

Electrophysiological Responses of the Rice Leaffolder, *Cnaphalocrocis medinalis*, to Rice plant Volatiles

Authors: Sun, Xiao, Liu, Zhuang, Zhang, Aijun, Dong, Hai-Bo, Zeng, Fang-Fang, et al.

Source: Journal of Insect Science, 14(70) : 1-14

Published By: Entomological Society of America

URL: <https://doi.org/10.1673/031.014.70>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.



Electrophysiological responses of the rice leaffolder, *Cnaphalocrocis medinalis*, to rice plant volatiles

Xiao Sun^{1a}, Zhuang Liu^{1b}, Aijun Zhang^{2c}, Hai-Bo Dong^{1d}, Fang-Fang Zeng^{1e}, Xiang-Yu Pan^{1f}, Yongmo Wang^{1g}, Man-Qun Wang^{1h}

¹Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China

²Invasive Insect Biocontrol and Behavior Laboratory, Plant Sciences Institute, USDA-ARS, Beltsville, MD 20705-2350

Abstract

The rice leaffolder, *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae), is one of the most destructive pests of rice. Electrophysiological responses of this species to 38 synthetic volatiles known to be released from rice plants (Poaceae: *Oryza* spp.) were studied using the electroantennogram (EAG) method. Compounds that elicited the strongest EAG responses for each physiological condition were selected for EAG dose-response tests at five concentrations. These compounds included: methyl salicylate, heptanol, linalool, cyclohexanol, and 2-heptanone for one-day-old male moths; heptanol, hexanal, (Z)-2-hexen-1-ol, and nonadecane for one-day-old females; methyl salicylate, heptanol, (E)-2-hexen-1-ol, and (Z)-2-hexen-1-ol for three-day-old males; linalool, heptanol, (E)-2-hexen-1-ol, 2-heptanone, and hexanal for three-day-old females; 2-heptanone, cyclohexanol, linalool, heptanol, and methyl salicylate for five-day-old virgin females; and methyl benzoate, (Z)-2-hexen-1-ol, heptanol, linalool, and hexanal for five-day-old mated females. Female and male *C. medinalis* exhibited broad overlap in their EAG responses, and there was no clear difference between male and female EAG responses to different compounds. Statistical analyses revealed that both volatile compound chemical structure and *C. medinalis* physiological condition (age, sex, and mating condition) had an effect on EAG response.

Keywords: electroantennogram**Abbreviations:** EAG, electroantennogram**Correspondence** ^a sunxiao19860310@126.com, ^b 294231362@qq.com, ^c zhanga@ba.ars.usda.gov, ^d donghaibonihao@163.com, ^e n119882006@163.com, ^f kingchys_pan@126.com, ^g ymwang@mail.hzau.edu.cn, ^h mqwang@mail.hzau.edu.cn**Editor:** Jurgen Ziesmann was editor of this paper.**Received:** 1 July 2012 **Accepted:** 1 February 2013 **Published:** 20 May 2014**Copyright:** This is an open access paper. We use the Creative Commons Attribution 3.0 license that permits unrestricted use, provided that the paper is properly attributed.**ISSN:** 1536-2442 | Vol. 14, Number 70**Cite this paper as:**

Sun X, Liu Z, Zhang A, Dong H-B, Zeng F-F, Pan X-Y, Wang Y, Wang M-Q. 2014. Electrophysiological responses of the rice leafroller, *Cnaphalocrocis medinalis*, to rice plant volatiles. *Journal of Insect Science* 14(70). Available online: <http://www.insectscience.org/14.70>

Introduction

The rice leafroller, *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae), is a migratory rice pest that is widely distributed in humid tropical and temperate regions of Asia, Oceania, Australia, and Africa (Khan et al. 1988; Kawazu et al. 2001). It has recently become widespread throughout the major rice-growing regions of Asia and is one of the most damaging pests of rice (Poaceae: *Oryza* spp.) (Sogawa and Kiyota 1995; Inoue et al. 2004). The larvae damage plants by folding the leaves and scraping the green leaf tissues within the fold, causing yield loss by reducing photosynthetic activity (Nathan et al. 2004). Chemical treatments against larvae are often impracticable due to the larvae's cryptic feeding habit. Effective control strategies must be based on controlling or killing adults.

Despite extensive research on *C. medinalis* response to sex pheromones and implementing related behavioral manipulation techniques (Ganeswara Rao et al. 1993; Ganeswara Rao et al. 1995; Kawazu et al. 2002; 2005; 2009), little is known about their host-plant volatile selection. Many phytophagous insects use odors as cues for orientation to food resources for their nutrition, mating location, or depositing their offspring (Jyothi et al. 2002; van Der Goes et al. 2006; Gallego et al. 2008; Fettig et al. 2009; Hu et al. 2009; Zhuge et al. 2010). Ramachandran et al. (1990) ex-

amined the electroantennogram (EAG) response of two-day-old *C. medinalis* males and four-day-old *C. medinalis* females to a spectrum of volatile chemicals and identified several compounds that elicit high EAG responses. However, many of the compounds used are not present in rice plants. It has also been shown that the attractiveness of a volatile may be dependent on environmental conditions (e.g., photoperiod or temperature) or the physiological condition of the individual (e.g., age or mating condition), not just the nature of the chemical (reviewed by Anton et al. 2007). Therefore, it is necessary to investigate the EAG responses of *C. medinalis* to volatile compounds known to be released from rice plants based on their physiological condition.

