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**Top-down control by *Harmonia axyridis* mitigates  
the impact of elevated atmospheric CO<sub>2</sub> on a plant-  
aphid interaction**

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**Running title:** *Multi-trophic interactions in an elevated CO<sub>2</sub> environment.*

## Abstract

- 1) This study investigated the impact of elevated atmospheric CO<sub>2</sub> (390 or 650  $\mu$ 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<sub>2</sub> 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<sub>2</sub> enrichment. On the resistant cultivar, aphid colonisation increased from 14% in ambient CO<sub>2</sub> to 70% in elevated CO<sub>2</sub> 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<sub>2</sub>.
- 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<sub>2</sub>. We do, however, demonstrate the potential for top down control to mitigate the effect of global climate change on pest populations.

## 59 **Introduction**

60 By 2100, atmospheric CO<sub>2</sub> concentrations are predicted to double pre-  
61 industrial levels of 280  $\mu\text{mol/mol}$  (Meehl *et al.*, 2007). There is growing  
62 interest in understanding how insect herbivores found on crops will respond to  
63 such global climate change, particularly in the context of achieving food  
64 security (Gregory *et al.*, 2009). While there is expanding literature on the  
65 effects of elevated atmospheric CO<sub>2</sub> concentrations (eCO<sub>2</sub>) on plant-  
66 herbivore interactions (Robinson *et al.*, 2012; Zavala *et al.*, 2013), only a few  
67 studies have addressed crop cultivars with genetic resistance to insect pests  
68 (e.g. Zavala *et al.*, 2008; Sun *et al.*, 2013). Moreover, to date these studies of  
69 crop resistance have largely overlooked the indirect effects of eCO<sub>2</sub> on the  
70 natural enemies of crop pests. It is these organisms which will ultimately  
71 determine the net effect of eCO<sub>2</sub> on pest population dynamics (Robinson *et al.*,  
72 2012). Given the need to increase food production by 50% by 2050 while  
73 using less resources and pesticides (Royal Society, 2009), understanding  
74 how climate change will affect ecosystem services such as predation of  
75 herbivorous pests, and the underlying mechanisms, is of paramount  
76 importance (A'Bear *et al.*, 2014).

77 In the absence of trophic interactions, plants, which rely on CO<sub>2</sub> assimilation  
78 for energy, generally respond positively to eCO<sub>2</sub>, with 25-38% increases in  
79 biomass being reported for C<sub>3</sub> plants (Stiling & Cornelissen, 2007; Robinson  
80 *et al.*, 2012). Within plant tissue, carbohydrates generally increase and  
81 nitrogen content is either diluted due to increased carbohydrates or  
82 reallocated, resulting in an average 19% increase in plant C:N ratio (Robinson  
83 *et al.*, 2012), ultimately altering many aspects of plant chemistry (Stiling &  
84 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub>, 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<sub>2</sub>, 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<sub>2</sub> 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<sub>2</sub>; 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub> 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<sub>1</sub>) Raspberry plants, like most C<sub>3</sub> plants, respond positively to elevated levels of atmospheric CO<sub>2</sub>. 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<sub>2</sub>) Aphid abundance will be distributed according to plant resistance with more aphids on the susceptible cultivars. Under eCO<sub>2</sub> aphid abundance and size will increase on less resistant cultivars (Martin & Johnson, 2011).

H<sub>3</sub>) Predation levels will increase to compensate for changes in prey quality. Consumption of prey from eCO<sub>2</sub> 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*<sup>™</sup> 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<sup>-1</sup>.m<sup>-2</sup>, 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 µmol/mol) and two at elevated (650 ± 50 µmol/mol) atmospheric CO<sub>2</sub> levels. A CO<sub>2</sub> sensor (Vaisala GMW22) was mounted in each chamber and connected to a controller unit (Mitsubishi Micro-controller AL2-24MR-D). Once CO<sub>2</sub> levels fell below the target concentration (390 µmol/mol and 550 µmol/mol, respectively), CO<sub>2</sub> 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  $18 \pm 1^\circ C$ , 16h photoperiod using the cultivar Malling Landmark (also  $A_1$  resistance) as a culture plant. The aphid population had been randomly



divided and maintained in either ambient or elevated CO<sub>2</sub> conditions for at least five generations before the experiment.

