

# Attraction of the tea aphid, *Toxoptera aurantii*, to combinations of volatiles and colors related to tea plants

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Accepted: 11 May 2012

**Key words:** tea shoot volatiles, green leaf volatiles, (*Z*)-3-hexen-1-ol, colored sticky boards, olfactory cues, visual cues, electrophysiology, orientation mechanism, Hemiptera, Aphididae, *Camellia sinensis*, Theaceae

## Abstract

The tea aphid, *Toxoptera aurantii* Boyer (Hemiptera: Aphididae), is a major pest of the tea plant, *Camellia sinensis* (L.) O. Kuntze (Theaceae). The attraction of the aphids to different colors and volatile compounds from tea shoots was investigated. Fourteen compounds were identified using gas chromatography-mass spectrometry from headspace samples of intact tea shoot volatiles (ITSV). Electrophysiological and behavioral responses of winged tea aphids to ITSV as well as to the full blend of 14 synthetic compounds, to a partial mixture of green leaf volatiles (GLV) included in the 14 compounds, and to individual synthetic compounds were studied by using electroantennography (EAG) and a Y-tube olfactometer. The various tea volatiles and blends were strongly active, with ITSV being the strongest. In the greenhouse and in tea plantations, sticky boards of six different colors strongly attracted tea aphids in flight, with 'rape-flower yellow' and 'Chinese olive-yellow-green' being the most attractive. Furthermore, these two boards in combination with ITSV attracted winged tea aphids more strongly than their corresponding colored sticky boards alone. In the greenhouse, plastic models of tea seedlings baited with (*Z*)-3-hexen-1-ol or the GLV mixture significantly attracted winged tea aphids in flight. This study demonstrates that green leaf volatiles from tea shoots are attractive to the tea aphid. The combination of these volatiles with the color light yellow or green, and the shape of tender tea shoots result in orientation flight and landing of winged tea aphids on host tea shoots.

## Introduction

Aphids comprise a large group of important insect pests on many agricultural and forest plants distributed throughout tropical and temperate zones (Blackman & Eastop, 2007). The aphids seek appropriate host plants by means of either olfaction (Pickett et al., 1992; Quiroz et al., 1997; Quiroz & Niemeyer, 1998; Park et al., 2000; Pettersson et al., 2007) or visual cues (Zhang & Zhong, 1983; Irwin et al., 2007; Pettersson et al., 2007), or both (Powell et al., 2006). An aphid in the appetitive mode

during flight will descend if it visually detects a host (Zhang & Zhong, 1983; Powell et al., 2006; Irwin et al., 2007), and if the aphid's olfactory organs perceive suitable plant volatiles, it will orient toward the host and land. Some insects, e.g., grape berry moth, *Paralobesia viteana* (Clemens), use both olfactory and visual cues for landing (Cha et al., 2008; Brévault & Quilici, 2010), and visual cues were shown to enhance the response of the plant bug *Lygus hesperus* (Knight) to host-plant volatiles (Blackmer & Cañas, 2005).

The tea aphid, *Toxoptera aurantii* (Boyer) (Hemiptera: Aphididae), occurs throughout the growing areas of the Chinese tea plant, *Camellia sinensis* (L.) O. Kuntze (Theaceae), and is one of the most destructive pest insects in tea plantations/gardens in southern China (Zhang & Zhong, 1983). Tender tea shoots are attractive to tea aphids and

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are strongly preferred as primary feeding sites compared to mature (older) leaves (Han & Chen, 2002). Feeding by tea aphids can seriously damage tender tea shoots that provide the raw materials for high-quality commercial teas. In this study, the attraction of tea aphids to colors and various volatiles from tea shoots was investigated to better understand orientation mechanisms of the aphids toward host plants and to provide insights for developing efficient monitoring in the integrated management of the pest.

## Materials and methods

### Plants and insects

The tea seedlings used in the study were grown from tea seeds (cultivar Longjing 43) by immersing them in warm water at 25 °C for 24 h. The seeds soon sprouted into tea seedlings that were cultured in distilled water. When

seedlings grew around 12 cm in height with one bud and two leaves, each seedling was held in a 5-ml cup filled with distilled water to be used in subsequent tests. A large batch of winged female tea aphid adults was collected from tea plantations in Meijiawu Village (30°3'N, 120°2'E), Hangzhou City, Zhejiang, China. Tea aphids were reared on tea seedlings at 25 °C, 80% r.h., and L18:D6 photoperiod. Tea aphid nymphs were placed on tea seedlings, one aphid per seedling. Under the experimental conditions, adults developed from eggs in about 15 days, and when winged aphids developed, the 2-day-old alate virginoparae were used in all experiments.

### Collection and identification of volatiles from intact tea shoots

In a previous study (Han & Chen, 2002), the major volatiles were identified from tea shoots and assigned numbers (Table 1). Number 14 was a mixture of green leaf volatiles (GLV) with C<sub>5</sub>–C<sub>6</sub> compounds (at 1:1 ratio)

**Table 1** Mean ( $\pm$  SD;  $n = 14$ ) tea aphid electroantennogram response ( $\mu$ V) elicited by different doses of synthetic compounds and mixtures associated with tea plants

