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Development of a new variety-rating system for sugarcane smut using improved statistical methods

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Abstract Sugar Research Australia has helped the Australian sugarcane industry to manage diseases through the development of disease-resistant varieties. Candidate varieties are screened for resistance to major diseases, before release to the industry, and data collected from screening trials are used to predict the disease rating of each variety. A linear mixed model is fitted to the combined historical and most recent trial and predictions of the average disease ratings for each standard, and candidate varieties are obtained from the model. Using the predicted ratings from current data, both standard and candidate varieties are assigned to one of three resistance groups, i.e. resistant, intermediate, or susceptible. Varieties in each resistance group are presented in a tabular form, but problems have been identified in the two-stage analysis and the tabular report. To rectify the problems, this study aimed to (a) account for trial data variation associated with environmental and biological parameters, (b) replace the resistance groups with a confidence interval, and (c) develop an easily grasped visual illustration that indicates the predicted resistance of a variety plus variation in trial data. The 10-year historical data used in the study consisted of 10 top commercial varieties, nine standard varieties and a combination of six newly released varieties and advanced clones. A Box-Cox transformation was applied to the ratings and then a weighted linear mixed model was fitted to the data. Several combinations of parameters in the model were used, such as trial name or trial year as a random effect and trial confidence as a weight variable. Predicted average ratings and the 95% confidence interval (CI) for the predicted average ratings were calculated from the models. As a visual representation of the predictions from the best model, a scatter plot with the confidence interval (as error bars) was used. The visual reports were presented at the 2019 industry meetings following support by the Regional Variety Committees.

Key words Disease rating, sugarcane smut, linear mixed model, screening trials

INTRODUCTION

New varieties increase industry productivity and profitability and protect the industry from new threats that develop in the challenging crop environment. In many cases, resistant varieties are the only option to efficiently manage sugarcane diseases. In Australian sugarcane, most diseases are managed through the cultivation of resistant varieties; examples include Fiji leaf gall, sugarcane smut, pachymetra root rot, leaf scald and orange rust. Disease-resistant varieties reduce the need for pesticides and are adopted at no direct cost by growers. Disease-resistance data have been providing reliable disease ratings for sugarcane varieties for years. Sugar Research Australia Limited (SRA) plant breeders, the adoption team, researchers and the sugar industry in general are the main clients for the SRA varietal-resistance screening program.

In screening trials, stalks of varieties from various stages of the breeding programs are inoculated with pathogens and planted in the glasshouse and/or in the field (Bhuiyan *et al.* 2010). A set of standard varieties with known disease ratings are included in every screening trial; these are assessed for symptoms or pathogen populations after 12 weeks (nematodes) to 14 months (leaf scald) post-inoculation, depending on the disease.

In the current SRA breeding program, all potential varieties must be screened for resistance to the major diseases at least twice before release. Each year, advanced clones (maximum propagation and accelerated stage) and selected commercial varieties are screened for resistance assessment. Data from these trials are analysed by SRA biometricians. BSES (until 2010) traditionally used the recommended International Society of Sugarcane Technologists (ISSCT) method for assigning resistance ratings, based on a regression equation describing the relationship between the disease score of a set of standard varieties with known historical ratings (Hutchinson *et al.* 1971). This regression equation was then used to assign a rating to the test varieties. This method was endorsed by the Pathology Committee of the ISSCT as an accepted method (Hutchinson and Daniels 1971). Although disease resistance in sugarcane is a quantitative trait, ratings are provided as ordinal categories and represented by numbers (1 to 9) such as susceptible (7–9), intermediate (4–6) and resistant (1–3). In the susceptible category, very high disease incidence and severe yield loss are expected; they are usually not recommended for selection or cultivation. For the intermediate category, moderate level of disease incidence, and moderate yield loss is expected under favourable condition; for the resistant category little or no disease incidence, and no impact on yield.

