Article (refereed) - postprint

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1 Agricultural and Forest Entomology

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4 Top-down control by *Harmonia axyridis* mitigates

5 the impact of elevated atmospheric CO_2 on a plant-

6 aphid interaction

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Abstract

1) This study investigated the impact of elevated atmospheric CO_2 (390 or 650 μmol/mol) on raspberry genotypes varying in resistance to the large raspberry aphid (*Amphorophora idaei*) and the subsequent impacts on the coccinellid predator *Harmonia axyridis.*

2) CO_2 enrichment promoted plant growth, ranging from 30% in the partially susceptible cultivar to over 100% increase for the susceptible cultivar.

3) Aphid abundance and colonisation (presence-absence) on the susceptible cultivars were not influenced by CO_2 enrichment. On the resistant cultivar, aphid colonisation increased from 14% in ambient CO_2 to 70% in elevated CO_2 with a subsequent increase in aphid abundance, implying a breakdown in resistance. Inclusion of the natural enemy on the resistant cultivar, however, suppressed the increase in aphid abundance at elevated CO_2.

4) This study highlights how crop genotypes vary in responses to climate change; some cultivars can become more susceptible to aphid pests under elevated CO_2. We do, however, demonstrate the potential for top down control to mitigate the effect of global climate change on pest populations.

Introduction

By 2100, atmospheric CO_2 concentrations are predicted to double pre-industrial levels of 280 μmol/mol (Meehl *et al.*, 2007). There is growing interest in understanding how insect herbivores found on crops will respond to such global climate change, particularly in the context of achieving food security (Gregory *et al.*, 2009). While there is expanding literature on the effects of elevated atmospheric CO_2 concentrations (eCO_2) on plant-herbivore interactions (Robinson *et al.*, 2012; Zavala *et al.*, 2013), only a few studies have addressed crop cultivars with genetic resistance to insect pests (e.g. Zavala *et al.*, 2008; Sun *et al.*, 2013). Moreover, to date these studies of crop resistance have largely overlooked the indirect effects of eCO_2 on the natural enemies of crop pests. It is these organisms which will ultimately determine the net effect of eCO_2 on pest population dynamics (Robinson *et al.,* 2012). Given the need to increase food production by 50% by 2050 while using less resources and pesticides (Royal Society, 2009), understanding how climate change will affect ecosystem services such as predation of herbivorous pests, and the underlying mechanisms, is of paramount importance (A'Bear *et al.*, 2014).

In the absence of trophic interactions, plants, which rely on CO_2 assimilation for energy, generally respond positively to eCO_2, with 25-38% increases in biomass being reported for C_3 plants (Stiling & Cornelissen, 2007; Robinson *et al.*, 2012). Within plant tissue, carbohydrates generally increase and nitrogen content is either diluted due to increased carbohydrates or reallocated, resulting in an average 19% increase in plant C:N ratio (Robinson *et al.,* 2012), ultimately altering many aspects of plant chemistry (Stiling & Cornelissen, 2007). Plant resistance is multifaceted, involving direct (physical

85 and antibiotic) and indirect (volatile organic carbons to attract natural

86 enemies) mechanisms (Turlings *et al.*, 1990; Schaller, 2008). Modification of

87 plant defences in an enriched CO_2 atmosphere has been attributed to

88 changes in plant chemistry (Zavala *et al.*, 2008).

89 The response of herbivores to the indirect effects of eCO_2 are modulated by

90 feeding guild and the plant species (Robinson *et al.,* 2012). By feeding directly

91 on the phloem, aphids can circumvent many of the plant defences associated

92 with feeding on plants (Raven, 1983). A meta-analysis by Robinson *et al.*

93 (2012) found only 15 studies investigating the response of phloem-feeding

94 insects to eCO_2, somewhat surprising given the significant damage they can

95 cause to host plants (Zvereva *et al.*, 2010). Despite this, aphid abundance

96 and fecundity generally increases in eCO_2, suggesting a reduction in plant

97 resistance to aphid herbivory. Indeed several crop varieties have recently

98 been shown to become more susceptible to aphid herbivory under eCO_2, via

99 manipulation of host plant chemistry and down regulation of the ethylene

100 pathway (Guo *et al.*, 2013; Sun *et al.*, 2013) In the present study, we

101 investigated the effects of eCO_2 on red raspberry (*Rubus idaeus* L.)

102 susceptibility to the European large raspberry aphid (*Amphorophora*

103 *idaei* Börner). Martin and Johnson (2011) demonstrated that this system is

104 affected by eCO_2; in particular the authors found that a partially resistant

105 cultivar became more susceptible to *A. idaei.* That study did not however,

106 include higher trophic groups, which have the potential to moderate these

107 effects (Martin & Johnson, 2011).

108 The inclusion of higher trophic levels within the community may mitigate the

109 breakdown of aphid resistance. The impact of eCO_2 on the plant may,

110 however, transfer to herbivores on the host plant. Aphids feeding on host

111 plants with low C:N ratio may have a high nutritional value for predators

112 (Couture *et al.*, 2010), therefore in a high CO_2 environment, where the C:N

113 ratio is increased, predators may require greater numbers of prey to fulfil their

114 physiological demands. This is analogous to compensatory feeding seen in

115 herbivores (e.g. Watt *et al.*, 1995) and detritivores (e.g. Dray *et al.*, 2014).

