BSES Limited

FINAL REPORT – SRDC PROJECT BSS307

DEVELOPMENT AND IMPLEMENTATION OF NIR BASED PREDICTIVE TOOLS TO RATE SUGARCANE VARIETIES AGAINST SMUT AND FIJI LEAF GALL

by

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BSES Limited Publication
SRDC Final Report SD12004 **March 2012**

CONTENTS

EXECUTIVE SUMMARY

Sugarcane smut and Fiji leaf gall (FLG) are serious diseases and are responsible for significant yield losses. The screening processes for both are difficult and expensive (especially for smut, which at the beginning of this project necessitated overseas trials for resistance screening), and also suffer from inconsistent field responses and trial infestations. As a result, there has been much research conducted in an attempt to simplify screening processes to produce methods which could be utilised together with existing methods to improve the cost and effectiveness of selection processes.

Sugarcane smut is caused by the fungus, *Ustilago scitaminea*, and is one of the most serious diseases of sugarcane. An incursion was identified on the east coast of Australia at Childers in June 2006 and by 2010, had spread to the northern districts of Mulgrave, Tully and Burdekin. The average yield loss reported at the time of the incursion was 6% yield loss for each 10% increase in the percentage of infected plants (Croft *et al*., 2008a). Prior to this incursion, initial smut screening trials were conducted in Indonesia from 1998 and characterised 69% of Australian clones as susceptible (Croft et al., 2008b). At the time, it was considered inevitable that smut would reach the east coast of Australia and there was a pressing need to develop predictive methods based on NIR techniques to estimate varietal smut ratings. It was envisaged that such a tool could be employed within the breeding and selection program to accelerate the identification of smut resistant varieties.

The implementation of NIR tools for plant selection would improve the resource and cost efficiency of the breeding program in several ways. Application at an earlier stage than traditional resistance screening would increase the relative proportion and number of resistant clones that progress through various selection stages. The screening of larger populations of clones earlier in the program would accelerate the rate of genetic gain and provide for the production of greater numbers of resistant varieties on a shorter timescale than traditional methods could deliver.

The key to developing NIR methods for smut and FLG resistance was to correlate NIR spectra to data obtained from appropriate long term field testing for known varieties. The NIR methods concentrated on the sugarcane bud (the site of smut infestation) and the leaf (the site where the leafhopper discriminates between varieties for FLG). Chemometrics techniques of Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression analysis facilitated this process.

The research followed a cyclic regime of calibration development followed by validation or blind testing. The initial calibration data set was developed by obtaining NIR spectra of a particular set of sugarcane clones that are used extensively as “standards” in the traditional field trials. Within screening activities, the performance of an unrated clone is rated or compared against the performance of a set of “standard clones”. These standard clones display reproducible tendencies and are classified on a rating scale from 1-9, with 1 describing the highest resistance to smut and 9 exhibiting high levels of susceptibility. Blind testing developed calibrations central to this research and involved scanning cane of unknown identity and predicting the ratings.

This project originally aimed to complete a research program to develop NIR methods for both smut and FLG and to implement them within the BSES plant breeding program within the life of the project. These aims were subsequently proven to be optimistic and project milestones were renegotiated as the project progressed.

NIR calibrations were successfully developed to predict the resistance of sugarcane clones to smut and FLG. The smut calibrations were improved over the course of three separate validation trials and contain a large quantity of data. Their development and planned

implementation was hampered by instrument difficulties (which have been overcome), validation trial issues and a change in the importance of implementing predictive methods as the smut incursion rapidly spread throughout Australia after this project had begun. For the smut calibrations, all work beyond the successful proof of concept activities were transferred to the SmutBuster SRDC project (BSS325). This work has been included in the BSS325 Final Report and will only be mentioned briefly in this report. The authors believe that the smut calibrations could be further developed into a useful predictive tool for the plant breeding program

The FLG calibrations have not been subjected to rigorous blind testing and their relative worth cannot yet be judged. Extensive validation testing needs to occur before these calibrations could be considered for implementation as a screening tool. Work on the FLG calibrations suffered because of the over-riding importance to produce methods for estimating smut resistance. FLG was considered to be significantly less important, and due to the instrumental problems mentioned above, this component of the project was reduced to the completion of proof of concept activities only, with no progress attempted regarding implementation of the method. There do not appear to be significant drivers to progress this work further at this time.

1.0 BACKGROUND

The plant breeding cycle consists of two main areas, the creation of genetic variability (through crossing) and discrimination within the variability (selection). Along the 10-12 year journey to produce commercial varieties, a large number of clones (around 100,000 per year) are assessed primarily for yield and sugar content traits in early selection stages. They are also assessed for their pest/disease resistance, fibre quality and sugar quality traits, but this must be performed at a much later stage in the overall selection process as techniques for their evaluation are not suitable to be applied to larger numbers of plants and are therefore must be performed at this stage.

