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Repellency of lavender oil and linalool against spot clothing wax cicada, Lycorma delicatula (Hemiptera: Fulgoridae) and their electrophysiological responses

Changmann Yoon ^a, Sang-Rae Moon ^a, Jin-Won Jeong ^a, Youn-Ho Shin ^a, Sun-Ran Cho ^a, Ki-Su Ahn ^b, Jeong-Oh Yang^a, Gil-Hah Kim^{a,*}

^a Department of Plant Medicine, College of Agriculture, Life and Environment Sciences, Chungbuk National University, Republic of Korea ^b Chungbuk Provincial Agricultural Research & Extension Services, Cheongwon, Chungbuk 363–880, Republic of Korea

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ABSTRACT

This study was performed to investigate the repellent effect of 5 µl doses of ten essential oils (bergamot, chamomile, clary sage, fennel, lavender, lemongrass, majoram, peanut, pennyroyal, and peppermint) against Lycorma delicatula 4th nymphs using an olfactometer. Only lavender oil exhibited significant repellency. We then tested 10, 5, 2.5, and 1 µl doses of lavender oil against the nymphs and females of L. delicatula. The oil showed significant repellency at 10 and 5 µl, although the latter is less potent to 1st instar nymphs. At the lavender oil dose of 2.5 µl, only 3rd and 4th instar nymphs and females were significantly affected. None of the stages tested were affected by 1 µl. Chromatographic and mass spectrometric analyses of lavender oil detected linalool (42.2%), linalyl acetate (49.4%), terpinen-4-ol (5.0%), and caryophyllene oxide (3.4%). Among the four main components, only linalool showed repellency to all instar nymphs and females. No synergism was detected. Antennae of all instar nymphs and females showed electrophysiological responses only to linalool. In field studies using linalool, 4th nymphs and adults were highly repelled at a dose of 30 µl of lavender oil. The effect differed according to test plot and treatment dose.

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Introduction

The spot clothing wax cicada, Lycorma delicatula (White) (Hemiptera: Fulgoridae) originally inhabited subtropical and tropical regions, including mainland China and Southeast Asia. It has since spread to the mid-west areas of the Republic of Korea (Han et al., 2008). This pest was first reported in Korea in 1932 (Doi, 1932). but its occurrence was confused for much of the time due to lack of collection (Choi et al., 2011). It was concluded that L. delicatula did not occur in Korea (Kwon and Hur, 2001). However, since its reoccurrence throughout Seoul and Gyeonggi provinces after 2006, the pest has spread rapidly throughout Korea, and its increase in population density is especially damaging to vineyards (KFRI, 2007; Han et al., 2008; Park et al., 2009). The spread to the Republic of Korea may have been prompted by the warmer winter temperatures that are an ongoing consequence of global warming, which have allowed L. delicatula to overwinter (Han et al., 2008; Park et al., 2009; Lee et al., 2011).

Until now, research on the spot clothing wax cicada has focused mainly on ecology, behavior, and chemical control. The spot clothing wax cicada feeds on plants and excretes honeydew causing grey mold. When infestation is severe, the parasitized host plant dies. The mass-feeding and infestation of single host plants by thousands of pests can be abhorrent (Lee et al., 2009). A large number of easily observable annual and perennial plant species have been recognized as acceptable feeding and/or reproductive hosts. These include 38 woody plant species, including tree of heaven. Chinese cedrela, Indian guassiawood, American ivv. grapevine, and three herbaceous plant species (Park et al., 2009). Lee et al. (2009) reported that feeding behavior of L. delicatula was influenced by feeding stimulants in grapevines. L. delicatula nymphs are susceptible to commercially registered insecticides (Park et al., 2009). The organophosphate insecticide chlorpyrifos most effectively kills eggs of L. delicatula, but 1st and 2nd instar nymphs are highly susceptible to most insecticides (Shin et al., 2010). Once L. delicatula eggs have hatched from their egg masses containing 30–50 eggs, the pests are difficult to control using insecticides because of their high motility. Moreover, use of insecticides carries concerns about overuse and/or redundancy (Shin et al., 2010).

