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1   **Changed host plant volatile emissions induced by chemical interaction between unattacked**

2   **plants reduce aphid plant acceptance with intermorph variation**

3

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10

11   **Abstract**

12   Olfactory orientation by aphids is guided by specific volatile blends released from their

13   hosts. Host plants that co-exist with other plants may be less attractive for aphids due to volatile

14   interactions between neighboring plants which can lead to changes in their volatile emissions.

15   These changes in host plant volatile profiles induced by interactions between undamaged plants

16   could be used to manage aphid populations in crops. When potato plants are exposed to volatiles

17   from onion plants, the volatile profile of potato changes in relation to that of unexposed plants

18   with consistently greater quantities of two terpenoids released. We examined the host plant

19   searching behavior of aphids and showed that induced changes in plant volatile emissions affect

20   aphid behavior. We assessed olfactory responses of winged and wingless aphids, *Myzus persicae*

21   Sulzer (Hemiptera: Aphididae) to the changed volatile emissions. Both morphs were

22   significantly less attracted to odors of potato plants that had been exposed to volatiles from onion

23   than to odors of unexposed potato plants. Further, both morphs were significantly less attracted

24   to synthetic blends mimicking volatiles emitted by onion-exposed potato plants than to blends

25   mimicking non-exposed controls, and to single compounds emitted in greater quantities by

26   exposed potato. Aphid morphs were repelled differently depending on the concentration of odor

27   sources; winged aphids responded to higher doses than did wingless aphids. The aphid responses

28   to changes in plant volatile profiles induced by neighboring plants may facilitate refinement of

29   habitat manipulation strategies (e.g. intercropping) for integrated pest management to reduce

30   aphid occurrence in crops.

31   **Keywords**

32   Alatae, apterae, *Myzus persicae*, olfactory response, plant interaction, volatile chemicals

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35   **Key Message**

36   • Polyphagous aphids use plant odors in their host plant detection.

37   • Co-existence with other plant species may change volatile emission of aphid host plants.

38   • Green peach aphids are less attracted to hosts with changed volatile profiles.

39   • Winged and wingless aphids respond differently to those changes.

40   • Winged aphid responded to higher concentration of odour sources than wingless morphs.

41   • Habitat manipulation strategies within crop field (e.g. intercropping) can be developed to

42   disrupt aphid orientation and prevent their establishment as a pest.

43

44   **Author Contribution Statement**

45   VN conceived and designed the experiments. ID and AV performed the experiments. VN

46   analyzed the data. ID, VN and AV wrote the manuscript. All authors read and approved the

47   manuscript.

Introduction

The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), is a polyphagous aphid

pest with high ecological and agronomical importance worldwide, mainly because it is a vector

of viruses of many crops including potato (Solanaceae: *Solanum tuberosum* L.). *Myzus persicae*

has developed resistance to at least 70 synthetic compounds and various insecticide resistance

mechanisms have been reported worldwide (Silva et al. 2012). So far, there is no alternative to

insecticides to control populations of these insects, thus alternative strategies are highly sought

after. By understanding how aphids locate their host plants, it may become possible to develop

alternatives of controlling populations by taking advantage of functional cues in their host

location behavior. As an extremely polyphagous aphid it was previously considered that *M.*

persicae does not use olfactory cues from plants in its searching behavior (Hori 1999; Vargas et

al. 2005). However, in the last decade it has been shown that *M. persicae* may respond to plant

volatiles released from healthy and damaged plants (Eigenbrode 2002; Ngumbi et al. 2007;

Alvares et al. 2007; Boquel et al. 2011; Verheggen et al. 2013; Rajabaskar et al. 2013a; Ninkovic

et al. 2013). Thus, its olfactory responses to volatile signals may be an important target for

disrupting its host searching behavior.

      Plants release a variety of different volatile compounds providing herbivore insects with

information that allows them to discriminate between host and non-host plants. As well as using

volatiles to determine taxonomic identity, aphids can also use plant volatiles to discriminate

between the suitability of different plants within the same species (Webster 2012). The emission

of volatiles from plants is significantly changed in plants under stress caused by abiotic factors

(Gouinguene and Turlings 2002), mechanical damage (Piesik et al. 2010), pathogens (Rajabaskar

et al. 2013b), herbivory (Arimura et al. 2009) or co-existence with other con- and heterospecific

3

72   plants (Ninkovic et al. 2002; Ninkovic 2003; Le Guigo et al. 2010; Glinwood et al. 2011) than in

73   unstressed plants. These changes in their volatile profiles can play important roles in aphid

74   behavior and host plant search. Recently, Ninkovic et al. (2013) found that winged *M. persicae*

75   prefer the odor of unexposed potato plants to the odor of potato plants previously exposed to

76   onion plants. The exposure resulted in greater production of two terpenoids, (*E*)-nerolidol and

77   (3*E*, 7E) - 4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMTT), both of which are involved in

78   indirect defenses of plants against herbivorous insects (Degenhardt and Gershenzon 2000;

79   Pophof et al. 2005; Arimura et al. 2009). The changes in volatile emissions of host plants

80   induced by the exposure to volatiles from neighboring plants reduced plant acceptance of winged

81   aphids. Further this can be one of explanations for the reduced abundance of winged *M. persicae*

82   observed in the field where potatoes were intercropped with onion (Ninkovic et al. 2013).