For a screening tool to be adopted within a plant selection program for use on large numbers of samples, the method employed needs to be inexpensive, require minimal sample preparation, and be relatively rapid in producing a result. Spectroscopic methods fit this description nicely and have considerable cost and throughput advantages over traditional methods of analysis.

This research project began with the intention of building upon proof of concept work which had already been performed and reported demonstrating that near infrared (NIR) techniques could predict the resistance status of sugarcane varieties for both smut and FLG.

Smut background
Sugarcane smut is a devastating fungal based disease and is one of the most serious diseases of sugarcane. Smut was reported for the first time in Australia in July 1998 in the Ord River Irrigation Area, and eight years later, was identified on the east coast of Australia at Childers. It has since spread and has been observed in most commercial sugarcane growing districts.

Smut, which is caused by the fungus *Ustilago scitaminea* H. and P. Sydow, can cause yield losses of up to 50% in susceptible cultivars, as well as rendering subsequent ratoon crops unprofitable. Plant infection occurs when spores come into contact with buds on standing stalks or germinating buds in the soil (Comstock, 2000). The fungus penetrates the buds and the hyphae grow in association with the plant's meristem. Upon infection with the fungus, early symptoms can occasionally be recognised, such as rigid spindle leaves, thin shoots, narrow leaves, bud proliferation, stem and leaf galls and an overall grassy appearance. However, these symptoms are not reliable and can often be the result of other diseases or weather conditions. Actively infected sugarcane stalks producing whips provide the only distinct symptom associated with sugarcane smut.

Smut can be effectively controlled through the breeding of resistant varieties. The methods for determining levels of varietal resistance involve planting field trials with sugarcane setts that were inoculated with smut spores or by planting cultivars for evaluation within rows of infected cane and allowing natural spread of the disease to take place. Both methods have drawbacks but, in particular, they are limited geographically in terms of where such field trials can occur, they are slow to produce results (up to plant and two ratoon crops are required), and the results obtained can differ between methods (Ferreira and Comstock, 1989). This complexity and the urgent need to rate Australian varieties and advanced clones for resistance as part of a strategy to minimise the damage of a smut incursion led to this research project aimed at developing a rapid, indirect screening method to identify smut resistant cultivars.

Prior work at BSES hypothesised that NIR based methods could also provide a solution to rating varieties for smut resistance. The starting assumption was that the level of resistance/susceptibility of the sugarcane variety could be directly related to the interaction of the smut fungus with sugarcane buds. This led to an examination of bud surfaces by both traditional morphological approaches and by indirect surface analysis using spectroscopic techniques. These proof of concept activities were published, demonstrating the potential of NIR based methods for smut rating prediction (Churchill *et al.,* 2006).

FLG background

Fiji leaf gall is one of the Australian sugar industry's most serious diseases, and is a result of plant infection by the Fiji disease virus (FDV) which is transmitted in Australia by the sugarcane planthopper *Perkinsiella saccharicida* Kirkaldy (Smith, 2000). Past epidemics have threatened industry viability in sugarcane growing regions south of Proserpine, which produce around 2.3 million tonnes of sugar (43% of the total Australian crop). Current control methods include the planting of resistant varieties in regions where the disease is prevalent, and use of disease-free planting material. The disease can cause total crop loss and varietal resistance is now a prerequisite for the release of a variety in areas south of Proserpine.

Fiji leaf gall ratings are difficult to determine due to the lack of infection of appropriate field trials. Traditionally, new varieties have been screened for resistance by planting small plots of the varieties between rows of cane infected with Fiji leaf gall, and using natural populations of the planthopper spread the disease to the test varieties. However, many annual trials fail due to insufficient or excessive infection (Croft *et al.*, 2004). A new glasshouse/field method recently reported has produced encouraging results (Croft *et al.*, 2004), but this and traditional methods are relatively labour intensive, thereby limiting the number of varieties that can be screened per year.

During the selection process, approximately 15-30% of prospective new varieties are discarded due to Fiji leaf gall susceptibility, and as such, the overall costs of the plant breeding and selection program would be greatly reduced by being able to discriminate between clones and discard susceptible ones at an earlier stage. In addition, there are other intangible costs incurred including the reduction of overall genetic gain by restricting crosses to resistant parents.

Prior proof of concept work has successfully demonstrated that FLG resistance ratings could be predicted using NIR spectra of the leaf surface (Purcell *et al.* 2005). This project aimed to build upon the reported preliminary work to provide robust calibrations for subsequent demonstration, validation and implementation purposes.

