

## Attraction Response of Spot Clothing Wax Cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) to Spearmint Oil

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Attraction responses of plant essential oils were investigated, and the electrophysiological response to nymphs and adults of spot clothing wax cicada (*Lycorma delicatula*) was confirmed. Of the ten tested oils, only spearmint oil was found attractive. In dose responses of spearmint oil, second to fourth instar nymphs, as well as adults, were significantly attracted to a dose of 5  $\mu$ L; for nymphs, fourth instar nymph showed greatest attraction response (90.9%), and second and third instar nymphs showed mild attraction. At a dose of 10  $\mu$ L, fourth instar nymphs and adults were significantly attracted to spearmint oil. Only fourth instar nymphs were attracted to spearmint oil at 2.5  $\mu$ L. After analyzing spearmint oil using gas chromatography (GC) and GC/mass spectrometry, carvone constituent was found as a significant attractant for both nymphs and adults, except for first instar nymphs. Limonene did not show any attraction response. All constituents mixed with each other appeared to have an additive effect. In electrophysiological response to spearmint oil, antennae of only fourth instar nymphs and female adults responded to carvone. Therefore, spearmint oil may be effective as an attractant for control of *L. delicatula* populations. In a field test, fourth nymphs and female adults were highly attracted to 20  $\mu$ L of spearmint oil. This is the first report on attraction response of *L. delicatula* to spearmint oil in the laboratory and the field.

**Key words:** attraction, carvone, electrophysiological response, *Lycorma delicatula*, spearmint oil, spot clothing wax cicada

Spot clothing wax cicada *Lycorma delicatula* originated from subtropical regions of mainland China and South-East Asia. This pest was first reported in Korea in the 1930s, but was thought to be non-problematic [Choi *et al.*, 2011]. However, the pest has spread rapidly in Korea since being observed in Seoul and Gyeonggi provinces in 2006, with increasing population density fueling annually increasing plant damage [Han *et al.*, 2008; Park *et al.*, 2009]. Wide spread of these pests in Korea may have been prompted by the increasing winter temperature. Ongoing consequence of global warming has allowed spot clothing wax cicada to over-winter [Han *et al.*, 2008; Park *et al.*, 2009; Lee *et al.*, 2011].

Spot clothing wax cicada nymphs are characterized by

the presence of a white spot on their black body surface until the fourth instar nymph stage, at which time the body turns red. Adults have a gorgeous wing color. Identified host plants include 38 species of woody plants and 3 species of herb plants [Park *et al.*, 2009].

Spot clothing wax cicada aggregates on host plants for feeding and excretes the honeydew, causing grey mold. When the infestation is severe, the parasitized host plant is killed. The mass-feeding and infestation of single host plant by thousands of the pests can be abhorrent [Lee *et al.*, 2009]. The management of spot clothing wax cicada is economically important, especially concerning damaged or killed grapevines [Lee *et al.*, 2009; Shin *et al.*, 2010]. Therefore more effective strategies for controlling spot clothing wax cicada are necessary.

There have been several reports concerning *L. delicatula*. Park *et al.* [2009] investigated basic aspects of the insect ecology and evaluated their susceptibility to insecticides. Lee *et al.* [2009] reported that the grapevine was exposed to damage vulnerably by the feeding behavior

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of *L. delicatula*. The organophosphate insecticide, chlorpyrifos is an ovicidal compound that most effectively kills eggs of *L. delicatula*, and 1st and 2nd instar nymphs are highly susceptible to most insecticides [Shin *et al.*, 2010]. However, it is difficult to control the population of *L. delicatula* using insecticides, because spot clothing wax cicada has high reproduction ability (30-50 eggs per egg mass) and hatched nymph have high mortality. Moreover, spraying of insecticides carries concerns on overuse and/or recurrency [Shin *et al.*, 2010].

