BSES Limited

REVIEW OF MOTH-BORER RESISTANCE SCREENING

AND REPORT ON VISIT TO SASRI

by

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SUMMARY

Information on procedures for screening sugarcane varieties for resistance to moth borers and associated research was obtained by a search of literature and by a 2-week visit to SASRI (South African Sugarcane Research Institute).

Screening procedures are mainly of two types:

- in field plots where plants are infested by naturally occurring populations of moths, sometimes encouraged by planting of susceptible host plants near the experimental plots and/or augmented by the release of additional moth borers from laboratory culture
- in pots where plants are infested artificially, often using moth-borer eggs.

Each of these has advantages and disadvantages. Natural infestation of field plots allows the full range of resistance mechanisms – antixenosis, antibiosis and tolerance – to operate under commercial conditions but results may be subject to considerable experimental variation due to variable environmental conditions and inconsistent numbers of borers. Artificial infestation of potted plants allows the experimenter to control environmental variables and apply a constant infestation pressure but some components of resistance (especially ovipositional antixenosis) may be missed. While artificial infestation has been used in numerous studies to elucidate resistance mechanisms, it seems to be currently used as a routine method of screening varieties for borer resistance only at SASRI against *Eldana saccharina*. Molecular markers and near-infrared spectroscopy are techniques that could aid with identification of potentially resistant varieties.

Data collected in resistance trials always includes a measure of borer damage, typically a count of bored internodes, and sometimes a measure of larval performance such as number or weight of borers or number of emergence holes indicating successful production of adults. A few studies have included plants from which borers are excluded, usually with insecticide, which allows crop tolerance to be measured, or have estimated tolerance by rating plant response according to indirect measures such as side-shooting or stalk breakage.

Systematic screening of varieties ideally includes a set of standard varieties covering the range of expected responses from susceptible to resistant. Most screening programs rate varieties as susceptible, resistant or intermediate, but the program at SASRI rates varieties on the 1-9 scale familiar to plant pathologists, with the results weighted according to experimental precision. Molecular markers for resistance have been identified that can help with choice of parents in breeding programs.

There is some evidence of ovipositional antixenosis, larval antixenosis and antibiosis and plant tolerance as mechanisms of genotypic sugarcane resistance against different species of moth borers. Of these, antixenosis or antibiosis acting against early-stage larvae seems the most common, preventing larval penetration of the stalk or delaying penetration so that small larvae are exposed to abiotic and biotic mortality factors. Near-infrared spectroscopy (NIR) has potential as a method for predicting resistance that is associated with stalk surface chemistry. Phenotypic resistance may be altered by plant nutrition; water stress or increased levels of nitrogen may increase susceptibility while increased silicon may promote resistance.

SASRI has adopted an annual program of screening varieties from stages 4 and 5 of their 5-stage breeding program in pots in a shadehouse for resistance to *E. saccharina*. This method ensures uniform infestation of plants, allows better control of environmental variables and requires less labour than similar field trials. However, yield trials in stages 4 and 5 are also sampled for *E. saccharina* damage but with fewer variables measured than in the shadehouse. Plants in the shadehouse are assessed for damage as bored internodes and larval performance as number of live larvae. Length of bored internodes and weight of larvae and pupae were measured when the program was begun several years ago but were strongly correlated with the other two variables and so were dropped from the procedure. Six standard varieties are included in each screening trial and both standard and test varieties are rated on a 1-9 (resistant-susceptible) scale by a statistical method that weights the measured variables according to their variance and then calculates ratings according to the precision of the experiment; the latter calculation is fundamentally different from the method used by BSES plant pathologists.

I gained practical experience of the SASRI screening procedure during my visit to South Africa, working with Malcolm Keeping who leads the program for evaluating resistance to *E. saccharina*. I obtained specifications of the shadehouse to allow a suitable structure to be built at Ramu Agri-Industries. I also worked with other SASRI staff (plant breeding, biometry) involved in the *E. saccharina* resistance program and with other SASRI entomologists (Mike Way, Des Conlong, Graeme Leslie) involved in other research programs.

1.0 BACKGROUND

Moth borers are extremely injurious pests of sugarcane in most countries. Tunnelling by borer larvae within stalks may lead to direct loss of stalk weight, disruption of nutrient flow, stalk breakage, top death and side shooting, as well as introduction of pathogens, all of which contribute to reduced yield and quality of sugar (e.g. Milligan *et al.*, 2003). Australia is currently free of serious moth-borer species but is at risk of an incursion of damaging species from Papua New Guinea (PNG), including *Sesamia grisescens*, species of *Chilo* and the top borer *Scirpophaga excerptalis*.

In the event of an incursion of moth borers, varietal resistance is likely to be a major component of a long-term management strategy. Mass production of biological control agents, particularly parasitoids, is done in many countries but may not be economically viable in Australia. Insecticides are also used in some countries, including PNG, but aerial application of insecticides to sugarcane in Australia over large areas would potentially have environmental and social costs and may not be acceptable to the community. Insecticide use could be minimised by integration with varietal resistance and the development of variety-specific treatment thresholds (Posey *et al.*, 2006).

Project BSS331 was commenced in 2009 to reduce the risk of pest and disease incursions from PNG; for moth borers a primary aim is to develop efficient and reliable screening procedures and understand resistance mechanisms, facilitating a screening program beyond the life of the project. Various systems are used for evaluating varietal resistance to moth-borer species such as *Diatraea saccharalis* in the Americas and *Eldana saccharina* in Africa, but suitable procedures must be established for other borers in PNG which have different biologies and may invoke different resistance mechanisms.

Before undertaking the experimental program, it was important to review resistance screening and associated research that has been done in other countries. This review covers conventional sources of resistance, and not genetic modification. In addition, a visit was made to SASRI (South African Sugarcane Research Institute) where considerable work has been done by Dr Malcolm Keeping and colleagues on methodologies for evaluating varietal resistance to the moth borer *E. saccharina*, and on resistance mechanisms and induced resistance, especially as influenced by soil-applied silicon.

2.0 OBJECTIVES

- Review resistance-screening procedures and associated research on moth borers worldwide
- Visit SASRI, South Africa, to assess shadehouse and field-plot trials

3.0 RESISTANCE-SCREENING PROCEDURES

3.1 Natural infestation

Exposure of varieties to naturally occurring populations of moth borers is the most straightforward and commonly used method for evaluating resistance. It allows the full set of resistance mechanisms to operate: antixenosis, antibiosis and tolerance. However, it also brings in variability in infestation pressure both within and between trials. This variability is often minimised in dedicated resistance trials by intercropping with susceptible host plants

(e.g. sorghum or maize for *E. saccharina* and *D. saccharalis*) and by seeding trials with moth borers (e.g. Keeping, 1999; White *et al.*, 2001). Precision of field-based assessment of varietal resistance is also influenced by site characteristics such as soil type, moisture and climate (Keeping, 2006).

There is a vast literature on differences in damage among varieties for various moth borers: *Chilo agamemnon* (Besheit *et al*., 1998), *C. auricilius* (Kooner *et al*., 1976; Singh *et al*., 1993; Jena *et al*., 1996; Pandey *et al*., 1998; Sharma *et al*., 2007b), *C. infuscatellus* (Hansi and Rao, 1995; Parsana *et al*., 1995; Arvind and Sardana, 1996; Ahad *et al*., 1999; Karnatak *et al*., 1999; Umapathy *et al*., 1999; Thirumurugan *et al*., 2000, 2004; Abdullah *et al*., 2006; Faqir *et al*., 2008), *C. sacchariphagus* (David and Joseph, 1983; Jayanthi, 1988; Rajendran *et al*., 1996; Suhartawan, 1998; Thirumurugan *et al*., 2000, 2004; Arul and Prabagar, 2003; Muhammad *et al*., 2008), *C. tumidicostalis* (Borah, 1993; Gupta, 1997; Abdullah *et al*., 2006), *D. saccharalis* (Hensley and Long, 1969; Lourencao *et al*., 1982; White *et al*., 1996; Salvatore *et al*., 2008), *E. saccharina* (Nuss, 1991), *Eoreuma loftini* (Pfannenstiel and Meagher, 1991), *Scirpophaga excerptalis* (Agarwal *et al*., 1974; Samoedi, 1988; Chaudhary and Yadav, 1995a; Gupta, 1997; Pandey *et al*., 1997; Tanwar and Varma, 1997; Suhartawan, 1998; Singla, 1999; Abdul *et al*., 2003; Bhoopathi and Karnatak, 2003; Abdullah *et al*., 2006; Muhammad *et al*., 2008), *Sesamia botanephaga* (Ogunwolu, 1979), *S. cretica* (El-Amin, 1984) and *S. grisescens* (Kuniata, 2000).