116 There are, however, very few studies investigating the interacting effects of

117 bottom-up (host plant quality) and top-down (predation) on aphid abundance

118 in eCO_2, particularly for woody plants. By using a gradient of plant resistance

119 to aphid herbivory, this study aims to increase our understanding of how tri-

120 trophic interactions are impacted by an eCO_2 environment. We specifically

121 extend earlier research (Martin & Johnson, 2011) through inclusion of different

122 cultivars and also a natural enemy of the aphid. Since plant architecture and

123 habitat complexity are important considerations for assessing the realistic

124 efficacy of natural enemies (Langelotto & Denno, 2004) our study also used

125 larger, structurally complex plants compared to Martin and Johnson (2011).

126 We test the following hypotheses:

127 H_1) Raspberry plants, like most C_3 plants, respond positively to elevated

128 levels of atmospheric CO_2. The magnitude of the response will be cultivar

129 specific, with the biggest increases in biomass in the partially resistant and

130 resistant cultivars (Martin & Johnson, 2011).

131 H_2) Aphid abundance will be distributed according to plant resistance with

132 more aphids on the susceptible cultivars. Under eCO_2 aphid abundance and

133 size will increase on less resistant cultivars (Martin & Johnson, 2011).

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134 H$_3$) Predation levels will increase to compensate for changes in prey quality.

135 Consumption of prey from eCO$_2$ will increase development time and adult

136 mass of predators.

137

138 **Materials and Methods**

139 *Chambers*

140 Experiments were carried out in four controlled environment chambers

141 (approx. 4m x 10m) of the *GroDome™* climate change research facility at the

142 Centre for Ecology and Hydrology (CEH), Wallingford, UK. Chamber

143 environments were maintained at 18 ± 1°C, 50-70% relative humidity. When

144 photosynthetic active radiation (PAR) dropped below 400µmol.s^{-1}.m^{-2}, 12 x

145 400W halide bulbs positioned approximately 1m above the plants

146 supplemented natural daylight in each chamber. A 16h photoperiod was

147 maintained. Chamber air cycled with outside air approximately four times

148 every hour, the industry standard (Buffington *et al.*, 2013). Two of the

149 experimental chambers were maintained at ambient (390 ± 50 *µ*mol/mol) and

150 two at elevated (650 ± 50 *µ*mol/mol) atmospheric CO$_2$ levels. A CO$_2$ sensor

151 (Vaisala GMW22) was mounted in each chamber and connected to a

152 controller unit (Mitsubishi Micro-controller AL2-24MR-D). Once CO$_2$ levels fell

153 below the target concentration (390 *µ*mol/mol and 550 *µ*mol/mol,

154 respectively), CO$_2$ gas (BOC) was injected for 1-second followed by 30-

155 second delay, repeating until the target concentrations were reached.

156 *Host plant*

157 Three cultivars of European red raspberry (*R. idaeus*), varying in resistance to

158 aphid herbivory, were used in the experiment. Glen Ample possesses a

159 resistance gene (A_1), now largely ineffective following adaptation by aphid

160 biotypes (Birch *et al.*, 2004) and thus represents the plant least resistant to

161 herbivory. Glen Clova has partial resistance to aphid herbivory underpinned

162 by multiple genes (multi-genic) (McMenemy *et al.*, 2009). Octavia is highly

163 resistant to aphid herbivory, possessing two resistance genes (A_{10} and A_{k4a})

164 (Knight & Fernández-Fernández, 2008). Plants were grown from root-stock at

165 the James Hutton Institute (JHI), Dundee, UK. When approximately 1cm in

166 height, the plants were transferred to CEH where they were potted-out into 3L

167 pots filled with peat-based compost (Levington M3, no additional fertiliser) and

168 randomly allocated to CO_2 treatments. All plants were grown in ambient or

169 elevated CO_2 conditions for approximately five weeks prior to the experiment

170 commencing.

171 *Aphids*

172 The European large raspberry aphid (*Amphorophora idaei*) is a specialist

173 phloem-feeding herbivore, found only on the European red raspberry causing

174 direct and indirect (vectors four plant-viruses) economic damage to fruit crops

175 (McMenemy *et al.*, 2009). Insect herbivore biotypes are populations that differ

176 in their ability to utilize a certain trait of a plant genotype/cultivar (Smith,

177 2005). The large raspberry aphid biotype (Biotype 2) used in this experiment

178 can survive on raspberry cultivars possessing A_1 resistance genes and is the

179 most common biotype found in the UK (McMenemy *et al.*, 2009). The aphid

180 culture was initiated from field-collected aphids at JHI and maintained in the

181 laboratory for multiple generations. This aphid population was maintained

182 at 18 ± 1°C, 16h photoperiod using the cultivar Malling Landmark (also A_1

183 resistance) as a culture plant. The aphid population had been randomly

184 divided and maintained in either ambient or elevated CO_2 conditions for at
185 least five generations before the experiment.

186 *Ladybirds*

187 The aphidophagous harlequin ladybird (*Harmonia axyridis* Pallas), native to
188 Asia, was originally used throughout Europe and North America as a
189 biocontrol agent against aphids (Brown *et al.*, 2008). Now established, it is
190 one of the most common ladybird species (Tedders & Schaefer, 1994;
191 Colunga-Garcia & Gage, 1998; Brown *et al.*, 2008). Adult female ladybirds
192 were collected from lime trees (*Tilia* spp.) in Oxfordshire, UK. The population
193 was maintained in clear acrylic cages (30cm x 20cm x 15cm) at 18 ± 1°C and
194 16hr photoperiod. In culture, *H. axyridis* populations were fed pea aphids
195 (*Acyrthosiphon pisum* Harris), but starved for 24 hours prior to the experiment.

