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Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric CO_2 on a plant-aphid interaction

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Running title: *Multi-trophic interactions in an elevated CO_2 environment.*

Abstract

1) This study investigated the impact of elevated atmospheric CO_2 (390 or 650 *μ*mol/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 *μ*mol/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

and antibiotic) and indirect (volatile organic carbons to attract natural enemies) mechanisms (Turlings *et al.*, 1990; Schaller, 2008). Modification of plant defences in an enriched CO_2 atmosphere has been attributed to changes in plant chemistry (Zavala *et al.*, 2008).

The response of herbivores to the indirect effects of eCO_2 are modulated by feeding guild and the plant species (Robinson *et al.,* 2012). By feeding directly on the phloem, aphids can circumvent many of the plant defences associated with feeding on plants (Raven, 1983). A meta-analysis by Robinson *et al*. (2012) found only 15 studies investigating the response of phloem-feeding insects to eCO_2, somewhat surprising given the significant damage they can cause to host plants (Zvereva *et al.*, 2010). Despite this, aphid abundance and fecundity generally increases in eCO_2, suggesting a reduction in plant resistance to aphid herbivory. Indeed several crop varieties have recently been shown to become more susceptible to aphid herbivory under eCO_2, via manipulation of host plant chemistry and down regulation of the ethylene pathway (Guo *et al.*, 2013; Sun *et al.*, 2013) In the present study, we investigated the effects of eCO_2 on red raspberry (*Rubus idaeus* L.) susceptibility to the European large raspberry aphid (*Amphorophora idaei* Börner). Martin and Johnson (2011) demonstrated that this system is affected by eCO_2; in particular the authors found that a partially resistant cultivar became more susceptible to *A. idaei.* That study did not however, include higher trophic groups, which have the potential to moderate these effects (Martin & Johnson, 2011).

The inclusion of higher trophic levels within the community may mitigate the breakdown of aphid resistance. The impact of eCO_2 on the plant may,

however, transfer to herbivores on the host plant. Aphids feeding on host plants with low C:N ratio may have a high nutritional value for predators (Couture *et al.*, 2010), therefore in a high CO_2 environment, where the C:N ratio is increased, predators may require greater numbers of prey to fulfil their physiological demands. This is analogous to compensatory feeding seen in herbivores (e.g. Watt *et al*., 1995) and detritivores (e.g. Dray *et al*., 2014). There are, however, very few studies investigating the interacting effects of bottom-up (host plant quality) and top-down (predation) on aphid abundance in eCO_2, particularly for woody plants. By using a gradient of plant resistance to aphid herbivory, this study aims to increase our understanding of how tri-trophic interactions are impacted by an eCO_2 environment. We specifically extend earlier research (Martin & Johnson, 2011) through inclusion of different cultivars and also a natural enemy of the aphid. Since plant architecture and habitat complexity are important considerations for assessing the realistic efficacy of natural enemies (Langelotto & Denno, 2004) our study also used larger, structurally complex plants compared to Martin and Johnson (2011).

We test the following hypotheses:

H_1) Raspberry plants, like most C_3 plants, respond positively to elevated levels of atmospheric CO_2. The magnitude of the response will be cultivar specific, with the biggest increases in biomass in the partially resistant and resistant cultivars (Martin & Johnson, 2011).

H_2) Aphid abundance will be distributed according to plant resistance with more aphids on the susceptible cultivars. Under eCO_2 aphid abundance and size will increase on less resistant cultivars (Martin & Johnson, 2011).

H_3) Predation levels will increase to compensate for changes in prey quality. Consumption of prey from eCO_2 will increase development time and adult mass of predators.

Materials and Methods

Chambers

Experiments were carried out in four controlled environment chambers (approx. 4m x 10m) of the *GroDome™* climate change research facility at the Centre for Ecology and Hydrology (CEH), Wallingford, UK. Chamber environments were maintained at 18 ± 1°C, 50-70% relative humidity. When photosynthetic active radiation (PAR) dropped below 400µmol.s^{-1}.m^{-2}, 12 x 400W halide bulbs positioned approximately 1m above the plants supplemented natural daylight in each chamber. A 16h photoperiod was maintained. Chamber air cycled with outside air approximately four times every hour, the industry standard (Buffington *et al.*, 2013). Two of the experimental chambers were maintained at ambient (390 ± 50 *µ*mol/mol) and two at elevated (650 ± 50 *µ*mol/mol) atmospheric CO_2 levels. A CO_2 sensor (Vaisala GMW22) was mounted in each chamber and connected to a controller unit (Mitsubishi Micro-controller AL2-24MR-D). Once CO_2 levels fell below the target concentration (390 *µ*mol/mol and 550 *µ*mol/mol, respectively), CO_2 gas (BOC) was injected for 1-second followed by 30-second delay, repeating until the target concentrations were reached.

