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Epidermal silicon in sugarcane: Cultivar differences and role in resistance to sugarcane borer *Eldana saccharina*

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ABSTRACT

Silicon (Si) application can significantly increase resistance of plants to insect herbivory. In sugarcane, Si-mediated resistance to the lepidopteran stem borer *Eldana saccharina* involves reduced survival, feeding efficiency and stalk penetration. In a pot trial, this study examined: (1) the effect of calcium silicate treatment on the accumulation of amorphous epidermal Si at three sites on the sugarcane stalk where the borer may penetrate, and (2) whether the accumulation of epidermal Si at these sites in Si-treated and control cane plants varied between a borer-resistant (N33) and borer-susceptible (N11) cultivar. Sections of mature stalk were subjected to Energy Dispersive X-ray (EDX) microanalysis to locate and quantify Si accumulation in the stem epidermis. In both cultivars, Si-treated plants had increased silica in each epidermal tissue zone (internode, root band, leaf bud). X-ray mapping confirmed that Si accumulation was restricted mainly to the epidermis of the internode and root band, but was sparse in the underlying tissues. By contrast, there was no evident concentration of Si in the bud scale epidermis compared with the underlying bud tissue. We contend that these patterns of Si deposition, especially at the internode and root band, may explain the previously reported enhanced resistance of Si^+ sugarcane to penetration and feeding by *E. saccharina* at these sites. This is consistent with an hypothesis of increased mechanical hindrance to feeding in Si-treated plants. At all sites, epidermal Wt% of Si was higher in N33 plants (both Si^+ and Si^-) than in N11 plants, indicating that the higher total stalk Si recorded for N33 compared with N11 was expressed to an appreciable degree at the epidermal level. If amorphous Si increases mechanical resistance to stalk penetration, then the low Si content of the bud scale epidermis compared with the internode and root band epidermis may in part explain the observation that the leaf bud is a preferred entry point on the sugarcane stalk for *E. saccharina* larvae.

1. Introduction

The importance of silicon (Si) as a nutrient that promotes growth and development in many plant species, and that is especially beneficial for plants under a range of abiotic and biotic stresses, is now beyond doubt (Ma and Yamaji, 2006; Liang et al., 2007), although its essentiality as an element for higher plants is still contentious (Epstein and Bloom, 2005). Silicon is taken up by plants in the form of monosilicic acid ($Si(OH)_4$), which is the dominant form of Si in the soil solution at a pH of less than 9 (Jones and Handreck, 1967). Following its uptake and transport from the roots into the shoot, silicic acid is concentrated through water loss due to transpiration, or through active physiological processes, and polymerises first as colloidal silicic acid and finally, with further concentration, as silica gel ($SiO_2 \cdot H_2O$) (Ma and Takahashi, 2002), also referred to as opaline or amorphous Si. Once deposited, Si is no longer mobile within the plant and hence older tissues contain larger amounts (Ma and Yamaji, 2006).

Substantial differences in shoot Si content exist between plant species (between 0.1 and 10% of dry weight) (Ma and Takahashi, 2002). Within the angiosperms, these differences appear to be underlain by different modes of Si uptake (active and passive in accumulator species and rejective in non-accumulators) (Takahashi et al., 1990). Most of the active Si accumulators fall within the Poaceae (Ma et al., 2001), where earlier studies noted that species in this group may contain 10–20 times the concentration of Si in non-monocots (Jones and Handreck, 1967). Sugarcane (*Saccharum* species hybrids; Poaceae) absorbs more Si than any other mineral

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(Ayres, 1966) and gives strong yield responses to Si application when leaf Si levels drop below 1.0%, and may accumulate up to 2.0% in leaves and 2.5% in the leaf sheaths on good cane-growing soils (Savant et al., 1999).

Differences in Si-accumulating capacity exist between sugarcane cultivars (Savant et al., 1999; Keeping and Meyer, 2006). Detailed studies have shown that such differences are reflected in the density, form, pattern and degree of silicification of (especially silica) cells within the epidermis of the leaf blades, leaf sheaths and stem, where they may provide a useful means of differentiating between cultivars (Artschwager, 1930; Kaufman et al., 1979).

As for other Si accumulators, numerous benefits are associated with high uptake and deposition of Si in sugarcane; these include increased cane and sucrose yields (attributable to increased number of millable stalks and plant size), improved photosynthesis due to reduced leaf freckling, inhibition of sucrose inversion, amelioration of heavy metal toxicities, reduced moisture loss through evapotranspiration, and enhanced resistance to stem borers (see reviews by Savant et al., 1999; Meyer and Keeping, 2001).

