1 **Airborne interactions between undamaged plants of different cultivars affect insect**

2 **herbivores and natural enemies**

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11 **Abstract** This study investigated the effects of airborne interaction between different barley

12 cultivars on the behaviour of bird cherry-oat aphid *Rhopalosiphum padi*, the ladybird

13 *Coccinella septempunctata* and the parasitoid *Aphidius colemani*. In certain cultivar

14 combinations, exposure of one cultivar to air passed over a different cultivar caused barley to

15 have reduced aphid acceptance and increased attraction of ladybirds and parasitoids.

16 Parasitoids attacked aphids that had developed on plants under exposure more often than

17 those from unexposed plants, leading to a higher parasitisation rate. Ladybirds, but not

18 parasitoids, were more attracted to combined odours from certain barley cultivars than either

19 cultivar alone. The results show that airborne interactions between undamaged plants can

20 affect higher trophic levels, and that odour differences between different genotypes of the

21 same plant species may be sufficient to affect natural enemy behaviour.

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Introduction

Plants usually coexist with one another, and herbivores and their natural enemies may respond to combined characteristics of the plant individuals and to the result of interactions between them. Combining different plant species has often been found to reduce the incidence of pest herbivores and increase that of their natural enemies (Andow 1991). Although discussion of mixed cropping has generally focussed on plant species, there is increasing evidence that mixing different genotypes of the same species can affect organisms that use the plants as hosts (Power 1991; Mundt 2002; Ninkovic et al. 2002; Cadet et al. 2007). Chemical mechanisms have been tested in theories seeking to explain the effects of mixed cropping on herbivores and natural enemies (e.g. Uvah and Coaker 1984), however the role of direct chemical interaction between plants has not been widely considered.

Chemical interaction between plants can affect organisms at higher trophic levels through changes in host plant status. For example, chemicals released by herbivore or pathogen-damaged plants can induce a range of responses in receiving plants, including the activation of direct defences or attraction of natural enemies (Dicke et al. 2003; Baldwin et al. 2006). However, plants are exposed to chemicals released by neighbouring plants even when they are apparently undamaged. In allelopathy for example, plant substances that escape into the environment may affect the growth and development of neighbours (Rice 1984). Although allelopathy is an important issue in agricultural science, affecting many aspects of plant coexistence and competition (Weston and Duke 2003), investigation of its effects at higher trophic levels such as herbivores and their natural enemies has started only recently (Ninkovic et al. 2006). Increasing the diversity of plant genotypes may lead to an increase in the diversity of plant volatile chemicals released, if the genotypes differ in their volatile profiles. However, insect responses to diversity in plant volatile emissions have not been widely

51   studied, even though evidence suggests that volatile profiles can differ between genotypes of

52   the same species (Rapusas et al. 2003; Degen et al. 2004; Nissinen et al. 2005).

53

54   Previous studies have found that, in certain combinations of undamaged barley cultivars,

55   airborne exposure causes receiving plants to become less acceptable to aphids (Pettersson et

56   al. 1999; Ninkovic et al. 2002; Glinwood et al. 2007), and aphid acceptance is also reduced

57   when the cultivars are grown together in the field (Ninkovic et al. 2002). The current study

58   therefore tested whether such interactions between undamaged barley cultivars can also affect

59   orientation and foraging behaviour of aphid natural enemies. A tritrophic system was used,

60   consisting of the cereal aphid *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) and two of its

61   natural enemies with varying degrees of specialisation; the polyphagous ladybird *Coccinella*

62   *septempunctata* (L.) (Coleoptera: Coccinellidae) and the aphid parasitoid *Aphidius colemani*

63   Viereck (Hymenoptera: Aphidiinae).

64

65   **Materials and Methods**

66

67   **Plants**

68   Barley plants, *H. vulgare* L. (cvs. Barke, Scandium, Frieda and Prestige) were grown in plastic

69   pots (9 x 9 x 7 cm) in potting soil (Hasselfors Garden, Sweden) with six plants per pot. Plants

70   were at the early two-leaf stage (6 days after planting) at the beginning of exposure to air passed

71   over other plants, and at the mid two-leaf stage (11 days after planting) at the beginning of

72   bioassays. An extensive screening program with undamaged barley plants had shown that aphid

73   plant acceptance is reduced when Scandium is exposed to air from Barke, and when Prestige is

74   exposed to Frieda, but not when these cultivars are exposed to the same cultivar (V Ninkovic

75   unpublished). Thus plants sharing the same pot were not expected to interact with each other in

76   this way. Plants were grown in a glasshouse at 18–22˚C, with a L16:D8 light cycle, and the

77   different cultivars were kept at least 3m away from each other.

78

79   *Aphids*

80   Bird cherry-oat aphid *R. padi* was reared on barley (cv. Golf) in multi-clonal cultures in a

81   glasshouse with the same conditions as for plants. Aphids used in the experiments were

82   wingless, mixed-instar individuals, and were collected from the cultures immediately prior to

83   bioassay.

84

85   *Ladybirds*

86   Adult *C. septempunctata* were collected from natural habitats close to Uppsala, Sweden

87   (59°47' N and 17°39' E), and were reared in culture in cages with *R. padi* on barley (cv. Golf)

88   and flowering oilseed rape, *Brassica napus* L. at 21 ±1 ℃, a photoperiod of 16L:8D, and

89   relative humidity 60 ±10 %.

90

91   *Parasitoids*

92   A culture of *A. colemani* was established using mummies obtained commercially from

93   Biobasiq (Laholm, Sweden). This species has a wide host range, being recorded from 40

94   different aphid species (Starý 1975), but can be considered a food specialist in comparison to

95   the polyphagous *C. septempunctata*. Parasitoids were reared on *R. padi* on barley (cv. Golf)

96   under the same conditions as ladybirds, through at least two generations before use. Mummies

97   were removed from the culture attached to leaf pieces and kept in a small emergence cage

98   with honey solution (1:1 in water) as food. Males and females emerged, but only females

99   were used for experiments, and were 2-3 days old and assumed to be mated.