83

84   Many aphid species have several distinct morphs during their life cycle, which may

85   specialize on different ranges of host plants and have differing behavioral responses to these

86   plants (Powell and Hardie 2001). Numerous studies have confirmed that volatiles strongly

87   influence the searching behavior of aphids (Beyaert et al. 2010; Webster 2012), but most have

88   focused on only one morph, thus there is little information on between-morph differences in

89   responses to volatiles. The behavior and ecological functions of winged and wingless aphids

90   differ because of their morphological divergence. Aphids perceive plant odors with olfactory

91   sensilla (rhinaria) on their antennae. Secondary rhinaria are much more abundant in winged

92   aphids than in wingless insects, suggesting that these structures might be involved in host

93   location and mate selection (Blackman and Eastop 2000; Sun et al. 2012). Given that winged

94   virginoparae are mostly responsible for finding and colonizing new secondary hosts (Klingauf

95   1987; Blackman 1990), we hypothesize that winged morphs will show different behavioral

96   responses to plant volatiles than wingless morphs.

97

98   The aim of the study presented here was to investigate whether winged and wingless *M.*

99   *persicae* respond differently to changes in volatile emissions of their hosts. Through their higher

100 mobility, winged aphids have the ability for the establishment of new colonies, while wingless

101 aphids have greater impact in their population development due to their higher reproduction rate

102 (Dixon 1985). If both respond negatively, changes in volatile emissions of plants would be an

103 effective target in pest control. We studied aphid olfactory responses to volatile interactions of

104 onion and potato plants in laboratory experiments using living plants, synthetic blends of plant

105 volatiles and single volatile compounds. The synthetic blends were designed to emulate natural

106 odors from host plants with and without modification by exposure to volatiles from neighboring

107 onion plants.

108

109

110 **Materials and Methods**

111 *Plants and insects*

112 We obtained potato tubers (Solanaceae: *Solanum tuberosum*, L.cv. Sava) from Lantmännen,

113 Sweden, and onion bulbs (Amaryllidaceae: *Allium cepa*, L.cv. Stuttgarter Riesen) from Weibulls

114 Horto, Sweden. We placed them individually in potting soil (Special Hasselfors garden,

115 Hasselfors, Sweden) in plastic pots (8 x 8 x 8 cm) and grew plants in a greenhouse maintained at

116 18-22°C with 16 h:8 h light:dark cycles, using HQIE lamps to extend the natural photoperiod as

117 required. To prevent interaction among plants during the pre-experimental period, onion and

118   potato plants were grown in separate greenhouse chambers, and to avoid emissions of

119   mechanically damage-related volatiles, only visibly undamaged plants (aged three weeks) were

120   used in the experiments.

121

122   Adult winged and wingless individuals of *M. persicae* derived from a stock culture were

123   grown on potted rapeseed plants (Brassicaceae: *Brassica napus* L.) under similar conditions to

124   the test plants, but in different climate chambers. Production of winged aphids was induced by

125   crowding under long-day conditions. Winged aphids after first take-off were used for behavioral

126   experiments.

127

128   Potato plants were exposed to volatiles from onion plants in a series of 'two-chamber cage'

129   experiments (Ninkovic et al. 2002), as follows. We placed a series of clear perspex cages in a

130   greenhouse maintained at 18-22°C with 16 h:8 h dark cycles (as above). The cages were divided

131   into two 10 x 10 x 40 cm chambers (inducing and responding) connected by a 7 cm diameter

132   opening in the dividing wall. Air was introduced into the system through the inducing chamber

133   with an onion plant, passed through the hole in the dividing wall into the responding chamber

134   with a potato plant. From the responding chambers air was extracted through a tube attached to a

135   vacuum tank and then vented outside the room by an electric fan preventing that the plants

136   volatiles contaminate the greenhouse. The inducing chamber was left empty for the control

137   treatment. Airflow through the system was adjusted to $1.3 \, \text{l min}^{-1}$. Individual pots were watered

138   using an automated drop system (DGT Volmatic) and placed in separate Petri dishes in the

139   chambers to prevent root exudates affecting other plants. Exposure time was set to five days,

140 based on previous studies of volatile interactions between plants (Ninkovic et al. 2013). The

141 plants were used for olfactometer studies immediately after exposure.

142

143

144 *Olfactory bioassays with plants*

145 Olfactory responses of aphids were measured using a two-way airflow olfactometer consisting of

146 two stimulus zones, arms (length 4cm) directly opposite each other connected by a neutral

147 central zone (2.5 x 2.5 cm) separating them (Ninkovic et al. 2013). Airflow in the olfactometer

148 was set to 180 ml/min, which established discrete air currents in the side zones. Test aphids were

149 randomly collected from the cultures using a fine paintbrush and placed in Petri dishes with

150 moistened filter paper to prevent dehydration. The aphids were then left in the bioassay room for

151 at least 2 h to acclimatize prior to the experiments. A single aphid was then introduced into the

152 central zone of the olfactometer through a hole in the top and after an adaptation period of 10

153 min, the position of the aphid in the arms, defined as a visit, was recorded at three minute

154 intervals over a 30 min period. The accumulated number of visits of a single aphid in a single

155 arm after ten recordings was regarded as one replicate. Observations of individual aphids in the

156 central zone cannot conclusively be related to one of the arm zones and are therefore excluded

157 from the analysis. Data were expressed as mean of individual aphid visits per olfactometer arm

158 during observation period. To avoid pseudoreplication, each aphid was only tested once and a

159 clean olfactometer was used for each aphid. The test was terminated if an aphid did not move for

160 longer than 10 minutes and these individuals were not included in the analysis. The

161 olfactometers were washed with 10% Teepol L (TEEPOL, Kent, UK) and rinsed with 80%

162 ethanol solution and distilled water and left to air dry. If an aphid did not move for more than 10

163   min the test was terminated and data for these individuals were not included in the analysis.

