SHORT, NON-REFEREED PAPER

ELDANA SACCHARINA SEX APPEAL: A FUTURE METHOD TO MONITOR AND CONTROL THIS PEST?

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Abstract

The first step towards integrated pest management is the timely detection of pest infestation. Insect traps not only allow detection and monitoring of a pest problem but also provide estimates regarding pest population density. Monitoring pest populations with pheromone-baited traps is one of the most useful applications derived from basic research in chemical ecology. *Eldana saccharina* Walker (Lepidoptera: Pyralidae) has been an important pest throughout the South African sugar industry for more than 40 years. Nevertheless, a pheromone based monitoring system and the possibility of the use of pheromones in a mating disruption approach to control, remain elusive. In order to investigate the sex pheromone signal sent by *E. saccharina* males, laboratory based behavioural experiments have revealed different degrees of attraction depending on the sex, condition and age of the moth. Indeed, whereas females and males are both significantly attracted by calling males, only females significantly preferred the odour emanating from male hair pencils, and none reacted to extracts from these hair pencils. In addition, preliminary electroantennography experiments demonstrated that vanillin, previously identified as a major pheromonal compound of *E. saccharina*, is not detected by the moths. Therefore, a new investigation of the hair pencil gland extract is needed, which could lead to a breakthrough in the understanding of *E. saccharina* sexual attraction and behaviour.

Keywords: sugarcane, *Eldana saccharina*, sex pheromone, chemical ecology, behaviour, electrophysiology

Introduction

Pheromone research is one of the major applied fields in chemical ecology. Sex pheromones are becoming more widely used in agriculture to protect crops in a sustainable and environmentally friendly manner (review in Witzgall *et al.*, 2010). Indeed, pheromones are highly species specific and provide an effective tool to monitor or trap pests, or to reduce their reproduction through mating disruption. Although several papers have been published on the sexual behaviour of *Eldana saccharina* (Atkinson, 1981; Zagatti, 1981; Farine, 1983; Bennett *et al*., 1991, Burger *et al*., 1993), no applications in the field have been developed.

The aim of this study was to evaluate in the laboratory, the various findings previously obtained as a point of departure for the development of pheromone use in the field.

Materials and Methods

The anemotactic responses of three different groups of moths were tested: males, virgin females which had already laid eggs ('old females' hereafter) and virgin females which had not yet laid eggs ('young females' hereafter). Ten to fifteen moths in each category were individually stimulated by an odour stream coming from one arm of a Y-tube olfactometer, whereas the second arm stream was without odour (control). The time spent within the arms was recorded for 10 minutes using a data logger (Behave v1.0, O. Pfaehler). Data was analysed using the statistical software 'R'.

The three different odour sources used were: three live calling males, three pairs of hair pencils freshly removed from males and deposited on a filter paper, and 10 µL extract made from five pairs of hair pencils dipped in 500 µL dichloromethane. This last stimulus was also used in electroantennography experiments, in addition to synthetic compounds diluted in paraffin oil at three logarithmic dilutions.

For electrophysiological experiments, a single moth antenna mounted between two glass electrodes and connected to signal amplifiers allowed the recording of neuronal activity elicited by odorant stimuli.

Results

A preliminary study revealed that virgin females started to lay infertile eggs 2.2±1.2 days after their emergence (n=134). It was noted that females already laying infertile eggs, and then subsequently mated, laid fertile eggs after mating.

Both young and old females displayed similar levels of attraction to calling males (Wilcoxon test, W=26, $p<0.01$ and W=28.5, $p<0.05$, respectively; Figure 1A) and to hair pencil odour (Wilcoxon test, W=98, $p<0.05$ and W=69.5, $p<0.05$, respectively; Figure 1B).

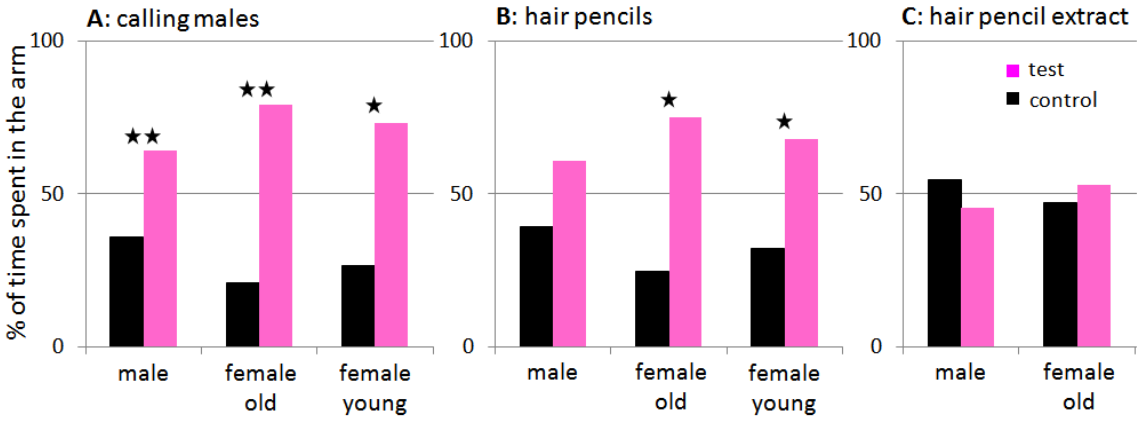

Figure 1. Percentage of time spent by *Eldana saccharina* moths of different sex in the arms of a Y-tube olfactometer. The pink histograms represent the arms baited with (A) three calling males, (B) three pairs of crushed hair pencils, and (C) dichloromethane extract from five pairs of hair pencils. Stars represent significant difference at $p<0.05$, measured by the Wilcoxon test.