Unfortunately, assigning a number to describe a resistance category has led to confusion that the symbol has a meaning that it was never intended to have. In 2010, BSES commissioned an external reviewer to develop an appropriate statistical method to estimate ratings for disease resistance that was statistically and practically reliable. The reviewer recommended the application of linear mixed model (Henderson *et al.* 1959) to disease scores to derive ratings.

In this method, the standard varieties are separated into groups based on the least significant difference test and the test varieties are placed in the group of best fit. A correlation is performed on the score of the standards in the trial to be analysed against the average score from all previous trials to determine if the most recent trial can be considered to provide a reliable estimate for the standards (Stringer *et al.* 2012). The method was more conservative than the Hutchinson method and grouped the 9-scale rating into three resistance rating groups, e.g. 2 (resistant), 5 (intermediate) and 8 (susceptible).

Nonetheless, ratings provided for varieties remained categorical with no indication of the reliability (level of confidence or indication of rating variability). Although the rating for a particular clone may change from trial to trial due to the environmental and other factors, ratings have been viewed as ‘fixed’, ‘definite’ and dependable, even with limited rating data. In the past, BSES/SRA supplied industry with ‘fixed ratings’ for canes grown commercially, and both the plant breeders and industry expected no or little change in the ratings for canes grown commercially. As more ratings data became available, and the predicted resistance of a variety changed, both plant breeders and industry became disillusioned and confused with the ratings applied to varieties.

Disease expression can vary due to many factors, such as inherited nature of the test clones, environmental conditions, variabilities in pathogen populations, nature of the pathogen, age of planting materials, crop management and other factors (Agrios 2004).

A controlling factor is also simply the number of tests applied to each new variety, with more reliable ratings expected with more tests. The previous analysis approach was statistically valid and provided accurate ratings provided that the level of infection in trials was fairly consistent over time. This has not been the case in recent years, with significantly higher values for smut incidence and severity observed in the long-term standards. A new analysis approach was developed to take into account variation in absolute infection levels and better reflect the relative performance of test clones compared to the standards.

The aims of our study were to: (a) account for the precision associated with varying number of resistance tests; (b) replace the resistance groups with numerical rating with a confidence interval; and (c) develop a visual report for disease ratings that is statistically and practically reliable.

METHODS

Model fit and model parameters

A set of sugarcane varieties and advanced clones that had been tested in smut-screening trials more than twice were selected for the analysis. The 10 major commercial varieties, six advanced clones whose ratings varied considerably from trial to trial (Problem clones) and 10 standard varieties (STDs) used in smut screening trials as checks were selected for the analysis (Table 1). Disease-rating data for sugarcane smut from 2007 to 2016 and 2007 to 2017 (most recent) were used for the analysis.

Table 1. Top 10 major varieties, newly released varieties and clones (Problem clones) and standard varieties selected for sugarcane smut rating.

Major variety	Problem clones	Standard variety
KQ228ɸ	KQ08-2180	NCo310
MQ239ɸ	QN02-1707	Q96
Q183ɸ	QS06-7324	Q124
Q200ɸ	QS06-8100	Q151
Q208ɸ	SRA8	Q155
Q231ɸ	SRA15ɸ	Q171
Q232ɸ		Q188ɸ
Q238ɸ		Q205ɸ
Q240ɸ		Q209ɸ
Q242ɸ		

Two sets of information were also considered in the analysis. The first one was the strength of the linear relationship between the ratings of STDs in the year 2017 and 2016 and the ratings of STDs in the historical data measured by the correlation coefficient. The second set of information was the confidence of the ratings partly based on unaccounted factors such as extreme weather conditions or low disease incidence. Rating confidences were assigned to “a”, “b” or “c” category, where an “a” denoted high confidence, “b” medium confidence and “c” low confidence.

