ASSESSMENT OF DAMAGE DUE TO *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE) IN SUGARCANE

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Abstract

The sugarcane stalk borer *Eldana saccharina* is the most injurious pest in the Zimbabwe sugar industry. With its increasing establishment and population build-up in the fields it was found necessary to conduct investigations into assessing current damage patterns and possible host plant mechanisms to add on to current management practices. The study was therefore aimed at variation in damage among sugarcane varieties and investigating morphological and biochemical traits associated with damage at the Zimbabwe Sugar Association Experiment Station. Rind total phenolics, condensed tannins, cane juice quality, root primordia diameter, primordia counts and pith expression were investigated for their association to damage. Damage was found to be highest in varieties ZN1L, ZN4 and ZN2E while varieties CP72-2086 and N14 had the least damage. Percentage Tunnel length bored ranged between 1.9-19.6%, % Stalk length red 4.6-33.4% and % Internodes bored between 6.9-32.2%. Of all the stalk characteristics assessed only cane juice purity was found to have a significant negative association with all damage parameters. ERC % cane was found to be associated only with % Internodes bored (r=-0.571, p<0.01) and not with % Stalk length red and % Tunnel length bored. Morphological traits assessed in this study also did not have a significant association with damage.

Keywords: sugarcane, *Eldana saccharina*, host plant resistance, damage, biochemistry, morphology

Introduction

The sugarcane stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is indigenous to Africa and occurs in a variety of host plants including numerous wetland sedges, grasses, and graminaceous crops such as sugarcane, maize, sorghum, millet and rice (Atkinson, 1980). Since the early 1970s, *E. saccharina* has persisted as the most injurious pest of sugarcane in southern Africa (Goebel *et al.*, 2005). When very young plants are attacked, 'dead hearts' occur, followed by plant tillering. Older plants and ratoon cane have their internodes bored by the larvae (Hill and Waller, 1988). Tunneling is usually not straight because borers feed along and across fibres. Infestation by *E. saccharina* results in less sugar being extracted from the cane because sucrose is inverted to glucose (Way and Goebel, 2005). In some cases, the affected sugarcane stalks turn reddish due to secondary infestation by red rot fungus (*Glomerella tucumanensis*), which is an opportunistic pathogen that further depletes sugars, and spreads to undamaged internodes (Anon, 2005). Annually, yield losses caused by *E. saccharina* have been estimated to be around 5000 tons of sugar (Mutambara-Mabveni, 2007). *E. saccharina* in the Zimbabwe sugar industry is mainly controlled by cultural management practices. Chemical control is still largely restricted to treatment of sugarcane setts during planting and is under further investigation in the Zimbabwe sugar industry. An

integrated pest management (IPM) approach with an emphasis on host plant resistance may provide a long term, economic and eco-friendly method of managing *E. saccharina.*

Research programmes that have been developed to investigate control options for *E. saccharina* in the South African sugar industry include host-plant resistance, biological control, and the use of insecticides (Leslie, 2003). Host-plant resistance represents the inherent ability of crop plants to restrict, retard or overcome pest infestations and thereby improve the yield and/or quality of the harvestable product (Dent, 1991). Ovipositional antixenosis was found to be unimportant in the *E. saccharina*-sugarcane interaction (Nuss and Atkinson, 1983; Mabulu and Keeping, 1999; Keeping and Rutherford, 2004). However, larval antixenosis and early stage antibiosis were found to be significantly effective as resistance mechanisms of sugarcane to the borer (Keeping and Rutherford, 2004). Agarwal (1969) described how morphological features have been found to obstruct or completely restrict the entry, penetration and feeding of insects on some sugarcane clones. The hardness of the rind and fibre content in the stalk are important factors for resistance to larvae (Agarwal, 1969). In South Africa, Rutherford *et al.* (1993) found evidence suggesting that a large proportion of the variation in sugarcane resistance to *E. saccharina* can be explained in terms of tannins, lignin and fibre content and that there appears to be a link between high fibre, high tannin, low flavonoid and unknown wax components. In Louisiana, pith, rind hardness and fibre content were observed to be associated with sugarcane resistance to another borer, *D. saccharalis* (White *et al.*, 2006).