Borer damage to detect differences is often estimated as an additional set of data from regional variety trials in the final stages of selection before release (e.g. Ramon *et al.*, 2007) rather than as part of dedicated resistance trials. Experimental design may be different if quantification of resistance is the primary aim; in particular, such studies often have greater replication (four or more) to allow for the inherent spatial variability of insect populations (Bessin *et al.*, 1990; Nuss, 1991; Meagher *et al.*, 1996; Legaspi *et al.*, 1999; Milligan *et al.*, 2003; Nibouche and Tibère, 2008).

The size of field plots varies greatly between studies, depending on the purpose of the work. Typically, larger multi-row plots are used when yield measurement is critical (e.g. Nibouche and Tibère, 2008), but small plots may be used if only damage is being estimated. At the extreme, Nibouche and Tibère (2009) compared clones in single-stool plots with high replication (20) and concluded that this design had similar efficiency to classical large-plot designs.

3.2 Artificial infestation

Artificial infestation of potted plants with moth-borer eggs or larvae provides a way of ensuring infestation pressure of sufficient intensity to discriminate among varieties, with all varieties receiving equal exposure, and also allows greater control of environmental variables. Environmental conditions can be manipulated to maximise experimental efficiency, e.g. potted plants for screening against *E. saccharina* are water-stressed to increase survival and growth of larvae (Nuss, 1991; Keeping, 2006) and moderate water stress also enhances survival and weight of *Eoreuma loftini* (Reay-Jones *et al.*, 2003). Infestation with eggs or neonate larvae is the most satisfactory procedure, as it allows resistance mechanisms to operate against vulnerable early instars. Infestation with eggs has been used for *D. saccharalis* (Jackson and Dunckelman, 1974) and is used for routine screening of varieties for resistance to *E. saccharina* (Keeping, 2006). Poor survival of eggs may introduce some variability into the results; for example, survival of *E. saccharina* from egg to mature larvae or pupae was 14% and 7% in trials in 2001 and 2002 (Keeping, 2006), which reflects in part the resistance mechanisms operating in the set of varieties but must also include a random component of background mortality. Pan and Hensley (1973) report an experiment to screen clones for resistance to *D. saccharalis* by infesting seedlings with

first instar larvae and counting subsequent dead hearts. A follow-up study showed a poor correlation between dead hearts in the seedling test and bored internodes of the same clones in a naturally infested field trial, but cluster analysis suggested that the seedling test might help to identify extremes in resistance or susceptibility (White and Dunckelman, 1989). White (1993a) infested a small number of varieties with first instar larvae at known positions on the stalk in order to assess larval establishment and its role in conferring resistance. Sosa (1995) evaluated a technique wherein stalks were infested with larvae of *D. saccharalis* by drilling a 5 mm hole and introducing third-instar larvae into each hole via a micro-centrifuge tube, using both intact and harvested stalks, and Albert *et al.* (2007) conducted a similar experiment with *C. sacchariphagus*.

Butterfield *et al.* (2007) describe the use of artificial infestation to estimate resistance to *E. saccharina* at a family rather than an individual-plant level. A mass-screening method was used with seedlings from 36 bi-parental crosses planted together in groups at a high density and then inoculated with eggs. The technique effectively discriminated among the average resistance of different families, and could be used as a tool in a recurrent-selection breeding strategy.

Any ovipositional antixenosis that might be present among cane varieties (e.g. Sosa, 1988) will not be detected using artificial infestation with eggs or larvae, as it removes moth choice from the infestation sequence. Resistance ratings to *E. saccharina* from field screening were in agreement with ratings from artificial infestation of potted plants for most varieties, but there are differences for a small number of varieties that might indicate differential oviposition (Keeping, 1999). Manual insertion of larvae into stalks bypasses other possible resistance mechanisms that may limit initial infestation of plants, and the validity of screening using this method would need to be confirmed for each borer of interest.

Plants have been caged with adults in some studies as a method for detecting ovipositional antixenosis (David and Joseph, 1984; Sosa, 1988; Meagher *et al.*, 1996; Keeping and Leslie, 1999) but this method seems not to have been used to routinely screen varieties for resistance. It is possible that uneven infestation pressure might arise due to external influences, such as presence of light sources or the cage walls themselves and their proximity to plants.

Currently, it seems that artificial infestation is being used routinely to screen varieties for resistance only against *E. saccharina* in South Africa (Keeping, 2006).

3.3 Measurements

Measurements taken to evaluate varietal resistance to moth borers relate to borer damage and borer performance (Keeping, 2006). The former includes numbers of bored stalks, numbers of bored internodes, lengths of larval tunnels and number of tunnels per stalk, while the latter includes numbers and weight of moth borer larvae or pupae and numbers of exit holes (e.g. Keeping, 1999, 2006; Bessin *et al.*, 1990b; Nibouche and Tibère, 2008). For *D. saccharalis*, larval entrance sites are clearly identifiable after leaf sheaths are removed, and so bored internodes can be identified without splitting the stalk (White *et al.*, 2001), but this does not apply to all moth borers. A subjective damage rating is also used for *D. saccharalis*, based on leaf-sheath feeding, side-shooting and death or breakage of tops (White *et al.*, 2001). A fixed number of stalks is usually sampled from each plot at time of harvest of the mature crop, e.g. 15 (Milligan *et al.*, 2003), 20 (Nuss, 1991; Legaspi *et al.*, 1999; Reay-Jones *et al.*, 2003) and 30 (Nibouche and Tibère, 2008).

There are few studies reporting a quantitative genetics approach to moth-borer resistance in sugarcane. White *et al.* (2001) and Milligan *et al.* (2003) investigated the heritability,

expected response to selection and genetic correlations among measures of borer-induced damaged commonly used for *D. saccharalis*. Percentage bored internodes was the most effective single trait to reduce borer damage, but a subjectively assessed damage rating (see above) was better if the cost of data collection was considered. The practice of screening at one location with four replicates was judged adequate.

Nibouche and Tibère (2008) compared seven damage measures used for screening for resistance to *C. sacchariphagus*. The percentage of bored stalks was the most cost-effective measurement of damage, being low-cost, as reliable as more detailed measures based on internode counts or stalk dissection, and highly correlated with all other damage measures. However, these authors noted that the percentage of bored stalks at harvest tends towards 100% when borer populations are high, possibly masking differences among varieties. In this circumstance, damage assessment could be performed earlier in crop growth if further studies showed that earlier assessment would give the same results.

As an alternative to measuring the length of borer tunnels, Albert *et al.* (2007) estimated the volume of tunnels made by *C. sacchariphagus* by injecting water into each hole and measuring the volume that they contained. They did this to account for the fact that tunnels made by the *C. sacchariphagus* are not uniform in diameter. An index of damage was calculated as the product of intensity of infestation (percentage of internodes bored) and average feeding volume on each variety.

Measurements of larval damage do not necessarily indicate how many adults are successfully produced from those plants. Bessin *et al.* (1990b) used emergence holes of *D. saccharalis* as a seasonal record of adult emergence, and calculated a relative survival index as the ratio of number of exit holes to number of bored internodes. This survival index was poorly correlated with percentage of bored internodes, indicating that a simple estimation of injury to different varieties does not take into account subsequent survival of larvae to adulthood. They argued that varieties should be chosen that not only have minimal damage but also slow the area-wide build up of moth populations, and that both resistance to injury and resistance to moth production should be considered when rating varieties. Similar work has been done for *E. loftini* (Reay-Jones *et al.*, 2003). However, selection of genotypes according to a moth production index may disadvantage productive varieties with more stalks per unit area (Milligan *et al.*, 2003).

The early-instar larvae of some moth borers leave feeding traces on leaves before boring into stalks. In a field survey for *C. sacchariphagus* in Mozambique, rating of varieties based on non-destructive estimation of feeding damage to the top four leaves of young sugarcane plants was in general agreement with ratings developed using a full destructive sampling to estimate internal stalk damage and borer performance (Conlong *et al.*, 2004). This suggested that a non-destructive method could be developed similar to that currently used for moth borers in maize and sorghum crops (e.g. van Rensburg, 1999). However, a subsequent study by Nibouche and Tibère (2009) indicated that leaf resistance is not the only resistance mechanism operating against *C. sacchariphagus* in sugarcane (see later).