A weighted linear mixed model was used for the data analysis, where ‘Variety/Clone’ was considered as a fixed effect while ‘Trial Name’ or ‘Trial Year’ was a random effect. As for the smut-screening trial, a set of 5–10 trials are being established in each year depending on the requirement from breeders or pathologists. Each trial is assigned with a ‘Trial Name’. As the data were highly unbalanced, the “Variety x Trial Name” interaction was not fitted. For the weight variable, the correlation coefficient (StdCorr) or the rating confidence was used. The rating confidences were coded in 3 different ways, (i) a=3, b=2, c=1, (ii) a=5, b=2, c=1, and (iii) a=10, b=5, c=2. Trials with zero StdCorr were dropped from the analysis. An observation with a larger weight means it contains relatively more information. This also means that observations in a Trial or a Year with a larger weight has greater influence in the model.

In summary, the parameters tested for a model were all possible combinations of the following:

- Data from 2007 to 2016 or data from 2007 to 2017
- Random factor of Trial Name or Trial Year
- Weight of StdCorr or one of the 3 rating confidences

Model assumptions

A Box-Cox transformation was used on the trait to satisfy the assumption of normally distributed errors. Lambda equal to 0.8 gave the residuals with a distribution closest to Gaussian. In addition, to account for heterogenous variances among trials, the error variance was partitioned either by Trial or Years.

Prediction

Using the fitted values from the weighted linear mixed model, the rating means and the 95% confidence interval of the rating means were estimated. Using the predicted values, the clones were assigned to 3-rating and 9-rating scales.

Model evaluation

To evaluate the fit of a model, we calculated:

- % mis-grp: percentage of observations where the predicted resistance group was different from the expected resistance group of the STD where resistance rating groups are rating 1–3 (resistant), 4–6 (intermediate), and 7–9 (susceptible).
- % mis-match: estimated the percentage of observations where the predicted rating is different from the expected rating of the STD where the established rating ranges from 1 to 9.
- -2 Res LL: Used for the Likelihood Ratio Test to compare nested models.

- Akaike information criterion (AIC) and Bayesian information criterion (BIC): AIC and BIC were used to compare (a) both nested and non-nested models, and (b) models with different error distribution. Model(s) with the lowest AIC and BIC values were selected.

Visualization

The estimated rating means and 95% confidence intervals (CIs) from the best model were back-transformed. A scatter plot with error bars was used for reporting purposes. Colour codes were used to highlight the resistance ratings of the clones, where green = resistant, yellow = intermediate and red = susceptible. Where a confidence interval limit went outside of the 1–9 rating scale, an arrowhead was denoted.

RESULTS

Model statistic

No differences were observed between the ratings estimated from the ‘2007 to 2016’ data series when compared to ‘2007 to 2017’ series. Analysis based on ‘Year’ as a random variable provided better prediction of ratings than that of ‘Trial’, as demonstrated by lower BIC and AIC values of the former. The scale of 1 to 9 ratings (9-rating scale) provided a better estimation compared to a 3-rating scale (2, 5 and 8 ratings), as indicated by the %mismatch statistics (<0.6 versus 0.8) (Table 2).

Weighted confidence (3=a, 2=b, 1=c) provided better estimation of ratings (close to the combined traditional rating) compared to others including coefficient correction of standards. The 9-rating scale has a 33% mis-match, while the 3-rating scale has a 78% mis-match. This shows that the 9-rating scale has a much better accuracy than the 3-rating scale.

Table 2. Fit statistics where random variable is ‘Trial Year’ and based on 2007 to 2017 rating data.

	Fit statistic					
	3-rating scale			9-rating scale		
Rating confidence	% mis-group	% mis-match	AIC	% mis-group	% mis-match	AIC
3=a, 2=b, 1=c	0	0.78	2384	0	0.33	2384
5=a, 3=b, 1=c	0	0.78	2385	0	0.33	2385
10=a, 5=b, 2=c	0	0.78	2386	0	0.33	2386

Combined analysis

The 3-rating scale, 9-rating scale, estimated means, and the CI of the mean are shown in Table 3. The back-transformed LS means and CIs were plotted in two formats. The back-transformed LS means are represented by a dot in Figure 1, and the error bars are the back transformed lower and upper limits of the 95% CI. Most of the problematic clones and some of the major clones have wider confidence intervals compared to the standard clones. The major clone Q231⌽ has the largest variation (95% back-transformed CI of the mean = [0.92– 5.28]).

