HOW DO CURRENT RATINGS OF SUGARCANE VARIETIES FOR RESISTANCE TO SMUT RELATE TO NATURAL INFECTION?

By

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Abstract

SUGARCANE SMUT, CAUSED by a fungus *Sporisorium scitamineum*, is an important disease of sugarcane in Australia. Sugarcane smut can be managed effectively through the propagation of resistant varieties. In Sugar Research Australia's (SRA) smut screening experiments, stalks of varieties from various stages of breeding programs are cut into one-eye setts and then dipped into a smut spore suspension (5×10^6 spores/mL water) for 10 min at 31 °C. After germination, the plants are transplanted to the field and disease incidence is measured in the plant crop and first and second ratoon crops. This method is effective for screening of a large number of varieties in a relatively short period (10–12 months) and is used in other countries. Although this method is widely accepted, it has some drawbacks: i) test plants are subject to very high disease pressure; and ii) it does not replicate natural infection. Three experiments were established in 2007, 2008 and 2009, to determine if the ratings obtained by artificial inoculation technique predict field resistance of varieties. All experiments were planted with 10 or 5 replicates of the test varieties planted between rows of infected Q205$^{\phi}$, and maintained until second ratoon. Highly susceptible varieties Q205$^{\phi}$ and Q157 had >40% infected plants in plant crops whereas little smut was observed in intermediate and resistant varieties. Average % of smut infected plants increased in all experiments from the plant crop (5–12%) to first ratoon (21–46%) and second ratoon (26–59%) crops. The correlation coefficient values between smut incidence in the natural infection experiments and the historical ratings obtained using dip-inoculation methods ranged from r = 0.82 to 0.72, indicating a good agreement between natural infection trials and dip inoculation ratings.

Introduction

Sugarcane smut can be managed effectively through the propagation of resistant varieties (Comstock, 2000). After detection of smut in Queensland in 2006, Sugar Research Australia (formerly BSES) commenced a large screening program to develop smut resistant varieties. In screening experiments, stalks of varieties from various stages of breeding programs are cut into one-budded setts and cleaned to remove sawdust and dirt.

Setts are then dipped into a smut spore suspension (approx. 5×10^6 spores/mL deionised water) for 10 min and maintained at 31^{o} C. After germination, the plants are transplanted to the field and disease incidence (percent of infected plants) are measured in a short duration plant crop, 3–4 months after planting, and then 3–4 months after ratooning.

Test varieties are classed as resistant (R), intermediate (I) or susceptible (S) based on incidence of disease symptoms relative to a set of standard varieties of known field reaction. These ordinal categories are represented by the numbers 1–3 (HR to R), 4–6 (IR to I) and 7–9 (S to HS), as proposed by Hutchinson and Daniels (1971) and recommended by the ISSCT Pathology Committee for sugarcane diseases.

This method is effective for screening large numbers of varieties in a relatively short period of time, and is widely used in other sugarcane growing countries for screening sugarcane varieties for smut resistance (Ferreira *et al.*, 1980). In Florida, significant correlation (~0.90) between artificial dip inoculation and natural infection was observed (Holder ,1985).

However, there is a concern that this method does not provide accurate ratings of varieties for all situations and there have been a number of varieties where the field observations have varied from the rating obtained using the dip inoculation method (Ladd *et al.*, 1974).

In nature, a sugarcane plant is infected by airborne spores landing on buds of standing stalks or on the soil where buds of germinating setts or young ratoon buds are infected (Lee-Lovick, 1978). The dip inoculation method approximates infection of buds on germinating setts. Some field infection does occur in the dip inoculation method, especially during the ratooning stage of the experiments.

Environment is also important in field reaction of varieties to a disease and for practical reasons the artificial dip inoculation method used in routine screening trial, is limited to only one environment, which may affect the rating of varieties.

The main objective of this research was to determine if the ratings obtained by the artificial inoculation technique were a good indication of the field resistance under natural conditions. Preliminary results of this work were published in Bhuiyan *et al.* (2010).