Assessment of borer damage alone is not sufficient to estimate crop loss caused by moth borers, or to detect differences in response to damage among varieties (tolerance). Regression methods comparing yields corresponding to different naturally occurring levels of damage among plots or fields have been used to evaluate varietal response. Legaspi *et al.* (1999) found that stalk weight, cane yield, sugar content, juice purity and sugar yield were all inversely related to percentage of bored internodes, while ash content increased with damage. A drawback with this method is that natural variation in intensity levels may be confounded with variation in agronomic factors or crop growth, and a better procedure to measure losses is to control the intensity of infestation by excluding borers from some plots, perhaps by using insecticide (e.g. Goebel and Way, 2007).

For *D. saccharalis*, Posey *et al.* (2006) note that yield data (in the absence of a borer-free comparison) are rarely used to measure the effect of cultivar on damage, because of the difference in yield potential among cultivars. The percentage of bored internodes, which has been used successfully since the 1960s, remains the most popular method to assess injury levels.

3.4 Experimental design and data analysis

For routine screening of varieties, a set of standard or control varieties covering the range of resistance levels from low to high can serve to calibrate the response of the test varieties, producing individual ratings that are standardised and independent of those of other varieties in the same trial (Keeping, 2006). Trial results must be interpreted with caution if the standard varieties do not perform as expected, or if there is inadequate discrimination among the standards. The current testing program against *E. saccharina* in a shadehouse uses six control varieties covering the range from the most susceptible to the most resistant (Keeping, 2006).

In resistance screening for diseases, it is customary to rate varieties according to a scale from 1 (resistant) to 9 (susceptible) (Hutchinson, 1970), and similar ratings can be applied to resistance to moth borers. Ratings developed by Keeping (2006) for resistance to *E. saccharina* were based on two damage variables, number of internodes bored and length of stalk bored, and two variables describing larval performance, numbers of larvae and pupae and their total weight. These variables, weighted according to their precision, were combined into a single response variable. A rating for each variety was then determined such that two units on the 1-9 unit scale were equivalent to a 95% confidence interval and the midpoint of the scale represented the mean of the six standard varieties. With this system, the ratings of the standard varieties can vary among trials, and will tend to cluster around the midpoint of the scale in trials that provide little discrimination. The current SASRI screening program continues to use this system, but two of the variables, length of stalk bored and total weight of larvae and pupae, are no longer measured because they were highly correlated with the other two variables (Keeping, pers. comm.). Alternatively, White (1993b) used cluster analysis to classify varieties according to their response to *D. saccharalis* as measured by percentage bored internodes and visual damage rating. Labels (resistant, susceptible) were placed on three or four discrete classes with the aid of varieties of known reaction to *D. saccharalis* included as standards. Many studies simply sort varieties according to their measured response variables, often percentage of bored internodes, or group them as susceptible, intermediate or resistant with little statistical analysis.

Borer damage is frequently measured as the proportion of bored internodes, and these data may then be compared among varieties by analysis of variance. As pointed out by Bessin *et al.* (1990a), the proportion of bored internodes will follow a binomial distribution and the data will likely violate the assumptions underlying analysis of variance. These authors evaluated several transformations and concluded that weighted least squares analysis of variance of logistic-transformed data was the most suitable statistical procedure. Other transformations are used; for example, Bessin *et al.* (1990b) transformed data on percentage bored internodes and relative larval survival using probits and applied a weighting according to the number of stalks examined, White (1993b) used the square root transformation for percentage data (usually less than 30%), and Legaspi *et al.* (1999) transformed percentages using arcsines.

3.5 Rapid methods

Screening of varieties for phenotypic resistance to moth borers is extremely time-consuming if destructive sampling is used, and is subject to uncertainty of infestation and environmental conditions. This limits the number of genotypes that can be screened using conventional biological assays.

Molecular markers could narrow down the list of genotypes of potential interest for resistance and aid in choice of parents in a breeding program. Butterfield *et al.* (2007) identified four molecular markers associated with phenotypic resistance to *E. saccharina*, and provided an empirical validation of improved efficiency of molecular over conventional breeding for this trait; currently SASRI uses six markers in its breeding program (Shailesh Joshi, pers. comm.). Selvi *et al.* (2008) identified three markers, two microsatellite markers and one RAPD (random amplified polymorphic DNA) marker associated with resistance to *S. excerptalis*.

Delay in penetration of young larvae into sugarcane stalks appears to be an important mechanism of resistance against *E. saccharina* (see later), suggesting that plant surface chemistry may be involved (Rutherford *et al.*, 1993). Rutherford *et al.* (1993) and Rutherford and van Staden (1996) correlated resistance levels and spectral data obtained on stalk surface wax using near-infrared (NIR) spectroscopy. Subsequent work indicated that NIR models were capable of predicting resistance ratings to *E. saccharina*, but a full calibration would require data over a range of sites, growing seasons and environmental conditions (Rutherford, 1998).

4.0 MECHANISMS

Knowledge of mechanisms and associated character traits could enhance the ability of sugarcane breeding and genetic engineering to increase levels of resistance in plant populations (Meagher *et al.*, 1996) and help with the design of realistic testing procedures (e.g. Keeping, 2006). A counter-argument is that time spent on mechanism studies could be better spent on screening, if identification of resistant varieties is the ultimate goal, and knowledge of mechanisms in known resistant varieties does not preclude the presence of unknown mechanisms in unstudied genotypes. Results on various moth-borer species are summarised below.

4.1 *Chilo agamemnon*

Differences in larval penetration by *C. agamemnon* were observed between two varieties, with NCo310 receiving more eggs but subsequently having smaller larval populations inside the stalk than GT54-9; more dead larvae were observed outside the stalks of NCo310 (Temerak, 1982). There was an inverse relationship between fibre content and damage caused by *Chilo* spp. among varieties in Egypt (Allam and Abou Dooh, 1995).

4.2 *Chilo auricilius*

Varieties resistant to *C. auricilius* had higher levels of fibre in leaf sheaths and stalks, more lignin and cellulose, lower sugar, more tannins and phenols, lower nitrogen and more potassium and phosphorus, compared with susceptible varieties (Sharma *et al.*, 2007a).

4.3 *Chilo infuscatellus*

Anatomical characteristics associated with resistance to *C. infuscatellus* included thick sclerenchyma of the leaf sheath, short distance between the vascular bundles and high compressive strength of stalks, together with various differences in chemical composition including a high level of silica and phenols and low number of amino acids (Kennedy and Nachiappan, 1992). However, this study evaluated only four varieties and much more work would be needed to confirm the generality of these associations and the relationships among them.

4.4 *Chilo sacchariphagus*

David and Joseph (1984) identified differences in speed of stalk penetration and weights of larvae and pupae among commercial varieties, as well as ovipositional antixenosis among species of *Saccharum* and related plants; however the latter was of doubtful significance in commercial sugarcane. Nibouche and Tibère (2009) identified two mechanisms of resistance against *C. sacchariphagus*, leaf resistance (see also Conlong *et al.*, 2004) and stalk resistance, both of which contributed to overall resistance quantified by stalk tunnelling. For this borer there was a positive genetic correlation between damage and cane yield, i.e., the more productive clones tended to be the ones more attacked, due to a relationship of stalk damage with stalk length and number (Nibouche and Tibère, 2008). This indicates a need to explore a wider source of genotypes for resistance mechanisms not negatively linked to desirable agronomic characters.

4.5 *Chilo tumidicostalis*

In a comparison of two varieties, larvae developed more slowly in excised stalk pieces of the more resistant variety, indicating antibiosis as one resistance mechanism (Gupta *et al.*, 2006).

4.6 *Diatraea saccharalis*

Resistance in NCo310 in comparison with a susceptible variety CP44-101 was due mainly to higher larval mortality, especially among young larvae (Kyle and Hensley, 1970). Establishment of larvae on NCo310 was inhibited by its tight leaf sheath (Coburn and Hensley, 1972). White (1993) also measured less successful stalk penetration on a resistant compared with a susceptible variety when artificially infested with neonate larvae. Rind hardness has been implicated as a resistance mechanism (Martin *et al.*, 1975), and White *et al.* (2006) determined that resistance was correlated with fibre content and target-internode rind hardness within a group of progeny from a bi-parental cross. However, fibre level is not consistently related to varietal resistance (Posey *et al.*, 2006). Ovipositional antixenosis appears not to be a general resistance mechanism (Kyle and Hensley, 1970), but pubescence adversely affected oviposition and first-instar mobility of *D. saccharalis* in a comparison of one pubescent and several glabrous varieties (Sosa, 1988, 1990). Tolerance may also be a mechanism of resistance, with some varieties apparently able to tolerate more larval tunnelling while maintaining yield (White and Hensley, 1987).