Although rind hardness has been found to impart resistance in sugarcane, its association with fibre and the well-established negative relationship between fibre % cane and recoverable sucrose makes it undesirable as a selection trait for resistance to the sugarcane stalk borer (Keeping and Rutherford, 2004). Plant surface chemistry is therefore relatively more important in varietal resistance mechanisms, although relatively understudied (Rutherford *et al.,* 1993; Keeping and Rutherford, 2004). Secondary plant substances involved in host-plant resistance include waxes, lignin and tannins, phenolics and flavonoids, among others (Thacker, 2002).

Young larvae do experience difficulty in penetrating cane stalks (Atkinson, 1980). The consequent delay in larvae penetrating the stalk exposes them to mortality factors such as predators, disease and harsh environments. The middle and base of stalks are mainly attacked, and most penetrations are around the node, especially the bud (Atkinson, 1980) and root primordia. Root primordia differ in size and density between different sugarcane varieties (Van Dillewijn, 1952) and were observed to be 'soft' areas as compared to the rest of the root band region along with the bud (pers. obs.). By demonstrating an association with genes or genetic regions, these morphological and biochemical traits in different sugarcane varieties can be introduced into breeding programme by crossing, or used to generate markers by crossing (Rae *et al.*, 2007).

Thus, there is a need to investigate whether there is an association between sugarcane stalk properties and damage by *E. saccharina*. This study was undertaken to ascertain the variation in damage and determine the role of morphological and biochemical properties associated with resistance to *E. saccharina* in 14 sugarcane varieties grown in the Zimbabwe sugar industry.

Materials and Methods

Plant and insect material

The investigation was conducted on 14 sugarcane varieties found in the Zimbabwe sugar industry. These included 10 Zimbabwe-Natal varieties (ZN1L, ZN2E, ZN3L, ZN4, ZN5, ZN6, ZN7, ZN8, ZN9 and ZN10), two Canal Point varieties (CP72-1312 and CP72-2086), one Natal variety (N14) and one Natal-Coimbatore variety (NCo376). The studies were conducted in a pot-plant trial establishment under 10% white shade cloth at Zimbabwe Sugar Association Experiment Station (ZSAES), during the period September 2007 to September 2008.

E. saccharina eggs were acquired from the South African Sugarcane Research Institute (SASRI) in July 2007. The eggs were kept in a sterile incubator at $25 \pm 5°C$. At the blackhead stage these eggs were cut out into batches of 50 on small tissue paper strips and kept in sterile petri dishes awaiting infestation.

Evaluation of sugarcane varieties for damage by *E. saccharina* through a pot-plant trial

Twelve varieties (CP72-1312, CP72-2086, ZN1L, ZN2E, ZN3L, ZN4, ZN5, ZN6, ZN7, ZN8, ZN9 and ZN10) of unknown resistance to *E. saccharina*, and two South African varieties of known resistance ratings (NCo376 and N14) were grown in pots at ZSAES in September 2007. These were grown under enclosures of white nylon netting supported by aluminium poles. The netting had 10% shading effect. Each pot (30 litres) contained 22 kg of soil mixture and 4 kg of crushed stone at the base of each pot. The soil comprised a mixture of soil (five wheelbarrows), sand (two wheelbarrows), manure (two wheelbarrows), 600 g of phosphate fertiliser (single superphosphate) and 250 g muriate of potash. After thorough mixing of the soil, two single-eyed setts of a particular variety were then planted per pot.

Each pot was restricted to three stalks. The pot plants were replicated six times for each variety. Pots were arranged in a randomised complete block design with the variety acting as the treatment. Three replications (pots) acted as the control (not infested) while the other three were infested. Each variety therefore consisted of 18 stalks, nine of which were infested and the other nine left as controls.