#### *Ladybirds*

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

#### *Experiment 1: Trophic interactions*

In a fully-factorial blocked design, 48 plants of each cultivar (susceptible, partially-resistant and resistant) were randomly assigned to the two atmospheric CO<sub>2</sub> (ambient and elevated) and subsequent predator (ladybird present or absent) treatments. This gave 12 replicates per treatment combination (cultivar x CO<sub>2</sub> x predator). The experiment was carried out September 2011 – September 2012 over a series of four runs to avoid pseudoreplication of CO<sub>2</sub> treatment. Each run comprised of three full replicates (n=36) of each treatment combination. Within each run the 18 plants were randomly distributed along a single bench inside each chamber. To prevent movement of flightless aphid nymphs between plants, individual pots were secured on circular plinths (10cm diameter x 3cm height) and placed in 50cm

x 50cm plastic trays filled with water (four plants per tray), ensuring the pots were above the water-line (see Johnson *et al.*, 2013 for details).

After five weeks growth in the CO<sub>2</sub> treatments, the height of each plant was measured and three adult large raspberry aphids were placed on the first fully unfurled leaf of each plant. Two weeks after aphid inoculation, the number of nymphs and adult aphids on each plant was counted and then a single adult female *H. axyridis* was introduced to the plants assigned to predator treatment. All plants were then placed within individual insect cages (25cm diam. x 65cm height, Insectopia, UK). The ladybirds remained on the plants for 72 hours, after which they were removed and the aphid population on each plant re-counted. Up to 10 adult aphids from each plant were collected at random, snap-frozen and freeze-dried. All aboveground plant material was destructively harvested and oven-dried for 48 hours at 70°C. Aphid and plant dry mass were recorded. Total soluble protein was determined from a subsample of the freeze-dried aphids using a protein assay kit (Thermo Scientific BCA Kit 23225) which used the Bradford (1976) method.

#### *Experiment 2: Ladybird development*

To provide aphid prey, 32 plants of the susceptible and partially resistant cultivar were randomly assigned to two CO<sub>2</sub> treatments across four controlled environment chambers (2 x ambient, 2 x elevated). Plants were inoculated with large raspberry aphid as in Experiment 1 and after four weeks aphids were collected daily and used as prey for the ladybird larvae in the trial. Eggs were laid in a series of clutches over a 5-day period from three randomly 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<sub>2</sub>). 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):

$$\text{MRGR} = (W_2 - W_1) / t,$$

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<sub>2</sub> 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<sub>2</sub> treatment (ambient 390  $\mu\text{mol/mol}$ , elevated 650  $\mu\text{mol/mol}$ ), 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<sub>2</sub> treatment (ambient 390  $\mu\text{mol/mol}$  and elevated 650  $\mu\text{mol/mol}$ ), 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> treatment also influenced the plant biomass, plants grown in eCO<sub>2</sub> achieving a greater dry mass compared to plants grown in ambient CO<sub>2</sub> (Table 1). The susceptible cultivar was the most responsive to eCO<sub>2</sub> with a 107% increase in dry mass compared to ambient CO<sub>2</sub> (Fig. 1). There was an 85% increase of dry mass of the resistant cultivar in eCO<sub>2</sub> compared to ambient. The partially susceptible cultivar was the least responsive to eCO<sub>2</sub>, 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

a factor of 10 (Fig. 2a). Atmospheric CO<sub>2</sub> enrichment significantly affected aphid abundance (Table 1b), but this varied between plant cultivars as indicated by the significant CO<sub>2</sub> x cultivar interaction (Table 1b). Altered population levels drove this effect of CO<sub>2</sub> enrichment on aphid abundance on the resistant plant cultivar. On the resistant cultivar, elevation of atmospheric CO<sub>2</sub> concentrations significantly increased the mean abundance of aphids (Fig. 2a). Furthermore, aphid colonisation of the resistant cultivar was markedly increased by CO<sub>2</sub> enrichment with 14% and 70% of plants supporting aphids under ambient and eCO<sub>2</sub> conditions, respectively ( $F_{1,5} = 7.9$ ,  $p = 0.05$ ). In contrast, aphid abundance on the susceptible and partially resistant cultivars were unaffected by manipulation of the CO<sub>2</sub> environment (Fig 2a, Table 1a).