4.7 *Eldana saccharina*

The major factor determining varietal resistance in *E. saccharina* is likely to be antixenosis or antibiosis to young larvae (Keeping, 2006). Larvae penetrate the stalk as first instars, often at the bud (Leslie, 1993), and a delay in penetration exposes these small larvae to

desiccation and predation. Varietal characteristics associated with resistance include rind hardness, fibre and the quantity and chemical composition of surface waxes (Rutherford *et al.*, 1993; Keeping and Rutherford, 2004). Some resistant varieties may have higher levels of endogenous silicon (Kvedaras and Keeping, 2007; Keeping *et al.*, 2009), which can enhance resistance levels when applied to silicon-deficient soils (see later). Drought-tolerant varieties are over-represented among varieties resistant to *E. saccharina* (Keeping and Rutherford, 2004), suggesting that this may be a factor in resistance; as noted earlier, water stress promotes survival and growth of *E. saccharina* larvae. Choice and no-choice tests of six susceptible or resistant varieties with caged moths did not show consistent antixenosis in terms of adult oviposition (Mabulu and Keeping, 1999), but the absence of ovipositional antixenosis under field conditions has not been confirmed (Keeping, 2006). Pre-trashing of sugarcane reduces infestation by *E. saccharina* by removing oviposition sites and exposing eggs and young larvae to predation and desiccation (Leslie 1989) so self-trashing varieties might be expected to similarly have reduced level of attack, but no difference among varieties with different trash habits was detected in a cage study (Keeping and Leslie, 1999). However, this may reflect an insufficient range of trashing habits among the varieties used and possible confounding of trash habit with other varietal characteristics such as rind hardness (Leslie, pers. comm.).

4.8 *Eoreuma loftini*

A diet-incorporation assay using leaf-sheath tissue indicated that larval antibiosis may operate against *E. loftini* in some varieties, with significant differences in larval development times and pupal weight, while larval-choice tests with leaf whorls indicated antixenosis, although the two mechanisms were not necessarily detected in the same varieties (Meagher *et al.*, 1996). Field measurements of resistance did not always agree with laboratory measurements, suggesting more the one mechanism may operate. Differences in oviposition were not consistently detected among up to six varieties grown in either pots or in the field and exposed to caged moths, suggesting ovipositional antixenosis is probably not a major resistance mechanism.

4.9 *Scirpophaga excerptalis*

Chaudhary and Yadav (1995a) screened 30 genotypes for resistance to *S. excerptalis* and found borer incidence was correlated with the number of midribs bored by young larvae. Incidence among genotypes was positively correlated with nitrogen in midribs, growing points and lamina tissue and negatively with phosphorus and potassium (Chaudhary and Yadav, 1995b). In a related study on structural constituents, borer incidence was negatively correlated with lignin in midribs but not in growing points or lamina, while correlations with cellulose, silica and ash were not significant (Chaudhary and Yadav, 1998). Mukunthan and Mohanasundaram (1998) characterised failed infestations of *S. excerptalis* as either Type 1, where larvae in the midrib failed to reach the spindle, or Type 2, where older larvae in the spindle failed to reach the meristem. Type 1 failure was frequent and was negatively correlated with the percentage of dead hearts among varieties; Type 2 failure was rare and not related to resistance. No antibiosis was detected among sugarcane varieties once larvae were established in the spindle (Mukunthan and Mohanasundaram, 1996).

4.10 *Sesamia nonagrioides*

Ovipositional antixenosis appears to be a significant component of resistance to this species, with more eggs laid on a susceptible cultivar than on resistant cultivars. However neonate

larvae did not show any difference in preference for susceptible or resistant varieties in choice or no-choice tests (Askarianzadeh *et al.*, 2005).

4.11 *Sesamia grisescens*

Young cane tends to be attacked regardless of variety, but in older cane those varieties showing some resistance produce a constriction in the internode below the bored internode, preventing rot from extending down the stalk (Kuniata, 2000).

4.12 Similarities and differences among species

The resistance rating of cane varieties may vary depending on the moth-borer species. For example, Conlong *et al.* (2004) found that varieties with high resistance to *E. saccharina* were not resistant to *C. sacchariphagus*. Presumably, this reflects different biologies and behaviours of different species. Neonate larvae of *E. saccharina* feed on the outside of sugarcane stalks until about the third instar, when they are able to penetrate the stalk (Kvedaras and Keeping, 2007). Conlong *et al.* (2004) also found evidence of changing resistance patterns among varieties depending on irrigation and on crop age. Of various resistance mechanisms, antixenosis or antibiosis acting against early stage larva seems the most common, preventing or delaying larval penetration of the stalk.

5.0 INDUCED RESISTANCE

Phenotypic resistance levels may be influenced by crop nutrition and water status. Incidence of *S. excerptalis* increases with increasing rates of nitrogen fertiliser (Saikia *et al.*, 1994) and plant levels of nitrogen were positively correlated with incidence of this pest (Chaudhary and Yadav, 1995b). Infestation of *C. auricilius* also increased with rising rates of nitrogen fertiliser (Singh *et al.*, 1983). Water stress increases the susceptibility of sugarcane to *E. saccharina* (Nuss, 1991) and *E. loftini* (Reay-Jones *et al.*, 2005), which may be due to accumulation of free amino acids in stressed plants (Reay-Jones *et al.*, 2005).

Extensive work has been conducted on the role of silicon in resistance of sugarcane to moth borers, especially *E. saccharina*. Keeping and Meyer (2000) reported reduced damage from *E. saccharina* and poorer larval performance when potted plants growing in river sand were treated with calcium silicate, while Anderson and Sosa (2001) recovered fewer *D. saccharalis* larvae from silicon-treated varieties. With regard to *E. saccharina*, applied silicon can partially offset the negative effects on damage and larval performance of applied nitrogen (Meyer and Keeping, 2005) and of water stress and varietal susceptibility (Kvedaras *et al.*, 2006, 2007). Silicon appears to suppress *E. saccharina* by delaying stalk penetration, so increasing exposure of larvae to external mortality factors, and by reducing larval growth and feeding damage (Kvedaras and Keeping, 2007). The mode of action of silicon includes increasing mechanical resistance to stalk penetration, while induction of biochemical defences in wounded stalks may also contribute (Kvedaras *et al.*, 2009; Keeping *et al.*, 2009).

6.0 VISIT TO SASRI

6.1 Resistance screening

Discussions were held with Dr Malcolm Keeping (Senior Entomologist) on biological and technical aspects of screening for resistance, particularly using potted plants in a shadehouse, Chandani Sewpersad (biometrician) on data analysis, Shailesh Joshi (plant breeder) on the plant breeding program and field measurements of *E. saccharina* infestation in breeding trials, and Stuart Rutherford (Manager Crop Protection Program) on near-infrared spectroscopy (NIR). Practical experience was also obtained in the shadehouse by participation in assessment of a pot trial. Physical measurements were obtained for the dimensions and materials used in the shadehouses at SASRI. Substantial progress was also made during the visit on the review of moth-borer screening with input from Malcolm Keeping.

The rationale behind screening for resistance using potted plants in a shadehouse rather than field plots is mainly that pot trials require much less labour than field trials, and allow much better control of environmental variables. Plants can all be water-stressed equally to encourage uniform moth-borer establishment and growth in susceptible varieties. An argument against this procedure is that any resistance based on moths not preferring a variety for oviposition (ovipositional antixenosis) will not be measured, as eggs are placed directly on to the plants. Mass-release of moths in the shadehouse was discussed as an alternative procedure, but there may be problems with uneven infestation due to external influences such as proximity of shadehouse walls to certain pots and external sources of directional lighting. The aim with artificial infestation is to remove sources of variation whenever possible.

Varieties in stages 4 and 5 of the 5-stage breeding program are screened for resistance in the shadehouse.

Pots (37 cm diam. at the top x 35 cm deep) are filled with river sand and planted with six pre-germinated single-eye setts of a given variety. Plants are sprayed with chlorpyrifos during early growth to keep them free of unwanted leaf-feeding insects, especially aphids. Water-stress is gradually imposed 1 month before the scheduled date for infestation of pots and insecticide application is ceased at that time. Stalks ideally have three to four green leaves when inoculated with *E. saccharina*, as a measure of water stress. Pots are thinned to five primary stalks to remove stalk number as a variable from the experiment and stalks are supported by string lines. Each pot is artificially infested with 200 eggs of *E. saccharina* from the culture held at SASRI. A portable logging device (Tempest®) keeps track of day-degrees above the developmental threshold calculated for *E. saccharina* (10°C) and pots are harvested when 500 d° are accumulated, corresponding to maturation of larvae. All stalks are split at harvest and numbers of internodes, bored internodes, and *E. saccharina* larvae and pupae are counted. When the method was first adopted at SASRI, staff also measured the length of bored internodes and the weight of larvae and pupae (total per pot) but these measurements are no longer taken because they were highly correlated with the other variables and added little to varietal discrimination. Varietal tolerance is not measured - stalks are not weighed and there are no uninfested pots for a comparison of yield with and without *E. saccharina*.

