# Global change and predator–prey interactions on a woody perennial

by

**William Thomas Hentley**

B.Sc. M.Sc.

Cardiff University
School of Biosciences

Submitted for the degree of Doctor of Philosophy

September 2014

# Contents

# Table of Figures

# Table of Tables

# Acknowledgements

Firstly I would like to thank my supervisory team (in alphabetical order, not preference), Rosie Hails, Scott Johnson, Hefin Jones and Adam Vanbergen. Their advice, encouragement and occasional free meals were invaluable; they developed me into a person that is capable of completing a thesis, well done!

I must thank the Centre for Ecology and Hydrology, without it I would not have been able to do my experiments or have a section in my CV for overcoming adversity. The staff and students at CEH have always been willing to help, I would particularly like to thank the facilities staff at CEH for putting up with my constant requests, they were very patient. Alison Dobson at the James Hutton Institute who grew all my raspberry plants for me, there would have been no PhD without her! The 'student body' has been invaluable throughout my PhD, it would have been a very difficult four years were it not for them. In particular I must thank, Leah Cuthbertson, Louise Colwill, Rory O'Connor, Dong O'Bear, Matt Dray, Steve Thomas, Tom Crowther, Caroline Schultz, Joe Rowland, Ruth Wade, Tom August, Sarah McCormack and Phil Martin who gave me so much support through the very low times of the PhD. I have to particularly thank the Colwills for giving me a place to stay when I ran out of money.

Throughout my PhD I have shared almost every experience, including supervisors and study system with Scott McKenzie. Scott has been my closest friend, my drinking partner and my best man, it wouldn't have been much fun without him, thank you. From the first year of my BSc at Sheffield University Andrew Beckerman has always believed in me, even when I didn't. I would not be where I am now were it not for his continued support and advice.

The support I have received from my family has been crucial throughout the PhD. My brother always encouraging me despite my protests and my mum's famous phrase 'keep buggering in son!' Finally I must thank my beautiful wife, Emily who, after my BSc, inspired me to chase a career in research. Emily has supported and somehow continued to love me throughout this PhD. We have experienced the highs and lows together and have come out fighting at the end. Little does she know this is only the beginning.

# Abstract

The impacts of global change on ecosystems from climate change and invasive species are likely to be complex. Rising atmospheric $CO_2$ concentrations, the associated climate forcing and greater frequency of extreme weather are serious challenges to natural ecosystems. In tandem with climate change, globalisation has led to the spread of invasive alien species around the globe that threaten to interrupt food web dynamics. Advancing understanding of the effects of global change on trophic interactions therefore requires study of interspecific and multi-trophic interactions.

The aim of this thesis was to examine how host-plant heterogeneity, native–invasive species interactions and climate change effects (elevated atmospheric $CO_2$ (e$CO_2$) or drought) influence trophic interactions. An experimental approach was used which centred on a study system comprising the European raspberry (*Rubus idaeus*), the herbivorous large raspberry aphid (*Amphorophora idaei*) and coccinellid beetle predators (native species: *Adalia bipunctata*, *Coccinella septempunctata;* invasive alien species *Harmonia axyridis*).

Under e$CO_2$, *R. idaeus* resistance to *A. idaei* was unchanged for two cultivars (Glen Clova, Glen Ample) partially susceptible to *A. idaei*, but significantly reduced for another (Octavia) with complete resistance in ambient climatic conditions. The inclusion of a coccinellid predator, however, mitigated the reduction in the resistance of Octavia by reducing aphid abundance. Behavioural responses to predation by *A. idaei* were also impaired under e$CO_2$ after feeding on Glen Ample. The role of natural enemies in controlling herbivore abundance in future climates is therefore crucial. Native coccinellid species are currently declining in much of Europe, attributed to the occurrence of the invasive species, *H. axyridis*. Despite the declines in native coccinellid species, it was found that behavioural modification to feeding by both native and invasive coccinellid species can, theoretically, result in coexistence.

Plant resistance in a future climate is likely to be modified significantly. Reduced resistance to aphid herbivory demonstrated here mirrors previous studies, highlighting the future importance of natural enemies to control aphid abundance. Changes to the abundance and behaviour of aphid prey and intraguild predators will modify the effectiveness of native and invasive natural enemies. Further mechanistic research is required to understand multi-trophic interactions in dynamic environments.

# General introduction

## 1.1 Global change

Global change refers to the broad suite of biophysical and socio-economic changes that are altering the biological, hydrological and biogeochemical properties of Planet Earth (Steflen *et al*., 2004). It encompasses a wide range of processes, which includes shifts in atmospheric composition, climate change, land use change and biological diversity. One of the processes that have led to global change is an increasing greenhouse effect, primarily caused by shifts in the composition of atmospheric gases. One such shift is the rapidly increasing concentrations of atmospheric carbon dioxide, which have been attributed unequivocally to anthropogenic processes (IPCC, 2013). Carbon dioxide ($CO_2$) is produced when fossil fuels are burnt; it is a by-product of many industrial processes. For at least 1000 years prior to the Industrial Revolution, atmospheric $CO_2$ concentrations were stable at around 270 parts per million (ppm) (IPCC, 2013). Present atmospheric $CO_2$ concentrations are at approximately 400 ppm (Tans, 2014) and are predicted to increase to over 550 ppm by 2050 over a range of different scenarios (IPCC, 2013). Increases to atmospheric $CO_2$ concentrations are generally uniform across the globe despite the emissions being localised (Ainsworth & Long, 2005).

The human population, the ultimate cause of rising $CO_2$, is predicted to reach over nine billion by 2050 (United Nations, 2013); ensuring food security for future generations at a time of significant global change is, therefore, crucial (The Royal Society, 2009). Increased concentration of atmospheric $CO_2$ is predicted to enhance C3 plant productivity by at least 20%; woody plants will be particularly responsive with an average 40% increase in photosynthetic rates (Ainsworth & Long, 2005). Most of these studies have, however, taken place with little consideration for consumer trophic level effects. Within a multi-trophic context, where different trophic levels may respond in different ways, the consequence of global change remains largely unknown (Long *et al*., 2004).

Crop productivity can be severely impacted by pests; the annual cost of controlling invertebrate pests in the United States of America is around $13.6 billion (Losey & Vaughan, 2006). Established mechanisms for controlling pest species, such as the growing of crop plants selectively bred for resistance, or biological control by natural enemies, can be disrupted by global change (Gregory *et al*., 2009). For example,

the impact of elevated atmospheric $CO_2$ ($eCO_2$) can have a significant impact on specific plant–herbivore interactions (Stiling & Cornelissen, 2007; Robinson *et al*., 2012). To date, these findings have been species-specific or context-dependent making generalisation difficult. A recent meta-analysis (Robinson *et al*., 2012) found that herbivore abundance is generally unaffected by increases in atmospheric $CO_2$; however, if split into feeding guilds, the abundance of phloem feeders increases under $eCO_2$ whereas other feeding guilds, such as leaf-miners, decrease in abundance (Robinson *et al*., 2012). If herbivore responses to global change are feeding guild- or species-specific, the knock-on effect to the associated higher trophic levels will be equally as idiosyncratic. If bottom-up (*e.g.* plant resistance) and top-down (*e.g.* regulation of herbivore populations by natural enemies) processes are disrupted, the pest status of many herbivore species may significantly change in future climate scenarios.

# 1.2 Factors driving herbivore abundance

Historically, the relative importance of top-down and bottom-up processes has been hotly debated. It could be argued the unpalatability of most plant material, to most herbivores, most of the time, suggests bottom-up processes are the most important (Ehrlich & Raven, 1964). The importance of natural enemies could equally be argued; they are active reducing agents of herbivore densities, thereby limiting the amount of defoliation that occurs (Hairston *et al*., 1960; Price *et al*., 1980; Hunter *et al*., 1997).

## 1.2.1 Bottom-up processes

Selective breeding for resistance to herbivory in crop species provides an opportunity to understand in more detail the processes involved in bottom-up control of insect herbivory. The focus of selective breeding has been on the overall phenotypic response of the plant (indicated by herbivore acceptance of, or damage caused on the plant in bioassays), rather than the underlying mechanisms determining host plant resistance. Consequently, the exact mechanisms that underpin most plant resistance remain unclear, but they are likely to consist of multiple processes that include physiological (Coley *et al*., 1985) and anatomical (Hanley *et al*., 2007) defence mechanisms. Host plant responses affecting herbivore colonisation can be manifested as modification to either the degree of host plant

acceptance by the herbivore or the herbivore's ability to digest 'defended' plant material (Powell *et al*., 2006).

### 1.2.1.1 Host plant acceptance

Antixenotic defences are host plant attributes that can alter insect herbivore oviposition, plant selection and feeding behaviour (Schaller, 2008). The composition of one such defence, epicuticular wax, can either deter (*e.g. Ostrinia nubilalis* (Hübner) (Udayagiri & Mason, 1997)) or stimulate (*e.g. O. latipennis* (Hübner) (Li & Ishikawa, 2006)) herbivore oviposition. The composition and thickness of this epicuticular wax layer, which protects the epidermal cells of most plant species, can also directly influence the decision of herbivores to accept or reject the plant as a suitable host to feed on (Schoonhoven *et al*., 2005). For instance, initial shallow probing by aphids of epidermal cells plays a major role in determining host plant acceptance (Powell *et al*., 2006); removal of the epicuticular wax can increase aphid colonisation and subsequent colony survival (Jackson & Dixon, 1996; Daoust *et al*., 2010).

Many plant species have small hair-like structures, trichomes, which act as an additional line of defence. Trichomes can both hamper herbivore movement on the plant and prevent feeding. This is clearly observed in aphids where trichomes can prevent effective stylet insertion (Levin, 1973). Some plant species possess glandular trichomes that secrete a toxic (Musetti & Neal, 1997) or repellent substance. Glandular trichomes on wild potato (*Solanum berthaultii* Hawkes) leaves, for example, secrete the repellent aphid alarm pheromone, (*E*)-β-farnesene (Gibson & Pickett, 1983).

### 1.2.1.2 Digesting plant material

Over 100,000 secondary metabolites have been identified as potentially playing a role impairing the ability of herbivores to digest plant material (Powell *et al*., 2006; Howe & Schaller, 2008). One such group of metabolites, protease inhibitors (PIs), have a significant role in plant defence (Schaller *et al*., 2008). Rarely fatal to insect herbivores (Rahbé *et al*., 2003), PIs reduce the efficiency of digesting plant material. This effect can cascade to subsequent generations by reductions in fecundity (Schaller *et al*., 2008). Many defence compounds are metabolically costly for the plant to produce (Chen, 2008) and tend to be induced when herbivore feeding is detected. Once the plant experiences herbivore stimuli (*e.g.* saliva), a series of signalling pathways are initiated within the plant tissue, ultimately resulting in

production of secondary metabolites specific to the attacking herbivore (Howe & Schaller, 2008). The signalling pathway initiated is determined by the attacking herbivore's feeding guild. Chewing herbivores, for example, initiate the jasmonic acid signalling pathway whereas phloem-feeding herbivores generally stimulate the salicylic acid pathway (Walling, 2000; Moran & Thompson, 2001; Staudt et al., 2010). Each pathway results in a different blend of secondary metabolites (Schaller, 2008). Phloem-feeding herbivores also circumvent many of the secondary metabolites associated with defence responses as phloem sap contains relatively low concentrations of them (Dungan et al., 2007). Compounds found in the phloem that can impact sap-feeding invertebrates, as well as other feeding guilds, include phenolics (Leszczynski et al., 1985), lectins (Down et al., 1996), glucosinolates (Kim & Jander, 2007) and alkaloids (Johnson, 2011). Herbivory can also stimulate the release of volatile organic compounds (VOCs) from the host plant (Tholl et al., 2006). These are any organic compounds with vapour pressures high enough to be vaporised into the atmosphere at ambient conditions (Koppmann, 2007). Once released into the atmosphere, other plants (Ton et al., 2007) or invertebrates (De Moraes et al., 1998; De Moraes et al., 2001) use VOCs as a cue to indicate an attacked (infested) plant.

## 1.2.2 Top-down processes

Within terrestrial food webs, herbivores are an important carbohydrate-, nitrogen- and lipid-rich food source for higher trophic levels. Some plant species recruit natural enemies to act as 'bodyguards' by offering morphological traits that provide refuges or food sources. In the ant–acacia mutualism, for example, ants live in the hollow thorns of the acacia, and in turn protect the plant from herbivores (Agrawal & Rutter, 1998). The provision of morphological traits to recruit natural enemies is limited to certain plant species due to the energetic cost incurred (Herms & Mattson, 1992). Nearly all plants, however, produce VOCs when attacked by herbivores. Herbivore-induced plant VOCs (HIPVs) can be used as a cue by aphid natural enemies to locate their potential prey (Mumm & Dicke, 2010). The blend of compounds within HIPVs can alter depending on the herbivore feeding guild (Staudt et al., 2010) and even species (Clavijo McCormick et al., 2012), providing a reliable cue for natural enemies. However, when the same herbivore prey attracts multiple natural enemies, competitive interactions can take place. Predatory arthropods are often associated with aggressive intraguild interactions due to their generalised

feeding habits (Snyder & Evans, 2006). Intraguild predation (IGP) involves the consumption of a predator by another predatory species that is competing for a similar, often limited, prey resource (Polis *et al*., 1989). These competitive interactions have the potential to significantly reduce the effectiveness of natural enemies at controlling herbivore abundance.

### 1.2.3 Synergy of bottom-up and top-down processes

In summary, herbivory may be regulated by utilising a combination of bottom-up (plant resistance) and top-down (natural enemies) processes (Hunter *et al*., 1997). Peach (*Prunus persica* (L.)) cultivars, for example, vary in their resistance to the aphid *Myzus persicae* (Sulzer). A resistant cultivar produced significantly more HIPVs than susceptible cultivars (Staudt *et al*., 2010); thus, in this case, plant resistance, a bottom-up process, is responsible for moderating a top-down process, highlighting that these two processes for control are not mutually exclusive and should be considered together (Walker & Jones, 2001). The combination of plant resistance and natural enemies can have synergistic effects on herbivore abundance (Bilu & Coll, 2007). Plant defences against herbivory can also influence the efficiency of natural enemies (Fordyce & Agrawal, 2001; Rutledge *et al*., 2003). For example, trichomes interfere with the foraging behaviour and survival of predatory coccinellid beetles. Similarly, epicuticular wax can reduce the foraging efficiency of aphid natural enemies, as seen on the common pea (*Pisum sativum* L.) where parasitoid and predator (Coccinellidae spp.) efficiency is reduced when thick deposits of wax are present on the leaves (Rutledge *et al*., 2003). Additionally, some herbivore species exploit the negative effect trichomes have on their natural enemy by preferentially feeding on leaves with high trichome density, despite the difficulty of consuming such plant material (Fordyce & Agrawal, 2001).

