Bulletin of Insectology ***70*** *(2): 273-282, 2017*
ISSN 1721-8861

Using herbivore-induced plant volatiles to attract lacewings, hoverflies and parasitoid wasps in vineyards: achievements and constraints

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Abstract

Plants produce volatile organic compounds (VOCs) as an adaptive response to abiotic and biotic stresses. The feeding behaviour of phytophagous arthropods can elicit the production of VOCs in the plant that can be used by predators and parasitoids to locate their prey. These VOCs have been classified as herbivore induced plant volatiles (HIPVs), which are considered highly-detectable synomones helping natural enemies to locate the host habitat. In two vineyards in Tuscany (Central Italy) we tested the attractiveness of sticky traps baited with two synthetic HIPV blends shown previously to be attractive to insect predators (Chrysopidae and Syrphidae) and parasitoids (Braconidae and Ichneumonidae). We also used Malaise traps to investigate the presence of the target insects in the studied areas. White sticky traps baited with a blend of methyl salicylate, acetic acid and 2-phenylethanol were strongly attractive to adult lacewings (Chrysopidae) of the genus *Chrysoperla*, but not to lacewings of the genus *Pseudomallada*. On the other hand, yellow sticky traps baited with a blend of geraniol and 2-phenylethanol were not attractive to Syrphidae. Both blends captured a relatively small number of Ichnemonoidea. The effective use of HIPVs to attract lacewings, hoverflies and parasitoid wasps in the field is discussed, focussing on existing constraints and possible future developments.

Key words: plant volatiles, Chrysopidae, Syrphidae, Ichneumonoidea, sticky traps, Malaise traps.

Introduction

Plants have evolved communication systems based on the production of volatile cues as an adaptive response to abiotic and biotic stresses (Baldwin *et al*., 2006). The source of the stress influences the quantity and composition of the emitted bouquet of volatile organic compounds (VOCs), which could be defined as a chemical vocabulary containing more than 1,000 words (Dudareva *et al*., 2004).

VOCs mediate intra- and interspecific interactions among plants and between plants and other organisms, particularly arthropods of different trophic levels (Baldwin *et al*., 2006; Pichersky *et al*., 2006; Hare, 2011). The feeding behaviour and/or the egg laying of phytophagous arthropods can elicit the production of VOCs in the plant, which can then be used by predators and parasitoids to locate their prey (Price *et al*., 1980; Dicke and van Loon, 2000; Cusumano *et al*., 2015).

These VOCs have been classified as herbivore induced plant volatiles (HIPVs) (Dicke and Sabelis, 1988; Takabayashi *et al*., 1995; Karban and Baldwin, 1997; Baldwin *et al*., 2006; Kessler and Halitschke, 2007). HIPVs usually include green-leaf volatiles (GLVs, C6 aldehydes, alcohols and acetates), terpenes and aromatic compounds (Pichersky *et al.*, 2006). Although the ecological roles and the behavioural interactions mediated by HIPVs are not fully understood, volatile-mediated interactions have a considerable potential to influence the structure and dynamics of ecosystems by inducing indirect host plant resistance, repelling phytophages and/or by attracting and concentrating the natural enemies of phytophages into a specific location (Turlings and Ton, 2006; Rodriguez-Saona *et al.*, 2012).

Various laboratory and field studies have reported contradictory and variable results regarding the attractiveness of HIPVs to specific target species. Indeed, several factors influence interactions among organisms and the role of volatile compounds can change depending on the environmental context, developmental stage of the target species, as well as the number of compounds perceived by the organism (Hare, 2011; Kaplan, 2012).

HIPVs can increase the diversity and the density of beneficial insect species within many fruit and vegetable crops (Vinson, 1977; Bernasconi Ockroy *et al*., 2001; James and Price, 2004; James, 2005; Yu *et al*., 2008; Orre *et al*., 2010; Simpson *et al*., 2011a), and in some cases, HIPVs can also decrease pest numbers and crop damage (Khan *et al*., 1997; James and Price, 2004; Simpson *et al.*, 2011b). Conversely, in Mexican maize fields, von Mérey *et al*. (2011) found more insect pest damage and only a slight effect on parasitoid attraction when synthetic GLVs were applied.

HIPVs are not only a resource for biological control, but also one of the most interesting and controversial new topics in agricultural research (Hare, 2011; Kaplan, 2012). Various ways of using HIPVs have been proposed. Natural sources of HIPVs have successfully been employed in Kenya, intercropping *Melinis minutiflora* Beauv. (Poaceae) in maize fields. In this 'push and pull' study, HIPVs produced by *M. minutiflora*, significantly repelled stem-borers, decreasing the level of infestation in the main crop and also increasing the larval parasit-

ism of stem-borers by *Cotesia sesamiae* (Cameron) (Hymenoptera Braconidae) (Khan *et al.*, 1997). In grapes, *Brassica* and sweet corn, Simpson *et al*. (2011b; 2011c) successfully used synthetic HIPVs to recruit biocontrol agents (BCAs) and intercropping flowers as a source of food and shelter for enhance BCA establishment in an 'attract and reward' experiment.

However, it has also been hypothesized that the use of HIPVs could cause undesirable side effects, as concentrating natural enemies in a treated area could weaken the defenses of the surrounding areas (Vinson, 1977; Gross, 1981; Jones *et al*., 2011). HIPVs may also stimulate plants to produce other VOCs. These VOCs could attract other insects as non-target natural enemies (James and Price 2004, Toth *et al*., 2006) or phytophages (von Mérey *et al*., 2011), thus influencing the dynamics of the ecosystem.

Methyl salicylate (MeSa) is one of the most tested HIPV compounds, which is commercially available as PredaLure® and used to recruit the natural enemies of agricultural pests (Rodriguez-Saona *et al*., 2011; Gadino *et al*., 2012). Many field studies have shown the attractiveness of MeSa to various natural enemies, such as Coleoptera (Coccinellidae), predaceous Heteroptera (Anthocoridae), Diptera (Syrphidae, Empididae and Sarcophagidae), parasitic Hymenoptera (Braconidae, Encyrtidae and Mymaridae) and Neuroptera (Chrysopidae) in apple orchards (Zhu and Park, 2005; Jones *et al*., 2011), in hops (James and Price, 2004) and in vineyards (James and Price 2004; James *et al*., 2005; Gadino *et al*., 2012). Other compounds that have shown attractiveness to natural enemies are geraniol and 2-phenylethanol. Geraniol attracts Braconidae and Sarcophagidae (James, 2005), 2-phenylethanol (contained in Benallure®) was found to attract *Coleomegilla maculata* (DeGeer) (Coleoptera Coccinellidae) and *Chrysoperla carnea* (Stephens) (Neuroptera Chrysopidae), and some species of Diptera Syrphidae (Kaplan, 2012).