Host plant

Three cultivars of European red raspberry (*R. idaeus*), varying in resistance to aphid herbivory, were used in the experiment. Glen Ample possesses a

resistance gene (A_1), now largely ineffective following adaptation by aphid biotypes (Birch *et al.*, 2004) and thus represents the plant least resistant to herbivory. Glen Clova has partial resistance to aphid herbivory underpinned by multiple genes (multi-genic) (McMenemy *et al.*, 2009). Octavia is highly resistant to aphid herbivory, possessing two resistance genes (A_{10} and A_{k4a}) (Knight & Fernández-Fernández, 2008). Plants were grown from root-stock at the James Hutton Institute (JHI), Dundee, UK. When approximately 1cm in height, the plants were transferred to CEH where they were potted-out into 3L pots filled with peat-based compost (Levington M3, no additional fertiliser) and randomly allocated to CO_2 treatments. All plants were grown in ambient or elevated CO_2 conditions for approximately five weeks prior to the experiment commencing.

Aphids

The European large raspberry aphid (*Amphorophora idaei*) is a specialist phloem-feeding herbivore, found only on the European red raspberry causing direct and indirect (vectors four plant-viruses) economic damage to fruit crops (McMenemy *et al.*, 2009). Insect herbivore biotypes are populations that differ in their ability to utilize a certain trait of a plant genotype/cultivar (Smith, 2005). The large raspberry aphid biotype (Biotype 2) used in this experiment can survive on raspberry cultivars possessing A_1 resistance genes and is the most common biotype found in the UK (McMenemy *et al.*, 2009). The aphid culture was initiated from field-collected aphids at JHI and maintained in the laboratory for multiple generations. This aphid population was maintained at 18 ± 1°C, 16h photoperiod using the cultivar Malling Landmark (also A_1 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 –

30 eggs) was collected and split randomly between the four diet treatments (cultivar x CO_2). There were 30 individual ladybird replicates per treatment combination, 120 in total. Eggs were placed individually into plastic pots (2cm height x 3cm diameter) in a constant temperature room at 18°C, 16 hours photoperiod. Upon eclosion from egg, each larva was provided with 10 – 15 aphids daily, any aphids not consumed from the previous day were removed. Time to each larval instar was recorded. To establish the effect of diet treatment on relative growth rate, a random sample of 11 individuals from each treatment combination (44 in total) were selected and weighed every day until pupation (Sartorius ME36S microbalance). Mean relative growth rate (MRGR) was calculated following Gotthard *et al.* (1994):

$$MRGR = (W_2 - W_1) / t,$$

where W_1 is the initial weight, W_2 the final weight and t is the number of days for each life-stage. Mass of all individuals were recorded at pupation and emergence. Adult dry mass was recorded after emerged adults were snap frozen and freeze dried (Heto PowerDry PL3000).

Statistical analysis

All data were analysed using generalised linear mixed models (GLMM) using PROC GLIMMIX (SAS Institute, version 9.01).

Experiment 1

Hypotheses 1 and 2. Initial aphid abundance (counts) was modelled using a Poisson error distribution and log-link function. Aboveground plant dry mass, change (delta) in aphid abundance, aphid dry mass and total soluble protein content were modelled using a normal (Gaussian) error distribution with identity-link function. Random effects were experimental run and chamber

nested within run for all models. Models of aphid abundance had an additional, observation-level random effect fitted to account for over-dispersion within the count data (Elston *et al.*, 2001). While chamber accounted for little variation in the data it represented an important structural random effect (i.e. CO_2 treatment was applied at the chamber level) and was thus retained in all models.

