Aphid alarm pheromone alters larval behaviour of the predatory gall midge, *Aphidoletes aphidimyza* and decreases intraguild predation by anthocorid bug, *Orius laevigatus*

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Running title: Aphid alarm pheromone affects IG prey behaviour

27 **Contribution of authors**

28 Conceived and designed the experiments: MH, WWW. Performed the experiments: MH, EH.

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30 WWW.

31

32 **Abstract**

33 Intraguild predation is the killing and consuming of a heterospecific competitor that uses

34 similar resources as the prey, and also benefit from preying on each other. We investigated the

35 foraging behaviour of the gallmidge, *Aphidoletes aphidimyza*, a predator of aphids used for

36 biological control, that is also the intraguild prey for most other aphid natural enemies. We

37 focus on how aphid alarm pheromone can alter the behaviour of the gallmidge, and predation

38 by the anthocorid bug *Orius laevigatus*. We hypothesized that gallmidges would respond to the

39 presence of (*E*)-β-farnesene (EBF) by leaving the host plant. Since feeding by *Aphidoletes*

40 gallmidge larvae does not induce EBF emission by aphids, this emission indicates the presence

41 of an intraguild predator. We found that gallmidge larvae reduced their foraging activities and

42 left the plant earlier when exposed to EBF, particularly when aphids were also present.

43 Contrastingly, gallmidge females did not change the time visiting plants when exposed to EBF,

44 but laid more eggs on plants that had a higher aphid density. Lastly, EBF reduced the number

45 of attacks of the intraguild predator, *O. laevigatus*, on gallmidge larvae, potentially because

46 more gallmidges stopped aphid feeding and moved off the plant at which point *O. laevigatus*

47 predated on aphids. Our work highlights the importance of understanding how intraguild

48 predation can influence the behaviour of potential biological control agents and the impact on

49 pest control services when other natural enemies are also present.

50

51 **Key words:** *Aphis fabae,* competition, intraguild predation, *Orius laevigatus,* signalling.

52 **Introduction**

53 Intraguild predation (IGP) is the killing and consuming of a heterospecific competitor that uses

54 similar resources as the prey, and also benefit from preying on each other. IGP has been shown

55 for a number of invertebrate and vertebrate species pairs (Polis et al., 1989; Rosenheim et al.,

56 1995; Rosenheim, 1998; Raymond et al., 2000; Snyder & Ives, 2001; Rieger et al., 2004;

57 Sergio et al., 2007; Martinou et al., 2010; Ferreira et al., 2011; Perdikis et al., 2014). The

58 aggressor is referred to as the intraguild predator (IG predator), the victim is the intraguild prey

59 (IG prey), and the common resource is an extraguild prey (Lucas et al., 1998). IGP not only

60 provides an additional food resource for IG predators, but may also reduce inter- or

61 intraspecific competition, so that it is sometimes considered to be an extreme form of

62 competition. As IG prey populations may suffer substantial mortality due to IGP (Lucas et al.,

63 1998; Dixon, 2000; Sato et al., 2005), there is evidence that in many cases IG prey tend to

64 avoid habitats where the IG predators are already or potentially present (Nakashima et al.,

65 2004; Sarmento et al., 2007; Frago & Godfray, 2014). Such habitat selection has been shown

66 both for IG prey females in their choice of suitable oviposition sites, and for IG prey offspring

67 in their choice of feeding sites. Examples are aphid-feeding ladybirds and lacewings (Ruzicka,

68 1998, 2001b, a; Agarwala et al., 2003; Sato et al., 2005), aphid hymenopteran parasitoids

69 (Nakashima et al., 2004), dragonflies and damselflies (Ferris & Rudolf, 2007; Mortensen &

70 Richardson, 2008), several species of tree frogs (*Hyla*) (Rieger et al., 2004) and various bird

71 species (e.g. Sergio *et al*. 2007).

72 While visual detection of IG predators may be common in vertebrates, invertebrate IG prey

73 may also use chemical cues associated with the presence of IG predators for habitat selection

74 (Dicke & Grostal, 2001). For example, oviposition-deterring compounds in the tracks of larvae

75 of ladybird species (Coleoptera: Coccinellidae) deter females of conspecific or heterospecific

76 ladybirds from laying eggs (Hemptinne et al., 2001; Ruzicka, 2003, 2006). Hydrocarbons left

77   on the plant by foraging adult ladybirds *Coccinella septempunctata* and *Adalia bipunctata* also

78   lead to patch-leaving behaviour of a number of aphid parasitoid species (Nakashima et al.,

79   2006). In addition to these non-volatile ladybird tracks, volatile cues have been implicated in

80   the IGP avoidance behaviour of the ladybird *Cycloneda sanguine*, but the compounds involved

81   have not yet been identified (Sarmento et al., 2007). We still know little about how IG prey

82   decide to avoid or to leave a patch where the risk of IGP is high. For habitat choice by IG prey,

83   any chemical cue emitted by an IG predator is a candidate cue to avoid contact with a particular

84   IG predator species. In addition, chemical compounds emitted by the (extraguild) prey, when

85   preyed upon, would also indicate the presence of a predator, but would not be specific to a

86   predator species. The use of such unspecific signals has not been described for IGP systems.