Synthetic insecticides enable a more rapid and easier control. However, development of resistance and environmental contamination remain as problems. An alternative to synthetic insecticides may be the essential oils [Choi and Kim, 2004; Kang *et al.*, 2009]. Essential oils are secondary metabolites that are naturally produced during plant metabolism. The constituents (volatile compounds) of essential oils are recognized to have low toxicity to humans and animals and are environmentally safe [Katz *et al.*, 2008; Mohamed and Abdelgaleil, 2008]. Good insect bioactivity in aspects such as fumigation, inhibited feeding, growth regulation, and repellent behavior have been reported [Bouda *et al.*, 2001; Coloma *et al.*, 2006; Negahban *et al.*, 2007; Tandon *et al.*, 2008; Nerio *et al.*, 2009]. Until now, essential oils have been studied more for their potential as repellents than as attractants. For instance, caraway and clove bud oil showed repellent activity to the bean bug, *Riptortus pedestris* [Yang *et al.*, 2009]; however, there are no reports on attraction activity of essential oils. Attraction response of insects has been mainly focused on pheromones [Cork and Hall, 1998].

The present study investigated the attraction response of selected essential oils against *L. delicatula* during each developmental stage and to bioassay major essential oil components using gas chromatography (GC) and GC combined with mass spectrometry (GC/MS). In addition, the electrophysiological responses of the antennae of *L.*

*delicatula* nymphs and adults were investigated using GC with an electroantennographic detector (GC-EAD).

## Materials and Methods

**Test insects.** Third to 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 grapevine orchard in Yongam-dong, Cheongju from December, 2009 to May, 2010. The insects were reared at  $25 \pm 2^\circ\text{C}$ , 50-60% relative humidity, and a light:dark photoperiod of 16:8 h. Collected eggs were hatched in a rearing cage (27×30×46 cm), and the hatched eggs were provided with the cut branch of 'tree of heaven' (*Ailanthus altissima*) as food. The cut branch was sealed with a piece of cotton and fix tightly into vial glass neck. A vial was filled with distilled water to provide moisture to the branch. The insects were successively maintained at each developmental stage including adult stage and then used when necessary. All treatments were replicated three times.

**Essential oils and terpene compounds.** Ten plant essential oils were tested for the attraction response of *L. delicatula*: cinnamon bark (French Korean Aromatics Co., Yongin, Korea), black pepper, caraway, cadamone, coriander, eucalyptus, neem, pine needle, sage, and spearmint (JinArome Co., New York, NY). Major compounds of essential oils such as carvone (96% purity) and limonene (97%) (Sigma-Aldrich, St. Louis, MO) were used.

**Attraction test using olfactometer.** An olfactometer (internal diameter: 10 cm; length: 40 cm; angle between arms:  $180^\circ$ ) was used for the response experiments (Fig. 1). The apparatus has a pair of arms, each of which bears a sample container at the end. The olfactometer design allows the insects to choose one or the other arm after crawling up the interior wall in response to different odor

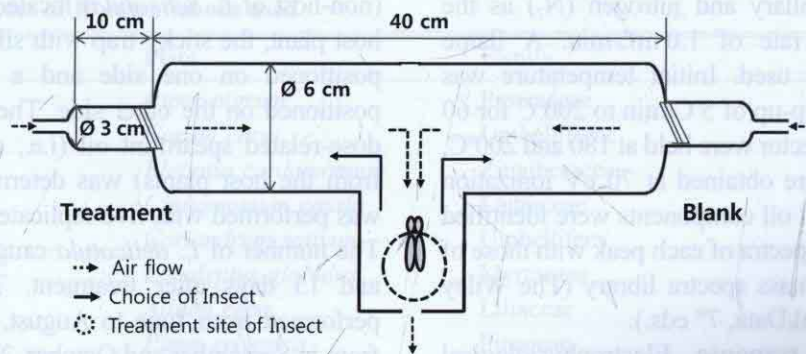


Fig. 1. Olfactometer apparatus design. The air flow on treatment and blank spaces did not influence the insect treatment site in the initial stage.

stimuli. 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 into the chamber at a speed of 100 mL/min using a Medipump<sup>®</sup> vacuum pump (Thomas, Sheboygan, WI). Each oil or monoterpene was dispensed at an appropriate dose on Whatman No. 2 filter paper (9 cm-diameter, 1/4) using a micro-applicator PAX 100-3 (Burkard, Uxbridge, UK). One filter paper was placed in each container of each arm, and the other container remained empty. Attraction response of *L. delicatula* nymphs and adults to the essential oils or monoterpene was evaluated by recording the chosen arm, and "no choice" was recorded when *L. delicatula* nymphs and adults did not enter either arm (within 10 cm from one end of arm) of the olfactometer within 5 min. To avoid carry-over contamination by previously tested volatiles, the olfactometer was replaced with a new one arm after testing five insects. 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 performed using the previously described conditions of temperature, relative humidity, and photoperiod. All experiments were performed using 40 replicates, except 20 replicates were used at the screening stage. In all experiments first to fourth instar nymphs and adults of *L. delicatula* were used. Data on the number of insects were analyzed by a binomial sign test [Zar, 1996]. Olfactory response (%) was calculated using the following formula:  $[\text{Treatment} \div (\text{Treatment} + \text{Blank})] \times 100$ , where 'treatment' is the number of insects attracted to odor-treated side.

