SHORT, NON-REFEREED PAPER

UNDERSTANDING THE CHEMICAL ECOLOGY OF STIMULO-DETERRENT DIVERSION AS A BASIS FOR SUGARCANE PEST CONTROL: *ELDANA SACCHARINA* VS *MELINIS MINUTIFLORA*

HARRACA V[1,2], DU PISSANIE J[1], RUTHERFORD R S[1] AND CONLONG D E[1,2]

[1]*South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, 4300, South Africa*
[2]*School of Biological and Conservation Sciences, Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg Campus, P/Bag X01, Scottsville 3610, South Africa*
vincent.harraca@sugar.org.za stuart.rutherford@sugar.org.za
jennyjennysmith@gmail.com des.conlong@sugar.org.za

Abstract

For more than 40 years, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) has been an important sugarcane pest in South Africa. In the South African Sugarcane Research Institute (SASRI) Area Wide Integrated Pest Management (AW-IPM) approach, a stimulo-deterrent diversion system or ‘Push-Pull’, using indigenous plants to attract (pull) or deter (push) this pest is being investigated. However, basic knowledge about the behaviour and ecology of *E. saccharina* is needed to understand and improve the creation of a resilient agro-system. This preliminary study focused on the influence of the push-plant *Melinis minutiflora* on the behaviour of *E. saccharina* larvae and moths. In laboratory bioassays, the odour of this plant significantly attracted *E. saccharina* larvae, but repelled moths. Electroantennogram recordings demonstrated that *E. saccharina* adults are able to detect hexenyl acetate, ethyl acetate and linalool but not indole, β-caryophyllene and β-farnesene (all natural volatiles of *M. minutiflora*). When testing single compounds, β-caryophyllene, emitted in large amounts by this plant, by itself attracted *E. saccharina* larvae. In contrast, high levels of hexenyl acetate were shown to repel larvae. We hypothesised that this molecule is also partly responsible for the repulsion of the moth. The identification and isolation of these and other compounds attracting larvae and repelling moths will enhance the stimulo-deterrent diversion strategy as part of the AW-IPM strategy against *E. saccharina*.

Keywords: Eldana saccharina, push-pull, *Melinis minutiflora,* chemical ecology, behaviour, electrophysiology

Introduction

Eldana saccharina Walker (Lepidoptera: Pyralidae) is the major pest of South African sugarcane. Due mostly to its cryptic habits, traditional pest management approaches have never fully controlled this borer (Conlong, 1997). As it is indigenous, the stimulo-deterrent diversion (or push-pull) strategy was considered a viable alternative management strategy. This followed the effective push-pull system model demonstrated in Kenyan maize fields to control a related borer species (Khan *et al.,* 1997, Cook *et al.,* 2007). Here *Melinis* *minutiflora* is used to repel borer adults from maize fields, and also to attract larval parasitoids into this agro-ecosystem (Khan *et al.*, 1997, Cook *et al.,* 2007).

Plants continuously emit volatile compounds that provide cues for insects to be either attracted to (pulled) or repelled by (pushed) the plants. While under pest attack, plants emit additional volatile compounds (Arimura *et al.,* 2005) that parasitoids and predators use as attractants as they signal the presence of herbivorous hosts on the plant (Turlings *et al.,* 1990, Dicke *et al.,* 1993). *Eldana saccharina* is effectively biologically controlled in its natural host plants (mainly Cyperaceae; Conlong, 1990). In contrast, the borer proliferates in sugarcane as no natural parasitoids are found attacking them in this habitat (Conlong and Hastings, 1984). *Melinis minutiflora* is used in the Kenyan push-pull system to provide missing volatiles for host plant and parasitoid selection (Khan *et al.,* 1997; Cook *et al.,* 2007). This model was considered applicable for testing *E. saccharina*/sugarcane agro-ecosystem.

However, Kasl (2004) and Barker (2008) showed that the role of odour in host plant selection by *E. saccharina* moths is reduced. In contrast, Kasl (2004) demonstrated that neonate larvae actively discriminated between potential host plants. This led to the hypothesis that neonate larvae are responsible for the active choice of host plant, as they seem more responsive to plant odours (Conlong *et al.,* 2007). It is thus important that specific knowledge about these plant/insect interactions be gained before application.

Materials and Methods

The anemotactic response of the insects stimulated by plant volatiles were tested in Y-tube and 4-way olfactometers, each testing arm being loaded with one specific volatile/mix. One *E. saccharina* neonate larva or moth was subjected in the olfactometers to either volatiles from a growing plant covered by an oven bag, or a synthetic compound diluted in paraffin oil. In every experiment, one arm had no odour source (control). Time spent in, or number of entrances into three odour zones of an arm was recorded over five minutes for larvae and 10 minutes for moths. Software R was used for statistical analyses.

