agreement for the SmutBuster project includes a requirement to review the breeding strategy for smut resistance

> "We will review our smut resistance breeding strategy early in 2009."
> "Report on smut resistance breeding strategy delivered (by May 2009)"

In January 2009, BSES commissioned Professor Jeff Hoy from Louisiana State University, Professor Bob McIntosh, University of Sydney, and Dr Mac Hogarth, formerly with BSES and SRDC, to conduct the review.

Review of smut resistance breeding strategy

The terms of reference for the review were:
1. Review the impact of the smut incursion on the BSES-CSIRO breeding program
2. Review the response of the plant breeding program through the smut incursion and breeding strategy
3. Review the communication strategy and the adoption of smut resistant varieties by industry
4. Recommendations for improvement to the current program
5. Any other comments on the BSES-CSIRO breeding program

As part of the review, we were asked specifically to consider the SmutBuster project as required by SRDC.

o **TOR1. Review the impact of the smut incursion on the BSES-CSIRO breeding program**

In 2006, 70-80% of the sugarcane crop was susceptible to smut, creating the potential for a catastrophic economic loss. In addition, about 80% of the parent collection was classified as susceptible, and the loss of this parental material would have a prolonged and severe effect on the ability of the sugarcane improvement program to maintain and improve productivity.

This was recognised in the 1990s, and BSES, with funding from SRDC, developed a smut incursion plan. In 1998, smut was found in the Ord River Irrigation Area (ORIA), and the smut incursion plan was put into action.

In addition, BSES had already obtained funding from SRDC to conduct smut resistance tests in Indonesia. After the smut incursion in WA, BSES staff visited Indonesia and negotiated a substantial increase in the number of varieties that could be tested annually for smut resistance. It was agreed that up to 250 varieties could be tested annually. In 1999, CSR, CSIRO and the WA Department of Agriculture, with the assistance of BSES and SRDC, commenced smut resistance screening work in the ORIA as well.

Both commercial and parent varieties were tested for resistance. Therefore, when the smut incursion was identified in Queensland, there was a great body of knowledge about the vulnerability of the commercial crop and the susceptibility of the parent collection.

The knowledge about the response of existing commercial varieties to the disease enabled the industry to respond rapidly by identifying smut resistant varieties to replace susceptible ones. These varieties were made available to growers in affected areas, so that the proportion of susceptible varieties could be quickly reduced.

The sugarcane improvement program commenced breeding for smut resistance after the incursion in WA, and the pre-emptive work on disease testing facilitated the making of smut-resistant crosses. In 2000, BSES breeders and pathologists set a target that at least 50% of the crosses should come from resistant and intermediate crosses. In 2004, this was adjusted to 50% intermediate and 25% resistant x resistant crosses. This was almost achieved in 2005 and has been exceeded since.

The impact of the smut incursion on the sugarcane improvement program was reduced substantially by the outstanding pre-emptive work that had been done between 1998 and 2006 in Indonesia and the ORIA.

o   **TOR2. Review the response of the plant breeding program through the smut incursion and breeding strategy**

The sugarcane improvement program was already responding pre-emptively to a possible smut incursion when smut arrived in Queensland in 2006. In addition, prior to the smut incursion in Queensland, BSES had approved the planting of crosses with high breeding value in WA for selection of resistant varieties and parents. Following the incursion, a decision was made to undertake those tests near Bundaberg.

In response to the incursion, the BSES-CSIRO sugarcane improvement program has:
•   Initiated the smut screening program using inoculation techniques at the dedicated smut farm established near Bundaberg.
•   Initiated research on the epidemiology of smut to assist in the decision process for variety release.
•   Built a third photoperiod house at Meringa specifically to make crosses between smut-resistant parents.
•   Commenced the SmutBuster program involving 400 crosses annually using parents with high breeding value but with smut-susceptibility levels exceeding those that are acceptable.
➢   Some 30,000 seedlings are planted each year.
➢   The aim of the program is to identify varieties with transgressive segregation for resistance to smut while retaining the elite characteristics of the Australian germplasm. This

would accelerate the development of elite resistant parents for the crossing program. The core program would use this germplasm as well as more exotic sources of resistance.