164   Before introducing each test insect the olfactometer was rotated 180° to avoid positional bias.

165   The number of replicates, individual aphids tested, varied between 15 and 21 per experiment.

166

167   In tests with plants we compared the aphids' preferences for odors from: (a) a potato plant

168   previously exposed to an onion plant versus an unexposed potato plant, (b) a combination of an

169   unexposed potato and an onion plant versus two unexposed potato plants, (c) an unexposed

170   potato plant versus an onion plant, (d) an unexposed potato plant versus soil with no plants, and

171   (e) an onion plant versus soil with no plants. The two-chamber cages containing plants used as

172   odor sources were connected directly to the arms of the olfactometer (Markovic et al. 2014). For

173   (b), the two plants on each side of the olfactometer were in separate cages and connected to the

174   inlet of the olfactometer by y-connectors to prevent interaction. The airflow in all olfactometer

175   tests was set to 180 ml/min driven by a sucking pump. The pots with soil but no plants were used

176   to account for variations in moisture levels between the chambers with and without plants.

177

178   *Olfactory bioassays with chemicals*

179   We also investigated whether winged and wingless aphids respond differently to the odors of

180   plants and to synthetic blends mimicking volatile profiles of onion-exposed and unexposed

181   potato plants. For the latter, we used serial dilutions of synthetic blends based on previous

182   chemical analyses of the volatile profiles of potato plants (Ninkovic et al. 2013). The blend

183   mimicking volatiles of onion-exposed potato plants consisted of 0.05 ng/µl (*E*)-2-hexenal; 0.04

184   ng/µl (*Z*)-3-hexen-1-ol; 0.48 ng/µl myrcene; 0.03 ng/µl limonene; 0.225 ng/µl linalool; 0.25

185   ng/µl (*Z*)- 4,8-dimethyl-1,3,7-nonatriene; 1.24 ng/µl (*E*)- 4,8-dimethyl-1,3,7-nonatriene; 0.95

186 ng/µl α-copaene; 0.03 ng/µl α-cedrene; 0.2 ng/µl (*E*)-caryophyllene; 0.09 ng/µl (*E*)-β-farnesene;

187 0.2 ng/µl (*E*)-nerolidol; 0.5 ng/µl (3*E,7E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. The blend

188 mimicking unexposed plants consisted of 0.056 ng/µl (*E*)-2-hexenal; 0.05 ng/µl (*Z*)-3-hexen-1-

189 ol; 0.54 ng/µl myrcene; 0.03 ng/µl limonene; 0.085 ng/µl linalool; 0.3 ng/µl (*Z*)- 4,8-dimethyl-

190 1,3,7-nonatriene; 0.67 ng/µl (*E*)- 4,8-dimethyl-1,3,7-nonatriene; 0.7 ng/µl α-copaene; 0.03 ng/µl

191 α-cedrene; 0.155 ng/µl (*E*)-caryophyllene; 0.1 ng/µl (*E*)-β-farnesene; 0.03 ng/µl (*E*)-nerolidol;

192 0.125 ng/µl (3*E,7E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. We compared aphid olfactory

193 responses to these synthetic blends by adding them in 10 µl micro caps to small pieces of filter

194 paper, allowing them to evaporate for 30 s, and placing the pieces into 2.5 mm diameter glass

195 tubes connected to holes in the sides of the olfactometer arms. Test concentrations in the

196 olfactometer were 1/100, 1/10, 1, 10 and 100 times the reported amount of volatiles emitted from

197 the plants during a period of 24 hours.

198

199   Since onion-exposed potatoes emit significantly more (*E*)-nerolidol and TMTT than

200 unexposed potatoes, according to Ninkovic et al. (2013), we also investigated responses of

201 winged and wingless aphids to these compounds, individually, at five concentrations (0.01, 0.1,

202 1, 10, and 100 ng μl^{-1}) in a series of dose-response olfactometer experiments with redistilled n-

203 hexane as a control.

204

205 ***Statistical analyses***

206 Wilcoxon matched pairs tests were used to compare the number of aphid visits to each

207 olfactometer arm in the olfactory bioassays using Statistica version 10 software (StatSoft Inc.,

208 2011), setting a significance level of $p = 0.05$.

209

210

Results

The main achievements of the study are summarized graphically in Figure 1.

213

Olfactory responses of the aphids to odors from plants

According to numbers of recorded visits to the olfactometer arms both winged and wingless *M. persicae* aphids significantly preferred the odor of unexposed potato plants to the odor of onion-exposed potato plants ($Z = 3.57$, $p = 0.0004$, n = 18 and $Z = 2,012$, $p = 0.04$, n = 17, respectively) (Fig. 2). The *p*-values indicate that winged *M. persicae* were more sensitive to the difference in volatile emissions from their host plant than the wingless morphs. In addition, neither winged nor wingless *M. persicae* preferred the odor of unexposed potato plants to those of associated potato and onion plants ($Z = 1.85$, $p = 0.06$, n = 18 and $Z = 1.136$, $p = 0.3$, n = 15, respectively). However, while winged *M. persicae* preferred the odor of unexposed potato plants to the odor of onion plants ($Z = 3.42$, $p = 0.0006$, n = 19), wingless morphs showed no preference for these options ($Z = 1.704$, $p = 0.09$, n = 17). Both winged and wingless morphs significantly preferred the odor of unexposed potato plants, their secondary host, to the odor of soil with no plants ($Z = 3.51$, $p = 0.0005$, n = 20; and $Z = 2.09$, $p = 0.036$, n = 20, respectively). The odor of onion plants did not repel the aphids; neither winged nor wingless *M. persicae* showed a preference between onion plants and soil with no plants ($Z = 0.852$, $p = 0.4$, n = 21; and $Z = 0.327$, $p = 0.7$, n = 18, respectively).