196 *Experiment 1: Trophic interactions*

197 In a fully-factorial blocked design, 48 plants of each cultivar (susceptible,
198 partially-resistant and resistant) were randomly assigned to the two
199 atmospheric CO_2 (ambient and elevated) and subsequent predator (ladybird
200 present or absent) treatments. This gave 12 replicates per treatment
201 combination (cultivar x CO_2 x predator). The experiment was carried out
202 September 2011 – September 2012 over a series of four runs to avoid
203 psudoreplication of CO_2 treatment. Each run comprised of three full replicates
204 (n=36) of each treatment combination. Within each run the 18 plants were
205 randomly distributed along a single bench inside each chamber. To prevent
206 movement of flightless aphid nymphs between plants, individual pots were
207 secured on circular plinths (10cm diameter x 3cm height) and placed in 50cm

208   x 50cm plastic trays filled with water (four plants per tray), ensuring the pots

209   were above the water-line (see Johnson *et al.*, 2013 for details).

210   After five weeks growth in the CO_2 treatments, the height of each plant was

211   measured and three adult large raspberry aphids were placed on the first fully

212   unfurled leaf of each plant. Two weeks after aphid inoculation, the number of

213   nymphs and adult aphids on each plant was counted and then a single adult

214   female *H. axyridis* was introduced to the plants assigned to predator

215   treatment. All plants were then placed within individual insect cages

216   (25cm diam. x 65cm height, Insectopia, UK). The ladybirds remained on the

217   plants for 72 hours, after which they were removed and the aphid population

218   on each plant re-counted. Up to 10 adult aphids from each plant were

219   collected at random, snap-frozen and freeze-dried. All aboveground plant

220   material was destructively harvested and oven-dried for 48 hours at 70°C.

221   Aphid and plant dry mass were recorded. Total soluble protein was

222   determined from a subsample of the freeze-dried aphids using a protein assay

223   kit (Thermo Scientific BCA Kit 23225) which used the Bradford (1976)

224   method.

225   *Experiment 2: Ladybird development*

226   To provide aphid prey, 32 plants of the susceptible and partially resistant

227   cultivar were randomly assigned to two CO_2 treatments across four controlled

228   environment chambers (2 x ambient, 2 x elevated). Plants were inoculated

229   with large raspberry aphid as in Experiment 1 and after four weeks aphids

230   were collected daily and used as prey for the ladybird larvae in the trial. Eggs

231   were laid in a series of clutches over a 5-day period from three randomly

232   selected mating pairs of Harlequin ladybirds. Each clutch (approximately 15 –

233 30 eggs) was collected and split randomly between the four diet treatments

234 (cultivar x CO_2). There were 30 individual ladybird replicates per treatment

235 combination, 120 in total. Eggs were placed individually into plastic pots (2cm

236 height x 3cm diameter) in a constant temperature room at 18°C, 16 hours

237 photoperiod. Upon eclosion from egg, each larva was provided with 10 – 15

238 aphids daily, any aphids not consumed from the previous day were removed.

239 Time to each larval instar was recorded. To establish the effect of diet

240 treatment on relative growth rate, a random sample of 11 individuals from

241 each treatment combination (44 in total) were selected and weighed every

242 day until pupation (Sartorius ME36S microbalance). Mean relative growth rate

243 (MRGR) was calculated following Gotthard *et al.* (1994):

244 MRGR = (W_2 – W_1) / t,

245 *where W_1 is the initial weight,* W_2 the final weight and t is the number of days

246 for each life-stage. Mass of all individuals were recorded at pupation and

247 emergence. Adult dry mass was recorded after emerged adults were snap

248 frozen and freeze dried (Heto PowerDry PL3000).

249 *Statistical analysis*

250 All data were analysed using generalised linear mixed models (GLMM) using

251 PROC GLIMMIX (SAS Institute, version 9.01).

252 *Experiment 1*

253 Hypotheses 1 and 2. Initial aphid abundance (counts) was modelled using a

254 Poisson error distribution and log-link function. Aboveground plant dry mass,

255 change (delta) in aphid abundance, aphid dry mass and total soluble protein

256 content were modelled using a normal (Gaussian) error distribution with

257 identity-link function. Random effects were experimental run and chamber

258 nested within run for all models. Models of aphid abundance had an

259 additional, observation-level random effect fitted to account for over-

260 dispersion within the count data (Elston *et al.*, 2001). While chamber

261 accounted for little variation in the data it represented an important structural

262 random effect (i.e. CO_2 treatment was applied at the chamber level) and was

263 thus retained in all models.

264 Potential explanatory variables included raspberry cultivar (susceptible (Glen

265 Ample), partially resistant (Glen Clova), resistant (Octavia)), CO_2 treatment

266 (ambient 390 μmol/mol, elevated 650 μmol/mol), predator treatment (ladybird

267 present or absent) and plant biometrics (height, dry mass). Of the original 144

268 plants, 12 died at various stages during the experiment and were not included

269 in the analysis. Aphid total soluble protein content was modelled separately

270 using a normal (Gaussian) error distribution with identity-link function.

271 *Experiment 2*

272 Hypothesis 3. Relative growth rate, development time and pupal mass of

273 ladybirds were modelled using a normal (Gaussian) error distribution with

274 identity-link function. Random terms were parent identity and the experimental

275 chamber in which the aphid prey was reared. When repeated measures were

276 used (relative growth rate) an observation-level random effect was added to

277 the R-side of the random structure. Raspberry cultivar (susceptible and

278 partially resistant), CO_2 treatment (ambient 390 μmol/mol and elevated 650

279 μmol/mol), sex upon emergence as adult and larval instars (relative growth-

280 rate only) were fitted as potential explanatory variables.