## 1.3 Impact of global climate change on herbivory

Numerous studies report how climate variation impacts plant–herbivore interactions; relatively few, however, have addressed the response of these herbivores within a multi-trophic context (including natural enemies etc.). Changes to plant–herbivore–natural enemy interactions can have far-reaching implications for a range of ecosystem services (*e.g.* pest management, pollination and decomposition) that

underpin crop security (Gregory *et al*., 2009), sustainable agriculture (Newton *et al*., 2011) and ecosystem functioning (Loreau *et al*., 2001).

The influence of global climate change, on insect herbivores, directly and indirectly (*i.e.* plant-mediated), has been well studied, and several general patterns of response identified (Table 1.1), despite certain perturbations (*e.g.* atmospheric $CO_2$ concentration and mean temperature) receiving more attention than others (*e.g.* UV-B, soil moisture). In the absence of predation, herbivore responses appear to be feeding guild specific (Table 1.1). For example, under $eCO_2$, the reduced nutritional value (*i.e.* lowered concentration of leaf protein) of plant tissue (Lincoln *et al*., 1986) often results in compensatory feeding, especially by free-living chewing herbivores. High concentrations of carbon-based defence compounds in plant tissue can, however, confound such compensatory feeding by reducing digestion efficiency of plant material (Stiling & Cornelissen, 2007). Sap-feeding herbivores can circumvent many of the defence compounds and therefore show little response to $eCO_2$ (Robinson *et al*., 2012). There are several reviews on plant–herbivore interactions and climate change which cover this in more detail (Table 1.1) and further coverage is beyond the scope of the thesis.

**Table 1.1** Effects of abiotic stressors associated with global change (increased temperature, $CO_2$, UVB radiation, increase and decrease in moisture) on the performance (*e.g.* reduced development rate or increased abundance) of aboveground herbivore feeding guilds. General trends identified as "+" positive response, "-" negative response and "?" unknown. References citing examples of the prevailing trends are given where possible. Where no review article or meta-analysis exists, primary literature (*) is referenced.

| Abiotic stressor | | Chewer | Phloem | Galling and mining | Reference |
|---|---|---|---|---|---|
| Temperature | | + | + | + | Bale *et al*., 2002 |
| $CO_2$ | | - | + | - | Stiling & Cornelissen, 2007; Robinson *et al*., 2012 |
| Moisture | Increase | ? | + | ? | Huberty & Denno, 2004 |
| | Decrease | - | + | ? | |
| UVB | | - | ? | - | Ballare *et al*., 2011 |
| Ozone | | + | + | ? | Valkama *et al*., 2007* |

In addition to changes to the abiotic environment, plant–herbivore interactions are embedded in a complex multi-trophic network of interacting species. In conjunction with bottom-up processes, species at higher trophic levels, such as natural enemies, are responsible for regulating herbivore populations (Price *et al.,* 1980). To predict how climate change will affect plant–herbivore interactions, we must incorporate an understanding of how they will affect, and are affected by, higher trophic levels (Price *et al*., 1980; Sentis *et al*., 2014). If climate change were to have no net effect on herbivore populations, but reduce the effectiveness of natural enemies, the pressure from herbivory on plant species would increase (Thomson *et al*., 2010). Of the empirical examples illustrating the effects of global change on plant–herbivore–natural enemy interactions (Table 1.2), one generic observation is that the responses are contingent on the environmental context, rather than the herbivore feeding guild (as was seen for herbivores in the absences of natural enemies; Table 1.1). Under an elevated ozone environment, for example, natural enemies show no response or decreased consumption of herbivores (positive effect on herbivore – Table 1.2 or no response), whereas under $eCO_2$ or elevated temperature, natural enemies show either no response or increased consumption of herbivores (negative effect on herbivores – Table 1.2). The mechanisms underpinning the responses of natural enemies to climate change remain unclear, but the outcome for herbivores is often reversed when natural enemies are included (Table 1.1 vs Table 1.2).

There is also a clear bias within the literature towards studies that investigate the impact of global climate change on the efficiency of parasitoids regulating herbivore abundance (text in bold – Table 1.2). One logistical reason for this is that many parasitoid species are commercially available as biocontrol agents and their efficiency at herbivore control in ambient conditions is well established. Predator species, such as coccinellids, also have a significant role in natural biological control (Schmidt *et al*., 2003), but there remains a dearth of information on how these predatory species will respond to climate change.

The direct and indirect effects of climate change on trophic interactions are often hard to distinguish. For example, temperature increases can directly alter invertebrate metabolism, but also indirectly affect invertebrates by altering host plant quality as a resource (Bale *et al*., 2002). Other aspects of climate change such as $eCO_2$ and altered precipitation have few documented direct impacts on invertebrate herbivores (Lincoln *et al*., 1993; Masters *et al*., 1998); instead most of the observed

responses are via changes to the plant. To understand the impact of climate change on tri-trophic linkages better, a detailed understanding of the indirect impacts is required before the additional complication of direct effects can be included. The opposing responses to herbivores in the absence (Table 1.1) and presence (Table 1.2) of their natural enemies to $eCO_2$ and altered precipitation create an ideal opportunity to study contrasting responses to climate change.

## 1.3.1 Elevated atmospheric $CO_2$

The impact of $eCO_2$ on bottom-up processes such as plant resistance can be differentially altered depending on plant species (Hughes & Bazzaz, 2001) or genotype (Martin & Johnson, 2011). For example, a study involving five aphid species and their responses to elevated atmospheric $CO_2$ found that while the abundance of one species (*M. persicae*) increased and another (*Acyrthosiphon pisum* (Harris)) decreased, the other three species (*Aphis nerii* Boyer de Fonscolombe*, A. oenotherae* Oestlund and *Aulacorthum solani* (Kaltenbach)) did not respond to $CO_2$ treatment (Hughes & Bazzaz, 2001). Each aphid species, however, was cultured on a different host plant species, suggesting that host plant effects may be driving the aphid-specific responses to $eCO_2$. Therefore, climate change effects on tertiary trophic levels and above may depend on the response of plant to the novel environment.

The dilution of nitrogen-based compounds within plant tissue under $eCO_2$ can elicit changes in secondary plant chemistry (Roth & Lindroth, 1995). Elevated $CO_2$ conditions can modify the composition of HIPVs (Yuan *et al*., 2009), reducing the ability of natural enemies to locate and identify their prey (Vuorinen *et al*., 2004). Natural enemies tend to use multiple signals (*e.g.* semiochemicals originating from the prey (Hatano *et al*., 2008)) to locate suitable prey. The loss of one such signal may therefore not result in any overall change in predation or parasitism. For example, $eCO_2$ has been shown to increase both parasitism and predation of the aphid *Sitobion avenae* (Fabricius) (Chen *et al*., 2007). Increased predation rate may be the result of *S. avenae* being unresponsive to the alarm pheromone, (*E*)-β-farnesene, of its conspecifics (Sun *et al*., 2010), allowing predators to consume more aphid prey within a given time.

## 1.3.2 Altered precipitation

Climate forcing, the result of changes in atmospheric composition, is likely to result in the increased frequency of extreme climatic events (IPCC, 2013), such as extended periods of drought (Christensen *et al*., 2007). In general, drought conditions appear to reduce the efficiency of natural enemies, increasing the performance of the herbivores, the opposite to what is seen under $eCO_2$ (Table 1.2). While the exact mechanism for this phenomenon remains unclear, it may be linked, as with $eCO_2$, to altered prey quality, mediated by changes within the plant. For instance, the reduced rate of *Aphidius ervi* Haliday parasitism on the aphid *Rhopalosiphum padi* (L.) under laboratory-based drought conditions was linked to a shift in aphid demography towards larger, and thus less suitable, aphids as hosts (Aslam *et al*., 2013). Drought conditions can also increase the ability of herbivores to defend themselves against parasitoid attack (*e.g.* encapsulation of the parasitoid egg(s) as seen in the mealy bug *Phenacoccus herreni* Cox & Williams (Calatayud *et al*., 2002)). For shorter periods of drought, it is unknown how changes to the plant can alter colonisation rate of both herbivores and natural enemies in the field.

## 1.3.3 Invasive species interactions

Anthropogenic migration, transport and commerce (Mack *et al*., 2000), in addition to the processes involved in global change, have led to many species expanding their habitat range (Parmesan, 2006). For example, an alien predatory lizard (*Anolis carolinensis* (Voigt)) found on Japanese islands consumes native honeybees. The reduction of the native bee population has facilitated the dominance of invasive honeybees, which themselves prefer to pollinate invasive plant species. This has markedly altered the local pollination webs (Abe *et al*., 2011). Predatory species that expand to new habitats can directly compete with endemic predatory species for resources (Walther *et al*., 2009) which can lead to top-down regulation of herbivore abundance being impaired by the antagonistic relationship between the native and invasive species. The red imported fire ant, *Solenopsis invicta* Buren, invasive to North America, is an example of a species that can be both beneficial, predating pest species in agro-ecosystems, and detrimental, halving the abundance of other beneficial predatory arthropods by indiscriminately consuming them in addition to pest species (Eubanks *et al*., 2002).

**Table 1.2** The effect of component aspects of global change on interspecific interactions between herbivores and their natural enemies. Net effect on prey performance in the presence of an antagonist; '+' positive response, '-' negative response, '=' no effect. Only studies that gave a clear indication of aphid performance were included. Only primary literature referenced. Parasitoid species names are in bold. Areas highlighted by dashed line are the focus of this thesis.

| Climate change component | [Reference] Herbivore – Natural enemy | Outcome | Effect on herbivore |
|---|---|---|---|
| Elevated $CO_2$ | [1]*Myzus persicae, Brevicoryne brassicae - Hippodamia convergens,* **Diaeretiella rapae** | Negligible effects on parasitism / predation | = |
| | [2]*Aphis gossypii - Leis axyridis* | Increased coccinellid predation | - |
| | [3]*Aphis gossypii -* **Lysiphlebia japonica** | Increased parasitism rates | - |
| | [4]*Lymantria dispar -* **Cotesia melanoscela** | Negligible effects on parasitism | = |
| | [5]*Malacosoma disstria -* **Compsilura concinnata** | Negligible effects on parasitism | = |
| | [6]Multiple leafminer - **multiple parasitoid species** | Increased parasitism rates | - |
| Elevated temperature | [7]Multiple species – **multiple parasitoid species** | Increased parasitism rates | - |
| | [8]*Myzus persicae -* **Aphidius matricariae** | Increased parasitism rates | - |
| | [9]*Acyrthosiphon pisum -* **Aphidius ervi, Aphidius eadyi,** *Hippodamia convergens* | Negligible effects on parasitism / predation | = |
| | [10]*Acyrthosiphon pisum -* **Aphidius ervi,** | Reduced resistance to parasitism | - |
| | [11]*Myzus persicae -* **Aphidius matricariae** | Negligible effects on parasitism | = |
| | [12]*Euphydryas aurinia,* **Cotesia bignelli** | Populations changed but remained in synchrony | = |
| Elevated $O_3$ | [13]*Cepegillettea betulaefoliae - multiple natural enemy species* | Negligible effects on parasitism or predation | = |
| | [14]*Malacosoma disstria -* **Compsilura concinnata** | Reduced parasitoid survivorship | + |
| | [15]*Plutella xylostella -* **Cotesia plutellae** | No effect on parasitism | = |
| Increased UV | [16]*Acyrthosiphon pisum -* **Aphidius ervi** | No effect on parasitism | = |
| Decreased precipitation | [16]Multiple Species | Reduced abundance of natural enemies | + |
| | [17]*Rhopalosiphum padi -* **Aphidius ervi** | Negative effects on parasitism | + |
| | [18]*Phenacoccus herreni -* **Apoanagyrus diversicornis, Aenasius vexans, Acerophagus coccois** | Reduced abundance of natural enemies | + |
| | [19]*Hepialus californicus - Heterorhabditis marelatus* | Reduced nematode predation | + |
| | [20]Multiple species | Variable precipitation reduced parasitism | + |

[1]Stacey & Fellowes (2002b), [2]Chen *et al*. (2005a), [3]Sun *et al*. (2011b), [4]Roth & Lindroth (1995), [5]Holton *et al*. (2003), [6]Stiling *et al*. (1999), [7]Hance *et al*. (2007), [8]Bezemer & Jones (1998), [9]Stacey & Fellowes (2002a), [10]Guay *et al*. (2009), [11]Bannerman *et al*. (2011), [12]Klapwijk *et al*. (2010), [13]Awmack *et al*. (2004), [14]Holton *et al*. (2003), [15]Pinto *et al*. (2008), [16]Trotter *et al*. (2008), [17]Aslam *et al*. (2013), [18]Calatayud *et al*. (2002), [19]Preisser & Strong (2004), [20]Stireman *et al*. (2005).

General introduction

The use of natural enemies to control herbivore pests plays an ever increasing role within integrated pest management in agricultural systems, particularly with many traditional pesticide products being withdrawn due to new environmentally-driven legislation (Van Driesche, 2008; Chandler *et al*., 2011). Most work, to date, has regarded tri-trophic interactions as part of a linear food chain (Polis & Strong, 1996). In reality, species interactions are embedded in a web of direct and indirect interactions between and within trophic levels (Tylianakis *et al*., 2008). A single aphid species will, for example, have multiple predator and parasitoid natural enemies, resulting in competition between natural enemies for aphid prey (Schmidt *et al*., 2003). Intraguild predation occurs when multiple natural enemies that share the same prey also consume each other (Polis *et al*., 1989), and can have an important role in ecological processes such as food web stability, coexistence of resource competitors and the suppression of a shared resource (Borer *et al*., 2007). This process is most explored and best understood between predators and parasitoids (Cardinale *et al*., 2003; Traugott & Symondson, 2008). When a parasitoid has oviposited within a herbivore, an "attack window" is created when the predator can consume both herbivore and developing parasitoid (Snyder & Ives, 2001). It is, therefore, of benefit to the parasitoid to avoid ovipositing if it detects chemical traces emanating from a predator (Frago & Godfray, 2014). Despite this form of biotic interference, the synergistic relationship between parasitoids and predators still results in suppression of herbivore abundance greater than either natural enemy acting alone (Snyder *et al*., 2004; Harvey & Eubanks, 2005; Chacon *et al*., 2008).

A further example is the predatory coccinellid *Harmonia axyridis* (Pallas). This beetle has spread from its native range in Asia, mostly as a result of being used as a biological control agent, into continental Europe and USA (Brown *et al*., 2008a). In the UK, however, *H. axyridis* has never been used as a biological control agent. It was first recorded during 2004, its origin unknown. Since then it has spread to become the dominant coccinellid. Correlative evidence suggests that, as the abundance of *H. axyridis* has increased, endemic coccinellid populations have declined in numbers (Brown *et al*., 2011; Roy *et al*., 2012). Some native species are able to compete against the invading species; the native green lacewing, *Chrysoperia carnea* (Stephens), for example, is superior to the invasive *H. axyridis* during intraguild predation scenarios (Nedved *et al*., 2013).