Melinis minutiflora leaf volatiles were collected on an adsorbent column and directly injected into a gas chromatograph equipped with a chromatoprobe kit and mass spectrometer allowing volatile compound identification.

The moth's antennae were mounted between two glass electrodes in order to record the neuronal activity elicited by the volatile stimuli on an electroantennogram. The stimuli consisted of 10 µL of specific synthetic compounds diluted in paraffin oil through six logarithmic steps.

Results

In both olfactometers, *E. saccharina* larvae significantly preferred *M. minutiflora* leaf odour over the blank air control. Of these, 57% entered and stayed for the test period in the odour zones of the arm of the Y-tube olfactometer (Wilcoxon test, V=330, $p<0.05$; Figure 1A) and 34% in the odour zones of the 4-way olfactometer (G-test, $\chi^2=27$, $p<0.001$; Figure 1B). However, adult moths responded oppositely. Males (15±18%, n=4) and females (13±13%, n=6), avoided the arm of the Y-tube olfactometer baited with *M. minutiflora* leaf volatiles (Wilcoxon test, V=100, $p<0.001$; Figure 1C).

Larvae spent variable amounts of time in odour fields of the 4-way olfactometer baited with different synthetic compounds, and concentrations of compounds (Figure 1C). β-caryophyllene was significantly attractive (Wilcoxon test, V>150, $p<0.05$) when diluted less than 10^8 times. Hexenyl acetate was significantly repellent (Wilcoxon test, V=47.5, $p<0.05$) when diluted 10^4 times. At any of the four dilutions tested, larvae spent similar times in odour fields with methyl jasmonate or not baited.

