



9 **Abstract** – The sesquiterpene (*E*)- $\beta$ -farnesene (*E* $\beta$ f) is the primary component of the alarm  
10 pheromone of most aphid species. It is released in response to physical stress including attack  
11 by natural enemies and causes aphids to cease feeding and disperse. *E* $\beta$ f also acts as a  
12 kairomonal cue for aphid natural enemies. In the present study, we collected the headspace  
13 volatiles released by aphid colonies of different sizes. GC-MS analysis demonstrated the  
14 presence of *E* $\beta$ f in absence of predator attack. A quadratic relationship was found between the  
15 released (*E*)- $\beta$ -farnesene amounts and aphid colony size. Behavioural impact of aphid alarm  
16 pheromone toward *E. balteatus* female oviposition behaviour was also demonstrated in this  
17 work. These results highlight the primary role of the small but continuous release of aphid  
18 alarm pheromone in mechanisms of decision-making by aphid predators during their foraging  
19 and egg-laying behaviour.

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21 **Key words:** Semiochemicals, *Episyrphus balteatus*, *Myzus persicae*, aphid alarm pheromone,  
22 (*E*)- $\beta$ -farnesene

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## 24 **Introduction**

25 (E)- $\beta$ -Farnesene (E $\beta$ F), the main component of the aphid alarm pheromone was identified in  
26 16 aphid species, alone or associated with other molecules (Francis *et al.* 2005). Previous  
27 behavioural studies have demonstrated the kairomonal role of E $\beta$ F in various aphid natural  
28 enemies, including ladybeetles, hoverflies and parasitic hymenoptera (Du *et al.* 1998; Al  
29 Abassi *et al.* 2000; Harmel *et al.* 2007; Verheggen *et al.* 2007, 2008).

30 In the hoverfly *E. balteatus*, there is a good evidence from laboratory and field studies for the  
31 existence of a positive density-dependent response to aphid colony size in term of oviposition  
32 (Dixon 1959; Chandler 1968; Itô and Iwao 1977; Bargen *et al.* 1998; Scholz and Poehling  
33 2000; Sutherland *et al.* 2001; Almohamad *et al.* 2006). However, there is only little work on  
34 the role of odour cues in predatory hoverflies attraction (Laubertie *et al.* 2006; Verheggen *et*  
35 *al.* 2008 ; Almohamad *et al.* 2007 ; 2008).

36 The present study aims to understand the role of chemical cues released from non-preyed  
37 aphid colonies on decision-making processes that lead to oviposition in female *E. balteatus*.  
38 In order to evaluate the olfactory signal released by the tested colonies, we quantified the  
39 volatile organic compounds released in their headspaces. The oviposition behaviour of *E.*  
40 *balteatus* was subsequently investigated with respect to *M. persicae* colonies of different  
41 sizes.

42

## 43 **Materials and Methods**

44 **Plants and insects** - Broad bean plants (*Vicia faba* L.) were grown in 30 x 20 x 5 cm plastic  
45 trays filled with a mix of perlite and vermiculite (1/1) and maintained in controlled  
46 environment growth rooms (16:8 Light: Dark ; 20  $\pm$  1°C). Two aphid species, namely *M.*  
47 *persicae* and *Megoura viciae* Buckton were taken from stock rearing on *V. faba*, in separate  
48 air-conditioned rooms under the same conditions as above. Adult *E. balteatus* were reared in

49 75 x 60 x 90 cm cages and were provided with bee-collected pollen, sugar and water. Broad  
50 beans infested with *M. viciae* were introduced into the cages for 3 h every two days to allow  
51 oviposition. Hoverfly larvae were mass-reared in aerated plastic boxes (110 x 140 x 40 mm)  
52 and were fed daily *ad libitum* with *M. viciae* as standard diet.

53 **Leaf disc system** – The leaf disc-system consisted of (1) a circular piece of *V. faba* leaf, (2)  
54 aphids and (3) 7 ml of an agar diet (agar 1% solution w:w), placed in a 25 mm diameter Petri  
55 dish, to reduce desiccation. Leaves were infested with different quantities of *M. persicae* and  
56 were kept for 24h in a controlled conditions incubator (16:8 Light: Dark;  $20 \pm 1^\circ\text{C}$ ) before  
57 testing.

