Supplementary material for Song, Beizhou, Yinping Liang, Sizhou Liu, Linfeng Zhang, Guangbo Tang, Teng Ma, and Yuncong Yao—Behavioral responses of *Aphis citricola* (Hemiptera: Aphididae) and its natural enemy *Harmonia axyridis* (Coleoptera: Coccinellidae) to non-host plant volatiles. Florida Entomologist 100: 411–421.

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Abstract

Plant volatiles can act as chemical signals that influence the behavior and distribution of insects. Although considerable information has been acquired on the effects of plant volatiles emitted from plants on herbivorous insects and their natural enemies, practical implementation of this knowledge is still lacking. We investigated 3 aromatic plant species, French marigold, *Tagetes patula* L. (Asteraceae), ageratum, *Ageratum houstonianum* Mill. (Asteraceae), and catnip, *Nepeta cataria* L. (Lamiaceae), to test their effectiveness in repelling or attracting spirea aphid, *Aphis citricola* van der Goot (Hemiptera: Aphididae), and its natural enemy, the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in the field and the laboratory. We found that intercropping apple trees *Malus* spp. (Rosaceae) with aromatic plants in an orchard significantly reduced the number of *A. citricola* aphids present, but had the opposite effect on *H. axyridis*. In addition, the association between *H. axyridis* and *A. citricola* numbers was strengthened when the intercropping included French marigold. Using an H-tube olfactometer, we found that *A. citricola* was repelled by French marigold and catnip, whereas *H. axyridis* was attracted most by French marigold. Volatile analysis revealed that the sesquiterpenes D-limonene and terpinolene and the alcohol 2-ethyl-1-hexanol were the most abundant volatile compounds released by French marigold and catnip. *Harmonia axyridis* was significantly attracted by 12.5 μL/L D-limonene, 50 μL/L terpinolene, and 25 μL/L of a 1:1 mixture of the 2 compounds, but was repelled by higher concentrations of D-limonene. The results suggest that aromatic plants increase the resistance of apple trees to *A. citricola* both directly, by reducing the population of *A. citricola* through chemical repulsion, and indirectly, by increasing the *H. axyridis* population through chemical attraction.

Key Words: aphid; aromatic plant; repellency; attractancy

Resumen

Los volátiles de las plantas pueden actuar como señales químicas que influyen en el comportamiento y distribución de los insectos. Aunque se ha adquirido bastante información sobre los efectos de los volátiles vegetales emitidos por las plantas sobre los insectos herbívoros y sus enemigos naturales, todavía falta la aplicación práctica de estos conocimientos. Se investigaron 3 especies de plantas aromáticas, clavel de moro, *Tagetes patula* L. (Asteraceae), ageratum, *Ageratum houstonianum* Mill. (Asteraceae) y menta de gato, *Nepeta cataria* L. (Lamiaceae), para probar su efectividad en repeler o atraer al pulgón spirea, *Aphis citricola* van der Goot (Hemiptera: Aphididae), y su enemigo natural, la mariquita asiática de multicolores, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), en el campo y en el laboratorio. Se encontró que al intercalar manzanos *Malus* spp. (Rosaceae) con plantas aromáticas en un huerto redujeron significativamente el número de áfidos de *A. citricola* presentes, pero tuvieron el efecto opuesto en *H. axyridis*. Además, la asociación entre *H. axyridis* y el número de *A. citricola* se fortaleció cuando el clavel de moro fue intercalado. Usando un olfatómetro tubo-H, encontramos que *A. citricola* fue repelido por el clavel de moro y la menta de gato, mientras que *H. axyridis* fue atraído más por el clavel de moro. El análisis volátil reveló que los sesquiterpenos D-limoneno y terpinoleno y el alcohol 2-etil-1-hexanol eran los compuestos volátiles más abundantes liberados por el clavel de moro y la menta de gato. *Harmonia axyridis* fue atraído significativamente por 12,5 μL / L de D-limoneno, 50 μL / L de terpinoleno y 25 μL / L de una mezcla 1:1 de los 2 compuestos, pero fue repelido por mayores concentraciones de D-limoneno. Los resultados sugieren que las plantas aromáticas aumentan la resistencia de los manzanos a *A. citricola* tanto directamente, reduciendo la población de *A. citricola* mediante la repulsión química, como indirectamente, aumentando la población de *H. axyridis* a través de la atracción química.

Palabras Clave: áfido; planta aromática; repelencia; atraccion

Supplementary Tables

Table S1. Relative amount (% of internal standard peak area) of volatile compounds released by French marigold.