Among South African sugarcane cultivars, increased Si uptake due to soil amendments with calcium silicate significantly enhanced resistance to the stem borer, *Eldana saccharina*. This was especially evident in borer susceptible cultivars treated with Si (Si^+) and subjected to water stress at a late stage in their growth (Kvedaras et al., 2007a). Stalk Si content was positively associated with treatment level and negatively associated with stalk borer damage (Keeping and Meyer, 2006), indicating that the concentration of Si in the stalk played some role in augmenting resistance. Stalk penetration by early instar larvae was delayed in Si^+ plants, particularly at the internode and in Si^+ cultivars with high endogenous resistance, leading to greater mortality and reduced weight gain of larvae (Kvedaras and Keeping, 2007); this, together with the positive effect of Si^+ on rind hardness of the internode and node (Kvedaras and Keeping, 2007; Kvedaras et al., 2007b), suggests that mechanical resistance to stalk penetration and larval feeding is an important component of Si-mediated resistance.

Eldana saccharinaa is the most destructive pest in South African sugarcane, where it has been managed largely through the planting of resistant cultivars (Keeping, 2006), cropping practices (Carnegie and Smaill, 1982), and most recently, insecticide use (Leslie, 2003). Amendment of Si-deficient, acidic soils with calcium silicate to neutralise soil acidity and ameliorate Al toxicity, while simultaneously enhancing resistance to the borer in susceptible cultivars (Keeping and Meyer, 2006), represents a third option that is now available to growers.

In this study, we attempt to further clarify the mechanism/s whereby Si enhances plant resistance to this particular pest, while providing greater insight into how Si may contribute to resistance of higher plants to insect herbivores in general. To this end, the study examined: (1) the effect of applied Si on the concentration of amorphous epidermal Si at three sites on the sugarcane stalk where *E. saccharina* may penetrate, and (2) whether the concentration of epidermal Si at these positions in Si-treated (Si^+) and control (Si^-) cane plants varied between borer-resistant and borer-susceptible cultivars. We then relate observed changes or variations in deposited amorphous Si at the epidermal level to changes in total stalk Si, and, by reference to previously published studies, to changes in resistance to *E. saccharina* when sugarcane was grown under the same conditions and fertilised with Si at the same rates as in the present study (Keeping and Meyer, 2006; Kvedaras and Keeping, 2007; Kvedaras et al., 2007a,b). The three sites on the stalk surface on which our study focussed were the internode, root band and leaf bud (more specifically the leaf bud scale, which forms a protective structure covering the bud proper) (see Fig. 1). These sites were chosen: (a) because *E. saccharina* uses these sites to penetrate the lower part of the stalk when infesting sugarcane in southern Africa (Leslie, 1993) and (b) to allow direct comparison with our previous studies (Kvedaras and Keeping, 2007; Kvedaras et al., 2007b), where the effects of Si-treatment on borer penetration, performance and damage at these sites were examined in detail.

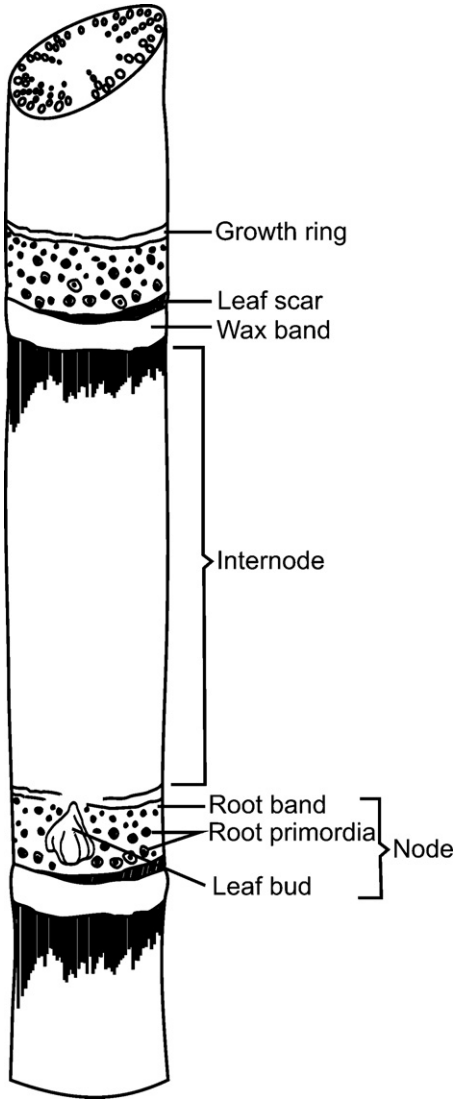

Fig. 1. Section of a sugarcane stalk showing various zones and anatomical features used for obtaining tissue sections for ESEM/EDX and for taking rind hardness measurements (after Kvedaras et al., 2007a).