The comparison of varieties is based on number of internodes bored rather than percentage bored. Malcolm believes that using percentages would bias the results against varieties with a smaller number of longer internodes, and that it is important not to confound resistance with other varietal traits such as number of internodes or number of tillers.

We discussed the method of measuring length of tunnelling by larvae, whether it should be just the tunnels or should also include the associated red rot within the stalk. When Malcolm was measuring length of bored internodes in his early trials, he did not include the extra rotten tissue because he believes that he is selecting for resistance to the insect and not to associated fungi. However, workers in field studies of crop loss often measure length of reddening or number of red internodes (Mike Way, pers. comm.) as this may be the most relevant variable affecting yield. The measurements collected should reflect the purpose of the experiment. We also discussed the strong correlation between total weight of larvae and pupae and number of bored internodes in pot trials, and whether this correlation would have been as strong if average weight rather than total weight per pot had been the variable analysed, but no conclusion was reached on this.

Data are input into Excel by the biometry group. ANOVAs are conduced in Excel, but additional analyses (tests of normality, covariance and REML) are performed in GenStat. The statistical analysis and calculation of resistance ratings still follows that described by Keeping (2006). It includes both plant response and larval performance variables which are weighted according to the precision with which they are measured. The methodology for allocating ratings is very different from that used routinely by plant pathologists in BSES. Whereas the ratings of the most susceptible and resistant varieties in our pathology tests are pinned at fixed values, in SASRI's method the ratings of all standard varieties are allowed to drift depending on the precision of the experiment and the statistical discrimination among varieties; in a poor experiment that provides zero discrimination, all standard varieties would end up with a rating of 5. The calculations of SASRI are such that a difference of 2 on the 1-9 rating scale corresponds to a 95% confidence interval. It was not clear to me why that should be so and SASRI staff were unable to enlighten me further; the originator of the statistical method (Mike Butterfield) has moved on.

Malcolm has tried other analytical techniques such as cluster analysis, but his concern with this method in particular is that it does not take into account that variables are not all measured with the same precision.

In addition to the dedicated screening trials for *E. saccharina* in the shadehouse, plant breeding staff also check varieties for moth-borer damage in field trials in stages 4-5 of their program. Stalks are cut by hand and weighed, and a sample is then split to assess borer damage, while another sample goes to the laboratory for sugar analysis. These trials assess bored internodes only, but ratings are developed from these measurements. The plant breeding group is using molecular markers to choose parents that may increase resistance levels within the breeding program, and currently have six markers correlated with resistance to *E. saccharina*.

The variety database held by SASRI contains ratings from shadehouse experiments, dedicated field trials, and variety yield trials, and these are all considered when an average rating is allocated to a variety (similar to BSES disease ratings).

We discussed the value of mechanism studies, and agreed that they can assist with the design of bioassays and may suggest alternative, rapid methods of identifying potentially resistant varieties. However, an argument against focussing on varieties possessing known resistance mechanisms is that resistant genotypes possessing new mechanisms may be ignored.

NIR could potentially be used to predict resistance to *E. saccharina*, and SASRI obtained good correlations in initial experiments. However, a substantial dataset would be needed to calibrate the method, and it is not currently being used.

Extensive work has been done at SASRI on the effect of silicon on borer resistance. Although a very large effect has been measured in pots in silicon-deficient soil, results have been inconsistent in the field due to variable uptake. If silicon trials are done in PNG, a method is needed for measuring the Si in leaves or stalks to ensure that it has been taken up by the plants. Currently, SASRI is investigating the interaction of Si and the defence-signalling compounds jasmonate and salicylate as reflected in plant resistance to *E. saccharina*.

The shadehouses used for pot experiments at SASRI vary in size, the smallest measuring 14 m x 14 m, with a sloping roof of height 3.0-4.1 m. Pots are aligned in rows of two, with each pair of rows 1.7 m apart centre to centre, allowing a good walkway between them. Pots are close together within each row, but there is a work area of 1-2 m at the end of each row. Pots sit on coarse gravel and are irrigated by constant-pressure drippers. Plants in adjacent pots touch when they are fully grown, but isolation of individual pots is not necessary as all receive the same initial density of eggs. The shadehouse frame is a simple structure of treated timber posts embedded in concrete. The roof is clear polycarbonate sourced from Australia. Walls are shadecloth, either white (preferred) or green, with 40% construction (= 40% wind reduction). The shadecloth is intended to exclude moths and not moth-borer parasites, which are not significant in South Africa. Considerable effort was made to cover all holes in the first shadehouses that were built, by covering joins with a second layer of shadecloth, but that is no longer deemed necessary. A set of photographs is attached showing shadehouse construction and layout of the pots.

A 14 x 14 m shadehouse at SASRI contains 8 dual rows of pots of 25 pots each, a total of 400 pots. Pots at each end are guards, allowing 368 experimental pots or about 60 varieties x 6 replicates. Perhaps a guard row should also be added at each side of the layout to avoid differential effects of sunlight and wind.

My participation in sampling of a pot trial in the shadehouse for *E. saccharina* and its damage demonstrated two things, that people well-practiced in splitting of sugarcane stalks make it look easy, and a knife I bought in Argentina for the purpose may be good for slicing up llamas but is unsuitable for sugarcane; a thin-bladed knife has been donated to the project by SASRI.

Suggestions for developing a shadehouse-screening method at Ramu Agri-Industries in PNG include the following:

- maintain quality control of eggs from the culture (assess hatching success)
- begin by optimising experimental conditions for good discrimination among varieties; particularly number of eggs per plant (perhaps plot F values from ANOVAs against number of eggs) and number of replicates;
- in initial experiments, allow plants to tiller normally and check for correlations among variables – should plants be pruned to a constant number of stalks?

A day-degree accumulation for timing harvest might not be possible in PNG because thermal requirements of *S. grisescens* larvae are probably unknown, but larval development could be monitored in a set of pilot pots that are sampled every few days.

We discussed existing results of varietal resistance against borers at Ramu. Malcolm was concerned that results for dead hearts and yield were extremely variable, such that apparently large differences between treatments (sprayed/unsprayed) were not statistically significant, and he questions the usefulness of these variables unless their precision can be increased. The current sample of 10 stalks per plot is probably too small to estimate dead hearts, and this number could easily be increased. He also expressed concern as to how to interpret results of resistance trials in which multiple borer species occur together and possibly interfere with each other; controlled experiments with artificial infestation in a shadehouse may offer a solution.

For new field trials in PNG, Malcolm believes that counts of eggs will be variable and that two replicates (as in the Sesamia trial planted in 2009) will not be sufficient; he suggests abandoning the sprayed/unsprayed comparison in that trial so as to increase replication to four. He also questions the desirability of tolerance, a characteristic that the sprayed/unsprayed comparison is designed to measure, as it does not suppress the pest population. Another issue with insecticide spraying as a trial procedure in PNG is that it is impossible to know which of the multiple borer species is responsible for any yield increase. Malcolm suggests that the trials in PNG should have fewer varieties and ask fewer questions, but get definite answers. He also believes that current plot size is too small for yield and that a 10-stalk sample is too few for assessing percentage bored stalks and dead hearts. Possible variables to measure include number of stalks, total length of stalk, length of damaged stalk, number of internodes per stalk. internodes damaged per stalk, number of larvae and pupae per stalk, and weight of larvae and pupae per stalk. He suggests measuring as many variables as possible in the initial stages of the project and then deciding what is important.

6.2 Other activities

The role of *Wolbachia* in population dynamics of *E. saccharina* was discussed with Deborah Sweby. *Wolbachia* is a bacterium found in many insects. An extensive survey found *Wolbachia* in *E. saccharina* in east Africa but not in South Africa, and there was also a difference between *E. saccharina* populations in *Cyperus papyrus*, a native host, and sugarcane. The significance of these findings is still being assessed.

An insecticide trial against sugarcane thrips was inspected with Graeme Leslie. Insecticides being evaluated in this trial are suSCon® Maxi (controlled-release imidacloprid), liquid imidacloprid and carbofuran granules. The trial is a factorial experiment with insecticides and rates combined with four planting times from September to December, as there is a window of crop age (up to 4 months old) at the time of peak thrips populations during December-January when plants are particularly susceptible; thrips numbers decline greatly after March for reasons not yet known. Graeme has not had great success with insecticide trials, and does not think thrips numbers are a good indicator of treatment success as they can change quickly; damage may be a better indicator.