Materials and methods

General method

Three experiments were established in 2007, 2008 and 2009 at the SRA (formerly BSES) smut research farm at Kinkuna, approximately 20 km south of Bundaberg, Queensland, to investigate the natural infection of different varieties by smut. Each experiment was run for three years in plant, first and second ratoon crops. A total 47 of varieties were included in these experiments.

The smut ratings for the varieties had already been established by the dip inoculation method in experiments conducted in Bundaberg, Queensland (Bhuiyan *et al.*, 2010). Most varieties had been screened multiple times and an average rating was obtained. Disease-free stalks of sugarcane were collected from propagation blocks at the BSES (SRA) Bundaberg Experiment Station. The plant materials were long hot water treated at 50^{o} C for 30 min before planting to eliminate any systemic infection of smut.

Experiment 1: natural infection 2007

The experiment was established in October 2007 with eight commercial and two advanced varieties. Rows of inoculated smut-susceptible variety Q205$^{\phi}$ were planted between rows of test plots as spreader rows.

Test varieties were planted in 5 m × 1.5 m single-row plots with 10 replications using a randomised complete block design. Both spreader rows and test rows were planted with a whole stalk planter.

The experiment was harvested in November 2008 (plant crop) and in October 2009 and 2010 (first ratoon and second ratoon crops, respectively). Disease incidence (percent of infected plants) was recorded during inspections at 1–3 monthly intervals in each crop.

Experiment 2: natural infection 2008

The second natural infection experiment was established in September 2008. Thirty-two commercial varieties and two advanced varieties were included in this experiment. Rows of Q205$^{\phi}$ inoculated with smut, as in the previous experiment, were planted between rows of the test plots as spreader rows. Test varieties were planted in 5 m × 1.5 m single-row plots with five replications using a randomised complete block design. The trial was inspected for smut before harvesting in October 2009, 2010 and 2011 in plant, first ratoon and second ratoon crops, respectively.

Experiment 3: natural infection 2009

The third experiment was established in September 2009 with 22 varieties following the same design as experiment 2. The experiment was inspected for smut before harvesting in October 2010, 2011 and 2012 in plant, first ratoon and second ratoon crops, respectively.

Statistical analysis and disease rating

The incidence of smut per plot was calculated as (*diseased plants/total number plants*) × 100. A linear mixed model was fitted to all datasets using ***proc mixed*** in SAS version 9.4 (SAS Institute, Cary, NC). For all experiments, crop class (plant, first and second ratoon), clone and their interactions effects were treated as fixed effects.

Block (replication) and the error term (residual) were treated as a random effects. All possible interactions of the fixed effects were also included in the model. A logit transformation was applied to the data prior to analysis as $proportion = \frac{total\ diseased\ plant+0.5}{total\ plant-total\ diseased\ plant+0.5}$ (Xu and Ridout, 1998).

Estimated logit values were then back-transformed before presenting in the results. For the appropriate significant factors, protected-mean comparisons of all possible pairwise differences of the means were tested at alpha = 0.05 using Fisher's protected LSD test.

Research overseas and in Australia suggests that if a sugarcane clone expresses >30% smut incidence it is deemed to be susceptible (Ladd *et al.*, 1974; Whittle, 1978). Each clone was assigned to a smut rating category based on a simplified smut rating scale from Ladd *et al.* (1974) (Table 1).

Table 1—Percent of smut infection, smut ratings and smut rating categories.

Smut incidence (%)	Smut rating	Rating category
0–2	1	Highly resistant (HR)
3–10	2–3	Resistant (R)
11–15	4	Intermediate resistant (IR)
16–20	5	Intermediate (I)
21–30	6	Intermediate susceptible (IS)
31–60	7	Susceptible (S)
61>	8–9	Highly susceptible (HS)

Results

In all three experiments, there were significant differences of smut incidence among crop classes, test varieties and their interactions (data are not presented).

Experiment 1: natural infection 2007

Except for the highly susceptible variety Q205$^{\phi}$, little disease was observed in any clone in the plant crop of experiment 1 (Table 2). Overall smut incidence in the plant crop was 5%, which increased to 21% and 44% in the first and the second ratoon crops, respectively.