87   Aphids (Hemiptera: Aphididae) are attacked by a large number of predators and parasitoids,

88   and hence IGP within the guild of aphid natural enemies is frequent (Lucas, 2005). One

89   effective aphid predator that is used frequently in aphid biocontrol is the predatory gallmidge

90   larvae, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) (Markkula et al., 1979;

91   Boulanger et al., 2019). The rather small and defenceless larvae of *A. aphidimyza* suffer from

92   IGP by many other aphid predators, in particular ladybird larvae and predatory anthocorid bugs

93   (Lucas et al., 1998; Christensen et al., 2002). Larvae of *A. aphidimyza* are furtive predators and

94   extract the aphids' body contents on site without stimulating any significant increase in quick

95   predator avoidance behaviour (e.g. aphid dropping); however, attacks may result in an increase

96   in aphid walking (slow predator avoidance behaviour). There is evidence that *A. aphidimyza*

97   larvae leave patches where it could become prey to other predators (Lucas et al., 1998; Lucas &

98   Brodeur, 2001). Lucas *et al.* (1998) studied IGP among three common aphid predator species,

99   *A. aphidimyza*, *Chrysoperla rufilabris* and *Coleomegilla maculata lengi*, in the presence and

100   absence of extraguild prey *Macrosiphum euphorbiae* to characterize the levels and symmetry of

101   IGP among the various stages of the predators. They found that *A. aphidimyza* is more

102 vulnerable to IGP than the other two predators. In addition, they realized that sessile and low

103 mobility stages such as larval or pupal stages of all predator species are more vulnerable to

104 IGP.

105 One important compound that mediates aphid-predator interactions is the aphid alarm

106 pheromone (*E*)-β-farnesene (EBF), that is emitted by an aphid when attacked by a predator

107 (Bowers et al., 1972; Kislow & Edwards, 1972). EBF triggers various behavioural reactions: an

108 aphid may become more alert, withdraw the stylet or drop off the host plant (Montgomery &

109 Nault, 1977; Humphreys & Ruxton, 2019). As EBF is only emitted after attack, it is an

110 indication for predatory activity in the aphid colony (Hatano et al., 2008).

111 In this paper, we use synthetic EBF to investigate if aphid alarm pheromone affects the

112 searching behaviour of the gallmidge *A. aphidimyza*. Such use of EBF to indicate the presence

113 of an IG predator would be interesting as this would be the first example of the use of an

114 unspecific (extraguild) prey alarm signalling for the avoidance of IGP. In particular, we test if

115 (1) *A. aphidimyza* larvae change their behaviour in aphid colonies when exposed to EBF, (2)

116 non-predatory adult females of *A. aphidimyza* change movement or oviposition behaviour in

117 aphid colonies when exposed to EBF, or at two different densities of aphids in the presence of

118 EBF, and lastly (3) EBF mediates changes in IGP of *Orius laevigatus* (Hemiptera:

119 Anthocoridae) on *A. aphidimyza.*

120

121 **Materials and methods**

122 *Experimental conditions*

123 Black bean aphids, *Aphis fabae*, were reared on, and experiments were conducted, on four-

124 week-old broad bean, *Vicia faba*, in 10 cm diameter pots covered with air-permeable

125 cellophane bags (L x W = 39 x 18.5 cm, Armin Zeller, Nachf. Schütz & Co, Langenthal,

126 Switzerland) to prevent the scape of experimental insects. These bags have to be air-permeable

127   because it was needed to remove the extra moisture from the bags. Aphids were originally

128   collected near Jena (Thuringia, Germany) on *Vicia faba*.

129   For the experiments, aphid replicate (isofemale) lines were initiated by placing single aphid

130   females on new plants. Descendants of a single foundress were used among treatments in each

131   experiment to account for maternal effects (Kunert & Weisser, 2003) and were always tested

132   on the same day (i.e. one aphid replicate line was used only once for each treatment). The

133   experiments were conducted at 20˚C, with a photoperiod of 16:8 L:D and about 75% relative

134   humidity.

135

136   *Rearing of experimental predators*

137   The predatory midge, *A. aphidimyza*, was obtained as pupa from a commercial supplier (Katz

138   Biotech Services, Germany). Adults were hatched by placing the pupae into a dark growth

139   chamber for 48 hours at 20˚C. To obtain gallmidge larvae, adult *A. aphidimyza* were released

140   on aphid-infested plants for laying eggs. Nine days after eclosion, the larvae reached the third

141   instar (maintained on plants with *A. fabae* as a food source) and were then used in the

142   experiment. To obtain gravid females, couples of newly-hatched female and male flies from

143   pupae stage were kept separately in test tubes (diameter 50 mm, height 100 mm) for 24 hours to

144   encourage mating and gravid females were subsequently used for experiment.

145   The predatory minute bug, *O. laevigatus*, were obtained as adults from the same commercial

146   supplier. Adults were kept in a dark growth chamber at 10˚C (according to the Katz Biotech

147   AG company's instruction for short-term storage of adult *O. laevigatus*, those should be stored

148   in a cool (8-10 ºC) and dark place) and fed with *A. fabae* until they were used in the

149   experiments.

150

151   *Experiment I - Larval behaviour*

152   This experiment tested the effect of EBF on the behaviour of *A. aphidimyza* larvae, in the

153   absence or presence of aphids. Thus, the experiment had two treatments with two factor levels

154   each in a 2x2 factorial design. One predatory *A. aphidimyza* gallmidge larva was released either

155   on an aphid-free plant or a plant infested with 10 third/fourth nymphal instars of the aphid *A.*

156   *fabae*, and these were exposed to either EBF (Bedoukian Research Inc., Danbury, CT, USA) or

157   to n-hexane as a control.