➢ Initiated research to evaluate inoculation methods for original seedlings so susceptible plants can be eliminated rapidly.

➢ Initiated a number of methods to characterise smut response, e.g. histological and chemical assay methods, NIR, and molecular markers in comparison to traditional field-based assessments.

➢ Comparisons of response to inoculation vs natural infection.

➢ Comparison of response ratings based on incidence or severity data.

➢ Evaluation of the use of fungicides for prevention of infection following hot-water treatment during variety propagation.

The response by the sugarcane improvement program to the incursion has been rapid, comprehensive and, in our opinion, entirely appropriate. This was enabled by the pre-emptive activities undertaken as a consequence of risk analysis prior to the incursion.

In particular, the SmutBuster sub-program is well-planned, innovative and supported by appropriate research input. We believe it has a high probability of achieving significant progress in producing elite, smut-resistant lines.

o **TOR3. Review the communication strategy and the adoption of smut resistant varieties by industry**

There are good communication channels in the sugar industry, and these were used effectively by BSES to inform the industry about the significance of the smut incursion and the steps that would have to be taken to control the disease both prior to and after the incursion in Queensland. Various communication approaches were used including meetings with growers, printed materials, radio interviews, weekly teleconferences, and DVDs sent to all growers.

Variety guides were kept updated with the latest smut information, and this information is now on-line in QCANESelect. We understand that this information is frequently accessed by growers.

Growers were advised about the resistance levels of the varieties available to them and were encouraged to plant varieties with resistant or intermediate responses. Varieties recommended by BSES were readily adopted by growers except where there were significant constraints beyond their control such as in the Herbert where the increase in seed was delayed by one year due to flooding.

The communication strategy was very effective. The industry was well-informed at all stages of the incursion and has shown a willingness to adopt the new varieties.

o **TOR4. Recommendations for improvement to the current program**

As the response program has only been in operation for two years, there is insufficient data to make definitive statements about the success or otherwise of the program. However, some data are available, and the results so far seem promising.

For example, consistent trials at the smut farm have provided ratings for response to smut for parent varieties and advanced clones under selection. There has also been progress in

the inoculation of seedlings for the SmutBuster program. It appears likely that this program will accomplish the objective of obtaining adequate frequencies of resistant clones from crosses involving the part of the population classified as susceptible. This is because the different genes leading to resistant responses can be accumulated in some offspring of the crosses.

One issue that needs to be addressed is the number of series of seedlings in the SmutBuster program.

Recommendation 1: One more series of SmutBuster crosses should be initiated. An assessment of progress should then be made to decide when to curtail the program.

Being a logarithmic scale, the method for rating smut response in disease is non-linear. As a result, ratings 1 to 5.9 are used for varieties with per cent infection of 0 to about 30% and ratings 6 to 9 are used for the remainder up to 100%. Varieties rated >6 are classified as susceptible. This is a conservative rating scale necessitated by the urgency of the threat. It is, therefore, likely that varieties classified as "susceptible" will have genes for resistance to smut as well as desirable agronomic genes. At present, many susceptible varieties are being used as parents in the SmutBuster program but these susceptible varieties might not be used in the core program. This is entirely appropriate as it enables the sugarcane improvement program to make rapid progress in breeding resistant varieties while continuing to access the favourable agronomic genes in parents classified as susceptible. However, when the SmutBuster program concludes, the sugarcane improvement program will need to decide whether any of the varieties classified as susceptible should continue to be used in the crossing program. This decision should be based on the level of resistance in SmutBuster crosses involving the susceptible parents, the relative agronomic performance of SmutBuster crosses, and the outcome of epidemiological research on rates of disease increase in different production areas.

Recommendation 2: The panel recognises the significant value inherent in crossing to and among varieties classified as susceptible, and the further use of smut-susceptible varieties is likely to be justified beyond the life of the SmutBuster program.