Below temperatures of about 25°C, *C. medinalis* females reach sexual maturity three days after emergence and begin mating with males, which reach sexual maturity one day after emergence (Kawazu and Tatsuki 2002). The adults suck nectar or honeydew secreted by aphids as supplementary food and have lifespans of five to eight days. Oviposition usually begins within 24 hrs of mating. *C. medinalis* tends to lay eggs scattered either on front or back of light or dark green rice leaves. Single eggs can be laid by both virgin and mated females, but unfertilized eggs cannot hatch (Zhang and Wang 1987).

This study investigates the EAG responses of *C. medinalis* to volatile compounds known to be released from intact rice plants (Lou et al. 2005; Wechgama et al. 2008; Lu et al. 2010; Yan et al. 2010) based on the age of males and females and the mating condition of females. In addition, dose-dependent EAG response’s relationships to selected compounds were investigated. This research is therefore an important step towards understanding the role of olfaction in manipulating the behavior of *C. medinalis*.

Materials and Methods

Insects

C. medinalis pupae were collected from an experimental plot in Wu’xue County of Hubei Province in China (115° 33 E; 29° 51 N) in 2010. Sexed pupae were kept inside culture dishes (20 cm diameter) in an environmental chamber (25 ± 1°C, 75 ± 5% RH, 16:8 L:D photoperiod) until the moths emerged. Emerged moths were fed with a 10% sucrose solution in wooden cages (30 x 30 x 30 cm) separate from the larvae. To obtain mated moths, newly emerged male moths and four-day-old female moths were paired in the same cage and allowed to mate. Moths used for

EAG studies were of six different physiological conditions: one- and three-day-old male and female moths and five-day-old virgin and mated females.

Synthetic compounds

Synthetic compounds used in EAG studies were purchased from commercial sources (Fluka, Sigma-Aldrich, www.sigmaaldrich.com; Table 1). These compounds belonged to different chemical classes: green leaf volatiles (hexanal, (E)-3-hexen-1-ol, (E)-2-hexenal, (E)-2-hexen-1-ol, (Z)-2-hexen-1-ol , heptanol, 2-heptanone), alkanes (octane, tetradecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane, heneicosane), hydroxyl and carbonyl compounds (cyclohexanol, nerolidol, (E)-2-penten-1-ol, dodecanal), aromatic compounds (p-cymene, benzaldehyde, N-phenyl-1-naphthylamine, methyl benzoate, methyl salicylate), and terpenoids (terpinene-4-ol, (1R)-(+)-α-pinene, sabinene, α-terpinene, γ-terpinene, β-myrcene, (-)-(Z)-caryophyllene, (-)-α-cedrene, linalool, lonone, (Z)-farnesene, α-terpineol, cedrol, R-(+)-limonene; Table 1). Compounds were stored in accordance with the instructions for use and used within the period of validity.

Electroantennogram recordings

The receptivity of antennae from male and female moths to plant volatile compounds was determined by EAG. EAG measurements were made using a commercially available electroantennographic system (Model CS-05; Syntech, www.syntech.nl) consisting of a dual electrode MTP-4 probe for antenna fixation, a CS-05 stimulus controller, and an IDAC box for data acquisition. Antennae were carefully removed at the base, and several terminal segments at the distal end were excised before attaching them to electrodes with Spectra 360 conductive gel (Parker, www.parkerlabs.com).

Table 1. List of compounds tested for <i>Cnaphalocrocis medinalis</i> EAG response to rice (Poaceae: <i>Oryza</i> spp.) volatile compounds.					
Code	Compounds	Purity	Code	Compounds	Purity
1	CK		21	Methyl benzoate	99.50%
2	Cyclohexanol	99%	22	(-)-(E)-caryophyllene	98.50%
3	Linalool	97%	23	Dodecanal	95%
4	Tetradecane	99.80%	24	Methyl salicylate	99%
5	Hexadecane	99.80%	25	γ-Terpinene	95%
6	Heptadecane	99%	26	Octane	98%
7	Octadecane	99.80%	27	2-Heptanone	99%
8	Nonadecane	99.80%	28	N-Phenyl-1-naphthylamine	98%
9	Eicosane	99%	29	R-(+)-Limonene	98%
10	Heneicosane	99%	30	Benzaldehyd	99%
11	(-)-α-Cedrene	99%	31	(Z)-2-penten-1-ol	99%
12	Nerolidol	98%	32	(1R)-(+)-α-pinene	99%
13	Cedrol	99%	33	Hexanal	99%
14	(Z)-Farnesene	99.80%	34	Lonone	99%
15	Terpinene-4-ol	95%	35	Heptanol	96%
16	α-Terpineol	95%	36	(Z)-3-hexen-1-ol	99%
17	α-Terpinene	95%	37	(E)-2-hexen-1-ol	99%
18	β-Myrcene	99.80%	38	(E)-2-hexenal	99%
19	Sabinene	97%	39	(Z)-2-hexen-1-ol	99%
20	p-Cymene	98%			