Eco-friendly control using essential oils is an attractive alternative control of L. delicatula. Essential oils are secondary metabolites produced in plant metabolism. They are composed of volatile compounds which have a peculiar aromatic fragrance (Mohamed and Abdelgaleil, 2008). The compounds have low toxicity to humans and are environmentally safe (Katz et al., 2008). Moreover, many kinds of plant essential oils are

^{*} Corresponding author at: Department of Plant Medicine, Chungbuk National University, Cheongju 361–763, Republic of Korea. Tel.: +82 43 261 2555; fax: +82 43 271 4414.

E-mail address: khkim@chungbuk.ac.kr (G.-H. Kim).

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highly repellent to arthropods (Nerio et al., 2010). The repellent effects have been investigated for their efficacy in the control of medical, stored-product and agricultural insect pests (Odalo et al., 2005; George et al., 2009; Yang et al., 2009; Zapata and Smagghe, 2010). Methods such as fumigation, feeding deterrent, growth regulation, attraction and repellent behavior have been studied (Gonzalez-Coloma et al., 2006; Negahban et al., 2007; Tandon et al., 2008; Nerio et al., 2009;). But, use of essential oils in the field is still hindered by a lack of constant efficacy and maintenance in nature.

This study was performed to survey plant essential oils having repellency to *L. delicatula* nymphs and females. Ten essential oils were bioassayed for repellency using an olfactometer. Their constituents were analyzed using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). In addition, the electrophysiological responses of the antennae of *L. delicatula* nymphs and females were investigated using GC with an electroantennographic detector (GC–EAD). Finally, we applied essential oils in the field to determine the possibility of using it to control *L. delicatula* nymphs and adults.

Materials and methods

Test insects

Third and fourth instar nymphs of *L. delicatula* were collected near Chungbuk National University, Cheongju in July, 2009. *L. delicatula* eggs were collected from near Chungbuk National University and from a vineyard in Yongam-dong, Cheongju from December 2009 to May 2010. The insects were reared at 25 ± 2 °C, relative humidity of 50–60%, and a photoperiod of 16:8 h (light:dark). Experiments were done after hatching of the eggs in a rearing cage ($27 \times 30 \times 46$ cm) provided with the cut branch of 'tree of heaven' (*Ailanthus altissima*) as food. The cut branch was sealed with a piece of cotton and fixes tightly with vial glass neck each other. A vial was filled with distilled water to provide moisture to the branch.

Essential oils and terpene compounds

Ten plant essential oils were used to test repellent efficacy against *L. delicatula*: lemongrass, majoram (Charabot, Grasse, France), pennyroyal (Hasegawa, Chiba-yachiyo, Japan), bergamot, chamomile, clary sage, fennel, lavender, peanut, and peppermint (JinAromatics, Anyang, Korea). Major compounds of some essential oils were also tested, including caryophyllene oxide (99% purity), linalool (97%), and linalyl acetate (97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and terpinen-4-ol (93%) was obtained from Wako (Osaka, Japan).

Olfactory response

An olfactometer (internal diameter: 10 cm; length: 40 cm; angle between arms: 180°) was designed for the response experiments. The apparatus has a pair of arms, each of which bears a sample container at the end. The olfactometer design allows insects to choose one of the two arms in response to different odor stimuli after crawling up the interior wall. Pressurized air was purified by filtration through a charcoal and silica gel before entering the sample containers. This air was then pushed through the two arms (treated side and blank side) into the chamber at a speed of 100 mL/min using a Medipump® vacuum pump (Thomas, Sheboygan, WI, USA) and pulled out through the above hole in the center of both arms. The air flow through the two arms did not influence the bottom area of the olfactometer, the initial inoculation site of a test insect. Each oil or compound was dispensed at an appropriate dose on Whatman No. 2 filter paper (9 cm-diameter, ¼) using a PAX 100-3 micro-processor controller (Burkard, Uxbridge, UK). One filter paper was placed in the container of one arm, and the other container remained empty. Repellency to L. delicatula was evaluated by recording the chosen arm. L. delicatula that did not enter either arm (within 10 cm from one end of arm) of the olfactometer within 5 min was recorded as "no choice." To avoid carry-over contamination by previously tested volatiles, the olfactometer was replaced after testing five insects. Also, after each test, the olfactometer was rinsed with ethanol and distilled water, and allowed to dry for 2 h in a 100 °C dry oven before re-use. All tests were done using the previously described conditions of temperature, relative humidity, and photoperiod. All experiments were performed using 40 replicates, except for 20 replicates in a screening stage. Experiments used 1st to 4th nymph instars and female adults of *L. delicatula*. Bioassays were compared using the binomial sign test (Zar, 1996). Olfactory response (%) was calculated as using following formula: [Blank \div (Treatment + Blank)] \times 100.