Recommendation 3: The level of resistance in SmutBuster crosses should be assessed by ratooning the inoculated disease trials rather than ploughing those trials out after the plant crop.

Recommendation 4: An assessment of the relative agronomic performance of SmutBuster crosses should be made by including a random sample of SmutBuster crosses in Stage 1 of the core program.

Evidence exists that some varieties have many diseased plants with low severity in each plant and, consequently, the crop suffers minimal production loss. Research is being conducted to determine if an index relating smut incidence and severity would provide a more meaningful rating of the effect of disease on production in a commercial situation. This obviously also has relevance for the rating of varieties for use as parents.

Recommendation 5: Continue epidemiological research related to disease expression, severity, and rates of disease increase in ratoon crops.

Recommendation 6: The research comparing incidence and severity in inoculated tests should be continued in all smut resistance screening trials.

We were shown data indicating that the age and position of buds on a stalk had a significant effect on the response to inoculation. This is an important issue and requires further investigation.

Recommendation 7: Evaluate the effect of age and bud position on response to inoculation to improve the reliability and consistency of results from smut screening trials.

At present, an accelerated testing regime is being used incorporating a 4-month plant crop followed by assessment in the ratoon crop at 3-4 months. Trials have been planted in which "normal" crops are grown with 12-month plant crops followed by normal ratoons.

Recommendation 8: We support the continuation of experiments to confirm that the accelerated methods of assessment give a true reflection of smut development under conventional farming practices.

Research is under way in the SmutBuster program on different methods to evaluate and select for resistance. Two methods attempt to evaluate the extent of pathogen infection and two are evaluations of disease resistance in the plant. The methods are:

• A histological method to detect the extent of fungal development in bud tissue of resistant and susceptible varieties. This research is underway but data are very limited, and it is impossible to draw any conclusions about the potential of this method.
• An assay for pathogen development based on ergosterol concentration was mentioned but no data were presented.
• A comprehensive program to investigate the potential of molecular markers for parental selection for a range of economic traits including smut resistance. Progress to date is not particularly encouraging for using molecular markers as a stand alone method for selection of smut-resistant varieties. We were impressed with some of the evidence showing that molecular markers could be useful for introgressing alien chromosomes.
• Investigations using Near Infra-red Spectroscopy (NIR) on bud tissue as a means of predicting smut response have provided very encouraging results. The research is continuing to strengthen the association between NIR measures and smut disease ratings from inoculated tests.

Recommendation 9: Preliminary research already under way on pathogen assays using tissue staining and other approaches should be continued under the auspices of SmutBuster, but their potential should be critically evaluated in June 2010.

Recommendation 10: Due to the complex nature of the sugarcane genome, the progress of molecular marker research was expected to be difficult, and this was reflected by progress for selection of markers for smut resistance. Therefore, unless further progress can be made, specific investment for smut resistance selection should be curtailed.

Recommendation 11: We recommend that 400 preliminary selections from the CAT stage for one region be tested using the NIR bud scanning method and in inoculated trials on the smut farm. Selections from the two methods should be compared in a further inoculated and NIR trial. This should be continued for several cycles to establish the utility of the NIR method for evaluating smut response.

o **TOR5. Any other comments on the BSES-CSIRO breeding program**

We were impressed by the professionalism and dedication of the staff of the sugarcane improvement program. The collaboration between BSES-CSIRO and the Indonesian Sugar Research Institute and between BSES-CSIRO and the WA Department of Agriculture has clearly been very beneficial to the response to the smut incursion. The collaboration between scientists from BSES-CSIRO and colleagues overseas and within Australia has also been of great benefit to the program.

Acknowledgements

We appreciate the opportunity to review an outstanding program. We would like to acknowledge the assistance of Dr Ross Gilmour, Mr Barry Croft, Dr Mike Cox, Dr Nils Berding, Dr Shamsul Bhuiyan, Mr Trevor Willcox, Dr Scott Hermann, Dr Michael O'Shea, and BSES staff at Bundaberg and Brisbane.