Potential explanatory variables included raspberry cultivar (susceptible (Glen Ample), partially resistant (Glen Clova), resistant (Octavia)), CO_2 treatment (ambient 390 *μ*mol/mol, elevated 650 *μ*mol/mol), predator treatment (ladybird present or absent) and plant biometrics (height, dry mass). Of the original 144 plants, 12 died at various stages during the experiment and were not included in the analysis. Aphid total soluble protein content was modelled separately using a normal (Gaussian) error distribution with identity-link function.

Experiment 2

Hypothesis 3. Relative growth rate, development time and pupal mass of ladybirds were modelled using a normal (Gaussian) error distribution with identity-link function. Random terms were parent identity and the experimental chamber in which the aphid prey was reared. When repeated measures were used (relative growth rate) an observation-level random effect was added to the R-side of the random structure. Raspberry cultivar (susceptible and partially resistant), CO_2 treatment (ambient 390 *μ*mol/mol and elevated 650 *μ*mol/mol), sex upon emergence as adult and larval instars (relative growth-rate only) were fitted as potential explanatory variables.

During the analysis of both experiments, explanatory variables were added in a forward stepwise fashion until a minimum adequate model was obtained (Crawley, 2002). F-ratio and p-values adjusted for other fitted terms (SAS type

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

Results

Experiment 1

Hypothesis 1 - Plant responses

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

Hypothesis 2 - Aphid responses

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

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

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

400 Top-down regulation of agricultural pest species by natural enemies is
401 becoming increasingly important as use of conventional chemical pesticides
402 becomes progressively more difficult under stricter legislation (such as
403 European Union Regulation (EC) No 1107/2009) (Van Driesche, 2008). This
404 is particularly true for crops grown under cover (e.g. glasshouse, polytunnel),
405 an increasingly common practice for enhancing productivity (Johnson *et al.*,
406 2010, 2012, Wittwer & Castilla, 1995), since natural enemies work more
407 effectively in closed environments (McMenemy *et al.*, 2009). Even in non-
408 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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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 ± 27.512	*Susceptible*	*14.940*		
	Partially resistant	*25.221*		
Experimental run = 6.28 ± 7.84	*Resistant*	*20.671*		
	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*		
Chamber (experimental run) = 0	*Partially resistant*	*4.197*		
Experimental run = 0.13±0.12	*Resistant*	*0.71*		
	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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664 Table 2. GLIMMIX results summary for ladybird responses (a) relative growth
665 rate and b) pupal mass), in relation to rearing conditions of their aphid prey
666 (CO_2 and raspberry cultivar), larval instar and gender. Significant variables in
667 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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	Martin & Johnson		Hentley *et al*	
Cultivar	eCO_2 impacts on plants	eCO_2 impacts on aphids	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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Figure 1. Aboveground plant dry mass of three raspberry cultivars in response to ambient and elevated CO_2. Data are least square mean ± S.E.

Figure 2. The effect on aphid abundance of cultivar resistance, CO_2 treatment and presence a) or absence b) of ladybird predation. Ambient (white bars) and elevated (grey bars) atmospheric CO_2 levels. Letters above bars denote significant differences. Aphid abundance for resistant cultivar scaled using a second y-axis to make treatment effects clearer. Data are mean ± S.E.

Figure 3. Least square mean for relative growth rate of larval stages of the ladybird *H. axyridis* fed aphid prey from susceptible (dashed line and triangle) or partially resistant (solid line and circle) raspberry cultivars. Data are least square mean ± S.E.