Although most HIPV studies have investigated single compounds, blends of HIPVs may be more suitable for predators and parasitoids (Hare, 2011; Kaplan, 2012). In various North American apple orchards, Jones *et al*. (2011; 2016) achieved a greater attraction of green lacewings by combining different compounds. They also demonstrated the high effectiveness of the blend made up of acetic acid, 2-phenylethanol and methyl salicylate in capturing various species of *Chrysoperla* (Chrysopidae), and of geraniol mixed with 2- phenylethanol to capture *Eupeodes* (Syrphidae) in apple, pear and walnut orchards (Jones *et al*., 2016). Within these families, there are important generalist predators of small arthropods, and some of them are commercially reared and sold as biological control agents (Waage *et al*., 1984; Daane *et al*., 1996).

Given the important predaceous activity of Chrysopidae and Syrphidae in the vineyard (Chambers, 1988; Belcari and Raspi, 1989; Daane *et al*., 1996; Daane and Yokota, 1997; Szentkiralyi, 2001), the same blends reported in Jones *et al*. (2016) were tested for the first time in the vineyard agroecosystem. Since MeSA and geraniol were reported as attractive on Braconidae and Ichneumonidae (Kaplan, 2012), we also studied the attractiveness of the tested blends on those families, which include important parasitoid species feeding on grapevine pests (Bagnoli and Lucchi, 2006; Moreau *et al*., 2010; Scaramozzino *et al*., 2017).

Materials and methods

Experimental area

In two vineyards located in the province of Pisa, Tuscany, Italy (vineyard 1: 43°35'47.265"N 10°32'12.695"E; vineyard 2: 43°35'42.795"N 10°34'18.241"E) (figure 1) we selected two homogeneously squared experimental areas of approximately 1.5 ha, about 3 km away from

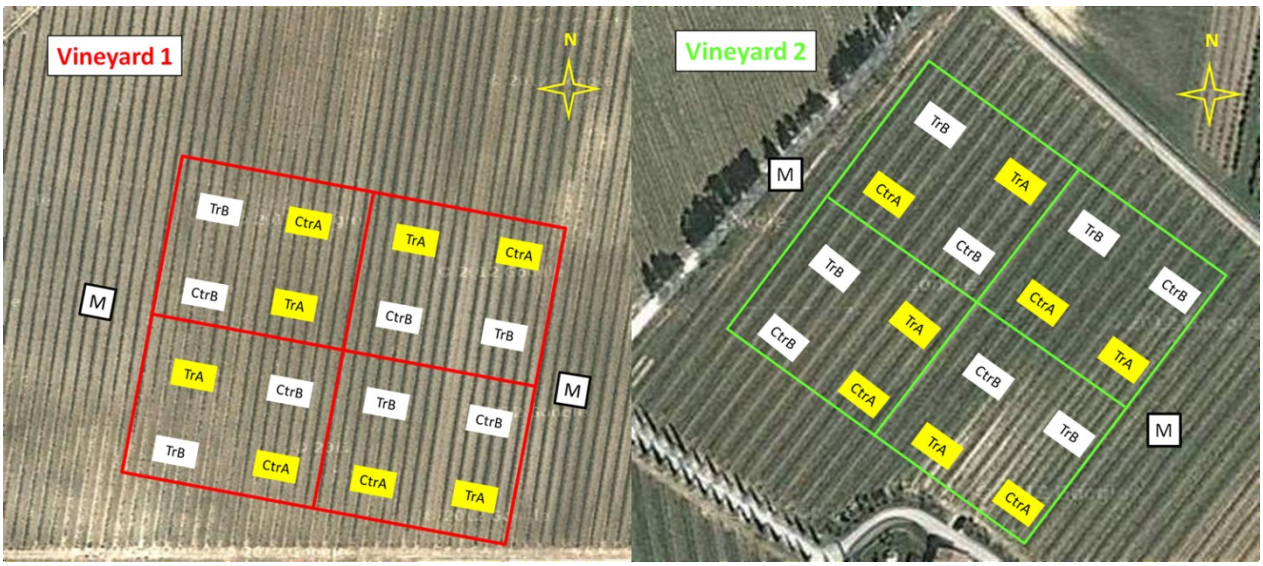

Figure 1. The experimental areas (vineyard 1 and 2) with four treatments and four replications, in a completely randomized design. TrA: yellow sticky traps baited with GER + PE; TrB: white sticky traps baited with MeSa + AA + PE; CtrA: unbaited yellow sticky traps; CtrB: unbaited white sticky traps; M: Malaise traps positions.

Figure 2. A): yellow sticky trap baited with PE + GER; **B)**: white sticky trap baited with MeSa + AA + PE.

each other. The vineyards, located in a rural landscape, were similar in size (about 30 hectares each), plant density (about 4,500 plants/hectare), variety (Sangiovese), canopy training system and plant age, but different in terms of pest management and soil tillage. In vineyard 1, a superficial soil tillage was performed between the rows in summer and a conventional control had been continuously adopted for 12 years against pests (1-2 sprays with organophosphate insecticide per year) and diseases (on average 5 sprays per year with sulfur, dimetomorph and/or cymoxanil, and/or mancozeb and/or phosetil-Al). In contrast, vineyard 2 was organic, with permanent grass and soil cover between the rows. In this vineyard, mating disruption has been used continuously for the last twelve years to control the grapevine moth *Lobesia botrana* (Denis et Schiffermuller) (Lepidoptera Tortricidae), with no additional insecticide sprayings.

Treatments

Following the same procedure recently described by Jones *et al*. (2016), we tested two different HIPV blends using sticky traps as the capture device. Treatment A (TrA), which is designed to be attractive to Syrphidae, in particular adults of the genus *Eupeodes*, was conducted with yellow sticky traps (23 × 14 cm, Back-Folded Yellow Card, Alpha Scents Inc., OR, USA) baited with a combo lure containing 2 ml of geraniol (GER) + 1 ml of 2-phenylethanol (PE). Unbaited yellow sticky traps served as the control (CtrA).