158   To obtain experimental aphids, eight adult aphids from a replicate line were placed on four new

159   broad bean plants (two adult aphids on each plant) to produce 10-12 offspring within 24h after

160   which time the adults were removed from the plant. The four plants were randomly allocated to

161   one of the four treatments. After six days, 10 offspring were left on the plant and used in the

162   experiment.

163   A single larva of *A. aphidimyza* was starved for five hours before being placed on a second

164   fully expanded leaf of each plant. The plant was then covered by a cellophane bag (Figure 1).

165   The cellophane bag had no connection with the plant, and there was a space between the plant

166   and the bag, so, the cellophane bag had no effect on larval foraging behaviour. Immediately

167   after placing the bag on the plant, EBF solution (500 ng in three µl n-hexane) or only three µl

168   n-hexane were applied, using a glass syringe (10 µl, Hamilton), through a small hole in the

169   cellophane bag to a piece of filter paper (1 x 1 cm) held by a wire that was inserted into the soil

170   (Kunert et al., 2005). The distance between the filter paper and the plant was approximately 5

171   cm (Figure 1). For the next 15 minutes, the behaviour of *A. aphidimyza* larva was observed at

172   every one minute such that a snapshot of behaviour was taken once every minute (in total 15

173   observations) without removing the cellophane bag using a desk table magnifier with 20x

174   magnification. Larvae displayed one of the following behaviours when being on the plant:

175   larval movement (crawling) on the leaf, larval movement on the stem, no movement (resting),

176   head circulation (alert behaviour while not moving on the plant, also used as defence behaviour

177 (cf. Messelink et al., 2011), and feeding (predatory behaviour). We also noted when a larva was

178 off the plant. Aphid behaviour was also observed for one minute after the application of EBF or

179 n-hexane, and noted when they moved off the plant. We calculated the proportion of time

180 points (N=15) doing a particular behaviour for using in the analyses. Finally, we calculated the

181 *time up to the first attack* as the number of observations before the first attack of a larva on an

182 aphid was observed. In total 15 replicates were used in the experiment (15 x 4 treatments = 60

183 experimental units).

184

185 *Experiment II- Female behaviour*

186 This experiment tested the effect of EBF on the behaviour of gravid *A. aphidimyza* females, at

187 two different densities of aphids. As preliminary experiments had shown that female *A.*

188 *aphidimyza* only lays eggs on aphid-infested plants, females were released on plants infested by

189 either 50 (high-density) or five (low-density) aphids. The different densities were chosen to test

190 the effect of EBF on females over a broader range of aphid densities. Thus, the experiment had

191 also two treatments with two factor levels each, in a 2x2 factorial design. Female *A. aphidimyza*

192 and aphids were exposed to either EBF (500 ng in three µl n-hexane) or three µl n-hexane as a

193 control three times in the experiment: at the beginning and after 8 and 16 h.

194 To obtain low-density aphid colonies a single adult of *A. fabae* was introduced on a new bean

195 plant and allowed to produce offspring for 24 hours. Five offspring were left on the plant. To

196 obtain high-density colonies, 10 adult aphids from the same line were at the same time

197 introduced to another plant for one day after which all aphids except about 50 (48-52) offspring

198 were removed from the plant. The plants were used in the experiment when the offspring were

199 six days old. Plants were again covered with cellophane bags.

200 To start the experiment, a single mated female of *A. aphidimyza* (17 days old) was released into

201 the cellophane bag using a glass tube (diameter 15 mm, height 120 mm). Immediately

202 afterwards, EBF or n-hexane was applied using a glass syringe (10 µl, Hamilton) onto a piece

203 of filter paper (1 x 1 cm) fixed with a wire that was inserted into the soil. The behaviour of the

204 female was observed at every one-minute for 10 minutes (in total 10 observations): Movement

205 on the plant, immobile on the plant, immobile off the plant (on the cellophane bag) or flying off

206 the plant. After 24 hours, the total number of eggs laid on the plant was counted. In addition,

207 aphid walking behaviour was recorded for one minute after the application of solutions. In total

208 27 replicates were used in the experiment (27 x 4 treatments = 108 experimental units).

209

210 *III- Effect of EBF on IGP*

211 To assess the effect of EBF on IGP of *A. aphidimyza* by *O. laevigatus*, four third-instar larvae

212 of *A. aphidimyza* were starved for five hours before being placed on a leaf of an experimental

213 plant with a group of eight black bean aphids covered with a cellophane bag. Immediately after

214 placing the larvae on the plant, EBF solution (500 ng in three µl n-hexane) or only 3 µl n-

215 hexane were applied (for details see experiment I).

216

217 After five minutes, the behaviour of the four *A. aphidimyza* larvae was classified: feeding on

218 aphids, moved off the plants, or still on the plant but not feeding. Aphid behaviour was also

219 observed for one minute after the application of EBF, to assess if aphids were walking away

220 from the feeding site or dropped from the plant. After these five minutes, an *O. laevigatus*

221 female was introduced, using a fine paintbrush, near the aphid colony where most *A.*

222 *aphidimyza* larvae were also present. The behaviour of the *O. laevigatus* was then observed

223 once a minute for 15 minutes. We noted if the *O. laevigatus* was walking on the plants, whether

224 it was immobile or whether it was attacking an *A. aphidimyza* larvae or an aphid. In total 13

225 replicates were used in the experiment (13 x 2 treatments = 26 experimental units).