I participated in the harvest of another thrips trial with Mike Way, measuring crop loss. Sugarcane thrips is a recently established pest in South African and is causing considerable concern among growers, as damage symptoms are very visible in small cane, but the yield loss that it can cause is unknown. Mike is attempting to measure losses by comparing yields of insecticide-treated and untreated plants. Stalks were cut by hand and left in bundles, which were subsequently weighed using a tractor-mounted grab. Results have not yet been examined, but numbers of thrips were low at the time of harvest and apparently had not been very high even early in crop growth.

Push-pull strategies for management of *E. saccharina* were examined with Des Conlong. Des is encouraging growers to plant ‘pull’ plants, the preferred natural hosts *Cyperus papyrus* and *C. dives*, around the margins of canefields and ‘push’ plants, repellent plants which are primarily *Melinus minutiflora* (molasses grass), within the fields, to move moths out of fields into the surrounding habitat. We also looked at work Des is doing with breeding of biological control agents of weeds, including terrestrial weeds such as *Cromolaena* (Siam weed) and aquatic weeds such as salvinia and water hyacinth. SASRI is to become the primary supplier of weed biological control agents in South Africa. Des believes that this fits in well with the overall SASRI strategy of habitat management as a primary way of managing pests of sugarcane.

Des also discussed new work he is doing with sterile male releases (sterile insect technique, SIT) for moth-borer control. A relatively low dose of radiation is needed to sterilise *E. saccharina* and radiation biology studies are now being done with *C. sacchariphagus*, a biosecurity risk to South Africa. *E. saccharina* will be targeted in isolated areas in the midlands, while eradication of *C. sacchariphagus* will be attempted in Mozambique, and perhaps later Mauritius and Réunion, if the biology studies are promising. An X-ray irradiator is to be purchased to replace the existing cobalt machine. Des was agreeable to the idea of importing borers of interest to Australia, such as *S. grisescens*, into South Africa under quarantine, to conduct radiation biology studies; this could be done by a Master's student funded by the Australian industry.

While at SASRI I presented a seminar covering general aspects of the Australian sugar industry, structure of BSES Limited, Australian sugarcane pests and associated research projects.

7.0 EXPECTED OUTCOMES

The results of the literature review and discussions at SASRI will inform planning and implementation of screening of varieties for resistance to moth borers, especially *S. grisescens*, in Papua New Guinea beginning in 2009 (BSS331). A shadehouse to be constructed at Ramu Agri-Industries will be modelled on the shadehouse at SASRI and a program to test the feasibility of pot screening for resistance to *S. grisescens* and other moth borers will be modelled on what works in South Africa.

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9.0 REFERENCES

Abdul K, Muhammad A, Khan MA and Amjad A. 2003. Varietal resistance in sugarcane against top borer, *Scirpophaga nivella* F. *Pakistan Entomol*. 25, 41-44.

Abdullah M, Biswas MM and Siddiquee MNA. 2006. Response of some promising sugarcane clones to major insect pests of sugarcane. *Indian Sugar* 56, 23-28.

Agarwal RA, Butani DK and Tiwari CB. 1974. Resistance of sugar cane varieties to top borer *Tryporyza nivella* (Fabricius). *Inter. Sugar J.* 76, 263-266.

Ahad MA, El-Taj HF and Hossain MA. 1999. Screening of sugarcane varieties against early shoot borer. *Bangladesh J. Training Devel.* 12, 49-51.

Albert S, Thirumalai M, Krishnamurthi, M, Ramya M, Lourdusamy A and Gopinathan MC. 2007. Evaluation of tolerance and susceptibility against internode borer of sugarcane. *Sugar Tech* 9, 308-311.

Allam AI and Abou Dooh AM. 1995. Strategies for breeding varietal resistance to sugarcane stalk borer (*Chilo* spp). *Proc. Int. Soc. Sugar Cane Technol*. 22, 604-609.

Anderson Dl and Sosa O Jr. 2001. Effect of silicon on expression of resistance to sugarcane borer (*Diatraea saccharalis*). *J. Amer. Soc. Sugar Cane Technol.* 21, 43-50.

Arul JS and Prabagar AC. 2003. Evaluation of promising varieties for growth, yield, quality and internode borer incidence in South Tamil Nadu. *Proc. Ann. Convention Sugar Technol. Assoc. India* 65, A155-A167.

Arvind M and Sardana HR. 1996. Reaction of some promising genotypes of sugarcane to shoot borer, *Chilo infuscatellus* Snellen incidence under alkaline conditions. *Coop. Sugar* 28, 129-130.

Askarianzadeh AR, Moharramipour S, Kamali K and Fathipour Y. 2005. Antixenosis resistance to stem borer *Sesamia nonagrioides* (Lef.) in sugarcane varieties. *Iran. J. Agric. Sci.* 36, 485-492.

Besheit SY, Abo-Dooh A, Beshay MG, Ali MK and El-Kareem HAA. 1998. Evaluation and borer sensitivity of some new Egyptian promising sugarcane varieties. *Egypt. J. Agric. Res.* 76, 191-201.

Bessin RT, Moser EB, Reagan TE and White WH. 1990. Analysis of percent bored internode data collected from sugarcane borer varietal resistance evaluations. *J. Amer. Soc. Sugar Cane Technol.* 10, 8-22.

Bessin RT, Reagan TE and Martin FA. 1990. A moth production index for evaluating sugarcane cultivars for resistance to the sugarcane borer (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 83, 221-225.

Bhoopathi R and Karnatak AK. 2003. Screening of sugarcane germplasm to *Scirpophaga excerptalis* Walker *Hieroglyphus banian* Fabricius in Tarai region of Uttaranchal. *Ann. Agric. Res.* 24, 679-680.

Borah BK. 1993. Response of certain sugarcane varieties to plassey borer, *Chilo tumidicostalis* Hmpsn. in Assam. *Indian Sugar* 43, 363-364.

Butterfield MK, Keeping MG and Sewpersad C. 2007. Estimating genetic parameters and the efficacy of molecular breeding for resistance to the stalk borer *Eldana saccharina*. *Proc. Int. Soc. Sugar Cane Technol*. 26, 691-698.

Chaudhary JP and Yadav SR. 1995a. Screening of promising sugarcane genotypes against top borer *Scirpophaga excerptalis* Walker (Lepidoptera: Pyralidae) under semi-natural conditions – II. *Indian Sugar* 45, 173-182.

Chaudhary JP and Yadav SR. 1995b. Effect of nitrogen, phosphorus, potash and sulphur present in mid-ribs, growing points and leaf blades of sugarcane genotypes on the incidence of top borer, *Scirpophaga excerptalis* Walker (Lepidoptera: Pyralidae): V. *Indian Sugar* 45, 671-682.

Chaudhary JP and Yadav SR. 1998. Effect of different levels of cellulose, lignin, silica and ash contents available in mid-ribs, growing points and leaf blades of sugarcane genotypes on incidence of top borer, *Scirpophaga excerptalis* Walker (Lepidoptera: Pyralidae)-VIII. *Cooperative Sugar* 29, 337.

Coburn GE and Hensley SD. 1972. Differential survival of *Diatraea saccharalis* larvae on 2 varieties of sugarcane. *Proc. Int. Soc. Sugar Cane Technol*. 14, 440-444.

Conlong DE, Sweet P and Piwalo J. 2004. Resistance of southern African varieties of sugarcane to *Chilo sacchariphagus* (Lepidoptera: Crambidae) in Mozambique, and development of a non-destructive field resistance rating system. *Proc. S. Afr. Sugar Technol. Assoc.* 78, 297-306.

David H and Joseph KJ. 1983. Nature and extent of losses in sugarcane caused by the internode borer, *Chilo sacchariphagus indicus* (Kapur). *Proc. Sugar Technol. Assoc. India* 47, Ag. 19-30.

David H and Joseph KJ. 1984. Mechanism of resistance in sugarcane against the internode borer, *Chilo sacchariphagus indicus* (Kapur). *Proc. Sugar Technol. Assoc. India* 48, 43-58.

El-Amin EM. 1984. Relative susceptibility of seven sugar cane varieties to the stem borer, *Sesamia cretica* Led., under conditions of natural infestation at Sennar, Sudan. *Beitrage zur tropischen Landwirtschaft und Veterinarmedizin* 22, 73-77.

Faqir G, Muhammad N and Inayatullah. 2008. Resistance in some promising varieties of sugarcane to different borers. *Sarhad J. Agric*. 24, 269-272.

Goebel F-R and Way MJ. 2007. Crop losses due to two sugarcane stem borers in Reunion and South Africa. *Proc. Int. Soc. Sugar Cane Technol*. 26, 805-814.