A continuous stream of moistened air was then blown at the antennae through a glass tube (0.8 cm diameter) positioned 1 cm away. Solutions of synthetic compounds were diluted in distilled paraffin oil. Test compounds (10 µL, 10 µg/µL) were applied to a piece of filter paper (5 mm × 20 mm), which was inserted into a Pasteur pipette. The tip of the pipette was inserted about 3 mm into a small hole in the wall of a glass tube (8 mm diameter, 12 cm long) directed at the antennal preparation. An air stimulus controller was used for air and odor delivery. A constant flow of 2 L/min of active carbon-filtered air was passed over the antennae through the open end of the glass tube. 200 mL/min of air was applied through the Pasteur pipette into the main airflow for 0.5 sec, creating an odor stimulation of the antenna. The response to the reference standard, (Z)-3-hexen-1-ol, was measured at the beginning and end of each recording session to correct for the loss of sensitivity of the preparation. It was assumed that the decrease in sensitivity was linear with time for the correction. Data were then normalized to the standard as follows (Gouinguene et al. 2005):

$$rEAG = \frac{EAG(A)}{EAG(std1) + \frac{EAG(std2) - EAG(std1)}{RT(std2) - RT(std1)} \times (RT(A) - RT(std1))}$$

Where rEAG means relative EAG response; EAG(A) is the amplitude (mV) of the EAG response to compound A; EAG(std1) is the EAG response to the standard at the beginning of the recording; EAG(std2) is the EAG response to the standard at the end of the recording; T(A) is the time elapsed before stimulation with compound A; T(std1) is the time of the first stimulation; T(std2) denotes the time of the final stimulation.

Comparison of EAG responses to different compounds

The compounds included in this study (Table 1) were tested individually as olfactory stimuli. Antennae were stimulated twice with each substance at 30 sec intervals. Fifteen replicates were carried out for each sex, and the sequence of exposure of each antenna to stimulus was randomly defined.

Dose-response relationship

Four or five compounds that elicited the strongest EAG responses for each sex were selected for EAG dose-response tests. Five doses (0.01, 0.1, 1, 10, and 50 µg/µL) dissolved in liquid paraffin were tested for their electrophysiological activity. Stimulations were made in order of concentration, with the lowest dose first and with 30 sec intervals between stimuli. The liquid paraffin was used as a blank control, and 10 µg/µL of (Z)-3-hexen-1-ol was used as a reference standard. Ten replicates were carried out for each sex.

Data analyses

Data were analyzed statistically using SPSS 11.0 for Windows (IBM, www.ibm.com). The relationship of male and female EAG responses was examined by Tukey's multiple comparison analysis. Paired-sample *t*-tests were used to analyze the EAG differences between males and females or virgin females and mated females.

Results

EAG activity of synthetic compounds

Statistical analyses (ANOVA) revealed that both the chemical structure of volatile compounds and the physiological condition of *C. medinalis* influenced EAG responses. For the six different physiological conditions of *C. medinalis* investigated, all rice plant compounds elicited significantly greater EAG

responses than the solvent controls at doses of 10 $\mu\text{g}/\mu\text{L}$ ($P < 0.01$; Figure 1). There were no significant differences in response to stimulation with the reference standard between one-day-old males and females ($t = 0.93$, $P > 0.05$), between three-day-old males and females ($t = 1.07$, $P > 0.05$), or between five-day-old virgin and mated females ($t = 1.38$, $P > 0.05$). However, there was a significantly different EAG response to some compounds between males and females.

For one-day-old moths, the highest EAG responses of virgin males were elicited by methyl salicylate, heptanol, linalool, cyclohexanol, and 2-heptanone. The highest EAG responses of virgin females were elicited by heptanol, hexanal, (Z)-2-hexen-1-ol, and nonadecane. For the three-day-old moths, the strongest EAG responses were elicited by methyl salicylate, heptanol, (E)-2-hexen-1-ol, and (Z)-2-hexen-1-ol for males, and linalool, heptanol, (E)-2-hexen-1-ol, 2-heptanone, and hexanal for females. The highest EAG responses of five-day-old virgin females were elicited by 2-heptanone, cyclohexanol, linalool, heptanol, and methyl salicylate, while the five-day-old mated females exhibited significantly larger EAG responses to hexanal, (Z)-2-hexen-1-ol, heptanol, linalool, and methyl benzoate (Figure 1).

All green-leaf volatiles elicited stronger antennal responses than most of the alkanes, terpenoids, aromatic compounds, and hydroxyl and carbonyl compounds, and did not elicit significantly different EAG responses between all the physiological conditions of *C. medinalis*.

Statistical analyses revealed that one-day-old *C. medinalis* males exhibited significantly greater EAG responses to nerolidol ($t = 3.62$, $P < 0.01$), dodecanal ($t = 3.35$, $P < 0.01$),