100

Airborne exposure of barley plants

102 Barley plants of one cultivar were exposed to air passed over plants of different cultivars

103 inside clear Perspex cages divided into two separate chambers connected by an opening as

104 previously described (Pettersson et al., 1999; Ninkovic et al. 2003; Glinwood et al. 2004,

105 2007). Pots were placed in Petri dishes to prevent interaction via roots, and watered via an

106 automated water drop system delivering 22 ml daily at 08:00 (2 hours into the photoperiod).

107 Control treatments consisted of two-chamber cages with a pot of barley plants in the rear

108 chamber and an empty front chamber. Five or six exposure cages were used for exposed plants

109 and a corresponding number for control plants. These were placed alternately on a bench in a

110 glasshouse at 18–22 °C, with a LD 16:8 h cycle. The exposure period was 5 days, based on

111 previous studies of airborne interactions between barley cultivars (Ninkovic et al. 2003;

112 Glinwood et al 2007). For all olfactometer experiments, at the end of the exposure period

113 plants were carefully transported inside exposure cages which were then connected to the

114 olfactometer

115

116 To produce infested plants and aphids for experiments, individual plants in pots to be exposed

117 were enclosed in transparent polystyrene tubes (50 ml, 12 cm x 3 cm diameter) and infested

118 with 30 *R. padi* (instars two to four). Plants were left overnight for aphids to settle before the

119 tubes were removed. Pots were then haphazardly assigned to exposed or unexposed

120 treatments and placed inside the exposure chambers. A small plastic ring coated with liquid

121 Teflon around the base of the plant (but not touching it) prevented aphids leaving.

122 Experiments on aphid settling, and ladybird and parasitoid olfaction were independent from

123 one another i.e. did not use the same plant material.

124

125

126 *Statistical analysis of behavioural experiments*

127 All statistical tests were carried out in the Statistica statistical package (Statsoft Inc. 2005).

128 Data were subjected to tests for homogeneity of variances and, where distributions were

129 found to significantly deviate from normal, nonparametric tests were applied.

130

131 *Aphid plant acceptance*

132 A no-choice settling test was used to measure aphid acceptance of experimental plants, as

133 described previously (Ninkovic et al. 2002; Glinwood et al. 2004, 2007). Ten wingless *R. padi*

134 (larval instars 2-4) were placed inside a polystyrene tube (described above) around the second

135 leaf and the number of aphids settled (not walking) on the leaf was recorded after 2 hours, since

136 this is sufficient time for aphids to settle and reach the phloem (Prado and Tjallingii 1997). Four

137 plants per pot (and therefore per exposure cage since each cage held a single pot) were randomly

138 selected for the test, giving 24 replicates per treatment. Data were expressed as proportions and

139 analysed by two-way ANOVA with exposure cage and aphid settling as factors.

140

141 *Olfactometry*

142 Olfactometry was used to test the olfactory responses of ladybirds and parasitoids to barley

143 cultivars that had been exposed to air passed over a different cultivar, and responses to odour

144 mixtures from different cultivars.

145

146 *C. septempunctata* was tested in two-way airflow olfactometer with an airflow of 300 ml/min,

147 previously described by Ninkovic and Pettersson (2003). An adult ladybird was placed in the

148 olfactometer for 10 minutes and its position recorded at 2 minute intervals. The observation

149 frequency method (Ninkovic and Pettersson 2003) was used as it gives a reliable measure

150 irrespective of whether the behavior is characterized by frequent short visits or few long visits

151 in the olfactometer arm. The accumulated number of observations in the arm zones after ten

152 observations was regarded as one observation. If an insect did not move between three

153 consecutive observations (was motionless) the replicate was discarded and a new one started

154 with a fresh insect. Data were analysed with Wilcoxon matched pairs tests. Each experiment

155 was replicated with 20 individual ladybirds, using five olfactometers simultaneously with the

156 positions of the treatment arms alternating. Thus five separate exposed and treated pots of

157 plants were used in the experiments (each pot for four experimental replicates, and each pot

158 from a separate exposure cage exposed during the same period in the glasshouse) to control

159 for variation in plant status.

160

161 To test for *C. septempunctata* preference for any particular cultivar, a four-way olfactometer

162 of similar construction as the two-way design was used. Experiments were performed in the

163 same way, with five separate olfactometers and plant sources simultaneously and 20

164 individual ladybirds. In all olfactometry experiments, equipment was cleaned between

165 experiments and precautions were taken account for positional bias in placement of odour

166 stimulus arms. Data were analysed by Friedmans ANOVA.

167

168 *A. colemani* was tested using a two-way airflow olfactometer described by Glinwood et al.

169 (2003) with an airflow of 250 ml/min. A female parasitoid was placed in the olfactometer

170 and, during 10 minutes, the amount of time spent by the insect in the arms was recorded. This

171 parameter was considered more suitable than that used for ladybirds since parasitoids moved

172 more rapidly. Twenty five parasitoids were used in each experiment. After every five

173 replicates, exposed and unexposed plants were replaced with new plants that had been

174 exposed in different exposure cages during the same period in the glasshouse. The mean

175   amount of time spent by parasitoids in the arms was analysed using Wilcoxon matched pairs

176   tests.

177

178   To test for *A. colemani* preference for any particular cultivar, a four-way olfactometer was

179   used. Twenty parasitoids were tested in the experiment. After every five replicates, the

180   exposed and unexposed plants were replaced with new plants grown at the same time in the

181   glasshouse. The mean amount of time spent by parasitoids in the arms was analysed by

182   Friedmans ANOVA.

183

184   In order to test the longevity of the attractiveness of exposed plants to ladybirds, a set of

185   plants was exposed in the glasshouse and, after 5 days exposure, the emitting barley plants

186   were removed from the exposure cages. A subset of exposed and unexposed plants was

187   removed and tested immediately in the olfactometer (Day 0). The remaining plants were left

188   in the exposure cages without emitting plants, and subsets were tested at 1, 4 and 7 days after

189   removal of emitter plants.

