

# Response of aphid predators to synthetic herbivore induced plant volatiles in an apple orchard

Respuesta de áfidos depredadores a compuestos volátiles sintéticos en un huerto de manzana

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Suggested citation:  
GENCER, N. S.; KUMRAL, N. A.; ALTIN, I.; PEHLEVAN, B. 2019. Response of aphid predators to synthetic herbivore induced plant volatiles in an apple orchard. *Revista Colombiana de Entomología* 45 (2): e7953. <https://doi.org/10.25100/socolen.v45i2.7953>

Received: 22-Feb-2017  
Accepted: 03-May-2019  
Published: 9-Jan-2020

Revista Colombiana de Entomología  
ISSN (Impreso): 0120-0488  
ISSN (En línea): 2665-4385  
<http://revistacolombianaentomologia.univalle.edu.co/>

Open access



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Publishers:  
Sociedad Colombiana de Entomología  
SOCOLEN (Bogotá, D. C., Colombia)  
<http://www.socolen.org.co>  
Universidad del Valle (Cali, Colombia)  
<http://www.univalle.edu.co/>

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- SOCOLEN y Universidad del Valle - Univalle

**Abstract:** The indirect defence compounds termed herbivore induced plant volatiles (HIPVs), which are used to attract beneficial fauna, are one of the most effective biological control tools for the aggregation of natural enemies of key pests. The aim of this study was to test the attraction of three aphid predators of the Chrysopidae, Coccinellidae and Syrphidae families using synthetic formulations of four main HIPVs [methyl salicylate (MeSa), benzaldehyde (B), linalool (L) and farnesene (F)] alone and in binary combinations (MeSa + B; MeSa + F; MeSa + L; F + B; B + L; F + L) in an apple orchard in the Bursa province of Turkey. This study was the first demonstration of the attraction of these aphid predators to single and binary combinations of synthetic HIPVs in an apple orchard. A larger number of coccinellids were captured using single treatments of both B and F than with other HIPV combinations. Furthermore, the chrysopid individuals studied were significantly attracted to traps baited with single HIPVs. In addition, the binary combination of MeSa + L significantly attracted more Syrphids than in both single treatments and control traps. Thus, some of the HIPV's tested were found to have potential value for the congregation of aphid predators in apple orchards.

**Keywords:** Synthetic HIPVs, predators, biological control, monitoring, aphid, Chrysopidae, Coccinellidae, Syrphidae, Neuroptera, Coleoptera, Diptera.

**Resumen:** Los compuestos de defensa indirectos, denominados compuestos volátiles de plantas inducidos por herbívoros (HIPV por su sigla en inglés), al atraer fauna benéfica, son una de las herramientas de control biológico efectivas para la agregación de enemigos naturales de plagas claves. El objetivo de este estudio fue evaluar la atracción de formulaciones sintéticas de cuatro HIPV principales: [salicilato de metilo (MeSa), benzaldehído (B), linalool (L) y farneseno (F)], solos y en combinaciones binarias (MeSa + B; MeSa + F; MeSa + L; F + B; B + L; F + L) a tres depredadores de áfidos de las familias Chrysopidae, Coccinellidae y Syrphidae en una plantación de manzanas de la provincia Bursa, en Turquía. Este estudio representa la primera evidencia de la atracción que ejercen combinaciones únicas y binarias de HIPV sintéticas a depredadores de áfidos en un manzano. Relativamente, un mayor número de coccinélidos fue capturado por los tratamientos individuales de B y F en comparación con otras combinaciones de HIPV. Los individuos de crisópidos fueron más atraídos significativamente a las trampas cebadas con estos HIPV solos. Además, la combinación binaria de MeSa + L atrajo significativamente más sírfidos que sus tratamientos únicos y trampas de control. Por lo tanto, se encontró que algunos de los HIPV evaluados tenían un valor potencial en la atracción de depredadores de áfidos en los manzanos.

**Palabras clave:** HIPVs sintéticos, depredadores, control biológico, supervisión, áfido, Chrysopidae, Coccinellidae, Syrphidae, Neuroptera, Coleoptera, Diptera.