NIR methods and background

The use of NIR spectroscopy coupled with the application of chemometrics has allowed the development of fast, reliable, non-destructive routine analysis in many fields. Generally, these types of analysis have considerable advantages over traditional methodologies including cost, throughput, non-destructive sample preparation and analysis. NIR spectroscopy is a technique used to measure the vibrational energy levels associated with chemical bonds within a sample. The spectrum obtained is unique, and can be considered to be like a fingerprint. From the spectrum, mathematical treatments can be applied to obtain information about the sample, and from a large, background database of spectral and analytical data, inferences can be made regarding molecular concentrations. Our study was quite different in that it has used NIR spectra to attempt to correlate with varietal ratings for smut and FLG. As outlined above, proof of concept activities have been published that proved that NIR techniques were capable of producing correlations to sugarcane smut (Churchill *et al.,* 2006) and FLG ratings (Purcell *et al.,* 2005).

This project was designed to progress these proof of concept activities and produce NIR calibrations of sufficient quality to support implementation within the plant breeding program as predictive tools.

2.0 OBJECTIVES

This project sought to take existing research outcomes and use them to develop and implement new predictive tools for varietal selection against smut and Fiji leaf gall. These tools would improve the delivery of resistant varieties in shorter timeframes, and reduce the cost of varietal selection.

Specifically, this project aimed to:

- build upon existing proof-of concept research that has demonstrated that varietal resistance to smut and Fiji leaf gall can be correlated with near infrared (NIR) spectroscopic measurements;
- develop prototype methods with acceptable prediction errors and assess them for suitability;
- optimise and blind-test prototype methods in conjunction with plant breeding and pathology staff;
- optimise and automate (where possible) systems for statistical data treatment, including spectral collection, chemometrics data treatment and tools for rating clones;
- devise a total on-farm or on-station application for implementation within the BSES-CSIRO Joint Venture for Plant Improvement to deliver significant efficiency gains and cost reductions over traditional screening methods.

Good progress has been made towards the development of accelerated NIR based tools for plant breeding application. This project built upon existing proof-of concept research which demonstrated that varietal resistance to smut and Fiji leaf gall could be correlated with near infrared (NIR) spectroscopic measurements.

Predictive tools for smut ratings

- This research was progressed from a “proof-of-concept” stage by conducting multiple sampling trips to expand the calibration set during 2007, using a series of varieties commonly used in traditional smut field trials. Sampling methodology was developed by scanning buds from the middle of the stalk region (identified by counting the number of buds below the apical meristem) using a fibre-optic probe. A finalised protocol was established which required the scanning of buds 15 and 16 using an angle of 90° - this procedure produced the most reproducible scanning sites which correlated with the smut ratings of the individual varieties.
- Confidence in the calibration models was improved by continuously adding new data, especially data which incorporated geographical diversity, and samples which allowed a relatively evenly distribution across the full rating range of 1-9.
- Formal methodologies were prepared to encompass all aspects of using the NIR techniques. This included sample preparation, NIR scanning procedures, data processing protocols and procedures to report predicted smut ratings. Staff training was conducted where necessary regarding sample preparation, NIR instrument operation, system monitoring and basic diagnostic testing. Systems were also developed to provide for automatic backup and semi-automatic handling of data and the overall procedure was used successfully at four three different BSES sites (Woodford, Bundaberg, Meringa, Burdekin), demonstrating the robustness of the methods developed.
- Trial work established the maximum likely throughput for applying NIR methods within the breeding program. Discussions with BSES plant breeding staff led to the target of 50 samples per day as an acceptable minimum throughput. Existing estimates regarding the cost of rating clones for smut are in the order of $80 per clone. The NIR methods would produce a method that would be significantly cheaper than this figure.

- Models developed were validated in three separate large scale trials. Blind testing of the developed calibrations was central to this research project and involved scanning cane of unknown smut ratings and predicting the smut rating. The NIR operator was not aware of the identity of the variety, and by definition, did not know the smut rating.
- Some of the results from the large scale validation trials were outlined within BSS307 Milestone Reports, with remaining work transferred to the SmutBuster SRDC project BSS325 during 2009. Results after this time have been reported in detail within the BSS325 reporting process.
- The most recent blind validation trial used a method where two different calibrations were used in parallel, one which performed better on clones with high smut ratings, and another which performed better on low smut ratings. This combined approach does a good job at the resistant and intermediate parts of the rating scale (misclassifying only 15% of these clones as susceptible), but successfully identifies only one-third of the susceptible clones.