GC–MS analysis

The constituents of essential oils having a repellent effect were analyzed using a model 6890 N gas chromatograph (Agilent Technology, Santa Clara, CA) and a model 7890A/5975C GC/MS apparatus (Agilent Technology) equipped with a splitless injector. Column chromatography was performed using a DB-WAX GC column (0.25 mm \times 30 m, thickness 0.25 µm; J&W Scientific, Folsom, CA) fused with a silica capillary and nitrogen (N₂) as the carrier gas at a flow rate of 1.0 mL/min. A flame ionization detector was used. The initial temperature was 35 °C, followed by a ramp-up of 5 °C/min to 200 °C for 60 min; the injector and detector were held at 180 °C and 200 °C, respectively. Spectra were obtained at 70 eV ionization voltage and the essential oil components were identified by comparing the mass spectra of each peak with those of authentic samples in a mass spectra library (The Wiley Registry of Mass Spectral Data, 7th eds.).

Electrophysiological response

Electrophysiological analyses of the essential oils were performed using a GC–EAD system consisting of a model 6890N gas chromatograph (Agilent Technologies) and an EAD setup (electroantennograph; model MP-15 probe/manipulator; model CS-55 stimulus controller; data acquisition interface box, serial IDAC-232; Syntech, Hilversum, The Netherlands). The end of the column was split into two paths at a 1:1 ratio using a Y splitter (Agilent Technology). One of the paths was connected to a flame ionization detector (FID), while the other path was connected to an EAD via an interface device. Odor samples were prepared at a 1:9 ratio of odor sample:ethanol. One microliter of 10% doses of odor sample was injected splitless at 100 °C, followed by opening the split vent after 1 min and heating the oven at a rate of 20 °C/min to 150 °C or at a rate of 25 °C/min to 200 °C. The end temperature was held for 5 min. A DB-WAX bonded-phase fused-silica

Table 1

Olfactory responses of *L. delicatula* 4th instar nymphs to 10 essential oils at a dose of $5 \,\mu$ l.

| Essential oil | Number | of nymphs | % ^a | P-value ^b | |
|---------------|--------|-----------|----------------|----------------------|--------|
| | Blank | Treatment | No choice | | |
| Bergamot | 7 | 7 | 6 | 50.0 | n.s. |
| Chamomile | 8 | 7 | 5 | 53.3 | n.s. |
| Clary sage | 7 | 7 | 6 | 50.0 | n.s. |
| Fennel | 6 | 9 | 5 | 40.0 | n.s. |
| Lavender | 13 | 2 | 5 | 86.7 | 0.0037 |
| Lemongrass | 6 | 11 | 3 | 35.3 | n.s. |
| Majoram | 5 | 9 | 6 | 35.7 | n.s. |
| Peanut | 7 | 9 | 4 | 43.7 | n.s. |
| Penny royal | 7 | 9 | 4 | 43.7 | n.s. |
| Peppermint | 6 | 10 | 4 | 37.5 | n.s. |

^a Olfactory response (%) = Blank \div (Blank + Treatment) \times 100.

^b The data was analyzed using binomial sign tests to evaluate the differences from 50:50 responses. n = 20, P < 0.05, n.s. (not significant) P > 0.05.