A dose of 14.2 g of ammonium nitrate and 12.3 g of muriate of potash were applied to each pot once every fortnight for two months and once a month thereafter as per agronomic recommendations. The pots were irrigated with 2-3 litres of water daily in three portions. Soil moisture was assessed using fingers and irrigation was rescheduled when the soil was saturated, particularly on rainy or humid days. The frequency of irrigation was reduced one month before artificial infestation with *E. saccharina*. The netting was sprayed every two weeks with cypermethrin and the plants were sprayed with dimethoate when aphid or mealy bug populations were seen to be significantly high on the plants. Spraying was halted one month before inoculation with *E. saccharina* to ensure that no residue remained on the plants. The temperature in the enclosures was monitored and recorded daily using a minimum-maximum thermometer so that degree-days could be estimated and calculated. Degree-days were calculated each day of 24 hours, and the figures accumulated as follows:

$$\frac{\text{Day average temperature + minimum temperature}}{2} - 10°C \quad\quad (1)$$

(10°C was taken as the insect threshold temperature below which growth does not occur.)

Artificial infestation

Plants were infested with 50 *E. saccharina* eggs by placing them inside dead leaf sheaths near the base of the stalk. Hatching larvae were allowed to develop over 400 degree-days to ensure that the larvae would have developed to the fifth or sixth instar stage by harvest. Plants were infested at 10 months maturity.

Trial harvest

The trial was harvested 400 degree-days after infestation. Each stalk was dissected, and the following measurements made from each stalk:

(i)   % tunnel length bored (%TLB)
(ii)   % stalk length red (%SLR)
(iii)   % internodes bored (%Int. bored)
(iv)   Number of larvae.

Biochemical parameters

Five stalks of each of the 14 sugarcane varieties were harvested at nine months of age from the same field (Shumba block at ZSAES) in October 2007. The bottom third of these stalks were cut, packed in polythene bags and frozen prior to analysis in the laboratory. The cane stalks were split longitudinally into four strands and the pith was scraped off using a sharp knife, leaving the rinds (Figure 1). The rinds were further cut into small pieces to facilitate homogenisation. Forty grams of each variety were homogenised in 200 ml of 50% methanol. The extract was then sieved using a muslin cloth before centrifuging at 3000 rpm for 10 minutes. The supernatant was frozen (<0°C) prior to analysis.

Each fraction of the supernatant (0.2 ml) was mixed with 0.5 ml of (1N) Forlin-Ciocalteau reagent and 2.5 ml of 2% sodium carbonate solution. The mixture was vortexed and allowed to stand at room temperature for 40 minutes. Absorbance was then measured at 725 nm in a spectrophotometer (Spectronic 20® Genesys™). The total phenolic content was calculated and expressed as microgrammes of gallic acid equivalents (GAE) per 100 millilitres of solution (mg/100 ml). The total phenolic concentrations in sugarcane fractions were subsequently calculated from these data using the standard curve equation:

$$(y = mx + c) \qquad (2)$$

where y = absorbance
       x = gallic acid concentration (per ml).