Treatment B (TrB), which is possibly attractive to Chrysopidae, consisted of white sticky traps (18 × 19 cm, Plastic Delta Insert, Alpha Scents Inc., OR, USA), baited with a blend of three different lures containing 2 ml of methyl salicylate (MeSa), 1 ml of acetic acid (AA), and 1 ml of PE, respectively. Unbaited white sticky traps served as the control (CtrB). All chemicals for the lures were obtained from Sigma-Aldrich, St Louis, MO, USA.

In each experimental area, four replicates of the two treatments and the relative controls were placed in a 4 × 4 grid, adopting a completely randomized design. The traps were folded longitudinally to increase their visibility through the rows and fastened between two supporting horizontal wires inside the plant canopy while placed 30 m from each other (figure 2). Lures were attached to the upper wire, as near as possible to the trap, but at a sufficient distance to prevent the risk of contact with the glued trap surface in case of strong winds. They were deployed in the fields on 11 June 2013 and were active until 1 October 2013.

The sticky panels were changed weekly and the lures monthly. When serviced, traps were covered with a transparent plastic film and stored in a freezer (−20 °C) until the captured insects were identified. Only specimens belonging to Chrysopidae, Syrphidae, Braconidae and Ichneumonidae were identified. Chrysopidae and Syrphidae were identified at the genus level, while the Hymenoptera were identified at the subfamily level, ensuring adequate information on the group's compositional and functional biodiversity (Loni and Lucchi, 2014a).

Structure of insect community

Malaise traps capture flying insects randomly and continuously by interception, and provide a reliable description of the insect community structure in different habitats through time and space (Malaise, 1937; Burgio and Sommaggio, 2007; Loni and Lucchi, 2012; Sommaggio and Burgio, 2014). To evaluate the presence and the community structure of the target insects in the studied areas, we deployed two Malaise traps in each vineyard: one in the centre and one on the border of the vineyard as described by Fraser *et al*. (2007) and by Loni and Lucchi (2012; 2014a; 2014b). Malaise traps were located outside the opposite side of the HIPV treated area (figure 1). Malaise trapping occurred during the same period as the sticky panels. Every two weeks we collected and stored the captured insects in a 70% ethanol solution. They were then identified under a stereo-microscope. All Chrysopidae, Syrphidae, Braconidae and Ichneumonidae were classified as belonging to the same taxonomic levels as adopted for the sticky traps.

Statistical analysis

As the vineyards were managed differently, analyses were performed separately for the two experimental units. The total number of each category of captured insects from each treatment was summarized adopting the cumulative insect days index (CID), providing a weekly trend of the data (Shearer *et al*., 2016).

CIDs were calculated as the summary of all the average population densities over the entire sampling period, between two consecutive dates of sampling:

$CID = \sum 0.5\ (Pa + Pb)\ D_{a\text{-}b}$

where Pa and Pb are the population densities (mean insects/per traps) at times a and b respectively, $D_{a\text{-}b}$ is the number of days comprised between time a and b. CID values were log (x + 1) transformed to assume the normality of data distribution and to analyze the sampling with zero captures.

Transformed data were analyzed by single factor ANOVA analysis by considering treatments as fixed factors. Mean values were separated by least statistical differences (LSD) with a P value of 0.01.

Both Malaise and sticky traps exerted their capture activity on the same area. We assumed that the population of a specific taxon would show the same relative abundance for both capture devices. To compare the relative abundances among the Malaise and sticky traps, we used the Pearson χ^2 test. A χ^2 comparison was only performed for *Eupeodes* by considering as variables, in a 2 × 2 table, the total captures of specimens belonging to this genus and the total captures of Syrphidae.

Table 1. Number of Chrysopidae adults caught on sticky traps in two vineyards for treatments (TrA; TrB) and controls (CtrA; CtrB) and in Malaise traps (identification at genus level).

Chrysopidae Genus	Vineyard 1						Vineyard 2					
	Sticky traps					Malaise traps	Sticky traps					Malaise traps
	TrA	TrB	CtrA	CtrB	Total		TrA	TrB	CtrA	CtrB	Total	
Chrysoperla	96	522	11	12	641	4	19	174	12	9	214	3
Pseudomallada		10	4		14	17	11	4	11	5	31	4
Total	96	532	15	12	655	21	30	178	23	14	245	7

Table 2. Number of Syrphidae adults caught on sticky traps in two vineyards for treatments (TrA; TrB) and controls (CtrA; CtrB) and in Malaise traps (identification at genus level).

Syrphidae Genus	Vineyard 1						Vineyard 2					
	Sticky traps					Malaise traps	Sticky traps					Malaise traps
	TrA	TrB	CtrA	CtrB	Total		TrA	TrB	CtrA	CtrB	Total	
Baccha												1
Chrysotoxum	1				1	5		2	2	1	5	
Epistrophe						4						1
Episyrphus	5	1		4	10	6	6	9	3	8	26	9
Eristalinus		1			1	1	1				1	
Eristalis	1	2	1	2	6		4	4	3		11	3
Eumerus												2
Eupeodes	5	6	2	19	32	6	15	32	20	27	94	17
Hammerschmidtia						14						5
Helophilus	1	2			3					1	1	1
Heringia							1	5		3	9	
Lejogaster												6
Melangyna		1			1							7
Melanogaster						5						1
Melanostoma		1			1	149		1		3	4	96
Merodon						1	1			1	2	
Milesia										1	1	
Paragus						426		1		1	2	155
Parasyrphus												3
Pipizella						4		1			1	34
Sphaerophoria		7		9	16	1114	6	36	13	30	85	985
Xanthogramma												1
Total	13	21	3	34	71	1735	34	91	41	76	242	1327

Results

Sticky panel traps

In the two vineyards we collected a total of 1,479 specimens (848 in vineyard 1 and 631 in vineyard 2) belonging to the four insect families under study.

C h r y s o p i d a e

Chrysopidae were the most abundant group captured in each vineyard, with 655 specimens in vineyard 1 and 245 in vineyard 2. They were represented almost completely by the genus *Chrysoperla*. The other captured genus was *Pseudomallada*, which accounted for only 2% and 12% of specimens, respectively in vineyards 1 and 2 (table 1). TrB was significantly attractive to the *Chrysoperla* genus, but not to *Pseudomallada*, in both experimental areas ($P < 0.001$; F 78.59; df 3, 56). In addition, TrA was significantly attractive to *Chrysoperla* in vineyard 1, but not in vineyard 2 (table 5).