281 During the analysis of both experiments, explanatory variables were added in

282 a forward stepwise fashion until a minimum adequate model was obtained

283 (Crawley, 2002). F-ratio and p-values adjusted for other fitted terms (SAS type

11

284 III) are presented and, where multiple comparison tests (i.e. SAS Least-
285 Square means) were used to test for treatment effects, a Bonferroni correction
286 was applied. Two-way interactions (e.g. between cultivar, predator and CO_2
287 treatments in Experiment 1) are reported only when statistically significant
288 ($p<0.05$).

289

290 **Results**

291 *Experiment 1*

292 *Hypothesis 1 - Plant responses*

293 Aboveground biomass varied significantly among the raspberry cultivars
294 irrespective of CO_2 treatment (Table 1). Plants partially-resistant to aphid
295 herbivory had the greatest dry mass, followed by the resistant cultivar (Fig. 1).
296 The susceptible cultivar had the lowest dry mass, almost half that of the
297 partially resistant cultivar (Fig. 1). CO_2 treatment also influenced the plant
298 biomass, plants grown in eCO_2 achieving a greater dry mass compared to
299 plants grown in ambient CO_2 (Table 1). The susceptible cultivar was the most
300 responsive to eCO_2 with a 107% increase in dry mass compared to ambient
301 CO_2 (Fig. 1). There was an 85% increase of dry mass of the resistant cultivar
302 in eCO_2 compared to ambient. The partially susceptible cultivar was the least
303 responsive to eCO_2, increasing in dry mass by 30%.

304 *Hypothesis 2 - Aphid responses*

305 There was a highly significant effect of cultivar on aphid abundance before the
306 onset of the predation treatment (Fig. 2a, Table 1b). While there were similar
307 numbers of aphids on the susceptible and partially-resistant cultivars, as
308 expected, the aphid abundance on the resistant cultivar was lower by almost

309 a factor of 10 (Fig. 2a). Atmospheric CO_2 enrichment significantly affected

310 aphid abundance (Table 1b), but this varied between plant cultivars as

311 indicated by the significant CO_2 x cultivar interaction (Table 1b). Altered

312 population levels drove this effect of CO_2 enrichment on aphid abundance on

313 the resistant plant cultivar. On the resistant cultivar, elevation of atmospheric

314 CO_2 concentrations significantly increased the mean abundance of aphids

315 (Fig. 2a). Furthermore, aphid colonisation of the resistant cultivar was

316 markedly increased by CO_2 enrichment with 14% and 70% of plants

317 supporting aphids under ambient and eCO_2 conditions, respectively ($F_{1,5}$ =

318 7.9, p = 0.05). In contrast, aphid abundance on the susceptible and partially

319 resistant cultivars were unaffected by manipulation of the CO_2 environment

320 (Fig 2a, Table 1a).

321 The presence of a ladybird predator significantly reduced aphid abundance on

322 all cultivars (Fig. 2a versus Fig. 2b; Table 1c). Moreover, while CO_2

323 enrichment increased aphid herbivore colonisation and abundance on the

324 resistant cultivar, once ladybird predation was introduced this CO_2 effect was

325 nullified (Table 1c, Fig. 2b). On the susceptible and partially-resistant

326 cultivars, the number of aphids consumed by the ladybird did not significantly

327 vary with CO_2 treatment (Fig. 2b). CO_2 treatment did not affect adult aphid dry

328 mass or total protein content ($F_{1,2}$ = 0.25, p = 0.667 and $F_{1,2}$ = 1.44, p = 0.353,

329 respectively). Aphid total soluble protein was greater when reared on the

330 susceptible cultivar than the partially resistant cultivar ($F_{1,100}$ = 11.6, p =

331 0.001).

332 *Experiment 2*

333   Hypothesis 3. The mean relative growth rate over the full duration of ladybird

334   development was not affected by the prey source environment (CO_2: $F_{1,2}$ =

335   1.03, p = 0.42 and cultivar: $F_{1,24}$ = 0.78, p = 0.38). Relative growth rate was

336   stage-specific with the earlier instars having a much lower mean growth rate

337   than the later instars. When fed aphids from the partially resistant cultivar, the

338   mean relative growth rate of fourth instar ladybird was significantly increased

339   (Fig. 3, Table 2a). When fed aphids reared on the partially resistant cultivar,

340   fourth instar ladybird larvae had significantly higher relative growth rate

341   compared to their siblings fed aphids reared on the resistant cultivar (Fig.3).

342   There was no significant effect of CO_2 treatment on relative growth rate of

343   ladybird larvae (Table 2.a). Despite the significant effect of cultivar on fourth

344   instar larval growth rate, duration of development from egg to adult was not

345   affected by the cultivar or CO_2 treatment ($F_{1,80}$ = 0.29, p = 0.59 and $F_{1,2}$ =

346   0.61, p = 0.44, respectively) aphid prey was reared in. Similarly, pupal mass

347   and adult mass were not affected by the rearing conditions of the aphid prey

348   (Table 2b). Pupal and adult mass was, however, affected by adult sex:

349   females were significantly heavier than males (Table 2b).