Predictive tools for FLG ratings

- Proof of concept work reported outside this project was repeated with similar conclusions drawn – it was possible to predict FLG ratings from sugarcane leaf NIR spectra. New data was able to be used together with the earlier data to provide an improved calibration model.
- An investigation into the NIR spectral wavelengths responsible for describing the most variance in the model revealed some specific spectral ranges where future calibration model development should focus.
- Instrument stray light effects were uncovered at higher wavenumbers which were affected the spectral quality of leaf NIR spectra. These effects are primarily due to stray light within the sampling environment (not part of the sampled plant surface) which is being reflected back into the optical probe. Subsequent work using an artificially darkened scanning environment circumvented most of these effects.
- Calibration development using the earlier uncovered wavelength ranges (largely aimed at reducing moisture effects) and a dark scanning environment produced calibrations with a Standard Error of Prediction (SEP) of 1.7 rating units. Based on work conducted with smut ratings, this level of error was acceptable for the current state of development of this calibration.
- Further sampling and data collection added more data to this calibration model and provided a Standard Error of Cross Validation (SECV) of 1.5 rating units. The model contained data on a total of 38 clones which were sampled from three separate environments (not all were available at each environment). This provided confidence that the calibration model was able to provide adequate predictions despite the presence of environmental differences such as soil type, crop class and nutrient status. The project work on FLG predictive methods ceased at this point.
- Project milestones which were intended to add further data, blind validate the resultant calibration models and implement the NIR method within the plant breeding program were removed from the project. This was due to instrumental difficulties which were experienced towards the end of the project, and the reduced importance placed on the successful development of FLG predictive methods by the plant breeding program.
- Progress in the overall development and implementation of a predictive method for FLG remains “parked” at this point. Steps remaining would be the addition of more data to add to the robustness of the calibration models, blind validation of the models, assessment of NIR outcomes and the subsequent development of a routine method for implementation.

3.0 METHODOLOGY

3.1 Smut ratings

Generation of calibration models for smut ratings

Earlier work reported by Churchill *et al.* (2006) was repeated and verified that NIR calibrations could be constructed to predict sugarcane smut ratings. The preferred conditions for NIR scanning was to scan the mid-buds of the stalk (typically buds from internodes 15 and 16 counting down from the apical meristem), using a fibre optic probe contact angle of 90º. These initial calibrations were slowly built up with more data which required sampling trips to locations such as Bundaberg, Woodford and the Burdekin.

This process is a methodical one and involves the validation of any new data, and if successful, reincorporation of this data into the original calibration set, and recalculation of new calibrations. The cycle is then repeated for each subsequent sampling as additional data is incorporated into the calibrations. The overall intent of this process is to produce a calibration which is robust enough to deal with the environmental variation as well as identify the differences in smut ratings which are largely a genetic or varietal effect. The fact that a satisfactory calibration model can be constructed by sampling across multiple regions, years, crop classes, soil types and probably nutrient status, leads us to conclude that these environmental effects are not significant when assessed against the overall model performance.

The calibration produced at the end of this sampling process is shown in Figure 1 as a validation plot of NIR predicted FLG ratings against traditional FLG ratings. The final version (on the right) is compared against an earlier version which contains less data. It can be clearly seen that the right-hand model exhibits better calibration model characteristics and should perform better during subsequent validation.

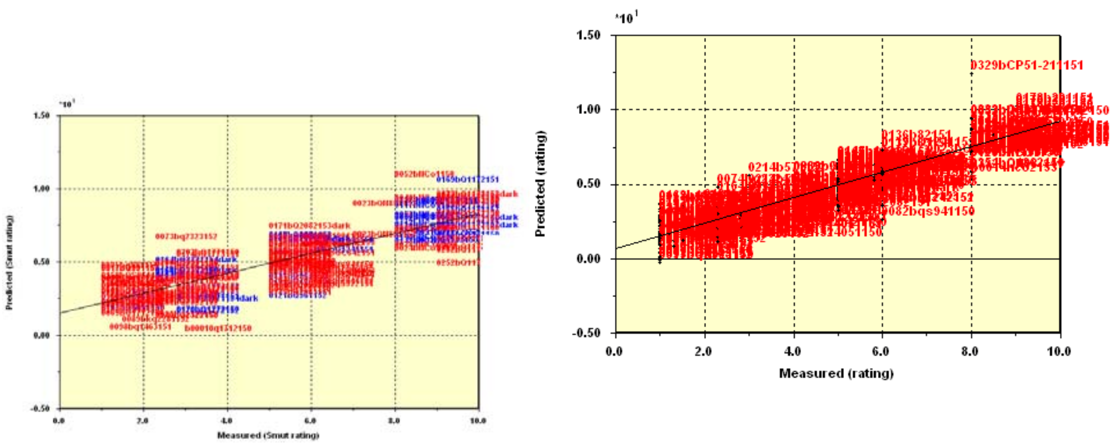

Figure 1 - Partial least squares plots of the prediction models used for the Woodford and Burdekin (left) and Bundaberg (right) validation experiments. The Bundaberg model has had significantly more data built into it, and exhibits better intercept and slope characteristics.