S y r p h i d a e

A total of 71 Syrphidae were captured in vineyard 1 versus 242 in vineyard 2. The most abundant genus was *Eupeodes*, which was caught in both areas and on all the baited and unbaited traps, followed by *Sphaerophoria* and *Episyrphus*. The three mentioned genera accounted for more than 80% of the total number of hoverflies caught in each vineyard (table 2). The CID test was performed only for the genera *Eupeodes* and *Spaerophoria* since their numbers allowed a statistical approach (table 5). In both vineyards no significant differences were found between TrA and CtrA, although in vineyard 1 significant differences emerged between TrA vs CtrB, TrB vs CtrA and CtrA vs CtrB ($P < 0.001$; F 13.15; df 3, 56). In vineyard 2, significant differences were found between TrA vs TrB , TrA vs CtrB and CtrA vs CtrB ($P < 0.001$; F 10.5; df 3, 60) (table 5). More *Sphaerophoria* were captured on the white traps than on the yellow traps, but no differences were found between the treatments and their control. In addition, no significant differences were found for the genus *Eupeodes* in vineyard 1 ($P = 0.02$; F 4.3; df 3, 40) and in vineyard 2 ($P = 0.04$; F 3.05; df 3, 44) (table 5).

B r a c o n i d a e a n d I c h n e u m o n i d a e

Eleven and twelve subfamilies of Braconidae, represented by 88 and 78 specimens were captured in vineyards 1 and 2, respectively. Microgastrinae, Alysiinae and Meteorinae were the most abundant subfamilies in vineyard 1, and Cheloninae, Microgastrinae and Alysiinae were the most abundant subfamilies in vineyard 2 (table 3).

A total of 34 adults of Ichneumonidae belonging to 11 subfamilies were captured in vineyard 1, and 66 in vineyard 2. The most abundant subfamilies were Cryptinae and Metopiinae in vineyard 1, and Cryptinae and Ichneumoninae in vineyard 2 (table 4).

With regard to Braconidae, significant differences were found in vineyard 1, between TrA vs CtrA and CtrB ($P < 0.001$; F 7.6; df 3, 56), and in vineyard 2 between TrA vs CtrB and CtrA vs CtrB ($P < 0.001$; F 9.7; df 3, 60). No statistically significant differences were found for the Ichneumonidae captured in vineyard 1 ($P = 0.1$; F 2.12; df 3, 56) and in vineyard 2 ($P = 0.07$; F 2.45; df 3, 60) (table 5).

Table 3. Number of Braconidae adults caught on sticky traps in two vineyards for treatments (TrA; TrB) and controls (CtrA; CtrB) and in Malaise traps (identification at subfamily level according to Sharkey, 1997).

Braconidae Subfamily	Vineyard 1						Vineyard 2					
	Sticky traps					Malaise traps	Sticky traps					Malaise traps
	TrA	TrB	CtrA	CtrB	Total		TrA	TrB	CtrA	CtrB	Total	
Agathidinae						10	2				2	79
Alysiinae	1	14	4	3	22	189	2	4	6	2	14	561
Aphidiinae						88			1		1	405
Braconinae	3	3	2		8	126	1		3		4	48
Cheloninae	5	1		1	7	80	5	5	9	1	20	27
Doryctinae			1		1	4						6
Euphorinae	1	2		1	4	74	2	3	3	1	9	158
Helconinae	1	2			3	33			1		1	18
Homolobinae						128	2		2		4	38
Hormiinae						7						8
Macrocentrinae	1				1	13						2
Meteorinae	7	6	1	2	16	4	1		1		2	4
Microgastrinae	12	6	3	1	22	621	6	5	6	2	19	433
Miracinae						3	1				1	2
Neoneurinae						1						1
Opiinae	1		1		2	52						70
Rogadinae		2			2	59	1				1	35
Total	32	36	12	8	88	1492	23	17	32	6	78	1895

Table 4. Number of Ichneumonidae adult caught on sticky traps in two vineyards for treatments (TrA; TrB) and controls (CtrA; CtrB) and in Malaise traps (identification at subfamily level according to Broad, 2016).

Ichneumonidae Subfamily	Vineyard 1						Vineyard 2					
	Sticky traps					Malaise traps	Sticky traps					Malaise traps
	TrA	TrB	CtrA	CtrB	Total		TrA	TrB	CtrA	CtrB	Total	
Anomaloninae						18			1		1	5
Banchinae						28	1		3		4	24
Brachycyrtinae						2						
Campopleginae	1		1		2	291	2	1	2		5	363
Cremastinae						5	1				1	4
Cryptinae	4	4	3	1	12	422	8	3	6	3	20	361
Ctenopelmatinae						4						15
Cylloceriinae						2						5
Diplazontinae		1		1	1	37	5	1			6	106
Hybrizontinae						59						51
Ichneumoninae	2		1		3	51	9	1	2	2	14	74
Mesochorinae		2			2	40		4			4	27
Metopiinae	5	2	1		8	49	2			1	3	107
Ophioninae						3						5
Orthocentrinae				1	1	27						181
Pimplinae	1		3		4	95	2	1		1	4	45
Tersilochinae						13						30
Tryphoninae				1	1	9	3			1	4	34
Total	13	9	9	4	34	1154	33	11	14	8	66	1437

Table 5. Mean (± SE) cumulative insect-days for insect groups captured by sticky traps in vineyards 1 and 2.