Odor resource	Compound number	Compound dose (g ml <sup>-1</sup> )			
		10 <sup>-2</sup>	10 <sup>-4</sup>	10 <sup>-6</sup>	
Green leaf volatiles	( <i>E</i> )-2-hexenal	1	17.0 $\pm$ 1.7	64.0 $\pm$ 6.4	105.0 $\pm$ 11.1
	n-hexanol	2	47.0 $\pm$ 4.8	56.0 $\pm$ 5.6	93.0 $\pm$ 9.5
	( <i>Z</i> )-3-hexenyl acetate	5	18.0 $\pm$ 1.8	44.0 $\pm$ 4.5	48.0 $\pm$ 5.0
	( <i>Z</i> )-3-hexen-1-ol	16	55.0 $\pm$ 5.6	98.0 $\pm$ 9.9	283.0 $\pm$ 29.0; 212.0 $\pm$ 21.5 <sup>1</sup>
Average of C <sub>6</sub> compounds			34.3 $\pm$ 3.5	65.5 $\pm$ 6.7	132.3 $\pm$ 13.1
	2-penten-1-ol	3	65.0 $\pm$ 6.5	66.0 $\pm$ 6.6	75.0 $\pm$ 7.5
	1-penten-3-ol	6	18.0 $\pm$ 1.9	26.0 $\pm$ 2.8	36.0 $\pm$ 3.5
Average of C <sub>5</sub> compounds	n-pentanol	4	26.0 $\pm$ 2.5	35.0 $\pm$ 3.6	71.0 $\pm$ 7.1
			36.3 $\pm$ 3.6	42.3 $\pm$ 4.3	60.7 $\pm$ 6.1
Average of C <sub>5</sub> and C <sub>6</sub> compounds			35.1	55.6	101.6
Monoterpenes	Linalool	7	25.0 $\pm$ 2.4	47.0 $\pm$ 4.8	57.0 $\pm$ 5.6
	Geraniol	8	9.0 $\pm$ 0.9	34.0 $\pm$ 3.6	36.0 $\pm$ 3.6
Average			17.0	40.5	46.5
Aromatics	Methyl salicylate	9	37.0 $\pm$ 3.6	47.0 $\pm$ 4.7	49.0 $\pm$ 4.9
	Benzaldehyde	10	39.0 $\pm$ 3.8	44.0 $\pm$ 4.4	55.0 $\pm$ 5.4
	Benzoic acid	11	18.0 $\pm$ 1.8	23.0 $\pm$ 2.3	26.0 $\pm$ 2.6
	Benzylalcohol	12	18.0 $\pm$ 1.7	18.0 $\pm$ 1.7	27.0 $\pm$ 2.6
	Indole	13	50.0 $\pm$ 4.9	57.0 $\pm$ 5.9	60.0 $\pm$ 5.9
Average			32.4	37.8	43.4
Mixtures	GLV <sup>2</sup>	14	71.0 $\pm$ 7.0	73 $\pm$ 7.2	75 $\pm$ 7.5
	FB <sup>3</sup>	15	79.0 $\pm$ 7.8	83 $\pm$ 8.3	121 $\pm$ 12.0
Average			75.0	78	98

<sup>1</sup>The distal primary rhinaria on the sixth segments of tea aphid antennae were exposed to the odor (283  $\pm$  45  $\mu$ V;  $n = 15$ ), then covered with glass electrodes and re-exposed (212  $\pm$  21  $\mu$ V), resulted in reduction in EAG response by 25.1%.

<sup>2</sup>Green leaf volatiles (GLV) blend with equal amounts of compounds 1–7 and 16.

<sup>3</sup>Full blend (FB) with equal amounts of compounds 1–13 and 16.

including nos. 1–7 and 16. Number 15 was the full blend (FB), a mixture of all 14 chemicals (at 1:1 ratio) including nos. 1–13 and 16. The chemicals were purchased from Sigma-Aldrich, St Louis, MO, USA, in chromatographic purities, and used in all assays. Chemical solutions were prepared with distilled hexane, except for the electrophysiological tests in which the solvent was liquid paraffin. We used the common air entrainment system described by Han & Chen (2002) to collect volatiles from fresh tea shoots, each being about 7 cm long with one bud and three leaves. The aeration extract was prepared for bioassays and assigned as no. 17, i.e., intact tea shoot volatiles (ITSV).

#### Y-tube olfactometer bioassays

Aphids were tested 2 days after becoming alates (winged adults). The Y-tube olfactometer and test procedures were the same as described by Han & Chen (2002). A vial filled with 1 ml odor extract/solution was placed in one jar, whereas another vial with 1 ml distilled hexane alone was placed in the second control jar, with both vials remaining open. For one replicate, 20 of the winged tea aphids were tested individually for each type of odor source. After 5 min, the olfactometer arm chosen by the aphid was recorded. After every 10 tea aphids that were tested, the Y-tube was cleaned with ethanol and dried, and then the treatment arms were reversed to avoid possible positional effects. Five replicates were performed on each odor source, i.e.,  $20 \times 5 = 100$  aphids were tested. Differences between the numbers of aphids choosing an odor source vs. the numbers of aphids choosing the control were analysed with  $\chi^2$  tests. Both Y-tube assays and the electrophysiological tests (next section) were conducted between 09:00 and 14:00 hours. The ambient temperature ranged from 18 to 20 °C, relative air humidity from 80 to 85%, and illumination from 3 400 to 3 600 lux.

#### Electrophysiology

The electroantennographic (EAG) procedure was similar to that reported in previous studies (Park et al., 2000; Han & Chen, 2002; Han & Han, 2007). The antenna was excised and inserted into the glass capillary reference electrode filled with Ringer's solution. The tip of the antenna was cut and placed inside the glass capillary recording electrode (filled with Ringer's solution). Aliquots of 20  $\mu$ l of standard solution of each test compound or mixture were applied to a filter paper strip (5  $\times$  60 mm), and solvent was allowed to evaporate for 30 s before the strip was inserted into a glass Pasteur pipette (15 cm long) used to dispense the test volatiles. Chemical stimuli were tested in an increasing order of concentration ( $10^{-6}$ ,  $10^{-4}$ , and  $10^{-2}$  g ml $^{-1}$ ), and each stimulation was followed by a

2-min purge period of charcoal-filtered air to ensure recovery of antennal receptors. To determine the role of distal primary rhinaria on the sixth segment of the tea aphid antenna in the overall EAG, we also measured the aphid EAGs to (*Z*)-3-hexen-1-ol with the distal primary rhinaria being immersed into glass electrode solution, and compared them with those recorded when the distal primary rhinaria were re-exposed. Each compound/mixture was tested on 15 individual antennae. The signals were passed through an amplifier (Syntech CS-05; Syntech, Hilversum, The Netherlands), displayed on an oscilloscope, and stored in a computer using Syntech Software. The EAG dose-response data of each compound or mixture were compared and tested by ANOVA followed by Duncan's new multiple range test (at  $\alpha = 0.05$ ).