Very low levels of smut (<3%) were observed in two resistant varieties, Q232$^{\phi}$ and Q151, included in this experiment throughout the three crop years.

Except for intermediate and intermediate susceptible Q190$^{\phi}$ and QS94-91, low levels (<10%) of smut were observed in intermediate resistant (smut rating 4–6) varieties over the three crop classes and these can be assigned to the resistant (R) category under natural infection. The varieties Q190$^{\phi}$ and QS94-91 had 69% and 93% smut respectively at second ratoon, and can be assigned to the highly susceptible (HS) category.

High levels of smut (59–98%) were observed in all susceptible varieties at the end of the second ratoon crop. Very low levels of smut were observed on the resistant (R and HR) varieties over the three crop cycles.

Table 2—Percent of smut in plant (P), first (1R) and second (2R) ratoon crops with average rating based on dip inoculation method and rating category assigned from the natural infection in the 2007 trial.

Clone	P [1]	1R	2R	Dip method rating [2,3]	Natural infection rating[4]
Q205$^{\phi}$	42.9a	99.3a	98.1a	9 (HS)	HS
QS94-91	0.5b	14.6b	93.2ab	6 (IS)	HS
Q190$^{\phi}$	0.5b	9.8bc	68.9b	5 (I)	HS
Q138	0.5b	29.2b	65.8b	7 (S)	HS
Q188$^{\phi}$	0.5b	23.4b	58.8bc	7 (S)	S
Q135	0.7b	4bcd	7.3cd	5 (I)	R
Q242$^{\phi}$	0.5b	6.3bc	4.2d	4 (IR)	R
Q232$^{\phi}$	0.5b	0.7cd	2d	3 (R)	R
Q151	0.7b	0.5d	0.6d	1 (HR)	HR

[1]Each data point is mean of 10 replications. Means followed by the same letter are not significantly different according to Fisher's protected LSD test (P=0.05)
[2]Rating based on dip inoculation, 1–9 scale where 1 is highly resistant and 9 is highly susceptible
[3]Rating category, HS= highly susceptible, S=susceptible, IS= intermediate susceptible, I = intermediate, IR=intermediate resistant, R= resistant and HR= highly resistant
[4] Ratings based on natural infection second ratoon crop

Experiment 2: natural infection 2008

Only 12% smut was observed in the plant crop of experiment 2, followed by 46% and 59% in the first and the second ratoon crops respectively.

n the plant crop, a high level (>30%) smut was observed in the four susceptible (HS and S) varieties, Q205$^{\phi}$, Q157, Q229$^{\phi}$ and QS96-6037 (Table 3). Out of 15 resistant varieties, 12 (80%) varieties maintained low levels (~20%) of smut until the second ratoon.

Three 'resistant' varieties Q177$^{\phi}$, Q212$^{\phi}$, and Q219$^{\phi}$ showed high levels of smut incidence (32% to 77%) and were rated susceptible to highly susceptilble by this method, and another two 'resistant' varieties, KQ236$^{\phi}$ and Q200$^{\phi}$ showed some smut (10%–13%) and were rated intermediate resistant, and Q226$^{\phi}$, showed moderate levels of smut (22.4%), and was rated intermediate (I).

All intermediate resistant varieties (4–6 rating) had very little smut in the plant crop. Three intermediate resistant varieties, Q234$^{\phi}$ Q142 and QS96-2787 had high levels of smut (33 to 99%) and were assigned to the highly susceptible (HS) or susceptible (S) categories based on natural infection in the second ratoon crop.

Experiment 3: natural infection 2009

An average of 7% smut was observed in the plant crop of experiment 3, followed by 24% and 26% in the first and the second ratoon crops respectively.

In the plant crop, a high level (>30%) of smut was observed in three highly susceptible (smut rating 8–9) varieties Q205$^{\phi}$, Q157, and Q224$^{\phi}$ (Table 4).