	Cumulative Insect Days per trap					
Vineyard 1	*Chrysoperla*	Syrphidae	*Eupeodes*	*Sphaerophoria*[1]	Braconidae	Ichneumonidae
TrA	20.6 ± 14.1 b	2.4 ± 2.9 bc	1.4 ± 0.7	-	6.3 ± 4.9 a	2.7 ± 3.0
TrB	115.8 ± 55.6 a	4.3 ± 3.3 ab	1.7 ± 1.7	1.66 ± 1.6	6.3 ± 9.7 ab	2.1 ± 2.2
CtrA	2.4 ± 2.9 c	0.7 ± 0.9 c	0.6 ± 0.9	-	2.1 ± 3.1 bc	2.1 ± 1.6
CtrB	2.8 ± 4.6 c	6.6 ± 5.2 a	4.9 ± 5.3	2 ± 2.4	1.7 ± 3.4 c	0.8 ± 0.9
F	78.59	13.15	4.3	2.97	7.6	2.12
df	3, 56	3, 56	3, 40	14	3, 56	3, 56
P	< 0.001	< 0.001	0.02	0.48	< 0.001	0.1
Vineyard 2	*Chrysoperla*	Syrphidae	*Eupeodes*	*Sphaerophoria*	Braconidae	Ichneumonidae
TrA	4.1 ± 4.3 b	7.2 ± 8.8 b	3.9 ± 4.3	1.4 ± 1.5 d	4.7 ± 2.8 ab	7 ± 8.9
TrB	37.7 ± 19.1 a	20.1± 10.3 a	9.5 ± 6.7	8.1 ± 6.7 ab	2.9 ± 4.5 bc	2.4 ± 2.2
CtrA	2.5 ± 3.8 bc	8-9 ± 8.8 b	5.5 ± 6.5	2.8 ± 2.2 bcd	6.2 ± 4.8 a	3.1 ± 2.5
CtrB	1.9 ± 3.1 c	16.7 ± 8.3 a	8.1 ± 4.2	6.9 ± 5.8 bc	1.2 ± 1.6 c	1.7 ± 1.6
F	40.66	10.5	3.05	6.9	9.7	2.45
df	3, 60	3, 60	3, 44	3, 56	3, 60	3, 60
P	< 0.001	< 0.001	0.04	< 0.001	< 0.001	0.07

Means in a column for each vineyard followed by different letter are significantly different ($P \leq 0.01$). Data natural log ($x + 1$) transformed, actual means reported.

[1] As regards the genus *Sphaerophoria* in vineyard 1, a t-test was performed because specimens were captured only in two treatments.

Malaise traps

Malaise traps captured a total of 4,402 specimens belonging to the four insect families under study in vineyard 1 and 4,666 in vineyard 2. Syrphidae was the most abundant family in vineyard 1, followed by Braconidae and Ichneumonidae. On the contrary, the most abundant family in vineyard 2 was Braconidae, followed by Ichneumonidae, Syrphidae and Chrysopidae.

Chrysopidae

Chrysopidae represented just a small fraction of the total captures in both vineyards. In total, Malaise traps captured 21 adults of the genus *Pseudomallada* and 7 of the genus *Chrysoperla* (table 1).

Syrphidae

The largest number of specimens were captured in vineyard 1 and subdivided into 12 genera. Vineyard 2

showed a higher richness, with a collection of 17 genera (table 2). In both experimental areas, the three most abundant genera were *Sphaerophoria*, *Paragus* and *Melanostoma*, which accounted for more than 90% of the total captures (table 2).

Braconidae and Ichneumonidae

We found specimens belonging to 17 subfamilies of Braconidae. Microgastrinae, Alysiinae, Homolobinae and Braconinae were the most abundant in vineyard 1, accounting for more than 71% of the total number of braconids captured, whereas Alysiinae, Microgastrinae, Aphidiinae and Euphorinae were the most represented in vineyard 2, accounting for more than 82% of the total number of braconids captured (table 3).

We also collected specimens belonging to 9 subfamilies of Ichneumonidae in vineyard 1, and 11 in vineyard 2. Cryptinae and Campopleginae were the most abundant subfamilies in both areas, though in vineyard 2, Orthocentrinae, Metopiinae and Diplazontinae provided a notable contribution (table 4).

Community structures in the Malaise and sticky traps

The Malaise traps intercepted more insects than the sticky panels. The only contrasting data regarded the populations of Chrysopidae and Syrphidae of the genera *Eupeodes*, *Eristalis* and *Eristalinus*, whose total captures were more abundant in the sticky traps (tables 1 and 2). It is known that Malaise traps are not efficient in sampling common species belonging to the genus *Eristalis* and *Eristalinus*, which are more caught by yellow traps (Burgio and Sommaggio, 2007). Captures of *Eupeodes* were much more abundant in the sticky traps of both areas compared with the captures in Malaise traps (Vineyard 1: $P < 0.001$; χ^2 214; df 1; vineyard 2: $P < 0.001$; χ^2 207; df 1).

Discussion

Similarly to what Jones *et al*. (2016) observed in apple, pear and walnut orchards, our results are consistent with the hypothesis that the HIPV blend MeSa + AA + PE (TrB) is effective for manipulating *Chrysoperla* spp. behaviour and attracts adults to traps in the vineyard as well. This blend shows a great specificity, as it is not attractive to *Pseudomallada* spp., Syrphidae, and Ichneumonidae. The GER + PE blend (TrA), which is designed to be attractive to Syrphidae, particularly to *Eupeodes* (Jones *et al.*, 2016), was found to be active only for *Chrysoperla* spp., at least in vineyard 1, thus showing poor reliability and specificity. TrA and TrB attracted significantly more Braconidae than their respective controls in vineyard 1 but not in vineyard 2. However, the small number of captures in both vineyards does not allow to rely on such difference and requires further investigations. Malaise traps, which have not been reported as being used in previous HIPV studies, were useful in increasing our knowledge regarding the taxon richness in the experimental fields and in supporting our results in the use of the tested blends. Malaise trap captures revealed an abundant presence of Braconidae and Ichneumonidae in both vineyards, further highlighting the low efficacy of the sticky traps baited with the tested blend to attract appreciably these taxa.

A limited number of Chrysopidae belonging to the genera *Chrysoperla* and *Pseudomallada* were captured in the Malaise traps, despite being numerous in the sticky traps. We speculate that the low captures could be due to the inadequacy of the passive Malaise traps to intercept lacewings, as already observed by Carvalho and Souza (2000), Vas *et al*. (2001) and Oliveira *et al*. (2012) especially when compared with the numbers of Chrysopidae captured on baited sticky traps.

Conversely, the abundance of Syrphidae in the Malaise traps was very high, confirming their reliability to intercept adults of this family (Burgio and Sommaggio, 2002). In both vineyards, Malaise traps captured almost the same genera of Syrphidae as the sticky traps and some differences were principally due to the poorly represented genera (< 5 specimens captured). The large number of adults belonging to the genera *Sphaerophoria* and *Melanostoma* in the Malaise trap samples confirmed their abundance in a rural landscape, as already reported by Burgio and Sommaggio (2002; 2007). The occurrence of large populations of *Sphaerophoria* in our experimental areas was also reflected to some extent by the sticky-trap captures.