226

227   *Statistical analysis*

228   Results are presented as means ± standard error in all cases. All data were analysed in R v3.2.0

229   using RStudio v 0.98.977. Data for the first (larval behaviour) and second (female behaviour)

230   experiment was analysed using GLMs with quasibinomial error distribution for the response

231   variables with proportion data. Here, we used the number of instances of a particular behaviour

232   bound as one variable to the total number of instances, using the cbind function in R. In these

233   experiments, we also analysed the movement of aphids (number of aphids moving within one

234   min of EBF application) and the number of eggs laid by the female *A. aphidimyza* and here, we

235   used a GLM with quasipoisson error distribution for count data. For experiment three, we also

236   ran models using aphid movement and *O. laevigatus* attack rate on aphids to test the relative

237   importance of each variable in the model. The time to first attack by *A. aphidimyza* larvae was

238   analysed using a standard linear model with normal error distribution. Full models were first

239   run, including block as a factor, and then a backwards stepwise model was used to obtain the

240   minimum adequate model.

241

242   **Results**

243   *Experiment I- Larval behaviour*

244   The *A. aphidimyza* larvae exhibited more instances of alert behaviour (head circulation, an alert

245   and orientation behaviour of the gallmidge larvae) and movement on the stem when EBF was

246   present, but this was dependent on the presence of aphids (Table 1). For example, head

247   circulation was most frequent when there were no aphids and EBF was present (17.8 ± 2.7 % of

248   instances in this treatment), and least when there were no aphids and no EBF (5.8 ± 2.2 % of

249   instances; Figure 2). Larval movement on the stem and off the plant was more frequent on

250   plants with aphids and EBF alarm pheromone (Figure 2); in this treatment, aphids were also

251   more likely to move off the plant (18.7 ± 2.1 % move when EBF was present compared to only

252 3.1 ± 0.9 % when EBF was absent). In the other treatments, there was little movement on the

253 stem or off the plant and therefore on these we observed more instances of movement on the

254 leaf and feeding on aphids (Figure 2). There was no significant difference in the time to first

255 aphid feeding instance between the EBF (11 ± 1.78 min, n=4) and control treatments (8.78 ±

256 1.16 min, n=9) ($F_{1,11}$ = 1.11, P = 0.314), although only 13 replicates, in which larvae were

257 feeding, could be evaluated.

258

259 *Experiment II- Female behaviour*

260 The behaviour of the adult female *A. aphidimyza* was strongly affected by aphid density (Table

261 2). Females spent more time on the plant when the aphid density was high and this did not vary

262 with the EBF treatment, which had very little effect on female behaviour (Table 2; Figure 3). In

263 total 57 out of 108 females laid eggs in the experiment. Females laid significantly more eggs in

264 the high aphid density treatments, with no effect of EBF (Table 2, Figure 3).

265

266 The addition of EBF increased aphid movement and the response was dependent on aphid

267 density with more instances of aphid walking in the high-density treatment with EBF (Table 2,

268 Figure 3). The number of instances of aphid movement was the same for the high and low

269 aphid densities with no EBF with five instances across all replicates compared to 74 instances

270 across all replicates when EBF was present (Figure 3). Thus, while there were more aphids in

271 the high-density treatment potentially leading to a higher chance of aphid movement, without

272 EBF the aphids moved very little in either density treatment.

273

274 *III- Effect of EBF on IGP*

275 Consistent with the previous experiments, aphids were observed to walk away and drop off the

276 plant more often in the presence of EBF ($F_{1,24}$=227.6, P<0.001). The behaviour of *A.*

277   *aphidimyza* larvae was consistent with the results from experiment I, with larvae only leaving

278   the plant when EBF was present ($F_{1,24}=26.67$, P<0.001) and also feeding on aphids for less time

279   with EBF present ($F_{1,24}=28.27$, P<0.001) (Figure 4). By including aphid movement into the

280   model as a covariate for movement of *A. aphidimyza* larvae off the plant, we see that EBF

281   treatment still significantly explains more of the variation ($F_{1,23} = 49.70$, P<0.001) than does

282   aphid movement ($F_{1,23} = 13.23$, P<0.001).

283

284   There was no effect of EBF on the time that *O. laevigatus* spent either immobile ($F_{1,24}=0.98$,

285   P=0.333) or walking on the plant ($F_{1,24}=0.02$, P=0.883) (Figure 4). Attacks of *O. laevigatus* on

286   *A. aphidimyza*, were, however, more frequent when EBF was not present ($F_{1,24}=9.21$, P=0.006),

287   and when EBF was present *O. laevigatus* attacked more aphids ($F_{1,24}=3.45$, P=0.076) (Figure

288   4). By including aphid movement and *O. laevigatus* attack rate on aphids into the model as a

289   covariate for the attack rate of *O. laevigatus* on *A. aphidimyza* we found that EBF again

290   explains a significant amount of variation ($F_{1,22} = 6.54$, P=0.018) above that explained by aphid

291   movement ($F_{1,22} = 6.39$, P=0.019) or *O. laevigatus* attack rate on aphids ($F_{1,22} = 13.85$,

292   P=0.001).

293

294   **Discussion**

295   We found that the larvae of the predatory gallmidge *A. aphidimyza* responded to the presence

296   of EBF with non-predatory adults not responding. The aphids themselves responded strongly to

297   EBF by moving off the plant, which may have also led the larvae to also move off the plant

298   since they only did this in response to EBF when aphids were present. In accordance, the larvae

299   showed less movement on the leaves as they moved onto the stem and consequently off the

300   plant when both aphids and EBF were present. Therefore, EBF presence plus aphid movement

301   off the plant together had a stronger (non-additive) effect on the probability of a larva leaving a

302 plant. Larval feeding was also disrupted by EBF, with more feeding occurring when there was

303 no EBF and less when there was, again likely influenced by aphid movement off the plant. The

304 increased probability of moving off a plant in the presence of both aphids and EBF was also

305 found to be related to a reduced probability of being preyed upon by intraguild predators, such

306 as *O. laevigatus*. This suggests that the plant-leaving behaviour also serves to reduce the risk of

307 intraguild predation.