2. Materials and methods

2.1. Trial establishment and silicon treatments

A potted sugarcane trial (24 pots) was established in a 'shade house' (14 m × 25 m × 3.3 m) with transparent polycarbonate roofing and walls of 40% green shade cloth, at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KwaZulu-Natal. Sugarcane transplants were produced from single budded setts, cut from mature stalks of two commercial cultivars, resistant (N33) and susceptible (N11) to *E. saccharina* Walker (Lepidoptera: Pyralidae) attack. One-month-old transplants of each cultivar were planted into 25 l PVC pots containing 31 kg (dry weight) of clean, sieved and thoroughly leached river sand, which allowed control of the nutrient supply. Pots were arranged, using the random function in Microsoft® Office Excel 2003, in a split-plot design, where whole plot was 'cultivar' and sub-plot was 'silica'. There were six

replicates for each cultivar, each pot containing four transplants of one cultivar.

Before planting, half the pots were treated (Si^+) with 124 g (equivalent to 10 t/ha; 4000 ppm) of wollastonite (i.e. calcium silicate, $CaSiO^3$; 7.9% Si; 60% plant available Si), and the other half left untreated (Si^-). The calcium silicate was mixed thoroughly with the sand, dampened and the filled pots left to stand for one week before planting on 06 December 2005. All pots were treated monthly with 16 g 4:1:1 (44) N:P:K fertilizer alternating with ammonium sulphate (250 g per 25 l water) at 500 ml per pot plus Hygrotech® Hydroponic Nutrient Mixture for seedlings (25 g per 25 l water) at 500 ml per pot, to ensure that all micronutrients (including Ca) were not limiting to the plants in both controls and treatments. Provision of adequate calcium (Ca) in the controls was important, as its levels would have been higher in the treatments due to the use of $CaSiO^3$ as a source of Si. The above fertilizing regime in all such pot trials (Keeping, 2006; Keeping and Meyer, 2006) has consistently ensured that leaf Ca levels are within the acceptable range of 0.15–0.39% for plant nutrition (Anon., 2008).

Plants were drip irrigated using tap water (3 ppm Si) at 1.3 l water per pot per day throughout the period of the trial. Insecticide spray was applied monthly (chlorpyriphos at 2 ml/l water or alpha-cypermethrin at 1 ml/l water) to prevent feral infestations of *E. saccharina* and other pests. The trial was terminated in March 2007.

2.2. Monitoring of stalk Si status

The Si % dry matter (DM) in stalks was determined when the cane was 14 months old (23 January 2007), using material remaining after the sections for the Environmental Scanning Electron Microscopy/Energy-Dispersive X-ray analysis (ESEM/EDX or EDAX; see below) had been cut from the individual stalks. For this reason, samples were taken from between three and six replicates rather than from all replicates in each treatment combination. Stalk Si% was determined by dry ashing using the procedures of Fox et al. (1967).

2.3. SEM/EDX analysis of stalk epidermal Si

Collection of stalk samples for ESEM/EDX analysis was conducted from 23 January to 23 March 2007. For each analysis, fresh material harvested on the same or preceding day was used. A maximum of four stalks were removed from different (randomly selected) pots of each treatment combination (cultivar and Si treatment) on any one day. Only mature primary stalks (rather than secondary tillers) that were uniform in height and appearance were selected. For each stalk, the node closest to 30 cm from the cut stalk base was identified and the internode above and below it cut at a point close to the neighbouring nodes. Hence, each segment of stalk consisted of a node accompanied by a section of internode on either side. The basal part of the stalk was used because larvae of *E. saccharina* preferentially attack the lower third of the stalk in southern African sugarcane (Mazodze and Conlong, 2003). The stalk segment was immediately sealed in a labeled zip-lock plastic bag, placed on ice in a cool box, then transferred to a fridge (4 °C) within 20 min of harvest. During transport to the SEM facility, stalk material was kept on frozen ice bricks in a 'cool bag' and transferred again to a fridge at the facility.