58 **Influence of aphid colony size on syrphid oviposition rate** – In no-choice experiments, a  
59 single *E. balteatus* female was introduced in a net cage and allowed to lay eggs for 3h on the  
60 leaf disc supporting a *M. persicae* colony made of 25, 75 or 125 individuals. The leaf disc  
61 system was placed on a Plexiglass holder at a height of 20 cm. This setup was previously  
62 shown as an efficient method to evaluate the oviposition behaviour of the hoverfly *E.*  
63 *balteatus* in response to aphid-infested plants (Almohamad *et al.* 2006). The number of eggs  
64 laid (oviposition rate) on the leaf disc was counted. This experiment was replicated 10 times  
65 for each aphid colony size.

#### 66 **Collection and analysis of volatile chemical emissions**

67 **Volatile collection system** – Volatile chemicals were collected using a push/pull volatile  
68 collection system consisting of a glass air-collection chamber (Schott®, 12 cm base-diameter,  
69 35 cm high) placed inside an incubator set at  $21 \pm 1^\circ\text{C}$ , and previously washed with hot water  
70 and *n*-hexane. The leaf disc system was placed on a Plexiglass holder similar to those used in  
71 the above-mentioned bio-assays. Incoming air was pushed through an in-line activated  
72 charcoal filter before entering the glass chamber at a flow of 200 ml/min. The volatile-  
73 enriched air was then pulled through an adsorption trap containing 40 mg SuperQ® (Alltech,

74 Deerfield, IL, USA). Six replicates were conducted for each aphid colony size and four  
75 replicates for the control (i.e. an aphid-free leaf disc system). Volatiles were collected during  
76 3h. Filters were eluted with 150  $\mu$ l of *n*-hexane and nonyl acetate (400 ng) was added to each  
77 sample as internal standard. The extracted samples were directly stored in a freezer at  $-80^{\circ}\text{C}$   
78 until GC analysis. Identification and quantification were performed respectively by GC-MS  
79 and GC-FID.

80

81 **Statistical analyses** – Regression analysis was used to correlate aphid density with number of  
82 eggs laid by female *E. balteatus* and amounts of released E $\beta$ F. All analyses were performed  
83 using Minitab® software (14.2 version, Minitab Inc, State College, PA, USA).

84

## 85 **Results and Discussion**

86 E $\beta$ F emission from non-preyed *M. persicae* was demonstrated in the present study by volatile  
87 collection and subsequent GC-MS analyses. Regression analysis revealed that there was a  
88 significant quadratic relationship between the amount of released E $\beta$ F (Y) and *M. persicae*  
89 colony sizes (X) ( $F_{2,23} = 14.89$ ;  $P < 0.001$ ;  $r^2 = 0.9997$ ). This relationship can be represented as  
90  $Y = 0.0143X^2 - 0.2265X$  (Fig. 1). Other chemical compounds were also identified, such as  
91 hexanal, 3-methyl-2-pentanone,  $\beta$ -terpinene, 6-methyl-5-hepten-2-one and limonene. These  
92 latter compounds were found to be released from leaf-disc system and they were not  
93 specifically induced by the presence of *M. persicae* colony on leaf disc. Geranyl acetone was  
94 also identified and its released amount was slightly decreasing with aphid colony size.

95 E $\beta$ F constant emission in absence of predators can have both positive and negative impacts.  
96 Aphids would have advantage to release small amounts of alarm pheromone in the case of  
97 crowded colonies to cause dispersion of the individuals and to help preserving the host plant.  
98 This effect would complete the already well-known effect of winged-morph induction

99 described by Kunert et al (2005). However, a constant emission of alarm pheromone increases  
100 the risk of the releasing colony to be located by a natural enemy.

101 According to previous observations, nearly no egg was laid in absence of aphids on the leaf  
102 disc (Fig. 1). The number of eggs laid (Y) increased significantly and linearly with the size of  
103 the aphid colony (X) ( $F_{1,39} = 94.12$ ;  $P < 0.001$ ;  $r^2 = 0.9996$ ), according to  $Y = 0.3475X + 0.1$ .

104 Several studies already suggested that the oviposition rate of syrphid females was a function  
105 of aphid densities (Chandler 1968; Itô and Iwao 1977; Bargen *et al.* 1998; Scholz and  
106 Poehling 2000; Sutherland *et al.* 2001; Almohamad *et al.* 2006). Our regression analysis  
107 suggests an adaptive oviposition behaviour leading the emerging larvae to locate immediately  
108 sufficient food resources. According to Bargen *et al.* (1998), the number of eggs laid by  
109 female hoverflies does not only depend on aphid quantity on the plant, but also on the  
110 presence and quantity of oviposition-eliciting substances emitted from the prey and the  
111 infested plant. Previous observations have indeed shown that predatory hoverflies oviposit in  
112 response to volatile compounds emitted from aphids and their liquid secretions such as  
113 honeydew (Dixon 1959; Bombosch and Volk 1966; Budenberg and Powel 1992; Shonouda  
114 1998; Verheggen *et al.* 2008).