715 Figure 1.

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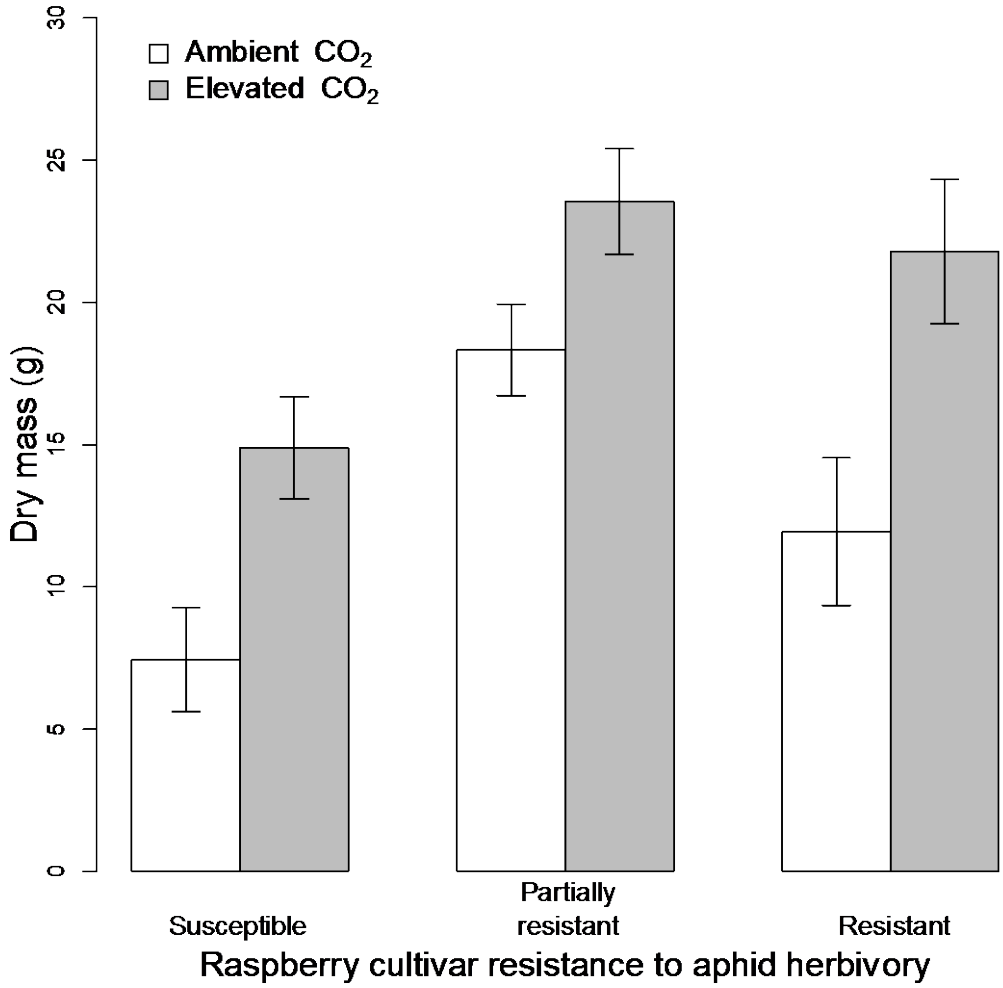

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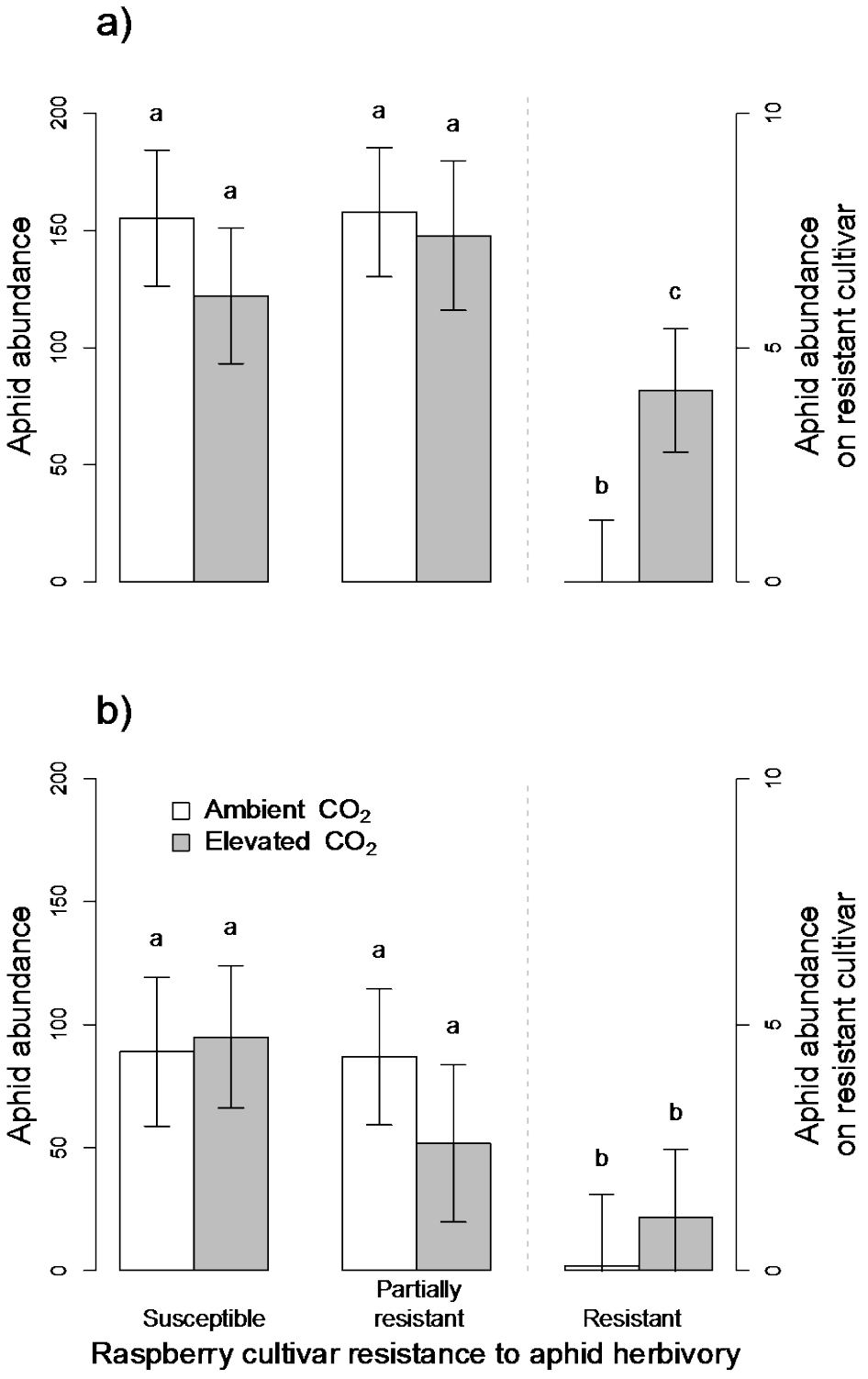
730 Figure 2.