350

351   **Discussion**

352   The fertilising effect of CO_2 enrichment is predicted to increase plant biomass

353   and productivity (Ainsworth & Long, 2005; Robinson *et al.*, 2012), particularly

354   for woody plants (Curtis & Wang, 1998). This study confirms this, with all

355   three raspberry cultivars showing increased biomass in response to elevated

356   atmospheric CO_2. This was also seen for the raspberry cultivars investigated

357   by Martin and Johnson (2011) (summarised in Table 3), suggesting that this

358   response is common to the species as a whole. On the two susceptible

359 cultivars, aphid populations were unaffected by the increased plant biomass

360 associated with elevated CO_2. Aphid colonisation and subsequent abundance

361 was greater on the resistant cultivar grown in elevated CO_2, suggesting a

362 reduction in resistance to aphid herbivory in the novel environment.

363 Plant biomass in ambient conditions was not correlated with resistance to

364 herbivory; the partially resistant cultivar had the greatest biomass, followed by

365 the resistant and susceptible cultivars. The extent to which plant biomass

366 increased under CO_2 enrichment varied with cultivar. The partially resistant

367 cultivar, with the greatest biomass in ambient CO_2, was the least responsive

368 (30% increase in biomass), suggesting that it is already close to its maximum

369 growth capacity under ambient CO_2. The 85% increase in biomass of the

370 resistant cultivar under eCO_2 suggests this cultivar to be particularly

371 responsive to eCO_2. In eCO_2 aphid colonisation was significantly higher on

372 the resistant cultivar, but aphid numbers remained very low despite a

373 significant increase from ambient conditions. The resistant cultivar used in this

374 study, Octavia, is the successful crossing of two aphid resistance genes, A_{10}

375 and A_{k4a}. Previous work by Martin and Johnson (2011) found the A_{10} was

376 robust to changes in CO_2 concentrations. This implies that CO_2 enrichment

377 may be modifying the function of the A_{k4a} resistance gene. This, however,

378 remains an untested hypothesis and is only one possible explanation.

379 Raspberry cultivars possessing the A_{10} resistance gene can show significant

380 variation in minor genes associated with aphid resistance, which may modify

381 the responses to elevated CO_2 (Hall, 2009). Even in cultivars possessing the

382 same resistance gene, it seems their genotypic background can modify

383 resistance expression at elevated CO_2. For example with two cultivars

384 possessing the A_1 resistance gene either becoming more susceptible to

aphids (Table 3, Martin & Johnson, 2011) or unaffected, as reported here. Similarly, expression of anti-herbivore defences among individuals from the same population of common milkweed (*Asclepias syriaca* L) vary considerably when grown in elevated CO_2 (Vannette & Hunter, 2011).

Without a detailed mechanistic understanding of raspberry resistance to *A. idaei*, the reason why aphid numbers increased in elevated CO_2 remains speculative. Resistance to *A. idaei* in raspberry is thought to be the result of antibiosis reducing colonisation and antixenosis reducing individual performance (Mitchell, 2007). The observed increase in colonisation rate suggests the former defence may be impaired in elevated CO_2. Increasing CO_2 levels have been shown to suppress the production of jasmonates and increase the production of salicylic acid, affecting specific signalling pathways related to plant defence (Zavala *et al.*, 2013). In particular, the down regulation of jasmonates has been linked to increased aphid abundance in elevated CO_2 (Sun *et al.*, 2013).

Top-down regulation of agricultural pest species by natural enemies is becoming increasingly important as use of conventional chemical pesticides becomes progressively more difficult under stricter legislation (such as European Union Regulation (EC) No 1107/2009) (Van Driesche, 2008). This is particularly true for crops grown under cover (e.g. glasshouse, polytunnel), an increasingly common practice for enhancing productivity (Johnson *et al.*, 2010, 2012, Wittwer & Castilla, 1995), since natural enemies work more effectively in closed environments (McMenemy *et al.*, 2009). Even in non-covered agricultural crops, top-down regulation of herbivore populations is,

409 however, important and increasingly encouraged (Stiling & Corneilissen,

410 2005, Van Driesche, 2008).

411 Predation by a natural enemy mitigated the breakdown of resistance to aphid

412 herbivory, returning the aphid population to its "ambient" state. There was no

413 evidence for a transfer of bottom-up effects across multiple trophic levels. The

414 CO_2 environment host plants were exposed to, did not affect the size or total

415 protein content of aphids living on them. Moreover, the CO_2 environment their

416 prey had been reared in did not influence the development of the next

417 generation of ladybirds. Similar to other studies, we found the effect of

418 elevated CO_2 on prey quality weak or non-existent (Salt *et al.*, 1995; Stacey &

419 Fellowes, 2002, Chen *et al.,* 2005) and subsequent predator generations were

420 also unaffected (Chen *et al.,* 2005).

421 The influence of bottom up processes, such as the effect of plant genotype on

422 prey quality, had a much more significant effect than eCO_2 on ladybird

423 development. Plant cultivar significantly affected total protein content in

424 aphids. When reared on the susceptible cultivar, aphids had a greater total

425 protein content than aphids reared on the partially resistant cultivar.

426 Unexpectedly, the opposite was observed for the mean relative growth rate of

427 ladybird larvae. Larvae fed aphid prey from the susceptible cultivar had

428 significantly lower growth rate than larvae fed aphids from the partially

429 susceptible cultivar. The underlying reason for this remains unclear, but the

430 higher protein content of aphids on the susceptible cultivar may reflect greater

431 fitness and behavioural responsiveness of these individuals in addition to their

432 nutritional value as prey. These individuals may be able to better resist attack

433 by ladybirds using behavioural strategies (e.g. kicking, evasion) and thereby

434 impose extra fitness costs (e.g. handling time) on ladybirds (Dixon, 2000).