To determine the condensed tannin, 0.5 ml of the supernatant, 2.5 ml of HCL-Methanol reagant and 2.5ml of Vanillin reagant were added. The mixture was left to stand for 60minutes at room temperature. After incubation absorbencies were read at 500nm in a spectrophotometer (Spectronic 20® Genesys™). Catechin was used as a standard and the blank contained 50% methanol instead of plant sample. Catechin equivalents in each fraction were subsequently calculated using equation (2), where y = absorbance, x = catechin concentration (per ml).
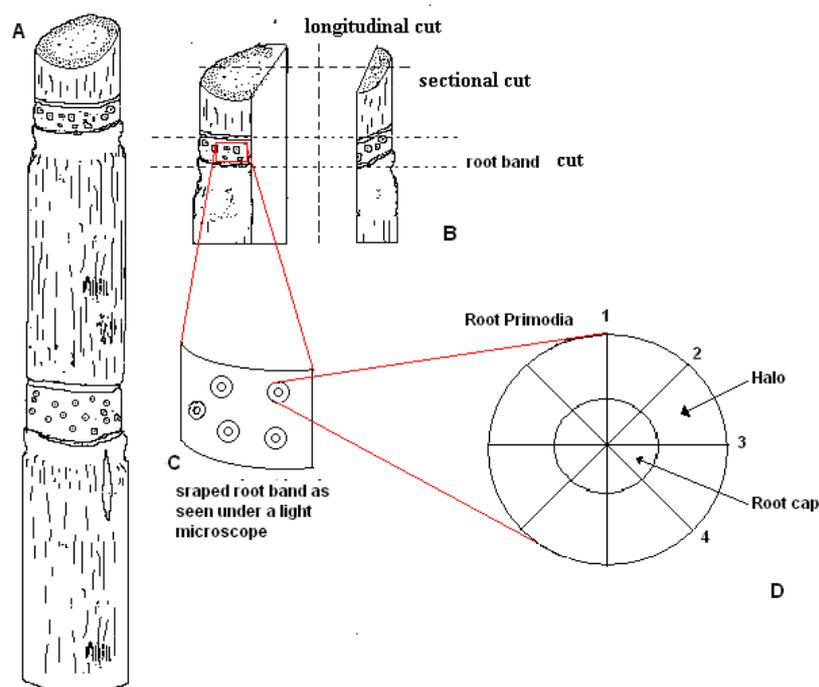
Figure 1. Preparation of primordia samples and measurements: A = section of stalk to be used, B = segment of stalk showing longitudinal, cross-sectional and root band cuts, C = root band section after scraping the ground tissue using blades, and D = typical root primordia showing halo region, root cap region, and the four diameter measurements for both halo and root cap.

Cane quality analysis

Cane quality measurements were carried out at the ZSAES chemistry laboratories based on methods described in the Laboratory Manual for South African Sugar Factories. Pol, fibre, juice purity and ERC % cane were measured in the 14 sugarcane varieties from the pot plant establishment for both the infested and uninfested cane samples.

Morphological characters

Primordial sizes were estimated on five month old cane from the same field (Zebra block, ZSAES) in September 2007. Three stalks of each variety were used for the assessments. The bottom third of these stalks were cut and frozen in plastic polythene bags before further analysis.

The cane stalks were split longitudinally into four strands (Figure 1) and the ground tissue was scraped off using blades from a Deluxe Knife Set (Cole Pamer) leaving only the rind material. The root bands from these strands were cut and further sectioned. Drawings of these sections were made at ×80 magnification using an Olympus SZ60 dissection microscope fitted with a drawing tube. At least 200 randomly chosen primordia for each variety were examined. Four measurements of the diameters of the halo of each primordia (Figure 1D) were taken from the drawings and the corresponding means calculated. Primordia counts were assessed from 10 month old cane from the pot plant establishment. These counts were done on whole stalks and a mean count calculated. Pith expression was also evaluated from these

cane samples. Pith expression was calculated as the proportion of the pith relative to the diameter of the stalk. This was assessed on uninfested cane samples from the pot plant trial.

Data analysis

Data on damage parameters of the varieties were analysed using ANOVA to test for significance of differences among varieties using statistical software Minitab 12.22. The treatment means were compared using Tukey's multiple comparisons test. Prior to analysis, % Tunnel length bored, % Stalk length red and % Internodes bored were arcsine transformed. The parameter 'Total Larvae' was transformed using the function log (x+1). These damage parameters were subsequently correlated with morphological and biochemical parameters.