Specimens for ESEM were prepared from each of the three structures/tissue zones mentioned in the Introduction, i.e. internode, leaf bud and root band. The reader is referred to Van Dillewijn's (1952) text for anatomical descriptions of these structures. Briefly, the sugarcane stalk is composed of joints, with each joint consisting of an internode and basal node; the latter is where the leaf is inserted and thus bears the leaf bud. Also on the node, the root band, with associated root primordia, is bounded by the growth ring and leaf scar (after leaf loss) on its upper and lower sides, respectively (see Fig. 1).

The following protocols were used for specimen preparation: (1) Internode: a cross-section *ca.* 2 mm thick was cut with a sharp razor blade within the section below the node (Fig. 1) that coincided as closely as possible with the midpoint of the internode. A wedge from the perimeter to the centre of the stalk representing about 1/8th of the circular section was then cut and mounted onto an aluminum specimen stub; the latter was covered in a thin layer of colloidal graphite to provide adhesion for the specimen and to eliminate charging; (2) Leaf bud: a 1–2 mm vertical section (i.e. perpendicular to the long axis of the stalk) was cut from the bud, to produce a cross-section through the bud scale and underlying bud tissue. Mounting was performed as above; (3) Root band: a cross-section *ca.* 2 mm thick was cut through the root band, with tissue wedges and mounts prepared as for internode specimens.

Specimens were examined under a Philips XL30 ESEM, equipped with an EDAX PHOENIX Ver. 3.2 digital microanalyzer, at an accelerating voltage of 15 kV and at a working distance of 10 mm. The chamber pressure was in the range of 0.9–1.0 torr, temperature 25 °C and RH about 5%. Under these working conditions of low chamber pressure, low RH and short working distance, studies have indicated that scattering of the primary electron beam away from the target and the consequent loss in resolution, should be reduced for EDX using the ESEM (Carlton, 1997).

For the majority of specimens, three 'spot' determinations of relative Si concentration (Wt%; see below) were performed at well-separated positions along the epidermis, using the EDAX ZAF (see description below) standardless quantification program over a scanning time of 100 live seconds, with a dead time of about 30%. Efforts were made to ensure that all specimens were processed as rapidly as possible to minimise loss of moisture and its possible impact on accurate determination of Si.

For standardless quantification, the *k*-ratio (i.e. the X-ray intensity ratio between unknown and standard), which is a good estimate of concentration, is calculated from the measured peak intensity of the element/s of interest. The calculated k-ratio is then corrected by the ZAF method to control for the effects of other elements in the unknown (sample) material and normalised to 100%. Therefore, the elemental composition of a sample is calculated as:

$$\mathrm{Wt\%} = Z \cdot A \cdot F \cdot \left(\frac{I^{\mathrm{Meas}}}{I^{\mathrm{Std}}} \right)$$

where Z, A and F are the matrix correction factors for the atomic number, absorption and fluorescence effects, respectively, and I^{Meas} and I^{Std} are the measured and standard intensities of the element involved. Since for standardless analysis I^{Std} is not available, EDAX uses a *calculated* standard, the complex formula for which is provided in Kusemann (2000), multiplied by a Standardless Element Coefficient (SEC) factor; the various benefits of using the latter factor are also detailed in Kusemann (2000).

X-ray Si mapping (using the same ESEM/EDAX system) to investigate qualitative differences in the distribution of Si within the epidermis and underlying tissues, was performed for a total of 29 specimens (at 43 min per scan), to obtain between one and four maps per Si treatment/cultivar combination. Since opaline silica is essentially immobile once deposited, drying of specimens over the prolonged scans is unlikely to have affected the localization of Si deposits within tissues.

2.4. Statistical analyses

Analyses were performed using Genstat 8 for Windows (Genstat, 2005). For the sake of clarity, all figures were plotted using untrans-