#### Greenhouse and field experiments

To test the attractiveness of different colors to alate virginopara, six color treatments were prepared (Table 2) using double-sided sticky boards (25  $\times$  25 cm). The colors were selected to mimic tea leaf colors from green to yellow with aid of a chroma meter (Model CR-300; Meinengda, Hong Kong) and a chromagram (see Table 2) (Term Laboratory of Translation & Edit & Publishing Committee of Chinese Academy of Sciences, 1957; Byers, 2006).

**Table 2** Brightness, chroma, and RGB of the six colors of sticky boards used

Color type <sup>1</sup>	Absolute color space <sup>2</sup>			RGB color space <sup>3</sup>		
	L	a	b	R	G	B
Rape-flower yellow	83.77	– 8.19	+ 50.73	225	211	111
Chinese olive yellow-green	72.89	– 37.20	+ 45.04	135	195	92
Calcedony green	75.20	– 52.17	+ 30.08	85	208	127
Malachite green	49.02	– 50.46	+ 11.72	0	136	95
Blue green	73.96	– 36.82	+ 21.41	119	199	141
Snow-white	92.96	– 5.09	+ 6.16	230	237	223

<sup>1</sup>Colors were judged according to a Chromagram described by the Term Laboratory of Translation and Edit and Publishing Committee of Chinese Academy of Sciences (1957).

<sup>2</sup>In absolute color space, the brightness and chroma of the various colored sticky boards were measured by a chroma meter.

'L' = brightness; 'a' = first chroma coordinate, with '+' indicating red, and '-' indicating green; 'b' = second chroma coordinate, '+' indicating yellow, and '-' indicating blue.

<sup>3</sup>This absolute color space is the same as the CIE-L\*a\*b color space which can be converted to the more common RGB (red, green, blue) color space (Byers, 2006: www.chemical-ecology.net).

**Test A – attraction of flying tea aphids to colored sticky boards under both greenhouse and tea garden conditions**

In a greenhouse at the same environmental conditions as the olfactometer tests, sets of six sticky boards were placed in a hexagon, with each board of a different color and 15 cm from the hexagon center point (Figure 1A). Ten tea seedlings as described earlier with tea aphids were placed in the hexagon center. Every day, about 100 alate aphids emerged on these seedlings. After 72 h, the alate aphids that had flown to the sticky boards were recorded. The test was replicated five times. Concurrently, in the greenhouse another experiment with the same test procedure was conducted; however, there the distance of each color board to the center point was doubled to 30 cm (Figure 1B).

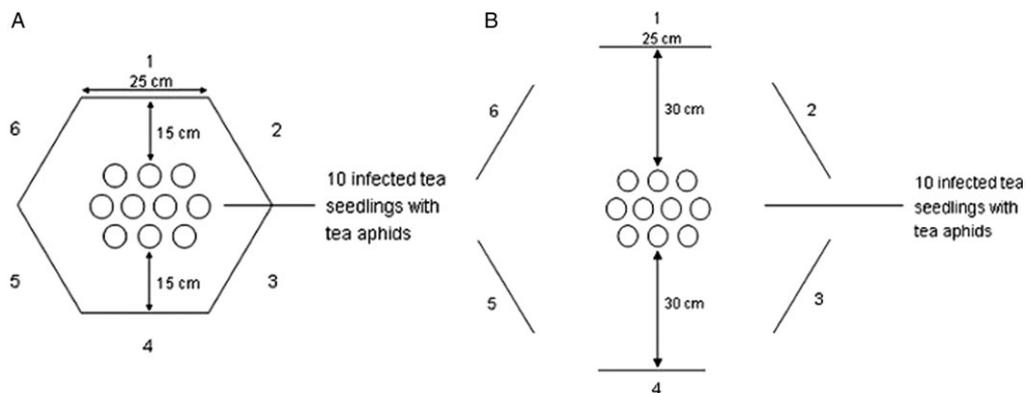
Field experiments were carried out at the experimental tea plantation of the Tea Research Institute of the Chinese Academy of Agricultural Sciences (Hangzhou, China), where plants were 30 years old, 90 cm in height with 150 cm between rows and 33 cm between plants within a row, and the breadths of the tea clumps were 110–120 cm. Trials were carried out from 20 to 30 April, 2010, when temperature was between 15 and 22 °C, the relative humidity ranged from 65–80%, and the wind was generally  $<0.5 \text{ m s}^{-1}$  during the day. In the first experiment, a total of 30 sticky boards (five traps for each color) were placed in the plantation, with about 7 m between neighboring traps, to compare the effects of colors on tea aphid attraction. Each sticky board bottom was placed 90 cm above ground by hanging on a garden stick (Figure 2A). This height was chosen because tea plants grown commercially are usually kept at a height below 90 cm. In the same

tea plantation, a second experiment, ca. 200 m from the first experiment, with the same test procedure was conducted; however, sticky boards (bottoms) were set up at 60 cm above ground (Figure 2B). ANOVA followed by Duncan's new multiple range test (at  $\alpha = 0.05$ ) was used to determine the difference in the numbers of alate aphids trapped on different colored sticky boards.

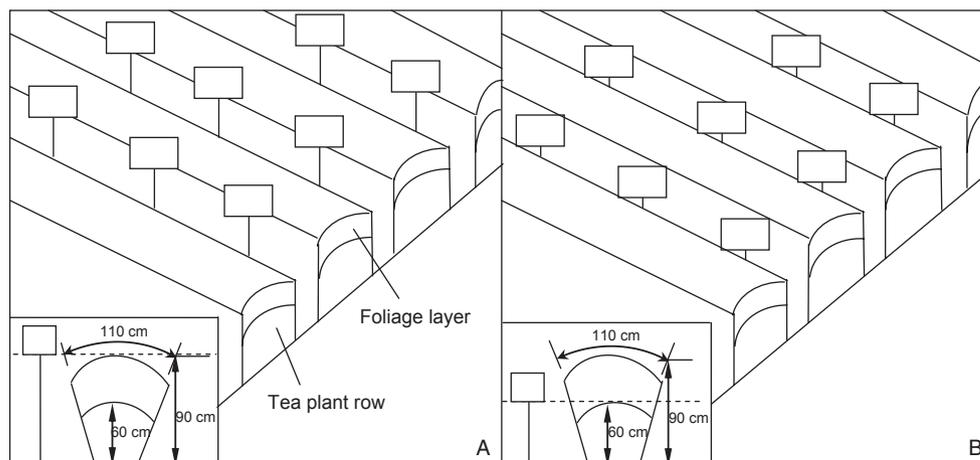
**Test B – attraction of flying tea aphids to colored sticky boards combined with tea plant volatiles in the greenhouse**