Gupta MK. 1997. Varietal reaction of sugarcane to top borer (*Scirpophaga excerptalis* Walker) and plassey borer (*Chilo tumidicostalis* Hampsn) infestation. *J. Agric. Sci. Soc. NE India* 10, 140-142.

Gupta MK, Singh KM and Bhuyan U. 2006. Life table for *Chilo tumidicostalis* Hmpsn. (Lepidoptera: Crambidae) on susceptible cultivars of sugarcane. *J. Plant Prot. Environ.* 3, 52-57.

Hansi KJ and Rao BR. 1995. Response of sugarcane varieties to incidence to shoot borer (*Chilo infuscatellus* Snell). *Indian Sugar* 45, 689-690.

Hensley SD and Long WH. 1969. Differential yield responses of commercial sugarcane varieties to sugarcane borer damage. *J. Econ. Entomol*. 62, 620-622.

Hutchinson PB. 1970. A standardized rating system for recording varietal resistance to sugarcane diseases. *Int. Soc. Sugar Cane Technol. Path. Newsletter* 5, 7.

Jackson RD and Dunckelman PH. 1974. Relative resistance of *Saccharum spontaneum* clones to the sugarcane borer. *Proc. Int. Soc. Sugar Cane Technol*. 15, 513-515.

Jayanthi R. 1988. Evaluation of *Saccharum* clones for resistance to the sugar cane internode borer. *Sugar Cane Spring Suppl*, 17-18.

Jena BC, Patnaik NC and Parida AK. 1996. Intensity of stalk borer attack in sugarcane. *Indian Sugar* 46, 537-540.

Karnatak AK, Sanjeev V and Kanaujia KR. 1999. Host plant resistance in some sugarcane varieties against shoot borer, *Chilo infuscatellus* (Snellen). *Shashpa* 6, 93-95.

Keeping MG. 1999. Field screening of sugarcane varieties for resistance to the stalk borer, *Eldana saccharina* (Lepidoptera: Pyralidae). *Proc. S. Afr. Sugar Technol. Assoc.* 73, 102-103.

Keeping MG. 2006. Screening of South African sugarcane cultivars for resistance to the stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Afr. Entomol*. 14, 277-288.

Keeping MG, Kvedaras OL and Bruton AG. 2009. Epidermal silicon in sugarcane: Cultivar differences and role in resistance to sugarcane borer *Eldana saccharina*. *Env. Exper. Bot*. 66, 54-60.

Keeping MG and Leslie GW. 1999. Are self-trashing varieties of sugarcane resistant to the stalk borer *Eldana saccharina* (Lepidoptera: Pyralidae)? *Proc. S. Afr. Sugar Technol. Assoc.* 73, 100-101.

Keeping MG and Meyer JH. 2000. Increased resistance of sugarcane to *Eldana saccharina* Walker (Lepidoptera: Pyralidae) with calcium silicate application. *Proc. S. Afr. Sugar Technol. Assoc.* 74, 221.

Keeping MG and Rutherford RS. 2004. Resistance mechanisms of South African sugarcane to the African stalk borer *Eldana saccharina* (Lepidoptera: Pyralidae): a review. *Proc. S. Afr. Sugar Technol. Assoc.* 78, 307-311.

Kennedy FJS and Nachiappan R. 1992. Certain anatomical, physical and chemical basis for differential preference of early shoot borer (*Chilo infuscatellus* Snellen) in sugarcane. *Med. Fac. Landbouww. Univ. Gent* 57, 637-644.

Kooner BS, Sandhu JS and Singla ML. 1976. Studies on the varietal susceptibility and control of stalk borer (*Chilo auricilia* Ddgn.) in the Punjab. *Indian Sugar* 25, 859-861.

Kuniata LS. 2000. Integrated management of sugarcane stemborers in Papua New Guinea. In: *Sugarcane Pest Management Strategies in the New Millenium* (eds PG Allsopp and W Suasa-ard) pp 37-50. Int. Soc. Sugar Cane Technol., Brisbane.

Kvedaras OL and Keeping MG. 2007. Silicon impedes stalk penetration by the borer *Eldana saccharina* in sugarcane. *Entomol. Exp. Appl.* 125, 103-110.

Kvedaras OL, Keeping MG, Goebel FR and Byrne MJ. 2006. Silicon and water stress synergy in resistance of sugarcane cultivars to the stalk borer, *Eldana saccharina* (Lepidoptera: Pyralidae). *Proc. S. Afr. Sugar Technol. Assoc.* 80, 291-296.

Kvedaras OL, Keeping MG, Goebel FR and Byrne MJ. 2007. Water stress augments silicon-mediated resistance of susceptible sugarcane cultivars to the stalk borer *Eldana saccharina* (Lepidoptera: Pyralidae). *Bull. Entomol. Res*. 97, 175-183.

Kyle ML and Hensley SD. 1970. Sugarcane borer host resistance studies. *Proc. Louisiana Academy of Science* 23, 55-67.

Legaspi JC, Legaspi BC Jr, Irvine JE, Johnson J, Meagher RL Jr and Rozeff N. 1999. Stalkborer damage on yield and quality of sugarcane in Lower Rio Grande Valley of Texas. *J. Econ. Entomol*. 92, 228-234.

Leslie GW. 1989. Effects of pre-trashing sugarcane on oviposition, and on predation of eggs and moths of *Eldana saccharina* (Lepidoptera: Pyralidae). *Proc. S. Afr. Sugar Technol. Assoc.* 63, 192-196.

Leslie GW. 1993. Dispersal behaviour of neonate larvae of the pyralid sugarcane borer *Eldana saccharina*. *Proc. S. Afr. Sugar Technol. Assoc.* 67, 122-126.

Lourencao AL, Rossetto CJ, Germek EB, Igue T, Rezende JAM and Pereira JCVNA.1982. The response of sugarcane clones to *Diatraea saccharalis* (Fabr., 1794). *Bragantia* 41, 145-154.

Mabulu LY and Keeping MG. 1999. Glasshouse tests for ovipositional antixenosis of South African sugarcane varieties to *Eldana saccharina* (Lepidoptera: Pyralidae). *Proc. S. Afr. Sugar Technol. Assoc.* 73, 104-106.

Martin FA, Richard CA and Hensley SD. 1975. Host resistance to *Diatraea saccharalis* (F.): Relationship of sugarcane internode hardness to larval damage. *Environ. Entomol*. 4, 687-688.

Meagher RL Jr, Irvine JE, Breene RG, Pfannenstiel RS and Gallo-Meagher M. 1996. Resistance mechanisms of sugarcane to Mexican rice borer (Lepidoptera: Pyralidae). *J. Econ. Entomol*. 89, 536-543.

Meyer JH and Keeping MG. 2005. The impact of nitrogen and silicon nutrition on the resistance of sugarcane varieties to *Eldana saccharina* (Lepidoptera: Pyralidae). *Proc. S. Afr. Sugar Technol. Assoc.* 79, 363-367.

Milligan SB, Balzarini M and White WH. 2003. Broad-sense heritabilities, genetic correlations, and selection indices for sugarcane borer resistance and their relation to yield loss. *Crop Sci*. 43, 1729-1735.

Muhammad M, Muhmmad A, Chattha AA and Khan HWA. 2008. Screening of early and medium/late maturing sugarcane varieties/lines against borer complex infestation in central Punjab, Pakistan. *J. Agric. Res. (Lahore)* 46, 373-378.

Mukunthan N and Mohanasundaram M. 1996. The development of top borer in different host plants. *Sugar Cane* 6, 11-13.

Mukunthan N and Mohanasundaram M. 1998. Failures of attack by the top borer, *Scirpophaga excerptalis* Wlk. in relation to resistance in sugarcane genotypes. *Insect Sci. Applic*. 18, 293-300.

Nibouche S and Tibère R. 2008. Damage assessment for selection of resistance to the spotted stalk borer and genetic correlations for resistance and yield components in sugarcane. *Plant Breeding* 127, 38-42.

Nibouche S and Tibère R. 2009. Genotypic variation of resistance to the spotted stalk borer *Chilo sacchariphagus* (Bojer) in sugarcane: evidence of two distinct resistance mechanisms. *Plant Breeding* 128, 74-77.

Nuss KJ. 1991. Screening sugarcane varieties for resistance to Eldana borer. *Proc. S. Afr. Sugar Technol. Assoc.* 65, 92-95.

Nuss KJ, Bond RS and Atkinson PR. 1986. Susceptibility of sugarcane to the borer *Eldana saccharina* Walker and selection for resistance. *Proc. S. Afr. Sugar Technol. Assoc.* 60, 153-155.