230

Olfactory responses of the aphids to plant volatiles

232   Winged *M. persicae* only showed statistically significant responses to the mixture of synthetic

233   volatiles at high doses: 10 ng µl^{-1} (Z = 2.97, *p* = 0.003, n = 19) and 100 ng µl^{-1} (Z = 2.43, *p* =

234   0.015, n = 21) (Fig. 3a), while wingless morphs reacted only to the lowest test doses: 0.01 ng µl^{-1}

235   (Z = 2.68, *p* = 0.007, n = 21) and 1 ng µl^{-1} (Z = 2.52, *p* = 0.01, n = 21) (Fig. 3b). However, both

236   winged and wingless *M. persicae* showed a clear ability to discriminate between the synthetic

237   blends of onion-exposed and unexposed potato plants.

238

239   As shown in Fig. 4, winged morphs visited the olfactometer arm containing the highest

240   doses of (*E*)-nerolidol significantly less than the control: 100 ng µl^{-1} (Z = 2.56, *p* = 0.01, n = 17)

241   and 10 ng µl-1 (Z = 2.46, *p* = 0.01, n = 18), while wingless morphs showed this preferential

242   response to four of five used concentrations: 100 ng µl^{-1} (Z = 2.72, *p* = 0.007, n = 18), 10 ng µl-1

243   (Z = 3.18, *p* = 0.002, n = 18), 1 ng µl^{-1}, (Z = 2.11, *p* = 0.04, n = 18) and 0.1 ng µl-1 (Z = 2.11, *p*

244   = 0.04, n = 19).

245

246   When TMTT was used as the test volatile, winged morphs visited the arm offering it at the

247   highest tested concentration, 100 ng µl^{-1} (Z = 2.63, *p* = 0.009, n = 17), significantly less often

248   than the control, while wingless morphs significantly responded in this manner to 10, 0.1, and

249   0.01 ng µl^{-1} doses of TMTT (Z = 1.99, *p* = 0.046, n = 19; Z = 2.46, *p* = 0.01, n = 20; and Z =

250   2.64, *p* = 0.008, n = 18, respectively) (Fig. 5).

251

252

253   **Discussion**

11

254 The importance of plant volatiles in searching behavior of extremely polyphagous aphids such as

255 *M. persicae* for a host plant is still unclear. Some studies have shown the unimportance of

256 olfactory cues for this aphid (e.g. 1959; Hori, 1999; Vargas et al., 2005). However our results

257 and some others have clearly shown that *M. persicae* responds to olfactory cues released from

258 one of its secondary hosts (Eigenbrode et al. 2002; Ngumbi et al. 2007; Amarawardana et al.

259 2007; Ninkovic et al. 2013). The presented results also show that both winged and wingless *M.*

260 *persicae* morphs can detect slight changes in concentrations and profiles of host plant's volatiles,

261 including changes induced by exposure to volatiles from another plant. These findings

262 demonstrate that volatile signals may be stimulants in the process of plant acceptance even for

263 polyphagous aphids, confirming that aphids are heavily dependent on olfactory cues when

264 searching for a suitable host in complex habitats (Pettersson et al. 2007; Webster 2012).

265

266

267 *Olfactory responses of aphids to odors from plants*

268 Host plants that co-exist with other plants may be less attractive and suitable for aphids due to

269 induced resistance from their association with neighboring plants (Dahlin and Ninkovic 2013).

270 Ninkovic et al. (2013) found that the migration of *M. persicae* into a potato field was

271 significantly reduced when potato plants were sown together with onion plants due to the change

272 in volatile emissions from potato plants making them less attractive for winged aphids. Our

273 results confirm this interactive effect of volatiles from different plants for both winged and

274 wingless *M. persicae*. Similar effects have been observed for the response of the wingless

275 oligophagous aphid *Rhopalosiphum padi* L. (Hemiptera: Aphididae) to barley plants exposed to

276 volatiles from weeds (Glinwood et al. 2004; Ninkovic et al. 2009; Dahlin and Ninkovic 2013) or

277 other barley cultivars (Ninkovic et al. 2002; Kellner et al. 2010). These volatile interactions can

278 reduce the attraction of potential host plants for wingless aphids as well as for flying, host-

279 seeking aphids, which may have profound consequences for the establishment of new colonies

280 by wingless aphids and hence aphid numbers (Wiktelius 1989; Ninkovic and Åhman 2009).

281

282   In other studies it has been speculated that intercropped plants may mask olfactory cues

283 used by herbivores to find their hosts (Randlkofer et al. 2010; Finch and Collier 2012). However,

284 we found that onion plants did not mask the odor of potato plants for *M. persicae*; a mixture of

285 odors from onion and potato plants was as attractive as the odor of only potato plants. This

286 suggests a mechanism based on volatile exchange between plants rather than odor masking. The

287 ability to locate and recognize host plants is essential for the survival of aphids; they can detect

288 slight changes in volatile emissions of their secondary hosts, even changes induced by interaction

289 with neighboring plants. The changes in volatile emission have informative value for the aphids

290 in terms of the host plant quality (Pickett and Glinwood 2007). The ability of onion to induce

291 changes in potato volatile emission, reducing their attraction for *M. persicae*, can have a great

292 potential as aphid control agent. Thus, intercropping with onion plants or plants that emit similar

293 volatiles may substantially improve integrated pest management in potato fields. Volatile

294 compounds released from living plants could also be used in integrated pest management

295 strategies against other pests such as thrips (Egger and Koschier 2014).