## Introduction

The herbivore-induced plant volatiles (HIPVs) released when a plant is damaged by any herbivore, are an indirect defense mechanism in plants with elicits top-down control of damaging pests through recruitment of natural enemies (Paré and Tumlinson 1999; Howe and Jander 2008). Thus, the volatiles play an important role as signals in tritrophic level interactions among plants, herbivores and natural enemies (Rodríguez-Saona *et al.* 2011). Based on previous analytical results, plants contain more than 1000 HIPVs and different volatile blends occurred by mixing hundreds of these volatiles can be released (Kaplan 2012).

The choice of compound or compound combinations is crucial decision for attracts to target natural enemy species. Nevertheless, several HIPVs have been often studied and these experiments focused on some compounds i.e. methyl salicylate (MeSa),

benzaldehyde (B), linalool (L) and farnesene (F). For example, the traps with MeSa attracted some natural enemy species in a Washington hop yard (James 2005). In the field conditions, eulophid parasitoids demonstrated greater numbers on the traps including MeSa in a sweetcorn plantation (Simpson *et al.* 2011). MeSa is commercially available with the name Predalure™ and is used to attract beneficial insects such as lacewings, lady beetles, syrphids, *Orius* spp. into the agricultural lands. Based on olfactometer bioassays in a laboratory trial, linalool was remarkably attractive to *Harmonia axyridis* Pallas, 1773 (Coleoptera: Coccinellidae) (Xie *et al.* 2008). In a non-agricultural system, Kessler and Baldwin (2001) demonstrated that predation on *Manduca sexta* (L., 1763) (Lepidoptera: Sphingidae) eggs by *Geocoris pallens* Stål, 1854 (Hemiptera: Geocoridae) increased when plants of *Nicotiana attenuata* Torr. ex Wats. (Solanales: Solanaceae) were treated with linalool. In a laboratory study, the attractiveness of benzaldehyde, which is a plant volatile released from flowers, ripening fruit and plants damaged by aphids, to three natural enemy species *Coccinella septempunctata* L., 1758 (Coleoptera: Coccinellidae), *Aphidius* sp. (Hymenoptera: Braconidae) and *Chrysoperla sinica* (Tjeder, 1968) (Neuroptera: Chrysopidae) was demonstrated via electroantennogram and olfactometer tests by Han and Chen (2002). Furthermore, benzaldehyde was found to be attractive to *Orius tristicolor* (White, 1879) (Hemiptera: Anthracoridae), *Stethorus punctum picipes* Casey, 1899 (Coleoptera: Coccinellidae), and flies in the families Tachinidae and Sarcophagidae in a field study (James 2005). It has been revealed that farnesene, which is the aphid alarm pheromone and a common ingredient of HIPV blends from some plants, including apple trees, showed a kairomone effect on *Adalia bipunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) (Francis *et al.* 2004). In a field study by James (2005), the parasitic mymarid wasp, *Anagrus daanei* Triapitsyn, 1998, was attracted to farnesene. Furthermore, the attraction of natural enemies by other synthetic HIPVs has been demonstrated under laboratory and field conditions, and their potential value in pest management has been recognized (James 2005; James and Grasswitz 2005; Kahn *et al.* 2008; Gurr and Kvedaras 2010; Lee 2010; Orre *et al.* 2010). In recent years, field studies have been realized by testing an HIPV (or a mixture of HIPVs) with a trap to determine the diversity and abundance of the attracted natural enemy species (Maeda *et al.* 2015; Lucchi *et al.* 2017; Yu *et al.* 2017).

Thus, while the synthetic HIPVs have been tested, there is less work with binary combinations of them in the field conditions. Furthermore, until our knowledge, there is only one study on the effectiveness of HIPVs in apple orchards (Jones *et al.* 2011), although many studies have been done in a variety of crop systems including hops (James 2005), grapes (James and Price 2004), pears (Scutareanu *et al.* 1997), cherries (Toth *et al.* 2009), corn and sorghum (Kahn *et al.* 2008), sweetcorn (Simpson *et al.* 2011), strawberries (Lee 2010), brassicas (Orre *et al.* 2010), cranberries (Rodriguez-Saona *et al.* 2011) and vineyards (Gadino *et al.* 2012). In this study, we used four synthetic HIPVs (MeSa, B, L and F) and all their binary combinations to test attraction to predators of aphids in an apple orchard.