Validation work for the smut rating calibrations

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

This section of the report describes work activities to validate the developed NIR methods for smut ratings 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.

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. This data was 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.

The two regression models on their own 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. We favour a combined approach with these models, 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 overpredicts 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 1. 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 – 30 resistant, 185 intermediate and 85 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 1 - Results using the combined model approach for predicting the smut ratings of 300 clones from the Bundaberg CAT

	ACTUAL RATINGS			
PREDICTED	Resistant	Intermediate	Susceptible	**Total**
R	5	8	16	**29**
I	34	38	116	**188**
S	8	7	68	**83**
Total	**47**	**53**	**200**	**300**

Clearly, these results show that the NIR models do a reasonably good job for resistant clones but do not perform as well for the intermediate and susceptible clones. Applying this model as a screening tool would result in 83 clones being removed, of which 68 are known to be susceptible (shaded cells). In order to improve the performance in differentiating intermediate from susceptible clones, we 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 1).

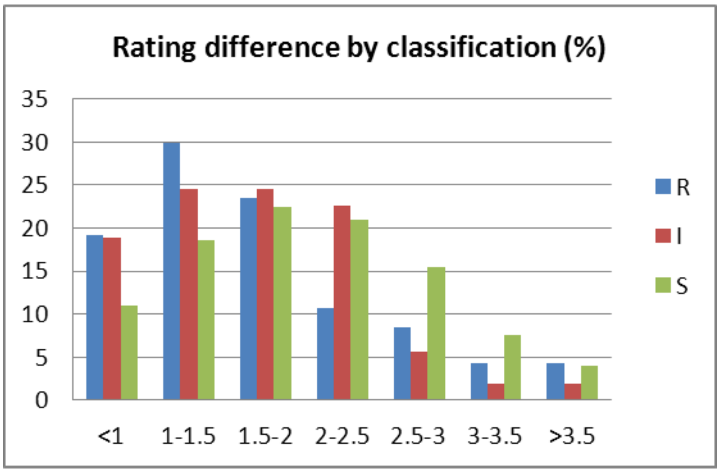

Figure 2 - Plot of the percentage of observation for each class against the differences between the two NIR models

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 2.

Table 2 - Results using the combined model approach with the additional rule for predicting the smut ratings of 300 clones from the Bundaberg CAT

	ACTUAL RATINGS			
PREDICTED	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 if used as a screening program, 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.

Future work required

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

- Automation of crude data treatment using a statistical approach. The crude data was 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 2. There are some opportunities to further improve the outcomes from this data, but these improvements must be validated in a separate trial.

3.2 FLG ratings

Generation of calibration models for FLG ratings

Prior work on developing NIR predictions for FLG varietal ratings was published by Purcell *et al.* (2005) and showed that acceptable NIR predictions could be produced using a set of 10 standard clones which possessed reliable resistance ratings. This spectral data was re-examined together with new data and produced an almost identical PLS plot to that shown reported above. This highlighted the fact that the spectral data was of high quality and that the earlier relationships found appear to be robust.

As explained above for smut ratings, a similar set of sampling exercises were undertaken for the model development for the prediction of plant ratings for Fiji leaf gall. Sampling was conducted in Woodford, Kallangur and Bundaberg. Models were constructed by sampling clones across the same range of environmental variables and as above, their performance characteristics were acceptable, leading to the same assumption that these variables are not significant with regard to overall model performance.

During calibration development, a series of stray light effects were observed, most likely from the reflection of light back from the leaf surface. Spectra were run in a normal laboratory operating conditions and again in a darkened environment created by covering the sampling platform, sample and fibre optic probe with a black non-reflective cloth. The conclusion drawn was that NIR spectra collected from the darkened environment were of better quality and contained less spectral variation at higher wavenumbers than those from the “normal” environment. This method of NIR scanning was used for all subsequent FLG work.

The calibration produced at the end of this sampling process is shown in Figure 3 as a validation plot of NIR predicted FLG ratings against traditional FLG ratings. The calibration has a Standard Error of Prediction of 1.7 rating units, which although a little high, should reduce with time as more data is added to the calibration. It is worth noting that the successful development of the smut calibrations began from a similar sized calibration set to this example and had a similar SEP value which provides confidence that a successful calibration can also be developed for FLG ratings.