Within a rating category, there were some interesting changes with the new ratings; almost all the ratings were either the same or lower compared to 9-scale traditional rating (Table 3). Overall, new varieties and problem clones had larger CI values compared to the standard varieties.

DISCUSSION

Most of the new ratings are in close agreement with the current ratings and remained within the same broader resistance categories. One of the major benefits of the new approach is that it accounts for differences in the infection levels between trials and over time. This is important. In the last few years, hotter and drier conditions have favoured smut infection, resulting in higher severity and incidence values for all clones including the long-term standards. The traditional analysis approach resulted in new clones being rated as more susceptible than they should be because they exhibited high levels of infection compared to trials in previous years. The new analysis approach accounts for temporal differences and better reflects the relative performance of new test clones compared to the standards.

Table 3. Comparison of new smut resistance ratings (LSMeans±CI) with traditional smut ratings (3- and 9-rating scale) using the major varieties, problem clones and standard varieties, based on 2007 to 2017 rating data.

Clone	Category	Smut rating				
		Traditional ratings		LSMeans		
		Rating (2,5,8)	Rating (1–9)	Rating (LS means)	Lower limit	Upper limit
KQ08-2180	Problem	5	6	6	4.36	6.89
KQ228Φ	Major	5	6	5	4.08	5.84
MQ239Φ	Major	2	3	3	1.85	3.96
NCo310	Standard	8	8	8	7.47	8.38
Q96	Standard	5	4	4	3.61	4.39
Q124	Standard	5	6	4	3.78	4.61
Q151	Standard	2	3	2	1.87	2.56
Q155	Standard	5	6	4	3.85	4.65
Q171	Standard	2	1	2	1.85	2.54
Q183Φ	Major	2	3	3	1.90	3.38
Q188Φ	Standard	8	7	7	6.97	7.90
Q200Φ	Major	5	6	5	4.53	5.83
Q205Φ	Standard	8	9	8	7.73	8.62
Q208Φ	Major	5	4	4	3.45	4.38
Q209 Φ	Standard	8	7	7	6.35	7.44
Q231Φ	Major	2	3	3	0.92	5.23
Q232Φ	Major	5	4	4	2.88	4.16
Q238Φ	Major	2	3	3	1.79	3.53
Q240Φ	Major	2	1	2	1.44	3.01
Q242Φ	Major	5	4	4	3.10	5.05
QN02-1707	Problem	5	6	5	2.47	5.32
QS06-7324	Problem	5	6	5	3.26	5.93
QS06-8100	Problem	5	6	5	3.31	5.99
SRA8	Problem	5	6	5	3.49	5.73
SRA15Φ	Problem	5	7	6	3.74	6.47