cedrol ($t = 3.35$, $P < 0.01$), heneicosane ($t = 2.70$, $P < 0.05$), cyclohexanol ($t = 2.70$, $P < 0.05$), *N*-phenyl-1-naphthylamine ($t = 2.70$, $P < 0.05$), (-)- α -cedrene ($t = 2.78$, $P < 0.05$), sabinene ($t = 2.63$, $P < 0.05$), and γ -terpinene ($t = 2.26$, $P < 0.05$) than three-day-old males. Analogously, one-day-old females showed significantly greater EAG responses to hexadecane ($t = 2.98$, $P < 0.05$), octadecane ($t = 2.70$, $P < 0.05$), and nonadecane ($t = 2.70$, $P < 0.05$) than five-day-old virgin females. One-day-old males showed significantly greater EAG responses to the linalool ($t = 3.62$, $P < 0.01$), 2-heptanone ($t = 2.32$, $P < 0.05$), and methyl salicylate ($t = 2.64$, $P < 0.05$) than one-day-old females. In contrast, one-day-old females showed significantly greater EAG responses to (1R)-(+)- α -pinene ($t = 3.80$, $P < 0.01$), p-cymene ($t = 4.07$, $P < 0.01$), terpinene-4-ol ($t = 3.10$, $P < 0.01$), hexadecane ($t = 2.41$, $P < 0.05$), nonadecane ($t = 2.19$, $P < 0.05$), benzaldehyde ($t = 2.65$, $P < 0.05$), α -terpinene ($t = 2.17$, $P < 0.05$), and β -myrcene ($t = 2.30$, $P < 0.05$) than one-day-old males. In addition, the data also showed that five-day-old mated females exhibited significantly greater EAG responses to hexadecane ($t = 3.83$, $P < 0.01$), nonadecane ($t = 3.73$, $P < 0.01$), octadecane ($t = 3.28$, $P < 0.05$), and heptadecane ($t = 2.24$, $P < 0.05$), which all belonged to the alkane group, than five-day-old virgin females.

Electroantennography dose-dependent responses

Based on the EAG results, compounds were selected that elicited the strongest responses in each of the six different *C. medinalis* physiological conditions tested in the EAG dose-response tests. The amplitude of the EAG response increased with dose for all compounds until the concentration of the compound was increased from 10 to 50 $\mu\text{g}/\mu\text{L}$. Between 10 to 50 $\mu\text{g}/\mu\text{L}$, the amplitude of the EAG response

decreased, indicating saturation of receptors at 10 $\mu\text{g}/\mu\text{L}$ (Figures 2–7). These compounds were methyl salicylate, linalool, and cyclohexanol for one-day-old males; *N*-nonadecane for one-day-old females; methyl salicylate for three-day-old males; linalool for three-day-old females; linalool and methyl salicylate for five-day-old virgin females; and linalool and methyl benzoate for five-day-old mated females.

In most cases, no difference was observed in the six physiological groups' EAG responses to a given compound. However, at a dose of 50 $\mu\text{g}/\mu\text{L}$, three-day-old males were more sensitive to heptanol and (E)-2-hexen-1-ol than three-day-old females.

Discussion

Plant volatiles (green leaf volatiles, monoterpenes, and other groups) were chosen based on previous studies demonstrating that they are emitted from undamaged and insect-infested plants (Takabayashi et al. 1994; Loughrin et al. 1995; Röse et al. 1996; Paré and Tumlinson 1998; Rodriguez-Saona et al. 2002; Blackmer et al. 2004; Williams et al. 2005). *C. medinalis* antennae of both sexes and various physiological conditions respond to a wide range of volatiles belonging to different chemical classes: green leaf volatiles, alkanes, terpenoids, aromatic compounds, hydroxyl, and carbonyl.

Compounds that elicited strong EAG responses included (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, (E)-2-hexena, (Z)-2-hexen-1-ol, heptanol, hexanal, cyclohexanol, linalool, cyclohexanol, nonadecane, and methyl salicylate. These compounds could play an important role in host-plant location. Additionally, these compounds have previously been reported to elicit high EAG responses (Ramachandran et al.

1990), except for (Z)-3-hexen-1-ol, hexanal, (Z)-2-hexen-1-ol and (E)-2-hexena. Cyclohexanol and nonadecane were not previously tested. Some compounds that have been reported to elicit high EAG responses, such as terpinene-4-ol and α -terpineol (Ramachandran et al. 1990), did not elicit high EAG response in *C. medinalis* in our experiment.

Female and male *C. medinalis* exhibited broad overlap in their EAG responses to individual plant odors, and there was no clear pattern of difference between responses of female and male antennae to different compounds. For example, strong EAG responses were elicited by heptanol in *C. medinalis* moths of all six physiological conditions. Likewise, (Z)-2-hexen-1-ol elicited strong EAG responses in all of the females, while all of the tested males exhibited sensitivity to methyl salicylate. Females showed significantly greater response than males to 2-heptanone and nonadecane, while males showed significantly greater response than females to linalool and methyl salicylate. In most cases, there were also differences between the male and female EAG responses to some plant odors at higher doses. Different EAG responses between females and males might be indicative of sex-specific differences in the importance of certain volatile cues in host-finding (Light et al. 1988). It has been reported that for many phytophagous insects, even though females and males can detect the same range of volatiles, there are differences between the EAG responses of the two sexes (Hansson et al. 1989; Ramachandran et al. 1990; Chinta et al. 1994; Raguso et al. 1996; Groot et al. 1999; Zhang et al. 2000; Fraser et al. 2003). Indeed, while both (Z)-3-hexenol (Z3-6:OH) and manuka oil emitted by ash foliage attracted *Agrilus planipennis*, the former was more attractive to males and the latter had a greater effect on females (Grant et al. 2010). For *C. medinalis*, different EAG

responses between sexes may be related to possible differences in the antennal morphology of the sexes. There are morphological differences between the type, size, and number of antennal sensilla in *C. medinalis* females and males. The antennae of males are longer and larger than those of females, there are more sensilla basiconic and sensilla coeloconica in males than females, and sensilla styloconica II are longer and thinner in males and exhibit surface longitudinal grooves, in contrast to their smooth surface in females (Sun et al. 2011). In contrast, Mercader et al. (2008) reported no significant sexual differences in the EAG responses of *Papilio glaucus* and *P. canadensis* to host-plant odor.