We also tested the attraction of flying tea aphids to rape-flower yellow and calcedony green sticky boards with and without host-plant volatiles in the greenhouse. For each color, 10 infested tea seedlings were evenly spaced in a line extending 150 cm with a parallel line on one side consisting of five colored sticky boards (25 × 25 cm), while on the other side a parallel line had five treated sticky boards [each baited with a glass vial loaded with 1 ml of ITSV (no. 17) solution] (Figure 3). The sticky boards were 30 cm apart (center to center) and the two parallel lines of colored sticky boards were 30 cm apart (Figure 3A and C). A sticky board was replaced if many aphids were trapped. The numbers of alate aphids captured on the sticky boards were recorded over a 72-h period. The same experimental protocol was repeated for both colors in another experiment, except that the distance employed between the treated and control board lines was increased to 60 cm (Figure 3B and D).

The differences in the numbers of aphids trapped between the sticky boards baited with host-plant volatiles and the un-baited control boards for each color or distance were determined by paired t-test at  $\alpha = 0.05$ .



**Figure 1** Diagrams of colored sticky boards in the greenhouse, (A) about 15 cm, or (B) about 30 cm from 10 tea aphid-infested tea seedlings. Colored sticky boards were: 1, snow-white; 2, rape-flower yellow; 3, Chinese olive yellow-green; 4, calcedony green; 5, malachite green; and 6, blue green.



**Figure 2** Diagrams of the six colored sticky boards (colors as in Figure 1) in relation to tea plant rows and foliage layers in tea gardens at trap heights of (A) 90 cm and (B) 60 cm.

#### Test C – attraction of flying tea aphids to plastic models of tea seedlings baited with tea plant volatiles in the greenhouse

In the greenhouse, 16 tea seedlings with aphids were placed together to form a  $12 \times 12$  cm square to act as a source of winged aphids (Figure 4). Twenty healthy tea seedlings with no aphids were set together to form a square at 1 m to the left of the aphid source. In addition, groups of 20 plastic model tea seedlings were each placed 1 m to the right, front, and back of the central aphid source (Figure 4). The plastic model tea seedlings were manufactured in Hangzhou Toy Factory using plastic, with their color, shape, and size similar to the healthy tea seedlings. Within each of the three tea model areas, either 10 vials each with 1 ml (*Z*)-3-hexen-1-ol (no. 16) solution at  $10^{-6}$  g ml $^{-1}$ , 10 vials each with 1 ml GLV (14) solution at  $10^{-6}$  g ml $^{-1}$ , or 10 vials each with 1 ml hexane alone were set up in each tea model position, respectively. All vial openings were sealed with Parafilm<sup>®</sup> and pricked once by an insect needle, such that the test solutions lasted for about 72 h. Solutions that were nearly empty in any vial were replaced. Over a period of 120 h at intervals of 12 h, the numbers of alate aphids that landed in each section (flying from the aphid sources) were counted and then removed. Four experimental set-ups as above, but with each having different randomized treatment positions were performed simultaneously in different rooms (closures) within the same greenhouse (Figure 4). During this test, the greenhouse was closed, the air was static, and fluorescent lamps provided a light source.

The numbers of aphids that landed during the entire trapping period (120 h) in each of the four treatment areas were analyzed using ANOVA followed by Duncan's new multiple range test at  $\alpha = 0.05$ .

## Results

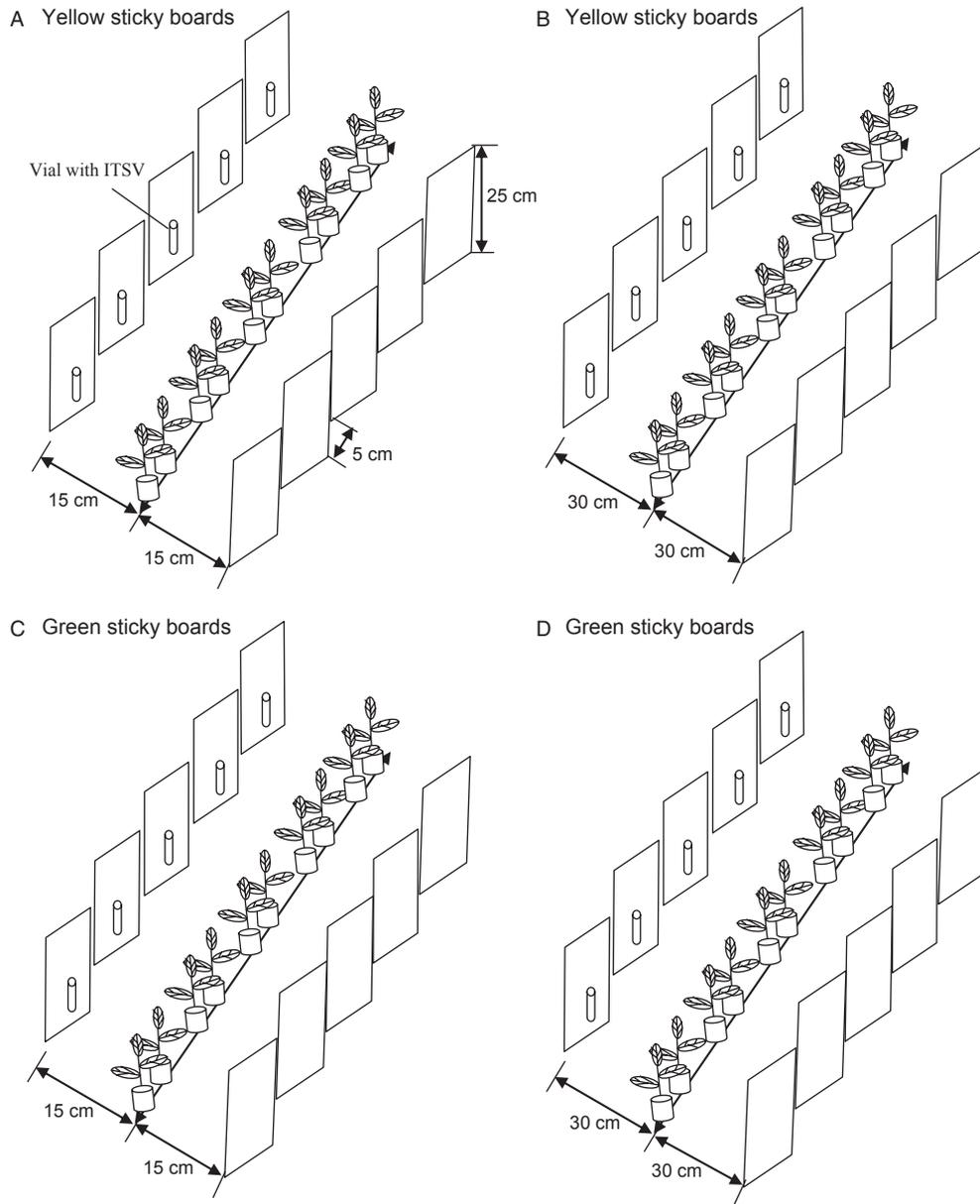
#### Attraction of winged tea aphids to tea plant volatiles in an olfactometer