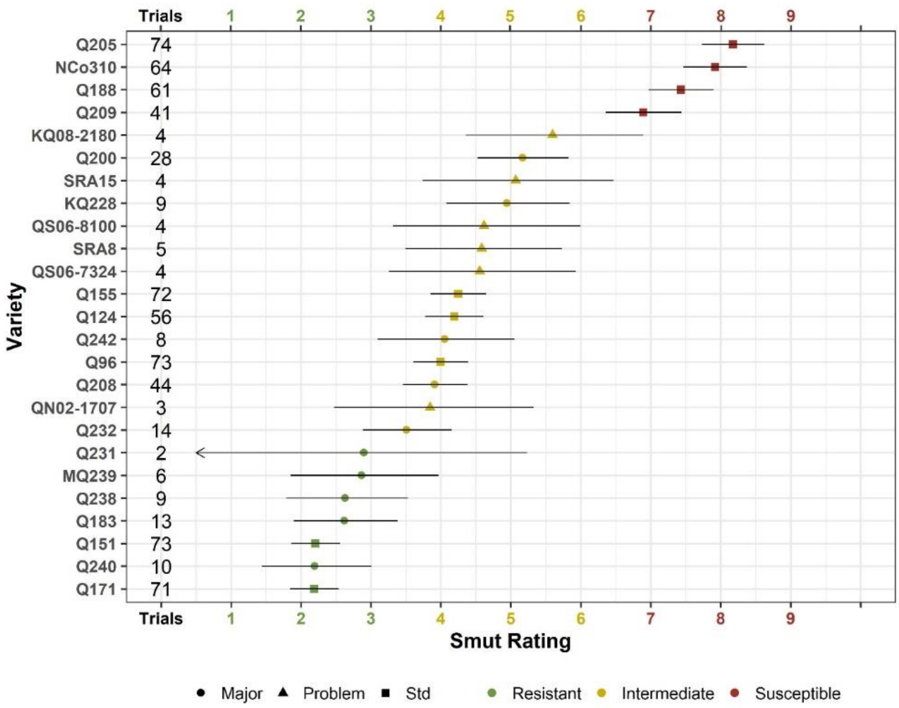

Figure 1. Rating of selected varieties/clones using the new analysis incorporating 95% Confidence Intervals (CI). An arrowhead indicates that the CI limit is beyond rating 1. The number in the plot besides a variety/clone denotes the number of times this variety/clone has been tested in the screening smut trials.

Most of the new varieties and ‘Problem clones’ had wider a confidence interval (CI) compared to the standard varieties. The standard varieties have generally been in every smut resistance trial from 2007 and their rating confidence interval is narrower (close to the mean) because of the much larger data set available for them (providing better rating precision). New varieties and ‘Problem clones’, which were in a limited number of trials, tend to have wider ratings confidence intervals. For example, the rating for Q231$^{\Phi}$ has the widest confidence interval, largely becasue it has only been tested in two trials (Figure 1). There is a possibility that with some varieties, three tests may provide a more precise predicted rating. Further analyses are needed to show how rating precision varies with clone and number of resistance tests. Variation among varieties could be due to the inherent nature of the test clones – interacting with the prevailing environment and the pathogen population (Madden *et al.* 2007). We hope to extend these analyses to other diseases as well.

Some changes were made to ratings for widely grown commercial varieties. The ratings for KQ228$^{\Phi}$ and Q200$^{\Phi}$ increased from resistant (2) to intermediate (5). In commercial fields, little smut has been is typically observed in KQ228$^{\Phi}$, whereas moderate to heavy smut infection is often observed in Q200$^{\Phi}$. Other changes included the ‘intermediate-susceptible’ clone QN02-1707 moving to the more resistant ‘intermediate’ category. Q208$^{\Phi}$, the most widely grown variety remained ‘intermediate-resistant’ to smut. In two natural infection trials (2007 to 2012) in Bundaberg, the two varieties KQ228$^{\Phi}$ and Q200$^{\Phi}$ exhibited little smut infection up to third ratoon in one trial, and moderate infection in a second trial (Bhuiyan *et al.* 2018). Rating of ‘intermediate-resistant’ varieties is always a challenge due to variation in disease between trials. The influence of variety, environment and growth of the cane could influence the levels of bud infection. The interaction between environmental conditions and varietal resistance may play a role in the differential expression of smut in these canes. Recently, moderate levels of smut were observed in two ‘intermediate-resistant’ varieties (Q252$^{\Phi}$ and SRA8$^{\Phi}$) in the Burdekin region. We believe unusually dry and warm conditions for the last few years created favourable condition for smut infection in the intermediate-resistant varieties in the Burdekin region (Hoy *et al.* 1991). More research is required to understand the interaction of the environment, sugarcane varieties and the smut fungus under natural conditions.

The new method for the application of resistance ratings has replaced the previous combined analysis. Overall feedback from the industry has been very positive as the current predicted resistance rating is presented with this system, plus a graphical illustration of the precision of those ratings. Data from other disease resistance tests are being analysed, ratings will be presented with the new ratings system. Ratings for pachymetra root rot are expected to be available in this format in 2020.

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