**GC-MS analysis.** The constituents of essential oil (spearmint oil) with an attraction response were analyzed using a model 6890N gas chromatograph (Agilent Technology, Santa Clara, CA) and a model 7890A/5975C GC/MS apparatus (Agilent Technology) equipped with a splitless injector. The column chromatography was performed using a DB-WAX GC column (0.25 mm×30 m, thickness 0.25 µm; J&W Scientific, Folsom, CA) fused with a silica capillary and nitrogen (N<sub>2</sub>) as the carrier gas at a flow rate of 1.0 mL/min. A flame ionization detector was used. 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 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, 7<sup>th</sup> eds.).

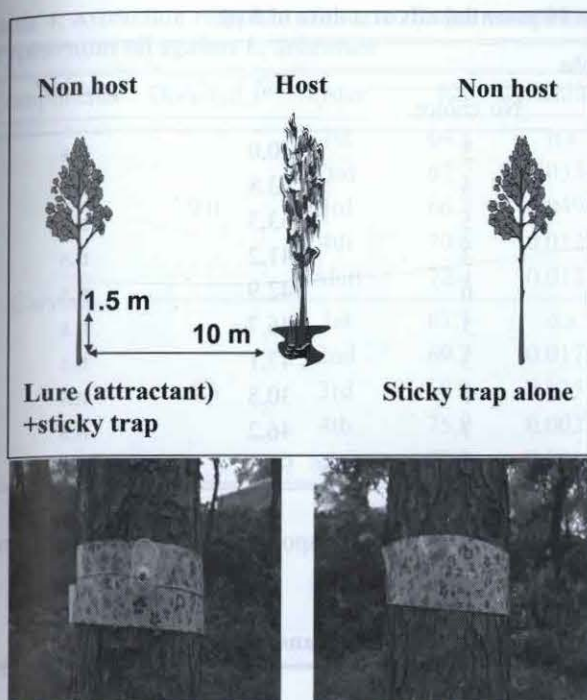
**Electrophysiological response.** Electrophysiological

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, 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), and the other path was connected to an EAD via an interface device. One microliter of an odor sample was injected splitless at 100, followed by opening the split vent after 1 min, and heating the oven at a rate of 20°C/min to 150°C or 25°C/min to 200°C. The end temperature was held for 5 min. A DB-WAX bonded-phase fused-silica capillary column was used for the analyses (0.25 mm×30 m, J&W Scientific) with a film thickness of 0.25 µm.

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). One electrode was connected into the cutting area of each head, and the other electrode was closely connected with antenna to the outlet of a capillary filled with Ringer solution (154 mM NaCl, 5.5 mM KCl, 1.4 mM CaCl<sub>2</sub>). EAG signals were recorded using the GC/EAD32 2005, Ver 3.74.4 program (Syntech) on a personal computer that included an 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 functioning as a rubber lure and a sticky trap was used to evaluate the attraction response of the tested essential oils against *L. delicatula* nymphs and adults in the field. Before the test, the septum was filled with an essential oil and maintained at 4°C for 24 h to fully absorb the oil. Field experimental conditions are depicted in Fig. 2.