In most cases, the numbers of aphids choosing odors of individual compounds or blends of compounds were higher than the numbers of aphids choosing the corresponding controls (Figure 5). However, statistically significant attractions were only recorded for ITSV (no. 17), FB (15), GLV (14), (*Z*)-3-hexen-1-ol (16), (*E*)-2-hexenal (1), linalool (7), and methyl salicylate (9) (Figure 5). Benzaldehyde (10) and benzoic acid (11), among many others, appeared to be unattractive (Figure 5).

#### Electroantennography responses of winged tea aphids to tea shoot volatile compounds

**Response profile.** Electroantennographic responses of the tea aphids in our study ranged from 9 to 283  $\mu$ V, but usually between 50 and 150  $\mu$ V. Among the individual compounds tested, (*Z*)-3-hexen-1-ol (no. 16) elicited the strongest EAG responses by the tea aphid, followed by (*E*)-2-hexenal (1), *n*-hexanol (2), 2-penten-1-ol (3), *n*-pentanol (4), indole (13), and methyl salicylate (9). EAG values elicited by benzoic acid (11) and benzylalcohol (12) were very low. EAG values elicited by solvent were zero. The blend odor sources (especially no. 15) also elicited very strong EAG responses (Table 1).

**Dose-response relationships.** Within the volatile concentration range tested ( $10^{-2}$ ,  $10^{-4}$ , and  $10^{-6}$  g ml $^{-1}$ ), EAG responses by the tea aphids increased as the volatile dosage decreased (Table 1). The average ( $\pm$  SD) EAG values elicited by nos. 1–16 were different among the three

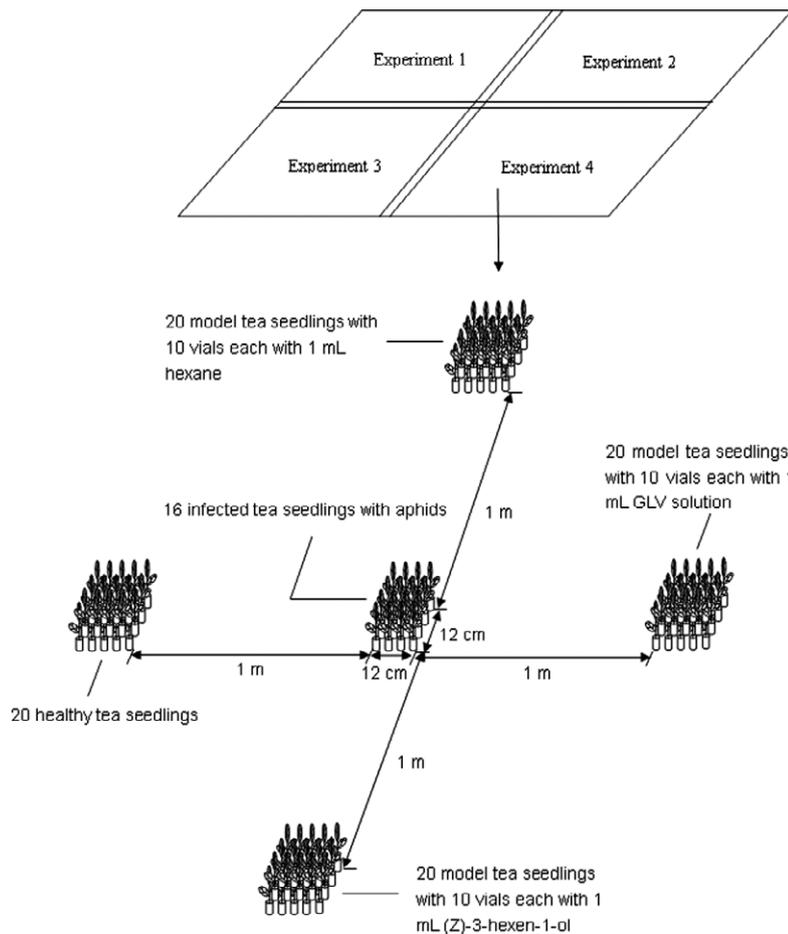


**Figure 3** Diagrams of the trapping tests on attraction of the flying tea aphids to (A, B) rape-flower yellow sticky boards or (C, D) calcedony green sticky boards, with or without tea plant volatiles in the greenhouse. ITSV, intact tea shoot volatiles.

concentrations (ANOVA:  $F_{2,45} = 3.96$ ,  $P = 0.026$ ), i.e.,  $37.0 \pm 21.9$ ,  $50.9 \pm 22.1$ , and  $76.1 \pm 61.5 \mu\text{V}$  at  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-6} \text{ g ml}^{-1}$ , respectively. At  $10^{-2} \text{ g ml}^{-1}$ , EAG responses elicited by FB (no. 15) and GLV (14) exceeded the average of EAG values elicited by single compounds of nos. 1–13 and 16 by 156.0 and 130.1%, respectively; at  $10^{-4} \text{ g ml}^{-1}$ , EAG responses by FB and GLV exceeded the average values of 1–13 and 16 by 76.6 and 55.3%, respectively; and at  $10^{-6} \text{ g ml}^{-1}$ , EAG values

by FB and GLV exceeded the individual compound averages by 66.0 and 3.0%, respectively (Table 1).