Differences in EAG responses were noted with respect to the age of the moths. One-day-old males showed significantly greater EAG responses than three-day-old males to dodecanal and cyclohexanol. One-day-old females showed significantly greater EAG responses to nonadecane than five-day-old virgin females. There are very few reports about age-dependent changes in olfactory-guided behavior to host plants. However, some male moths have been shown to change their responsiveness to sex pheromones with age. When testing the behavioral responsiveness of *Agrotis ipsilon* males to sex pheromone, clear changes were found with age; freshly hatched males did not react to the conspecific female's pheromone, but after a few days some males did respond and the best responses were obtained within about five days (Gadenne et al. 1993). The response to the male-produced aggregation pheromones changed with adult age in *Schistocerca gregaria*, and adults were indifferent to aggregation pheromones from three to four weeks of age (Ignell et al. 2001). Newly emerged *C. medinalis* were likely more sensitive to plant volatiles due to higher nutritive needs. This could also make it easier for

one-day-old males to find mates. Some plant volatiles are perceived in a synergistic manner and employed by insects to locate mates and increase reproduction (Reddy and Guerrero 2004). Interactions between pheromones and plant volatiles have also been observed in the laboratory and field (Dickens et al. 1993; Light et al. 1993; Reddy and Guerrero 2000).

Mating condition also affected female EAG response. The data show that five-day-old mated females exhibited significantly greater EAG responses than five-day-old virgin females to hexadecane, nonadecane, octadecane, and heptadecane, which all belonged to the alkane group. The greater response of mated females may be adaptive, given that these compounds are associated with the presence of host eggs. Many insects undergo significant changes in their general physiology during mating; this plasticity in host recognition and acceptance is suggested to be very important in insect speciation (Berlocher and Feder 2002; Linn et al. 2003; Tasin et al. 2007). Mated herbivorous females were thought to be more likely to search for their host plant in order to lay their eggs (Anton et al. 2007). It has been reported that only mated *Lobesia botrana* females were attracted by grapevine plant volatiles (Masante-Roca et al. 2007).

The amplitude of some volatiles decreased when the concentration of the compound was increased from 10 to 50 $\mu\text{g}/\mu\text{L}$, indicating saturation. The EAG responses to lower doses of the extract may well correspond to attraction, whereas the EAG responses to higher doses may correspond to repellency or behavioral inhibition. This proposal is consistent with the view that the mechanisms of host-plant selection in insects are largely a matter of gradation and balance between chemicals rather than clearly definable and different cues (Schoonhoven et al. 2005). This idea suggests that *C.*

medinalis may rely on various EAG-active compounds in particular ratios to find the plant for feeding or for oviposition. Bruce et al. (2005) argued that the ratio between ubiquitous plant volatiles should be seen as the most prevalent mechanism mediating host-plant recognition. Many studies demonstrate that host location can be identified by the particular blend of electrophysiologically active volatiles identified from the headspace of an insect's host plant (Guerin et al. 1983; Nojima et al. 2003; Hern and Dorn 2004; Tasin et al. 2007; Tasin et al. 2010). Also, an EAG response does not necessarily mean there is a behavioral response. Behavioral bioassays showed that not all higher EAG-active compounds, when tested individually, affected behavioral activity of *Batocera horsfieldi* (M-Q Wang, personal observation). EAG-active compounds could be attractive or repellent to an insect. It has been demonstrated that the green leaf volatile Z-3-hexen-1-ol, which elicited strong EAG responses in *Arhopalus tristis*, was a repellent that reduced attraction and oviposition (Suckling et al. 2001). Based on this electrophysiological data, *C. medinalis* EAG and behavioral responses to the tested mixtures of compounds should be studied further.

Acknowledgements

We acknowledge technical assistance from Mr. J-M Zhang of Zhejiang Academy of Agricultural Sciences with EAG experiments. This study was supported and funded by the Special Fund for Agro-scientific Research in the Public Interest of China (201403030), Huazhong Agricultural University Scientific & Technological Self-innovation Foundation, and The Industry Project of Ministry of Agriculture of China (200903051).

References

- Anton S, Dufour MC, Gadenne C. 2007. Plasticity of olfactory-guided behaviour and its neurobiological basis: lessons from moths and locusts. *Entomol. Exp. Appl.* 123: 1–11.
- Berlocher SH, Feder JL. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? *Ann. Rev. Entomol.* 47: 773–815.
- Blackmer JL, Rodriguez-saona C, Byers JA, Shope KL, Smith JP. 2004. Behavioral response of *Lygus hesperus* to conspecifics and headspace volatiles of alfalfa in a Y-tube olfactometer. *J. Chem. Ecol.* 30: 1547–1564.
- Bruce TJA, Wadhams LJ, Woodcock CM. 2005. Insect host location: a volatile situation. *Trends Plant Sci.* 10: 269–274.
- Chinta S, Dickens JC, Aldrich JR. 1994. Olfactory reception of potential pheromones and plant odors by tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae). *J. Chem. Ecol.* 20: 3251–3267.
- Dickens JC, Smith JW, Light DM. 1993. Green leaf volatiles enhance sex attractant pheromone of the tobacco budworm, *Heliothis virescens* (Lep.: Noctuidae). *Chemoecology* 4: 175–177.
- Fettig CJ, McKelvey SR, Dabney CP, Borys RR, Huber DPW. 2009. Response of *Dendroctonus brevicornis* to different release rates of nonhost angiosperm volatiles and verbenone in trapping and tree protection studies. *J. Appl. Entomol.* 133: 143–154.
- Fraser AM, Mechaber WL, Hildebrand JG. 2003. Electroantennographic and behavioral responses of the sphinx moth *Manduca sexta*

to host plant headspace volatiles. *J. Chem. Ecol.* 29: 1813–1833.