435 Mitchell *et al.* (2010) reported that *A. idaei* showed less 'dropping behaviour'

436 and suggested they may rely more on such behavioural resistance to

437 parasitoid attack when feeding on susceptible cultivars, so this explanation is

438 at least credible.

439 Confining aphids on plants necessitated use of potted plants in closed

440 chambers, which may be argued to give artificially high plant growth

441 responses to CO_2 (Ainsworth *et al.,* 2008). Given, however, that > 90% of

442 raspberry production takes place in closed polytunnels which buffer

443 environmental fluctuations (Johnson *et al.*, 2010; Johnson *et al.*, 2012), this is

444 perhaps a less relevant concern in this system as chambers have similar

445 effects. Moreover, our use of large pots and potting media minimised

446 restrictions to root growth the potential for hypoxic conditions, as advocated

447 by Passioura (2006).

448 This study highlights the importance of considering multiple trophic levels

449 when trying to understand pest dynamics and ecosystem responses to future

450 climates. Increasing atmospheric CO_2 has the potential to impair plant

451 defences against herbivory which may have important implications for agro-

452 ecosystems. We demonstrate that higher trophic levels may, however, partly

453 mitigate this reduction in plant defences by controlling herbivore numbers on

454 the affected plants. The longer-term effects of elevated CO_2 on tri-trophic

455 interactions remain however little understood. This study provides an

456 empirical demonstration of how the net level of plant herbivory under elevated

457 CO_2 depends on both the interaction between the herbivore and the natural

458 enemy.

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463 Acknowledgements

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657　Table 1. GLIMMIX results summary for a) plant dry mass, b) initial aphid

658　abundance and c) change in aphid abundance-post predation in relation to

659　biotic and abiotic environment. Significant variables in bold retained in final

660　model. MPE = multiple parameter estimates.

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Response variable	Explanatory variables	Estimate	$F_{(ndf, ddf)}$	P
a) Plant dry mass	**CO_2**		**$12.85_{(1,3)}$**	**0.0377**
	Ambient	*12.830*		
	Elevated	*20.671*		
Random effect estimate:	**Cultivar**		**$20.37_{(2,121)}$**	**<0.0001**
Chamber (experimental run) = 28.73	*Susceptible*	*14.940*		
± 27.512	*Partially resistant*	*25.221*		
	Resistant	*20.671*		
Experimental run = 6.28 ± 7.84	Initial aphid abundance	20.666	$0.33_{(1,123)}$	0.568
b) Initial aphid abundance	**CO_2**		**$14.49_{(1,2)}$**	**0.063**
	Ambient	*-1.555*		
	Elevated	*0.71*		
Random effect estimate:	**Cultivar**		**$148.67_{(2,128)}$**	**<0.0001**
Replicate = 0.46±0.081	*Susceptible*	*4.203*		
	Partially resistant	*4.197*		
Chamber (experimental run) = 0	*Resistant*	*0.71*		
Experimental run = 0.13±0.12	Dry mass	0.888	$0.88_{(1,121)}$	0.349
	CO_2 * Cultivar	**MPE**	**$8.89_{(2,128)}$**	**0.0002**
c) Delta aphid abundance	CO_2		$0.3_{(1,2)}$	0.639
Random effect estimate:	*Ambient*	*-0.678*		
Chamber (experimental run) = 0	*Elevated*	*-4.747*		
Experimental run = 468.36±429.36	**Cultivar**		**$10.64_{(1,125)}$**	**<0.0001**
	Susceptible	*-1.99*		
	Partially resistant	*6.21*		
	Resistant	*-2.84*		
	Dry mass	2.062	$0.6_{(1,123)}$	0.439
	Predator treatment	**MPE**	**$33.55_{(1,125)}$**	**<0.0001**
	Control	*0.04*		
	Ladybird	*-2.84*		
	Cultivar * predator treatment	**MPE**	**$7.37_{(2,1.25)}$**	**0.0009**

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Table 2. GLIMMIX results summary for ladybird responses (a) relative growth

rate and b) pupal mass), in relation to rearing conditions of their aphid prey

(CO_2 and raspberry cultivar), larval instar and gender. Significant variables in

bold retained in final model. MPE = multiple parameter estimates

668

Response variable	Explanatory variables	Estimate	$F_{(ndf, ddf)}$	P
a) Relative growth rate	CO_2		$0.79_{(1,2)}$	0.385
	Ambient	*4.793*		
	Elevated	*4.715*		
Random effect estimate:	**Cultivar**		**$3.07_{(1,23)}$**	**0.093**
Parent = 0.001±0.007	*Susceptible*	*5.195*		
Replicate = 0.252±0.135	*Partially resistant*	*4.670*		
Chamber = 0	**Larval instar**	**MPE**	**$637.22_{(3,60)}$**	**<0.0001**
	1st	*0.022*		
	2nd	*0.727*		
	3rd	*2.033*		
	4th	*4.67*		
	Larval instar * Cultivar	**MPE**	**$3_{(3,60)}$**	**0.038**
b) Pupal mass	CO_2		$0.03_{(1,2)}$	0.884
	Ambient	*35.725*		
	Elevated	*35.846*		
Random effect estimate:	Cultivar		$0.02_{(1,92)}$	0.898
Parent = 1.463±1.863	*Susceptible*	*35.831*		
Chamber = 0	*Partially resistant*	*35.767*		
	Sex		**$26.30_{(1,93)}$**	**<0.0001**
	Male	*0.868*		
	Female	*1.603*		

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680   Table 3. Comparison of plant and aphid responses to elevated atmospheric CO_2 (eCO_2) found by Martin & Johnson, 2012 and the

681   findings of this study.