## 1.4 Aphid herbivory

Aphid herbivores are considered one of the most common pest species in agro-ecosystems (Way & Cammell, 1970). Despite a high level of variability in herbivore responses to global change, phloem-feeding insects are an exception by generally benefiting from climate change (Table 1.1). Aphid herbivory can significantly reduce growth, reproduction and photosynthesis of their host plants (Crawley, 1989; Zvereva et al., 2010). In addition to the direct damage caused to plants, aphids act as vectors of various plant viruses (Pickett et al., 1992; Miles, 1999). For example, infection of rice with *Rice tungro virus* causes an annual estimated $1.5 billion loss (Hull, 2002). *Tomato spotted wilt virus*, which infects multiple plant species such as tomato (*Solanum lycopersicum* L.), peanuts (*Arachis hypogaea* L.) and tobacco (*Nicotiana tabacum* L.) causes an estimated $1 billion loss in crop yield (Hull, 2002). Beyond agro-ecosystems, aphid herbivory plays an important role in the dynamics of communities and ecosystems (Huntly, 1991).

Despite the clear detrimental impact aphids can have on their host plant, aphid herbivory is only explored in some 5% of the literature on plant–herbivore interactions (Zvereva et al., 2010). Taxonomically, phloem-feeding insects are less diverse than folivores, but can exploit a greater diversity of habitats (Root, 1973; Zvereva et al., 2010). Aphids have evolved specialised syringe-like mouthparts to pierce and feed on plant vascular tissues. The mouthparts consist of the stylet bundle, elongated maxillae and mandibles that penetrate the phloem with the aid of a modified labium (Dixon, 1973). To aid feeding, a stylet sheath is formed by gel saliva secreted from the tip of the stylet in small beads; once a bead is secreted, the stylet will probe further, eventually forming a complete sheath. The exact function of the sheath remains unknown, but it is likely to aid penetration between plant cells and prevent initiating a wound response by protecting surrounding plant cell walls from damage from the stylet. During probing, enzymes in the watery saliva break down bonds between cells, again, avoiding a wound response in the plant associated with cell damage (Miles, 1999). On reaching the phloem, watery saliva containing high concentrations of $Ca^+$ binding proteins is then secreted in large quantities to prevent sieve plate occlusion, another plant defence mechanism (Will et al., 2009).

Phloem sap contains high concentrations of simple sugars, but amino acids and other nutrients are limited. The ratio of essential : non-essential amino acids in phloem sap varies from 1:4 to 1:20; considerably lower than the 1:1 ratio typical of other animals (Douglas, 2006). Aphids consume large quantities of phloem and rely on symbiotic gut bacteria (*Buchnera* spp.) to acquire sufficient concentrations of limited essential amino acids required for growth and reproduction, while the excess sugars are excreted as honeydew.

Aphids undergo both parthenogenesis and sexual life stages (Dixon, 1985). In spring, during bud-burst, eggs from the previous year hatch to form wingless females called stem-mothers or fundatrices (Dixon, 1973). This viviparous wingless female produces live offspring by parthenogenesis and continues throughout most of the season while the host plant remains suitable, allowing for rapid colonisation of the host plant. Aphids respond to periods of overcrowding, poor host plant quality, predator risk or seasonal cues by producing winged viviparous females or alates, which can migrate to new host plants (Dixon, 1973). During autumn when the length of darkness increases, winged males and oviparous winged females are produced which mate and lay overwintering eggs.

## 1.5 Study system

The European red raspberry (*Rubus idaeus* L.) is a relatively small but economically valuable crop; UK raspberry production in 2012 was worth £80 million (FAOSTAT, 2014). The European large raspberry aphid (*Amphorophora idaei* (Börner)) is a pest found only on *R. idaeus*. In addition to the direct effects of aphid herbivory (reduced plant vigour, wilting and contamination of fruit crop), *Amphorophora idaei* is known to transmit at least four plant viruses (McMenemy *et al*., 2009). The combination of aphid herbivory and the viruses they carry causes substantial detrimental effects on this economically valuable crop. Virus transmission can take place within two minutes of the commencement of aphid feeding (McMenemy *et al*., 2009); prevention of aphid feeding is, therefore, crucial. Failing this, aphid populations must be maintained at low numbers on infested plants to limit the spread of winged aphids to otherwise healthy plants.

## 1.5.1 *Amphorophora idaei*

McMenemy *et al*. (2009) published a detailed review of the biology of the European large raspberry aphid. Adult aphids range from 2.5 to 4.1 mm in length with long antennae and pale green body colour (Fig. 1.1). Like most aphid species (see Section 1.4) the large raspberry aphid undergoes parthenogenetic reproduction during the spring and summer months. *A. idaei* can vector four viruses of the raspberry mosaic disease complex: *Black raspberry necrosis virus* (BRNV), *Raspberry leaf mottle virus* (RLMV), *Raspberry leaf stop virus* (RLSV) and *Rubus yellow net virus* (RYNV).

**Figure 1.1** Adapted from McMenemy *et al*. (2009). (A) General morphology of apterous female *Amphorophora idaei*, dorsal view showing: 1: tibia, fore tibia darkened; 2: tarsus, darkened; 3: cauda, longer than basal width; 4: siphunculus, swollen at distal end; 5: antenna, long with dark pigmentation; 6: compound eye; and 7: antennal tubercle, well developed. (B) Seasonal life cycle of *A. idaei* as might typically occur on commercial raspberries in the U.K.



General introduction
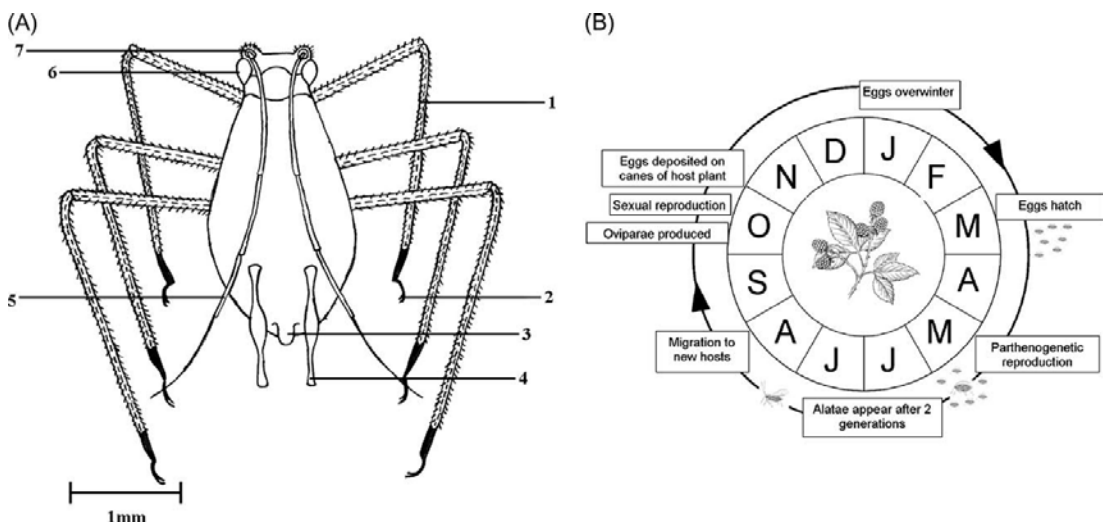
## 1.5.2 UK raspberry breeding – a brief history

The intensification of raspberry growing over the last century initiated a push to breed cultivars with resistance to aphid herbivory. Resistance is currently assessed using small (8–10 cm) plants grown in aphid free conditions. These plants are then loaded with 2 or 3 adult aphids, and plants are assessed 7–10 days later. Plants free of aphids after this period are deemed resistant (Jones *et al*., 2000). The strongest forms of resistance to *A. ideai* are conferred by single dominant genes that are passed on during crossing, allowing for rapid and accurate selection of resistant progeny (Hall *et al*., 2009). There are lower levels of resistance to aphid herbivory that are controlled by a complex of genes, which are little understood and hard to select for during breeding (McMenemy *et al*., 2009). In UK-bred cultivars, a resistance gene called $A_1$ has been one of the most successful against *A. ideai*. The $A_1$ resistance gene was derived from crossbreeding North American red raspberry cultivars with UK cultivars.

The strong selection pressure acting on large raspberry aphid populations feeding on resistant cultivars led to the emergence of aphid populations ($A_1$ breaking biotypes) adapted to overcome the plant resistance (McMenemy *et al*., 2009). Aphid virulence to the $A_1$ resistance gene involves the inheritance of a single dominant allele, which rapidly spread through *A. ideai* populations in the UK (Briggs, 1965). By the 1990s, 75% of the large raspberry aphid population possessed virulence genes to overcome $A_1$ resistance (Hall *et al*., 2009). $A_1$ was not the only resistance gene identified in UK cultivars, others ranged from $A_2 – A_{10}$, but only cultivars possessing $A_1$ and $A_{10}$ became commercially available. The most recent (*circa* 1980) resistance gene, $A_{10}$, was derived from *Rubus occidentalis* L., the North American black raspberry. There is evidence that this resistance is also being broken by certain large raspberry aphid biotypes (McMenemy *et al*., 2009).

The resistance genes in the cultivar Octavia include the resistance genes from an $A_{10}$ cultivar and a cultivar from Germany with the resistance gene $A_{k4a}$. Octavia was released commercially in 2002 and is currently the most resistant cultivar available (McMenemy *et al*., 2009). Reducing *A. idaei* abundance by plant resistance is the most widespread method of control, but other management strategies are required with increasing occurrence of resistance breaking aphid biotypes. One such alternative strategy is 'trapping' aphids between bottom-up processes such as plant

resistance and top-down processes such as natural enemies (Price *et al*., 1980). In the past, combining bottom-up and top-down processes has been difficult to implement (Ode, 2006) but soft fruit production is increasingly reliant on protected environments (*e.g.* polytunnels (McMenemy *et al*., 2009)); natural enemies may become more efficient in enclosed spaces which limit their dispersal ability (Johnson *et al*., 2012).

## 1.5.3 Impacts of global change on the study system

Of the few existing studies, $eCO_2$ has been shown to increase aphid abundance on one raspberry cultivar (Martin & Johnson, 2011). This study suggests that the $A_1$ resistance gene in raspberries may be impaired under $eCO_2$ conditions, whereas plants with either no resistance or $A_{10}$ resistance remain unaffected. The precise mechanisms of resistance against the large raspberry aphid are undocumented; therefore identifying how $eCO_2$ is impairing aphid resistance is difficult. Several aspects of plant chemistry that impact plant resistance can be altered under $eCO_2$. For example, the carbon : nitrogen (C:N) ratio within plant tissue generally increases under $eCO_2$ (Bezemer & Jones, 1998). Although strong changes to raspberry leaf C:N ratio were not  seen over a short period of exposure to $eCO_2$ (Martin & Johnson, 2011), an increase of the C:N ratio can alter the relative abundance and structure of carbon-based compounds.

Epicuticular wax which makes up part of raspberry antixenotic defences mainly consists of fatty acids, alcohols and terpenoids (Shepherd *et al*., 1999), therefore any shifts to the structure and abundance of carbon-based compounds will impact on epicuticular wax function. Additionally, $eCO_2$ has been reported to impair the jasmonic acid signalling pathway in plant tissue (Zavala *et al*., 2008) and modification of induced defence responses, such as the release of HIPVs, has been reported in other plant species. The composition of HIPVs from herbivore-induced damage to the white cabbage (*Brassica oleracea* L.), for example, was altered under $eCO_2$ (Vuorinen *et al*., 2004), preventing the parasitoid from locating its herbivore host. Evidence from predatory invertebrates suggests that they may not be impacted by $eCO_2$ to a similar extent as parasitoids (Chen *et al*., 2007). This could be the result of predatory invertebrates not relying on HIPVs when locating their prey, and instead relying on other cues such as the presence of honeydew (Purandare & Tenhumberg, 2012). Some predatory species have been negatively affected by $eCO_2$ through changes to aphid prey quality (Gao *et al*., 2010); changes

to the nutritional value of the host plant may also affect the nutritional value of aphids as prey (Aqueel & Leather, 2012).

## 1.5.4 Natural enemies

Few studies have investigated the interaction between the large raspberry aphid and its natural enemies. Parasitoids are the most common form of natural enemy reported from field sampling of raspberries; the species recorded generally belong to four families; Cynipidae, Chalcididae, Encyrtidae and Braconidae (Dickson, 1979). A more recent study found that of three common parasitoid species (*Aphidius colemani* Viereck, *Aphelinus abdominalis* Dalman and *A. ervi*), only one species, *A. ervi*, successfully parasitized *A. ideai* (Mitchell, 2007). Parasitism rates by *A. ervi* were significantly higher on raspberry cultivars with intermediate resistance to *A. ideai* compared to cultivars with low or high levels of resistance. To combine bottom-up and top-down processes effectively, a compromise needs to therefore be made in reduced plant resistance, allowing for increased efficiency of natural enemies. There have been no studies investigating the effectiveness of predators such as coccinellids at aphid control on *Rubus* species. Some coccinellid species, such as *Adalia bipunctata* (L.), are commercially available as biological control agents (Koppert, Kent, UK), and are known to control populations of other aphid species such as *A. pisum* (Ferrer *et al*., 2008) and *M. persicae* (Blackman, 1967). With changes to legislation controlling pesticide use (Van Driesche, 2008), public opposition to its use (Crane *et al*., 2006) and the potential effects of climate change, there is an urgent need to use the integration of bottom-up and top-down processes, as described above, to control the large raspberry aphid.

# 1.6 Thesis rationale

Changes to atmospheric composition as a result of anthropogenic processes, and the associated climate forcing (IPCC, 2013), have the potential to alter biotic community composition significantly. The complex network of interactions that comprise natural communities makes predicting the impact of abiotic change difficult (Tylianakis *et al*., 2008). A first step to understanding, and therefore predicting, the impact of abiotic change of natural communities is to characterise the response of simple trophic communities. Agro-ecosystems are often viewed as three trophic level communities, consisting of primary producer (crop), primary consumer

(herbivore) and secondary (herbivore natural enemy) consumer (Loucks, 1977; Colfer & Rosenheim, 2001). Once the underpinning mechanisms behind the response to abiotic change are understood in these relatively simple systems, disentangling the complex network of interactions that comprise semi-natural systems becomes potentially more achievable.

Using a host plant–herbivore–natural enemy as a model system for understanding the effect of abiotic change allows for an enhanced understanding of the response of semi-natural systems. The raspberry–aphid–natural enemy system presents an ideal opportunity to explore how the future of integrating bottom-up and top-down processes to control herbivory will be affected by climate change. Food crops, and especially long-lived perennial food crops such as *Rubus* spp., have not been widely studied in terms of climate change and trophic interactions.

## 1.7 Overall aim

The aim of this thesis is to examine how host-plant heterogeneity, native–invasive species interactions and climate change effects ($eCO_2$ or drought) influence trophic interactions. Specifically, the following avenues will be explored:

1)  The role of bottom-up and top-down processes in ambient conditions;
2)  How increasing levels of atmospheric $CO_2$ and drought can impact bottom-up and top down processes.
3)  Whether native and invasive species interactions associated with global change can interrupt top-down processes.