**Structure-activity relationships.** The average EAG values at the doses  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-6} \text{ g ml}^{-1}$  of green leaf volatiles were the largest, compared with the averages of other compounds; the lowest average of EAG values was elicited by monoterpenes (Table 1). For green leaf volatiles,  $C_6$  compounds were more active than  $C_5$



**Figure 4** Diagram of four experiments to investigate attraction of tea aphids residing on 16 infested tea seedlings (center) and flying to healthy tea seedlings and plastic seedling models with different tea plant volatiles or solvent control in the greenhouse. GLV, green leaf volatiles.

compounds. The important ingredients of tea aroma, 2-penten-1-ol (no. 3) and 1-penten-3-ol (6), are reciprocal isomers, but at doses of  $10^{-6}$ ,  $10^{-4}$ , and  $10^{-2}$  g ml $^{-1}$  the average EAG values elicited by 2-penten-1-ol (3) exceeded those by 1-penten-3-ol (6) by 261.1, 153.8, and 108.3%, respectively (paired *t*-test: *t* = 18.57, 21.36, and 27.03, respectively; all d.f. = 28, *P* < 0.0001). Apparently, the double-bond position is more stimulating at *C*<sub>2</sub> than at *C*<sub>1</sub>.

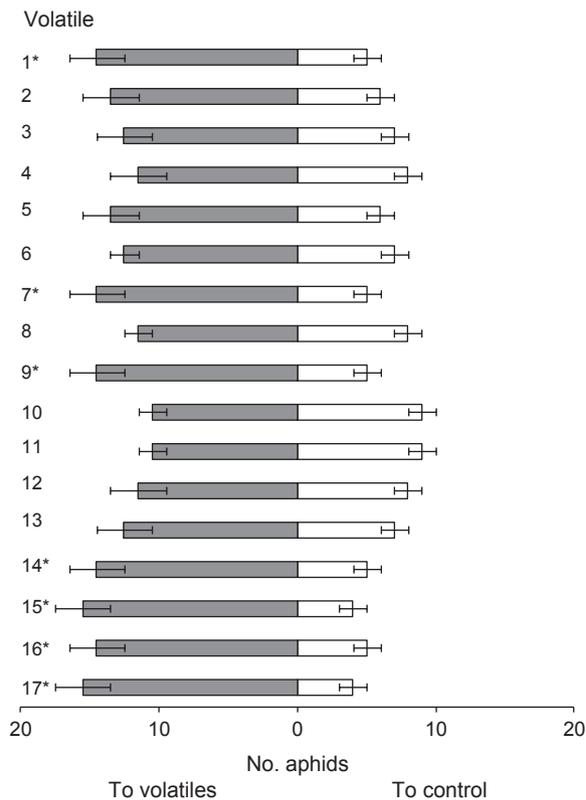
The primary rhinaria on the aphid's antennae appears important for receiving semiochemicals because covering the distal sixth segment (primary rhinaria) of the tea aphid antenna by inserting this into the glass electrode reduced the EAG response to (*Z*)-3-hexen-1-ol (no. 16) at  $10^{-6}$  g ml $^{-1}$  by 25.1% (Table 1).

#### Attraction of flying tea aphids to colored sticky boards in the greenhouse and tea gardens

Under the greenhouse conditions, the rape-flower yellow, olive yellow-green, and chalcedony green-colored traps were the most attractive, followed by malachite-green,

blue-green, and snow-white (Figure 6A). Significant differences in the numbers of aphids trapped on the six colors of sticky boards were observed (one-way ANOVA followed by Duncan's new multiple range test:  $F_{5,24} = 40.06$ , *P* < 0.0001). When the distance from the center of the aphid source to each one of six colored sticky boards was increased from 15 to 30 cm, the overall numbers of alates trapped on 30 boards (six colors, five replicates) were significantly reduced, however, the differences in the numbers of aphids on the six colored boards was still significant (ANOVA:  $F_{5,24} = 62.51$ , *P* < 0.0001) (Figure 6B).

In the tea plantation (garden), the rape-flower yellow, olive yellow-green, and chalcedony green boards also trapped the most winged tea aphids (Figure 6). There were significant differences in trap catches among the six colored boards (one-way ANOVA followed by Duncan's new multiple range test:  $F_{5,24} = 97.14$ , *P* < 0.0001) (Figure 6C). Trap boards hung at 90 cm (close to the tender leaves) above ground caught overall much more winged aphids than did the ones hung at 60 cm height; moreover, the



**Figure 5** Mean ( $\pm$  SD) number of tea aphids responding to an odor source (volatile) vs. a control (hexane). For each odor source, 20 aphids were tested individually (five replicates). Odor sources: (1) (*E*)-2-hexenal, (2) *n*-hexanol, (3) 2-penten-1-ol, (4) *n*-pentanol, (5) (*Z*)-3-hexenyl acetate, (6) 1-penten-3-ol, (7) linalool, (8) geraniol, (9) methyl salicylate, (10) benzaldehyde, (11) benzoic acid, (12) benzylalcohol, (13) indole, (14) green leaf volatiles blend comprised of equal amounts of volatiles 1–7 + 16, (15) full blend comprised of equal amounts of volatiles 1–13 + 16, (16) (*Z*)-3-hexen-1-ol, and (17) intact tea shoot volatiles. \* indicates significant preference ( $\chi^2$  test:  $P < 0.05$ ).

differences in the numbers of aphids trapped by the six colored boards hung at 90 cm was significant (ANOVA:  $F_{5,24} = 101.54$ ,  $P < 0.0001$ ) (Figure 6D).