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Cultivar	Martin & Johnson eCO_2 impacts on plants	eCO_2 impacts on aphids	Hentley *et al* eCO_2 impacts on plants	eCO_2 impacts on aphids
Malling Jewell (susceptible)	197% increase growth rate	None		
Glen Lyon – A_1	41% increase in growth rate	Increase in abundance and adult mass		
Glen Ample – A_1			107% increase in dry mass	None
Glen Clova – multi			30% increase in dry mass	None
Glen Rosa – A_{10}	186% increase in growth rate	None		
Octavia – A_{10} and A_{K4}			85% increase in dry mass	Increase in aphid colonization and abundance

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685   Figure 1. Aboveground plant dry mass of three raspberry cultivars in response

686   to ambient and elevated CO_2. Data are least square mean ± S.E.

687

688   Figure 2. The effect on aphid abundance of cultivar resistance, CO_2 treatment

689   and presence a) or absence b) of ladybird predation. Ambient (white bars)

690   and elevated (grey bars) atmospheric CO_2 levels. Letters above bars denote

691   significant differences. Aphid abundance for resistant cultivar scaled using a

692   second y-axis to make treatment effects clearer. Data are mean ± S.E.

693

694   Figure 3. Least square mean for relative growth rate of larval stages of the

695   ladybird *H. axyridis* fed aphid prey from susceptible (dashed line and triangle)

696   or partially resistant (solid line and circle) raspberry cultivars. Data are least

697   square mean ± S.E.