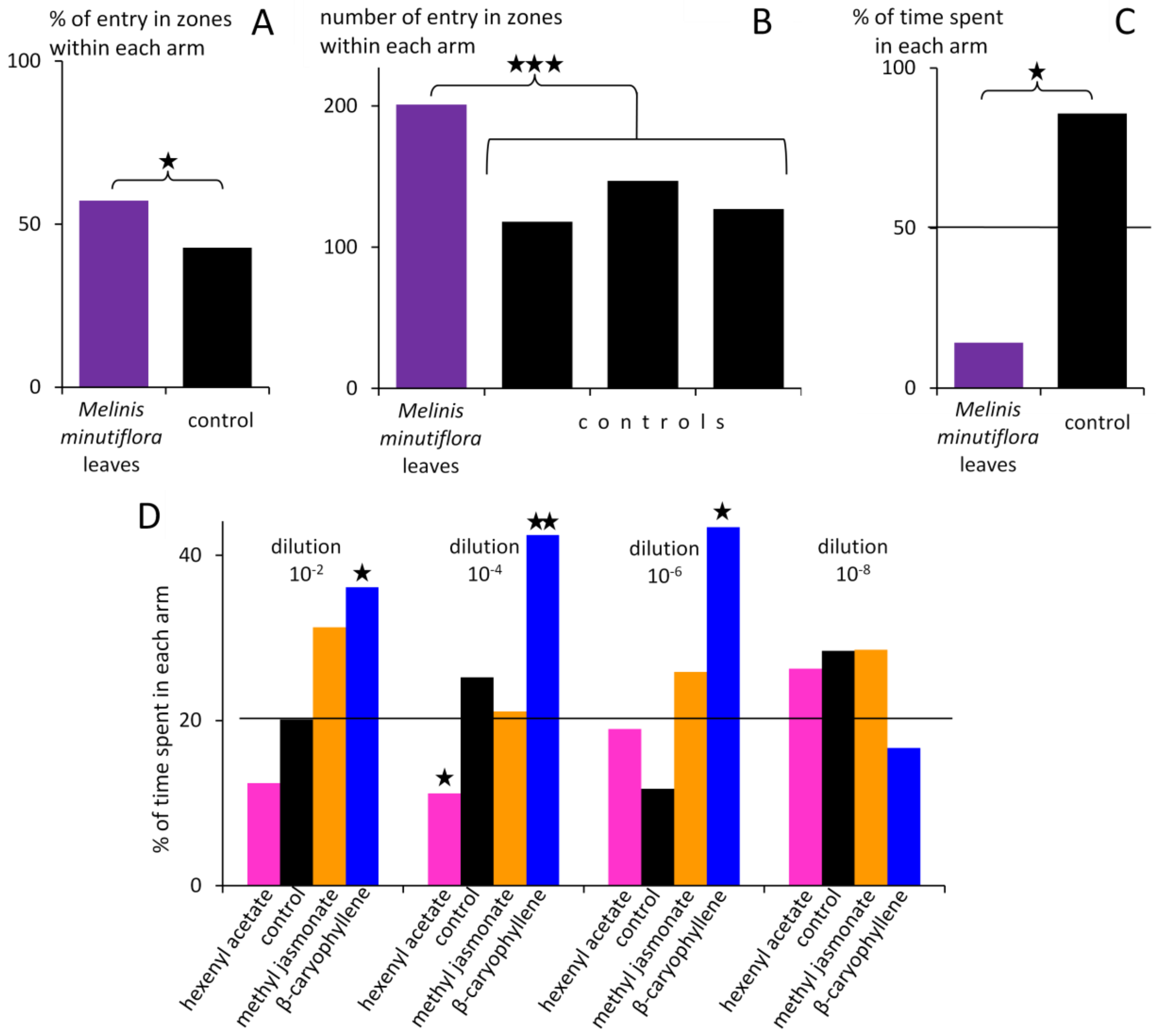

Figure 1. Behavioural experiments on *Eldana saccharina* larvae (A,B,D) and moths (C) in a Y-tube olfactometer (A,C) and a 4-way olfactometer (B,D) to assess the influence of volatiles emanating from *Melinis minutiflora* (A,B,C) and of synthetic compounds (D). Stars indicate statistically significant differences.

Compounds recovered by air entrainment of *M. minutiflora* were identified as mostly common monoterpenes and sesquiterpenes (Figure 2A).

Of the 13 synthetic common plant compounds tested at six dilutions, only six gave relevant electroantennogram responses, three of them being recovered in *M. minutiflora* volatile collections (Figure 2B).