The presence of a ladybird predator significantly reduced aphid abundance on all cultivars (Fig. 2a versus Fig. 2b; Table 1c). Moreover, while CO<sub>2</sub> enrichment increased aphid herbivore colonisation and abundance on the resistant cultivar, once ladybird predation was introduced this CO<sub>2</sub> effect was nullified (Table 1c, Fig. 2b). On the susceptible and partially-resistant cultivars, the number of aphids consumed by the ladybird did not significantly vary with CO<sub>2</sub> treatment (Fig. 2b). CO<sub>2</sub> treatment did not affect adult aphid dry mass or total protein content ( $F_{1,2} = 0.25$ ,  $p = 0.667$  and  $F_{1,2} = 1.44$ ,  $p = 0.353$ , respectively). Aphid total soluble protein was greater when reared on the susceptible cultivar than the partially resistant cultivar ( $F_{1,100} = 11.6$ ,  $p = 0.001$ ).

## *Experiment 2*

Hypothesis 3. The mean relative growth rate over the full duration of ladybird development was not affected by the prey source environment ( $\text{CO}_2$ :  $F_{1,2} = 1.03$ ,  $p = 0.42$  and cultivar:  $F_{1,24} = 0.78$ ,  $p = 0.38$ ). Relative growth rate was stage-specific with the earlier instars having a much lower mean growth rate than the later instars. When fed aphids from the partially resistant cultivar, the mean relative growth rate of fourth instar ladybird was significantly increased (Fig. 3, Table 2a). When fed aphids reared on the partially resistant cultivar, fourth instar ladybird larvae had significantly higher relative growth rate compared to their siblings fed aphids reared on the resistant cultivar (Fig.3). There was no significant effect of  $\text{CO}_2$  treatment on relative growth rate of ladybird larvae (Table 2.a). Despite the significant effect of cultivar on fourth instar larval growth rate, duration of development from egg to adult was not affected by the cultivar or  $\text{CO}_2$  treatment ( $F_{1,80} = 0.29$ ,  $p = 0.59$  and  $F_{1,2} = 0.61$ ,  $p = 0.44$ , respectively) aphid prey was reared in. Similarly, pupal mass and adult mass were not affected by the rearing conditions of the aphid prey (Table 2b). Pupal and adult mass was, however, affected by adult sex: females were significantly heavier than males (Table 2b).

## Discussion

The fertilising effect of  $\text{CO}_2$  enrichment is predicted to increase plant biomass and productivity (Ainsworth & Long, 2005; Robinson *et al.*, 2012), particularly for woody plants (Curtis & Wang, 1998). This study confirms this, with all three raspberry cultivars showing increased biomass in response to elevated atmospheric  $\text{CO}_2$ . This was also seen for the raspberry cultivars investigated by Martin and Johnson (2011) (summarised in Table 3), suggesting that this response is common to the species as a whole. On the two susceptible

cultivars, aphid populations were unaffected by the increased plant biomass associated with elevated CO<sub>2</sub>. Aphid colonisation and subsequent abundance was greater on the resistant cultivar grown in elevated CO<sub>2</sub>, suggesting a reduction in resistance to aphid herbivory in the novel environment.

Plant biomass in ambient conditions was not correlated with resistance to herbivory; the partially resistant cultivar had the greatest biomass, followed by the resistant and susceptible cultivars. The extent to which plant biomass increased under CO<sub>2</sub> enrichment varied with cultivar. The partially resistant cultivar, with the greatest biomass in ambient CO<sub>2</sub>, was the least responsive (30% increase in biomass), suggesting that it is already close to its maximum growth capacity under ambient CO<sub>2</sub>. The 85% increase in biomass of the resistant cultivar under eCO<sub>2</sub> suggests this cultivar to be particularly responsive to eCO<sub>2</sub>. In eCO<sub>2</sub> aphid colonisation was significantly higher on the resistant cultivar, but aphid numbers remained very low despite a significant increase from ambient conditions. The resistant cultivar used in this study, Octavia, is the successful crossing of two aphid resistance genes, A<sub>10</sub> and A<sub>k4a</sub>. Previous work by Martin and Johnson (2011) found the A<sub>10</sub> was robust to changes in CO<sub>2</sub> concentrations. This implies that CO<sub>2</sub> enrichment may be modifying the function of the A<sub>k4a</sub> resistance gene. This, however, remains an untested hypothesis and is only one possible explanation. Raspberry cultivars possessing the A<sub>10</sub> resistance gene can show significant variation in minor genes associated with aphid resistance, which may modify the responses to elevated CO<sub>2</sub> (Hall, 2009). Even in cultivars possessing the same resistance gene, it seems their genotypic background can modify resistance expression at elevated CO<sub>2</sub>. For example with two cultivars possessing the A<sub>1</sub> 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<sub>2</sub> (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<sub>2</sub> 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<sub>2</sub>. Increasing  
395 CO<sub>2</sub> 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<sub>2</sub> (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,

however, important and increasingly encouraged (Stiling & Cornelissen, 2005, Van Driesche, 2008).