296

297 ***Olfactory responses of aphids to odors from synthetic blends and single volatile compounds***

298 Aphids perceive plant odors through highly specialized olfactory receptor neurons. The

299 similarity of the responses of both winged and wingless *M. persicae* to the synthetic blends of

300   volatiles and living plants we observed suggests that blends of chemicals can adequately

301   represent the fragrances of onion-exposed and unexposed potato plants. For both aphid morphs

302   the synthetic blend of exposed plants was significantly less attractive than the blend from

303   unexposed potato plants, but winged and wingless morphs were sensitive to different doses of

304   the blends. Flying aphids should be able to discriminate different plant odors from greater

305   distance than wingless morphs that tend to migrate locally within a reduced range of host

306   (Wiktelius 1989). Alate aphids possess more olfactory sensilla than apterous forms, thus alate

307   aphid forms of *Sitobion avenae* have higher sensitivities and selectivity to leaf odor components

308   than apterous (Yan and Visser 1982).

309

310   Aphids recognize and locate their hosts by detecting emissions of characteristic blends of

311   volatile compounds, and respond more strongly to blends than to individual components (as

312   reviewed in Bruce and Pickett 2011). However, concentrations of single compounds in blends

313   can play a critical role in aphids' acceptance of host plants. We found that winged and wingless

314   aphids responded negatively to both the single compounds (*E*)-nerolidol and TMTT, which are

315   released in higher amounts by potato plants that have been exposed to onion plants (Ninkovic et

316   al. 2013), and to synthetic blends made of these compounds together with other compounds

317   released by potatoes. Thus, TMTT and (*E*)-nerolidol might act as signals for the location of

318   aphid host plants, but as repellents if the emission of these chemicals is increased in fields by

319   diverse plant associations (Hedge et al. 2011; Kos et al. 2013; Markovic et al. 2014).

320

321   Our finding that winged aphids responded to relatively high concentrations of single

322   volatile compounds released by plants, is in accordance with previous reports (Webster et al.

323 2010; Hori 1998), while wingless individuals responded to low concentrations of the synthetic

324 blend of volatiles, and TMTT. A possible explanation for this is that small changes in odor

325 emissions of host plants are more important for wingless aphids than huge deviations. Wingless

326 aphids are usually in closer contact with plants and their odors. Huge concentrations of odors

327 from exposed plants are overwhelming and unrecognizable for them, and thus these odors are

328 meaningless and without benefit for wingless aphids that usually have low intention to leave a

329 host plant. However, (*E*)-nerolidol was effective at a wide range of doses, repelling wingless

330 aphids across the range of test concentrations. Morphs of other aphid species such as *Sitobion*

331 *avenae* (F.) and *R. padi* also reportedly have differing capacities to detect volatile

332 semiochemicals (Yan and Visser 1982; Quiroz and Niemeyer 1998). An important difference

333 between winged and wingless aphids is the greater abundance of secondary rhinaria on the

334 antennae of winged morphs (Pickett et al. 1992), but the function of these organs is still

335 unknown. Pickett and colleagues (1992) suggested that they might play a role in host searching

336 behavior and mate selection, but they have no proven role in the detection of plant odors (Hardie

337 et al. 1994; Park et al. 2000; Park and Hardie 2004).

338

339 Integrated pest management based on plant-insect relationships is a promising method as

340 alternative strategy to decrease the excessive reliance on insecticides. By understanding how

341 different aphid morphs locate their host plants, it is possible to control populations by taking

342 advantage of functional cues in their host location behavior. Through manipulation of botanical

343 composition in crop fields (e.g. by intercropping), aphid orientation may be disrupted reducing

344 their abundance and prevent their establishment as a pest. Based on our findings about the

345 volatile interactions between plants and between plants and insects, functional biodiversity in

346 agro-ecosystems can be exploited as a component of integrated pest management. Application of

347 volatile chemicals in the field might be difficult; the effects depend on correct concentration and

348 emission rate and applications might have to be repeated. We recommend rather using living

349 plants that emit permanently the "right dose".