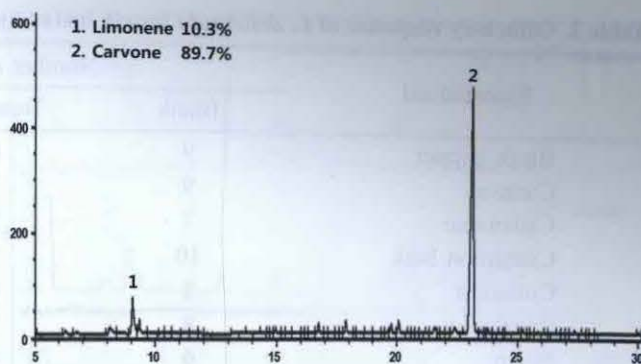
Based on a host plant (tree of heaven) and two plants (non-host of *L. delicatula*) located within 10 m from the host plant, the sticky trap with silicone sleeve septa was positioned on one side and a sticky trap only was positioned on the other side. The attraction response of dose-related spearmint oil (i.e., attract of *L. delicatula* from the host plants) was determined. The experiment was performed with five replicates in two different sites. The number of *L. delicatula* caught was recorded 5, 10, and 15 days after treatment. The investigation was performed from June to August, 2010 for nymphs and from in September and October, 2009 for adults.



**Fig. 2. Field condition for testing of attractant.** (Above) Diagram of experiment for the setting of silicone sleeve septum and sticky trap. (Below) A picture of a set silicone sleeve septum and sticky trap in a tree.

### Results and Discussion

**Attraction response of spearmint on each developmental stage.** Of the ten tested oils, only spearmint oil showed attraction response, with a high attraction ratio (94.1%,  $p < 0.0001$ ). The attraction responses of spearmint oil at each developmental stage of *L. delicatula* and adult females are shown in Table 3. The attraction response was highest in the fourth instar nymphs (90.9%,  $p < 0.0001$ ), followed by female adult (78.1%,  $p < 0.001$ ). Second and third instar nymphs were only marginally significant (68.8%,  $p < 0.05$  and 70.0%,



**Fig. 3. GC profile of spearmint oil.** DB-WAX capillary column (I.D. 0.25 mm, 30 m long, 0.25 mm film thickness) (Temp., 35 to 200°C at 5°C/min).

$p < 0.05$ , respectively). First instar nymphs did not show any attraction response. When treated with 10  $\mu$ L of spearmint oil, fourth instar nymphs, as well as female adults, revealed significant differences (76.7%,  $p < 0.01$  and 71.0%,  $p < 0.05$ , respectively), but only the fourth instar nymphs showed a significantly different attraction response (75.8%,  $p < 0.01$ ) when treated with 2.5  $\mu$ L of the oil. The other essential oils did not show any attraction responses. The bioassay results of essential oils can vary (i.e., attractant or repellent response) depending on the developmental stage of the insect and the treatment dose; pine needle appears to have repellent activity rather than attraction activity (Table 1). In the present study, among all tested doses of spearmint oil, 10, 5, and 2.5  $\mu$ L produced attraction responses.

Spearmint oil is widely used in medical supplies, foodstuffs, and as an ingredient of aromatic fragrances [Song *et al.*, 1998; Mohamed and Abdelgaleil, 2008]. Its insecticidal and insect fumigant actions have also been described [Soliman and Badaea, 2002; Choi and Kim, 2004; Han *et al.*, 2006]. The identified bioactivities of *Mentha* spp. mint plants include antifeeding, repellent, and insecticidal activities [Hori, 2003; Papachristos and

**Table 1. Name and sources of 10 essential oils used**

Essential oil	Plant	Family	Source
Black pepper	<i>Piper nigrum</i>	Piperaceae	JinArome (USA)
Caraway	<i>Carum carvi</i>	Umbelliferae	JinArome (USA)
Cadamone	<i>Elettatia cardamomum</i>	Zingiberaceae	JinArome (USA)
Cinamon bark	<i>Cinnamomum cassia</i>	Lauraceae	FKA (Korea)
Coriander	<i>Coriandrum sativum</i>	Umbellifera	JinArome (USA)
Eucalyptus	<i>Eucalyptus globulus</i>	Myrtaceae	JinArome (USA)
Neem	<i>Allium cepa</i>	Liliaceae	JinArome (USA)
Pine needle	<i>Pinus sylvestris</i>	Pinaceae	JinArome (USA)
Sage	<i>Salvia officinalis</i>	Labiatae	JinArome (USA)
Spearmint	<i>Mentha spicata</i>	Labiatae	JinArome (USA)