Predation by a natural enemy mitigated the breakdown of resistance to aphid herbivory, returning the aphid population to its “ambient” state. There was no evidence for a transfer of bottom-up effects across multiple trophic levels. The CO<sub>2</sub> environment host plants were exposed to, did not affect the size or total protein content of aphids living on them. Moreover, the CO<sub>2</sub> environment their prey had been reared in did not influence the development of the next generation of ladybirds. Similar to other studies, we found the effect of elevated CO<sub>2</sub> on prey quality weak or non-existent (Salt *et al.*, 1995; Stacey & Fellowes, 2002, Chen *et al.*, 2005) and subsequent predator generations were also unaffected (Chen *et al.*, 2005).

The influence of bottom up processes, such as the effect of plant genotype on prey quality, had a much more significant effect than eCO<sub>2</sub> on ladybird development. Plant cultivar significantly affected total protein content in aphids. When reared on the susceptible cultivar, aphids had a greater total protein content than aphids reared on the partially resistant cultivar. Unexpectedly, the opposite was observed for the mean relative growth rate of ladybird larvae. Larvae fed aphid prey from the susceptible cultivar had significantly lower growth rate than larvae fed aphids from the partially susceptible cultivar. The underlying reason for this remains unclear, but the higher protein content of aphids on the susceptible cultivar may reflect greater fitness and behavioural responsiveness of these individuals in addition to their nutritional value as prey. These individuals may be able to better resist attack by ladybirds using behavioural strategies (e.g. kicking, evasion) and thereby

impose extra fitness costs (e.g. handling time) on ladybirds (Dixon, 2000). Mitchell *et al.* (2010) reported that *A. idaei* showed less 'dropping behaviour' and suggested they may rely more on such behavioural resistance to parasitoid attack when feeding on susceptible cultivars, so this explanation is at least credible.

Confining aphids on plants necessitated use of potted plants in closed chambers, which may be argued to give artificially high plant growth responses to CO<sub>2</sub> (Ainsworth *et al.*, 2008). Given, however, that > 90% of raspberry production takes place in closed polytunnels which buffer environmental fluctuations (Johnson *et al.*, 2010; Johnson *et al.*, 2012), this is perhaps a less relevant concern in this system as chambers have similar effects. Moreover, our use of large pots and potting media minimised restrictions to root growth the potential for hypoxic conditions, as advocated by Passioura (2006).