Table 2

Repellency of lavender oil against *L. delicatula* nymphs and females using a T-tube olfactometer.

| Dose ($\mu l/cm^2$) | Instar | Number of L. delicatula | | | % ^a | P-value ^b |
|-----------------------|--------|-------------------------|-----------|-----------|----------------|----------------------|
| | | Blank | Treatment | No choice | | |
| 10 | 1st | 20 | 14 | 6 | 58.8 | n.s. |
| | 2nd | 22 | 11 | 7 | 66.7 | 0.0401 |
| | 3rd | 23 | 9 | 8 | 71.9 | 0.0100 |
| | 4th | 25 | 7 | 8 | 78.1 | 0.0011 |
| | Adult | 24 | 8 | 8 | 75.0 | 0.0035 |
| 5 | 1st | 26 | 8 | 6 | 76.5 | 0.0015 |
| | 2nd | 24 | 9 | 7 | 72.7 | 0.0068 |
| | 3rd | 25 | 7 | 8 | 78.1 | 0.0011 |
| | 4th | 24 | 6 | 10 | 80.0 | 0.0007 |
| | Adult | 24 | 7 | 9 | 77.4 | 0.0017 |
| 2.5 | 1st | 17 | 15 | 8 | 53.1 | n.s. |
| | 2nd | 18 | 12 | 10 | 60.0 | n.s. |
| | 3rd | 20 | 10 | 10 | 66.7 | 0.0494 |
| | 4th | 22 | 9 | 10 | 71.0 | 0.0147 |
| | Adult | 23 | 10 | 7 | 69.7 | 0.0175 |
| 1 | 1st | 15 | 16 | 9 | 48.4 | n.s. |
| | 2nd | 16 | 15 | 9 | 51.6 | n.s. |
| | 3rd | 19 | 12 | 9 | 61.3 | n.s. |
| | 4th | 23 | 13 | 4 | 63.9 | n.s. |
| | Adult | 17 | 13 | 10 | 56.7 | n.s. |

^a Olfactory response (%) = Blank \div (Blank + Treatment) \times 100.

capillary column (0.25 mm \times 30 m, J&W Scientific) with a film thickness of 0.25 μ m was used for analyses.

To identify the electrophysiological response of *L. delicatula* antennae, the excised heads of *L. delicatula* were placed on the antenna holder of an EAG probe (Syntech) and one electrode was connected to the cut area of each head. The other electrode was connected with the antenna to the outlet of a capillary filled with Ringer solution (154 mM NaCl, 5.5 mM KCl, 1.4 mM CaCl₂). The EAG signals were recorded using the GC/EAD32 2005, Ver 3.74.4 program (Syntech) on a personal computer that included a MP-15 probe/micromanipulator, data acquisition interface box (serial IDAC-232), and a CS-55 stimulus air controller. First to fourth instar nymphs and female adults were used.

Field experiment

A silicone sleeve septum TM (# 14/20, Korea Ace Scientific Co., Seoul, Korea) functioning as a rubber lure and sticky trap (Fly catcher TM; 4.5×60 cm, Daegil Chemicals Co., Gimhae, Korea) was used to evaluate



Fig. 1. GC profiles of lavender oil. DB-WAX capillary column (I.D. 0.25 mm, 30 m long, 0.25 mm film thickness) (Temp., 35 $^\circ$ C to 200 $^\circ$ C at 5 $^\circ$ C/min).

the repellency of lavender oil against *L. delicatula* nymphs and adults in the field. For release control, it requires the pretreatment of the septum to fully absorb the essential oils. The septum was treated with essential oils and put into an insect breeding dish (100×40 mm, SPL Lifesciences, Pocheon, Korea). The dish was sealed with parafilm and then maintained at 4 °C for 24 h.