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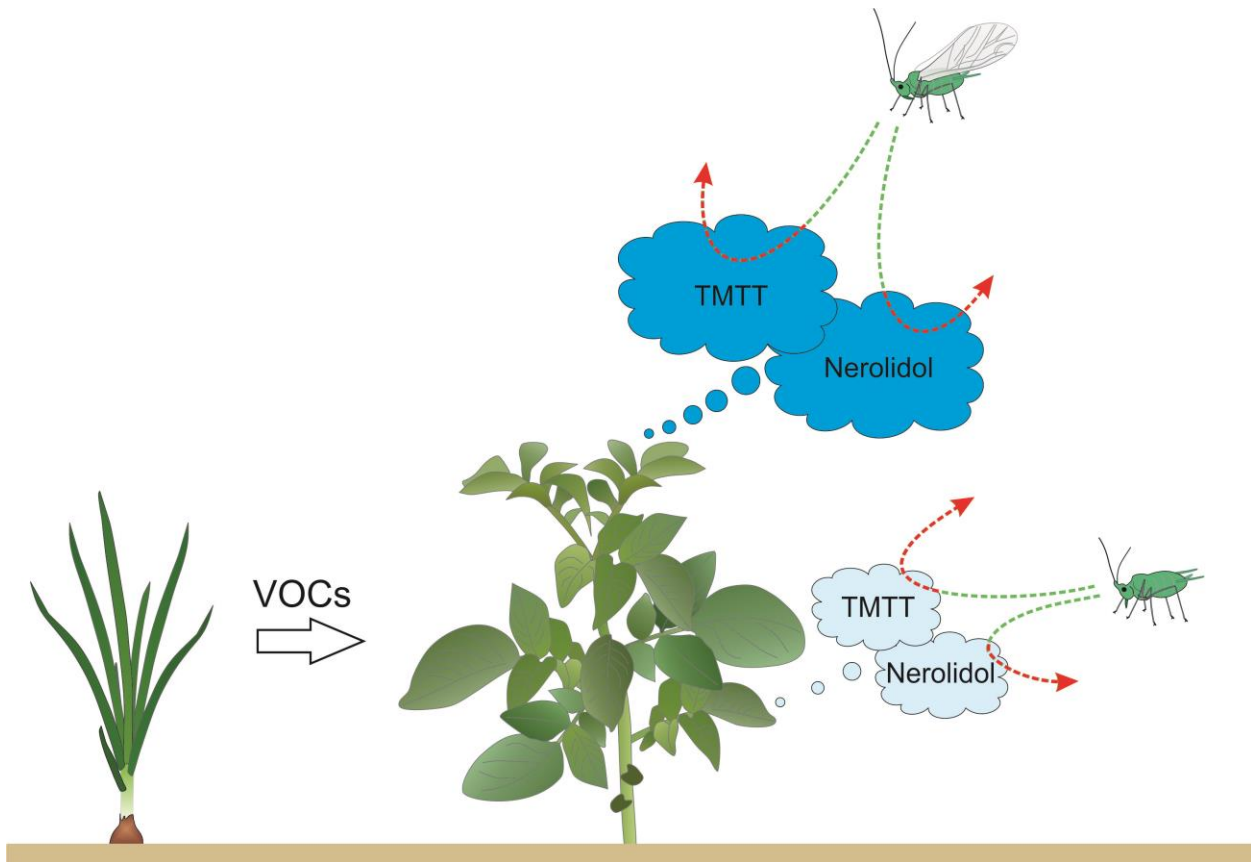492

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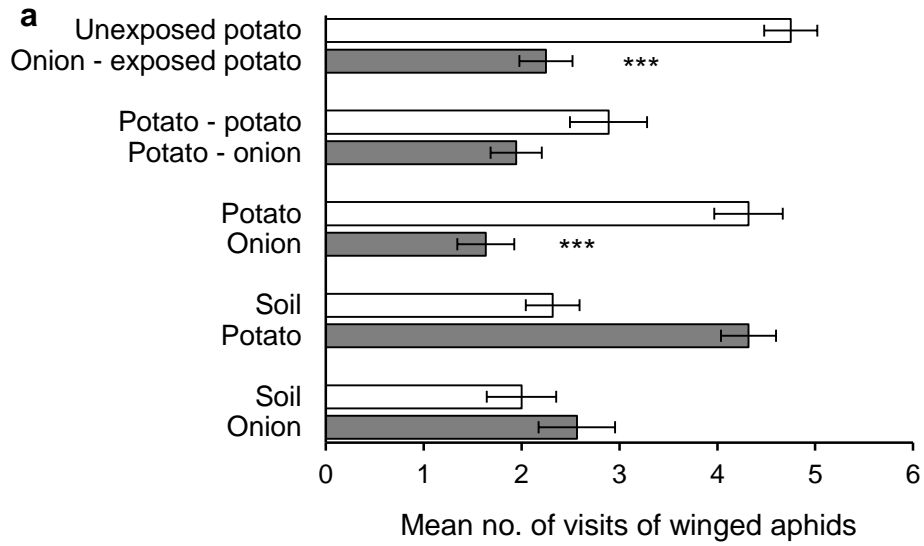498

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505 **Fig. 1** Volatile organic compounds (VOCs) from onion plants induce changes in the volatile

506 emission of neighboring potato plants with a higher release of the terpenoids TMTT and

507 nerolidol. High doses of these compounds repelled winged aphids, while wingless aphids were