Gadenne C, Renou M, Sreng L. 1993. Hormonal control of sex pheromone responsiveness in the male black cutworm, *Agrotis ipsilon*. *Experientia* 49: 721–724.

Gallego D, Galián J, Diez JJ, Pajares JA. 2008. Kairomonal responses of *Tomicus destruens* (Col., Scolytidae) to host volatiles α -pinene and ethanol. *J. Appl. Entomol.* 132: 654–662.

Ganeswara Rao A, Krishnaiah K, Hall DR, Cork A, Reddy DDR. 1993. Evaluation of sex pheromone components of rice leaf folder. *Cnaphalocrocis medinalis* Guenee. *Curr. Sci. India* 65: 355–356.

Ganeswara Rao A, Reddy DDR, Krishnaiah K, Beevor PS, Cork A, Hall DR. 1995. Identification and field optimisation of the female sex pheromone of the rice leaf folder, *Cnaphalocrocis medinalis* in India. *Entomol. Exp. Appl.* 74: 195–200.

Gouinguene S, Pickett JA, Wadham LJ, Birkett MA. 2005. Antennal electrophysiological responses of three parasitic wasps to caterpillar-induced volatiles from maize (*Zea mays mays*), cotton (*Gossypium herbaceum*), and cowpea (*Vigna unguiculata*). *J. Chem. Ecol.* 31: 1023–1038.

Grant GG, Ryall KL, Lyons DB, Abou-Zaid MM. 2010. Differential response of male and female emerald ash borers (Col., Buprestidae) to (Z)-3-hexenol and manuka oil. *J. Appl. Entomol.* 134: 26–33.

Groot AT, Timmer R, Gort G, Lelyveld GP, Driifhout FP, van Beek TA, Visser J. 1999. Sex-related perception of insect and

plant volatiles in *Lygocoris pabulinus*. *J. Chem. Ecol.* 25: 2357–2371.

Guerin PM, Stadler E, Buser HR. 1983. Identification of host plant attractants for the carrot fly, *Psila rosae*. *J. Chem. Ecol.* 9: 843–861.

Hansson BS, van der Pers JNC, Löfqvist J. 1989. Comparison of male and female olfactory cell response to pheromone compounds and plant volatiles in the turnip moth, *Agrotis segetum*. *Physiol. Entomol.* 14: 147–155.

Hern A, Dorn S. 2004. A female-specific attractant for the codling moth, *Cydia pomonella*, from apple fruit volatiles. *Naturwissenschaften* 91: 77–80.

Hu J, Angeli S, Schuetz S, Luo Y, Hajek AE. 2009. Ecology and management of exotic and endemic Asian longhorned beetle *Anoplophora glabripennis*. *Agr. Forest Entomol.* 11: 359–375.

Ignell R, Couillaud F, Anton S. 2001. Juvenile-hormone-mediated plasticity of aggregation behaviour and olfactory processing in adult desert locusts. *J. Exp. Biol.* 204: 249–259.

Inoue H, Kamiwada H, Fukamachi S. 2004. Seasonal changes in adult density and female mating status of the rice leaf roller *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae) in paddy fields of Southern Kyushu, Japan. *Japanese J. Appl. Entomol. Zool.* 48: 177–183.

Jyothi KN, Prasuna AL, Sighamony S, Krishna Kumari B, Prasad AR, Yadav JS. 2002. Electroantennogram responses of