#### Attraction of flying tea aphids to combinations of colored sticky boards and tea plant volatiles in the greenhouse

At 15 cm from the aphid sources, the rape flower yellow boards with ITS<sub>V</sub> (no. 17) caught significantly more aphids (mean  $\pm$  SD =  $58 \pm 5$  aphids per trap) than did the rape-flower yellow boards alone ( $43 \pm 4$  aphids per trap; paired *t*-test:  $t = 5.50$ , d.f. = 8,  $P = 0.0006$ ). The same was also true for the calcedony green boards with and without ITS<sub>V</sub>, i.e.,  $49 \pm 4$  vs.  $42 \pm 4$  aphids per trap (paired *t*-test:  $t = 3.26$ , d.f. = 8,  $P = 0.012$ ).

At 30 cm from the aphid sources, the rape-flower yellow boards with ITS<sub>V</sub> caught significantly more aphids than did the rape-flower yellow boards alone ( $48 \pm 4$  vs.  $39 \pm 3$  aphids per trap; paired *t*-test:  $t = 3.81$ , d.f. = 8,  $P = 0.0052$ ), whereas the calcedony green boards with and without ITS<sub>V</sub> had similar aphid catches, i.e.,  $40 \pm 3$  and  $38 \pm 3$  aphids per trap, respectively (paired *t*-test:  $t = 0.98$ , d.f. = 8,  $P = 0.36$ ).

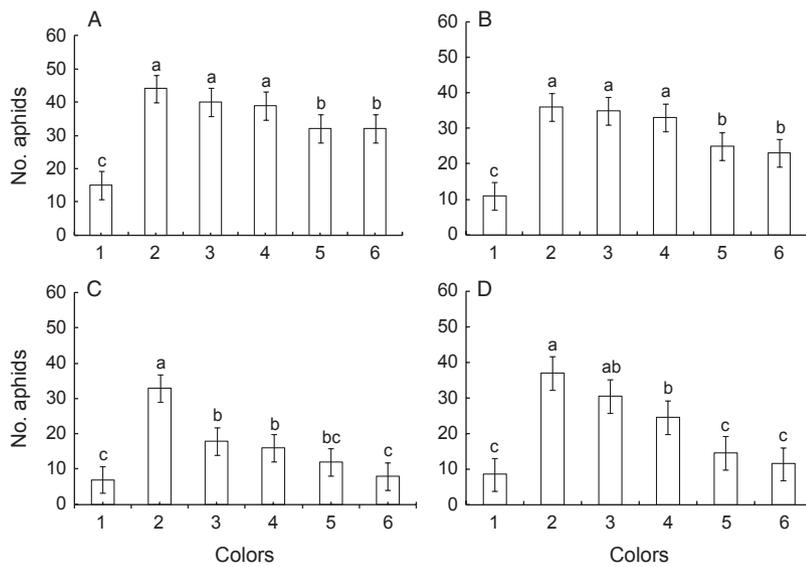
#### Attraction of flying tea aphids to plastic models of tea seedlings with tea plant volatiles in the greenhouse

The plastic models of tea seedlings baited with hexane alone were attractive to the tea aphids; however, addition of (*Z*)-3-hexen-1-ol or GLV to the plastic dummies (models) significantly increased its attraction to the tea aphids (Figure 7). GLV seemed to be more attractive than (*Z*)-3-hexen-1-ol, but both were less attractive than the real tea seedlings (Figure 7).

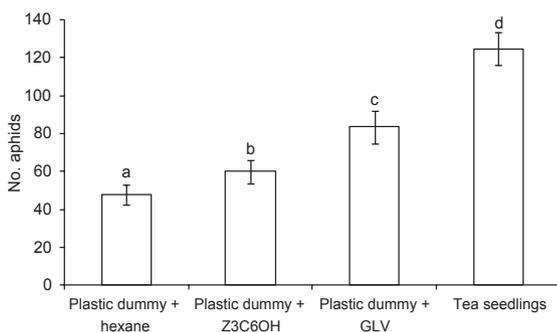
#### Discussion

This is the first detailed report on behavioral and EAG responses of the winged tea aphid, *T. aurantii*, to host-plant volatiles. Our results show that *T. aurantii* are able to perceive the plant volatile compounds tested, especially (*Z*)-3-hexen-1-ol, (*E*)-2-hexenal, *n*-hexanol, 2-penten-1-ol, *n*-pentanol, methyl salicylate, and indole. Earlier studies on other aphids reported similar results, e.g., (*E*)-2-hexenal elicited strong EAG responses by wheat aphid, *Sitobion avenae* (Fabricius) (Yan & Visser, 1982; Liu et al., 2003), pea aphid, *Acyrtosiphon pisum* (Harris) (van Giesse et al., 1994), oat bird-cherry aphid, *Rhopalosiphum padi* (L.) (Liu et al., 2003), and black bean aphid, *Aphis fabae* Scopoli (Webster et al., 2008). Among 80 volatile compounds tested, (*E*)-2-hexenal elicited the strongest EAG responses from vetch aphid, *Megoura viciae* Buckton (Visser & Piron, 1995). (*E*)-2-hexenal also elicited large EAG responses of the proximal primary rhinaria in *A. fabae* (Park & Hardie, 2004). (*Z*)-3-hexen-1-ol reportedly elicited strong EAG responses by various aphid species (Visser & Piron, 1995; Park & Hardie, 2003; Webster et al., 2008).