## 1.8 Thesis scope and outline

As an exploratory study in ambient conditions, **Chapter 2** reports on a study that investigates the role of raspberry plant resistance in modifying aphid abundance and distribution on the plant. Four raspberry cultivars were used to represent a gradient of resistance to herbivory by *A. idaei.* Of these four cultivars, three were completely susceptible to aphid herbivory, with no discernable difference in aphid abundance between cultivars, suggesting *A. idaei* has overcome resistance mechanisms in these cultivars. In contrast, the fourth cultivar, which was the most resistant, consistently had very few or no aphids.

Having determined the effectiveness of raspberry resistance to *A. idaei* in ambient conditions, **Chapters 3** and **4** explore possible modifications of the plant–aphid interaction under altered abiotic conditions (eCO$_2$). Given the apparent lack of effectiveness of plant resistance to *A. idaei* (**Chapter 2**), a natural enemy, the predatory coccinellid *H. axyridis* was included as a form of top-down control (**Chapter 3**) to complement bottom-up plant resistance under eCO$_2$ conditions. Previous studies have found that aphid escape response to mechanical damage is reduced when feeding in eCO$_2$. In **Chapter 4** this was studied further by looking at aphid escape responses to attack by a coccinellid predator, especially in relation to aphid alarm pheromone.

The predatory coccinellid used in **Chapters 3** and **4**, *H. axyridis*, is a recent invader to the UK. Recent evidence suggests *H. axyridis* is responsible for the decline of native coccinellids, possibly reducing the effectiveness of natural enemies to control *A. idaei* abundance. In **Chapter 5**, the interspecific interactions between native and invasive coccinellid predators are explored.

In a semi-field experiment, the impact of drought on the colonisation of herbivore and natural enemies on raspberry plants was investigated. This is reported in **Chapter 6**. Finally, the major results from the separate studies are discussed and synthesised in **Chapter 7**. Both the ecological and applied importance of the findings are explored, as well as a discussion of the limitations of the research and future directions.

# Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

## 2.1 Abstract

Plant resistance to aphid herbivory is one of the key factors controlling aphid abundance. Selective breeding has resulted in multiple plant genotypes that can vary in their resistance to herbivory. A plant genotype is deemed resistant when aphid abundance is lower than genotypes with no known resistance. This approach does not account for how plant resistance mechanisms can impact other aspects of herbivore populations such as individual size and distribution on the plant.

Four raspberry cultivars were used, representing four resistance genotypes, which created a gradient of resistance to herbivory by the large raspberry aphid (*Amphorophora idaei*). Variation in aphid abundance and distribution were recorded over a two-week period in addition to aphid impacts on plant biomass.

Three of the four resistance genotypes were ineffective against *A. idaei*, only the most resistant genotype was still inaccessible, with no aphids found after two weeks. Despite no impact of the other three resistance genotypes on *A. idaei* abundance, aphid distribution on the host plant was genotype specific and a shift in distribution towards the stem resulted in significant reductions in plant biomass.

This study demonstrates that although resistance to *A. idaei* is largely ineffective at limiting their abundance, other genotype specific mechanisms, such as quality of feeding sites, are acting to shape distribution on the plant. Aphid distribution can have a significant impact on the effectiveness of natural enemies. The important roles of integrating other ways of suppressing aphid herbivory, such as natural enemies, are discussed.

Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

20

## 2.2 Introduction

Phytophagous insects are one of the most diverse groups in the animal kingdom. Over 50% of all insects are classed as herbivores (Schaller, 2008). Plants have evolved various mechanisms of defence against insect herbivores, creating an arms race between the host plant and consumers. Plant defence strategies can have profound impacts on herbivore abundance but, like most other animals, insect herbivores are mobile and capable of changing their distribution in response to resource availability and / or quality.

Plants directly defend themselves against herbivory by utilising a combination of constitutive defences such as epicuticular wax or trichomes, and secondary metabolites such as protease inhibitors (Schaller *et al*., 2008). Selective breeding has produced multiple plant genotypes that vary in their ability to resist herbivory (Fritz & Simms, 1992), and has a clear impact on herbivore abundance (Dixon, 1985). When plant resistance, due to herbivore adaptation, no longer affects herbivore abundance, it is unclear if the remaining resistance mechanisms still impact other aspects of herbivore population dynamics (*e.g.* distribution on the host plant and individual size / mass). Intraspecific differences in plant genotype can cause changes to constitutive herbivore resistance, which, in turn, can affect herbivore distribution on the plant (Bond & Lowe, 1975; Jackson & Dixon, 1996).

The Optimum Defence Theory predicts the spatial distribution of plant defence chemicals based on two assumptions. The defences are beneficial, preventing damage to plant tissue; and also that these defences are energetically costly to produce (McKey, 1974). Therefore, the allocation of plant defences will depend how valuable the plant part is and the frequency it is attacked by herbivores. Younger leaves, for example, with their greater photosynthetic capacity (Harper, 1989), have greater investment of resources in defence chemicals than older leaves (McCall & Fordyce, 2010). This has been shown to be the case in the epicuticular wax of young Sitka spruce (*Picea sitchensis* (Bong)) needles where the tissue composition deters the green spruce aphid (*Elatobium abietinum* (Walker)), causing a feeding preference for older needles (Jackson & Dixon, 1996). Other aphid species, in contrast, feed preferentially on either growing or senescing leaves due to the increased flow of nitrogenous material into or out of these leaves, respectively (Dixon, 1986).

Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

21

A trade-off may exist for the aphid between avoiding antixenotic plant defences and the profitability of the feeding site. Whilst, at least nutritionally, leaves may represent a superior site, a sub-optimal site, such as the stem, may have reduced investment in plant defences, thus making it a more accessible feeding site. Plant defences influencing herbivore distribution have been observed in the bean aphid (*Aphis fabae* Scopoli), where, on aphid-susceptible bean (*Vicia faba* L.) cultivars, aphids aggregate on the leaves, but on resistant cultivars, the aphid populations are divided between the leaves and the stems (Bond & Lowe, 1975; Salyk & Sullivan, 1982). In woody plants, which often have physical defences such as trichomes (Levin, 1973), both physical and chemical defences act in tandem against herbivory. Some butterfly species, however, have evolved the ability to circumvent these defences (*e.g. Heliconius charithonia* (L.), (Cardoso, 2008)) or even use them as a refuge against natural enemies (*e.g. Battus philenor* (L.), (Fordyce & Agrawal, 2001)).

It is unclear what other impacts plant resistance may have on aphid populations that are adapting to resistance. Plant resistance may alter the distribution of aphids on the plant, which may, in turn, alter the impact of aphids on plant metrics such as biomass and growth rate. These subtle aspects of plant resistance remain little explored, but could significantly shape the nature and structure of herbivore populations. For example, a host plant that would appear susceptible to aphid herbivory (in terms of aphid abundance) could cause specific changes at both the level of individuals (*e.g.* altered body size / physiology) and the population (*e.g.* spatial shifts on the plant). Moreover, aphids can have feedbacks on their host plants, especially by changing source–sink relations. Aphid herbivory can, for example, cause a shift in resource allocation resulting in biomass gain in some areas of the plant and loss in others (Hackett *et al*., 2013). Additionally, whole-plant biomass has been shown to increase as result of aphid herbivory (Johnson *et al*., 2012).

In a microcosm approach, using red raspberry cultivars differing in resistance to the aphid herbivore *Amphorophora idaei* (Börner), this study set out to determine how plant resistance can 'shape' aphid population dynamics and how aphid herbivory, in turn, can modify the host plant. Three hypotheses were tested:

(H1) Aphid body size and population abundance are negatively correlated with increasing plant resistance to aphid herbivory.

(H2) Greater cultivar resistance results in aphids circumventing the defences associated with the leaves by preferentially aggregating on stems (Bond & Lowe, 1975).

(H3) Aphid herbivory stimulates relative growth rate and increases plant biomass with the effect most pronounced in susceptible cultivars (Johnson *et al*., 2012).

# 2.3 Materials and methods

## 2.3.1 Introduction

All plant growth, culturing and experiments were performed at The James Hutton Institute (JHI), Dundee, UK. The experiment took place in a temperature-controlled glasshouse kept at 18 ± 5°C. To maintain a daily 16 h photoperiod, 400 W metal halide bulbs, positioned above the plants, were used when photosynthetic active radiation (PAR) dropped below 400 $\mu$mol.s$^{-1}$.m$^{-2}$.

## 2.3.2 Host plant

Four cultivars of European red raspberry (*Rubus idaeus* L.), varying in resistance to aphid herbivory, were used. Malling Jewel has no recognised major resistance genes against the large raspberry aphid, making it completely susceptible to aphid herbivory. Glen Ample possesses a resistance gene ($A_1$), now largely ineffective following adaptation by aphid biotypes (Birch *et al*., 2004), and represents a plant partially susceptible to herbivory. Glen Clova also has partial susceptibility to aphid herbivory but, unlike Glen Ample, its resistance is underpinned by multiple genes (multi-genic), making it more resistant than Glen Ample and is therefore referred to as partially resistant (McMenemy *et al*., 2009). Octavia is highly resistant to aphid herbivory, possessing two major resistance genes ($A_{10}$ and $A_{k4a}$) (Knight & Fernández-Fernández, 2008).

Plants were grown from rootstock until approximately 1 cm in height (*circa* 4 weeks); at this stage the plants were transferred to 8 cm disposable pots. After two weeks of further growth, plants were transferred into 3 L pots filled with peat–sand–perlite mix compost (Sinclair Horticulture Ltd, no insecticide or additional fertiliser). Plants were placed in the experimental glasshouse 24 hours prior to the experiment and watered *ad libitum* throughout the experiment.

Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

23

### 2.3.3 Aphids

The European large raspberry aphid (*A. idaei*) is a specialist phloem-feeding herbivore, found only on the European red raspberry causing direct and indirect (as a vector of at least 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. The aphid population was maintained in a controlled environment at 18 ± 1°C and 16 h photoperiod using the cultivar Malling Landmark (also A1 resistance) as a culture plant.

### 2.3.4 Experimental design

Prior to aphid inoculation, the length of the primocane (first year growth in raspberries) and number of leaves were recorded. To prevent the movement of aphids between plants, a line of insect barrier glue was applied to each pot. A total of 200 plants were used, 50 per cultivar. 40 replicate plants from each cultivar were selected at random for inoculation with *A. idaei* (n = 160). The remaining 10 replicate plants of each cultivar were established as 'no aphid' controls, providing a baseline for plant performance in the absence of aphid herbivory (*e.g.* relative growth rate). Plants were arranged in the glasshouse using a randomised blocked design. Each block contained 20 plants, 5 plants of each cultivar comprising of 4 aphid inoculated plants and 1 control plant not inoculated with aphids. The experiment was staggered over five days (two blocks per day) to facilitate the logistics of measurement and harvesting.

Plants (n = 160) were inoculated with three teneral apterous adult female aphids; the remaining 40 plants had no aphids on them. Aphids were placed on the third leaf from the growing tip. On day 24, length of the primocane and leaf count, abundance and distribution of adult and nymphal aphids on the plant (leaf or stem), were non-destructively recorded by visual inspection. To estimate the effect of cultivar on aphid size, 10 plants per cultivar were randomly selected and 25 adult aphids taken from each plant. These aphids were stored in ethanol and hind-tibia length, an

Chapter 2: Population abundance and distribution of the large raspberry aphid
(*Amphorophora idaei*) in relation to host plant resistance and growth

24

indicator of overall aphid size (Johnson *et al*., 2003), measured at x10 magnification using a dissecting microscope (LEICA MZ125).

## 2.3.5 Statistical analysis

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

The response variables (aphid abundance, distribution, tibia length, and plant growth-rate and biomass) were modelled with experimental block as a random effect. The variable, experimental block encompassed both spatial and temporal variation within the experimental design and was chosen for a random effect. The aphid abundance model suffered from over dispersion; to account for this an observational level random term was used (Elston *et al*., 2001). Potential explanatory variables included raspberry cultivar (susceptible (Malling Jewel), partially susceptible (Glen Ample), partially resistant (Glen Clova) and resistant (Octavia)), aphid treatment and plant biometrics (height, dry mass). A rogue aphid, common in glasshouses, *Aulacorthum solani* (Kaltenbach), was found on some replicate plants, its presence / absence was included as a fixed effect in the models to account for influence this aphid may have had.

Counts of aphids (aphid abundance) were modelled with a Poisson error distribution and log-link function. Relative growth rate and plant biomass were analysed with normal error distribution and identity link function, no transformation of data was required. Aphid distribution between leaves and stem was captured using the log ratio (Log (aphids on stem +1) / Log (aphids on leaves +1)). A value of less than zero indicates the majority of the aphids were found on the stem, a value greater than zero on the leaves.

Temporal variation in the measurement of dry mass was accounted for within the models by the variable "Date dry mass" which was always fitted in conjunction with plant dry mass. Explanatory variables were added in a forward step-wise fashion until a minimum adequate model was obtained. Non-significant variables were dropped from the model. F-ratios and p-values adjusted for other fitted terms (SAS Type III) were presented and, where multiple comparison tests (SAS LSmeans) were used, a Bonferroni correction was applied. All two-way interactions between explanatory variables were considered but reported only when statistically significant ($p < 0.05$).