Adult *Eupeodes* spp. captured with Malaise traps were a small proportion of the total number of hoverflies captured in the two test vineyards. On the other hand, *Eupeodes* spp. captured by the sticky traps represented over 1/3rd of all Syrphidae captured on sticky traps in the test sites. These differences support the evidence that baited sticky traps are more suitable for catching *Eupeodes* spp. than Malaise traps.

In contrast with our findings, in a rural landscape environment Burgio and Sommaggio (2007) captured a similar number of *Eupeodes* spp. adults in Malaise traps and in unbaited yellow sticky traps (2.6% of total Syrphidae captured with Malaise and 2.3% with sticky traps). The same authors (Sommaggio and Burgio, 2014) observed that *Eupeodes* spp. represented just 5% of the total number of hoverflies captured with the Malaise traps in two vineyards in northern Italy. Even though difficult to prove, we could speculate that HIVPs blends could have played some role in attracting *Eupeodes* spp. into the plots hosting the sticky traps, where the overlap of odours in a relatively small area could have prevented *Eupeodes* adults from discriminating between baited and unbaited sticky traps, where they could have been attracted by the white colour of the traps more than by the blend.

The idea of attracting natural enemies to improve the biological control of crop pests in agroecosystems is appealing but despite the increasing knowledge of HIPVs, not much is known about their ecological role with regard to insect population dynamics and influence of environmental factors (Gish *et al*., 2015). Although synthetic volatiles such as MeSa, phenylacetaldehyde, iridodial and squalene can manipulate *Chrysoperla* and *Chrysopa* spp. population density in diverse habitats (James, 2003; James and Price, 2004; Toth *et al*., 2006;

Koczor *et al*., 2010; Jones *et al*., 2011; 2016), it is not clear whether they can be used to successfully improve biological control. A key role could thus be played by phytophages, as low prey densities could reduce the predators' efficiency and fitness (Jones *et al*., 2011).

Insect behaviour should also be considered. For example with *C. carnea*, newly emerged adults can fly up to 40 km in 2 hours with a favourable wind (Duelli, 1980), responding to "vegetative stimuli" (*sensu* Duelli) only at the end of the migratory flight, when they show a sedentary behaviour, flying at foliage level and start mating. Because of this behaviour, Chrysopids may be attracted to areas managed with HIPVs, where their larvae can perform biological control. Unfortunately, the new generation of adults might also migrate, thus undermining this strategy.

This research is just one piece of the puzzle in understanding how to better use HIPVs for the biological control of crop pests. Research is still needed to gain knowledge on the practical use of HIPVs in the field, the suitable release rates and related formulations, as well as the possible association of HIPVs with parasitoid sex pheromones aimed at enhancing attraction. HIPVs could be exploited to trap natural enemies in an agro-ecosystem in order to help IPM practitioners to understand their abundance and their sensitivity to different management programs (Jones *et al*., 2011). In line with Toth *et al.* (2006), we believe that funnel traps would be more suitable than sticky traps for this purpose, as they might be able to catch alive Chrysopidae.

On the other hand, spraying HIPVs directly onto grapevines (Simpson *et al*., 2011a) can also be problematic because, at least in Europe, the direct contact of the chemicals with the plant tissues may represent an obstacle for the registration process of these lures, due to the need of additional studies on ecotoxicology, degradation and residues on the plant and the environment. Deploying evaporating HIPVs in appropriate dispensers seems more feasible for concentrating natural enemies in the cultivated field, similar to the use of PredaLure® (AgBio, Westminster, CO, USA) in Western Oregon vineyards (Gadino *et al*., 2012).

In addition, releasing HIPVs with aerosol systems is an even more interesting approach since it has already been used to release synthetic pheromone (Casado *et al*., 2014). In this case, important issues such as release time, release rate and suitable doses can be overcome.

References

BAGNOLI B., LUCCHI A., 2006.- Parasitoids of *Lobesia botrana* (Den. & Schiff.) in Tuscany.- *IOBC-wprs Bulletin*, 29: 139-142.

BALDWIN I. T., HALITSCHKE R., PASCHOLD A., VON DAHL C. C., PRESTON C. A., 2006.- Volatile signaling in plant-plant interactions: "Talking Trees" in the genomics Era.- *Science*, 311: 812-814.

BELCARI A., RASPI A., 1989.- Un nuovo predatore di *Lobesia botrana* (Denn. & Schiff.) (Lepidoptera Tortricidae): *Xanthandrus comtus* (Harr.) (Diptera Syrphidae).- *Bollettino di Zoologia Agraria e di Bachicoltura*, 21: 185-192.

BERNASCONI OCKROY M. L., TURLINGS T. C. J., EDWARDS P. J., FRITZSCHE-HOBALLAH M. L., AMBROSETTI L., BASSETTI P., DORN S., 2001.- Response of natural populations of predators and parasitoids to artificially induced volatile emissions in maize plants (*Zea mays* L.).- *Agricultural and Forest Entomology*, 3: 201-209.

BROAD G., 2016.- Checklist of British and Irish Hymenoptera - Ichneumonidae.- *Biodiversity Data Journal*, 4: e9042.

BURGIO G., SOMMAGGIO D., 2002.- Diptera Syrphidae caught by Malaise trap in Bologna province and new record of *Neoascia interrupta* in Italy.- *Bulletin of Insectology*, 55 (1-2): 43-47.

BURGIO G., SOMMAGGIO D., 2007.- Syrphids as landscape bioindicators in Italian agroecosystems.- *Agriculture Ecosystems and Environment*, 120: 416-422.

CARVALHO C. F., SOUZA B., 2000.- Methods for rearing and production of chrysopids: biological control of pests: mass production and quality, pp. 91-110. In*: Controle biológico de pragas: produção massal e controle de qualidade* (BUENO V. H. P., Ed.).- Ufla, Lavras, Brazil.

CASADO D., CAVE F., WELTER S., 2014.- Puffer®-CM dispenser for mating disruption of codling moth: area of influence and impacts on trap finding success by male.- *IOBC-wprs Bulletin*, 99: 25-31.