308

309 Gallmidge head circulation movements were also increased after EBF application, particularly

310 when there were no aphids. This indicates the behaviour may be linked to IG predator

311 recognition. Head circulation is an alert behaviour response to search for additional cues on the

312 presence of an IG predator (Messelink et al., 2011). Predatory *A. aphidimyza* gallmidges are

313 stealthy predators, and the larvae approach their victims by inconspicuous creeping movements

314 and subdue them by injecting a paralyzing toxin, thereby deactivating behavioural defences of

315 the prey. Gallmidge feeding itself does not stimulate any significant increase in dropping

316 behaviour or movements of the remaining aphids in the colony (Klingauf, 1967; Lucas &

317 Brodeur, 2001). Thus, for gallmidge larvae, any increase in aphid plant-leaving behaviour on

318 the plant is evidence for the action of a *different* aphid predator on the plant (Lucas et al.,

319 1998). By leaving plants when aphids start to move around, gallmidge larvae not only decrease

320 the risk of becoming a victim of IGP, but this could also be a cue to leave due to diminishing

321 resources. While we did not inherently test this in our experiments, the slow and stealthy attack

322 method by *A. aphidimyza* larvae means they cannot feed on moving aphids and thus would be

323 negatively affected by increased aphid movement.

324 The behaviour of adult females of *A. aphidimyza* was not affected by the application of EBF.

325 Instead, females responded to increased aphid density on the plant by increasing residence time

326 and oviposition rate. Thus, females respond positively to the likelihood of increasing their

327 reproductive success (reviewed by Boulanger et al., 2019), but they do not react towards

328 possible risks for their offspring. A possible explanation for the lack of response, apart from a

329 possible inability to perceive EBF, is that EBF emission is not a good indicator for the future

330 risk of IGP for the gallmidge offspring. In another study, adult *A. aphidimyza* females also did

331 not respond to the presence of adult or larvae of the coccinellid IG predator *Coleomegilla*

332 *maculata* (Lucas & Brodeur, 1999). On the other hand, female gallmidges are able to recognize

333 the presence of conspecific gallmidge larvae. When aphid colonies were exposed to *A.*

334 *aphidimyza* larvae or to water extracts of larvae, female gallmidges laid significantly fewer

335 eggs in such colonies (Ruzicka & Havelka, 1998). These conflicting results need further

336 attention. It is possible that the time-delay between egg-laying and the hatching of the larvae

337 makes an avoidance of currently predator-occupied patches non-adaptive, as many aphid

338 predators stay only for a short time in aphid colonies (Minoretti & Weisser, 2000). However,

339 this may be unlikely since eggs are vulnerable to intraguild predation because of their small

340 size and immobility (Lucas, 2005). With respect to their ability of perceiving EBF, a number of

341 studies have suggested that female midges use honeydew as a cue in the process of prey

342 location and do not use plant volatiles or odours from the aphids themselves (reviewed by

343 Boulanger et al., 2019).

344

345 Intraguild predation is widespread in aphidophagous guilds and represents an important

346 mortality factor for aphid predators (Rosenheim et al., 1993; Müller et al., 1999; Arim &

347 Marquet, 2004; Lucas, 2005; Nedved et al., 2013; Yu et al., 2019). We have shown that the

348 presence of EBF not only alerts aphids but also results in a change in the behaviour of

349 predatory gallmidge larvae. To our knowledge, this provides the first example for a role of an

350 unspecific (extraguild) prey alarm signal in the avoidance of IGP by the intraguild prey.

351 Interestingly, in the interaction between aphids and gallmidges, EBF may be classified as a

352   synomone (Vet & Dicke, 1992) as it provides benefits to both the producer and the receiver of

353   the signal: for gallmidge larvae the risk of IGP is reduced while the leaving of gallmidges also

354   provides benefits for the aphids because their predation pressure is reduced. However, while

355   the aphid benefits from short-term reduced predation, it also suffers from reduced feeding that

356   will reduce its own reproductive efforts. Moreover, if the gallmidge is successful in avoiding

357   IG predation then this can benefit its population growth, therefore longer-term dynamics may

358   reveal a negative effect on aphids. It need to have in mind that in this study the synthetic EBF

359   have been tested, not a compound that is released by an organism.

360   The most important applied aspects of findings about IGP are their use in biological control and

361   conservation management (Müller & Brodeur, 2002; Boulanger et al., 2019). We note that in

362   this study synthetic EBF was used rather than aphid-derived EBF, and thus these interactions

363   require further study to understand how the levels produced by aphids in the field may impact

364   biological control and IGP effects. We showed that at higher aphid density adult gallmidges

365   were more likely to be on the plant and lay eggs, while the larvae were more likely to respond

366   to the alarm pheromone and follow aphids off the plant. In an agricultural field, this would

367   increase the number of larvae on plants with high aphid density, but also the larvae will

368   potentially follow the aphids as they move onto other plants after being disturbed by other

369   predators further increasing overall biocontrol. Field experiments and longer-term studies on

370   the community-level consequences will lead to a greater understanding of how IGP can be

371   managed in the field to maximise biological control success.