Retention time	Compound	Relative amount (%)
2.72	pentanal	1.22
3.28	1,6-hexanediol	1.76
4.19	(2*E*)-2-octene	1.52
4.49	hexanal	4.76
4.82	hexamethylcyclotrisiloxane	0.96
6.01	ethylbenzene	0.90
6.27	3,5-octadiyne	2.10
6.94	1,2-xylene	2.78
8.26	alpha-pinene	2.18
9.05	2-ethyl-hexanal	2.25
10.15	octamethylcyclotetrasiloxane	1.44
10.3	(6*Z*)-1,6,10-dodecatriene,7,11-dimethyl-3-methylene	1.35
10.82	4-methyl-1-(methylethyl)bicyclo[3.1.0]hexane	1.22
11.5	*p*-isopropyltoluene	3.59
11.67	D-limonene	16.73
11.78	2-ethyl-1-hexanol	25.90
11.98	ocimene	3.40
13.71	terpinolene	14.82
13.89	1-methyl-4-(1-methylethenyl)-benzene	7.76
14.65	4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene	1.32
15.29	(4*E*,6*Z*)-2,6-dimethyl-2,4,6-octoatriene	2.01

Table S2. Relative amount (% of internal standard peak area) of volatile compounds released by catnip.

Retention time	Compound	Relative amount (%)
4.2	(2*E*)-2-octene,	1.20
4.48	hexanal	2.62
5.92	leaf alcohol	1.49
6.01	ethylbenzene	8.04
6.27	o-xylene	9.05
6.8	3-heptanone	1.26
6.93	*p*-xylene	7.60
8.24	alpha-pinene	1.24
9.03	2-ethyl-hexanal	1.14
9.76	beta-pinene	1.00
10.15	octamethylcyclotetrasiloxane	6.41
10.27	beta-pinene	2.48
10.6	3-octanol	1.42
10.89	(3*Z*)-3-hexen-1-ol,1-acetate	2.91
11.09	1,3-dichlorobenzene	1.86
11.65	D-limonene	3.85
11.76	2-ethyl-1-hexanol	23.29
11.96	3,7-dimethyl-(3*E*)-1,3,6-octatriene	4.12
12.34	3,7-dimethyl-1,3,6-octatriene	4.76
13.69	terpinolene	7.75
14.49	nonyl aldehyde	0.95
14.68	phenethyl alcohol	2.59
15.28	1,5,5-trimethyl-3-methylene-cyclohexene,	1.83
15.69	2,2,4,4,6,6,8,8,10,10-decamethyl-cyclopentasiloxane	1.16

Table S3. Relative amount (% of internal standard peak area) of volatile compounds from air. "—" indicates a substance that was not identified.

Retention time	Compound	Relative amount (%)
3.04	pentanal	1.55
3.64	3-methyl-1-butanol	5.57
3.69	2-methyl-1-butanol	1.40
4.63	—	2.47
4.94	hexanal	5.03
5.11	—	1.17
5.31	hexamethylcyclotrisiloxane	1.79
6.53	ethylbenzene	1.02
6.8	*o*-xylene	1.29
7.35	5-methyl-3-hexanone	2.21
7.49	—	2.29
9.65	2-ethyl-hexanal,	2.02
10.75	octamethylcyclotetrasiloxane	11.49
12.41	2-ethyl-1-hexanol	27.35
15.4	phenethyl alcohol	1.99
16.35	decamethylcyclopentasiloxane	4.51
20.23	cinnamaldehyde	5.38
20.34	cinnamaldehyde	2.67
22.19	dodecamethylcyclohexasiloxane	18.80