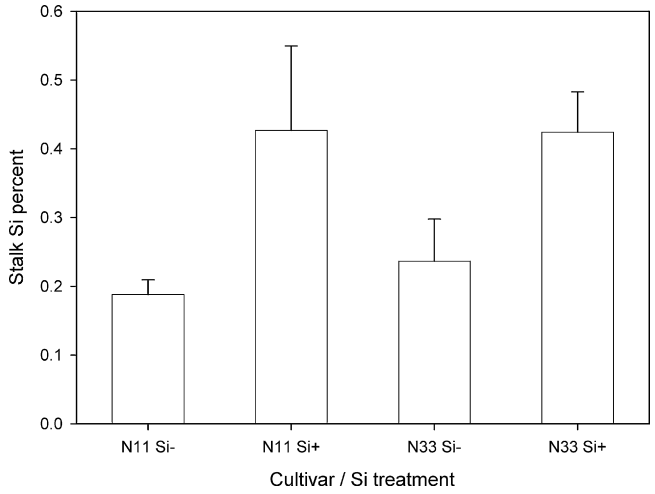

Fig. 2. Mean percent stalk Si obtained from samples taken on 23 January 2007 from each cultivar (N11, N33), treated (Si^+) at 10 t/ha and untreated (Si^-) with Si. Errors bars are SEs.

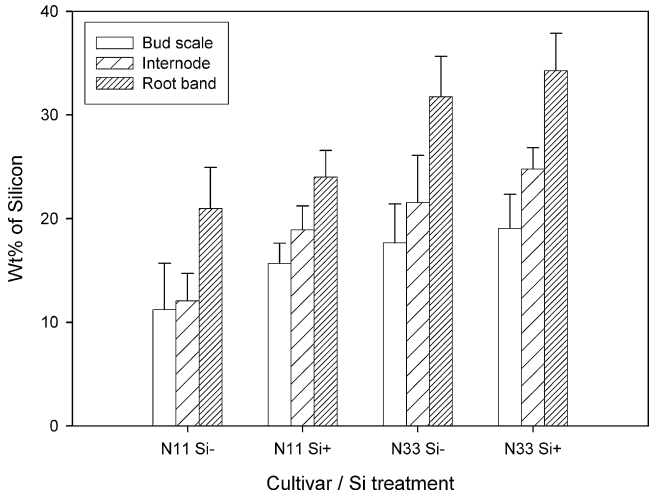

Fig. 3. Mean Si percent obtained from ESEM/EDX spot determinations on the epidermis of the bud scale, internode and root band tissue sections from each cultivar (N11, N33) treated (Si^+) at 10 t/ha and untreated (Si^-) with Si. Errors bars are SEs.

formed data, even where transformation was required for statistical analysis.

The data for total stalk Si met the assumptions of parametric analysis and were subjected to a two-way ANOVA, with Si and cultivar as main effects. As the EDX-determined Wt% (Si) data obtained from epidermal tissues did not meet the assumptions of ANOVA, a square root transformation was applied to normalize the distribution and stabilize variance. Differences in Wt% of Si among treatments were tested using a three-way REML analysis with Si, cultivar, and tissue type (internode, bud scale, root band) as fixed effects.

Within the REML procedure, individual Wt% spot determinations were analyzed at a 'sample-within-a-pot' level. The individual spot determinations were considered to be sub-samples within the experimental unit (i.e. pot), which allowed variation between samples from different stalks *within* each pot to be accounted for. Such sampling variation is due to random effects and not treatment effects. Identifying and eliminating this from the total variation in the data set reduced the error variance and increased the power of detection of significant treatment differences (Steel et al., 1997). REML analysis reports the Wald statistic, with probability levels based on the chi-square distribution.

Where appropriate, the Holm–Sidak multiple comparisons test was used to determine where any significant differences lay.

3. Results

3.1. Stalk Si status

Analysis of stalk material collected on 23 January 2007 showed a significant effect of Si treatment ($F_{1,15} = 5.5$; $p = 0.034$) in increasing total stalk Si% (Fig. 2), but no effect of cultivar or cultivar × treatment interaction.

3.2. Quantitative differences in epidermal Si concentration among cultivars, Si treatments and tissue types

There was a significant effect of cultivar ($X^2_1 = 4.68$; $P = 0.042$), Si treatment ($X^2_2 = 5.38$; $P = 0.03$) and tissue type ($X^2_3 = 29.16$; $P < 0.001$) on epidermal Wt% of Si; cultivar N33 and Si^+ plants had a higher overall Si content (Fig. 3). There were no significant interactions ($P > 0.48$) among these fixed effects. Holm–Sidak pairwise comparisons (comparison-wise error rate = 0.017) revealed that Wt% of Si was significantly higher in the root band epidermis than in the internodal or bud scale epidermis, while the latter two tissue types did not differ significantly (Fig. 3).