### Materials and methods

**Chemicals.** Synthetic formulations of four HIPVs, linalool (L) (97 % purity) (Acros Organics, Belgium), benzaldehyde

(B) (99.5 % purity) (Merck, USA), methyl salicylate (MeSa) (99 % purity) (Acros Organics, Belgium), farnesene (F) (95 % purity) (Sigma-Aldrich, USA) and a solvent that was mainly hexane (Merck, USA) (99.5 % purity) were used in this study. Single compounds (L, B, MeSa, F) and binary combinations of these compounds (MeSa + B; MeSa + F; MeSa + L; F + B; B + L; F + L) were prepared in hexane solutions. Each HIPV was diluted 1:1 in hexane.

**Traps.** Beneficial insects were caught at each site (including controls) by yellow sticky cards (28 x 23 cm, Trece Inc., USA) tied to an apple trunk 1.5 m above the ground. The mentioned ten combinations were investigated to determine the response of the insects to yellow sticky cards with 5 ml glass vials (containing 2 ml of candidate HIPV diluted in hexane) versus control cards including only 2 ml of hexane. The glass vials were 4.5 cm in length and 1.5 cm at the internal diameter of the opening. The opening of each vial was covered with a piece of cotton to directly prevent evaporation of the volatile compounds. The vials were embedded at the top of a two-sided sticky cards. The glass vials containing the volatile compounds were renewed weekly, and all traps were replaced weekly from early May to late July of 2013 (12 weeks).

**Experimental area and design.** The field experiment was conducted in a 1-ha apple orchard (5 year old, Granny Smith cv.) of the University of Bursa Uludag Agricultural Faculty in Bursa. Yellow sticky cards for each HIPV were spaced 15 m apart according to James (2005) and distributed in a randomized design. Three replicates for each of the ten treatments and also three replicates of the control were used. Insecticide and fungicide applications (Thiacloprid 350 g/L and Fluopyram 200 g/L + Tebuconazole 200 g/L) were kept to a minimum at all sites. More attention was paid to their use against the codling moth, *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae) and apple scab, *Venturia inaequalis* (Cooke) G. Winter, 1875 which are known to have minimal effects on beneficial arthropods. The predatory insects collected from traps for twelve weeks were counted weekly under a stereomicroscope (Leica, USA) in the laboratory.

**Data analysis.** The numbers of coccinellid, chrysopid and syrphid predators were compared using a repeated measures analysis with treatment, time, and treatment x time interaction as fixed effects, and each trap that was repeatedly sampled as the random subject effect. Then post-hoc comparison of the treatments was done by Tukey HSD test in SPSS (SPSS Inc. 2004). The mean insect numbers on traps with each HIPV combination vs. control were also compared by a pairwise Student t-test.

### Results

**Predator composition.** Three insect families (Chrysopidae, Coccinellidae and Syrphidae) were attracted to alone and binary combinations of the synthetic HIPVs in the apple tree orchard (Table 1). From the Chrysopidae family, only *Chrysoperla carnea* (Stephens, 1836) *sensu lato* has attracted and counted in traps. The most abundant family was Coccinellidae, with five species as follows: *Coccinella septempunctata* L., 1758, *Hippodamia variegata* (Goeze, 1777), *Oenopia conglobata* (L., 1758) *Psyllobora vigintiduopunctata* (L., 1758) and *Adalia bipunctata* (L., 1758) (Coleoptera: Coccinellidae).

**Table 1.** Total number of predatory insects caught on traps with four different synthetic HIPVs [linalool (L), benzaldehyde (B), methyl salicylate (MeSa), farnesene (F)] and their binary mixtures in an apple orchard during summer 2012.

Insect families	Synthetic formulations of four HIPVs										hexane (Control)	Total	$\chi^2$	P
	MeSa	B	F	L	MeSa + B	MeSa + F	MeSa + L	F + B	B + L	F + L				
Chrysopidae	26	67	61	44	28	34	33	31	30	16	22	392	64.739	< 0.01
Syrphidae	14	13	5	14	7	9	29	14	9	14	11	139	27.588	0.021
Coccinellidae	16	31	26	14	18	12	17	13	18	22	15	202	17.164	0.071
Total	56	111	92	72	53	55	79	58	57	52	48	733	54.617	< 0.01