Ogunwolu EO. 1979. Relative susceptibility of 3 sugarcane cultivars in Nigeria to attack by *Sesamia botanephaga* (Lepidoptera, Noctuidae). *J. Econ. Entomol*. 72, 11-12.

Pan YS and Hensley SD. 1973. Evaluation of sugarcane seedlings for resistance to the sugarcane borer, *Diatraea saccharalis*. *Environ. Entomol*. 2, 149-154.

Pandey KP, Singh RK, Singh SB and Singh RG. 1998. Resistance of sugarcane accessions against *Chilo auricilius*. *Sugar Cane* 4, 13-16.

Pandey KP, Yadav SA, Vinay K and Singh RG. 1997. Natural behaviour of promising sugarcane cultivars to top borer (*Scirpophaga excerptalis* Wlk) under field conditions. *Indian Sugar* 47, 187-188.

Parsana GJ, Malavia DD and Koshiya DJ. 1995. Relative susceptibility of different promising sugarcane varieties to shoot borer, *Chilo infuscatellus* (Snellen). *Gujarat Agric. Univ. Res. J.* 20, 182-184.

Pfannenstiel RS and Meagher RL. 1991. Sugarcane resistance to stalkborers (Lepidoptera, Pyralidae) in south Texas. *Fla. Entomol*. 74, 300-305.

Posey FR, White WH, Reay-Jones FPF, Gravois K, Salassi ME, Leonard BR and Reagan TE. 2006. Sugarcane borer (Lepidoptera: Crambidae) management threshold assessment on four sugarcane cultivars. *J. Econ. Entomol*. 99, 966-971.

Rajendran B, Gopalan M and Hanifa AM. 1996. Screening for field reaction of sugarcane to internode borer *Chilo sacchariphagus indicus* (Kapur). *Indian Sugar* 46, 257-261.

Ramon M, Mauriello F, Graterol Y, Perez M, Izarraga R and Mendoza C. 2007. Correlation between varietal traits and damage by sugarcane stemborers, *Diatraea* spp. (Lepidoptera: Crambidae). *Proc. Int. Soc. Sugar Cane Technol*. 26, 844-849.

Reay-Jones FPF, Showler AT, Reagan TE, Legendre BL, Way MO and Moser EB. 2005. Integrated tactics for managing the Mexican rice borer (Lepidoptera: Crambidae) in sugarcane. *Environ. Entomol*. 34, 1558-1565.

Reay-Jones FPF, Way MO, Sétamou M, Legendre BL and Reagan TE. 2003. Resistance to the Mexican rice borer (Lepidoptera: Crambidae) among Louisiana and Texas sugarcane cultivars. *J. Econ. Entomol*. 96, 1929-1934.

Rutherford RS. 1998. Prediction of resistance in sugarcane to stalk borer *Eldana saccharina* by near-infrared spectroscopy on crude budscale extracts: involvement of chlorogenates and flavonoids. *J. Chem. Ecol*. 24, 1447-1463.

Rutherford RS, Meyer JH, Smith GS and van Staden J. 1993. Resistance to *Eldana saccharina* (Lepidoptera: Pyralidae) in sugarcane and some phytochemical correlations. *Proc. S. Afr. Sugar Technol. Assoc.* 67, 82-87.

Rutherford RS and van Staden J. 1996. Towards a rapid near-infrared technique for prediction of resistance to sugarcane borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) using stalk surface wax. *J. Chem. Ecol*. 22, 681-694.

Saikia DK, Dutta SK and Dutta BC. 1994. Infestation of top borer and plassey borer in sugarcane as influenced by levels of nitrogenous fertilizers. *J. Appl. Biol*. 4, 44-48.

Salvatore AR, Lopez G, Willink E, Ahmed M and Varela L. 2008. Attack by the sugarcane stem borer caterpillar *Diatraea saccharalis* in Tucuman: analysis of the preharvest stage in the 2003 to 2007 seasons. *Avance Agroind*. 29, 12-13.

Samoedi D. 1988. Yield losses of commercial cane varieties by top borer. *Majalah Perusahaan Gula* 24, 1-6.

Selvi A, Mukunthan N, Shanthi RM, Govindaraj P, Singaravelu B and Prabu TK. 2008. Assessment of genetic relationships and marker identification in sugarcane cultivars with different levels of top borer resistance. *Sugar Tech* 10, 53-59.

Sharma BL, Saroj J and Chaudhary OP. 2007a. Biochemical basis of host plant resistance in sugarcane varieties against stalk borer, *Chilo auricilius* Dudgeon. *Coop. Sugar* 39 (1), 29-39.

Sharma BL, Saroj J and Chaudhary OP. 2007b. Screening of sugarcane genotypes against stalk borer, *Chilo auricilius* Dudgeon. *Coop. Sugar* 39 (4), 27-34.

Singh SP, Singh V and Singh VP. 1983. Incidence of stalk borer in sugarcane varieties as influenced by nitrogen levels and row spacings. *Indian Sugar Crops J*. 9, 18.

Singh D, Yadav SR, Singh H and Ahlawat DS. 1993. Screening of sugarcane genotypes against stalk borer *Chilo auricilius* Ddgn. infestation under natural conditions. *Haryana J. Agron*. 9, 135-139.

Singla ML. 1999. Resistance of sugarcane varieties to top borer and termite damage. *Insect Environ*. 5, 136.

Sosa O Jr. 1988. Pubescence in sugarcane as a plant resistance character affecting oviposition and mobility by the sugarcane borer (Lepidoptera: Pyralidae). *J. Econ. Entomol*. 81, 663-667.

Sosa O Jr. 1990. Oviposition preference by the sugarcane borer (Lepidoptera: Pyralidae). *J. Econ. Entomol*. 83, 866-868.

Sosa O Jr. 1995. Evaluation for resistance in sugarcane to the sugarcane borer *Diatraea saccharalis* (F.). *Proc. Int. Soc. Sugar Cane Technol*. 22, 594-598.

Suhartawan. 1998. Resistance of sugarcane varieties toward sugarcane moth borer. *Berita - Pusat Penelitian Perkebunan Gula Indonesia* 23, 16-19.

Tanwar RK and Varma A. 1997. *Scirpophaga excerptalis* Walker infestation in relation to its natural parasitoids and sugarcane cultivars in eastern Uttar Pradesh. *Uttar Pradesh J. Zool.* 17, 33-37.

Temerak SA. 1982. The susceptibility to *Chilo agamemnon* (Lepidoptera: Crambidae) in two commercial varieties of sugarcane and factors affecting intensity of infestation. *Bull. Soc. Entomol. Egypt* 64, 1-8.

Thirumurugan A, Shah SE and Venkatachalam SR. 2000. Screening of sugarcane clones for shoot borer and internode borer of sugarcane. *Coop. Sugar* 31, 965-966.

Thirumurugan A, Venkatachalam SR and Shah SE. 2004. Comparative performance of promising sugarcane clones for mid-late season and their level of resistance to borers. *Coop. Sugar* 35, 713-715.

Umapathy G, Rajendran B, Purushothaman RS and Babu C. 1999. Evaluation of sugarcane clones for their field reaction to early shoot borer, *Chilo infuscatellus* Snell. *Coop. Sugar* 30, 779-780.

White WH. 1993a. Movement and establishment of sugarcane borer (Lepidoptera: Pyralidae) larvae on resistant and susceptible sugarcane. *Fla. Entomol*. 76, 465-473.

White WH. 1993b. Cluster-analysis for assessing sugarcane borer resistance in sugarcane line trials. *Field Crops Res*. 33, 159-168.

White WH and Dunckelman JW. 1989. The response of sugarcane selections to sugarcane borer in the greenhouse and field. *J. Amer. Soc. Sugar Cane Technol*. 9, 56-61.

White WH, Miller JD, Milligan SB, Burner DM and Legendre BL. 2001. Inheritance of sugarcane borer resistance in sugarcane derived from two measures of insect damage. *Crop Sci*. 41, 1706-1710.

White WH, Tew TL and Richard EP Jr. 2006. Association of sugarcane pith, rind hardness, and fiber with resistance to the sugarcane borer. *J. Amer. Soc. Sugar Cane Technol.* 26, 87-100.

Figure 1 - Shadehouse at SASRI: top, external and internal view; centre, roof material (clear polycarbonate) and wall material (shadecloth); bottom, horizontal joining of cloth panels and early attempt to improve insect-proofing by covering join with second layer of cloth

Figure 2 - Pot trial in shadehouse at SASRI: top, pots and irrigation system; centre, frass of *Eldana saccharina* extruding from stalk and instrument for calculating accumulated day-degrees to determine when to harvest plants; bottom, slitting stalks to assess trial and tunnel of *E. saccharina* inside split stalk