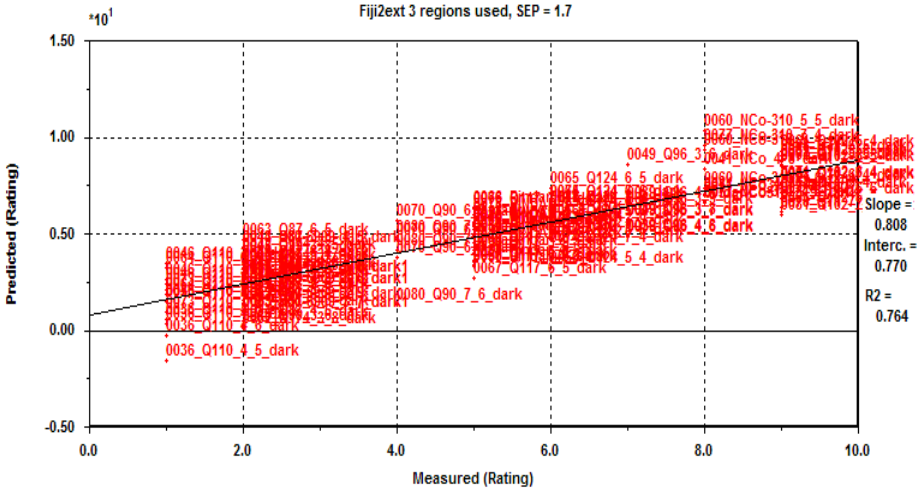

Figure 3 - Plot of predicted versus measured Fiji leaf gall ratings from NIR spectra collected in a darkened environment

Validation work for the FLG rating calibrations

Due to all the difficulties experienced with the FOSS XDS instrument research related to Fiji leaf gall was not progressed to the implementation stage.

The FLG calibration was validated during work conducted at BSES Bundaberg during October 2009. A total of 27 clones were selected for this experiment on the basis that they had previously exhibited reliable field based FLG ratings. From these 27 clones, a total of 235 spectra were collected and were subsequently subjected to chemometric analysis. Various regression models were constructed and compared (work not described here). From the full data set, 12 outliers were identified and removed from the analysis which described 9 significant principal components (PC). The model performance is shown in Figure 4 and contains a Standard Error of Cross Validation (SECV) = 1.49 and an R^2 = 0.71. As described in the appropriate milestone reports, this error is acceptable for a model at the current stage of development, and we anticipate that it will reduce as more data and larger numbers of varieties are added to the calibration set with time.

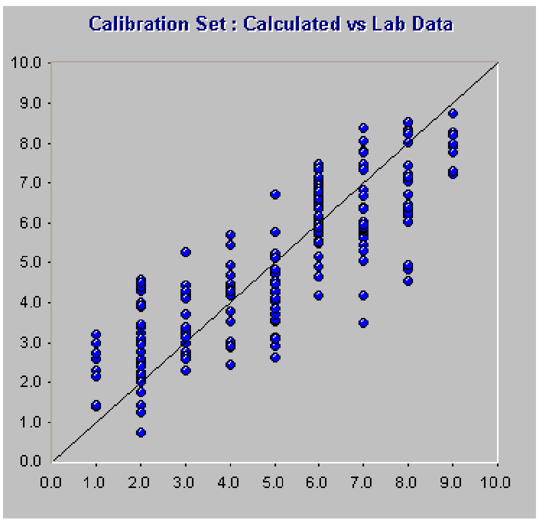

Figure 4 - Plot of predicted versus measured FLG rating from the Bundaberg validation work

The FLG calibrations were not validated any further due to instrument difficulties and a lower priority placed on the development of this predictive tool. As a result, the final milestone of the project was not attempted.

3.3 NIR instrument issues and difficulties

Unfortunately, progress within this project was hampered by the number of difficulties experienced with the FOSS XDS NIR instrument at BSES. Milestone delays during the course of this project were the result of investigations into various instrument non-compliance issues. Eventually, the existing instrument was replaced by the manufacturer. However, it became apparent during testing of the new instrument that some of the issues faced by the project were due to the design and functionality of the instrument rather than the problems that were experienced with the old instrument. In particular, we had observed problems with photometric stability and reproducibility with both the old and newly replaced XDS NIR instruments. Subsequent investigative work revealed that the photometric instability was due to noise associated with high amplifier gain (signal magnification). These issues were particularly identified at high wavelengths and were magnified due to the highly reflective surfaces of sugarcane buds, leading to high absorbance values and the subsequent amplifier gain problems.

The instrument difficulties experienced caused additional problems by creating uncertainty in the minds of the intended end users of the technology, the plant breeders. This has also contributed to the lack of implementation of technology, coupled with higher expectations regarding the quality of the data and recommendations produced.

It is important to stress that despite these difficulties, this does not cast doubt on the performance of the NIR calibrations outlined in this report. The instrument issues were more about the instrument not performing within designated instrument tolerances at specific wavelengths and at high absorbance levels. The NIR calibrations were investigated without the offending wavelengths present in the calibration, and there was no drop in predictive performance.