Supplementary Figures

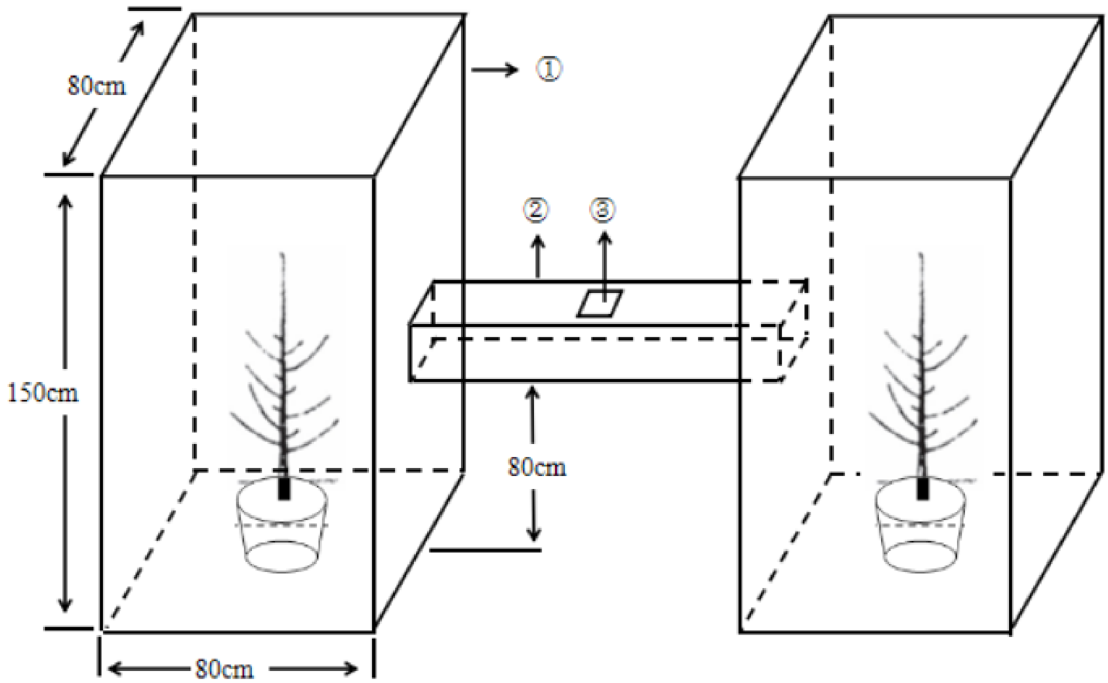

Fig. S1. H-tube olfactometer schematic diagram: ①: Plastic boxes (80 cm in length, 80 cm in width, 150 cm in height); ②: cross arm (20 cm in length, 8 cm in width, 8 cm in height); ③: hole used to introduce insects (5 cm in length, 5 cm in width).

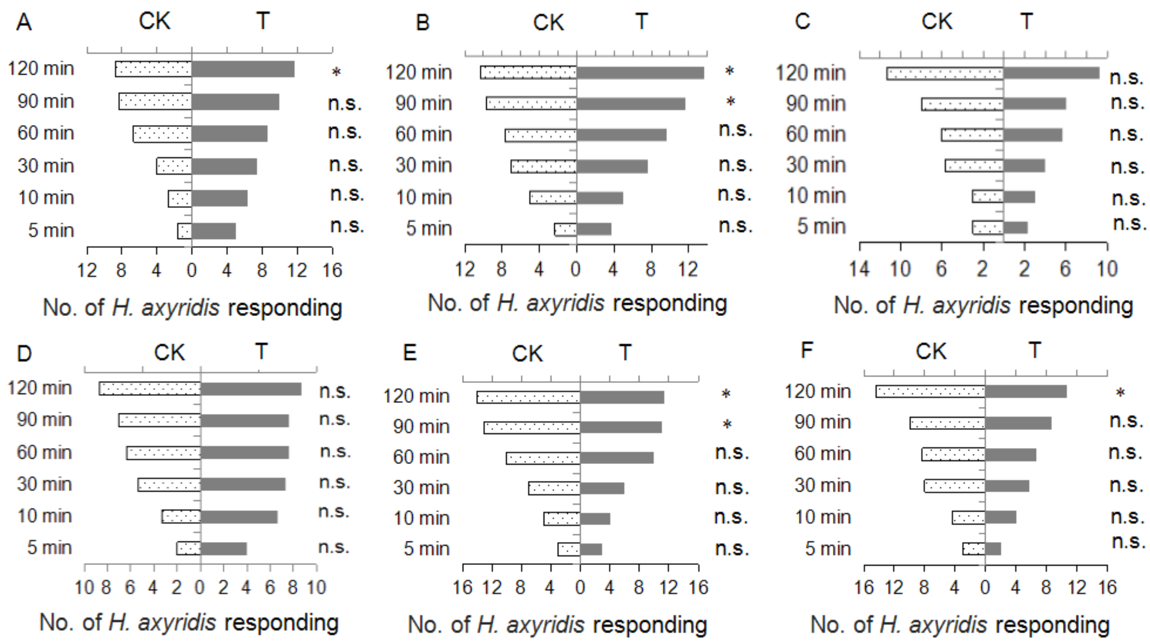

Fig. S2. Response of *Harmonia axyridis* adults to French marigold (*Tagetes patula*) (A, B, and C) and catnip (*Nepeta cataria*) (D, E, and F). A and D: no aphids, B and E: aphids present; C and F: aphids were applied for 2 h and removed. T: Apple trees + aromatic plants; CK: apple trees only. Asterisks represent level of significance (by paired *t*-test): * significant ($P < 0.05$); n.s. no significant difference.