190

191   The influence of odour mixing from two different cultivars on ladybirds and parasitoids was

192   investigated using pairs of cultivars that had been shown to increase natural enemy attraction

193   when exposed to each other i.e. Barke and Scandium, and Frieda and Prestige and pairs that

194   had not i.e. Frieda and Scandium and Barke and Prestige. Pots of six plants were contained in

195   separate exposure cages, which were connected to each other and to the olfactometer using a

196   Y-connector. Thus the olfactometer arm contained volatiles from two cultivars, but there was

197   no exchange of volatiles between the cultivars. To compensate for differences in biomass, the

198   binary mixture was tested against another two cages, both containing the same cultivar. In all

199   experiments, ladybirds and parasitoids were kept under olfactometer lighting for 30 minutes

200   prior to bioassay.

201

202   *Parasitoid attack rate*

203   Parasitoid attack rate was used to test for effects of airborne exposure of barley on parasitoid

204   host preference via aphid quality/behaviour. Thirty aphids from either exposed or unexposed

205   plants were placed in a Petri dish (9 cm) with filter paper lining and sides treated with liquid

206   Teflon to prevent aphids leaving the floor. Aphids between larval instars two and four were

207   used since these are often preferred by parasitoids (Liu et al. 1989), and separate paintbrushes

208   were used to handle aphids from exposed and unexposed plants. A single female parasitoid

209   was introduced and observed for 10 minutes, recording the following: the number of times the

210   parasitoid examined an aphid with its antennae but did not attack (number of antennations),

211   and the number of times the parasitoid struck an aphid with its ovipositor (number of attacks).

212   From these data, the following were calculated: the total number of contacts with aphids made

213   by the parasitoid (antennations + attacks) and the percentage of contacts that resulted in attack

214   (% attack). Ten parasitoids were tested against each treatment, using a new Petri dish and

215   group of aphids each time. Treatments were tested alternately over two consecutive days.

216   Means were compared by Mann-Whitney U tests.

217

218   To measure parasitoid oviposition/development, aphids were collected from the Petri dishes

219   after each replicate, and transferred to separate pots containing 10 barley plants of the cultivar

220   on which they had been exposed, each sealed in a perforated plastic bag (Cryovac). These

221   were kept for 14 days in a glasshouse at 20-24 ºC, and a photoperiod of L16:D8 hours. The

222   number of mummies formed from each group of aphids was recorded, and used to calculate

223 the mean percentage of attacks that led to the formation of mummies (% mummies). Means

224 were compared by Mann-Whitney U tests.

225

226 *Ladybird feeding*

227 Feeding was used to test for effects of airborne exposure of barley on ladybird host preference

228 via aphid quality/behaviour. Ladybird larvae were confined individually on barley plants (cv

229 Golf) with free access to *R. padi* until they became adult. Forty *R. padi* from either exposed or

230 unexposed plants were placed on filter paper in a 15cm Petri dish arena with lid and left for 1

231 hour before a ladybird in its first day of adult life was introduced. After 24 hours the number

232 of aphids that had been consumed was calculated. Fifteen arenas were used for each

233 treatment, placed alternately on a bench in a glasshouse at 20-22 ℃, and a photoperiod of

234 L16:D8 hours. The mean number of aphids consumed by ladybirds was compared using t-

235 tests.

236

237 **Results**

238

239 *Aphid settling on barley cultivars exposed to volatiles from another cultivar*

240 Aphid settling was significantly reduced on barley cultivar Scandium exposed to Barke

241 (ANOVA, $F_{1,36}= 13.7$, $P= 0.0007$) and on Prestige exposed to Frieda ($F_{1,36}= 9.5$, $P= 0.004$)

242 (Fig. 1 A and B), but not on Prestige exposed to Barke ($F_{1,36}= 0.06$, $P= 0.81$), or Scandium

243 exposed to Frieda ($F_{1,36}= 1.4$, $P= 0.23$), (Fig. 1 C and D). In no experiment was the exposure

244 cage factor significant.

245

246

247   *Ladybird and parasitoid olfactory response to barley cultivars exposed to volatiles from*

248   *another cultivar*

249   The finding of effects on aphid settling in receiving plants in certain cultivar combinations

250   were confirmed in independent experiments with ladybirds and parasitoids. Ladybirds were

251   observed significantly more often in olfactometer arms containing odour of barley plants of

252   cultivar Scandium exposed to Barke (Wilcoxon test, Z= 2.67, P= 0.007) and Prestige exposed

253   to Frieda (Wilcoxon test, Z= 2.42, P= 0.01) (Fig. 1 A and B), but not of Prestige exposed to

254   Barke (Wilcoxon test, Z= 0.22, P= 0.82) or Scandium exposed to Frieda (Wilcoxon test, Z=

255   0.47, P= 0.64) (Fig. 1 C and D).

256

257   Parasitoids spent significantly more time in olfactometer arms containing odour of barley

258   plants of cultivar Scandium exposed to Barke (Wilcoxon test, Z= 2.62, P= 0.008) and Prestige

259   exposed to Frieda (Wilcoxon test, Z= 3.70, P= 0.0002) (Fig. 1 A and B), but not of Prestige

260   exposed to Barke (Wilcoxon test, Z= 0.16, P= 0.32) or Scandium exposed to Frieda

261   (Wilcoxon test, Z= 0.18, P= 0.38) (Fig. 1 C and D).