372

373   **Disclosure**

374   The authors declare that they have no conflict of interest.

375

376   **Acknowledgments**

382

383   **References**

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518 Table 1. Summary of results from *Aphidoletes aphidimyza* larval behaviour experiment, using

519 plants with/without aphids and with/without exposure to EBF

	Aphid presence		Alarm pheromone		Aphid presence x alarm pheromone	
	F	P	F	P	F	P
Larval behaviour						
Head circulation	0.42	0.517	↑ 3.77	0.057	**9.24**	**0.004**
Movement on stem	↑ **8.66**	**0.005**	↑**39.63**	**<0.001**	**7.76**	**0.008**
Off plant	↑ **6.14**	**0.016**	↑**13.99**	**<0.001**	3.16	0.081
Movement on leaf	↓**10.14**	**0.002**	↓ **7.68**	**0.008**	8.79	0.004
Feeding	na	na	↓ **7.63**	**0.001**	na	na
Aphid behaviour						
Movement	na	na	↑**50.81**	**<0.001**	na	na

520 *Arrows show direction of effect, ↑ means more instances of this behaviour in either the*

521 *presence of aphids or with EBF alarm pheromone, ↓ means fewer instances of this behaviour.*

522 *N=60. Values in bold are significant at P<0.05*

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531 Table 2. Summary of results from *Aphidoletes aphidimyza* female behaviour experiment, using

532 plants with high/low aphid density and with/without exposure to EBF

	Aphid density		Alarm pheromone		Aphid density x alarm pheromone	
	F	P	F	P	F	P
Female behaviour						
Number of eggs laid	↑**10.87**	**0.001**	(1.88)	(0.173)	(0.43)	(0.511)
Movement on plant	↑**10.36**	**0.002**	↓ 3.42	0.067	(0.37)	(0.546)
Immobile on plant	↑**19.77**	**<0.001**	(0.11)	(0.742)	(0.12)	(0.727)
Off plant (immobile)	↓**24.16**	**<0.001**	(1.24)	(0.266)	(0.05)	(0.819)
Off plant (flying)	↓ 2.85	0.094	(0.31)	(0.581)	(0.06)	(0.810)
Aphid behaviour						
Movement	↑**30.82**	**<0.001**	↑**85.61**	**<0.001**	**4.63**	**0.034**

533 *Arrows show direction of effect, ↑ means more instances of this behaviour in either the high*

534 *aphid density or with EBF alarm pheromone, ↓ means fewer instances of this behaviour.*

535 *N=108. Values in brackets were removed from the minimum adequate model. Values in bold*

536 *are significant at P<0.05*

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544   **Figure legends**

545   **Figure 1.** The experimental unit, which shows setup for the experiments. A broad bean, *Vicia*

546   *faba*, in 10 cm diameter pots covered with air-permeable cellophane bags (L x W = 39 x 18.5

547   cm, Armin Zeller, Nachf. Schütz & Co, Langenthal, Switzerland) to prevent the scape of

548   experimental insects.

549

550   **Figure 2.** The behaviour of aphids and *Aphidoletes aphidimyza* larvae. Data given as the mean

551   number for the aphids and as the proportion of time spent (15 mins) among experimental

552   treatments for *Aphidoletes*: with aphids (hashed bars) and without (solid bars) aphids, and with

553   exposure to EBF alarm pheromone (grey bars) and without exposure to EBF alarm pheromone

554   (white bars). *Aphidoletes* behaviour was split into different movement behaviours (off plant, on

555   leaf, on stem and no movement) plus alert behaviour (head circulation) and predatory

556   behaviour (feeding). Different letters denote significant difference between treatments (P <

557   0.05). Error bars show ± 1SE.

558

559

560   **Figure 3.** The behaviour of aphids and *Aphidoletes aphidimyza* females. Data given as the

561   mean number for the aphids and eggs laid, and the proportion of time spent (10 mins) among

562   experimental treatments for other *Aphidoletes* female behaviour: with high density of aphids

563   (hashed bars) and low density of aphids (solid bars), and with exposure to EBF alarm

564   pheromone (grey bars) and without exposure to EBF alarm pheromone (white bars).

565   *Aphidoletes* female behaviour was split into different movement behaviours [movement on

566   plant, immobile on plant, off plant (immobile) and off plant (flying)] plus oviposition

567   behaviour (number of eggs laid). Different letters denote significant difference between

568   treatments (P < 0.05). Error bars show ± 1SE.

569

570   **Figure 4.** The behaviour of *Aphidoletes aphidimyza* larvae and *Orius laevigatus* adults, with

571   and without EBF alarm pheromone addition. Data given as the mean number of *Aphidoletes*

572   (total of four individuals) and the proportion of time spent (15 mins) for *Orius* behaviour.