Three species of Syrphidae, *Eristalis arbustorum* (L., 1758), *Myathropa florea* (L., 1758) and *Episyrphus balteatus* (De Geer, 1776) were identified. Most of the predators (94 %) were attracted to the traps baited with HIPVs (alone + binary combinations) however a small number of insects was counted in the control traps. Chrysopid species (54 %) was most often caught in the baited traps, followed by coccinellids (27 %) and syrphids (19 %). The orientation to HIPVs was highest in the benzaldehyde compound followed by farnesene and MeSa + L combination. Among the single synthetic HIPVs, benzaldehyde (67 and 31 insects/traps) and farnesene (61 and 26 insects/traps) were highly attractive to chrysopid and coccinellid species compared with the other baited traps. The largest numbers of syrphid captures occurred in the linalool and methyl salicylate baited traps. The majority of the chrysopids were attracted to the MeSa + F mixture (34 insects/traps), whereas the coccinellids were captured in traps baited with F + L (22 insects/traps). The syrphids were more attracted to MeSa + L blends (29 insects/traps) than traps with other single and binary combinations.

**Predatory catches.** In an apple orchard, the mean seasonal abundances of predatory families captured in sticky traps baited with single and binary combinations of synthetic HIPVs are presented in Figure 1.

In traps baited with MeSa, the density of chrysopids, coccinellids and syrphids peaked in the second week and then rapidly decreased in the third week. Similarly, the first population peak of all families was observed in the third and fourth weeks in traps baited with L. In traps baited with B, two high peaks of all predatory families were observed in the second and the fourth weeks. Additionally, the densities of chrysopids and coccinellids had a peak in the twelfth week. In the traps baited with F, a very similar population pattern was determined with the traps baited with B.

In the binary combination experiments, chrysopid density peaked three times in the traps with a MeSa + F mixture in the third, eighth and eleventh weeks. For coccinellid density, four population peaks were observed in the second, fifth, seventh and twelfth weeks. In the F + B traps, the predator densities showed a strong peak in the third-fourth weeks and then rapidly declined until the eleventh week. The population densities of the coccinellids and chrysopids reached a final peak in the eleventh week and the twelfth week, respectively. Similarly, a peak in the third week was observed for all predators in traps with the MeSa + B binary combination. However, the population density of chrysopids formed two peaks in the seventh and tenth weeks. In traps with the B + L mixture, the predator densities showed a similar population pattern with traps, including traps with only B, with the exception of the coccinellid peak in the eighth week. Similar

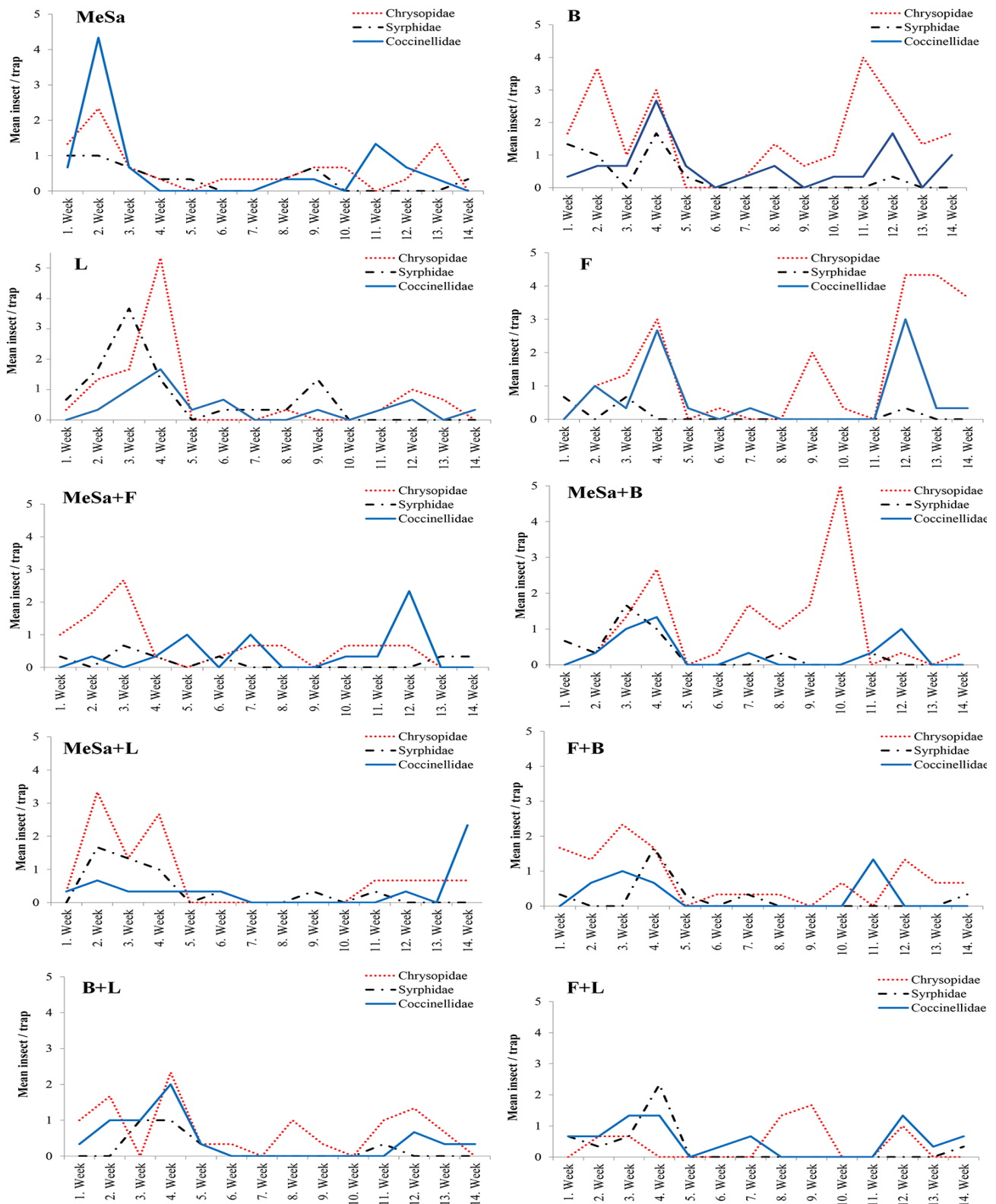
population patterns were found in the traps with L and the traps containing the MeSa + L mixture, F + L and the traps including only F.