262

263   In the combinations found to increase natural enemy attraction above, when receiving plants

264   were infested with aphids, ladybirds did not show a preference between plants exposed to an

265   undamaged barley cultivar or unexposed plants: Barke-Scandium- mean (±SE) observations

266   in odour of exposed plants 4.29 (0.47), unexposed plants 3.38 (0.43), Wilcoxon test Z= 1.0,

267   P= 0.31 and Frieda-Prestige: exposed 3.75 (0.49), unexposed 4.03 (0.45), Z= 0.31, P= 0.75. In

268   similar tests, parasitoids did not show a preference between exposed or unexposed plants in

269   the combination Frieda-Prestige- mean time (s) (±SE) in odour of exposed plants 177.3

270   (14.9), unexposed plants 179.1 (12.8), Wilcoxon test Z= 0.14, P= 0.88, however parasitoids

271 spent significantly longer in the odour of infested exposed plants in the combination Barke-

272 Scandium- exposed 188.0 (17.2), unexposed 139.1 (14.9), Z= 2.3, P= 0.02.

273

274 *Longevity of ladybird olfactory response to barley cultivars exposed to air passed over*

275 *another cultivar*

276 Ladybirds were observed significantly more often in olfactometer arms containing odour of

277 exposed plants up to seven days after removal of the emitting plant in the combination

278 Prestige exposed to air passed over Frieda, and up to four days in the combination Scandium

279 exposed to air passed over Barke (Table 1).

280

281 *Ladybird and parasitoid olfactory response to odour of barley cultivars*

282 There was no significant difference in the number of ladybird observations in olfactometer

283 arms when given a choice between the odour of four barley cultivars (mean number of

284 observations ($\pm$ s.e.) Frieda 2.15 (0.33), Prestige 2.30 (0.37), Barke 2.27 (0.46), Scandium

285 2.25 (0.46); Friedman ANOVA, $\chi^2 = 0.14$, df= 3, P= 0.98). No preference for the inducing

286 cultivars (Frieda or Barke) makes passive absorption/release of volatiles unlikely to be

287 responsible for the attraction to exposed plants reported above.

288

289 There were significant differences in parasitoid residence times in olfactometer arms when

290 given a choice between the above cultivars (Friedman ANOVA, $\chi^2 = 28.5$, df= 3, P< 0.0001)

291 (Fig. 2). Cultivar Frieda was significantly preferred by parasitoids (P< 0.01, Pair wise

292 Wilcoxon tests), while there were no significant differences between the other three cultivars

293 (P> 0.05, Pair wise Wilcoxon tests). In a separate test, parasitoids did not show a preference

294 between odour of cultivar Scandium and that of cultivar Golf, on which they had been reared

295 (Mean time (s) ($\pm$ s.e.) spent in odour of Golf: 113.1 (14.7), mean time spent in odour of

296   Scandium 105.1 (18.7), Wilcoxon test Z= 0.55, P= 0.58, n= 20). This decreases the likelihood

297   that the preference for Frieda was due to a conditioned response to chemical similarity of

298   Frieda with that of the rearing cultivar Golf.

299

300   *Ladybird and parasitoid olfactory response to mixed odour from barley cultivars*

301   In six of eight comparisons of cultivar combinations, ladybirds were observed significantly

302   more often in olfactometer arms with mixed odours of two barley cultivars compared with an

303   equal biomass of either cultivar alone (Fig. 3). Parasitoids were attracted to mixed odours in

304   only one of four comparisons (Fig. 4).

305

306   *Ladybird and parasitoid host selection behaviour with aphids from barley cultivars exposed*

307   *to air passed over another cultivar*

308   When aphids had fed on barley cultivar Prestige exposed to cultivar Frieda, several indicators

309   of parasitoid host preference were affected compared with aphids from unexposed plants

310   (Table 2). A similar pattern was observed when aphids had fed on cultivar Scandium exposed

311   to cultivar Barke, although the strength of the effects was lower and statistical significance

312   marginal in some cases (Table 2).

313

314   When given access to aphids that had fed on barley cultivar Scandium exposed to Barke,

315   ladybirds consumed significantly more aphids than when given access to aphids from

316   unexposed Scandium (Mean (± s.e.) number of aphids eaten exposed plant: 30.6 (2.2),

317   unexposed plant 21.3 (2.1), t-test P= 0.004, n= 15). There was no significant difference when

318   Prestige was exposed to Frieda (Mean (± s.e.) number of aphids eaten exposed plant: 25.3

319   (2.5), unexposed plant 21.2 (2.0), t-test P= 0.21, n= 15).

320

Discussion

The results show that both direct airborne interaction and odour mixing in genotypes of a single plant species can affect the behaviour of a herbivore and its natural enemies. The effects on aphid plant acceptance are in line with previous studies showing reduced aphid acceptance of exposed barley in specific binary combinations of undamaged cultivars (Pettersson et al. 1999; Ninkovic et al. 2002). In fact, a large-scale screening program involving 50 barley genotypes released over a period of 100 years indicates that these effects are released in 10-25 % of tested cultivar combinations (V Ninkovic unpublished). In the current study, all possible pair wise combinations were not tested, however cross-matching the receiving with the alternative emitting cultivars confirms previous observations that the combination of cultivars is important, rather than the emitting cultivar itself. Cultivar combinations in which aphid acceptance of exposed plants was reduced also resulted in olfactory attraction of both ladybirds and parasitoids to exposed plants. Exposure to volatiles from herbivore-damaged plants induces natural enemy attraction to neighbouring undamaged plants in some plant species (Dicke et al. 2003), and ladybirds were attracted to barley exposed to volatiles from weeds (Ninkovic and Pettersson 2003). The current study suggests that aphid natural enemies may respond to plants exposed to volatiles from undamaged plants of the same species.