CHAMBERS R. J., 1988.- Syrphidae, pp. 259-270. In: *Aphids, their biology, natural enemies and control* 2B (MINSK A. K., HARREWIJN P., Eds).- Elsevier, Amsterdam, The Netherlands.

CUSUMANO A., WELDEGERGIS B. T., COLAZZA S., DICKE M., FATOUROS N. E., 2015.- Attraction of egg-killing parasitoids toward induced plant volatiles in a multi-herbivore context.- *Oecologia*, 179 (1): 163-174.

DAANE K. M., YOKOTA G. Y., 1997.- Release strategies affect survival and distribution of green lacewings (Neuroptera: Chrysopidae) in augmentation programs.- *Environmental Entomology*, 26: 455-464.

DAANE K. M., YOKOTA G. Y., ZHENG Y., HAGEN K. S., 1996.- Inundative release of common green lacewings (Neuroptera: Chrisopidae) to suppress *Erythroneura variabilis* and *E. elegantula* (Homoptera: Cicadellidae) in vineyards.- *Environmental Entomology*, 25: 1224-1234.

DICKE M., SABELIS M. W., 1988.- Infochemical terminology: based on cost-benefit analysis rather than origin of compounds?- *Functional Ecology*, 2: 131-139.

DICKE M., VAN LOON J. J. A., 2000.- Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context.- *Entomologia Experimentalis et Applicata*, 97: 237-249.

DUDAREVA N., PICHERSKY E., GERSHENZON J., 2004.- Biochemistry of plant volatiles.- *Plant Physiology*, 135: 1893-1902.

DUELLI P., 1980.- Preovipository migration flights in the green lacewing, *Chrysopa carnea* (Planipennia, Chrysopidae).- *Behavioural Ecology and Sociobiology*, 7: 239-246.

FRASER M. E. S., DYTHAM C., MAYHEW J. P., 2007.- Determinants of parasitoid abundance and diversity in woodland habitats.- *Journal of Applied Ecology*, 44: 352-361.

GADINO A., WALTON V. M., LEE J. C., 2012.- Evaluation of methyl salicylate lures on populations of *Typhlodromus pyri* (Acari: Phytoseiidae) and other natural enemies in Western Oregon vineyards.- *Biological Control*, 63: 48-55.

GISH M., DE MORAES C. M., MESCHER M. C., 2015.- Herbivore-induced plant volatiles in natural and agricultural ecosystems: open questions and future prospects.- *Current Opinion in Insect Science*, 9: 1-6.

GROSS H. R., 1981.- Employment of kairomones in the management of parasitoids, pp. 137-150. In: *Semiochemicals: their role in pest control* (NORDLUND D. A., JONES R. L., LEWIS W. J., Eds).- Wiley, New York, USA.

HARE J. D., 2011.- Ecological role of volatiles produced by plants in response to damage by herbivorous insects.- *Annual Review of Entomology*, 56: 161-180.

JAMES D. G., 2003.- Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*.- *Journal of Chemical Ecology*, 29: 1601-1609.

JAMES D. G. 2005.- Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects.- *Journal of Chemical Ecology*, 31: 481-495.

JAMES D. G., PRICE T. S., 2004.- Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops.- *Journal of Chemical Ecology*, 30: 1613-1628.

JAMES D. G., CASTLE S. C., GRASSWITZ T., REYNA V. 2005.- Using synthetic herbivore induced plant volatiles to enhance conservation biological control: field experiments in hops and grapes, pp. 192-205. In: *Proceedings of the second international symposium on biological control of arthropods*, Davos, Switzerland, 12-16 September, 2005.

JONES V. P., STEFFAN S. A., WIMANA N. G., HORTON D. R., MILICZKY E., ZHANG Q., BAKER C. C., 2011.- Evaluation of herbivore-induced plant volatiles for monitoring green lacewings in Washington apple orchards.- *Biological Control*, 56: 98-105.

JONES V. P., HORTON D. R., MILLS N. J., UNRUH T. R., BAKER C. C., MELTON T. M., MILICKZY E., STEFFAN S. A., SHEARER P. W., AMARASEKARE K. G., 2016.- Evaluating plant volatiles for monitoring natural enemies in apple, pear and walnut orchards.- *Biological Control*, 102: 53-65.

KAPLAN I., 2012.- Attracting carnivorous arthropods with plant volatiles: the future of biocontrol or playing with fire?- *Biological Control*, 60: 77-89.

KARBAN R., BALDWIN I. T., 1997.- *Induced responses to herbivory*.- University of Chicago Press, Chicago, USA.

KESSLER A., HALITSCHKE R., 2007.- Specificity and complexity: the impact of herbivore-induced plant responses on arthropod community structure.- *Current Opinion in Plant Biology*, 10: 409-414.

KHAN Z. R., AMPONG N. K., CHILISWA P., HASSANALI A., KIMANI S., LWANDE W., OVERHOLT W. A., PICKETT J. A., SMART L. E., WADHAMS L. J., 1997.- Intercropping increases parasitism of pests.- *Nature*, 388: 631-632.

KOCZOR S., SZENTKIRÁLYI F., BIRKETT M. A., PICKETT J. A., VOIGHT E., TÓTH M., 2010.- Attraction of *Chrysoperla carnea* complex and *Chrysopa* spp. lacewings (Neuroptera: Chrysopidae) to aphid sex pheromone components and a synthetic blend of floral compounds in Hungary.- *Pest Management Science*, 66: 1374-1379.

LONI A., LUCCHI A., 2012.- Preliminary study on the suitability of Hymenoptera Braconidae as bioindicators of the pest management system in the vineyards.- *IOBC-wprs Bulletin*, 75: 119-122.

LONI A., LUCCHI A., 2014a.- Hymenoptera parasitoid, a suitable biodiversity resource for vineyard environmental discrimination.- *Journal of Agricultural Science*, 6: 36-106.

LONI A., LUCCHI A., 2014b.- Are higher taxa level assemblages suited to discriminate different habitats? A case study using Braconid subfamilies in three Tuscan vineyards.- *IOBC-wprs Bulletin*, 100: 81-85.

MALAISE R., 1937.- A new insect trap.- *Entomologisk Tidskrift*, 58: 148-160.

MOREAU J., VILLEMANT C., BENREY B., THIÉRY D., 2010.- Species diversity of larval parasitoids of the European grapevine moth (*Lobesia botrana*, Lepidoptera: Tortricidae): the influence of region and cultivar.- *Biological Control*, 54: 300-306.