**Trapping analysis.** There were noted significant effects among the different synthetic compounds or their combinations in attracting syrphids when these effects were analysed over the entire trapping period ( $F = 2.41$ ;  $df = 10.32$ ;  $P = 0.04$ ) (Fig. 2). Syrphid adults were significantly more prevalent in traps containing MeSa+L. The chrysopid population did not show any significant responses to the synthetic HIPVs ( $F = 1.93$ ;  $df = 10.32$ ;  $P = 0.09$ ). Traps baited with B, L, F and MeSa + L attracted significantly more chrysopid adults. Similarly, although the coccinellid population was not significantly attracted to the synthetic HIPVs ( $F = 1.44$ ;  $df = 10.32$ ;  $P = 0.23$ ), the traps baited with B and F captured relatively more coccinellid adults compared with the control traps.

Although there were not find any significant effects of synthetic HIPVs with respect to capturing chrysopids, significant effects were noted for the volatile-date interaction based on the repeated measures ANOVA analysis ( $F = 1.28$ ;  $df = 13.130$ ;  $P = 0.044$ ). Traps baited with B, L, F and MeSa + L attracted significantly more chrysopid adults than the control traps. In addition, pairwise t-tests showed that significantly more chrysopids were attracted to F-baited sticky traps compared with control traps ( $P = 0.011$ ). For attracting coccinellids and syrphids, the interaction between traps baited with HIPVs and date did not show significant effects ( $F = 1.24$ ;  $df = 13.130$ ;  $P = 0.072$ ,  $F = 1.15$ ;  $df = 13.130$ ;  $P = 0.16$ ).