**Table 2.1** GLIMMIX results summary for aphid abundance, tibia length, adult and nymph distribution in response to aphid and plant metrics. MPE – multiple model parameter estimates. Variables in bold retained in final model.

| Response variable | Explanatory variables | Estimate | $F_{(ndf, ddf)}$ | $P$ |
|---|---|---|---|---|
| a) Aphid abundance | Plant dry mass | -0.495 | $1.45_{(1,174)}$ | 0.2296 |
| *Random effect estimate (var):* | **Cultivar** | | **$132.42_{(3,179)}$** | **<0.0001** |
| *Replicate = 2.53 ± 0.29* | **Malling Jewel, Glen Ample, Glen Clova, Octavia** | **6.526, 6.477, 6.867, 0** | | |
| *Experimental block = 5.76E–18 ± 0* | | | | |
| | *A. solani* presence | | $1.15_{(1,175)}$ | 0.2843 |
| | Present, absent | 1.056, -0.657 | | |
| | **Date dry mass taken** | | **$21.06_{(1,162)}$** | **<0.0001** |
| | **11129, 11157** | **-1.112, 0** | | |
| b) Hind-tibia length | **Adult distribution** | **2798.27** | **$7.22_{(1,26)}$** | **0.0124** |
| *Random effect estimate (var):* | Cultivar | | $0.06_{(2,18)}$ | 0.9457 |
| *Experimental block = 842.9 ± 1935.7* | Malling Jewel, Glen Ample, Glen Clova | 0, 9.038, 11.614 | | |
| | Plant dry mass | | $2.58_{(1,27)}$ | 0.1196 |
| | *Aphid abundance* | | $0.63_{(1,26)}$ | 0.4351 |
| | *A. solani* presence | MPE | $0.23_{(1,27)}$ | 0.6336 |
| c) Adult distribution | **Plant dry mass** | **0.335** | **$41.54_{(1,113)}$** | **<0.0001** |
| *Random effect estimate (var):* | Cultivar | | $2.59_{(2,105)}$ | 0.0801 |
| *Experimental block = 0.0077 ± 0.01* | Malling Jewel, Glen Ample, Glen Clova | 0, 0.211, 0.159 | | |
| | *A. solani* presence | | $0.06_{(1,112)}$ | 0.7992 |
| | Present, absent | 0, 0.0329 | | |
| | **Date dry mass taken** | | **$7.66_{(1,107)}$** | **0.0066** |
| | **11129, 11157** | **0.254, 0** | | |
| d) Nymph distribution | **Plant dry mass** | **0.0344** | **$36.42_{(1,109)}$** | **<0.0001** |
| *Random effect estimate(var):* | **Cultivar** | | **$16.9_{(2,105)}$** | **<0.0001** |
| *Experimental block = 0.057 ± 0.045* | **Malling Jewel, Glen Ample, Glen Clova** | **0, 0.825, 0.805** | | |
| *Julian dry mass = 0.022 ± 0.061* | | | | |
| | *A. solani* presence | | $4.26_{(1,97)}$ | 0.0412 |
| | Present, absent | 0, -0.441 | | |
| | Julian dry mass taken | | $2.98_{(1,1.5)}$ | 0.0871 |
| | 11129, 11157 | 0.2579, 0 | | |

Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

26

**Figure 2.1** Partial residual plot, derived from GLIMMIX model showing the relationship between adult aphid tibia length (surrogate for size) on the linear predictor scale and adult distribution on the plant. Aphid distribution was modelled, and therefore displayed as log-likelihood, increasing value indicates shift in distribution towards the leaves.
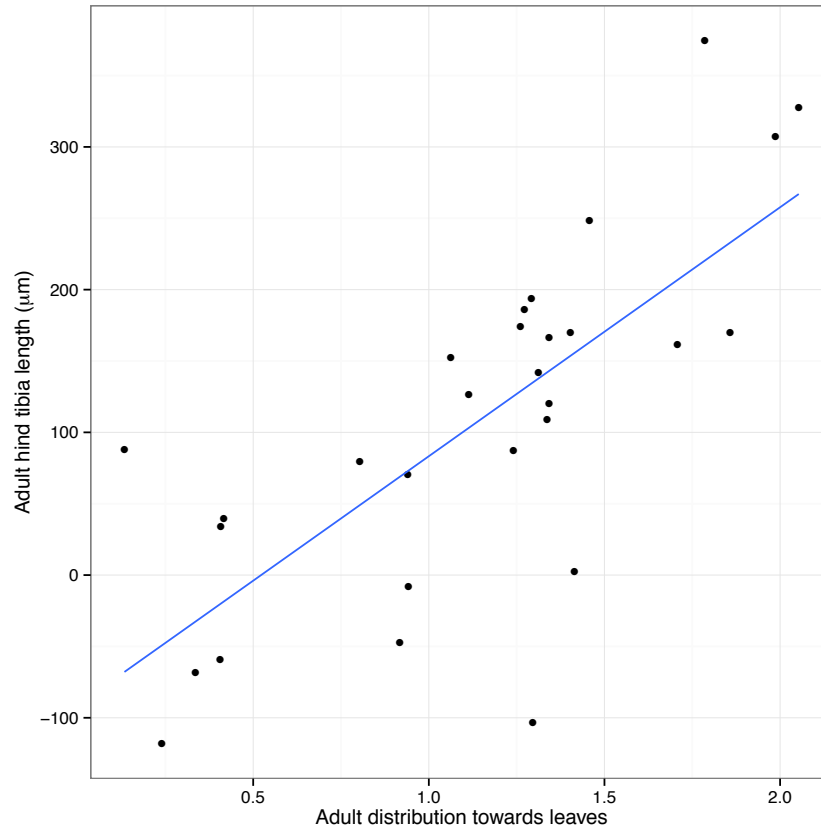


## 2.4 Results

Aphid abundance, as predicted, was significantly affected by raspberry cultivar (Table 2.1a). Aphid abundance on the resistant cultivar Octavia was over 10 times lower than the other susceptible cultivars. There was no significant variation in aphid abundance between the remaining three cultivars. Adult tibia length was positively linked to the distribution of adult aphids on the plant (Table 2.1b, Fig. 2.1).

Plants where the majority of aphids were found on the leaves (highest mean log ratio) also had the largest aphids. The smallest adults were found on plants with the lowest mean log ratio for stem and leaf. Neither aphid abundance nor raspberry cultivar had an impact on adult tibia length (Table 2.1b).

Overall, aphid adults and nymphs favoured leaves over stems (mean ± S.E.) log-ratio for stem and leaves of nymphs, 0.86 ± 0.06, and adults, 0.78 ± 0.04 (value > 0 indicates more aphids on leaves compared to stem). The distribution of adult aphids was positively correlated with plant dry mass (Table 2.1c, Fig. 2.2a). Plants with the greatest dry mass had the greatest aggregation of adult aphids on the leaves (*i.e.* greatest mean log-ratio), whereas smaller plants had a more even distribution of adult aphids between leaves and stem with a mean log-ratio closer to 0.

**Table 2.2** GLIMMIX results for plant growth rate and dry mass in relation to plant and aphid metrics. Variables in bold retained within final model.

| Response variable | Explanatory variables | Estimate | $F_{(ndf, ddf)}$ | $P$ |
|---|---|---|---|---|
| a) Growth rate | **Adult distribution** | **3.851** | **$15.43_{(1,187)}$** | **0.0197** |
| *Random effect estimate (var):* | **Cultivar** | | **$10.96_{(3,184)}$** | **<0.0001** |
| *Experimental block = 4.958 ± 3.252* | **Malling Jewel, Glen Ample, Glen Clova, Octavia** | **2.93, -3.941, 0.71, 0** | | |
| | Aphid treatment | | $2.88_{(1,183)}$ | 0.0911 |
| | *Not inoculated, inoculated* | *0, -1.874* | | |
| | *A. solani* presence | | $2.01_{(1,191)}$ | 0.158 |
| | *Present, absent* | *0, 2.215* | | |
| b) Plant dry mass | **Nymph distribution** | **-0.216** | **$9.79_{(1,187)}$** | **0.002** |
| *Random effect estimate (var):* | **Cultivar** | | **$20.37_{(3,185)}$** | **<0.0001** |
| *Experimental block = 2.004 ± 3.288* | **Malling Jewel, Glen Ample, Glen Clova, Octavia** | **2.441, -10.465, -12.125, 0** | | |
| | **Nymph distribution * Cultivar** | | **$3.92_{(3,187)}$** | **0.0096** |
| | **Malling Jewel * nymph** | **2.471** | | |
| | **Glen Ample * nymph** | **5.181** | | |
| | **Glen Clova * nymph** | **10.854** | | |
| | **Octavia * nymph distribution** | **0** | | |
| | *A. solani* presence | | $0.79_{(1,171)}$ | 0.374 |
| | *Present, absent* | *0, 2.251* | | |
| | Aphid treatment | | $0.03_{(1,179)}$ | 0.8704 |
| | *Not inoculated, inoculated* | *0, 0.367* | | |
| | **Julian dry mass** | | **$16.08_{(1,179)}$** | **<0.0001** |
| | **11129, 11157** | **-5.906, 0** | | |

Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

28

**Figure 2.2** Partial residual plot, derived from GLIMMIX model showing the impact of plant dry mass on a) adult and a) nymph distribution on the host plant and the impact of c) cultivar on nymph distribution (Octavia not shown due to very low aphid abundance). Aphid distribution modelled as log-likelihood along the linear predictor scale; positive value

Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

29
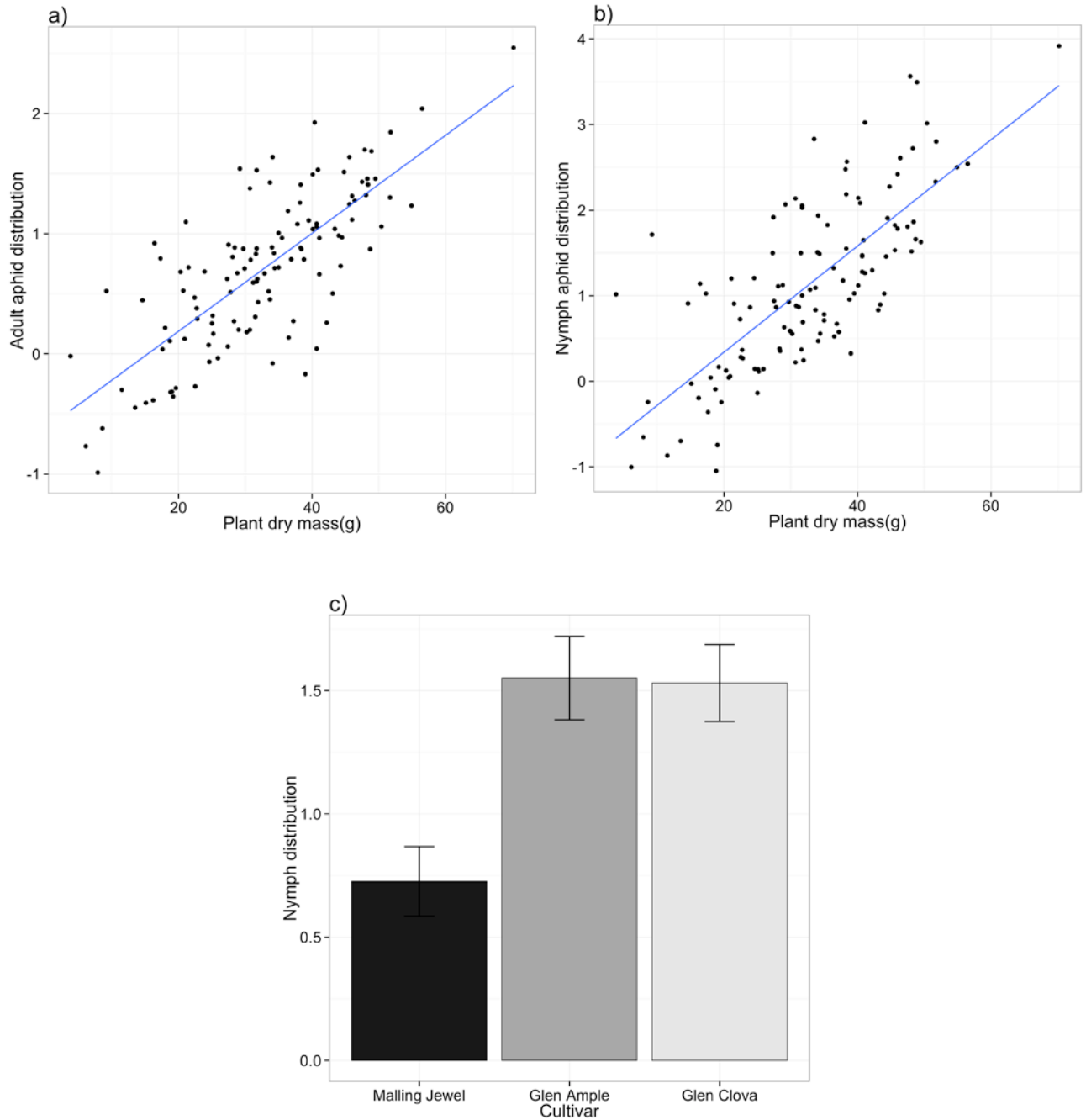
Nymph distribution was, as expected, closely aligned to adult distribution and showed a similar relationship with plant dry mass (Table 2.1c, Fig. 2.2b). Adult distribution did not differ across cultivars; nymph distribution, on the other hand, did, with nymphs on Malling Jewel being more equally distributed between leaves and stem compared to the other two susceptible cultivars (Fig. 2.1d).

Plant growth-rate differed between cultivars (Table 2.2a); the partially susceptible cultivar had a lower growth-rate than the susceptible, partially resistant and resistant cultivars. There was, however, no evidence that the presence of aphid herbivory had an effect on plant growth rate (Table 2.2a). Despite the presence of aphid herbivory having no impact on plant growth rate, adult distribution did significantly impact plant growth rate (Table 2.2a). Plants with the highest growth rate had the greatest abundance of adult aphids on the leaves compared to the stem. In contrast to growth rate, plant dry mass was affected by the interactive effect of nymph distribution and cultivar (Table 2.2b). For the three susceptible cultivars, there was a positive correlation between nymph distribution towards the leaves and plant dry mass. The gradient of this linear relationship steepened as plant resistance increased (Fig. 2.3).

**Figure 2.3** Partial residual plot, derived from GLIMMIX model showing the relationship between nymph distribution and plant dry mass across the four raspberry cultivars. Aphid distribution was modelled and therefore displayed as log-likelihood; increasing value indicates shift in distribution towards the leaves.

Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

31
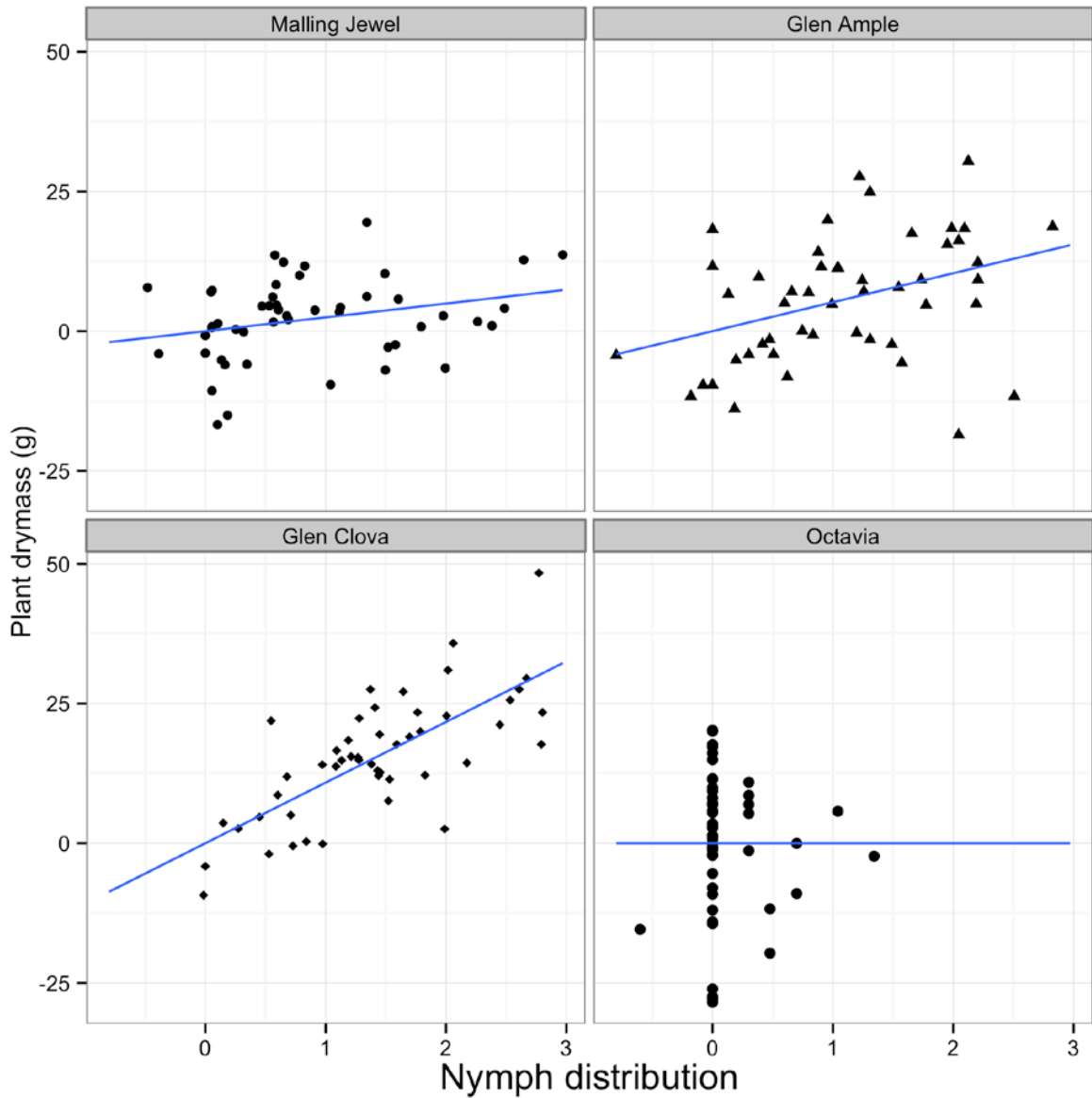
## 2.5 Discussion

As predicted (H1), plant resistance to aphid herbivory in the resistant cultivar Octavia resulted in very low or non-existent aphid numbers. Aphid abundance on the three, more susceptible, cultivars was not correlated with the predicated increasing gradient of plant resistance. Whilst there was no difference in aphid abundance between these three cultivars, there were intra-cultivar differences in aphid distribution. Many of these trends in aphid distribution were uniform across cultivars, suggesting host plant resistance does not modulate many aspects of aphid distribution. Some evidence was found that aphid distribution on the plant can have significant impacts on plant growth rate and dry mass that may ultimately influence the overall crop yield in a long-lived woody species such as the susceptible cultivars of *R. idaeus*.