Using two host plants (tree of heaven) located within 10 m of one another, the sticky trap with the silicone sleeve septa was positioned on one side and with a sticky trap only was positioned on the other side. The septum and sticky trap were placed in a tree 1.5 m above the ground. The repellency was determined. The experiment was done with five replicates in two different sites. The number of *L. delicatula* caught was recorded 5, 10 and 15 days after treatment. The investigation period

Table 3

Repellency of major components of lavender oil against *L. delicatula* using a T-tube olfactometer.

| Components | Dose (µl) | Instar | % ^a | P-value |
|----------------------|-----------|----------------|----------------|----------------|
| Linalyl acetate | 4.94 | 1st | 55.6 | n.s. |
| | | 2nd | 54.6 | n.s. |
| | | 3rd | 51.5 | n.s. |
| | | 4th | 61.8 | n.s. |
| | | Female | 59.4 | n.s. |
| | 2.47 | 1st | 52.8 | n.s. |
| | | 2nd | 48.4 | n.s. |
| | | 3rd | 54.6 | n.s. |
| | | 4th | 61.1 | n.s. |
| | | Female | 60.0 | n.s. |
| Linalool | 4.22 | 1st | 55.9 | n.s. |
| | | 2nd | 68.8 | 0.0251 |
| | | 3rd | 71.9 | 0.0100 |
| | | 4th | 73.7 | 0.0025 |
| | | Female | 74.2 | 0.0053 |
| | 2.11 | 1st | 66.7 | 0.0401 |
| | | 2nd | 71.9 | 0.0100 |
| | | 3rd | 75.0 | 0.0035 |
| | | 4th | 76.5 | 0.0015 |
| | | Female | 77.4 | 0.0017 |
| Terpinen-4-ol | 0.50 | 1st | 57.1 | n.s. |
| | | 2nd | 62.5 | n.s. |
| | | 3rd | 58.1 | n.s. |
| | | 4th | 60.0 | n.s. |
| | | Female | 62.1 | n.s. |
| | 0.25 | 1st | 58.8 | n.s. |
| | 0.23 | 2nd | 64.5 | n.s. |
| | | 3rd | 61.3 | n.s. |
| | | 4th | 59.4 | n.s. |
| | | Female | 60.0 | n.s. |
| Caryophyllene oxide | 0.34 | 1st | 48.3 | n.s. |
| caryophynche oxide | 0.54 | 2nd | 51.6 | n.s. |
| | | 3rd | 54.8 | n.s. |
| | | 4th | 60.0 | n.s. |
| | | Female | 61.3 | n.s. |
| | 0.17 | 1st | 51.4 | n.s. |
| | 0.17 | 2nd | 48.5 | n.s. |
| | | 3rd | 46.4 | |
| | | 4th | 58.8 | n.s. n.s. |
| | | Female | 58.1 | |
| Mixture ^c | 10.0 | 1st | 64.7 | n.s. |
| WIALUIC | 10.0 | 2nd | 70.0 | n.s. 0.0214 |
| | | 3rd | 70.0 | 0.0214 |
| | | 4th | 72.7 | 0.0068 |
| | | | | |
| | 5.0 | Female 1ct | 75.8 | 0.0023 |
| | 5.0 | 1st 2nd | 69.4 | 0.0144 |
| | | 2nd | 74.2 | 0.0053 |
| | | 3rd | 77.1 | 0.0017 |
| | | 4th Formale | 76.5 | 0.0015 |
| | | Female | 78.1 | 0.0011 |

^a Olfactory response (%) = Blank \div (Blank + Treatment) \times 100.

 $^{\rm b}$ The data was analyzed using binomial sign tests to evaluate the differences from 50:50 responses. n = 40, *P*<0.05, n.s. (not significant) *P*>0.05.

^c Mixture = linalyl acetate (49.4%) + linalool (42.2%) + terpinen-4-ol (5.0%) + caryophyllene oxide (3.4%).

^b The data was analyzed using binomial sign tests to evaluate the differences from 50:50 responses. n = 40, P < 0.05, n.s. (not significant) P > 0.05.

was from June 2010 to August 2010 for nymphs and from September 2010 to October 2010 for adults.