Prof. Jeff Hoy   Prof. Bob McIntosh

Dr DM Hogarth, AM

APPENDIX 8

BSES-CSIRO JV Response to Smut Review Report

Recommendation 1: One more series of SmutBuster crosses should be initiated. An assessment of progress should then be made to decide when to curtail the program.

We agree that one more series of crosses should be planted in 2010. The first CATs will be harvested in 2010 and we will be able to compare the rEGV means of SmutBuster and Core trials. In terms of deciding when to curtail the program, it would seem that an arbitrary decision will need to be made. If it appears little progress has been made, we would not continue but even if progress has been made, there may be little value in continuing the program once high-value smut resistant clones and parents dominate our populations. Then, greater gain will be made using the core, family selection-based program. SmutBuster clones will be compared with those arising from the core program as they progress through CATs and FATs. This will be simplified through the use of specific clone designations for SmutBuster clones.

Recommendation 2: The panel recognises the significant value inherent in crossing to and among varieties classified as susceptible, and the further use of smut-susceptible varieties is likely to be justified beyond the life of the SmutBuster program.

The crossing program will be altered when the SmutBuster crossing program comes to a close. We will reassess the smut disease-loss penalty applied in the estimation of $EBV (breeding value) especially for varieties rated moderately susceptible (Rating 7). The penalty will be re-evaluated based on data from the epidemiology study on the performance of varieties of intermediate susceptibility and from an assessment of the proportion of susceptible, intermediate and resistant progeny from the different resistant classes of crosses. This will allow a judgement on the efficiency of including these crosses in the routine breeding program.

Recommendation 3: The level of resistance in SmutBuster crosses should be assessed by ratooning the inoculated disease trials rather than ploughing those trials out after the plant crop.

The 3-eye sett population planted in 2008 will be ratooned in 2009 after selection and the percentage of susceptible clones will be estimated in the young ratoon crop (about 3 months of age). This will give us an estimate of the mean and range of the proportion of resistant clones in crosses between combinations of R, I and S parents. If necessary, this can be repeated in 2010.

Recommendation 4: An assessment of the relative agronomic performance of SmutBuster crosses should be made by including a random sample of SmutBuster crosses in Stage 1 of the core program.

We will attempt to include some SmutBuster crosses in the core program in 2010.

Dr Wei did some preliminary work that showed that the average $EBV of SmutBuster crosses was higher than that of core crosses, which provides some evidence of the potentially higher value of these crosses.

Recommendation 5: Continue epidemiological research related to disease expression, severity, and rates of disease increase in ratoon crops.

This is a high priority for the SRDC funded smut epidemiology project. Jeff Hoy conducted an independent review of this project and he made recommendations on the how this research should be progressed in commercial fields in the different regions of the industry.

The disease development trial at Bundaberg which includes 35 important commercial varieties and the natural spread trials at Bundaberg and Mackay will be continued. These trials will give valuable information about the disease development and disease severity of commercial varieties.

Recommendation 6: The research comparing incidence and severity in inoculated tests should be continued in all smut resistance screening trials.

This research will be continued in the 2008 routine screening trials and in the disease development trial. The data from these trials will be reviewed and a decision will be made on whether future ratings will be based on incidence, disease severity or a combination of these rating methods.

Recommendation 7: Evaluate the effect of age and bud position on response to inoculation to improve the reliability and consistency of results from smut screening trials.

Further research will be conducted on the effect of bud age (position) and how this influences resistance. We will also investigate how the maturity of the stalks used in smut resistance screening trials affects the observed resistance. Recommendations will be made on how we can manage this characteristic to obtain more consistent smut resistance ratings.

Recommendation 8: We support the continuation of experiments to confirm that the accelerated methods of assessment give a true reflection of smut development under conventional farming practices.