This study highlights the importance of considering multiple trophic levels when trying to understand pest dynamics and ecosystem responses to future climates. Increasing atmospheric CO<sub>2</sub> has the potential to impair plant defences against herbivory which may have important implications for agro-ecosystems. We demonstrate that higher trophic levels may, however, partly mitigate this reduction in plant defences by controlling herbivore numbers on the affected plants. The longer-term effects of elevated CO<sub>2</sub> on tri-trophic interactions remain however little understood. This study provides an empirical demonstration of how the net level of plant herbivory under elevated CO<sub>2</sub> depends on both the interaction between the herbivore and the natural 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	<b>CO<sub>2</sub></b>		<b>12.85<sub>(1,3)</sub></b>	<b>0.0377</b>
	<i>Ambient</i>	12.830		
	<i>Elevated</i>	20.671		
Random effect estimate: Chamber (experimental run) = 28.73 ± 27.512 Experimental run = 6.28 ± 7.84	<b>Cultivar</b>		<b>20.37<sub>(2,121)</sub></b>	<b>&lt;0.0001</b>
	<i>Susceptible</i>	14.940		
	<i>Partially resistant</i>	25.221		
	<i>Resistant</i>	20.671		
	Initial aphid abundance	20.666	0.33 <sub>(1,123)</sub>	0.568
b) Initial aphid abundance	<b>CO<sub>2</sub></b>		<b>14.49<sub>(1,2)</sub></b>	<b>0.063</b>
	<i>Ambient</i>	-1.555		
	<i>Elevated</i>	0.71		
Random effect estimate: Replicate = 0.46±0.081 Chamber (experimental run) = 0 Experimental run = 0.13±0.12	<b>Cultivar</b>		<b>148.67<sub>(2,128)</sub></b>	<b>&lt;0.0001</b>
	<i>Susceptible</i>	4.203		
	<i>Partially resistant</i>	4.197		
	<i>Resistant</i>	0.71		
	Dry mass	0.888	0.88 <sub>(1,121)</sub>	0.349
	<b>CO<sub>2</sub> * Cultivar</b>	<b>MPE</b>	<b>8.89<sub>(2,128)</sub></b>	<b>0.0002</b>
c) Delta aphid abundance	<b>CO<sub>2</sub></b>		0.3 <sub>(1,2)</sub>	0.639
Random effect estimate: Chamber (experimental run) = 0 Experimental run = 468.36±429.36	<i>Ambient</i>	-0.678		
	<i>Elevated</i>	-4.747		
	<b>Cultivar</b>		<b>10.64<sub>(1,125)</sub></b>	<b>&lt;0.0001</b>
	<i>Susceptible</i>	-1.99		
	<i>Partially resistant</i>	6.21		
	<i>Resistant</i>	-2.84		
	Dry mass	2.062	0.6 <sub>(1,123)</sub>	0.439
	<b>Predator treatment</b>	<b>MPE</b>	<b>33.55<sub>(1,125)</sub></b>	<b>&lt;0.0001</b>
	<i>Control</i>	0.04		
	<i>Ladybird</i>	-2.84		
	<b>Cultivar * predator treatment</b>	<b>MPE</b>	<b>7.37<sub>(2,1.25)</sub></b>	<b>0.0009</b>

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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<sub>2</sub> and raspberry cultivar), larval instar and gender. Significant variables in  
667 bold retained in final model. MPE = multiple parameter estimates

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

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680 Table 3. Comparison of plant and aphid responses to elevated atmospheric CO<sub>2</sub> (eCO<sub>2</sub>) found by Martin & Johnson, 2012 and the  
681 findings of this study.

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Cultivar	Martin & Johnson eCO <sub>2</sub> impacts on plants	eCO <sub>2</sub> impacts on aphids	Hentley <i>et al</i> eCO <sub>2</sub> impacts on plants	eCO <sub>2</sub> impacts on aphids
Malling Jewell (susceptible)	197% increase growth rate	None		
Glen Lyon – A <sub>1</sub>	41% increase in growth rate	Increase in abundance and adult mass		
Glen Ample – A <sub>1</sub>			107% increase in dry mass	None
Glen Clova – multi			30% increase in dry mass	None
Glen Rosa – A <sub>10</sub>	186% increase in growth rate	None		
Octavia – A <sub>10</sub> and A <sub>K4</sub>			85% increase in dry mass	Increase in aphid colonization and abundance