## Discussion

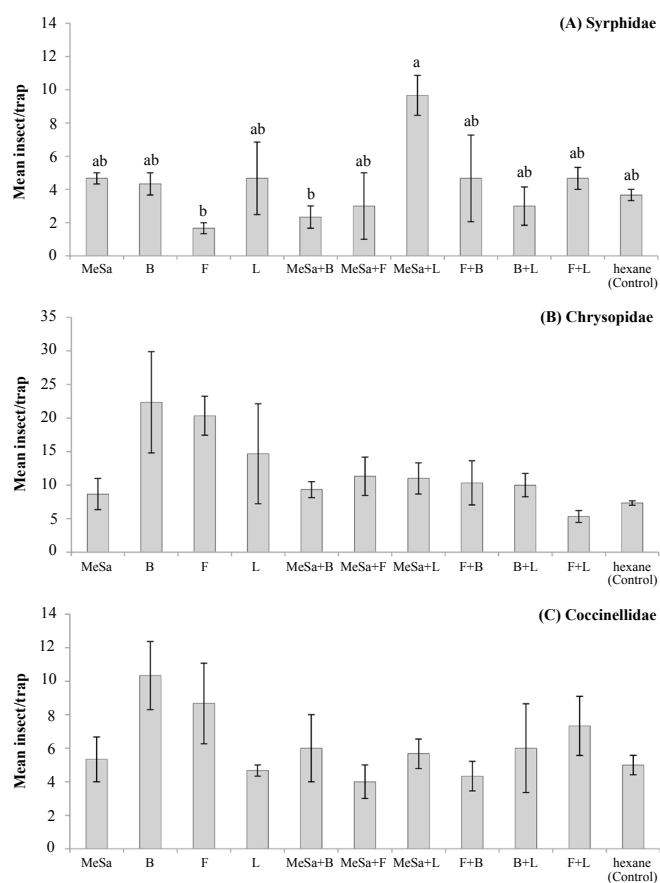
The current study demonstrated that important aphid predators, *C. carnea s.l.*, *C. septempunctata*, *A. variegata*, *O. conglobata*, *P. vigintiduopunctata*, *A. bipunctata* and *E. balteatus* were attracted by some HIPV combinations. These results conform with the findings of authors such as Aslan and Uygun (2005) and Rossi *et al.* (2006). B, F, L and the MeSa + L combination on sticky yellow traps attracted relatively more predator adults of the Coccinellidae, Chrysopidae and Syrphidae families than the control traps (Table 1). Only, the MeSa + L combination was significantly attracted to syrphid individuals (Fig. 2). The attraction of some species of the families Syrphidae to MeSa-baited traps was reported in earlier field-trapping studies (James 2005). However, the same author shows that some predator species from different orders such as *Chrysopa nigricornis* Burmeister, 1839 (Neuroptera: Chrysopidae), *S. p. picipes*, *O. tristicolor*, *G. pallens*, *Deraeocoris brevis* (Uhler, 1904) (Hemiptera: Miridae), *Hemerobius* sp. (Neuroptera: Hemerobiidae) were attracted by MeSa-baited traps. In addition, a study on corn proved



**Figure 1.** Mean numbers of syrphids, chrysopids and coccinellids trapped on yellow sticky traps containing different synthetic HIPVs [linalool (L), benzaldehyde (B), methyl salicylate (MeSa), farnesene (F)], and their binary combinations from May-July 2013.

that the systemic release of linalool by injured rice coincided with attractiveness for the parasitoid *Anagrus nilaparvatae* (Pang et Wang, 1985) (Hymenoptera: Mymaridae) (Xiao *et al.* 2012). But, there is no study about attraction to traps baited with a binary combination of MeSa + L.

In the current study, greater numbers of chrysopids were captured on the traps baited with B, F and L among single HIPVs than on the controls and other combinations. Based on weekly observation, traps baited with B, F and L and a binary combination of MeSa + L captured significantly more



**Figure 2.** Mean captures of (A) Syrphidae, (B) Chrysopidae and (C) Coccinellidae on yellow sticky traps with different synthetic HIPVs [linalool (L), benzaldehyde (B), methyl salicylate (MeSa), farnesene (F)], and their binary combinations versus traps including only hexane from May-July 2013. Grey bars indicate means of insect/trap. Thin bars show standard errors. Means followed by the different letter in a bar are significantly different (Tukey,  $P < 0.05$ ). Means which were not indicated by any letter are not significantly different (Tukey,  $P > 0.05$ ).

chrysopids than control traps including hexane based on the repeated measures ANOVA analysis. However, based on pairwise t-testing analysis, farnesene was statistically more attractive compared with control traps including hexane. These results were similar to those reported by Jones *et al.* (2011) in which benzaldehyde significantly attracted some lacewing species. The attractiveness of benzaldehyde has been ever demonstrated in *C. sinica* with electroantennogram and olfactometry tests (Han and Chen 2002). Additionally, attraction of *C. carnea* to traps including farnesene has been reported in a field study by Zhu *et al.* (1999). Their results were very similar to those reported in the current study in relation to the attraction of benzaldehyde to the families Chrysopidae. Also, another study showed that 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* (Lucchi *et al.* 2017).