The proximate reason for natural enemy attraction may be modification of the volatile profile of exposed plants, although the nature of this remains to be investigated. The close presence of a neighbouring plant may induce responses that could result in modified volatile release via changes in plant physiology. It has been shown, for example, that barley aerially exposed to undamaged plants of a different cultivar undergo reallocation of biomass resources (Ninkovic 2003). Plant stress responses to abiotic factors can also result in release of specific volatile

346   substances (Karl et al. 2008). Recently, interaction between plant volatile stress signals and

347   regulation of allelopathy has been shown (Bi et al. 2007), suggesting a link between these

348   plant behaviours. When plants were infested with aphids, natural enemies preference for

349   odour of exposed plants was lost or weakened. Natural enemies may use a hierarchy of cues

350   in host location (Morrison and King 2004) and, when presented with a very reliable and

351   detectable (*sensu* Vet and Dicke 1992) indicator of host presence, aphid-induced volatiles

352   (Ninkovic et al. 2001), responses to other plant signals may become redundant.

353

354   Although the interactions appear to be mediated by exchange of plant volatiles, alternative

355   mechanisms cannot currently be ruled out, such as the transfer of endo- or epiphytic

356   microflora. From the current data, it is also not possible to determine if insect responses to

357   exposed plants are due to induced chemical changes or passive adsorption. Aphids do not

358   show differential attraction or settling with any of the tested cultivars (Glinwood

359   unpublished). Ladybirds also showed no olfactory preference for any cultivar. Absorbed

360   volatiles may however have contributed to a more attractive ratio. Indeed, ladybirds were

361   attracted to binary combinations of cultivars compared to single cultivars. However, they

362   were attracted to combinations in which no effects were observed with exposed plants,

363   arguing against passive absorption and re-release. Parasitoids expressed a clear preference for

364   the odour of Frieda. However, for parasitoids Barke as well as Frieda caused exposed

365   cultivars to become more attractive. Further, parasitoids were not generally attracted to binary

366   combinations of cultivars. Odour of exposed plants remained attractive to ladybirds for up to

367   seven days after the end of exposure to the emitting cultivar, so although any absorbed odours

368   would have to be released over a relatively long period, this mechanism is one that will be

369   addressed by investigation of the plant's volatile emissions.

370

371   If the response of aphid natural enemies to odour of exposed plants has adaptive significance,

372   this may be related to the host quality of aphid prey. Once aphid natural enemies have located

373   suitable habitats, prey selection involves an assessment of host quality and, for parasitoids in

374   particular, this can be affected by the chemical and behavioural characteristics of the prey

375   (Vinson 1976). The current results suggest that there was no reduction in the quality of aphids

376   from exposed plants in terms of supporting parasitoid development, but that higher parasitoid

377   contact and attack rates were achieved. This could occur if aphids' behavioural defences (Liu

378   et al. 1984) were altered as a result of developing on exposed plants, allowing more efficient

379   prey handling. This may also explain why ladybirds ate significantly more aphids from

380   Scandium plants exposed to Barke (although this was not repeated in the combination Frieda-

381   Prestige). A similar result could also be obtained if aphids obtain a smaller size on exposed

382   plants. Host size can influence parasitoid choice (Liu et al. 1984), and might lead ladybirds to

383   consume more individual aphids within a set time period. The results suggest that there may

384   be a link between effects of plant airborne interaction on aphids and on their natural enemies,

385   and this is expressed via changes in aphid characteristics.

386

387   *C. septempunctata* is a polyphagous predator and, though aphids are an important foot source,

388   it has a broad diet that includes other small insects and pollen. It should thus favour

389   botanically diverse habitats, especially in the absence of aphid prey (Banks 1999; Elliot et al.

390   2002; Pettersson et al. 2008). In a previous study, more *C. septempunctata* were observed in

391   barley growing together with two common weeds than in weedless patches, and laboratory

392   studies showed both exposure of barley to weed volatiles, and mixing of barley and weed

393   odours were attractive to ladybirds (Ninkovic and Pettersson 2003). The current study

394   suggests specific odour diversity may represent an attractive stimulus, and that *C.*

395 *septempunctata* may be able to detect this chemical diversity even between genotypes of the

396 same species.

397

398 Botanical diversity has been found to enhance the effectiveness of herbivore natural enemies

399 in some systems (Russell 1989), which has been explained by the provisioning of alternative

400 resources (Root 1973). It is unlikely that cultivars of the same plant species fulfil this role for

401 a generalist predator (Pettersson et al. 2005, 2008). However, *C. septempunctata* could

402 potentially use odour diversity as an informational cue denoting botanical diversity. *A.*

403 *colemani* is more specialised in its prey range than a polyphagous ladybird. It would not be

404 expected to respond in the same way to cues potentially denoting habitats with varied plant

405 resources, and parasitoids did not show a consistent preference for the odours of barley

406 cultivar combinations that attracted ladybirds.

407

408 Only certain combinations of barley cultivar odours were more attractive to ladybirds,

409 suggesting that specific characteristics rather than odour diversity *per se* are important.

410 Further, in order to recognise odours mixtures at all, there would need to be differences in the

411 volatile profiles of the different cultivars. There is evidence for genotype-differences in

412 volatile profiles in apparently undamaged sweetpotato (Wang and Kays 2002), rice (Rapusa et

413 al. 2003), cotton (Elzen et al. 1986), pear (Scutareanu et al. 2003) and carrot (Nissinen et al.

414 2005). Several studies have also shown variability in herbivore-induced volatiles between

415 plant cultivars (Takabayashi et al. 1991; Loughrin et al. 1995; Degen et al. 2004).

416

417 This study shows that airborne interaction between cultivars of a single species can release

418 behavioural effects in herbivores and their natural enemies. Beneficial effects have been

419 achieved by mixing plant cultivars for control of aphids (Ninkovic et al. 2003), aphid-

420   transmitted plant viruses (Power 1991), fungal pathogens (Mundt 2002) and nematodes

421   (Cadet et al. 2007). Airborne plant-plant interaction may be an underestimated mechanism

422   contributing to such effects. In respect to the limitations of the results reported here, it should

423   be noted that while laboratory behavioural studies can show that an organism maintains a

424   particular response in its behavioural repertoire, the extent to which this response is expressed

425   in nature may vary depending upon other factors and can be demonstrated only through field

426   experiments. However this study suggests that airborne interaction between undamaged plants

427   can affect insects at higher trophic levels.