**Table 2. Olfactory response of *L. delicatula* fourth instar nymphs to 10 essential oils at a dose of 5  $\mu$ L**

Essential oil	Number of nymphs			% <sup>a</sup>	<i>p</i> -value <sup>b</sup>
	Blank	Treatment	No choice		
Black pepper	9	6	5	40.0	n.s
Caraway	9	7	4	43.8	n.s
Cadamone	7	8	5	53.3	n.s
Cinnamon bark	10	7	3	41.2	n.s
Coriander	8	6	6	42.9	n.s
Eucalyptus	8	7	5	46.7	n.s
Neem	9	8	3	47.1	n.s
Pine needle	9	4	7	30.8	n.s
Sage	7	6	7	46.2	n.s
Spearmint	1	16	3	94.1	<0.0001

<sup>a</sup>Olfactory response (%)=Treatment/(Blank+Treatment) $\times$ 100.

<sup>b</sup>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 3. Attraction response of various doses of spearmint oil against *L. delicatula* nymphs and adults**

Dose ( $\mu$ L)	Instar	Number of <i>L. delicatula</i>			% <sup>a</sup>	<i>p</i> -value <sup>b</sup>
		Blank	Treatment	No choice		
10	1st	18	18	4	50.0	n.s
	2nd	13	20	7	60.6	n.s
	3rd	11	20	9	64.5	n.s
	4th	7	23	10	76.7	0.0026
	adult	9	22	9	71.0	0.0147
5	1st	11	21	8	65.6	n.s
	2nd	10	22	8	68.8	0.0251
	3rd	9	21	10	70.0	0.0214
	4th	3	30	7	90.9	0.0001
	adult	7	25	8	78.1	0.0011
2.5	1st	14	16	10	53.3	n.s
	2nd	13	18	9	58.1	n.s
	3rd	12	19	9	61.3	n.s
	4th	8	25	7	75.8	0.0023
	adult	11	20	9	64.5	n.s
1	1st	19	15	6	44.1	n.s
	2nd	17	15	8	46.9	n.s
	3rd	17	18	5	51.4	n.s
	4th	16	17	7	51.5	n.s
	adult	15	18	7	54.6	n.s

<sup>a</sup>Olfactory response (%)=Treatment/(Blank+Treatment) $\times$ 100.

<sup>b</sup>Data were analyzed using binomial sign tests to evaluate the differences from 50:50 responses. *n*=40, *p*<0.05, 0.01, and 0.001, n.s (not significant) *p*>0.05.

Stamopoulos, 2004; Coloma *et al.*, 2006]. However, no attractive response has hitherto been described. Previous researches on attractants have mainly concentrated on pheromones [Cork and Hall, 1998], whereas bioactivity testing of plant essential oils have focused on the repellent response rather than the attraction response [Hori, 1998;

2003; Koschier and Sedy, 2003]. To the best of our knowledge, the present study is the first report concerning the attraction response of spearmint oil for *L. delicatula*.

Plant essential oils are complexes of volatile compounds produced as a secondary metabolite. Their production is related to the defense mechanism of plants against