3.3. Qualitative differences in distribution of Si between tissue types

The X-ray mapping procedure produced clear evidence that in both cultivars, and in treatments and controls, Si is deposited in relatively larger quantities within the epidermis of the internode and root band than in the underlying tissues; this is illustrated by the greater density of Si deposits within a band coincident with the epidermis in these two tissue zones. The clearest representations of this pattern of Si deposition are provided in Fig. 4C–F.

Within the bud scale epidermis, this greater density of deposits was not apparent (Fig. 4A and B) and did not differ in any obvious way from Si deposits in deeper bud tissue underlying the bud scale itself (Fig. 4A and B). The distribution of Si within the cortex, vascular bundles and parenchyma was comparatively sparse, with no evident concentration in particular tissues or cells. In some maps (e.g. Fig. 4D) deposits of Si appeared to be present in the area above the cuticle (i.e. in the region of the mounting stub); this is either a spurious artifact of scattering of the electron beam away from the target (sample), as mentioned under Section 2, or due to favourable orientation of the surface of the specimen towards the detector.

For both internode and root band, the epidermis was visible as a single layer of cells with distinctly thickened cell walls, underlain by several layers of cells (also with thickened walls) making up the rind (or cortex) between the epidermis and the vascular bundle sheaths (Fig. 4C and E). The waxy cuticle was also visible above the epidermis (Fig. 4A, C, and E). In Fig. 4A, the cuticle appears to be a very thick layer; however, this together with the apparent presence of Si in the cuticle (Fig. 4B), is probably due to the specimen surface being oriented towards the detector.

4. Discussion

Among the graminaceous crops, sugarcane and rice are prominent in their capacity to absorb Si from the soil solution and to accumulate it in the shoot where it may impart significant agronomic benefits, together with improved resistance to a range of biotic (i.e. pests and disease) and abiotic plant stresses (Savant et al., 1999; Datnoff et al., 2007). For South African sugarcane cultivars, studies have shown that uptake to produce >0.30% Si DM for stalk and >0.60% Si DM for leaf, is required to produce a sub-