428

432

433   **References**

434
435   Andow DA (1991) Vegetational diversity and arthropod population response. Annu. Rev.

436   Entomol. 36, 561-586.

437

438   Baldwin IT, Halitschke R, Paschold A et al (2006). Volatile signaling in plant–plant

439   interactions: talking trees in the genomics era. Science, 311, 812–815.

440

441   Banks JE (1999) Differential response of two agroecosystem predators, *Pterostichus*

442   *manarius* (Coleoptera: Carabidae) and *Coccinella septempunctata* (Coleptera: Coccinellidae),

443   to habitat-composition and fragmentation-scale manipulations. Can. Entomol. 131: 645–657.

444

445   Bi H, Zeng R, Su L et al (2007). Rice allelopathy induced by methyl jasmonate and methyl

446   salicylate. J. Chem. Ecol. 33: 1089-1103.

447

448   Cadet P, Berry SD, Leslie GW et al (2007) Management of nematodes and a stalk borer by

449   increasing within-field sugarcane cultivar diversity. Plant Pathol. 56: 526-535.

450

451   Degen T, Dillmann C, Marion-Poll F et al (2004) High genetic variability of herbivore-

452   induced volatile emission within a broad range of maize inbred lines. Plant Physiol. 135:

453   1928-1938.

454

455   Dicke M, van Poecke RMP, de Boer JG (2003) Inducible indirect defence of plants: from

456   mechanisms to ecological functions. Basic Appl.Ecol. 4: 27-42.

457

458   Elliott NC, Kieckhefer RW, Michels GJ Jr. et al (2002) Predator abundance in alfalfa fields in

459   relation to aphids, within-field vegetation, and landscape matrix. Environ. Entomol. 31: 253-

460   260.

461

462   Elzen GW, Williams HJ, Vinson SB (1986) Wind tunnel flight responses by hymenopterous

463   parasitoid *Campoletis sonorensis* to cotton cultivars and lines. Entomol. Exp. Appl. 42: 285-

464   289.

465

466   Glinwood RT, Pettersson J, Ninkovic V. et al (2003) Change in acceptability of barley plants

467   to aphids after exposure to allelochemicals from couch-grass (*Elytrigia repen*s). J. Chem

468   Ecol. 29: 259-272.

469

470   Glinwood R, Ninkovic V, Ahmed E. et al (2004) Barley exposed to aerial allelopathy from

471   thistles (*Cirsium* spp.) becomes less acceptable to aphids. Ecol. Entomol. 29: 188-195.

472

473   Glinwood RT, Gradin T, Karpinska B et al (2007) Aphid acceptance of barley exposed to

474   volatile phytochemicals differs between plants exposed in daylight and darkness. Plant

475   Signalling Behav. 2: 205-210.

476

477   Karl T, Guenther A, Turnipseed A et al (2008) Chemical sensing of plant stress at the

478   ecosystem scale. Biogeosciences 5: 1287-1294.

479

480   Loughrin JH, Manukian A, Heath RR et al (1995) Volatiles emitted by different cotton

481   varieties damaged by feeding beet armyworm larvae. J. Chem. Ecol. 21: 1217–1227

482

483   Liu S-S, Morton R, Hughes R (1989) Oviposition preferences of a hymenopterous parasitoid

484   for certain instars of its aphid host. Entomol. Exp. Appl. 35: 249-254.

485

486   Morrison LW, King JR (2004) Host location behavior in a parasitoid of imported fire ants. J.

487   Ins. Behav. 17: 367-383.

488

489   Mundt CC (2002) Use of multiline cultivars and cultivar mixtures for disease

490   management. Annu. Rev. Phytopathol. 40: 381-410.

491

492   Ninkovic V, Al Albassi A, Pettersson J (2001) The influence of aphid-induced plants

493   volatiles on ladybird beetle searching. Biol. Control 21: 191-195.

494

495   Ninkovic V, Olsson U, Pettersson J (2002) Mixing barley cultivars affects aphid host plant

496   acceptance in field experiments. Entomol. Exp. Appl. 102: 177-182.

497

498   Ninkovic V, Pettersson J (2003) Searching behaviour of sevenspotted ladybird,

499   *Coccinella septempunctata* – effects of plant-plant odour interaction. Oikos 100: 65-70.

500

501   Ninkovic V, Glinwood R, Pettersson J (2006) Communication between undamaged plants by

502   volatiles: the role of allelobiosis. In: Baluška F, Mancuso S, Volkmann D. (eds)

503   Communication in Plants: Neuronal Aspects of Plant Life, Springer-Verlag, Berlin

504   Heidelberg, pp 421-434.

505

506

507   Nissinen A, Ibrahim M, Kainulainen P et al (2005) Influence of carrot psyllid (*Trioza*

508   *apicalis*) feeding or exogenous limonene or methyl jasmonate treatment on composition of

509   carrot (*Daucus carota*) leaf essential oil and headspace volatiles. J. Agric Food Chem. 53:

510   8631-8638.

511

512   Pickett JA, Glinwood RT (2007) Chemical Ecology. In: Aphids as Crop Pests, van Emden

513   HF, Harrington R (eds), CABI, UK, pp 235-260.

514

515   Pettersson J, Ninkovic V, Ahmed E (1999) Volatiles from different barley cultivars affect

516   aphid acceptance of neighbouring plants. Acta. Agric. Scand. Sect. B 49: 152-157.

517

518   Pettersson J, Ninkovic V, Glinwood R, Birkett MA, Pickett JA (2005) Foraging in a complex

519   environment – semiochemicals support searching behaviour of the seven spot ladybird. Eur. J.