**Table 4. Attraction response of the two major components of spearmint oil against *L. delicatula***

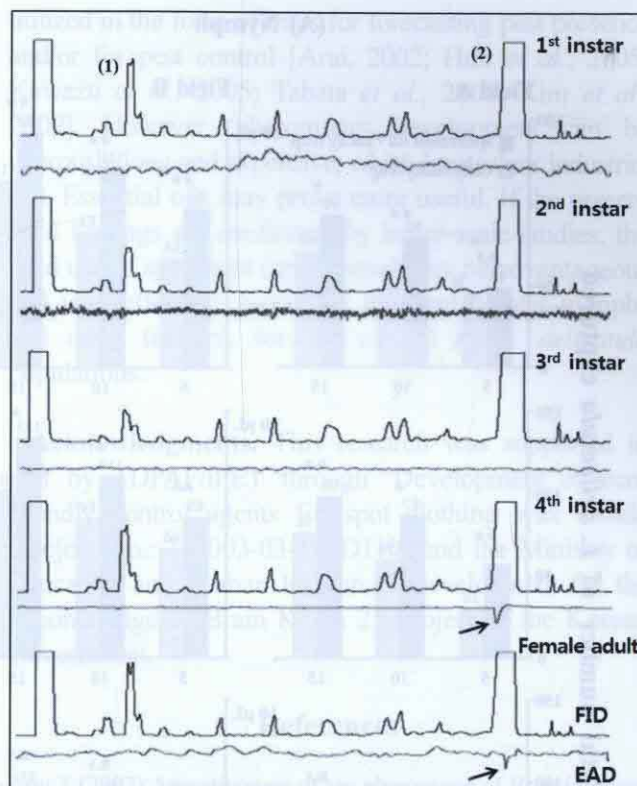
Components	Dose ( $\mu\text{L}$ )	Instar	% <sup>a</sup>	<i>p</i> -value <sup>b</sup>
Carvone	9.0	1st	64.5	n.s
		2nd	67.7	0.0354
		3rd	66.7	0.0494
		4th	70.6	0.0122
		adult	72.4	0.0121
	4.5	1st	61.1	n.s
		2nd	69.7	0.0175
		3rd	68.8	0.0251
		4th	75.8	0.0023
		adult	79.3	0.0012
Limonene	1.0	1st	54.5	n.s
		2nd	55.6	n.s
		3rd	54.6	n.s
		4th	44.1	n.s
		adult	48.5	n.s
	0.5	1st	48.6	n.s
		2nd	46.7	n.s
		3rd	50.0	n.s
		4th	42.4	n.s
		adult	44.1	n.s
Mixture <sup>c</sup>	10.0	1st	58.1	n.s
		2nd	62.5	n.s
		3rd	67.7	0.0354
		4th	69.7	0.0175
		adult	73.3	0.0081
	5.0	1st	66.7	0.0401
		2nd	70.6	0.0122
		3rd	70.0	0.0214
		4th	78.8	0.0007
		adult	80.7	0.0004

<sup>a</sup>Olfactory response (%) = Treatment/(Blank+Treatment) × 100.

<sup>b</sup>The data was analyzed using binomial sign tests to evaluate the differences from 50:50 responses. n=40, *p* < 0.05, 0.01, and 0.001, n.s (not significant) *p* > 0.05.

<sup>c</sup>Mixture (100%) = carvone (89.7%) + limonene (10.3%).

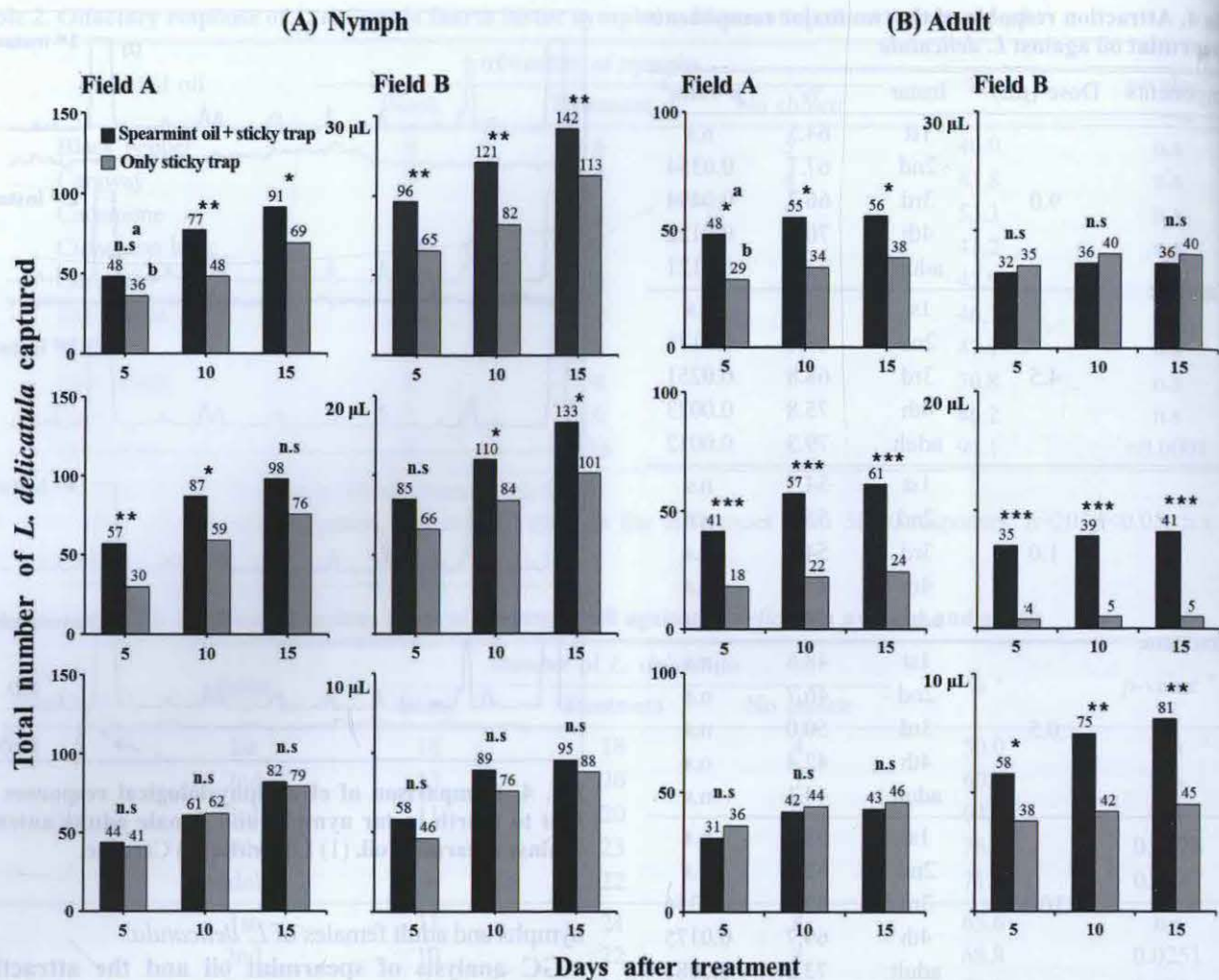
herbivore insects and insect behaviors affecting the selection of host plants, searching for ovipositional sites and/or evasion from an adverse environment [Visser, 1986]. Odors of plant essential oils could be repellent [Bakkali *et al.*, 2008]. Conceivably, plant essential oils may act as attractant of insects to the host plant, which then traps the insects. In this scenario, the plants could be exploited as a population monitor or to actively decrease the population density of the target species. The present preliminary results indicate that, in this regard, spearmint oil may have a value for exterminating fourth instar