Results and discussion

Repellent effect of lavender oil on each developmental stage

Essential oils having repellent activity have been described mainly relating to medical and stored product insect pests (Hori, 2003). However, no information has been available concerning the repellent activity of essential oils against *L. delicatula*. This study offers the first evidence of the repellent effect of selected essential oils on *L. delicatula* nymphs and female adults in laboratory and field experiments. Among the ten tested oils, only lavender oil displayed significant repellent activity (86.7%) (Table 1). Each nymph stage and female adults of *L. delicatula* were repelled by different doses of lavender oil (Table 2). All nymphs (except 1st instar nymphs) and female adults were significantly repelled at a dose of 10 µl (range 66.7% to 78.1%) and at a dose of 5 µl (range 72.7% to 80.0%). At a dose of 2.5 µl, repellent activity was lower, and only 3rd and 4th instar nymphs, and female adults displayed a significant reaction (range 66.7% to 71.0%). There was no repellent activity at a dose of 1 µl.

Within the same dose, third and fourth instar nymphs of *L. delicatula* were more affected compared to first and second instar nymphs, and fourth instar nymphs were most affected among all instar nymphs. The compounds are sensed by olfactory receptors that exist on several body regions, mostly the antennae. Olfactory receptors seem to be more sensitive to volatile compounds because their sensilla organs (mainly in the antennae) are more sensitive. In adults, repellency decreased but no differences were evident at a same

dose. The reason repellency of female adults decreased may be because other sensory responses developed for survival, such as feeding or oviposition. In this experiment, 4th instar nymphs were more responsive to lavender oil than the adults, although the difference was not significant.

Lavender oil also repelled Lasioderma serricone females in an olfactometer study (Hori, 2003) and Meligethes aeneus females (Mauchline et al., 2005). However, the similar results between these prior studies and the present studies are not due to plant essential oils, which can produce variable results depending on the test method, insect, or treatment dose. Essential oils from the family Linaceae, including lavender oil, have bioactivities that include feeding inhibition, repellence, and insecticidal action for various insect pests (Papachristos and Stamopoulos, 2004; Gonzalez-Coloma et al., 2006). Essential oils are secondary metabolites related to plant defense mechanisms and are a factor for selection of the host and ovipositional site (Visser, 1986). The response of an insect may be affected by these factors. Koschier and Sedy (2003) examined the effect of 1% lavender on Thrips tabaci female adults and reported a repellent effect on oviposition. Although lavender oil in this study displayed a repellent effect on all nymphs and adults, further development is required before we can perform field bioassay studies.

GC analysis of lavender oil and repellent effect of their constituents

Lavender oil was analyzed using GC and GC–MS. The results are presented in Fig. 1. Monoterpenes that were detected included (in order of prevalence) linalyl acetate (49.4%), linalool (42.2%), terpinen-4-ol (5.0%), and caryophyllene oxide (3.4%). Of these components, only linalool displayed a repellent effect against *L. delicatula* (Table 3). When



Fig. 2. Electrophysiological responses of the antennae of each nymphal stage and adult females to different components of lavender oil; (1) linally acetate, (2) linalool, (3) terpinen-4-ol, and (4) caryophyllene oxide.

linalool was used at a dose of 2.11 µl (half-strength of its proportion in the original oil), all nymphs and female adults were significantly repelled (range 66.7 to 77.4%). When the linalool dose was 4.22 µl, all nymphs (except 1st instar nymphs) and female adults were significantly repelled, but the activity was lower than the 2.11 µl dose. Hori (2004) reported that L. serricone female adults were repelled when treated with 1 µl of linalool, but were attracted when treated with 0.1 µl of linalool. α -Terpineol and (–)-perillyl alcohol also produces different results when used at different doses. In this study, the efficacy of lavender oil as a repellent was dose-dependent, although in a pattern that was the opposite of the pattern seen with lavender oil from Table 3 and from the pattern reported in Hori (2004). Clearly, further studies are needed to more precisely determine the effective doses of particular essential oils and their constituents. In this study, adult insects treated with 2.11 µl and 4.22 µl linalool displayed a repellent effect of 77.4% and 74.2%, respectively. Adults appeared more repelled by linalool than the nymphs, although the difference was not significant.

Linalool is one of the major components of lavender. It has a peculiar aromatic fragrance that has been widely exploited aesthetically and as a foodstuff (Letizia et al., 2003). Linalool is also a repellent. Hori (2004) revealed that, as one of the principal components of shiso oil, linalool displays repellent activity against *L. serricone*. Mauchline et al. (2008) reported repellent effects of lavender oil against *Meligenthes aeneus* female adults and showed the active compounds were linalool and linalyl acetate.