### 2.5.1 Individual and population performance

Firstly, this study raises questions as to the effectiveness of host plant resistance to aphid herbivory in raspberries. The most resistant cultivar (Octavia) demonstrated very strong resistance and nearly all plants were free of aphids at the end of the experimental period. Despite its strong resistance, this cultivar has not been embraced commercially due to its relatively low yield compared to other, more susceptible, cultivars (Hall *et al*., 2009). The remaining three cultivars used in this study represented a gradient of resistance, one that would be expected to be apparent in aphid abundance. This was not the case; aphid abundance across all three cultivars was the same. A potential explanation is that aphid populations have periodic exponential population growth and decline phases driven by biotic (*e.g.* intraspecific competition) and abiotic (*e.g.* drought) factors (Matis *et al*., 2009). At the time of aphid count, aphid populations on the three cultivars may have been at different stages of this cycle. The appearance of alate (winged) adults would have suggested, however, that the population was suffering from biotic or abiotic stress (Dixon, 1973) but none were found on any cultivar. It is, therefore, more likely that *A. idaei* was able to overcome $A_1$ and multi-genic forms of host plant resistance at the population-level. Lack of population-level response to host plant resistance was not mirrored in the behavioural response.

Chapter 2: Population abundance and distribution of the large raspberry aphid
(*Amphorophora idaei*) in relation to host plant resistance and growth

32

## 2.5.2 Cultivars and feeding site

Aphid abundance was greatest on the leaves compared to the stem for all three susceptible cultivars. Leaves may offer a superior feeding site with greater availability of nitrogenous material being transported from the roots to leaf tissue (Dixon, 1986) or, by positioning themselves underneath leaves, aphids avoid desiccation and predation. Cultivar type did not influence the distribution of adult aphids, but nymphs were influenced. More nymphs were found on the stem on cultivar Malling Jewel (no resistance) compared to the other two susceptible cultivars (Glen Ample and Glen Clova). Adult distribution on the plant, although not affected by cultivar, was found to be a good predictor of adult size, with larger individuals found on the leaves compared to the stem, possibly highlighting that leaves are indeed a superior feeding site. Adult size and stylet length are positively correlated, allowing larger adults found on the leaves to extract more resources than smaller aphids because of reaching phloem vessels deeper within the leaf tissue (Völkl, 1989). Individuals will be able to stay on a feeding site for longer, increasing the carrying capacity, and therefore aphid densities on leaves, while also forcing smaller nymphs to other sites, which may include the stem. Interspecific competition may play a role, with larger aphids being able to stand their ground to a greater extent than smaller individuals, causing smaller individuals to seek sub-optimal feeding sites.

## 2.5.3 Aphid impacts on plant growth

The presence of aphids had association with plant dry mass or growth rate. It is, therefore, more likely that plant dry mass or size had an impact on aphid distribution, rather than aphid distribution impacting plant dry mass. Across all cultivars, bigger plants had a lower proportion of adult aphids on the stem compared to shorter plants. Bigger plants may have had proportionally bigger leaves, or a greater abundance of leaves available for aphid feeding sites. The exception to this was Malling Jewel, which was the quickest growing, had the greatest final biomass, but nymph distribution was shifted towards the stem. Despite having the greatest biomass, Malling Jewel generally had smaller leaves compared to other cultivars (personal obs.). This suggests greater abundance of nymphs on the stem was the result of intraspecific competition driving nymphs to feed on alternative, sub-optimal feeding sites, such as the stem.

## 2.5.4 Conclusions

Aphid abundance on the three susceptible cultivars was far higher than that observed in the field (McMenemy *et al*., 2009). Plant resistance did not appear to operate along a gradient across the four cultivars used in this study. The resistant cultivar was simply inaccessible to *A. idaei*, but the other three cultivars appeared equally susceptible to aphid herbivory. In semi-natural- and agro-ecosystems, aphid herbivory would not take place without the influence of natural enemies which both directly (consumption) and indirectly (dropping behaviour) affect aphid densities. It is possible that any negative effect of herbivory can be mitigated by the actions of natural enemies. We found $A_1$ and multi-genic forms of resistance in raspberries were largely ineffective against a common biotype of *A. idaei*. These cultivars are, however, very different in morphological features such as epicuticular wax composition, and trichome density that possibly impacts the efficiency of natural enemies. Morphological features such as trichome can significantly impair foraging efficiency of natural enemies (Riddick & Wu, 2011). Raspberry cultivar Glen Ample, which has no trichomes, may be more 'resistant' to aphid herbivory in field conditions due to greater accessibility to predators.

While crop breeding for aphid resistance is an important feature of raspberry protection, it is just one factor within an integrated pest and disease management framework, which also includes 'beneficial' natural enemies of insect pests (Gordon *et al*., 1997). Insect herbivore populations, and potentially plant productivity, will therefore depend on how these natural enemies contribute to the overall 'resistance' of the crop. The inclusion of higher trophic levels in this kind of study would help to realistically evaluate the effectiveness of host plant resistance. A plant is deemed 'resistant' when aphid biotypes are unable to colonise it. With the global reduction in pesticides and increasing reliance on biological control, it remains unclear if the term 'resistance' should be updated to include non-lethal impacts of the host plant (*i.e.* aphid distribution) and what this ultimately means when higher trophic levels are considered concurrently.

Chapter 2: Population abundance and distribution of the large raspberry aphid (*Amphorophora idaei*) in relation to host plant resistance and growth

34

# Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

## 3.1 Abstract

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

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

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 $CO_2$.

This study highlights how crop genotypes vary in responses to elevated atmospheric $CO_2$; 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 elevated $CO_2$ associated with global climate change on pest populations.

## 3.2 Introduction

By 2100, atmospheric $CO_2$ concentrations are predicted to more than double pre-industrial levels of 280 ppm (IPCC, 2013). There is growing interest in understanding how insect herbivores found on crops will respond to the resulting 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 that 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 fewer resources and pesticides (The 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.

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 C3 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 to pests 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 responses 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

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

36

increase 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.*, 2013a; Sun *et al.*, 2013).

In the present study, the effects of $eCO_2$ on European red raspberry (*Rubus idaeus* L.) susceptibility to the European large raspberry aphid (*Amphorophora idaei* (Börner)) were investigated. Found only on *R. idaeus*, *A. idaei* is a specialist phloem-feeding herbivore, 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). 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 understanding of how tri-trophic interactions are impacted by an $eCO_2$ environment. Earlier research (Martin & Johnson, 2011) was expanded upon with the inclusion of different cultivars and also an aphid natural enemy. Since plant architecture and habitat complexity are important considerations for assessing the realistic efficacy of natural enemies (Langellotto & Denno, 2004) this study also used larger, structurally complex plants compared to Martin and Johnson (2011).

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

The following hypotheses were tested:

(H$_1$) Raspberry plants, like most C3 plants, respond positively (e.g. increased biomass) 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 reduced prey quality. Consumption of prey from $eCO_2$ will increase development time and adult mass of predators.

# 3.3 Materials and methods

## 3.3.1 Chambers

Experiments were carried out in four controlled environment chambers (approx. 4 m x 10 m) 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 400 µmol m$^{-2}$ s$^{-1}$, 12 x 400 W halide bulbs positioned approximately 1 m above the plants supplemented natural daylight in each chamber to maintain a 16 h photoperiod. 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 ppm) and two at elevated (650 ± 50 ppm) 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 and 550 ppm, respectively), $CO_2$ gas (BOC) was injected for 1 s followed by 30 s delay, repeating until the target concentrations were reached.

## 3.3.2 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 1 cm in height, the plants were transferred to CEH where they were potted-out into 3 L 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.

### 3.3.3 Aphids

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, 16 h 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 $CO_2$ conditions for at least five generations before the experiment.

### 3.3.4 Coccinellids

The aphidophagous harlequin coccinellid (*Harmonia axyridis* (Pallas)), native to Asia, was originally used throughout Europe and North America as a biocontrol agent against aphids (Brown et al., 2008a). Now established, it is one of the most common coccinellid species (Tedders & Schaefer, 1994; Colunga-Garcia & Gage, 1998; Brown et al., 2008a). Adult female coccinellids 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 16 h photoperiod. In culture, *H. axyridis* populations were fed pea aphids (*Acyrthosiphon pisum* (Harris)), but starved for 24 hours prior to the experiment.

### 3.3.5 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 $CO_2$ (ambient and elevated) and subsequent predator (coccinellid present or absent) treatments. This gave 12 replicates per treatment combination (cultivar x $CO_2$ x predator). The experiment was carried out from September 2011–September 2012

over a series of four runs to reduce pseudoreplication of $CO_2$ 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 (10 cm diameter x 3 cm height) and placed in 50 cm x 50 cm plastic trays filled with water (four plants per tray), ensuring the pots were above the waterline (see Johnson *et al*., 2013 for details).

After five weeks growth in the $CO_2$ 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 (25 cm diam. x 65 cm height, Insectopia, UK). The coccinellids remained on the plants for 72 hours, after which time 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 above-ground 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.

### 3.3.6 Experiment 2: Coccinellid development

To provide aphid prey, 32 plants of the susceptible and partially resistant cultivar were randomly assigned to two $CO_2$ treatments across four controlled environment chambers (2 x ambient, 2 x elevated). Plants were inoculated with three large raspberry aphids as in Experiment 1 and after four weeks aphids were collected daily and used as prey for the coccinellid 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 coccinellids. Each clutch (approximately 15–30 eggs) was collected and split randomly between the four diet treatments (cultivar x $CO_2$). There were 30 individual coccinellid replicates per treatment combination, 120 in total. Eggs were placed individually into plastic pots (2 cm height x 3 cm 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

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

40

the effect of diet treatment on relative growth rate, a random sample of 11 individuals from each treatment combination (44 in total) was selected and weighed every day until pupation (Sartorius ME36S microbalance). Mean relative growth rate (MRGR) was calculated following Gotthard *et al.* (1994):

MRGR = (ln($W_2$) – ln($W_1$)) / $t$,

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 was recorded at pupation and emergence. Adult dry mass was recorded after emerged adults were snap frozen and freeze dried (Heto PowerDry PL3000).

## 3.3.7 Statistical analysis

Data for Experiment 1 were analysed using generalised linear mixed models (GLMM) using PROC GLIMMIX (SAS Institute, version 9.01). Data analyses for Experiment 2 were performed within the R environment (R Core Team, 2014) using a GLMM (package lme4 – Bates *et al.*, 2014; and package lmerTest for F tests and approximation of degrees of freedom, Kuznetsova *et al.*, 2014).

### 3.3.7.1 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 ppm, elevated 650 ppm), predator treatment (coccinellid 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.

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

41

**3.3.7.2 Experiment 2**

Hypothesis 3. Relative growth rate, development time and pupal mass of coccinellids 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 ppm and elevated 650 ppm), 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). Two-way interactions (*e.g.* between cultivar, predator and $CO_2$ treatments in Experiment 1) are reported only when statistically significant (p<0.05).

# 3.4 Results

## 3.4.1 Experiment 1: Trophic interactions

### 3.4.1.1 *Hypothesis 1 – Plant responses*

Above-ground biomass varied significantly among the raspberry cultivars irrespective of $CO_2$ treatment (Table 3.). Plants partially resistant to aphid herbivory had the greatest dry mass, followed by the resistant cultivar (Fig. 3.1). The susceptible cultivar had the lowest dry mass, almost half that of the partially resistant cultivar (Fig. 3.1). $CO_2$ 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 3.). The susceptible cultivar was the most responsive to $eCO_2$ with a 107% increase in dry mass compared to ambient $CO_2$ (Fig. 3.1). 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%.

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

**Table 3.1.** GLIMMIX results summary for a) plant dry mass, b) initial aphid abundance and c) change in aphid abundance post-predation in relation to biotic and abiotic environment. Variables in bold retained in final model. MPE = multiple parameter estimates.

| Response variable | Explanatory variables | Estimate | $F_{(ndf, ddf)}$ | $P$ |
|---|---|---|---|---|
| a) Plant dry mass | **$CO_2$**<br>*Ambient, elevated* | *12.830, 20.671* | **$12.85_{(1,3)}$** | **0.0377** |
| *Random effect estimate (var):*<br>*Chamber (experimental run) = 28.73 ± 27.51* | **Cultivar**<br>**Susceptible, partially resistant, resistant** | *14.940, 25.221 20.671* | **$20.37_{(2,121)}$** | **<0.0001** |
| *Experimental run = 6.28 ± 7.84* | Initial aphid abundance | 20.666 | $0.33_{(1,123)}$ | 0.568 |
| b) Initial aphid abundance | **$CO_2^{\dagger}$**<br>*Ambient, elevated* | *-1.555, 0.710* | **$14.49_{(1,2)}$** | **0.063** |
| *Random effect estimate (var):*<br>*Replicate = 0.46±0.081*<br>*Chamber (experimental run) = 0* | **Cultivar**<br>**Susceptible, partially resistant, resistant** | *4.203, 4.197, 0.710* | **$148.67_{(2,128)}$** | **<0.0001** |
| *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 | $CO_2$<br>*Ambient, elevated* | *-0.678, -4.747* | $0.3_{(1,2)}$ | 0.639 |
| *Random effect estimate (var):*<br>*Chamber (experimental run) = 0* | **Cultivar**<br>**Susceptible, partially resistant, resistant** | *-1.99, 6.21, -2.84* | **$10.64_{(1,125)}$** | **<0.0001** |
| *Experimental run = 468.36±429.36* | Dry mass | 2.062 | $0.6_{(1,123)}$ | 0.439 |
| | **Predator treatment**<br>*Control, coccinellid* | *0.04, -2.84* | **$33.55_{(1,125)}$** | **<0.0001** |
| | **Cultivar * predator** | **MPE** | **$7.37_{(2,1.25)}$** | **0.0009** |

$^{\dagger}$ Retained in model due to interaction term.