Table 3—Percent of smut in plant (P), first (1R) and second (2R) ratoon crops with average rating based on dip inoculation method and rating category assigned from the natural infection in the 2008 trial.

Clone	P	1R	2R	Dip method rating [2,3]	Natural infection rating[4]
Q157	41.6 b	99.5a	99.4a	9 (HS)	HS
Q205$^{\phi}$	61.9 a	99.1ab	98.8ab	9 (HS)	HS
QS96-2787	20.7 c	89.6abcd	98.7ab	6 (IS)	HS
Q142	8.0 e	82.4abcde	98.6ab	4 (IR)	HS
QS96-6037	33.1 bc	80.5abcdef	96.8abc	8 (HS)	HS
Q120	1.4 e	27.7cdefghijk	85.8abcd	7 (S)	HS
Q220$^{\phi}$	2.9 e	55.2bcdefg	85.2abcd	7 (S)	HS
Q233$^{\phi}$	13.2 d	94abc	84abcde	7 (S)	HS
Q229$^{\phi}$	48.6 b	58.7bcdefg	81.9abcdef	7 (S)	HS
Q219$^{\phi}$	1.4 e	42.9cdefghi	76.1abcdefg	3 (R)	HS
Q138	1.0 e	52.9cdefg	53.3bcdefgh	7 (S)	S
Q201$^{\phi}$	6.9 e	16.2cdefghijk	52.6bcdefgh	7 (S)	S
Q230$^{\phi}$	1.8 e	31.4cdefghij	50.4bcdefgh	7 (S)	S
Q209$^{\phi}$	0.5 e	44.8cdefgh	50.4bcdefgh	7 (S)	S
Q212$^{\phi}$	1.1 e	54.3cdefg	44.8cdefgh	3 (R)	S
Q234$^{\phi}$	13.5 d	6.1efghijk	32.6cdefghi	6 (IS)	S
Q177$^{\phi}$	2.7 e	13.2defghijk	32.3cdefghi	3 (R)	S
Q226$^{\phi}$	1.5 e	2.3ghijk	22.4defghij	3 (R)	IS
Q237$^{\phi}$	1.5 e	11.3defghijk	18.4defghij	5 (I)	I
Q200$^{\phi}$	4.1 e	1.8ghijk	14defghij	3 (R)	IR
Q231$^{\phi}$	4.9 e	13.8defghijk	12.7defghij	4 (IR)	IR
Q135	1.0 e	0.9ijk	11defghij	5 (I)	IR
KQ236$^{\phi}$	2.7 e	4.6fghijk	10.1defghij	3 (R)	IR
Q199$^{\phi}$	4.0 e	1.1hijk	9defghij	3 (R)	R
Q183$^{\phi}$	4.1 e	1.2hijk	8.9defghij	4 (IR)	R
Q235$^{\phi}$	0.5 e	3.5ghijk	6.7efghij	2 (R)	R
Q208$^{\phi}$	1.0 e	1hijk	6.3efghij	4 (IR)	R
Q232$^{\phi}$	0.5 e	0.9hijk	5.8fghij	3 (R)	R
KQ228$^{\phi}$	0.6 e	8.1efghijk	4ghij	2 (R)	R
Q238$^{\phi}$	0.4 e	2.3ghijk	2.9hij	2 (R)	R
MQ239$^{\phi}$	0.4 e	0.6jk	0.6ij	3 (R)	HR
Q151	0.8 e	0.5jk	0.6j	1 (HR)	HR
Q171$^{\phi}$	1.0 e	0.5k	0.6j	1 (HR)	HR
Q240$^{\phi}$	0.5 e	0.5k	0.4j	3 (R)	HR

[1] Each data point is mean of 5 replications. Means followed by the same letter are not significantly different according to Fisher's protected LSD test (P=0.05)
[2] Rating based on dip inoculation, 1–9 scale where 1 is highly resistant and 9 is highly susceptible
[3] Rating category, HS= highly susceptible, S=susceptible, IS= intermediate susceptible, I = intermediate, IR=intermediate resistant, R= resistant and HR= highly resistant
[4] Ratings based on natural infection second ratoon

Most of the resistant (smut rating 1–3) and the intermediate (smut rating 4–6) varieties maintained <2% smut until the second ratoon and were assigned to the highly resistant or resistant category. The only exception was resistant variety KQ228$^{\phi}$ which had 24% of smut in the first and the second ratoon crops and assigned to intermediate susceptible (IS) category.