Results

Damage parameters
Damage varied significantly between the 14 commercial varieties ($p<0.05$). Varieties ZN1L, ZN2E and ZN4 were the most tunneled by the borer, while varieties CP72-2086, N14, NCo376 and ZN6 were the least tunneled (Table 1). There was a significant positive correlation between %TLB and %SLR ($r=0.98$, $p<0.05$). The proportion of internodes bored was least in varieties CP72-2086 and N14 while all the other varieties did not have a significantly different % Internodes bored. There was also a significantly positive correlation between % Internodes bored and the two damage parameters, %TLB and %SLR (Table 1 and Figure 2). The variety ZN4 carried the highest number of larvae (8.8 per stalk), followed by ZN1L with 8.3 larvae per stalk. The least number of larvae were recovered in varieties N14, ZN9, CP72-2086 and NCo376. The number of larvae per stalk for each variety was positively correlated with other damage parameters (Figure 2).

Table 1. Damage parameters of the fourteen sugarcane varieties grown in a pot-plant trial.

Varieties	%Tunnel Length Bored	%Stalk Length Red	% Internodes bored	Larvae per stalk
ZN1L	19.6c	33.4d	32.2b	8.3
ZN2E	15.5c	25.2cd	26.9ab	5.5
ZN3L	6.7ab	10.4abc	14.5ab	2.2
ZN4	15.7bc	29.5cd	32.2b	8.8
ZN5	8.9ab	14.9abcd	19.1ab	2.1
ZN6	4.3a	11.6abc	17.7ab	4.2
ZN7	8.9ab	22.5abcd	24.3ab	5.0
ZN8	9.7abc	16.7abcd	13.8ab	3.0
ZN9	11.4abc	17.4abcd	12.7ab	1.4
ZN10	6.7ab	14.6abcd	15.4ab	3.6
CP72-1312	6.6ab	13.3abc	19.9ab	3.2
CP72-2086	1.9a	4.6a	6.9a	1.6
N14	3.5a	7.1ab	9.7a	1.0
NCo376	5.4a	10.5abc	12.5ab	1.7

Mean values followed by the same letter in a column are not significantly different at $p\leq0.05$.

	%TLB	%SLR	% Int. Bored	Larval Number	Juice Purity	Juice Polarity	Fibre
%SLR	0.975 (0.000)						
%Int. Bored	0.840 (0.000)	0.918 (0.000)					
Larval Number	0.857 (0.000)	0.924 (0.000)	0.890 (0.000)				
Juice Purity	-0.758 (0.002)	-0.746 (0.002)	-0.737 0.003)	-0.613 (0.020)			
Juice Polarity	-0.382 (0.177)	-0.440 (0.115)	-0.500 (0.069)	-0.310 (0.281)	0.808 (0.000)		
% Fibre	-0.221 (0.448)	-0.122 (0.678)	0.062 (0.834)	0.077 (0.795)	0.106 (0.718)	0.041 (0.890)	
% ERC	-0.470 (0.090)	-0.517 (0.058)	-0.571 (0.033)	-0.388 (0.170)	0.876 (0.000)	0.991 (0.000)	0.030 (0.920)

Contents: Correlation
              (*P*-Value)

Figure 2. Pearson correlations of biochemistry parameters and damage parameters.

Biochemical and cane juice quality parameters

The Canal Point varieties had the highest total phenolic concentrations. Varieties N14, ZN5, ZN6 and ZN8 had the least concentrations with less than 40 mg/100 ml Gallic acid equivalents (Table 2). Variety N14, however, had the highest condensed tannins concentrations along with the Canal Point varieties, ZN4 and ZN2E. Both biochemical parameters were not significantly correlated with all the damage parameters.

Table 2. Concentration of total phenolics, condensed tannins and cane juice quality parameters.