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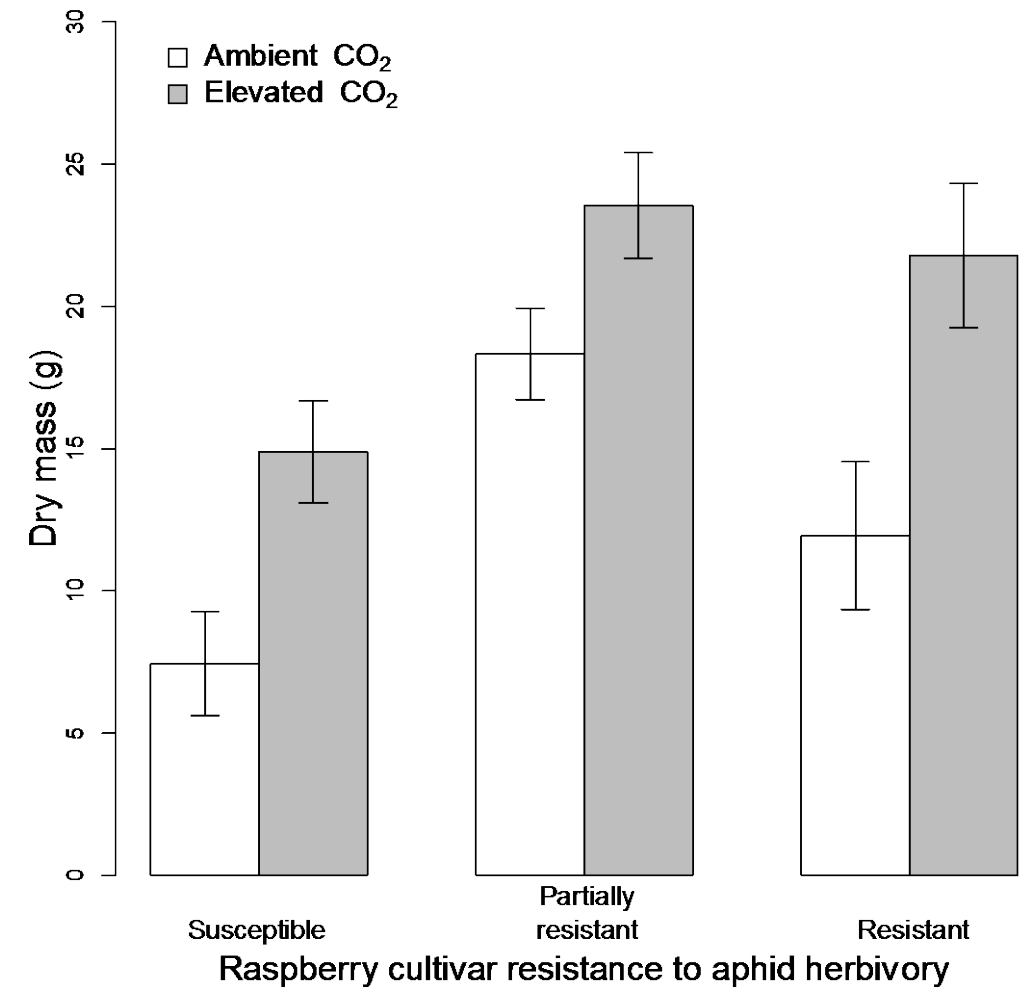
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Figure 1. Aboveground plant dry mass of three raspberry cultivars in response to ambient and elevated CO<sub>2</sub>. Data are least square mean  $\pm$  S.E.

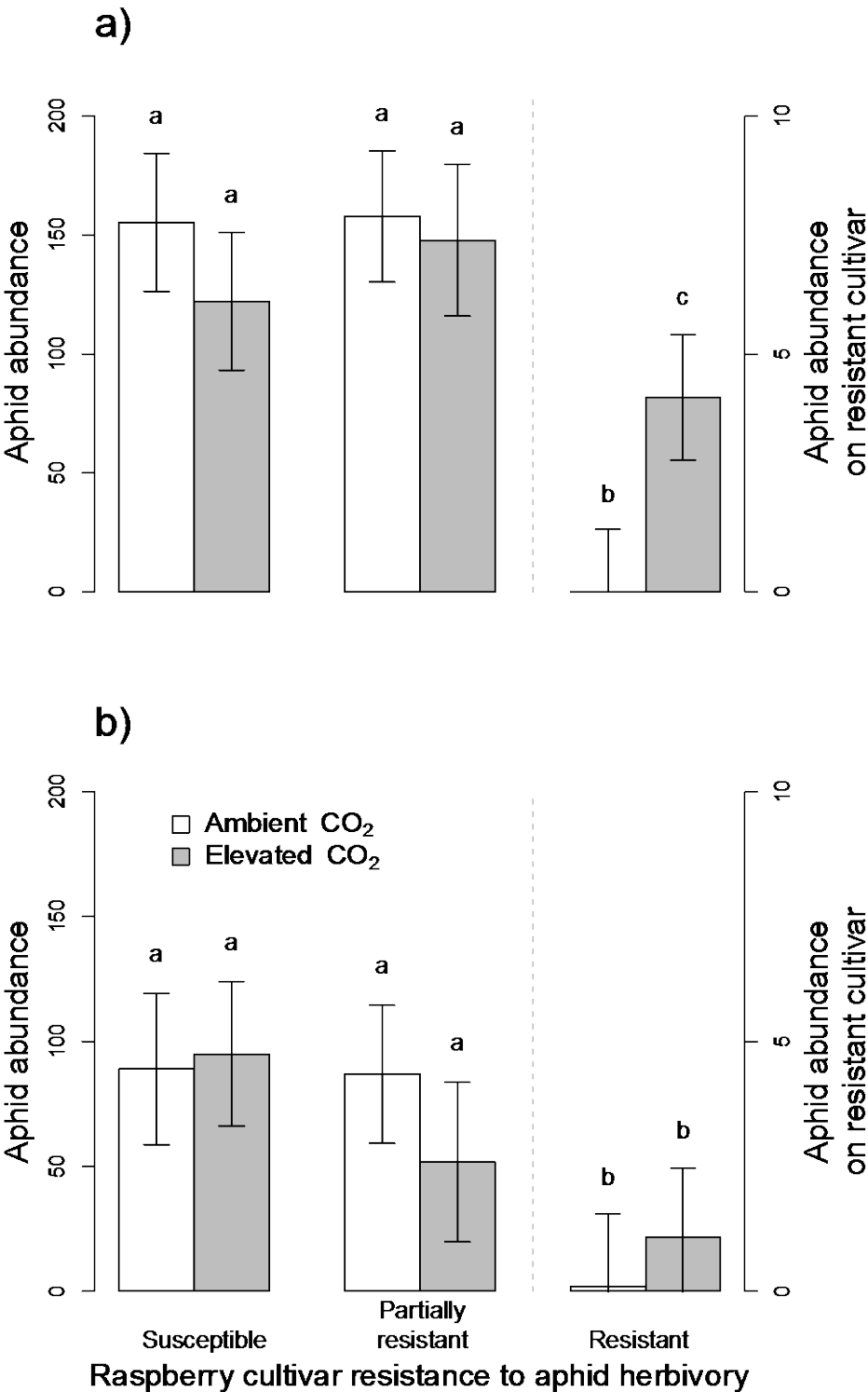
Figure 2. The effect on aphid abundance of cultivar resistance, CO<sub>2</sub> treatment and presence a) or absence b) of ladybird predation. Ambient (white bars) and elevated (grey bars) atmospheric CO<sub>2</sub> levels. Letters above bars denote significant differences. Aphid abundance for resistant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