Two susceptible varieties, Q211$^{\phi}$ and Q138 had ~20% smut and were assigned to intermediate, whereas the susceptible varieties Q188$^{\phi}$, Q218$^{\phi}$ and Q220$^{\phi}$ had little smut (<3%) and were assigned to resistant in this trial.

Table 4—Percent of smut in plant (P), first (1R) and second (2R) ratoon crops with current rating (based on dip inoculation) and rating category, and expected rating category under natural infection in the 2009 trial.

Clone	P	1R	2R	Dip method rating [2,3]	Natural infection rating[4]
Q157	50a	97.7a	97.7a	9 (HS)	HS
Q205$^{\phi}$	53.4a	96a	96a	9 (HS)	HS
Q167$^{\phi}$	6.4bc	65ab	65ab	8 (HS)	HS
Q217$^{\phi}$	0.5d	61.6ab	61.6ab	8 (HS)	HS
Q211$^{\phi}$	0.5d	19.7bcde	19.7bcde	7 (S)	I
Q209$^{\phi}$	6bc	52.3abc	52.3abc	7 (S)	S
Q224$^{\phi}$	30.2ab	84ab	84ab	8 (HS)	HS
Q138	0.5d	17.2bcdef	17.2bcdef	7 (S)	I
Q247$^{\phi}$	0.5d	1.6defg	1.6defg	3 (R)	HR
Q208$^{\phi}$	0.5d	0.6efg	0.6efg	4 (IR)	HR
Q242$^{\phi}$	0.5d	1.3defg	1.3defg	4 (IR)	HR
Q135	0.5d	0.9defg	0.9defg	5 (I)	HR
Q220$^{\phi}$	1cd	2.5cdefg	2.5cdefg	7 (S)	R
Q200$^{\phi}$	0.5d	0.9defg	0.9defg	3 (R)	HR
Q183$^{\phi}$	0.5d	0.6efg	0.6efg	4 (IR)	HR
Q244$^{\phi}$	0.6d	0.4g	0.4g	5 (I)	HR
Q241$^{\phi}$	0.5d	0.5fg	0.5fg	1 (R)	HR
Q188$^{\phi}$	0.5d	0.4g	0.4g	7 (S)	HR
Q218$^{\phi}$	0.5d	0.6efg	0.6efg	8 (HS)	HR
KQ228$^{\phi}$	0.5d	24.2bcd	24.2bcd	2 (R)	IS
Q245$^{\phi}$	0.5d	1.3defg	1.3defg	1 (HR)	HR
Q151	0.5d	0.5fg	0.5fg	1 (HR)	HR

[1]Each data point is mean of 5 replication. Means followed by the same letter are not significantly different according to Fisher's protected LSD test (P=0.05)
[2]Rating based on dip inoculation, 1–9 scale where 1 is highly resistant and 9 is highly susceptible
[3]Rating category, HS= highly susceptible, S=susceptible, IS= intermediate susceptible, I = intermediate, IR=intermediate resistant, R= resistant and HR= highly resistant
[4] Ratings based on natural infection second ratoon

All experiments combined

In most cases, the smut levels remained similar for a particular clone when planted in more than one natural infection experiments (Table 5), although, there were some exceptions. In some varieties, the expression of smut varied significantly among experiment

Susceptible variety Q188$^{\phi}$ had high levels of smut in experiment 1 but low levels in experiment 3, and assigned to highly resistant category. Similarly, susceptible variety Q220$^{\phi}$ had high levels of smut in experiment 2 (assigned to HS) but low levels of smut in experiment 3 (assigned to R).