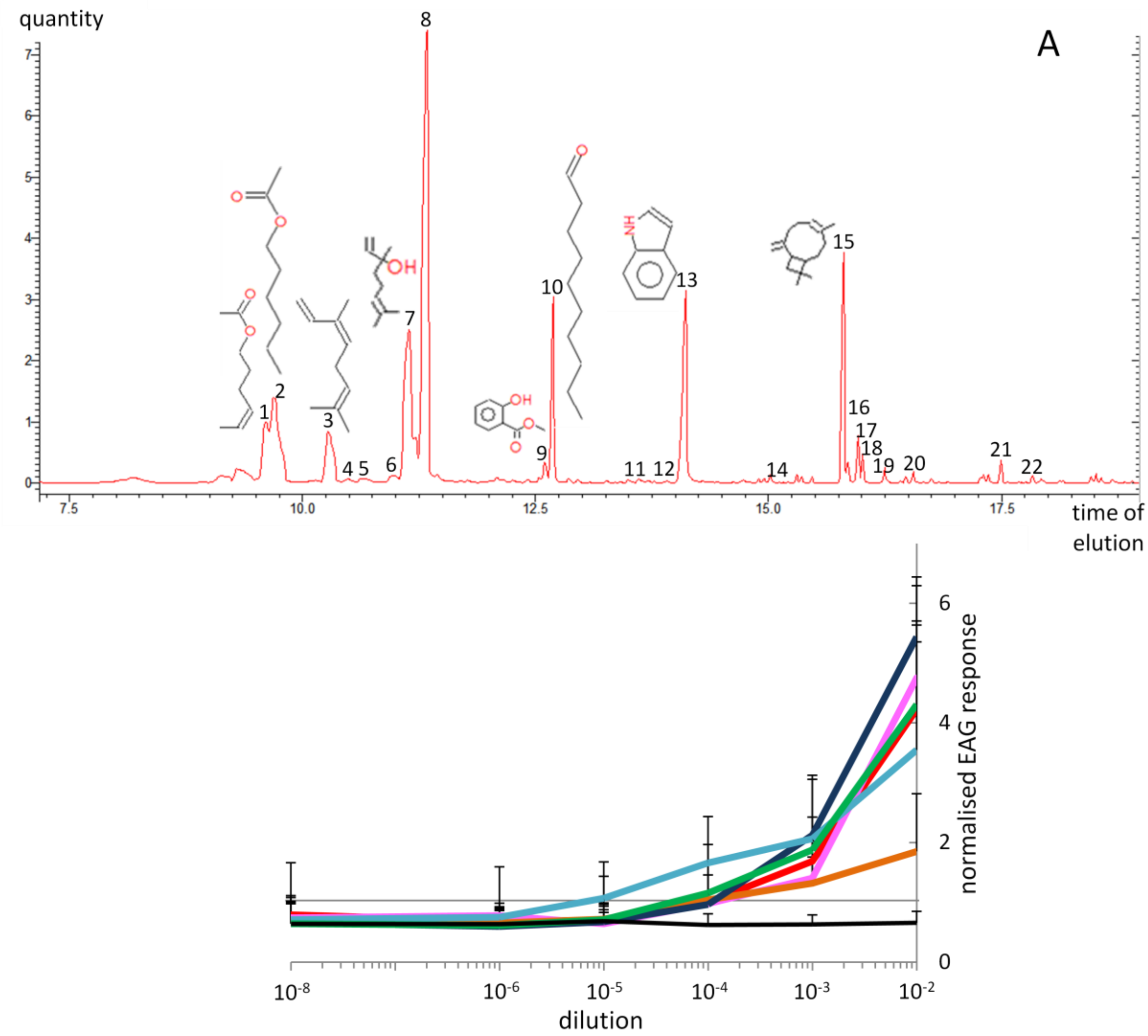

Figure 2. Air entrainment of the push-plant *Melinis minutiflora* (A) and electroantennogram (EAG) of synthetic compounds diluted in paraffin oil (B).

The compounds recovered in A were (1) hexenyl acetate, (2) ethyl acetate, (3) β-*cis*-ocimene, (4) (*E*)-2-octenal, (5) 1-octanol, (6) linalool oxide, (7) linalool, (8) 1,1-dimethyl-3-methylene-2-vinylcyclohexane, (9) methyl salicylate, (10) decanal, (11) (*E*)-2-decanal, (12) 3-methyl-6-oxo-hex-2-enyl ester acetic acid, (13) indole, (14) geranyl acetate, (15) β-caryophyllene, (16) α-bergamotene, (17) geranyl acetone, (18) β-farnesene, (19) α-caryophyllene, (20) germacrene D/cubebene, (21) *trans*-nerolidol, (22) caryophyllene oxide.

The compounds tested in B were benzaldehyde (red), hexenyl acetate (pink), ethyl acetate (brown) linalool (dark blue), myrcene (light blue), phenyl ethanol (green) and paraffin oil as control (black). The response to benzaldehyde diluted 10^3 times was used to normalise the other responses.

Discussion

Melinis minutiflora emits many more volatile compounds compared to sugarcane and papyrus (unpublished data, Figure 2A; Smith *et al.*, 2006). Most of these are indirect defensive compounds (Arimura *et al.*, 2005) usually emitted by a plant under attack from herbivores. However, these volatiles were highly attractive to *E. saccharina* larvae (Figure 1A and 1B), suggesting that the specific presence and/or concentration of these plant volatiles are major cues attracting larvae. This supports other unpublished results by the authors, where 65% of larvae exposed significantly preferred volatiles from leaves of an infested sugarcane stalk over volatiles from leaves of an uninfested stalk. Synthetic compounds carrying the same stress information via different pathways (see Arimura *et al.,* 2005) were tested to confirm this hypothesis. These showed that, whereas high amounts of the green leaf volatile hexenyl acetate were avoided by the larvae, β-caryophyllene on its own was very attractive (Figure 1D). Both compounds were detected in the odour of *M. minutiflora* (peaks 1 and 15 in Figure 2A), β-caryophyllene being emitted in much higher concentrations and probably being partly responsible for attraction of larvae to this plant. *Melinis minutiflora* could thus be regarded as an efficient pull-plant for larvae, especially for its propensity to release β-caryophyllene.

In contrast, *M. minutiflora* can clearly be used as a push-plant for *E. saccharina* moths (Figure 1C). Electrophysiological recordings of 13 synthetic compounds (Figure2B), six of which are emitted by *M. minutiflora* (Figure 2A), demonstrated that the moth can detect six compounds, including hexenyl acetate. In the absence of behavioural experiments with synthetic compounds, we can only suggest that moth avoidance of *M. minutiflora* is partly due to its ability to detect hexenyl acetate (which repels larvae) and not β-caryophyllene which is attractive to larvae (Figures 1D, 2A and 2B). We further suggest that female *E. saccharina* moths are not that 'un-maternal' (Conlong *et al.*, 2007), because even if they do not detect the positive volatile cues used by their larvae, they are able to detect compounds that repel their larvae and therefore avoid ovipositing on plants emitting these volatiles, or even on plants in the same environment. *Melinis minutiflora* is thus regarded as a useful component in an efficient push-pull system against *E. saccharina*, because of its ability through its chemical ecology, to attract larvae (pull-plant) and repel adults (push-plant) of this insect pest.

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