The disease development trial where varieties were inoculated by the dip method and will be grown for a conventional plant and ratoon crop will be compared with the same varieties inoculated by the same method but ratooned after 3-4 months. This experiment will tell us if the rapid ratooning gives the similar ratings compared to trials where the plants are grown in a more conventional method. This experiment will be repeated if necessary.

Recommendation 9: Preliminary research already under way on pathogen assays using tissue staining and other approaches should be continued under the auspices of SmutBuster, but their potential should be critically evaluated in June 2010.

A small experiment will be conducted where the tissue staining method will be assessed using the 10 standard varieties. The research will be evaluated by June 2010 to determine if they have potential for use in the selection program. This would require data suggesting that gain per unit cost is higher than normal screening methods.

Recommendation 10: Due to the complex nature of the sugarcane genome, the progress of molecular marker research was expected to be difficult, and this was reflected by progress for selection of markers for smut resistance. Therefore, unless further progress can be made, specific investment for smut resistance selection should be curtailed.

Currently, various analysis models are being investigated for the DArT marker data and their association with traits of interest. Current field trials including (i) progeny from bi-parental crosses and (ii) cultivars and parents for use in association mapping, will be completed and no further field trials will be planted. The results of the research that is almost complete will be analysed and the analysed results will be reviewed by mid-2010 to determine if potential

exists to use markers for indirect selection. At this stage, it would appear the cost is too high and, if this is verified, further work on markers for selection will be curtailed. This will require reallocation of funds to other more appropriate areas.

Recommendation 11*: We recommend that 400 preliminary selections from the CAT stage for one region be tested using the NIR bud scanning method and in inoculated trials on the smut farm. Selections from the two methods should be compared in a further inoculated and NIR trial. This should be continued for several cycles to establish the utility of the NIR method for evaluating smut response.*

The 400 tentative selections from 2007 CATs from one program will be assessed using NIR and the results compared with conventional smut inoculation methods. The clones selected as resistant by both methods and a sample of clones rated susceptible will be repeated in dip inoculation and screened by NIR in 2010 to confirm the results of the initial test. Depending on results, this will be repeated in 2010 and beyond.

APPENDIX 9

Integration of Sugarcane Breeding and Biotechnology
An Australian Perspective

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Program Leader | BSES Limited
May 2011

BSES Limited

Molecular markers - selection

Requirements

- Selection index to combine phenotypic and marker data for traits of interest
- Simulation of various marker incorporation strategies
  - Scenarios
  - Assumptions
    - Population size, genetic parameters, costs, marker effects
- Acknowledgements: Phil Jackson and Xianming Wei

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Molecular markers - selection

Conclusions

- In order to implement MAS, we require:
  - Medium to high marker effects
    - 20-30% for TCH/CCS, 40-50% for smut
  - Low genotyping costs
    - $5/genotype
- Implementation of MAS in selection is currently not viable
  - Marker effects too low
  - Genotyping costs too high

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APPENDIX 10

List of 31 parents selected for crossing in 2011 together with parentage and smut ratings

Clone	Smut rating	Female parent	Female smut rating	Male parent	Male smut rating
QS06-7026	1	Co1007	3	QA86-1005	5
QS06-7034	5	Co1007	3	QS91-7008	6
QS06-7063	5	CP92-1666	3	Q200	3
QS06-7065	5	CP92-1666	3	Q200	3
QS06-7103	4	F172	6	SP70-3370	4
QS06-7183	5	N27	7	QS87-7430	6
QS06-7316	4	Q208	4	QS91-7008	6
QS06-7329	5	Q220	6	QS87-8210	8
QS06-7556	4	QC82-954	6	F172	6
QS06-7565	4	QC83-626	3	Q200	3
QS06-7639	1	QC89-6019	2	N29	2
QS06-7644	1	QC89-6019	2	SP83-5073	1
QS06-7648	4	QC89-633	0	Q205	9
QS06-7668	1	QC90-289	6	SP78-3137	6
QS06-7701	1	QC90-6001	5	SP70-3370	4
QS06-7708	1	QC91-423	6	Q184	8
QS06-7837	1	QN79-183	7	QC95-6004	1
QS06-7880	1	QN80-3425	1	Q170	9
QS06-7900	1	QN80-3425	1	QC89-6002	8
QS06-8079	1	QN85-2770	8	QN83-657	7
QS06-8151	1	QN87-2109	4	HoCP85-845	1
QS06-8157	5	QN87-2109	4	Q166	8
QS06-8317	1	QN91-227	6	F172	6
QS06-8417	4	QN93-738	0	QN89-2449	7
QS06-8505	1	QN95-285	8	QN89-109	3
QS06-8704	1	QN97-1072	0	QN98-1762	0
QS06-9020	5	QS79-1226	8	QA88-1501	7
QS06-9059	1	QS87-7140	2	QN89-109	3
QS06-9085	1	QS87-7428	6	QN89-109	3
QS06-9165	4	SP70-3370	4	F172	6
QS06-9187	4	SP83-5073	1	QS90-7022	6