In such previous studies, each component of lavender oil has been bioassayed separately. However, the knowledge that various compounds are present in various proportions in plant essential oils makes it conceivable that a repellent effect involves combinations of the compounds. This requires further study. In this study, each component in lavender oil was bioassayed for its repellent effect. Treatment with doses of 10 µl and 5 µl were repellent (69.4–78.1%), albeit insignificantly. Plant essential oils naturally exist in various combinations and proportions. Depending on the combination and relative proportion, various synergistic activities may occur.

Electrophysiological response to the constituents of lavender oil

Olfactory responses of insects depend on the interaction of chemicals with antennal sensillae. In this study, the electrophysiological responses of *L. delicatula* were tested. Lavender oil was identified as a responsive compound in all nymphs and female adults using GC–EAD. Antennae of 1st to 4th instar nymphs and female adults responded to 10% doses of lavender and linalool (a monoterpene and the major component of lavender oil) (Fig. 2). However, there were no responses to linalyl acetate, terpinen-4-ol, or caryophyllene oxide.

Electroantennographs of all instar nymphs and female adults showed responses to linalool simultaneously with the GC peak. The EAD peak of females did not respond simultaneously with the GC peak and were delayed approximately one second. The peak and the peak maxima are delayed in the EAD signal. With the knowledge of the delay between the GC and EAD signals, an interpretation of complex chromatogram is possible. Significant EAG responses are observed using doses as low as sample. The large olfactory stimulation elicited by essential oils can be explained by the high sensitivity (or high numbers of olfactory receptors) of *L. delicatula* to linalool, as demonstrated by coupling GC–EAD recordings. High doses of essential oil reproducibly provoked a positive response. This is probably due to chemoreception of linalool on the antenna of *L. delicatula* adult.



Fig. 3. Repellent effects of lavender oil against nymphs and adult females at 10, 20 and 30 µl per sticky trap at two field sites. Studies were carried out from May 2010 to August 2010 (A) and from August 2010 to September 2010 (B). ^aThe data was analyzed using binomial sign tests to evaluate the differences from 50:50 responses. * *P*<0.05, ** *P*<0.01, *** *P*<0.001, and n.s. no significance.

The antennal responses to volatile compounds have been extensively studied, and they have been closely linked with the olfactory response (Heinbockel and Kaissling, 1996; Dotterl et al., 2006; Cook et al., 2007; Mauchline et al., 2008). The detection of volatile compounds by insects provides cues in the search for hosts and for ovipositional site (Bruce et al., 2005). This is closely related to the attractive/repellent nature of certain chemicals. Olfactory receptors are located on several body regions, predominantly in the antennae (Bruce et al., 2005; Visser, 1986).

Repellent effect: field study

The repellent effect of lavender oil seen in laboratory studies was tested under field conditions with *L. delicatula* nymphs and adults (Fig. 3). In nymphal stages, treatment with 20 μ l and 30 μ l lavender oil produced similar effects in two test plots through day 15 after treatment. Use of 10 μ l lavender oil produced different results at the two field sites: one was insignificant but the other was not. Repellency was tested against male and female adults. In adult stage, treatment with 20 μ l and 30 μ l lavender oil produced significant repellent effects. The efficacy was stronger using 30 μ l lavender oil. The repellent effect was not evident using 10 μ l.

The repellent effects of essential oils have been studied mainly on sanitary and stored-product insect pests (Odalo et al., 2005; George et al., 2009; Zapata and Smagghe, 2010) and arthropods (Nerio et al., 2010). The repellent capability of lavender oil has also been described for *L. serricone, Meligethes aeneus*, and *Thrips tabaci* (Hori, 2003; Koschier and Sedy, 2003; Mauchline et al., 2005). We identified repellent effects of lavender oil in laboratory bioassays and confirmed their effects in the field. The present study was novel in extending lab findings to the field. The findings indicate the potential of lavender oil, alone or in combination with as-yet unidentified compounds, in the field control of *L. delicatula*.

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