BSES has since purchased a new benchtop NIR instrument – an Antaris II instrument manufactured by ThermoScientific. The Antaris II differs from the XDS instrument in that it is a Fourier-Transform instrument, rather than using the somewhat older dispersive technology built into the XDS instrument. It offers many advantages which directly relate to this research project, particularly regarding energy throughput which requires less signal attenuation, and software to facilitate calibration transfer which will be important to move the calibrations from XDS format to the Antaris format.

4.0 OUTPUTS

Outputs have been described against the project objectives.

a) *Build upon existing proof-of concept research that has demonstrated that varietal resistance to smut and Fiji leaf gall can be correlated with near infrared (NIR) spectroscopic measurements;*
b) *Develop prototype methods with acceptable prediction errors and assess them for suitability;*

- For both smut and FLG, further work conducted within this project supported earlier findings that NIR methods could be developed to predict resistance ratings.
- NIR calibrations were developed to provide acceptable errors of prediction based upon expanded data sets.
- Satisfactory calibration models were constructed by sampling across multiple regions, years, crop classes, soil types and probably nutrient status, leading to a conclusion that environmental effects were not as significant as the varietal effect when assessed against the overall model performance.
- Processes were developed to optimise calibration performance – this included defining the substrate scanning site, scanning angles and scanning environment.

c) *Optimise and blind-test prototype methods in conjunction with plant breeding and pathology staff;*

- A series of validation trials was conducted where the methods developed were tested in blind situations.
- Staff training was conducted at regional locations when sampling trips and validation trials were conducted. This was performed in anticipation of future implementation where the method would be utilised at various sites. Upon training, local staff were able to generate data of the same quality as the original project staff, proving that there were no technical issues which would prevent implementation.

d) *Optimise and automate (where possible) systems for statistical data treatment, including spectral collection, chemometrics data treatment and tools for rating clones;*

- Protocols were developed to support the large scale use of the NIR calibrations – this included file naming nomenclature, automated backup procedures for spectral data, and definition of spectral data to enable capture within the large BSES NIR library based in Meringa.
- Routine methods of analysis were defined within the FOSS Vision software, enabling a semi-automated analysis of samples with an automatic system for data archiving, nomenclature and preliminary chemometric treatment.
- It was further intended to explore the means of classifying and incorporating this data into SpidNet, the BSES plant breeding database system, but this was not necessary once it became clear that the project was not going to progress to full implementation on the intended timescale.

e) *Devise a total on-farm or on-station application for implementation within the BSES-CSIRO Joint Venture for Plant Improvement to deliver significant efficiency gains and cost reductions over traditional screening methods.*

- Costs were examined for the current methods of screening clones (approximately $50-60 per clone) and the costs for the NIR methods compare very favourably with this.
- Method development studies determined that the NIR methods should be able to provide a throughput of approximately 100 clones per day. The minimum target at the beginning of the project was 50 clones per day which would provide an acceptable throughput for the breeding program.
- Final development of a system for onfarm application was not completed as the project outcomes were not to be delivered.

5.0 INTELLECTUAL PROPERTY AND CONFIDENTIALITY

The only intellectual property to be produced by this project relates to the NIR calibrations for predicting smut and FLG ratings. These calibrations are not currently commercially usable and require further development outside of this project in order to progress them towards potential implementation within plant breeding programs.

There are no confidentiality issues regarding this IP, and project outcomes have already been communicated through publication in peer reviewed journals which have included initial proof of concept work as well as subsequent validation work.

6.0 ENVIRONMENTAL AND SOCIAL IMPACTS

There are no immediate environmental or social impacts upon the completion of this project. Subsequent development and implementation of the NIR calibrations will eventually lead to the enhanced production of productive smut- and FLG-resistant varieties for the Australian sugarcane industry. Maintaining industry productivity through the application of resistant varieties is likely to provide large, positive environmental and social benefits.

7.0 EXPECTED OUTCOMES

The major anticipated outcome from this project was the development of NIR based predictive tools to assist in the selection and subsequent development of resistant varieties for smut and FLG. While the project research activities have been moderately successful, they have not resulted in the development of tools for implementation within selection processes. For reasons outlined in this report, the primary drivers for the implementation of these methods have reduced in importance and the project results were not successful enough to support progressing towards implementation. Work has ceased on the development of these tools but can be restarted in the future if required. Steps required to do so are addressed in Section 8.

Research has shown that NIR methods are capable of predicting the resistance status of sugarcane varieties for smut and FLG. Clearly, there is also the prospect of developing similar methods for other traits. At the outset of this project, there were discussions regarding other traits that would benefit from the development of NIR based predictive methods. Traits discussed and their relative importance included:

- Pachymetra - Medium
- Borers – Medium (but could be elevated to High)

- Nematode - Medium
- Water-use efficiency - Medium
- Ratoonability - Low
- Ramu stunt - Medium
- Exotic diseases – Medium
- Leaf scald - Low
- Downy mildew - Low

There has been no work performed on any of these traits to date. It is possible that some of these may become important enough to consider for the development of NIR methods. However, there is no current driver to warrant even preliminary investigations as yet.