- Apanteles obliquae* (Hym., Braconidae) to various infochemicals. *J. Appl. Entomol.* 126: 175–181.
- Kawazu K, Adati T, Yosiyasu Y, Sumiartha K, Susila W, Sudiarta P, Purwanto H, Tatsuki S. 2009. Sex pheromone components of the rice leaffolder, *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae), in Indonesia. *J. Asia-Pac. Entomol.* 12: 97–99.
- Kawazu K, Nagata K, Zhang Z, Sugie H, Tatsuki S. 2002. Comparison of attractiveness in Japan and China of three synthetic pheromone blends based on geographic variations in the rice leaffolder. *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 92: 295–299.
- Kawazu K, Setokuchi O, Kohno K, Takahashi K, Yoshiyasu Y, Tatsuki S. 2001. Sex pheromone of the rice leaffolder moth. *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae): Synthetic Indian and Philippine blends are not attractive to male *C. medinalis*, but are attractive to *C. pilosa* in the South-Western islands in Japan. *Appl. Entomol. Zool.* 36: 471–474.
- Kawazu K, Suzuki Y, Yoshiyasu Y, Castillon EB, Ono H, Vuong T, Huang FK, Adati T, Fukumoto T, Satsuki T. 2005. Attraction of *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae) males in Southeast Asia to female sex pheromone traps: Field tests in southernmost China, northern Vietnam and southern Philippines with three synthetic pheromone blends regarding geographic variations. *Appl. Entomol. Zool.* 40: 483–488.
- Kawazu K, Tatsuki S. 2002. Diel rhythms of calling behavior and temporal change in pheromone production of the rice leaffolder moth, *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae). *Appl. Entomol. Zool.* 37: 219–224.
- Khan ZR, Barrion AT, Litsinger JA, Castilla NP, Joshi RC. 1988. A bibliography of rice leaffolders (Lepidoptera: Pyralidae). *Insect Sci. Appl.* 9: 129–174.
- Light DM, Flath RA, Buttery RG, Zalom FG, Rice RE, Dickens JC, Jang EB. 1993. Host-plant green volatiles synergize the synthetic sex pheromones of the corn earworm and codling moth (Lepidoptera). *Chemoecol.* 4: 145–152.
- Light DM, Jang EB, Dickens JC. 1988. Electroantennogram response of Mediterranean fruit fly, *Ceratitidis capitata*, to a spectrum of plant volatiles. *J. Chem. Ecol.* 14: 159–180.
- Linn CJ, Feder JL, Nojima S, Dambroski HR, Berlocher SH, Roelofs WL. 2003. Fruit odor discrimination and sympatric host race formation in *Rhagoletis*. *Proc. Natl. Acad. Sci. USA* 100: 11490–11493.
- Lou Y-G, Ma B, Cheng J-A. 2005. Attraction of the parasitoid *Anagrus nilaparvatae* to rice volatiles induced by the rice brown planthopper *Nilaparvata lugens*. *J. Chem. Ecol.* 31: 2357–2372.
- Loughrin JH, Manukian A, Heath RR, Tumlinson JH. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.* 21: 1217–1227.
- Lu K, Li X, Zhou J L Jie X J, Qi S, Zhou Q. 2010. Influence of the herbivore-induced rice volatiles on fungal disease (in Chinese). *Chinese Sci. Bull.* 55: 2925–2930.

- Masante-Roca I, Anton S, Delbac L, Dufour M-C, Gadenne C. 2007. Attraction of the grapevine moth to host and non-host plant parts in the wind tunnel: effects of plant phenology, sex and mating status. *Entomol. Exp. Appl.* 122: 239–245.
- Mercader RJ, Stelinski LL, Scriber JM. 2008. Differential antennal sensitivities of the generalist butterflies *Papilio glaucus* and *P. canadensis* to host plant and non-host plant extracts. *J. Lepid. Soc.* 62: 84–88.
- Nathan SS, Chung PG, Murugan K. 2004. Effect of botanical insecticides and bacterial toxins on the gut enzyme of the rice leaf folder *Cnaphalocrocis medinalis*. *Phytoparasitica* 32: 433–443.
- Nojima A, Linn C, Morris B, Zhang AJ, Roelofs W. 2003. Identification of host fruit volatiles from hawthorn (*Crataegus* spp.) attractive to hawthorn-origin *Rhagoletis pomonella* flies. *J. Chem. Ecol.* 29: 321–336.
- Paré PW, Tumlinson J H. 1998. Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochemistry* 47: 521–526.
- Raguso RA, Light DM, Pichersky E. 1996. Electroantennogram responses of *Hyles lineata* (Sphingidae: Lepidoptera) to floral volatile compounds from *Clarkia breweri* (Onagraceae) and other moth-pollinated flowers. *J. Chem. Ecol.* 22: 1735–1766.
- Ramachandran R, Khan ZR, Caballero P, Juliano BO. 1990. Olfactory sensitivity of two sympatric species of rice leaf folders (Lepidoptera: Pyralidae) to plant volatiles. *J. Chem. Ecol.* 16: 2647–2666.
- Reddy GVP, Guerrero A. 2000. Behavioral responses of the diamondback moth, *Plutella xylostella*, to green leaf volatiles of *Brassica oleracea* subsp. *capitata*. *J. Agric. Food. Chem.* 20: 6025–6029.
- Reddy GVP, Guerrero A. 2004. Interactions of insect pheromones and plant semiochemicals. *Trends Plant Sci.* 9: 253–261.
- Rodriguez-Saona C, Crafts-brandner, SJ, Williams L, Paré PW. 2002. *Lygus hesperus* feeding and salivary gland extracts induce volatile emissions in plants. *J. Chem. Ecol.* 28: 1733–1747.
- Röse USR, Manukian A, Heath RR, Tumlinson JH. 1996. Volatile semiochemicals released from undamaged cotton leaves. *Plant Physiol.* 111: 487–495.
- Schoonhoven LM, Van Loon JJA, Dicke M. 2005. *Insect-plant biology*. Oxford University Press.
- Sogawa K, Kiyota H. 1995. Quantification and forecasting of the damage caused by the rice leaf folder. *Proc. Assoc. Pl. Prot. Kyushu* 41: 58–62. (in Japanese)
- Suckling DM, Gibb AR, Daly JM, Chen X, Brockerhoff EG. 2001. Behavioral and electrophysiological responses of *Arhopalus tristis* to burnt pine and other stimuli. *J. Chem. Ecol.* 27: 1091–1104.
- Sun X, Wang M-Q, Zhang G. 2011. Ultrastructural observations on antennal sensilla of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). *Microsc. Res. Tech.* 74: 113–121.
- Takabayashi J, Dicke M, Posthumus MA. 1994. Volatile herbivore-induced terpenoids

in plant-mite interactions: variation caused by biotic and abiotic factors. *J. Chem. Ecol.* 20: 1329–1354.