On the other hand, resistant variety KQ228$^{\phi}$ had low levels of smut in experiment 2 and moderate levels of smut in experiment 3. Similarly, susceptible variety Q138 had high levels of smut in experiments 1 and 2 but moderate levels of smut in experiment 3.

Table 5—Smut levels on clones planted more than one experiments.

Clone	Dip method rating[1]	% smut Experiment 1 (rating)	% smut Experiment 2 (rating)	% smut Experiment 3 (rating)
KQ228$^{\phi}$	R	–	4 (R)	24.2 (IS)
Q135	I	7.3 (R)	11 (I)	0.9 (R)
Q138	S	65.8 (HS)	53.3 (S)	17.2 (I)
Q151	HR	0.5 (HR)	0.6 (HR)	0.6 (HR)
Q157	HS	NA	99.4 (HS)	97.7 (HS)
Q183$^{\phi}$	IR	NA	8.9 (R)	0.6 (HR)
Q188$^{\phi}$	S	58.8 (S)	NA	0.4 (HR)
Q200$^{\phi}$	R	NA	14 (IR)	0.9 (HR)
Q205$^{\phi}$	HS	98.1 (HS)	98.8 (HS)	96 (HS)
Q208$^{\phi}$	IR	NA	6.3 (R)	0.6 (HR)
Q209$^{\phi}$	S	NA	50.4 (S)	52.3 (S)
Q220$^{\phi}$	S	NA	85.2 (HS)	2.5 (R)
Q242$^{\phi}$	IR	1.3 (HR)	NA	4.2 (R)

[1] Rating category, HS= highly susceptible, S=susceptible, IS= intermediate susceptible, I = intermediate, IR=intermediate resistant, R= resistant and HR= highly resistant

There were highly significant correlations between historical ratings from the dip inoculation method and natural infection, and the correlation coefficients varied from trial to trial 0.82 in the Experiment 1 to 0.72 in the Experiment 3 (Table 6).

Table 6—Pearson correlation coefficients between the historical dip inoculation ratings and the smut incidence in natural infection trials

	Natural infection		
Trial	Experiment 1	Experiment 2	Experiment 3
Dip inoculation	0.82	0.78	0.72
P value	*0.007*	*<0.0001*	*0.0001*

Discussion

The smut ratings of most of the varieties included in the natural infection experiments are in general agreement with the ratings obtained from the dip inoculation method used in current screening experiments. The three experiments showed high correlations (0.72 to 0.82) with the dip inoculation method, and are in agreement with the overseas studies. Ladd *et al.* (1975) reported a reasonably high correlation (r=0.72) between this two method with slight biased towards the dip inoculation method for overestimation of susceptibility. They found clones with less than 20% of plants infected by artificial inoculation had zero to 5% infection under natural conditions. In Florida, significant correlation (~0.90) between artificial dip inoculation and natural infection was observed (Holder ,1985).

Later, Comstock *et al.* (1987) found the correlations between the two methods varied from r=0.45 to 0.84. They hypothesised that differences in correlation values likely influenced by the types of clones included in the natural infection trials. In the trials where high number of susceptible and resistant clones were included, a high correlation coefficient occurred between the ratings obtained from the dip inoculation and the natural infection trials. On the other hand, when clones were selected from the intermediate or the intermediate susceptible categories the correlation coefficient between two methods were lower. The higher correlation coefficient value (r=0.82) in Experiment 1, and relatively low correlation coefficient values in the later experiments in our study confirms their hypothesis.

Dip inoculation is the most widely used method for screening varieties in sugarcane breeding programs (Lee-Lovick, 1978; Ferreira *et al.,* 1980). The dip inoculation method has been very effective in SRA smut screening program, and enabled to screen approximately 2 500 clones from various stages of selection programs within 10–12 months. This method has been successfully used in most of the sugarcane producing countries for more than 30 years. In Hawaii, breeding populations shifted from highly susceptible (36% resistant) to highly resistant (89% resistant) within a five year period using dip inoculation method (Comstock *et al.*, 1987).