**Fig. 4. Comparison of electrophysiological responses on first to fourth instar nymphs and female adults antenna against spearmint oil. (1) Limonene (2) Carvone.**

nymphs and adult females of *L. delicatula*.

**GC analysis of spearmint oil and the attraction response of its constituents.** Spearmint oil was analyzed using GC and GC-MS. The analyses revealed the predominance of carvone (89.7%) in spearmint oil, followed by limonene (10.3%) (Fig. 3). The attraction response of both compounds to *L. delicatula* was assessed; only carvone was attractive (Table 4). This response was dose-related but not dose-dependent. When carvone was applied at a dose of 4.5  $\mu\text{L}$  (half-strength of its proportion in the original oil), adults and all nymphs except the first instar stage were attracted (range 68.8-79.3%, *p* < 0.05-0.01). When treated at a dose of 9  $\mu\text{L}$ , all nymphs except the first instar, as well as adults, displayed significant differences (range 66.7-72.4%, *p* < 0.05-0.01), but no differences were evident at 4.5  $\mu\text{L}$ . Hori [2003; 2004] and Wang *et al.* [2006] reported that the response of an insect can vary depending on the treated dose of oil, but not in a dose-dependent manner. Also, the attraction responses in the present study differed according to the developmental stage. Both facets of the attractive response warrant further study. Zhu and Park [2005] reported on the interaction between an herbivore, soybean aphid (*Aphis glycines*), and a predator, seven-spotted ladybird (*Coccinella septempunctata*). They reported that



**Fig. 5.** Attraction response of spearmint oil to nymph and adult in two field plots. (A) The test was performed from May–August, 2010. (B) The test was performed in August and September, 2010. <sup>a</sup>The data was analyzed using binomial sign tests to evaluate the differences from 50:50 responses. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . <sup>b</sup>This number means the number of captured insects.

soybean plant benzaldehyde was the attractant, and that infested soybean plants excreted more methyl salicylate, which also acted as an attractant. Chapman *et al.* [1981] reported that plant volatiles such as carvone could attract aphids and *Cavariella aegopodii*. The collective data from the previous and present studies are consistent with the view that some volatile compounds can function as attractants in the insect-plant interaction.