In the present study, (*Z*)-3-hexen-1-ol elicited the largest EAG activity and the strongest behavioral responses in the olfactometer bioassay, as well as significantly improved the attraction of tea aphids to the plastic model tea seedlings. (*Z*)-3-hexen-1-ol and (*E*)-2-hexenal are the main aroma components released from fresh tea shoots (Li, 2000). These two volatile components may be important compounds that tea aphids use to locate their host tea plants. However, as the real tea seedlings were much more attractive to



**Figure 6** Mean ( $\pm$  SD) number of aphids per trap on the six colors of sticky boards at (A) 15 or (B) 30 cm distance from the central aphid source in the greenhouse, and at (C) 60 or (D) 90 cm height in the tea plantation (five replicates). Colors: (1) snow-white, (2) rape-flower yellow, (3) Chinese olive yellow-green, (4) calcedony green, (5) malachite green, and (6) blue green. Bars with different letters within the same test were significantly different (ANOVA followed by Duncan's new multiple range test:  $P < 0.05$ ).



**Figure 7** Mean ( $\pm$  SD) number of tea aphids landing on tea seedlings and plastic seedling models with different tea plant volatiles in the greenhouse during a 120-h testing period (four replicates). Bars with different letters were significantly different (ANOVA followed by Duncan's new multiple range test:  $P < 0.05$ ). GLV, green leaf volatiles.

aphids than the plastic models baited with (*Z*)-3-hexen-1-ol or GLV, other volatile compounds from tea shoots might be responsible for enhancing the aphid attraction. In some other insects, a blend of volatiles elicited stronger behavioral responses than did the individual compounds (Byers et al., 1985; Cha et al., 2008; Padmaja et al., 2010; Webster et al., 2010). Some studies have investigated the optimal combinations of host volatiles for efficient monitoring or mass-trapping of pests (Zhang et al., 1999; Hammack, 2003; Blackmer et al., 2004; Leskey et al., 2005; Pinero et al., 2006). Further field work on testing the synthetic blends of tea shoot volatiles at natural ratios is underway.

The color of fresh (tender) leaves and buds of tea plants has more yellow characteristics (i.e., light yellow-green) than the color of mature leaves (dark green). We found that tea aphids prefer yellow over green. The adults of mustard leaf beetle, *Phaedon cochleariae* Fabricius, prefer younger (green) over mature (yellow) leaves; the former contain more nutrition (Kühnle & Müller, 2011). Aphids seem to respond differentially to leaf colors (Döring & Hardie, 2007), shapes and sizes, and other visual factors in the host location process (Prokopy & Owens, 1983; Cornelius et al., 1999; Bostanian & Racette, 2001; Horridge, 2006; Brévault & Quilici, 2007). Additional behavioral bioassays on tea aphid responses to hues, chromas, lightness (values), and wavelength of the tea plant colors are needed. The hues of the dry finished product teas and the colors, aromas, and tastes of their soups are the key factors that determine the quality of the merchant teas. Furthermore, the hues and the colors of tea soups are normally measured using a chroma meter. Thus, we used the chroma meter to measure the color of sticky boards in the present paper (Table 2). The tomato fruit fly, *Neoceratitis cyanescens* (Bezzi), preferred the bright orange spheres, whose attractiveness relied more on the proportion of reflected light in the spectral region around 610 nm than brightness of color (Brévault & Quilici, 2007). The reflective wavelength seemed to be more important than the intensity for the mustard leaf beetle (Kühnle & Müller, 2011).

Visual characteristics together with odors are key components of the primary attraction mechanisms of some hemipteran insects (Vaishampayan et al., 1975; Meierdirk & Oldfield, 1985). In males of *P. cochleariae*, visual cues

may override olfactory cues; in females, both are equally important. Moreover, females are more sensitive to color discrimination, which reflect difference in ecological requirements and/or physiological abilities (Kühnle & Müller, 2011). During landing and oviposition, cabbage root fly, *Delia radicum* L. (Košťál, 1993), and diamondback moth, *Plutella xylostella* (L.) (Couty et al., 2006), use physical and chemical cues to locate their hosts. Combining olfactory and visual stimulants resulted in a stronger behavioral response of the parasitoid *Microplitis croceipes* (Cresson) than did the individual stimulants (Wäckers & Lewis, 1994). Recently, a syrphid fly, *Episyrphus balteatus* (De Geer), was found to use olfactory and not visual cues to find a pollen/nectar host plant (Primante & Dötterl, 2010).

In addition to the tea aphid, there are several other pests that damage tea plants (Hazarika et al., 2009). However, because tea is extremely important in the beverage industry, insecticidal control of tea pests should be reduced or even banned if possible. Any non-toxic approaches including trapping with color/odor lures should be encouraged in contemporary tea pest management practices. For example, yellow sticky boards have been used in Japanese tea fields for monitoring the destructive mulberry scale, *Pseudaulacaspis pentagona* (Targioni) population (Kaneko et al., 2006). In tea gardens, yellow sticky boards caught many tea leafhoppers, *Jacobiasca formosana* Paoli, spiny-blackfly, *Aleurocanthus spiniferus* (Quaintance), and black tea thrips, *Dendrothrips minowai* Priesner (Hsiao, 1997). To the best of our knowledge, this is the first investigation that combines tea shoot volatiles and colors to lure pests in tea plantations. In future, host-plant attractants could contribute to semiochemical-based monitoring and management practices of many species of pests (El-sayed et al., 2006; Germinara et al., 2011).

Our experiments indicate that tea aphids combine visual and olfactory modalities to recognize and select their host plants. The long-term goal of our research effort is to develop an efficient trapping system that will include a powerful plant volatile-based synthetic lure and an optimal color, size, and shape of sticky trap. This trapping system can be used to monitor aphid populations and mass-trap the winged aphids in tea fields as a way to improve management of tea aphids. Unlike the powerful species-specific sex pheromones or aggregation pheromones of insects (Witzgall et al., 2010), plant volatile attractants (kairomones) are normally less attractive and specific but they can be processed into the attract-and-kill formulations (Socorro et al., 2010) and used in pest management. More work is needed to optimize the kairomonal composition and ratios as well as dispenser technology for the tea aphid.

## Acknowledgments

This research were supported by a grant from the National Natural Science Foundation of China (31071744), two grants from the Zhejiang Provincial Natural Science Foundation of China (Y3100375, Y3090531), a fund from the Technological Project of Zhejiang Province of China (2011C22046), and a grant from Solidaridad Foundation of The Netherlands (SOL 2010/714). The authors thank two anonymous reviewers for their helpful comments and suggestions on the manuscript.

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