Tasin M, Bäckman AC, Coracini M, Casado D, Ioriatti C, Witzgall P. 2007. Synergism and redundancy in a plant volatile blend attracting grapevine moth females. *Phytochemistry* 68: 203–209.

Tasin M, Backman A-C, Anfora G, Carlin S, Ioriatti C, Witzgall P. 2010. Attraction of female grapevine moth to common and specific olfactory cues from 2 host plants. *Chem. Senses* 35: 57–64.

van Der Goes, van Naters W, Carlson JR. 2006. Insects as chemosensors of human sand crops. *Nature* 444: 302–307.

Wechgama K, Laopaiboom L, Laopaiboon P. 2008. Quantitative analysis of main volatile and other compounds in traditional distilled spirits from Thai rice. *Biotechnol.* 7: 718–724.

Williams L, Rodriguez-saona C, Pare PW, Crafts-brandner SJ. 2005. The piercing-sucking herbivores *Lygus hesperus* and *Nezara viridula* induce volatile emissions in plants. *Arch. Insect Biochem. Physiol.* 58: 84–96.

Yan F, Wang X, Lv J, Pang B-P, Lou Y-G. 2010. Comparison of the volatiles from rice plants infested by rice striped stem borer, *Chilo suppressalis* and rice leaf folder, *Cnaphalocrocis medinali*. *Entomological Knowledge* 47: 96–101. (In Chinese with English Abstract)

Zhang QH, Achlyter F, Birgersson G. 2000. Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): chemical and

electrophysiological analysis. *Chemoecol.* 10: 69–80.

Zhang ZS, Wang GM. 1987. *Control of rice leaffolders (Lepidoptera: Pyralidae)*. Shanghai Scientific & Technical Publishers. (In Chinese)

Zhuge P-P, Luo S-L, Wang M-Q, Zhang G. 2010. Electrophysiological responses of *Batocera horsfieldi* (Hope) adults to plant volatiles. *J. Appl. Entomol.* 134: 600–607.

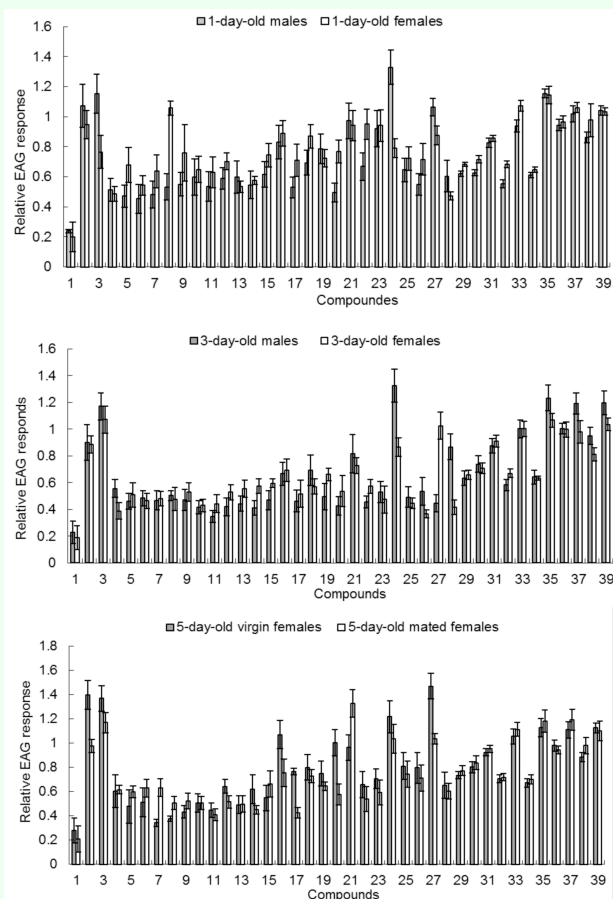


Figure 1. Relative EAG responses (mean \pm SE, $n = 15$) of *Cnaphalocrocis medinalis* to different compounds. Compounds numbers are: 1. CK; 2. cyclohexanol; 3. linalool; 4. tetradecane; 5. hexadecane; 6. heptadecane; 7. octadecane; 8. nonadecane; 9. eicosane; 10. heneicosane; 11. (-)- α -cedrene; 12. nerolidol; 13. cedrol; 14. (Z)-farnesene; 15. terpinene-4-ol; 16. α -terpineol; 17. α -terpinene; 18. β -myrcene; 19. sabinene; 20. p-cymene; 21. methyl benzoate; 22. (-)-trans-caryophyllene; 23. dodecanal; 24. methyl salicylate; 25. γ -terpinene; 26. octane; 27. 2-heptanone; 28. N-phenyl-1-naphthylamine; 29. R-(+)-limonene; 30. benzaldehyde; 31. cis-2-penten-1-ol; 32. (1R)-(+)-pinene; 33. hexanal; 34. Ionone; 35. heptanol; 36. (Z)-3-hexen-1-ol; 37. (E)-2-hexen-1-ol; 38. (E)-2-hexenal; 39. (Z)-2-hexen-1-ol. High quality figures are available online.