115 Along with the previous results of Verheggen et al. (2008) on the role of oviposition stimulant  
116 of synthetic E $\beta$ F, these results demonstrate the strong implication of aphid alarm pheromone  
117 in aphidophagous syrphid oviposition behaviour.

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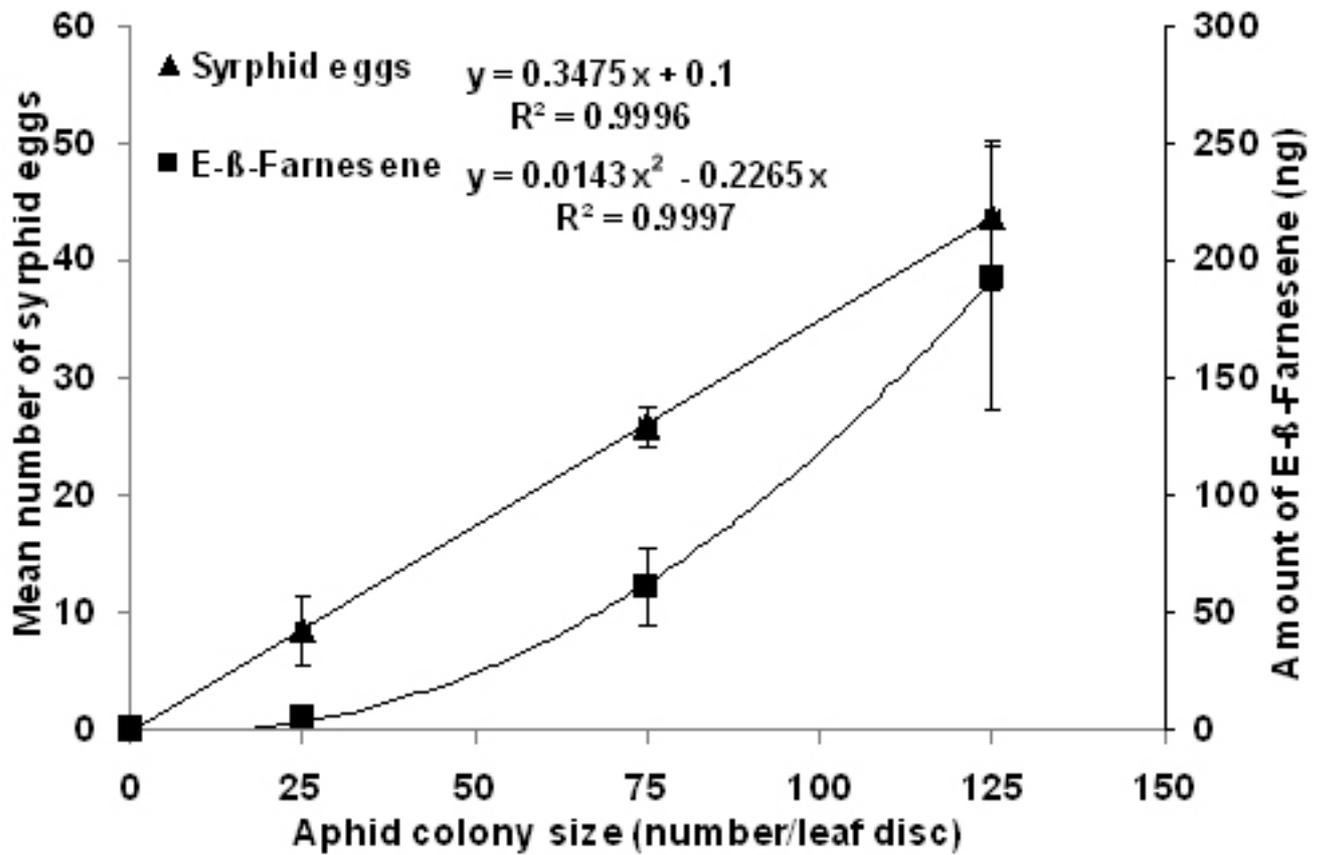
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177

178 **Figure legend**

179 **Figure 1.** Amount of (*E*)- $\beta$ - farnesene and mean number of *E. balteatus* eggs laid in response  
180 to increasing *M. persicae* colony size on broad bean leaf disc. Bars indicate standard errors of  
181 the means.  
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