Varieties	Total phenolics (mg/100 ml)	Condensed tannins (mg/100 ml)	Polarity	Purity	% ERC	Fibre
ZN1L	42.25	64.10	9.37	64.89	6.64	12.26
ZN2E	43.94	129.79	12.03	70.05	9.32	9.18
ZN3L	47.70	80.28	14.38	76.62	11.85	11.80
ZN4	28.17	111.83	10.77	70.59	8.29	11.31
ZN5	28.92	88.81	10.47	70.95	8.12	9.92
ZN6	31.17	45.08	13.86	78.52	11.47	15.10
ZN7	43.19	65.52	12.80	76.37	10.51	11.12
ZN8	32.11	32.72	14.03	80.27	11.95	10.41
ZN9	29.11	20.72	13.78	74.90	11.22	10.35
ZN10	41.41	90.59	11.31	74.89	9.17	9.47
CP72-1312	54.84	123.56	10.60	73.72	8.34	13.53
CP72-2086	64.04	139.79	13.18	79.37	11.09	11.39
N14	36.9	189.96	10.68	73.43	8.44	11.86
NCo376	49.01	70.50	12.22	76.83	10.10	9.82

Cane juice quality parameters (Polarity, Purity and ERC % cane) were found to be highest in varieties ZN8, ZN3L, ZN6 and CP72-2086 and lowest in the varieties ZN1L, ZN2E, ZN4, ZN5, CP72-1312 and N14 (Table 2). These three cane juice quality parameters were positively correlated with each other. Among the damage parameters, ERC % cane was to be only significantly negatively correlated to % Internodes bored (r=-0.571, p<0.05). Fibre was not significantly correlated to any of the other cane quality parameters, as well as damage. The highest fibre content was noted in ZN6 (15.10), while the least was noted in ZN2E (9.18) (Table 2).

Morphological parameters
Varieties ZN1L, ZN2E and ZN4 had large halo diameters (≥1.85 cm) although they had less primordia counts, whereas varieties ZN3L, ZN8, CP72-1312 and NCo376 had smaller halo diameters yet higher primordia counts (Table 3). Varieties ZN6 and CP72-2086 had the least pith expressions followed by varieties N14 and ZN4. The highest pith expression was noted in CP72-1312. There was, however, no association between the three morphological parameters investigated.

Table 3. Root primordia diameters, primordia count and pith expression for 14 sugarcane varieties assessed from field samples.

Variety	Root primordia diameter (cm)	Primordia counts	Pith expression
ZN1L	1.85	47.58	0.19
ZN2E	1.88	42.53	0.17
ZN3L	1.71	49.00	0.17
ZN4	2.08	38.82	0.15
ZN5	2.27	56.58	0.18
ZN6	1.54	63.14	0.01
ZN7	2.89	51.80	0.16
ZN8	1.66	54.20	0.19
ZN9	1.56	44.71	0.21
ZN10	1.89	37.69	0.26
CP72-1312	1.66	63.81	0.57
CP72-2086	1.81	54.44	0.04
N14	1.90	34.63	0.13
NCo376	1.82	39.80	0.21

Discussion

The results show that, generally, damage was highest in the varieties ZN1L, ZN4, and ZN2E while N14 and CP72-2086 had the least damage. There was a very strong positive correlation between most damage. Although %TLB and %SLR were found to be significantly correlated, some varieties seemed to be more susceptible to red rot fungus infestation. For instance, the variety ZN7 had greater %SLR than varieties ZN5, ZN8 and ZN9 although the latter varieties had greater %TLB. The same can also be discerned when comparing varieties ZN3L and NCo376. Also, although there was a significant positive correlation between the total number of larvae recovered and %TLB between the varieties, some varieties seemed more susceptible to boring than others. For instance, comparing varieties ZN3L and ZN5 it was found that, although ZN5 carried less larvae per stalk than ZN3L, it had a greater % Internodes bored. The same can also be seen when comparing ZN1L and ZN4, where the same % Internodes

were bored, but ZN4 had a greater larval load. It is worth noting that in some cases larvae would be recovered outside the stalk inside leaf sheaths before managing to bore into the stalk.