Two main constraints of the natural infection method compared with the dip inoculation method are that it requires (i) at least two ratoon cycles (3 years) to get a reliable result (Ferreira *et al.*, 1980) compared with less than one year (10–12 months) for the dip method and (ii) more than double the area for experimental establishment that limits the number of clones can be tested. The low levels of smut in the plant and the first ratoon crops in natural infection experiments is not unexpected and agrees with the experience from overseas that has shown that the best ratings from the natural infection method are obtained in the second ratoon crop (Holder, 1985).

Although, most of the ratings obtained using dip inoculation are in close agreement with natural infection, there were some differences. 'Resistant' varieties Q219$^{\phi}$ and Q212$^{\phi}$ had high levels of smut (>30%) in the 2008 natural infection trial, and Q188$^{\phi}$ had high levels of smut in experiment 1 but low levels in experiment 3. A few possible causes may be suggested:

(i) in the current dip inoculation method, a clone is screened at least three times before release. The final rating of this clone is based on the combined analysis of three or more screening experiments.

(ii) disease expression depends on interactions between test varieties, number and quality of smut spores (inocula), and environmental conditions. A resistant or intermediate variety may show a susceptible reaction if subject to stress such as drought and high temperatures. A susceptible clone may show little smut if the condition are not conducive for smut infection (Holder, 1985).

(iii) some clones may exhibit 'false' resistance or susceptibility when subject to dip inoculation method (Comstock *et al.,* 1987).

The high levels of smut in the varieties Q205$^{\phi}$ and Q157 in all experiments agree with the observations in commercial fields that these varieties are exceptionally susceptible. Likewise, highly resistant varieties Q171$^{\phi}$ and Q151 had very little smut in all experiments. All of the resistant varieties released as commercial varieties from the SRA breeding program maintained low levels of smut in commercial fields. There were considerable variations of smut ratings among intermediate varieties, ranging from resistant to susceptible.

Rating intermediate resistant varieties is always a challenge, and more likely to vary among experiments. The influence of variety, environment and growth of the cane could influence the levels of infection in buds. The interaction between environmental conditions and varietal resistance may play a role in the differential expression of smut in intermediate resistant varieties. Recently, moderate levels of smut were observed in two intermediate resistant varieties Q252$^{\phi}$ and SRA8 in the Burdekin region. We believe unusually dry and warm conditions for last few years created favourable condition for smut infection in the intermediate resistant varieties in the Burdekin region. More research work is required to understand the interaction of the environment, sugarcane varieties and the smut fungus under natural condition

Smut infection can occur in buds on standing stalks (Lee-Lovick, 1978) and smut whips on side-shoots developing from infected buds on standing stalks have been very common in the natural spread experiments reported in this paper and in commercial fields in Bundaberg (Croft *et al.*, 2008). The results from this study suggest that, if plant sources are inspected and any stalks with smut whips are discarded, the spread of smut in planting material can be minimised.

The increase in smut incidence between the plant and ratoon crop has been widely reported overseas (Comstock, 2000; Lee-Lovick, 1978). There is a significant varietal interaction between ratooning and smut incidence, with incidence increasing in older ratoons in some varieties and

stabilising or even decreasing in others. The mechanisms of resistance in dormant buds on setts, young germinating buds on setts and buds and young tillers in ratoon crops appear to differ among varieties. In the dip inoculation method, experiments are maintained until first ratoon and this allows for expression of symptoms in infected plants that have not developed symptoms in the plant crop and for secondary spread of infection into the first ratoon crop. The dip method therefore combines to some degree the direct effect of artificial dip inoculation and then natural spread between plots in the field.

Conclusions

The results from the three natural infection experiments (2007, 2008 and 2009) in Bundaberg indicated that the screening of sugarcane varieties for smut using the dip inoculation method is generally good at estimating smut resistance and gives results comparable with natural spread of the disease. The advantages of the current screening method over natural infection are that it requires a shorter time, relatively less area and is less costly. Although, most of the ratings obtained from dip inoculation are in close agreement with natural infection, care should be taken in the interpretation of the resistance of varieties from these results, in particular for the intermediate varieties which can vary significantly in different disease pressure, environments and seasons.

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