8.0 FUTURE RESEARCH NEEDS

There is clearly further work which can be done to build upon the progress made to date to develop smut and FLG predictive tools. However, the larger issue to answer is the likelihood of these tools being implemented.

For smut ratings, this project began at a time before smut was present in Australia and many of the logistical and economic factors behind the need for a predictive tool have reduced significantly in their importance. Research on the development of molecular markers for smut resistance has been similarly affected, and has also been largely unsuccessful. As smut is now virtually endemic within the Australian sugar industry, the ability to conduct field trials has increased and information is now much more freely available on the performance of varieties with respect to smut resistance. The importance of field based responses to smut will continue to be important and it is considered that the NIR based predictive methods are of a low enough priority to discontinue the work for now. If this method was to be pursued further in the future, the following research would be required.

- Transfer existing NIR calibrations onto a new NIR instrument platform. BSES now has an FT-NIR instrument (ThermoScientific Antaris II) which has considerable advantages over the older dispersive NIR instrument used within this project (FOSS XDS) which experienced problems.
- Confirm the operation of calibrations on the Antaris instrument, and conduct another blind validation trial.
- Examine validation data for this trial as well as that produced on the 2009 and 2010 validation trials. There may be opportunities to further improve the outcomes of these earlier trials based on both the new data and different analytical approaches.
- Finally, assuming a successful validation, the method would require fine-tuning to provide a method which can be readily implemented within the existing juice lab sampling and operations.

For FLG ratings, research on the predictive methods has ceased shortly after the proof of concept stage. As outlined earlier in this report, this was due to a combination of instrument difficulties and reduced priorities for the requirement of such a technique for FLG. No further information has been identified to change these opinions at the close of this project. In a similar way to that described above, further research into the FLG predictive method would involve the following work.

- Bulk up the data within the NIR calibrations with an extensive sampling and analysis program.
- Verify the calibration with a staged series of validation trials of increasing size.
- Examine validation data and identify any shortcomings.
- Assuming successful validation, fine-tune the method to produce a routine tool which can be readily implemented within the existing operations.

9.0 RECOMMENDATIONS

Clearly, this research offers substantial benefits if the findings outlined can be developed further towards a field-based application for screening. An effective screening tool that could give almost an instantaneous rating for the assessment of large numbers of plants would be a tremendous advance in breeding for resistance characteristics. Furthermore, such a tool could be used outside of regions where the pest/disease is prevalent, could be employed earlier in the plant selection process and would complement existing screening methods which are likely to be retained and used for smaller numbers of prospective varieties which were close to commercial release. All of this is prevalent upon two issues – being cost effective compared to existing screening methods and producing data of acceptable quality.

The NIR methods of predicting plant ratings have a clear, long term advantage regarding the costs required on a per clone basis to provide resistance ratings for sugarcane clones. Blind validation of the smut method showed that useful data could be obtained, although it would be preferred if it could perform better at removing susceptible clones. Early data on the FLG method indicates that it should perform similarly to the smut method upon further development, based on an analysis of SEP values at similar development stages.

We believe that the project should cease at the current stage. Clearly, it would not be a large job to resurrect the project work and continue the development of the NIR methods should there be an identified need to do so in the future. That decision will rest within the BSES Plant Improvement Program.

10.0 LIST OF PUBLICATIONS

- The following Poster and Oral presentation was presented at the BSES Mackay Field Day in May 2009

  DE Purcell, BJ Croft, MC Cox and MG O'Shea (2009). Predicting smut varietal ratings without field trials.

- The following Poster and Oral presentation was presented at the 14th International Conference on Near Infrared Spectroscopy (2009) held in Bangkok, Thailand.

  DE Purcell, S Kokot and MG O'Shea (2009). Rapid screening by near infrared spectroscopy for sugarcane smut resistance to improve breeding and selection outcomes.

- The following paper was presented at the 32nd ASSCT conference held in Bundaberg in May 2010 and was reprinted in the International Sugar Journal (Appendix 1).

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. *Proceedings of the Australian Society of Sugar Cane Technologists*, 32:366-374.

DE Purcell, JP Oxley, MC Cox, BJ Croft, MG O'Shea (2011). On-site rapid screening for sugarcane smut resistance using Near-Infrared (NIR) spectroscopy. *International Sugar Journal*, 113(1345):38-42.

- The following BSES Final Report was submitted to the SRDC in June 2011.

  MC Cox, BJ Croft and G Bonnett (2011). SRDC Project BSS325 SmutBuster: Accelerated Breeding of smut-resistant sugarcane varieties.

  BSS325 Supplementary Report information supplied September 2011 (Appendix 3).

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