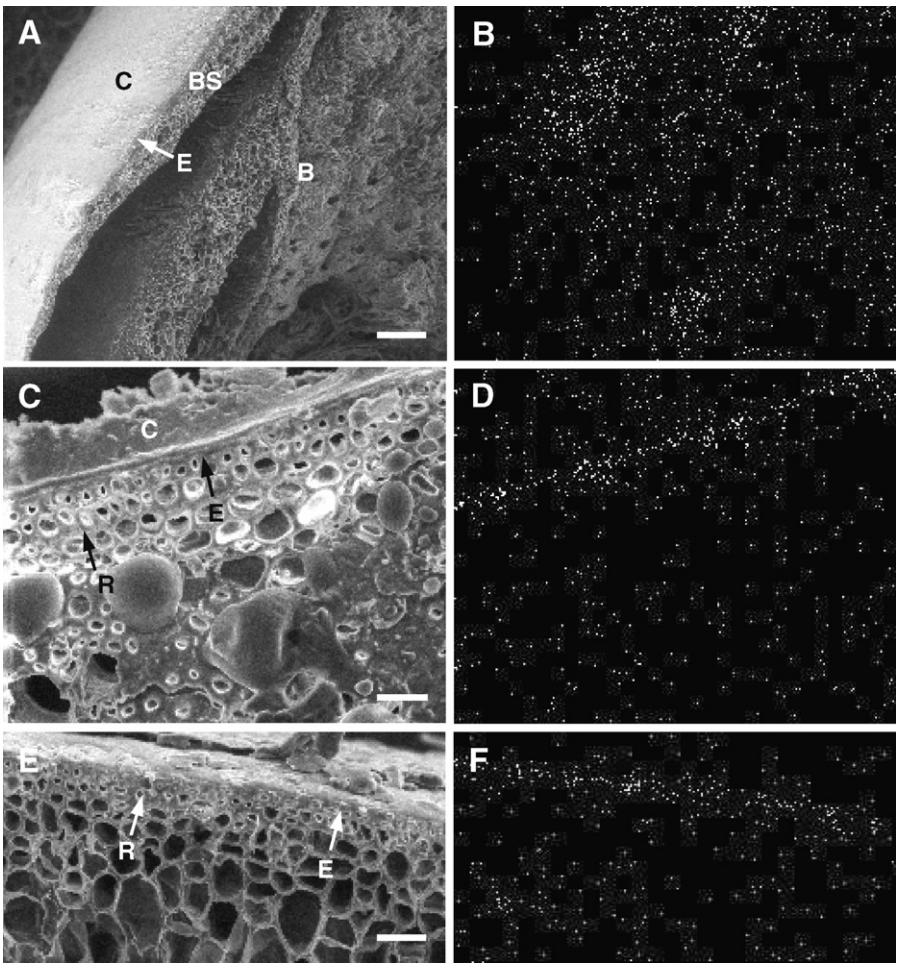

Fig. 4. Scanning electron micrographs (A, C and E) and corresponding Si X-ray maps (B, D and F) illustrating the distribution of Si within the leaf bud of Si^+ N11 (A and B), the outer internodal tissues of Si- N33 (C and D) and the root band of Si- N33 (E and F). The concentration of Si within the epidermis of the internode and root band is clearly shown by the higher density of deposits within the corresponding area of the X-ray map. B = bud; BS = bud scale; C = cuticle; E = epidermis; R = rind; V = vascular bundles. Scale lines: A: 200 μ; C: 30 μ; E: 50 μ.

stantial increase in resistance to *E. saccharina* (Keeping and Meyer, 2006; Kvedaras et al., 2007a). The Si treatments in the present study produced these levels (Fig. 2) in both cultivars.

EDX spot determinations showed quantitatively that on average Si^+ sugarcane plants had increased levels of silica compared with Si^- plants (Fig. 3) and a higher epidermal Wt% of Si in the borer-resistant cultivar N33 compared with the susceptible cultivar, N11 (Fig. 3). However, in contrast to earlier studies using the same cultivars and identical treatments (Kvedaras et al., 2007a,b), in this study N33 did not display significantly higher total stalk Si% than N11. X-ray mapping (Fig. 4) provided qualitative confirmation that Si accumulation was localized most notably in the epidermis of the internode and root band, while that in the underlying cortex, vascular bundles and parenchyma was sparser.

Substantial evidence now exists for the increased resistance of Si-treated plants to herbivorous insect attack (see Keeping and Kvedaras, 2008). Using the same cultivars (N11 and N33) as in the present study, Kvedaras and Keeping (2007) demonstrated that the elevated plant Si levels of Si-treated cane artificially infested with *E. saccharina* significantly reduced larval stalk penetration, especially during the first 72 h of feeding activity, as well as larval weight gain. They argued further that, along with a significant increase in rind hardness of Si^+ plants at the internode in both cultivars, the results provided correlative support for a physical role of amorphous Si in impeding initial stalk penetration at the internode. A delay in stalk penetration is likely to prolong exposure of early instar larvae to adverse environmental factors (e.g. predation and desiccation), thereby increasing mortality (Kvedaras and Keeping, 2007). However, this does not discount the probable role of induced biochemical defenses, such as those involved in the jasmonate (JA) pathway (Bower et al., 2005), following injury to the plant during borer feeding (Heinze et al., 2001) and which may be facilitated by soluble Si as suggested by Kvedaras et al. (2007a); such a response has been demonstrated in Si-fertilised wheat (Gomes et al., 2005) and cucumber (Correa et al., 2005), infested with aphids and whitefly, respectively.

We contend that in the present study, the increased quantity of amorphous Si deposited in the stalk epidermis of Si^+ sugarcane plants provides correlative evidence for their enhanced resistance to penetration and feeding by *E. saccharina* (earlier studies cited above). This is consistent with an hypothesis of Si-mediated resistance based on increased mechanical hindrance to herbivore feeding (Djamin and Pathak, 1967). Such an hypothesis has also been proposed and extensively debated for Si-mediated resistance of plants to fungal attack (Fauteux et al., 2005). The mechanism/s whereby Si may act in increasing plant resistance to insect attack include reduced digestibility and/or increased hardness and abrasiveness of plant tissues due to silica deposition, mainly as opaline phytoliths, in various tissues, including epidermal silica cells (Salim and Saxena, 1992; Massey et al., 2006). A mechanical basis to insect resistance is particularly important in grasses, which are characterised by high levels of opaline

silica, making them difficult to chew (Bernays and Barbehenn, 1987).

Sugarcane cultivars resistant to *E. saccharina* attack have also been found to have higher levels of total endogenous leaf and stalk Si in the absence of exogenously supplied Si (Keeping and Meyer, 2006; Kvedaras et al., 2007b). Results of this study indicate that cultivar differences in total stalk Si may be expressed to an appreciable degree at the epidermal level; Si- cane of resistant N33 had a higher Si content in the epidermis of each tissue type than Si^- cane of susceptible N11 (Fig. 3). This lends credence to the argument that differences in endogenous Si content (without Si provision) contribute to differences in stalk borer resistance between cultivars (Kvedaras and Keeping, 2007), and further, that such differences in Si-mediated resistance exist to a large extent due to the varying propensity of cultivars to deposit Si within the stalk epidermis.