Work on the interaction between insects and phenolic compounds, particularly flavonoids and tannins, has been carried out by workers elsewhere (Rutherford *et al*. 1993; Harbone and Grayer, 1994; Rutherford and van Staden, 1996 and Schoonhoven *et al.*, 2005). In sugarcane clones, a significant association between flavonoids and resistance to *E. saccharina* has since been found (Rutherford *et al.*, 1993; Rutherford and van Staden, 1996). In this study, however, no significant association was found between total phenolics, condensed tannins and damage. It is worth noting two important issues associated with this result. First, these plant compounds were assessed from the rind of cane stalks in this study, while damage parameters were compared to those assessed from the cortex. Condensed tannins, for instance, are known to be involved in antibiosis by reducing the growth rate of larvae (Harbone and Grayer, 1994). Secondly, these attributes were instantaneous measurements from one particular field, time and cane age. These environmental conditions might have differed from those in the pot and shade trial and hence not ideal for comparison. It would be essential in future work to further elucidate which phenolic compounds are involved in resistance and susceptibility by further isolation of the sugarcane extracts as well as analysis of more samples at different time intervals and between seasons. This may be a critical tool for routine analysis of sugarcane clones during breeding. It would, however, require more precise extraction methods such as High Performance Liquid Chromatography and Near Infrared Spectroscopy, among others (Meyer, 1998; Rutherford, 1998). A follow-up study investigating the changes in the levels of phenolic compounds before and after insect herbivory and also as the cane matures would be critical in establishing the relationship between sugarcane and *E. saccharina*. This is because plant biochemistry is known to change before and after herbivory, and even as plants mature (Schoonhoven *et al., 2005).

Cane juice polarity and fibre were found to have no significant association with damage. For instance, ZN6 with the most fibre was found to carry the heaviest larvae. This is contrary to earlier work done elsewhere in which fibre was found to be significantly associated with damage (Rutherford et al, 1993). Purity, however, was found to have a significant negative correlation with damage. This implied that as damage increased cane juice quality decreased. There was no significant association between %TLB and %SLR and ERC % cane. However, ERC % cane was significantly associated with %Int. bored. This implied that *E. saccharina* larval feeding or damage was probably not related to the amount of sugar in the cane varieties. Therefore, there could be other factors influencing larval feeding in cane, and characterisation of plant metabolite profiles in various indigenous hosts of *E. saccharina* is critical to establish any cues or deterrents shared between them and sugarcane.

The morphological parameters investigated in this study were not associated with damage. However, varieties CP72-2086 and ZN6 with the least pith expressions were also found to have the heaviest larvae. This probably suggested that low pith expression could impede tunneling, which could also be associated with more larval weight. Pith expression was found by other workers to influence damage in sugarcane (White *et al*, 2006). Although the bud and root primordia were the two most frequent entry sites, this appeared to be specific to the variety. In ZN7 and ZN2E, for instance, most of the larvae found their way into the stalks through stem cracks. Stem cracking is an inherent characteristic of these varieties. Most of the buds that had been bored through in the varieties CP72-1312, ZN3L and NCo376 had broken

apical dominance (shooting). This could have contributed to damage. It is also worth noting that the crop for the pot and shade trial was generally stressed prior to infestation.

There was no significant correlation between damage and morphological parameters investigated in this study. There was also no significant correlation between rind biochemistry (phenolics and tannins) and damage. Of the juice parameters assessed, purity had a significant negative association with damage while ERC % cane was associated only with % Internodes bored. Since *E. saccharina* larval damage was not found to be related to the amount of sugar it is worth further investigating the chemical parameters associated with feeding in sugarcane. Further work therefore needs to be done on sugarcane host plant resistance to elucidate more on these parameters and reveal more characteristics that might impart reasonable degrees of resistance, thereby reducing damage and improving yields. These characteristics would eventually need to be assessed for their various contributions to resistance before being factored in for breeding or pest management.

Acknowledgements

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