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

43

**Figure 3.1** Aboveground plant dry mass of three raspberry cultivars in response to ambient and elevated $CO_2$. Data are least square mean ± S.E.
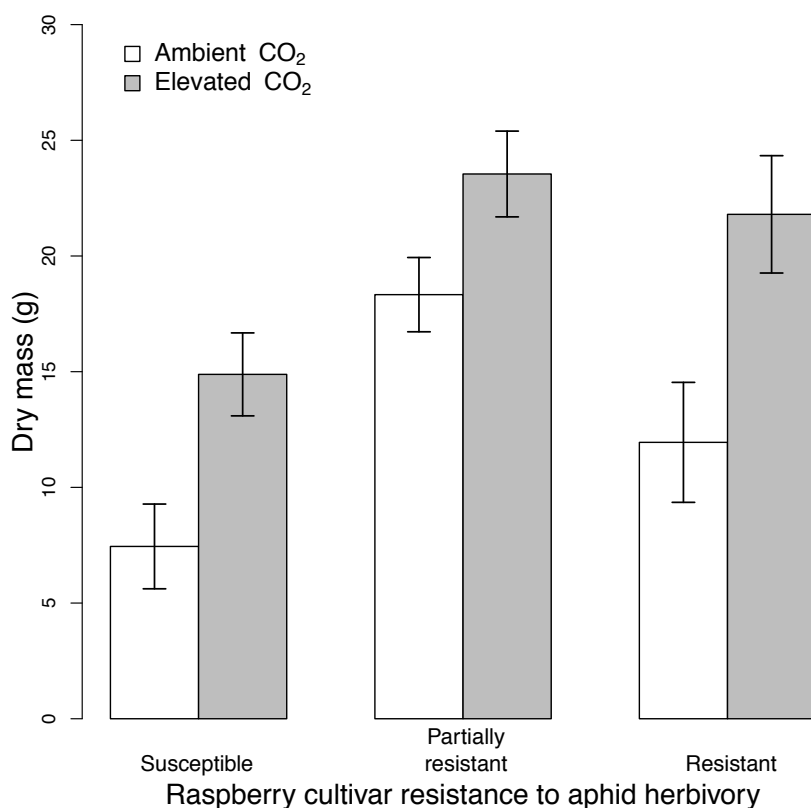


### 3.4.1.2 Hypothesis 2 – Aphid responses

There was a highly significant effect of cultivar on aphid abundance before the onset of the predation treatment (Fig. 3.2a, Table 3.1b). 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. 3.2a). Atmospheric $CO_2$ enrichment significantly affected aphid abundance (Table 3.1b), but this varied between plant cultivars as indicated by the significant $CO_2$ x cultivar interaction (Table 3.1b). Reduced aphid abundance drove this effect of $CO_2$ enrichment on the resistant plant cultivar. On the resistant cultivar, elevation of atmospheric $CO_2$ concentrations significantly increased the mean abundance of aphids (Fig. 3.2a).

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

44

**Figure 3.2** The effect on aphid abundance of cultivar resistance, $CO_2$ treatment and a) absence or b) presence of coccinellid 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.



Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

45
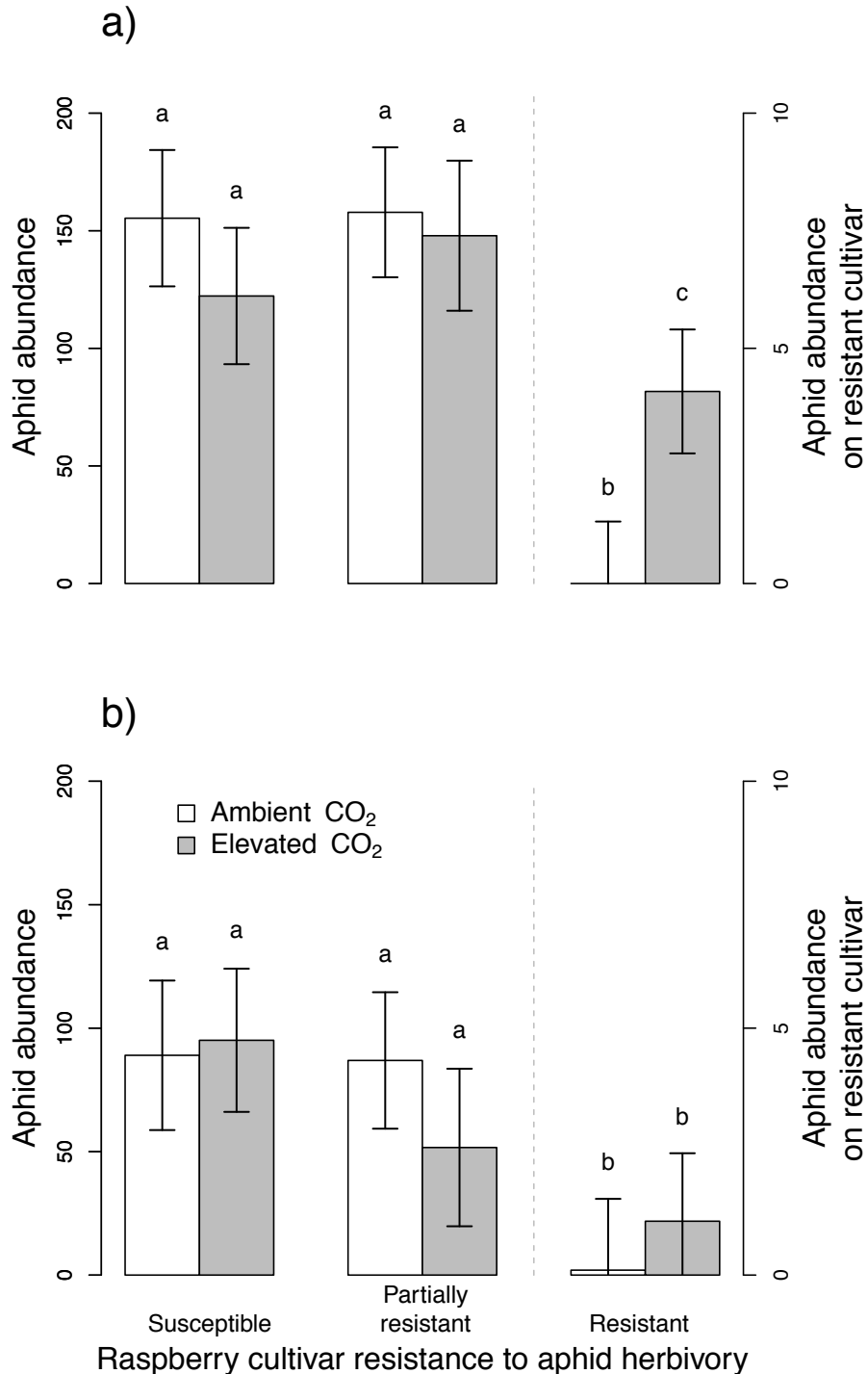
Furthermore, aphid colonisation of the resistant cultivar was markedly increased by $CO_2$ enrichment with 14% and 70% of plants supporting aphids under ambient and $eCO_2$ conditions, respectively ($F_{1,5} = 7.9$, $p = 0.05$). In contrast, aphid abundance on the susceptible and partially resistant cultivars was unaffected by manipulation of the $CO_2$ environment (Fig 3.2a, Table 3.1a).

The presence of a coccinellid predator significantly reduced aphid abundance on all cultivars (Fig. 3.2a versus Fig. 3.2b; Table 3.1c). Moreover, while $CO_2$ enrichment increased aphid herbivore colonisation and abundance on the resistant cultivar, once coccinellid predation was introduced, this $CO_2$ effect was nullified (Table 3.1c, Fig. 3.2b). On the susceptible and partially resistant cultivars, the number of aphids consumed by the coccinellid did not significantly vary with $CO_2$ treatment (Fig. 3.2b). $CO_2$ 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 ($F_{1,100} = 11.6$, $p = 0.001$).

**Figure 3.3** Least square mean ± S.E. for relative growth rate of larval stages of the coccinellid *H. axyridis* fed aphid prey from susceptible or partially resistant raspberry cultivars.
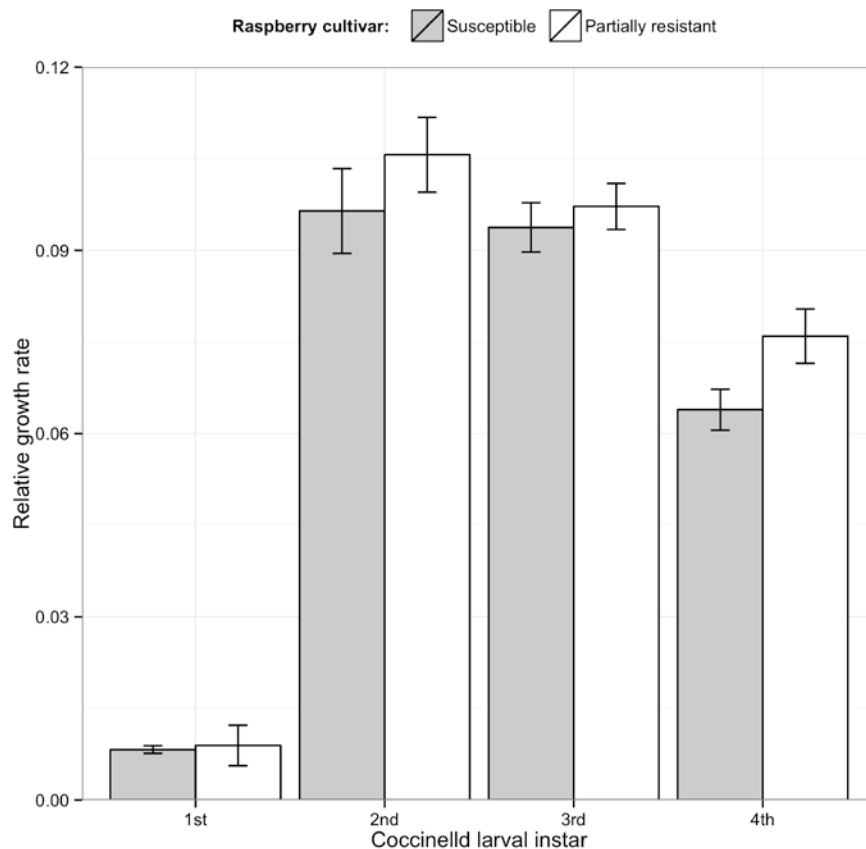
### 3.4.2 Experiment 2: Coccinellid development

Hypothesis 3. The mean relative growth rate over the full duration of coccinellid development was not affected by the prey source environment ($CO_2$: $F_{1,2}$ = 0.01, p = 0.94 and cultivar: $F_{1,24}$ = 0.02, p = 0.87). 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 coccinellid was significantly increased (Fig. 3.3, Table 3.2a). When larvae were fed aphids reared on the partially resistant cultivar, fourth instar coccinellid larvae tended to have higher relative growth rate compared to their siblings fed aphids reared on the resistant cultivar (Fig. 3.3). There was no significant effect of $CO_2$ treatment on relative growth rate of coccinellid larvae (Table 3.2.a). Despite the almost significant (p = 0.056) effect of cultivar on fourth instar larval growth rate, duration of development from egg to adult was not affected by the cultivar or $CO_2$ 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 3.2b). Pupal and adult mass was, however, affected by adult sex: females were significantly heavier than males (Table 3.2b).

## 3.5 Discussion

The fertilising effect of $CO_2$ 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 $CO_2$. This was also true for the raspberry cultivars investigated by Martin and Johnson (2011) (summarised in Table 3.3), 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 $CO_2$. Aphid colonisation and subsequent abundance was greater on the resistant cultivar grown in elevated $CO_2$, suggesting a reduction in resistance to aphid herbivory in the novel environment. Plant biomass in ambient conditions was not correlated with resistance to herbivory;

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

47

the partially resistant cultivar had the greatest biomass, followed by the resistant and susceptible cultivars.

**Table 3.2** GLIMMIX results summary for coccinellid (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. Variables in bold retained in final model. MPE = multiple parameter estimates

| Response variable | Explanatory variables | Estimate | $F_{(ndf, ddf)}$ | $P$ |
|---|---|---|---|---|
| a) Relative growth rate | $CO_2$ *Ambient, elevated* | *4.793, 4.715* | $3.14_{(1,1)}$ | 0.358 |
| *Random effect estimate (var):* | Cultivar *Susceptible, partially resistant* | *5.195, 5.195* | $4.05_{(1,21)}$ | 0.056 |
| *Parent = 0.0006±0.0001* | | | | |
| *Replicate = 0.001±0.0001* | | | | |
| *Chamber = 0.0002±0.0002* | **Larval instar** ***$1^{st}$, $2^{nd}$*** ***$3^{rd}$, $4^{th}$*** | ***0.022, 0.727*** ***2.033, 4.67*** | $\mathbf{181.38_{(3,75)}}$ | **<0.0001** |
| b) Pupal mass | $CO_2$ *Ambient, elevated* | *35.725, 35.846* | $0.03_{(1,2)}$ | 0.884 |
| *Random effect estimate (var):* | Cultivar *Susceptible, partially resistant* | *35.831, 35.767* | $0.02_{(1,92)}$ | 0.898 |
| *Parent = 1.463±1.863* | | | | |
| *Chamber = 0* | **Sex** ***Male, female*** | ***0.868, 1.603*** | $\mathbf{26.30_{(1,93)}}$ | **<0.0001** |

Plant biomass increased under $CO_2$ enrichment for all three cultivars. The partially resistant cultivar, with the greatest biomass in ambient $CO_2$, was the least responsive (30% increase in biomass), suggesting that it is already close to its maximum growth capacity under ambient $CO_2$. The 85% increase in biomass of the resistant cultivar under $eCO_2$ suggests this cultivar to be particularly responsive to $eCO_2$. In $eCO_2$ 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 cultivars with two different aphid resistance genes, $A_{10}$ and $A_{k4a}$. Previous work by Martin and Johnson (2011) found the $A_{10}$ resistance gene expression was robust to changes in $CO_2$ concentrations. This implies that $CO_2$

enrichment may be modifying the function of the $A_{k4a}$ resistance gene. This, however, remains an untested hypothesis and is only one possible explanation. For example, raspberry cultivars possessing the $A_{10}$ resistance gene can show significant variation in minor genes associated with aphid resistance, which may modify the responses to elevated $CO_2$ (Hall *et al*., 2009). Even in cultivars possessing the same resistance gene, it seems their genotypic background can modify resistance expression at elevated $CO_2$, for example, with two cultivars possessing the $A_1$ resistance gene either becoming more susceptible to aphids (Table 3.3, 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 antixenosis reducing colonisation and antibiosis 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 (Wittwer & Castilla, 1995; Johnson *et al*., 2010; 2012), 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 important and encouraged increasingly (Stiling & Cornelissen, 2005; Van Driesche, 2008).

