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RESEARCH ARTICLE

Electrophysiological responses of the rice striped stem borer *Chilo suppressalis* to volatiles of the trap plant vetiver grass (*Vetiveria zizanioides* L.)

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Abstract

Vetiver grass (*Vetiveria zizanioides* L.) was previously found to effectively attract female adults of *Chilo suppressalis* (Walker), an important pest of rice. To determine the volatile compounds involved in this attraction, electroantennography (EAG) responses to seven synthetic volatiles released from vetiver plants were examined. Our results indicated that the responses of *C. suppressalis* adult antennae to the different compounds varied widely. The compounds elicited strong EAG responses in female antennae were subsequently selected for further EAG response tests, namely, caryophyllene, β-ocimene, linalool and α-pinene. EAG responses to a combination of these four compounds did not differ significantly from the individual compounds. However, pair combination tests indicated that 0.01 μg μL^{-1} linalool and 50 μg μL^{-1} α-pinene, 50 μg μL^{-1} caryophyllene and 0.01 μg μL^{-1} linalool, 0.01 μg μL^{-1} β-ocimene and 0.01 μg μL^{-1} linalool, and 0.01 μg μL^{-1} β-ocimene and 50 μg μL^{-1} caryophyllene elicited significantly greater EAG responses in 3-day female moths compared to the 1-day female. These compound combinations and the corresponding ratios are probably playing an important role in attracting female adults of *C. suppressalis* to the vetiver grass.

Keywords: *Chilo suppressalis*, electroantennography (EAG), volatiles, vetiver grass, trapping mechanism, attraction

1. Introduction

Rice (*Oryza sativa* L.), the most important staple food in the world, is at risk of attack by stem borer, a persistent and chronic pest found in almost every rice field throughout the growing season. Of these stem borers, the striped stem borer *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) is most widely distributed (Qu *et al.* 2003) and attacks all rice growth stages from the seedling to maturity. *C. suppressalis* larvae cause damage by boring into the stem and feeding from within, causing "deadhearts" and "whiteheads" at the vegetative and reproductive stages, respectively (Pathak 1968; Rubia *et al.* 1996; Jiang and Cheng 2003; Lu *et al.* 2015). As a result, plants would fail to produce a productive panicle (Dale 1994; Rubia *et al.* 1996; Jiang and Cheng 2003). In recent years, *C. suppressalis* outbreaks have been observed in China probably because of changes in the rice cultivation system and wide adoption of hybrid varieties (Peng 2016). Control is primarily dependent on the application of insecticide and insecticide resis-

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tance is an important contributing factor in the outbreaks (Zhu *et al.* 1987; Su *et al.* 1996; Li *et al.* 2001; Zibaee *et al.* 2009; Hu *et al.* 2010; Su *et al.* 2014; Li *et al.* 2015). In the past few years, chlorantraniliprole has been widely used to control *C. suppressalis*, however, its field control efficacy has greatly decreased, leading to failed control in some areas in 2016. Effective and sustainable control strategies for *C. suppressalis* are therefore necessary.

Vetiver grass (*Vetiveria zizanioides* L.) is usually planted on hills and roadsides to prevent soil erosion. Recently it has been reported to be a repellent to a number of insect species (Berg 2006). Vetiver grass is also attacked by a few insects, which has raised concerns over its use as a refuge or trap plant for crop pests. Our previous study showed that vetiver grass attracts ovipostion of *C. suppressalis* adults (Zheng *et al.* 2009), and its use as a trap plant in paddy fields has been suggested as a method to control *C. suppressalis* populations in China (Zheng *et al.* 2009; Liang *et al.* 2015; Lu *et al.* 2015).

Plant volatiles have been known to affect insect behavior (Lou and Cheng 1997; Bruce *et al.* 2005). Most phytophagous insects use their antenna to identify odors as cues to find food resources, mates and places to deposit their offsprings (Gallego *et al.* 2008; Fettig *et al.* 2009; Hu *et al.* 2009; Zhuge *et al.* 2010; Sun *et al.* 2014). Zuo (2007) studied the electroantennography (EAG) responses of male and female *C. suppressalis*, and found strong responses to certain volatiles. If identified, these volatile compounds could be used to create a core lure to attract *C. suppressalis*, saving time, effort and costs.

Anton *et al.* (2007) suggested that the attractiveness of a volatile is dependent on physiological conditions of the individual. We examined the responses of *C. suppressalis* to vetiver volatile compounds based on their physiological conditions. In this paper we report our investigations on 1) dose-dependent EAG responses to selected vetiver grass volatile compounds; 2) the EAG responses to these compounds based on physiological conditions (1-day moths *vs.* 3-day moths); and 3) the compounds and corresponding compositions eliciting the strongest EAG responses in the females. The findings will provide a reference for further research aimed at the development of a core lure for *C. suppressalis*.

2. Materials and methods

2.1. Insects

Larvae (5th–6th instar) and pupae of *C. suppressalis* were collected from paddy fields in Xiaoshan District (120°12´E, 30°04´N), Hangzhou, China, in 2015. They were reared in an artificial atmospheric phenomena simulator with temperature of (27±1)°C, (70±5)% RH and a 16 h L:8 h D photoperiod until the moths emerged. To examine the gender-dependent effects of the volatile compounds, males and females were separated individually at the pupal stages and the newly emerged within 12 h (1-day moths) were collected separately. Remaining moths were paired to mate and fed with 10% honey solution until the third day (3-day moths).

2.2. Synthetic volatile compounds

Synthetic compounds used in the experiments were caryophyllene, methyl salicylate, α-pinene, β-ocimene, linalool, nonanal and camphor, all of which were commercially purchased (Table 1). The compounds were stored and used in accordance with the instructions provided. All these compounds have been detected in the volatiles of vetiver grass. *cis*-3-Hexen-1-ol was used as a reference check.

2.3. Antennal preparation, stimulation and EAG recordings

The receptivity of the antennae of male and female moths to the individual volatile compounds and the corresponding compositions found in vetiver grass were determined by the EAG. Antennae were carefully removed at the base with several terminal segments at the distal end excised before mounting them on the electrodes with Spectra 360 conductive gel. These preparations were viable for about 20–30 min. For the tests all substances were applied once per antenna and in a constant order as in Table 1, starting with caryophyllene. The reference check, *cis*-3-hexen-1-ol was applied twice on each antenna, defining the beginning and end of the test series to calibrate for any loss in sensitivity of the preparation (Eltz and Lunau 2005; Sun *et al.* 2014). With each stimulus, 3 μL of test solution was pipetted onto a fresh 3 mm×20 mm strip of filter paper. Test compounds

Table 1 List of compounds used to examine electroantennography (EAG) responses of *Chilo suppressalia* to vetiver grass volatile compounds

Compounds	Sample chemical purity (%)	Chemical company
Caryophyllene	≥98.5	Sigma Chemical Co., Ltd., USA
Methyl salicylate	99	Sigma Chemical Co., Ltd., USA
β-Ocimene	98	Aldrich Chemical Co., Ltd., USA
Linalool	97	Aldrich Chemical Co., Ltd., USA
Nonanal	97	Aldrich Chemical Co., Ltd., USA
Camphor	96	Aldrich Chemical Co., Ltd., USA
(+)-α-Pinene	≥99	Aldrich Chemical Co., Ltd., USA
cis-3-Hexen-1-ol	≥98	Aldrich Chemical Co., Ltd., USA