573   Different letters denote significant difference between treatments (P < 0.05). Error bars show ±
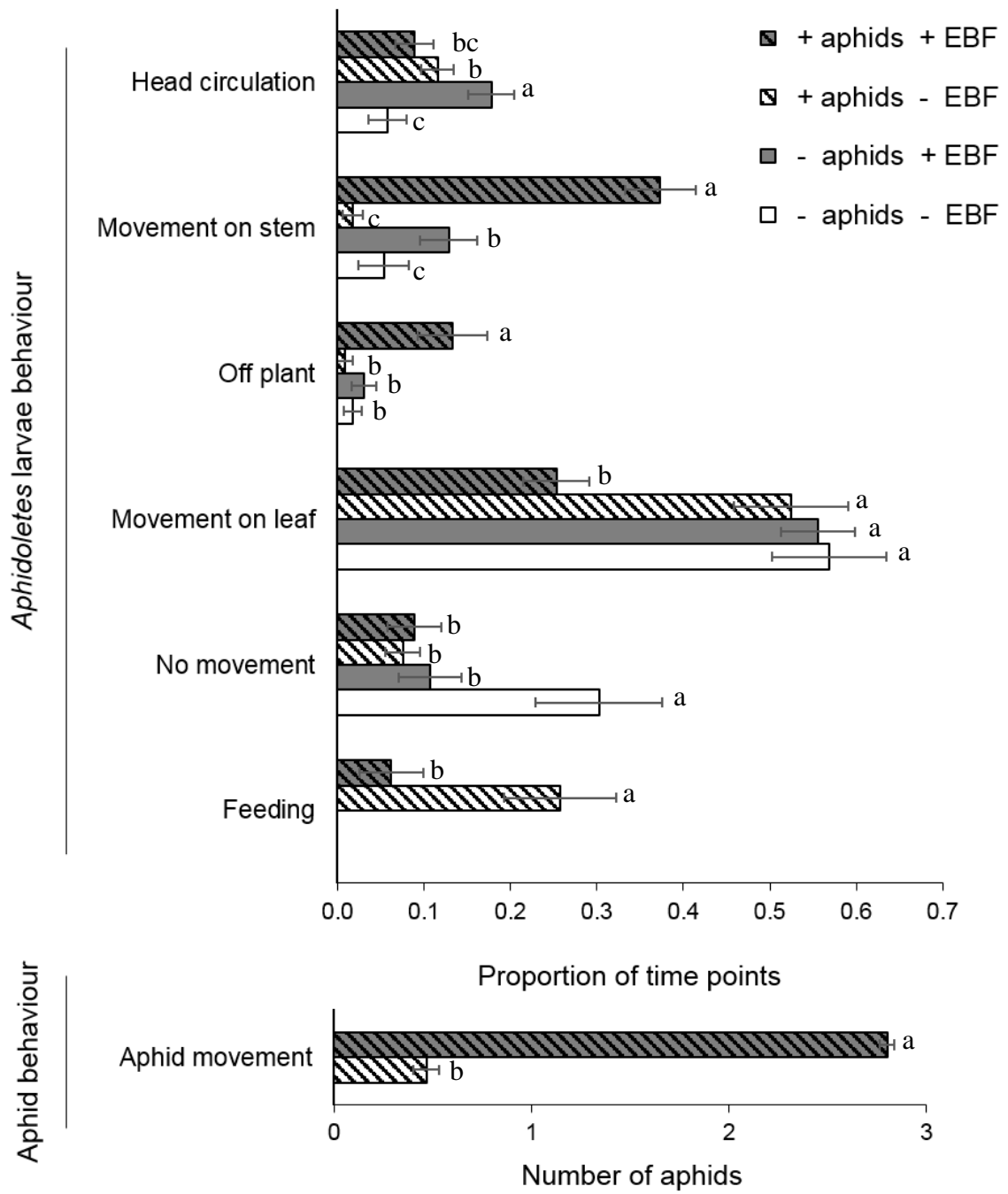