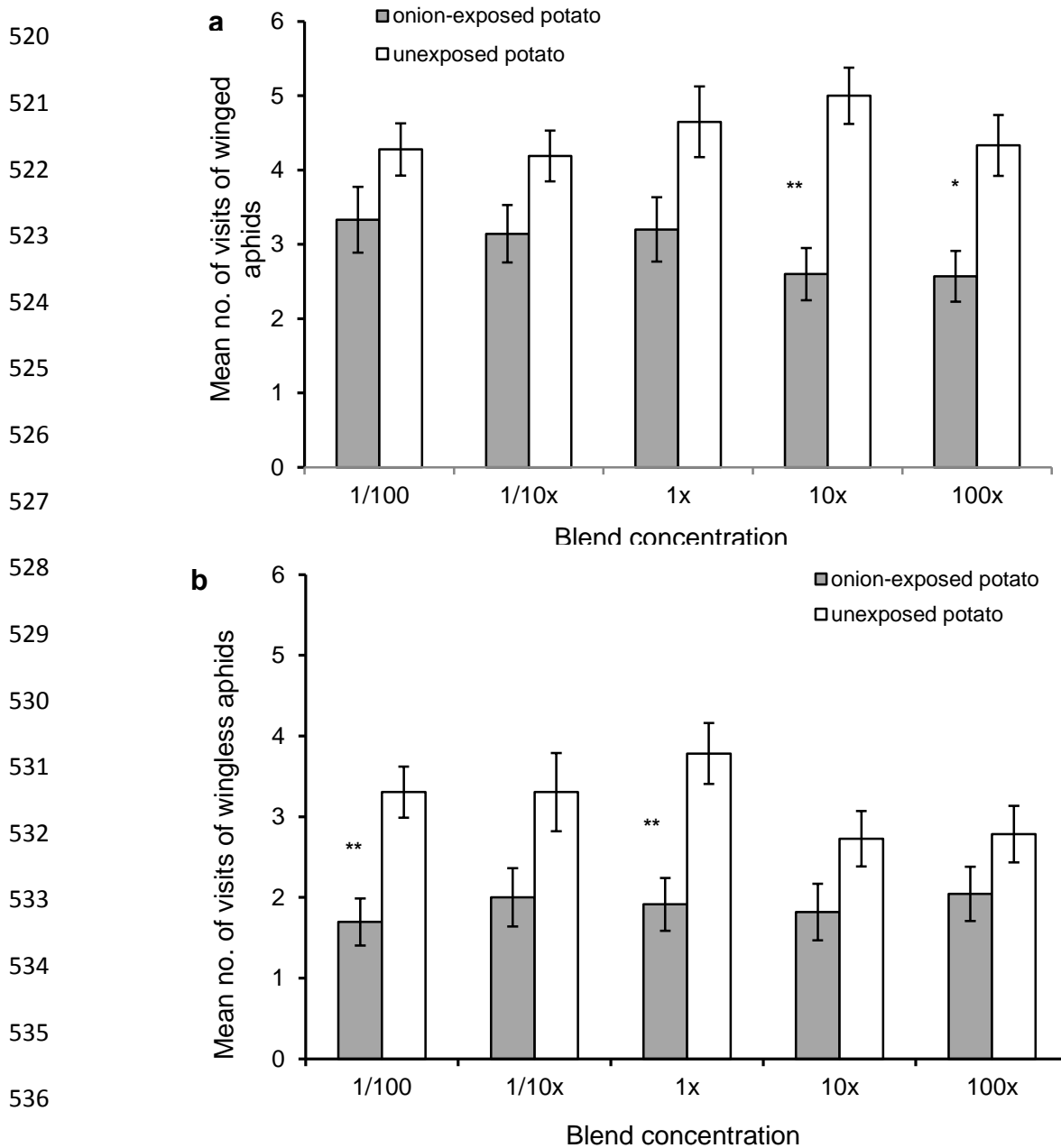508 repelled by low doses.

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Fig. 2 Behavioral responses of winged (a) and wingless (b) *Myzus persicae* to indicated choices

of volatiles from plants. Error bars indicate ± SE. Asterisks indicate preferences at significance