Figure 1.

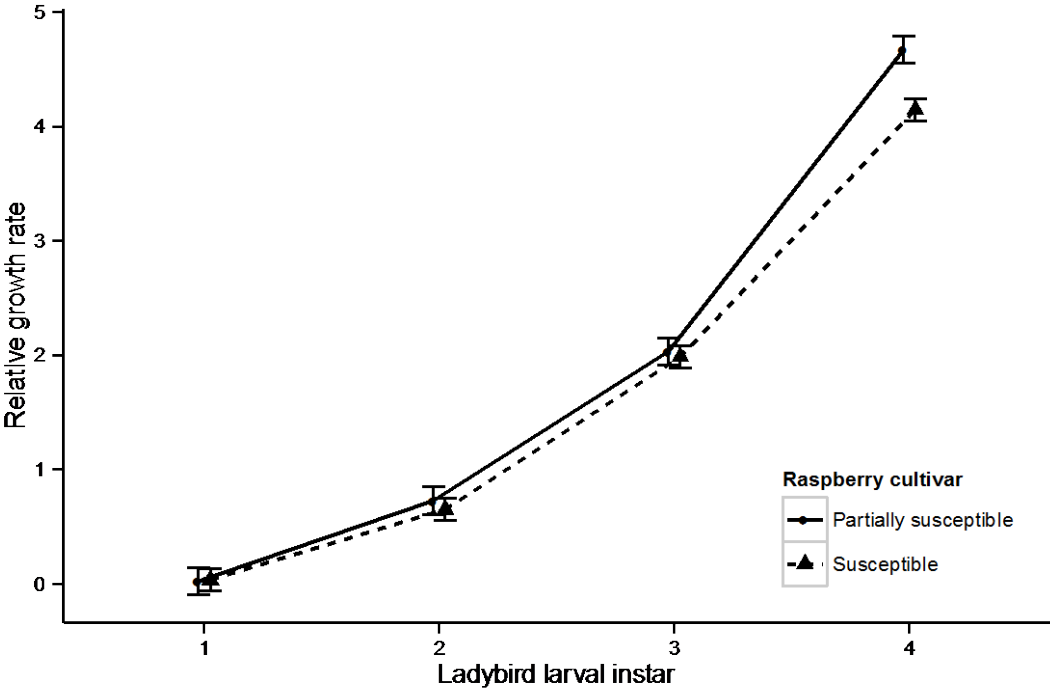






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735 Figure 3.



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