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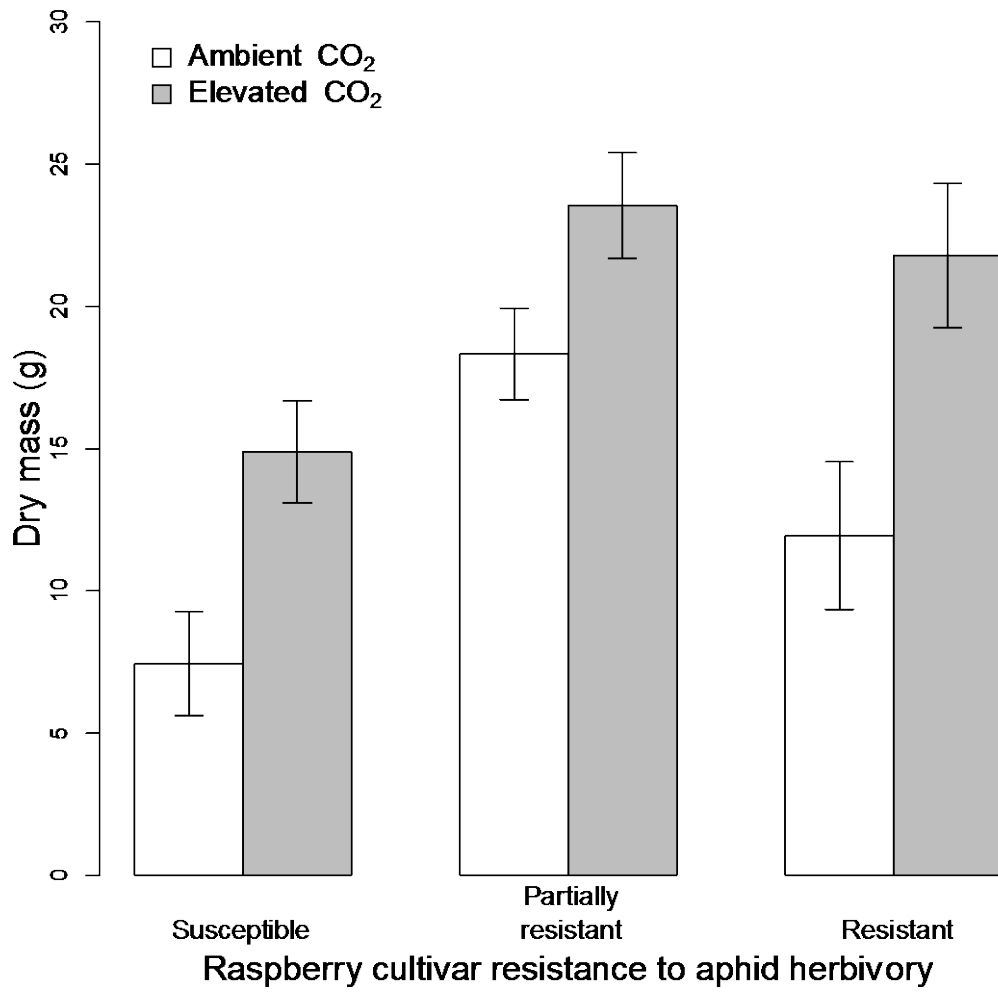704
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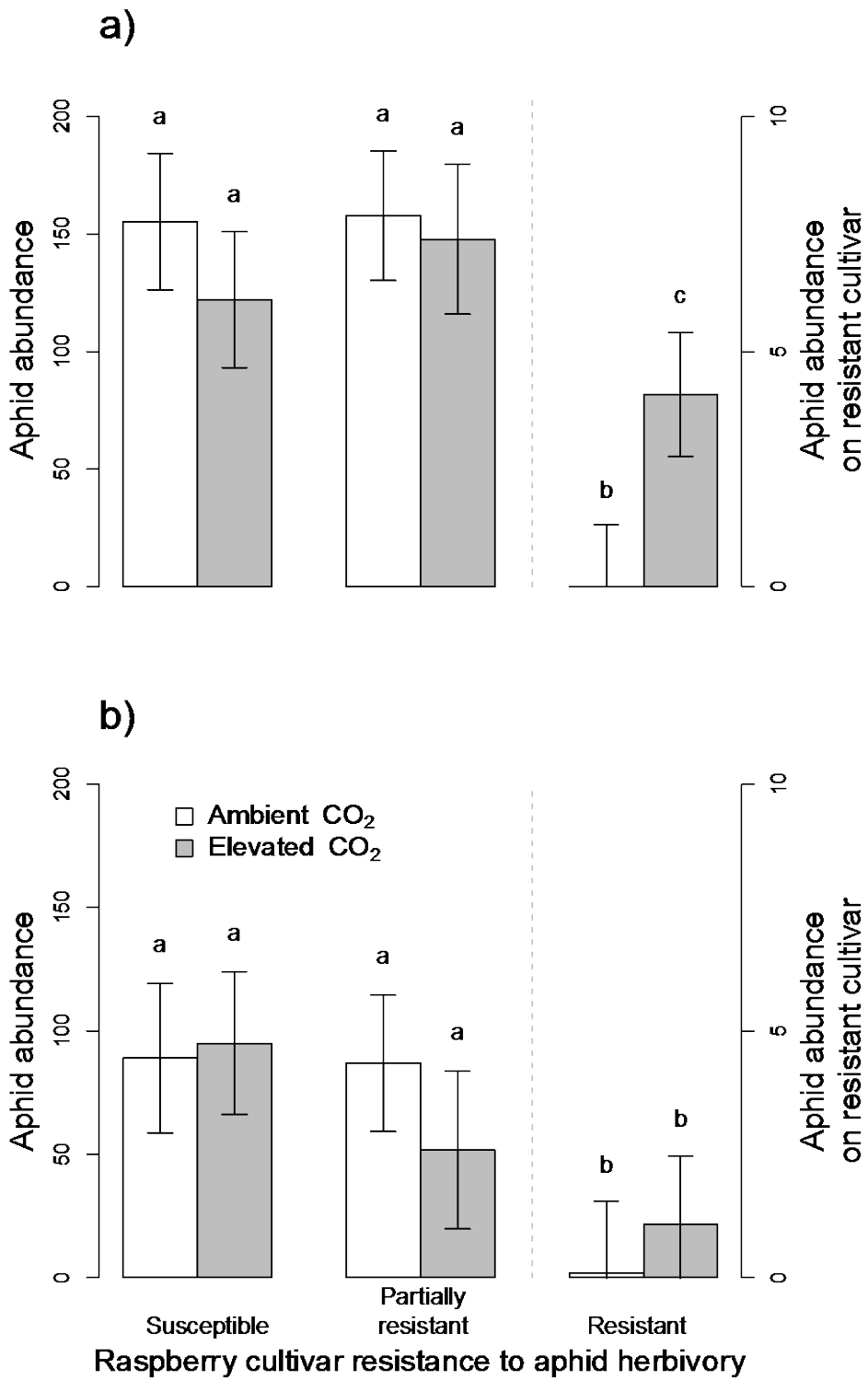
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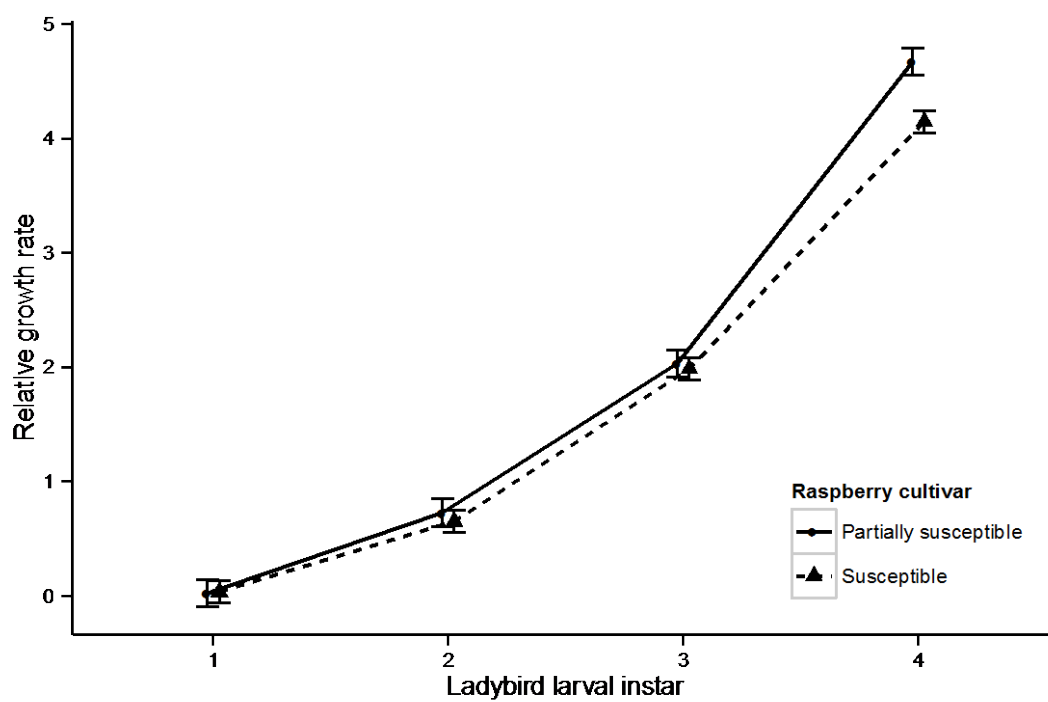722
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