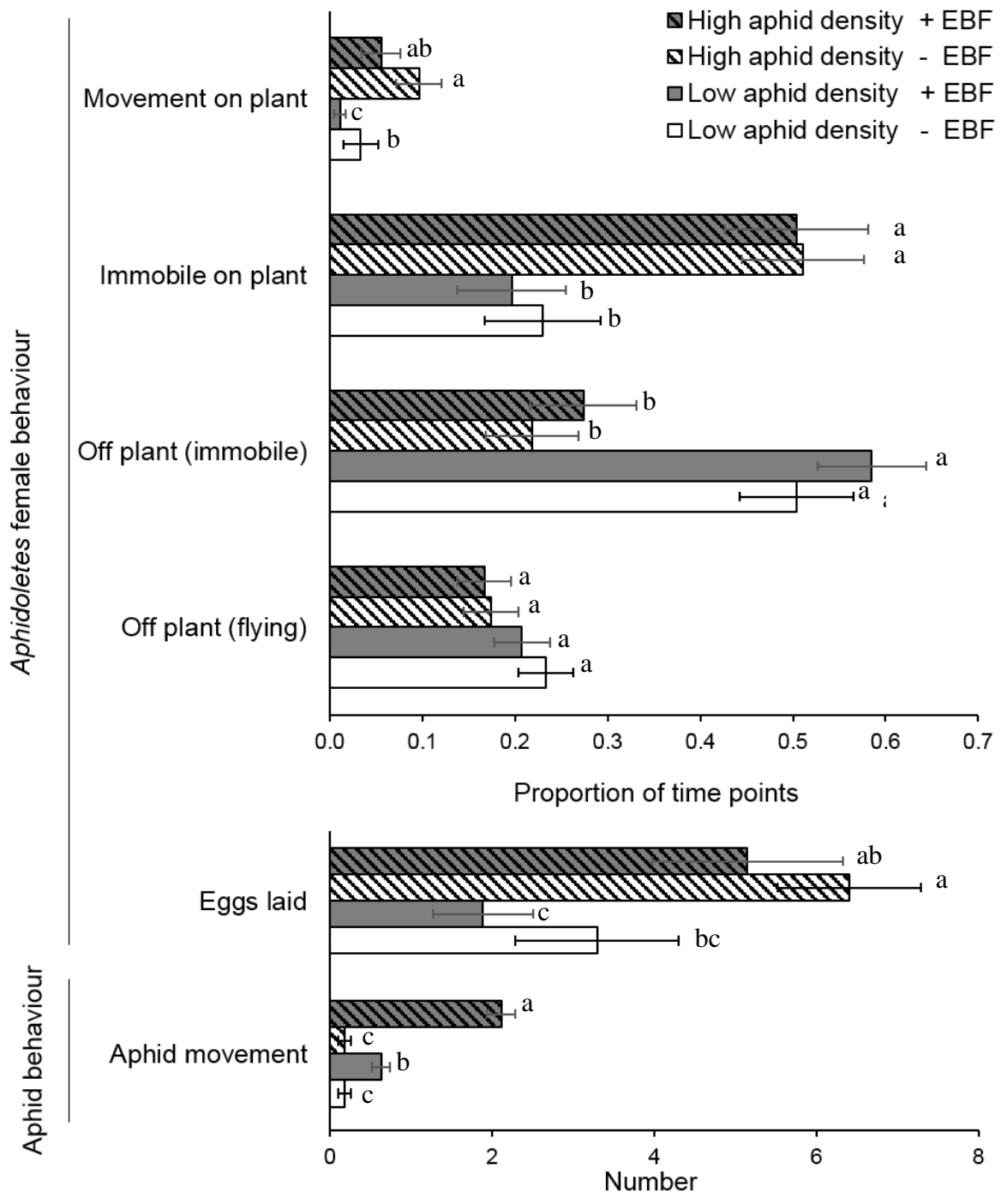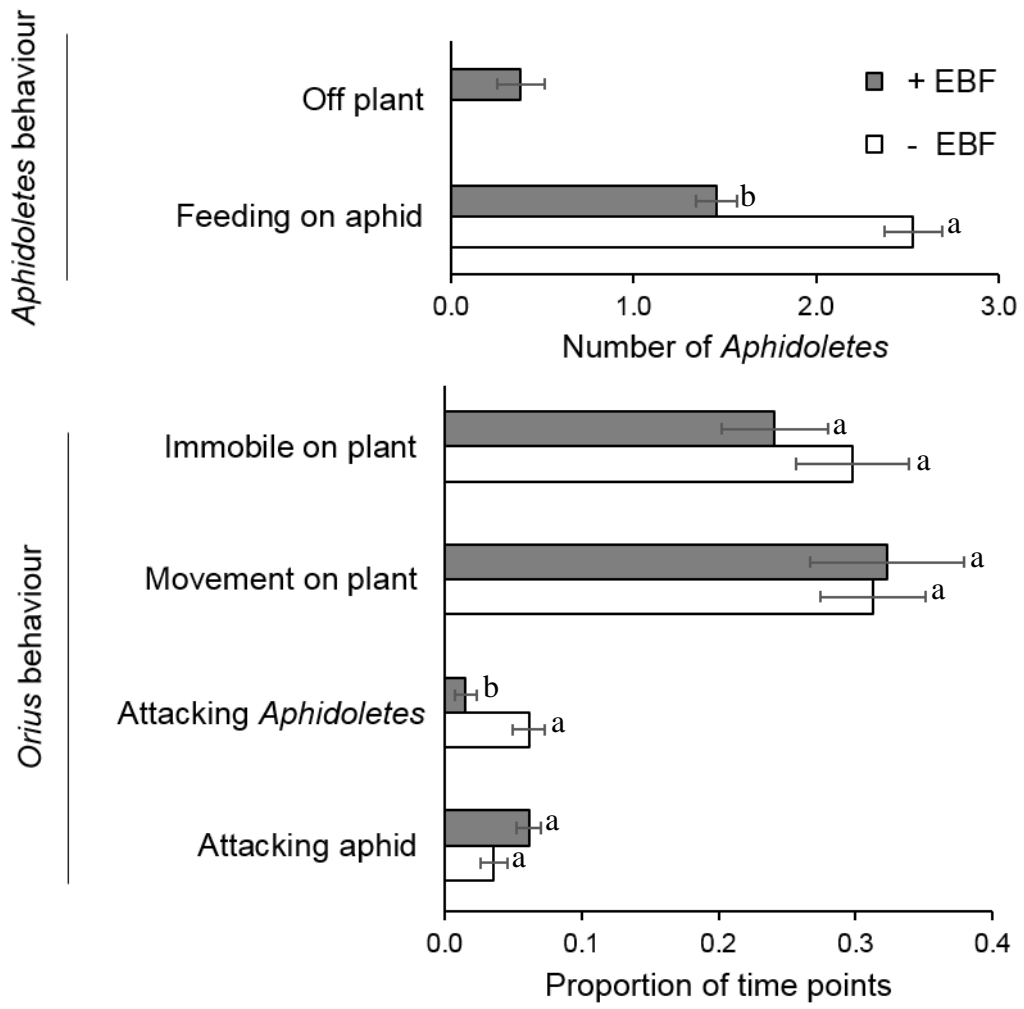
574   1SE.

Figure 1

Figure 2

Figure 3

Figure 4