520   Entomol. 102: 365-370.

521

522   Pettersson J, Ninkovic V, Glinwood R et al (2008) Chemical stimuli supporting foraging

523   behaviour of *Coccinella septempunctata* L (Coleoptera: Coccinellidae): volatiles and

524   allelobiosis – a minireview. App. Entom. Zool. 43: 315-321.

525

526   Power AG (1991) Virus spread and vector dynamics in genetically diverse plant populations.

527   Ecology 72: 232-241.

528

529   Rapusas HR, Bottrell DG, Coll M (1996) Intraspecific variation in chemical attraction of rice

530   to insect predators. Biol. Control 6: 394-400.

531

532   Root RB (1973) Organization of a plant–arthropod association in simple and diverse habitats:

533   the fauna of collards (*Brassica oeracea*). Ecol. Monogr. 43:95-124.

534

535   Russell EP (1989) Enemies hypothesis: a review of the effect of vegetational diversity on

536   insect predators and parasitoids. Environ. Entomol. 18: 590-599.

537

538   Scutareanu P, Bruin J, Posthumus MA et al. (2003) Constitutive and herbivore-induced

539   volatiles in pear, alder and hawthorn trees. Chemoecology 13: 63-74.

540

541   Starý P (1975) *Aphidius colemani* Viereck: its taxonomy, distribution and host range

542   (Hymenoptera, Aphidiidae). Acta Entomol. Bohemos. 72: 156-163.

543   Takabayashi J, Dicke M, Posthumus MA (1991) Variation in composition of predator-

544   attracting allelochemicals emitted by herbivore-infested plants: relative influence of plant and

545   herbivore. Chemoecology 2: 1–6.

546

547   Uvah III, Coaker TH (1984) Effect of mixed cropping on some insect pests of carrots and

548   onions. Entomol. Exp. Appl. 36: 159–167.

549

550   Vinson SB (1976). Host selection by insect parasitoids. Annu. Rev. Entomol. 21: 109-134.

551

552   Wang Y, Kays SJ (2002) Sweetpotato volatile chemistry in relation to sweetpotato weevil

553   (*Cylas formicarius*) behavior. J. Am. Soc. Hortic. Sci.127: 656-662.

554

555   Weston LA, Duke SO (2003) Weed and crop allelopathy. Crit. Rev. Plant Sci. 22: 367-389.

Fig. 1 Effect of airborne exposure of one barley cultivar to a different cultivar on *Rhopalosiphum padi* plant acceptance (settling) of exposed plants and orientation of *Coccinella septempunctata* and *Aphidius colemani* to odour of exposed plants in an olfactometer. Four cultivar combinations were used A) Scandium exposed to Barke, B) Prestige exposed to Frieda, C) Prestige exposed to Barke and D) Scandium exposed to Frieda. Experiments on aphid settling, and ladybird and parasitoid olfaction were independent from one another i.e. did not use the same plant material. For aphids N= 24 individual plants tested with 10 aphids per plant in each comparison, P values from ANOVA. For ladybirds and parasitoids N= 20 and 25 individuals tested in each comparison respectively, P values from Wilcoxon tests.

Fig. 2 Aphid parasitoid *Aphidius colemani* olfactory response to four barley cultivars. Mean (± se) residence time in the olfactometer arm containing the barley odour. N= 20. Bars with different letters are significantly different (at $P < 0.05$, Friedman ANOVA followed by pair wise Wilcoxon tests)

Fig. 3 Ladybird *Coccinella septempunctata* olfactory response to mixed odours of barley cultivars A- Scandium mixed with Barke, B- Frieda mixed with Prestige C- Scandium mixed with Prestige, D- Frieda mixed with Barke. Mean (± se) number of observations in the olfactometer arm containing the barley odour. N= 20 in all comparisons. P values from Wilcoxon test

Fig. 4 Aphid parasitoid *A. colemani* olfactory response to mixed odours of barley cultivars A- Scandium mixed with Barke, B- Frieda mixed with Prestige. Mean (± se) residence time (s) the olfactometer arm containing the barley odour. N= 22 in all comparisons. P values from Wilcoxon test

Table 1 Effect of airborne exposure of one barley cultivar to a different cultivar on ladybird *Coccinella septempunctata* olfactory orientation to the odour of exposed plants- influence of the number of days after the end of the plant exposure period. Ladybird response was measured as mean (±SE) number of observations into the arms of a two-way olfactometer. N= 20 individuals tested in each comparison.

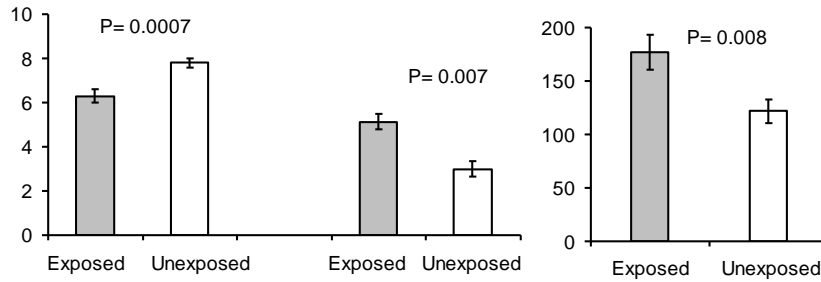| Barley cultivars emitting	exposed	Mean no. obs. in olfactometer arm exposed	unexposed	Wilcoxon test Z	P
Barke	Scandium				
0 days		5.05(0.40)	3.40 (0.35)	2.08	0.03
1 day		5.65 (0.39)	3.05 (0.32)	3.01	0.002
4 days		5.60 (0.35)	3.06 (0.29)	3.11	0.002
7 days		4.05 (0.46)	3.65 (0.45)	0.41	0.68
Frieda	Prestige				
0 days		4.80 (0.40)	3.30 (0.34)	2.11	0.03
1 day		5.35 (0.39)	2.70 (0.25)	3.39	0.0007
4 days		5.00 (0.34)	3.20 (0.32)	2.49	0.01
7 days		5.89 (0.38)	3.16 (0.36)	3.01	0.002

Table 2 Host attack behaviour of the aphid parasitoid *Aphidius colemani* against aphids from barley cultivar Prestige aerially exposed to cultivar Frieda and cultivar Scandium exposed to Barke. Number of contacts, antennations, attacks and % attacks are parameters of host seeking and host preference. Number and % of mummies formed are components of host suitability (see materials and methods for a definition of the parameters). Values are means (± SE) from 20 replicates. Values of U and P from Mann-Whitney U test.

