A novel method to determine larval mandibular wear of the African stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae)

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The African stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae), is the most significant indigenous pest on sugarcane in South Africa (Atkinson 1980; Conlong 2001). Currently, crop management and cultivar resistance in sugarcane remain the most widely accepted control methods for *E. saccharina* in South Africa (Nuss & Atkinson 1983; Keeping 2006). Following emergence, *E. saccharina* larvae graze up and down the sugarcane stalk or disperse onto dead leaf sheaths for several days until old enough and, presumably, with sufficiently strong mandibles to bore into the plant (Leslie 1993). As young larvae use their mandibles to chew through the sugarcane stalk, abrasion of the mandibles’ chewing surface is inevitable and could ultimately reduce feeding efficiency. It is recognized that fertilization with silicon (Si) can enhance the resistance of plants to insect attack, including that of sugarcane to *E. saccharina* (Keeping & Meyer 2002, 2006; Kvedaras et al. 2007). Testing the effects of this treatment highlighted a need to measure the extent of wear on larval mandibles.

While there have been studies that have reported wear of chewing mouthparts of insects, they have either failed to describe the method (Djamain & Pathak 1967), used a method which can only be applied to mandibles of the same size (Raupp 1985) or have not accounted for differences in mandibular size between individuals (Kokko et al. 1993). The objective of this study was to develop an accurate and repeatable method to measure the mandibular wear experienced by *E. saccharina* larvae fed on commercial sugarcane. It is anticipated that the method will be useful in quantifying mandibular wear in other adult and larval chewing insects. A method is described that uses a simple image analysis technique to determine the loss of feature on the chewing edge of the mandible.

Ten third instar *E. saccharina* larvae were randomly sampled from commercial sugarcane cultivars N26 and N11 grown at the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KwaZulu-Natal. Larvae were placed directly into 70 % alcohol until required.

Prior to analysis, the larvae were removed from the alcohol, allowed to air dry for 10 min and their heads removed under a light microscope. The labrum was removed and using a fine tipped dissecting needle, the mandibles were carefully pried apart to avoid chipping and air-dried for 1 h. The heads were mounted with their mandibles in a horizontal position using black, double-sided carbon tape (12 mm × 20 m, Structure Probe Inc., West Chester, U.S.A.), on standard aluminium scanning electron microscopy (SEM) stubs. Debris on the mandibles was removed using a fine dissection needle and a fine camel hair brush. The specimens were gold coated for 3.5 min (approx. 6 nanometres thickness) using a gold sputter-coater (Polaron E5100 coating unit and E5500 film thickness monitor, Polaron Equipment Limited, Watford Hertfordshire, U.K.). Specimens were viewed using a scanning electron microscope (Leo 1450 Scanning Electron Microscope, Cambridge, U.S.A.) and the images captured digitally.

Each mandible has two scissorial teeth and a proximal tooth, the mola. The degree of wear of a mandible was quantified using an algorithm to obtain a mean square error (MSE), as follows. The coordinates \((x,y)\) of the pixels in the digital image that represent the chewing edge of the mandible, from the distal to the proximal convex protrusion (distal scissorial teeth and mola tooth) (Fig. 1), were subjected to linear regression. As the chewing edge of each mandible is carefully orientated under a microscope in a vertical direction, the deviation in the X-axis is the most relevant measurement, hence the best-fitting straight line is calculated as:

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X = aY + b, \]

where \(X = \) horizontal pixel coordinate, \(a = \) function

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