The present study noted the first record of chrysopids responding to a binary combination of MeSa + L based on the repeated measures of ANOVA analysis. It is likely that a synergistic effect on the attraction of these HIPVs may be conferred, as evidenced by the greater capture rate of chrysopids during the second and fourth weeks. In a study on a strawberry field by Lee (2010), positive responses to

MeSa lures were found in Chrysopidae during July-August. Additionally, it was reported in an electrophysiological and behavioural study that a parasitoid wasp, *Anaphes iole* Girault, 1911 (Hymenoptera: Mymaridae), was most responsive to linalool (Williams *et al.* 2003).

This study showed that relatively greater numbers of coccinellids were caught in traps baited with B, F and F + L. Only the attraction of benzaldehyde obtained a nearly significant effect according to pairwise t-test analysis ( $P = 0.06$ ). Consistent with our results, Han and Chen (2002) demonstrated that benzaldehyde was attractive to *C. septempunctata* in the laboratory. Additional evidence for the coccinellid *S. p. picipes* captivation of benzaldehyde was revealed in a field study in a hop yard in Washington State (James 2005). Similarly, Zhu *et al.* (1999) showed that the attraction of farnesene was proven in the coccinellid *Coleomegilla maculata* De Geer, 1775 (Coleoptera: Coccinellidae) in laboratory tests. Verheggen *et al.* (2007) suggested that farnesene was indicated as having potential usefulness in a push-pull method, which repels target pests from a hostplant while luring them towards an attractive trap using *H. axyridis* as a bio-control agent in aphid infested plants. Furthermore, in a study conducted by Yu *et al.* (2017) linalool attract *Propylaea japonica* (Thunberg, 1781) (Coleoptera: Coccinellidae). Hence, our field study confirmed that farnesene is considered an important attractant for coccinellids.

In the present study, MeSa and L used alone attracted a small number of syrphid individuals. However, mixing of these two chemicals captured significantly more predators. In a field study by James (2005), although linalool and 3-octanone were among the 15 HIPVs tested that have reportedly failed to attract any insect families or species, the present study showed that linalool is a highly attractive semiochemical on syrphids when it is used together with methyl salicylate. This result was partially in accordance with Mallinger *et al.* (2011), who reported that significantly greater numbers of syrphid flies were caught on traps adjacent to the methyl salicylate lure in soybean fields. Similarly, James (2005) demonstrated that sticky traps baited with a synthetic version of methyl salicylate significantly attracted greater numbers of syrphids in Washington State hop yards. On the other hand, Dudareva and Pichersky (2006) revealed that the floral scent of a palm plant, *Prestoea schultzeana* (Burret) H. E. Moore which includes almost exclusively terpenoids, especially linalool, is visited frequently by syrphids. However, farnesene by itself appeared to be nearly inactive, with only small number of syrphids being caught over the trapping periods in the orchard. Previous studies have identified that the secondary plant compound farnesene repels some insects, such as aphids (Verheggen *et al.* 2007).

## Conclusions and recommendations

This study was the first demonstration of attraction of some aphid predators to single and binary combinations of synthetic HIPVs in an apple orchard. In summary, it was observed a great number of coccinellids and chrysopids in traps baited with benzaldehyde and farnesene. In addition, the binary combination of MeSa + L attracted more syrphids than single treatments of either agent. The tested HIPVs were found to have potential value in congregating aphid predators in apple orchards. Finally, it is essential to continue examining and

quantifying the direct benefits of attracting aphid predators as a pest management strategy, with the intent of enhancing biological control. In the future, for the species among the detected predatory species in this study, new studies should be conducted with novel single HIPVs and binary combinations of synthetic HIPVs. Additionally, to evaluate the regulatory effects on populations by these compounds, a new study that monitors the populations of both aphids and their predators when using the potential HIPV combinations should be conducted to confirm these findings.

### Acknowledgements

This study was supported by University of Bursa Uludag, Scientific Research Unit, Bursa, Turkey, Grant Project No: UAP (Z) - 2010/45.

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#### Origin and funding

*This study was supported by University of Bursa Uludag, Scientific Research Unit, Bursa, Turkey, Grant Project No: UAP (Z) - 2010/45.*

#### Author contribution

*N. S. Gencer and N. A. Kumral conceived and planned the experiment, N. S. Gencer and I. Altın carried out the experiment, N. S. Gencer and N. A. Kumral contributed to the interpretation of the results, N. A. Kumral and B. Pehlevan analysed the data and wrote the manuscript in consultation with N. S. Gencer. All authors wrote, read and approved the manuscript. The authors have declared that no conflict interest exist.*