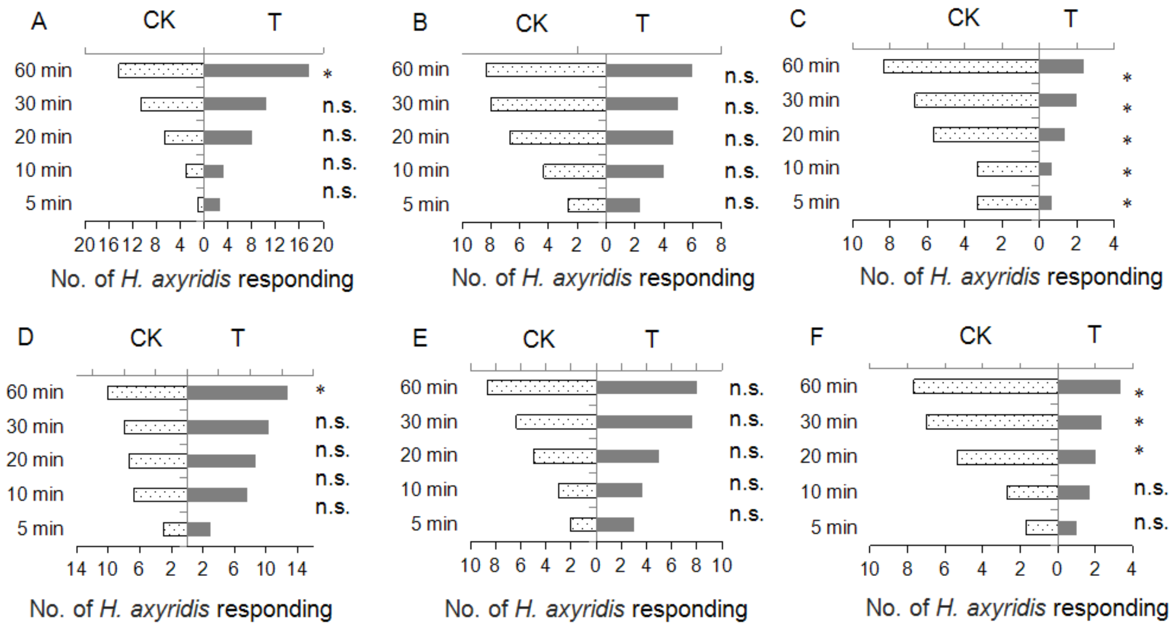

Fig. S3. Response of *Harmonia axyridis* adults to 12.5µL/L (A, D), 25µL/L (B, E), and 50µL/L (C, F) D-limonene (A, B, and C: no aphids; D, E, and F: aphids present). T: Apple tree + D-limonene; CK: apple tree + distilled water. Asterisks represent level of significance (by paired *t*-test): * significant ($P < 0.05$); n.s. no significant difference.