Several earlier studies have related the abundance and/or arrangement of silica and silica cells in the leaf or stalk epidermis to host plant resistance. For example, Djamin and Pathak's (1967) classic study of rice cultivars with varying resistance to the stem borer *Chilo suppressalis* (Walker), found that cultivars with higher silica content generally had more silica cells in the stalk epidermis. As in the case of *E. saccharina* in sugarcane (Kvedaras and Keeping, 2007), significantly more larvae were able to penetrate the stems of a low silica rice cultivar than those of a high silica cultivar.

Eldana saccharina demonstrates an entry site preference and increased performance at the root band or bud as opposed to the internode of a cane stalk, particularly in Si^+ cane (Kvedaras et al., 2007a,b). The localization of Si, particularly at the internode (as shown in this study) reveals how differences in the accumulation and concentration of Si in different epidermal tissue zones on the sugarcane stalk may influence the ability of *E. saccharina* larvae to penetrate the stalk surface. Studies have shown that rind hardness is greater at the internode compared with the nodal tissue, especially the bud (Kvedaras and Keeping, 2007; Kvedaras et al., 2007b). Silicon treatment has also been demonstrated to increase rind hardness at the internode in all cultivars trialed (Kvedaras et al., 2007a; Kvedaras and Keeping, 2007), and at the root band in N33 (Kvedaras and Keeping, 2007). Furthermore, the borer-resistant cultivars had significantly harder rind at the internode than the susceptible ones (Kvedaras and Keeping, 2007; Kvedaras et al., 2007a), an observation borne out by a significant correlation between rind hardness and resistance across 72 cultivars of varying susceptibility to *E. saccharina* (Keeping and Rutherford, 2004). Indeed, internode rind hardness appears to be important in plant resistance to sugarcane borers worldwide (David and Joseph, 1982; White et al., 2006).

In sugarcane, the distinct softness of the bud as determined from penetrometer readings (Kvedaras et al., 2007b), as well as the significantly lower Si content of the bud scale epidermis compared with the internode and root band epidermis (this study; Fig. 3), are consistent with the observation that the leaf bud is a preferred entry point into the stalk for *E. saccharina* larvae (Leslie, 1993). The bud tissue has a higher nitrogen content and is lower in fibre than the surrounding stalk tissue (R.S. Rutherford, unpublished results), making it a good initial food source for young larvae, although the occurrence of defensive compounds (chlorogenates and certain flavonoids) in relatively high concentrations in some resistant cultivars (Rutherford, 1998) may counteract this to some extent. However, while *E. saccharina* often enters through the bud, larvae quickly move into the central part of the stalk where they spend most of their time feeding. The bud likely provides the path of least resistance for larvae attempting to penetrate the stalk.

Despite the greater external hardness of the internode compared with the root band (Kvedaras et al., 2007b), the epidermis of the latter contained significantly more Si than the internodal epidermis in both cultivars (this study; Fig. 3). We cannot claim, therefore, that Si is the major factor contributing to rind hardness. Fibre (cellulose, hemicellulose and lignin) is probably a crucial component in this regard (more so in resistant cultivars), as indicated by a significant positive correlation between fibre% cane and internode rind hardness across the 72 cultivars mentioned above (Keeping and Rutherford, 2004). Fibre is also directly associated with resistance to *E. saccharina* (Rutherford et al., 1993). Collectively, therefore, results of the present and previous studies strongly suggest that hardness of the internodal epidermis is important in conferring resistance to *E. saccharina*, with both fibre and the accumulation of Si in this tissue as key components.

5. Conclusions

The relationship between insect herbivore attack and the deployment of Si-based defenses in plants is clearly a dynamic one. Where Si deters feeding by herbivores, or where consumption of high-Si tissue leads to reduced insect performance and fitness, selection may act on insects to avoid plant parts that are high in Si. Plants, on the other hand, may be selected to deploy such defenses where they are most effective in preventing colonisation and attack by insect herbivores. Where crop cultivars are subject to selection programmes for enhanced resistance to pests, such as stalk borers in sugarcane, the plant breeder may similarly be unconsciously selecting cultivars that deploy Si most efficaciously against the key pest/s.

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