OLIVEIRA S. A., AUAD M. A., SOUZA B., FONSECA M., RESENDE T., 2012.- Population dynamics of *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) in a silvo-pastoral system.- *International Journal of Biodiversity and Conservation*, 4: 179-182.

ORRE G. U. S., WRATTEN S. D., JOHNSSON M., HALE R. J., 2010.- Effects of an herbivore-induced plant volatile on arthropods from three trophic levels in brassicas.- *Biological control*, 53: 62-67.

PICHERSKY E., NOEL J. P., DUDAREVA N., 2006.- Biosynthesis of plant volatiles: nature's diversity and ingenuity.- *Science*, 311: 808-811.

PRICE P. W., BOUTON C. E., GROSS P., MCPHERON B. A., THOMPSON J. N., WEIS A. E., 1980.- Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies.- *Annual Review of Ecology and Systematics*, 11: 41-65.

RODRIGUEZ-SAONA C., KAPLAN I., BRAASCH J., CHINNASAMY D., WILLIAMS L., 2011.- Field responses of predaceous arthropods to methyl salicylate: a meta-analysis and case study in cranberries.- *Biological Control*, 59: 294-303.

RODRIGUEZ-SAONA C., BLAAUW B. R., ISAACS R., 2012.- Manipulation of natural enemies in agroecosystems: habitat and semiochemicals for sustainable insect pest control, pp. 89-126. In: *Integrated pest management and pest control - current and future tactics* (LARRAMENDY M. L., SOLONESKI S., Eds).- InTech, Rijeka, Croatia.

SCARAMOZZINO P. L., LONI A., LUCCHI A., 2017.- A review of insect parasitoids associated with *Lobesia botrana* (Denis & Schiffermüller, 1775) in Italy. 1. Diptera Tachinidae and Hymenoptera Braconidae (Lepidoptera, Tortricidae).- *ZooKeys*, 647: 67-100.

SHARKEY J. M., 1997.- Key to new world subfamilies of the family Braconidae, pp. 39-63. In *Manual of new world genera of the family Braconidae (Hymenoptera)* (WHARTON A. E., MARSH M. P., SHARKEY J. M., Eds).- The International Society of Hymenopterist, Washington, DC, USA.

SHEARER P. W., AMARASEKARE K. G., CASTAGNOLI S. P., BEERS E. H., JONES V. P., MILLS N. J., 2016.- Large-plot field studies to assess impacts of newer insecticides on non-target arthropods in Western US orchards.- *Biological Control*, 102: 26-34.

SIMPSON M., GURR G. M., SIMMONS A. T., WRATTEN S. D., JAMES D. G., LEESON G., NICHOL H., ORRE G. U. S., 2011a. Insect attraction to synthetic herbivore-insect plant volatile treated field crops.- *Agricultural and Forest Entomology*, 13: 45-57.

SIMPSON M., GURR G. M., SIMMONS A. T., WRATTEN S. D., JAMES D. G., LEESON G., NICHOL H., ORRE G. U. S., 2011b.- Attract and reward: combining chemical ecology and habitat manipulation to enhance biological control in field crops.- *Journal of Applied Ecology*, 48: 580-590.

SIMPSON M., GURR G. M., SIMMONS A. T., WRATTEN S. D., JAMES D. G., LEESON G., NICHOL H., ORRE G. U. S., 2011c.- Field evaluation of the "attract and reward" biological control approach in vineyards.- *Annals of Applied Biology*, 159: 69-78.

SOMMAGGIO D., BURGIO G., 2014.- The use of Syrphidae as functional bioindicator to compare vineyards with different managements.- *Bulletin of Insectology*, 67: 147-156.

SZENTKIRALYI R., 2001.- Lacewings in fruit and nut crops, pp. 172-238. In: *Lacewings in the crop environment* (MECEWEN P., NEW T. R., WHITTINGTON A. E., Eds).- Cambridge University Press, Cambridge, UK.

TAKABAYASHI J., TAKAHASHI S., DICKE M., POSTHUMUS M. A., 1995.- Developmental stage of herbivore *Pseudaletia separata* affects production of herbivore induced synomone by corn plants.- *Journal of Chemical Ecology*, 21: 273-287.

TÓTH M., BOZSIK A., SZENTKIRÁLYI F., LETARDI A., TABILIO M. R., VERDINELLI M., ZANDIGIACOMO P., JEKISA J., SZARUKÁN I., 2006.- Phenylacetaldehyde: A chemical attractant for common green lacewings (*Chrysoperla carnea* s.l., Neuroptera: Chrysopidae).- *European Journal of Entomology*, 103: 267-271.

TURLINGS T. J., TON J., 2006.- Exploiting scents of distress: the prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests.- *Current Opinion in Plant Biology*, 9: 421-427.

VAS J., ÁBRAHÁM L., MARKÓ V., 2001.- Methodological investigation on a Neuropteroidea community.- *Acta Phytopathologica et Entomologica Hungarica*, 36: 101-113.

VINSON S. B., 1977.- Behavioural chemicals in the augmentation of natural enemies, pp. 237-279. In: *Biological control by augmentation of natural enemies* (RIDGWAY R. L., VINSON S. B., Eds).- Plenum, New York, USA.

VON MÉREY G., VEYRAT N., MAHUKU G., LOPEZ VALDEZ R., TURLINGS T. C. J., D'ALESSANDRO M., 2011.- Dispensing synthetic green leaf volatiles in maize fields increases the release of sesquiterpenes by the plants, but has little effect on the attraction of pest and beneficial insects.- *Phytochemistry*, 72: 1838-1847.

WAAGE J. K., CARL K. P., MILLS N. J., GREATHEAD D. J., 1984.- Rearing entomophagous insects, pp. 45-66. In: *Handbook of insect rearing*, vol. 1 (SINGH P., MOORE R. F., Eds).- Elsevier, Amsterdam, The Netherlands.

YU H., ZHANG Y., WU K., XI W. G., YU Y. G., 2008.- Field-testing of synthetic herbivore-induced plant volatiles as attractants for beneficial insects.- *Environmental Entomology*, 37: 1410-1415.

ZHU A. J., PARK K. C., 2005.- Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator *Coccinella septempunctata*.- *Journal of Chemical Ecology*, 31: 1733-1746.

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Received May 18, 2017. Accepted September 7, 2017.