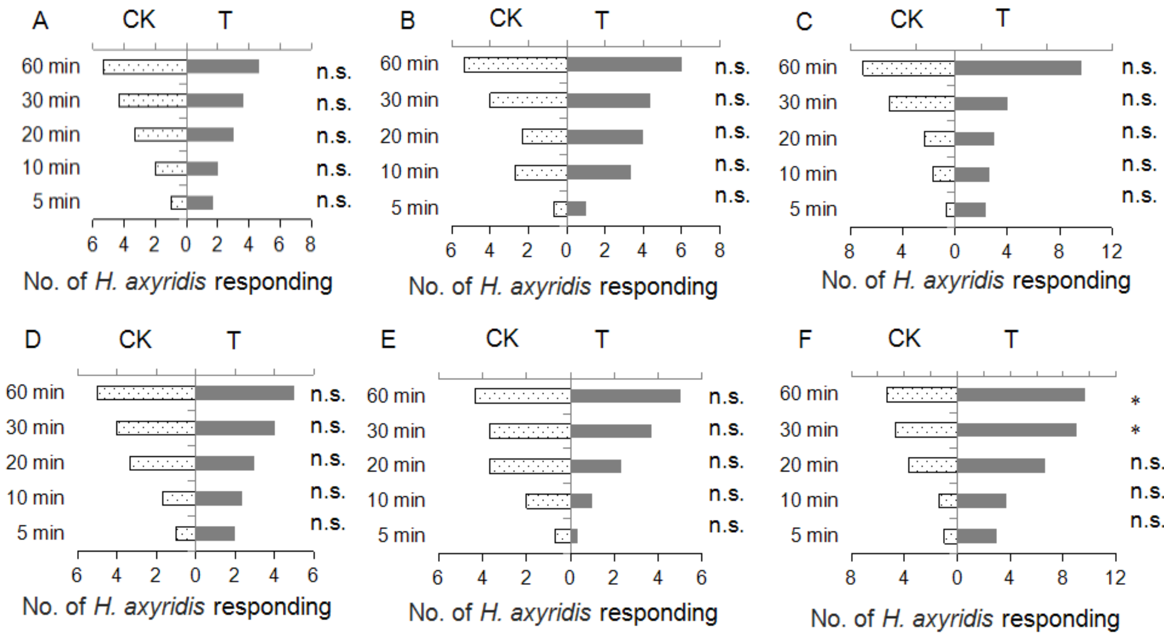

Fig. **S4**. Response of *Harmonia axyridis* adults to 12.5 μL/L (A, D), 25 μL/L (B, E), and 50 μL/L (C, F) terpinolene (A, B, and C: no aphids; D, E, and F: aphids present). T: Apple tree + terpinolene; CK: apple tree + distilled water. Asterisks represent level of significance (by paired *t*-test): * significant ($P < 0.05$). n.s. no significant difference.

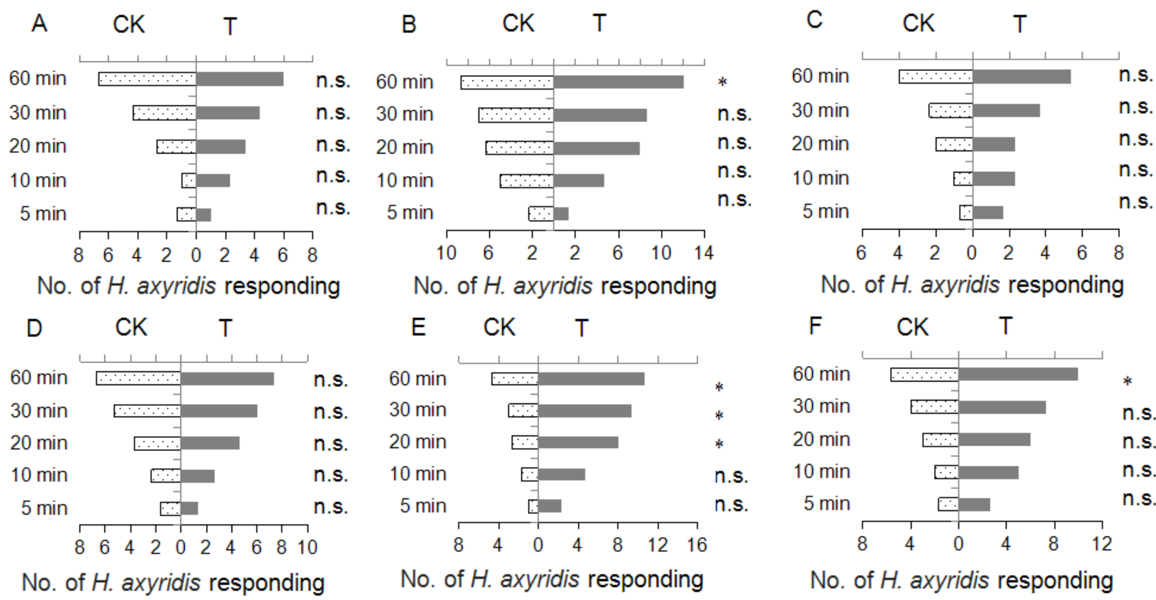

Fig. S5. Response of *Harmonia axyridis* adults to 12.5 µL/L (A, D), 25 µL/L (B, E), and 50 µL/L (C, F) 1:1 mixed D-limonene and terpinolene (A, B, and C: no aphids; D, E, and F: aphids present). T: Apple tree + terpinolene; CK: apple tree + distilled water. Asterisks represent level of significance (by paired *t*-test): * significant ($P < 0.05$); n.s. no significant difference.