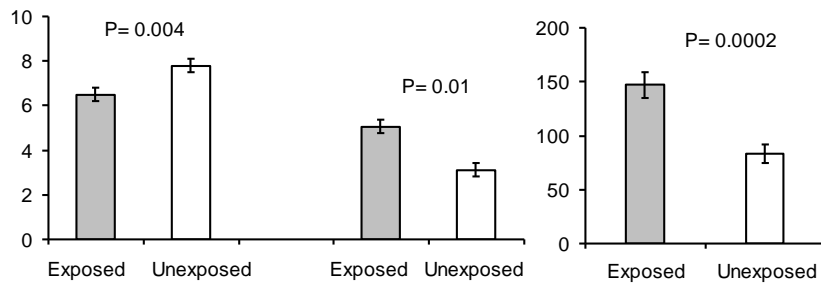| Aphids from	No. antennations	No. attacks	Total no. contacts	% attack	No. mummies	% mummies
Frieda-Prestige						
Exposed	9.7 (1.0)	17.5 (1.4)	27.2 (2.1)	64 (2.0)	5.8 (0.6)	32.5 (2.3)
Unexposed	8.7 (0.7)	11.4 (1.2)	20.0 (1.7)	57 (4.0)	3.8 (0.4)	32.6 (1.9)
U	162	97	109	170	108	189
P	0.31	0.004	0.01	0.43	0.01	0.75
Scandium-Barke						
Exposed	23.8 (2.3)	19.8 (3.3)	43.6 (4.1)	43 (3.0)	2.4 (0.3)	15.9 (3.1)
Unexposed	20.0 (1.6)	13.3 (1.0)	33.4 (2.4)	40 (2.0)	1.5 (0.2)	12.3 (1.7)
U	239	218	202	279	225	279
P	0.15	0.06	0.03	0.52	0.09	0.52

Fig. 1

Aphid settling (mean/plant) | Ladybird olfactory response (mean observations in arm) | Parasitoid olfactory response (mean time in arm (s))
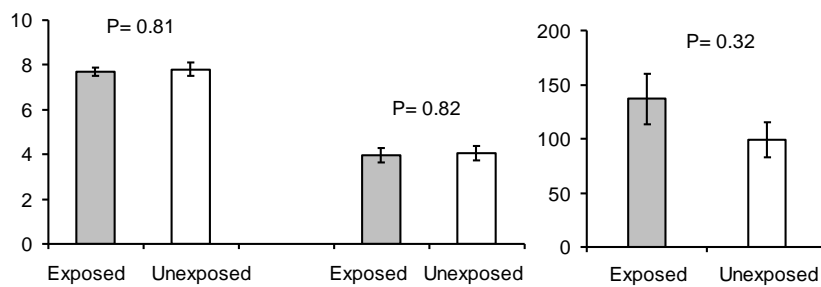
A) Barke - Scandium

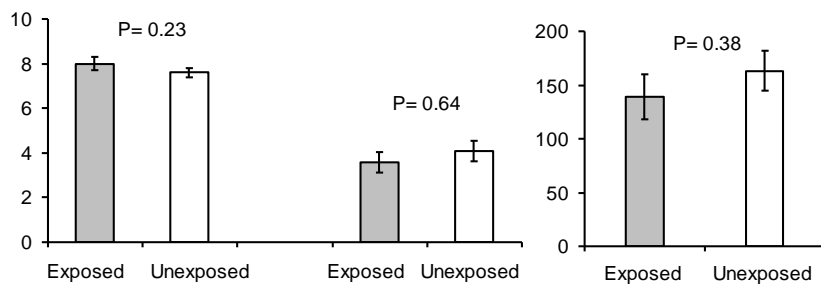B) Frieda - Prestige
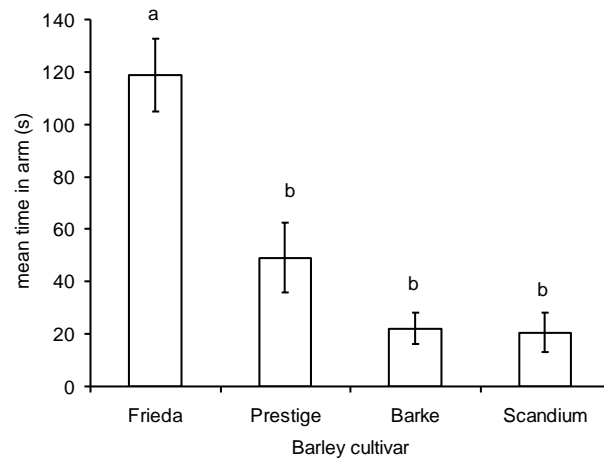
C) Barke - Prestige

D) Frieda - Scandium

Fig. 2

Fig. 3

Fig. 4

Mean time in olfactometer arm

A

P= 0.47

P= 0.18

Scandium + Barke Scandium + Scandium   Scandium + Barke Barke + Barke

B

P= 0.03

P= 0.72

Frieda + Prestige Frieda + Frieda   Frieda + Prestige Prestige + Prestige

Barley cultivar combination