In contrast, only males spent significantly more time in the arm baited by odours from other calling males (Wilcoxon test, W=26.5, p<0.01; Figure 1A). The extract of hair pencils did not induce any preferred choice in the Y-tube olfactometer (Figure 1C).

However, the hair pencil extract did elicit electroantennographic responses from antenna of both males (2.54±1.09, n=30) and females (2.93±0.93, n=132). Vanillin is one of the major components of this extract (data not shown, Burger *et al.* 1993), but except for guaiacol, none of the compounds with a structure related to vanillin elicited a strong neuro-electrical signal at any dilution tested (Figure 2).

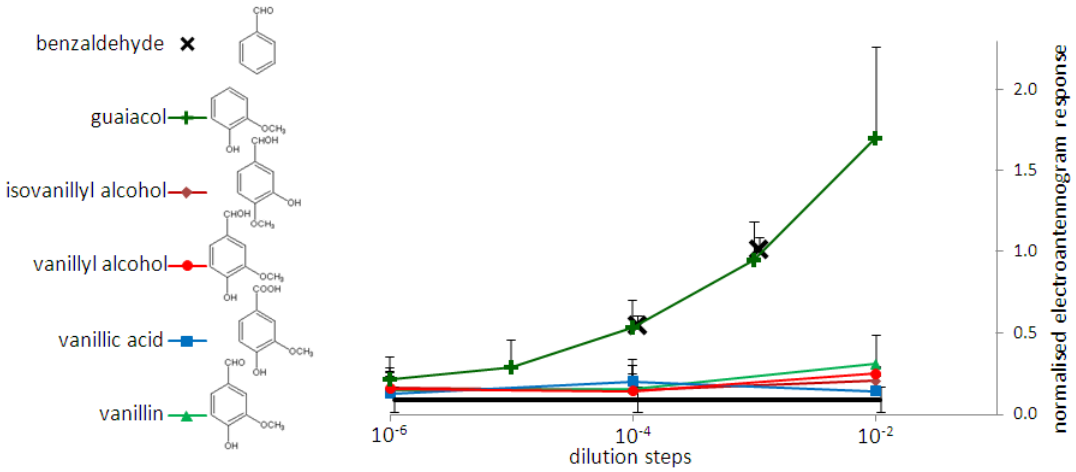

Figure 2. Electrophysiological responses recorded on *Eldana saccharina* antenna to vanillin associated compounds. Each point represents the mean antenna response with standard deviation (three females and three males) to different compounds diluted in paraffin oil (control, black line) at three to five logarithmic dilution steps. Responses were normalised using the response of benzaldehyde diluted 10^3 times as reference.

Discussion

It was previously thought that *E. saccharina* females are not able to reproduce after laying their first infertile eggs (Kasl, 2004). In this study, older virgin females were found able to locate a mate and to lay fertilised eggs (Figure 1A), confirming the results of Walton (2011).

The sexual behaviour of *E. saccharina* is complex as, in addition to odours emitted from two different glands, the males also produce sound to attract females (Zagatti, 1981; Bennett *et al.*, 1991). However, even if a synergistic effect between these three stimuli occurs (Burger *et al.*, 1993), the emission of the hair pencils may be those that carry information to a distance (Bennett *et al.*, 1991). In our experiments excised hair pencils significantly attracted *E. saccharina* females (Figure 1).

Considering the electroantennogram responses recorded for benzaldehyde, guaiacol (Figure 2) and other different plant compounds (Harraca *et al.*, 2011), we can conclude that both sexes of *E. saccharina* do not detect any of the compounds with a structure related to vanillin, which was found by Burger *et al.* (1993) to be common in their analysis of hair pencil gland extracts. Another compound in this extract may be

responsible for the electroantennogram response we recorded, but it did not elicit a behavioural response (Figure 1C). Solvent extraction may have weakened the attractiveness of the sexual signal by dilution, but pheromones are usually behaviourally active at very low doses (Witzgall *et al.*, 2010).

To avoid using solvent, a direct chemical analysis of the content of the hair pencil gland was recently conducted (data not shown). Results were promising with the detection of new compounds, among them amines. If these latest compounds are critical cues for *E. saccharina*, it may explain the prevalence of this pest in sugarcane of high nitrogen content. However, more experiments are needed to validate this hypothesis and to isolate the active compounds from the hair pencil extract. Thereafter, it may be possible to formulate an efficient odorant mixture which can reproduce the attraction level of the natural pheromone.

Acknowledgement:

The authors wish to thank Dr Andreas Jürgens of Biological and Conservation Sciences, UKZN, Pietermaritzburg, for his constructive discussions and help in the identification of pheromone components; also Angela Walton and the technicians of the Insect Unit at SASRI, who provided the insects. This work was supported by SASRI via the project 09CP04.

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