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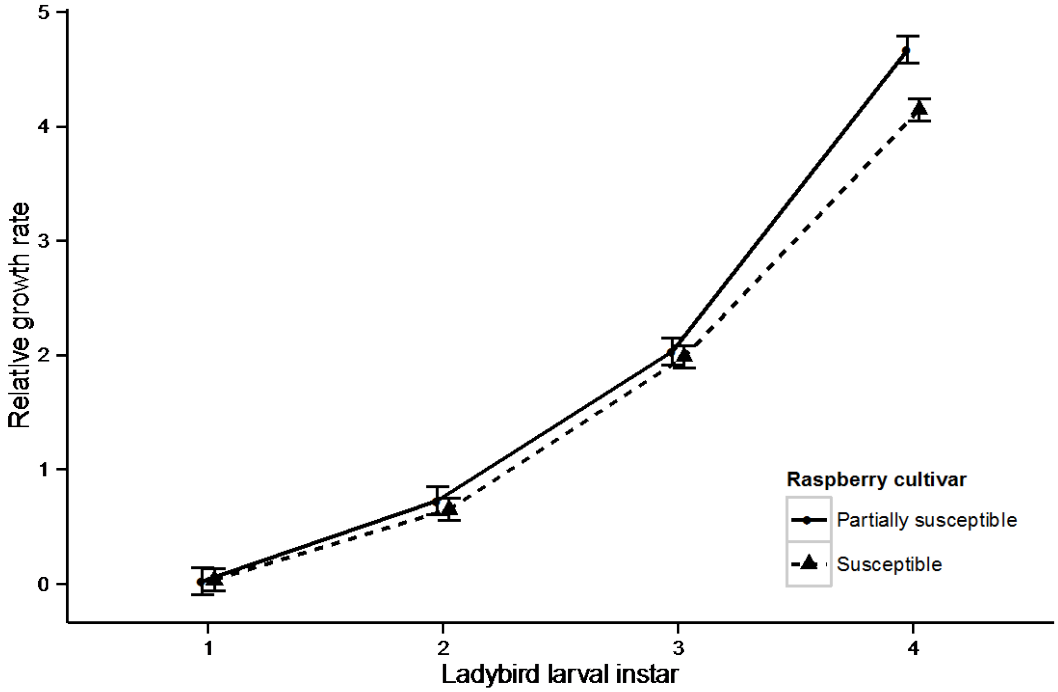
735 Figure 3.



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