APPENDIX 11

List of the top 30 SmutBuster clones from the 2006 series ranked on average plant crop rEGV together with individual trial performance and ranking

Clone	Average rEGV	rank	PNR09-28 rEGV	rank	RAC09-28 rEGV	rank	MUL09-236 rEGV	rank	MUL09-237 rEGV	rank	BIN09-25 rEGV	rank	BIN09-26 rEGV	rank
KQ228	11.96	1	11.96	1										
QS06-8032	11.56	2									11.56	5		
QS06-9075	11.09	3			10.61	9					11.56	4		
QS06-8676	10.82	4	10.75	2	10.39	25	10.77	8	10.52	14	11.67	3		
QS06-8885	10.76	5	10.21	10			11.11	1	9.97	59	11.77	1		
QS06-8055	10.69	6	9.68	40	11.25	1	10.83	5					11.01	4
Q232	10.69	7									10.68	29	10.70	9
QS06-8192	10.59	8	10.19	11	10.11	59			10.67	6	11.39	6		
QS06-8140	10.55	9	9.65	43	10.81	5			10.68	5	11.06	11		
QS06-7923	10.55	10	9.93	22	10.69	8	10.47	16			11.09	10		
QS06-7890	10.53	11	10.56	3	10.05	73			10.64	8			10.87	6
QS06-9128	10.52	12			10.10	63							10.94	5
QS06-9114	10.50	13			10.37	30					10.64	36		
QS06-8106	10.49	14			10.89	3					10.10	106		
QS06-7136	10.49	15	9.70	39	10.47	14	10.63	11					11.17	2
QS06-7938	10.48	16	10.08	15	10.42	21			10.57	12	10.84	20		
QS06-8380	10.48	17			10.38	27					10.57	41		
QS06-8120	10.47	18			10.11	60					10.83	21		
QS06-8686	10.44	19	9.39	82	10.33	34	11.03	2	10.96	2			10.49	16
QS06-8648	10.44	20	9.97	17	10.45	18	10.02	51	10.79	3	10.95	15		
QS06-8230	10.42	21	10.38	6	9.36	330			10.25	25	11.69	2		
QS06-7934	10.36	22	9.81	30	10.84	4	10.41	19			10.38	62		
QS06-8157	10.36	23			9.51	254	10.37	23			11.19	8		
QS06-7943	10.35	24	9.87	25	10.44	19			10.14	39	10.93	16		
QS06-8114	10.34	25	9.62	46	9.93	101	10.78	7					11.05	3
QS06-8119	10.33	26	9.88	24	10.30	36			10.15	35	10.98	14		
QS06-7319	10.31	27	10.03	16	9.85	124	10.60	12					10.78	7
QS06-9062	10.31	28	9.86	27			10.43	18	10.54	13	10.40	59		
QS06-8785	10.25	29	9.55	53	9.51	250	10.85	4	10.04	49	11.28	7		
QS06-8511	10.24	30	9.55	52	11.24	2					9.93	147		