In the present study, carvone played the primary role in the attraction response of spearmint oil. Because many compounds in other systems can act in a synergistic fashion, it may be more illuminating to study combinations of essential oil components, rather than each component in isolation. With this aim, various combinations of spearmint oil were tested. Each constituent (i.e., carvone and limonene) was mixed to reflect its natural proportion. Significant responses were produced in all nymphs and adults at 5 µL (range 66.7–80.7%,  $p < 0.05$ –0.01), with

especially pronounced responses noted from the fourth instar nymphs and adults. The attractive response of the carvone-limonene mixture was greater than that observed for carvone alone, but was not statistically significant. When 10 µL of the mixture was applied, third and fourth instar nymphs and adults displayed significant attraction (range 67.7–73.3%,  $p < 0.05$ –0.01). In an experiment where the olfactory responses of fourth instar *L. delicatula* nymphs to ten selected essential oils applied individually were measured, caraway oil, which is constitutionally very similar to spearmint oil, did not display attractive behavior (Table 2). However, a previous study using a mixture of carvone and limonene (56.1 and 43.9%, respectively) reported a pronounced difference [Yang *et al.*, 2009]. The dichotomy between the previous study and ours prompted an experiment where the attraction response of carvone and limonene was tested using a 9:1 ratio of the compounds, which accurately reflects their

natural proportions in spearmint oil. No attractive response was observed. Therefore, the major constituents and their proportion of caraway may not be suitable to attract the *L. delicatula*.

**Electrophysiological response to carvone and limonene.** Herbivore insects have an olfactory cue to select the host for oviposition. Similar to a predator's search for food, insects can recognize volatile compounds excreted by a host plant when infested [Bruce *et al.*, 2005]. These specialized compounds are likely related to the evasion or attraction of insects. These compounds are sensed by olfactory receptors that exist in several regions of the body surface, mostly in the antennae. Olfactory responses result from the interaction between the sensilla organ (mainly in the antennae) and volatile compounds [Visser, 1986; Bruce *et al.*, 2005]. The electrophysiological response of insect antennae has been well-studied, and insect behavior is highly related to the electrophysiological response [Heinbockel and Kaissling, 1996; Zhu and Park, 2005; Dotterl *et al.*, 2006; Cook *et al.*, 2007; Mauchline *et al.*, 2008]. In the present study, the electrophysiological response of *L. delicatula* antennae on each instar nymph and female adult to spearmint oil was examined using GC-EAD. Antenna of the fourth instar nymphs and female adults responded to a 10% dose of spearmint oil and to carvone (Fig. 4). However, the antennae from first to third instar nymphs were poorly responsive or nonresponsive to 10% spearmint oil. The reason why *L. delicatula* is attracted to spearmint oil is unknown. One reason can be guessed from the data of Tables 3 and 4. Second and third instar nymph did not respond to 10  $\mu$ L dose of spearmint and showed marginal significance to 5  $\mu$ L spearmint oil. They also showed marginally attracted second and third instar nymphs to 9 and 4.5  $\mu$ L of carvone. However, the antennae may interact with carvone differently depending on the stage of development.

**Field study of the attraction response.** The attraction response of spearmint was field tested. Field test was performed using fourth instar nymphs and adults. The results are depicted in Fig. 5. Nymphs treated with 30 and 20  $\mu$ L of spearmint oil displayed similar responses in both test plots until 15 day after treatment, but the response to 10  $\mu$ L of spearmint oil was insignificant. Adults treated with 30 and 10  $\mu$ L displayed responses that were not uniform with plot location. However, treatment with 20  $\mu$ L produced responses in the adults that were both significant and similar between the two test plots.

Plant essential oils, as in the present study, are not so much applied to attract the insect pest. To date, the attraction response has been studied mainly in signal materials such as pheromones rather than plant essential oils or volatile compounds. Pheromones are more widely

utilized in the form of traps for forecasting pest presence and/or for pest control [Arai, 2002; Huh *et al.*, 2005; Kawazu *et al.*, 2005; Tabata *et al.*, 2008; Kim *et al.*, 2009]. However, pheromones development can be onerously long and expensive, which limits their industrial use. Essential oils may prove more useful. If the present field findings are confirmed by larger-scale studies, the field use of spearmint (or carvone) may be advantageous and very effective, especially for fourth instar nymphs and adult females, for the control of *L. delicatula* populations.

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