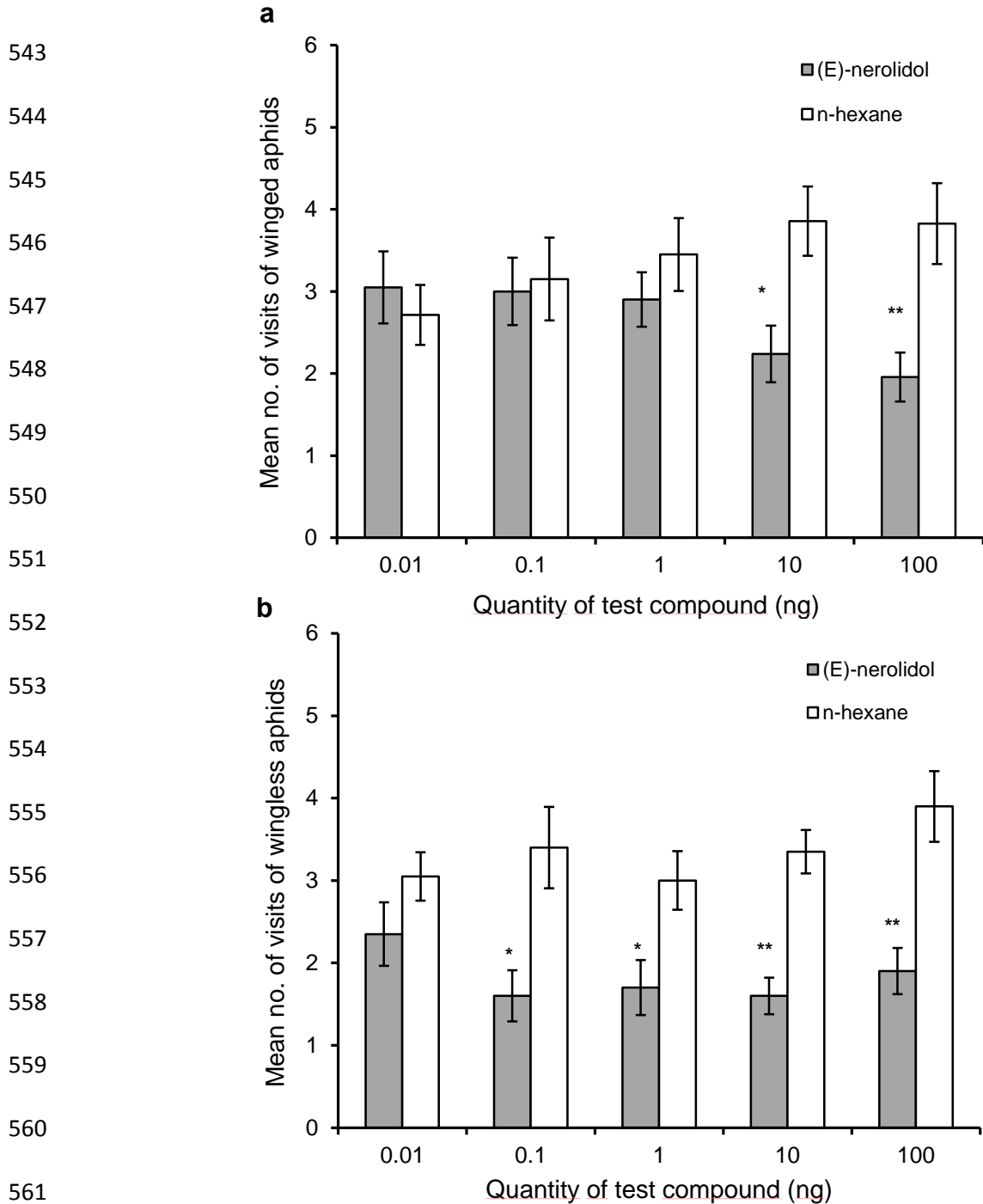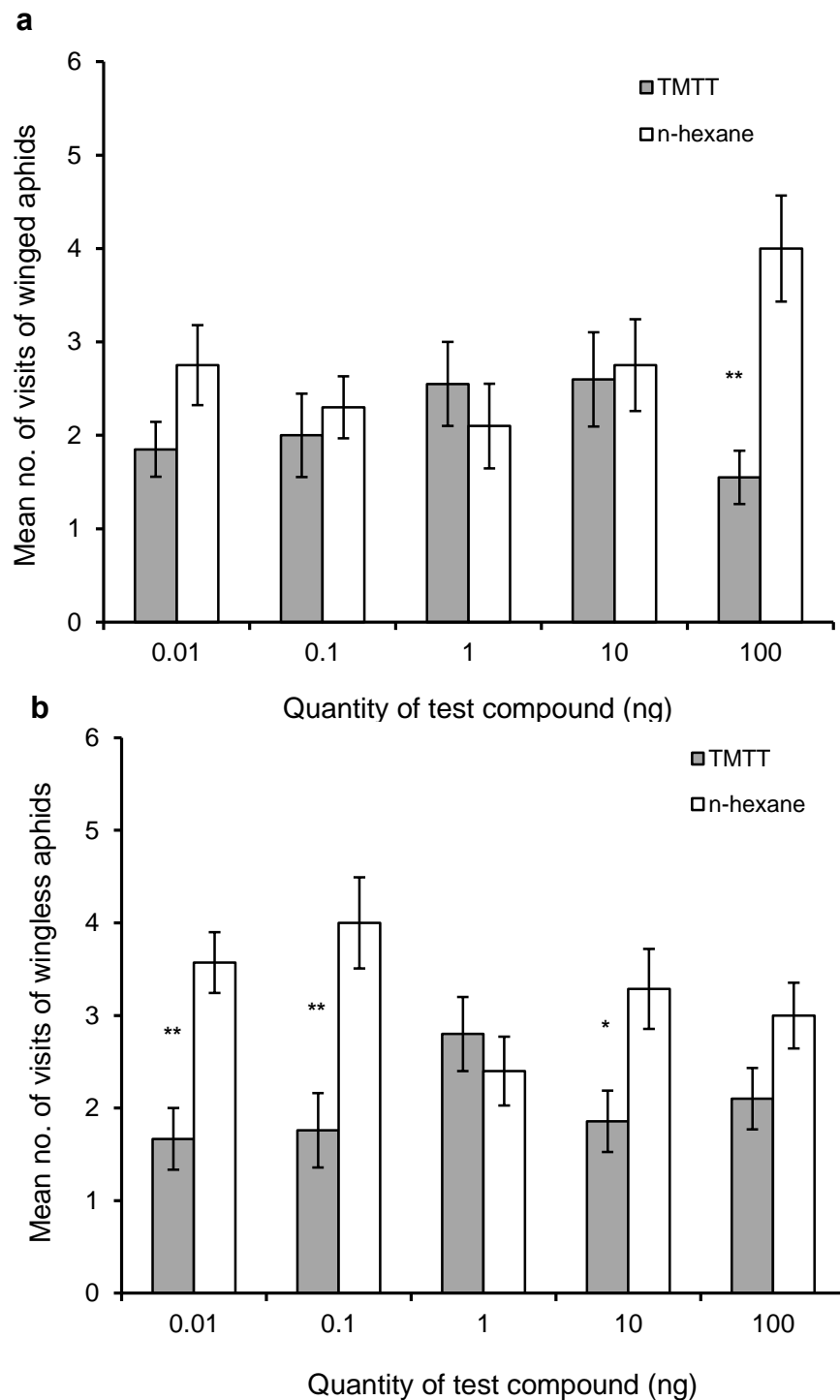levels of * $p \leq 0.05$, and *** $p \leq 0.001$ (Wilcoxon matched pairs test).

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Fig. 3 Preferential responses of winged (a) and wingless (b) *Myzus persicae* to synthetic blends of volatile organic compounds of potato plants that had been exposed (treatment) and unexposed (control) to onion plants. Synthetic blends were at 1/100, 1/10, 1, 10 and 100 times the original concentration of volatiles identified in potato headspace. Error bars indicate ± SE. Asterisks indicate preferences at significance levels of * $p \leq 0.05$, and *** $p \leq 0.001$ (Wilcoxon matched pairs test).

Fig. 4 Preferential responses of winged (a) and wingless (b) *Myzus persicae* to indicated amounts of (*E*)-nerolidol, a terpenoid released more strongly from potato plants when exposed to onion plants, vs. n-hexane controls. Error bars indicate ± SE. Asterisks indicate preferences at significance levels of * $p \leq 0.05$, and *** $p \leq 0.001$ (Wilcoxon matched pairs test).

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Fig. 5 Preferential responses of winged (a) and wingless (b) *Myzus persicae* to indicated amounts

of (3E, 7E) 4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMTT), a terpenoid released more

strongly from potato plants when exposed to onion plants, vs. n-hexane controls. Error bars

588   indicate ± SE. Asterisks indicate preferences at significance levels of * $p \leq 0.05$, and *** $p \leq 0$.

589   001 (Wilcoxon matched pairs test).

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