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

49

**Table 3.3** Comparison of plant and aphid responses to elevated atmospheric $CO_2$ ($eCO_2$) found by Martin and Johnson, 2011 and the findings of this study.

| Cultivar | Martin & Johnson (2011) | | This study | |
| | $eCO_2$ 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 |

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

50

Predation by a natural enemy mitigated the breakdown of plant resistance to aphid herbivory, returning the aphid population to its "ambient" abundance. There was no evidence for a transfer of bottom-up effects across multiple trophic levels. The $CO_2$ environment that the host plants were exposed to, did not affect the size or total protein content of aphids living on them. Moreover, the $CO_2$ environment their prey had been reared in did not influence the development of the next generation of coccinellids. Similar to other studies, we found the effect of elevated $CO_2$ on prey quality weak or non-existent (Salt *et al*., 1995; Stacey & Fellowes, 2002b; Chen *et al*., 2005a) and subsequent predator generations were also unaffected (Chen *et al*., 2005b).

The influence of bottom up processes, such as the effect of plant genotype on prey quality, had a much more significant effect than $eCO_2$ on coccinellid 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 coccinellid 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 resist attack by coccinellids using behavioural strategies (*e.g.* kicking, evasion) better and thereby impose extra fitness costs (*e.g.* handling time) on coccinellids (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. Surprisingly the relative growth rate of the first instar coccinellids was very low, especially considering the high growth rate of first instar larvae of studies (*e.g.* Mishra *et al*., 2012). This is possibly due to the difficulty of the first instars in attacking and consuming large aphid prey such as *A. idaei*.

Confining aphids on plants necessitated use of potted plants in closed chambers, which may be argued to give artificially high plant growth responses to $CO_2$ (Ainsworth *et al*., 2008). Given, however, that > 90% of raspberry production takes place in closed polytunnels which buffer environmental fluctuations (Johnson *et al*.,

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

51

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).

## 3.5.1 Conclusions

This study highlights the importance of considering multiple trophic levels when trying to understand pest dynamics and ecosystem responses to future climates. Increasing atmospheric $CO_2$ has the potential to impair plant defences against herbivory which may have important implications for agro-ecosystems. This study demonstrates 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 $CO_2$ on tri-trophic interactions remain however little understood. This study provides an empirical demonstration of how the net level of plant herbivory under elevated $CO_2$ depends on the interactions between the plant, the herbivore and the natural enemy.

Chapter 3: Top-down control by *Harmonia axyridis* mitigates the impact of elevated atmospheric $CO_2$ on a plant–aphid interaction

52

# Chapter 4: Elevated atmospheric CO$_2$ impairs aphid escape responses to predators and conspecific alarm signals

## 4.1 Abstract

Research into the impacts of atmospheric change on predator–prey interactions has mainly focused on density dependent responses and trophic linkages. As yet, the chemical ecology underpinning predator–prey interactions has received little attention. Group-living animals have evolved behavioural mechanisms to escape predation. Chemical alarm signalling between conspecific prey can be susceptible to environmental change if the physiology and behaviour of these organisms are affected by changes in dietary quality resulting from environmental change. Using *Rubus idaeus* plants, it is shown that elevated concentrations of atmospheric CO$_2$ (eCO$_2$) severely impaired escape responses of the aphid *Amphorophora idaei* to predation by the larvae of the coccinellid *Harmonia axyridis*. Escape responses to coccinellids were reduced by >50% after aphids had been reared on plants grown under eCO$_2$. This behavioural response was rapidly induced, occurring within 24 h of being transferred to plants grown at eCO$_2$ and, once induced, persisted even when aphids were transferred to plants grown at ambient CO$_2$. Escape responses were impaired due to reduced sensitivity to aphid alarm pheromone, (*E*)-β-farnesene, via an undefined plant-mediated mechanism. Aphid abundance often increases under eCO$_2$, however, reduced efficacy of conspecific signalling may increase aphid vulnerability to predation, highlighting the need to study the chemical ecology of predator–prey interactions under environmental change.

Chapter 4: Elevated atmospheric CO$_2$ impairs aphid escape responses to predators and conspecific alarm signals.

53

## 4.2 Introduction

Animals that live in groups have evolved numerous behavioural mechanisms for escaping predation, ranging from aggregation for protection (*e.g.* the 'selfish herd' hypothesis (Hamilton, 1971)) to more 'altruistic' alarm signalling to conspecifics (Zuberbuehler, 2009). The latter has evolved in many different invertebrate and vertebrate taxa, employing a variety of acoustic, visual and chemical cues to warn conspecifics of a predation risk (Ruxton *et al*., 2004). Alarm signalling between individuals via pheromones is particularly prevalent amongst insects (Blum, 1969).

Predicting how ecosystems will respond to climate change requires greater understanding of the impacts on community processes, like herbivore prey–predator interactions (Jamieson *et al*., 2012; Facey *et al*., 2014). Atmospheric carbon dioxide ($CO_2$) has reached 400 ppm and is predicted to increase to 421–936 ppm by 2100 (IPCC, 2013). This may have profound consequences for predator–prey interactions (Robinson *et al*., 2012; Facey *et al*., 2014). Experimental studies have hitherto focussed on density dependent population responses and trophic interactions (*e.g.* Chen *et al*., 2005a; Chapter 3). Elevated atmospheric $CO_2$ ($eCO_2$) could, however, also alter behavioural aspects of predator–prey interactions, such as how prey respond to conspecific chemical signals (*e.g.* pheromones) to avoid predators. Such communication could be indirectly altered by $eCO_2$ affecting the physiology of herbivorous prey via changes to their plant resources (Zavala *et al*., 2013).

Aphids emit the alarm pheromone, (*E*)-β-farnesene (*E*βF), to alert conspecifics of imminent attack (Bowers *et al*., 1972). This pheromone facilitates a variety of evasive tactics used by aphids, including cessation of feeding, walking from the signal source or dropping from the plant (Pickett *et al*., 1992; Vandermoten *et al*., 2012). Reduced evasion responses of aphids when physically disturbed by the experimenter have been shown under $eCO_2$ (*e.g.* squeezing with forceps, Awmack *et al*., 1997 or prodding the thorax, Mondor *et al*., 2004). While these studies did not explicitly link this to *E*βF, a subsequent study in which grain aphids (*Sitobium avenae*) were subjected to periodic release of *E*βF showed population declines at ambient atmospheric $CO_2$ ($aCO_2$), but no change at $eCO_2$ (Sun *et al*., 2010). They suggested that this was due to aphids becoming insensitive to *E*βF under $eCO_2$ (Sun *et al*., 2010). To date, however, the behavioural response of aphids to both *E*βF and predators when feeding on intact plants under $eCO_2$ has not been

Chapter 4: Elevated atmospheric $CO_2$ impairs aphid escape responses to predators and conspecific alarm signals.

54

investigated, previous studies have used excised leaves (Awmack *et al*., 1997; Mondor *et al*., 2004) or focused solely on the response to *E*βF (Sun *et al*., 2010).

The large raspberry aphid (*Amphorophora idaei* (Börner)) used in this study were feeding on red raspberry (*Rubus idaeus* L.) as a model system. Densities of *A. idaei* increase in response to $eCO_2$ on some *R. idaeus* genotypes (Martin & Johnson, 2011), but predation at $eCO_2$ can negate this increase (Chapter 3) potentially due to increased susceptibility of aphid prey. It was therefore hypothesised that aphids have diminished escape responses to predator attack under $eCO_2$, compared to $aCO_2$, which will be underpinned by reduced sensitivity to *E*βF.

## 4.3 Materials and Methods

### 4.3.1 Insects, plants and environmental chamber conditions

Insect cultures (full details of collection and maintenance given in Chapter 3) were reared for at least four generations at $aCO_2$ (390 ± 50 ppm) and $eCO_2$ (650 ± 50 ppm) conditions in four environmentally controlled chambers, two per $CO_2$ regime, all at 20 ± 4°C, 50 – 70% relative humidity and 16 h photoperiod. These chambers (full details in Chapter 3) were also used for growing plants. Forty *R. idaeus* (cv. Glen Clova) were grown from rootstock, at approximately six weeks old (1 cm height) plants were transferred to 3 L pots, and randomly assigned to the four chambers. To minimize chamber effects, plants were moved between corresponding treatment chambers once a week for five weeks prior to assays (*sensu* Bezemer *et al*., 1998; Johnson & McNicol, 2010).

### 4.3.2 Behavioural assays

Behavioural assays used intact plants exposed to the different $CO_2$ treatments for five weeks. Fully-crossed combinations (Fig. 4.1) of aphids and plants maintained under the two $CO_2$ regimes were tested in response to stimuli from the presence of (i) coccinellid (*Harmonia axyridis* (Pallas)) predators, (ii) 200 ng of *E*βF in 5 µl hexane solvent and (iii) control of 5 µl hexane (both Sigma-Aldrich, UK). For each assay, a leaf was randomly selected and 50 mixed-age, apterous aphids were confined to the underside of the leaf with a 20 mm diameter clip-cage for 24 h. The cage was then removed and the number of aphids feeding counted, aphids not feeding were removed prior to the onset of the assay. Assays proceeded as follows: (i) a single fourth instar coccinellid larva, starved for 24 h, was introduced onto the

Chapter 4: Elevated atmospheric $CO_2$ impairs aphid escape responses to predators and conspecific alarm signals.

leaf c. 2 cm from the aphids and observed for 5 mins. The coccinellid was replaced if it did not attack aphids after 5 mins. For the assays with (ii) $E\beta F$ and (iii) the control hexane only, 5 µl of the solution was placed c. 2 cm from the aphid colony and, again, observed for 5 mins for escape responses. Each assay was repeated 10 times. For each assay, the proportion of aphids that stopped feeding and showed predator avoidance behaviour (*e.g.* walking away, dropping) in response to stimuli (i–iii) was quantified.

**Figure 4.1** Schematic of behavioural assays using reciprocal treatments of aphids and plants maintained under $aCO_2$ and $eCO_2$
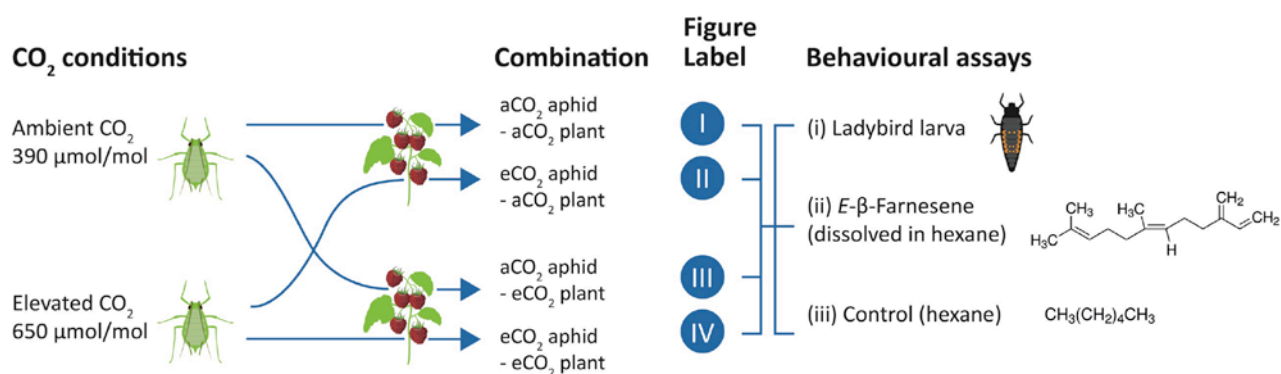


### 4.3.3 Statistical analysis

The proportion of aphids responding to stimuli was modelled with generalised linear mixed effect models fitting a binomial error distribution (GLIMMIX, SAS Institute).

The random effect was environmental chamber nested within date of bioassay. Mean temperature was fitted as a fixed effect to account for spatial (between chambers) and temporal (between days) variation during the experiment (Table 4.1f). A minimum adequate model was obtained with forward stepwise selection of fixed effects. F-ratio and p-values presented are adjusted for other significant fitted terms (SAS type III), two-way interactions are only reported where $p < 0.05$. Degrees of freedom were estimated with Sattherthwaite's approximation (Sattherthwaite, 1946).

**Table 4.1** Final GLMM results summary for aphid escape response to predator / ($E$)-β-farnesene stimulus and $CO_2$ regimes. Significant variables in bold.

| Response variable | Explanatory variables | Estimate | $F_{(ndf,ddf)}$ | p |
|---|---|---|---|---|
| % aphids per plant showing escape behaviours | a) Stimulus<br>*Hexane / Coccinellid / EβF* | *2.17 / 3.54 / 2.68* | **6.46**$_{(2,6)}$ | **0.0031** |
| | **b) Plant growing environment**[†]<br>*aCO₂/ eCO₂* | *2.74/2.68* | **15.28**$_{(1,6)}$ | **0.0089** |
| *Random effect Estimate (var) = 0.07 ± 0.07* | c) Aphid rearing environment<br>*aCO₂/ eCO₂* | *3.29/2.68* | **21.43**$_{(1,145)}$ | **<0.0001** |
| | **d)** Plant growing * aphid rearing environment<br>*aCO₂ plant * aCO₂ aphid / eCO₂ plant * aCO₂ aphid*<br>*aCO₂ plant * eCO₂ aphid / eCO₂ plant * eCO₂ aphid* | *4.04 / 2.68*<br>*2.68 / 2.68* | **38.40**$_{(1,38)}$ | **<0.0001** |
| | e) Aphid rearing environment * stimulus<br>*aCO₂ * Hexane / aCO₂ * Coccinellid / aCO₂ * EβF*<br>*eCO₂ * Hexane / eCO₂ * Coccinellid / eCO₂ * EβF* | *1.30 / 2.16 / 2.68*<br>*2.68 / 2.68 / 2.68* | **6.02**$_{(2,145)}$ | **0.0031** |
| | f) Mean temperature | -0.275 | 5.41 $_{(1,5)}$ | 0.0544 |

[†] Retained in model due to interaction term.

**Figure 4.2** Mean (± S.E.) percentage of aphids reared at $aCO_2$ (grey bars) or $eCO_2$ (white bars) showing escape responses to coccinellid larva *Harmonia axyridis*. Roman numerals refer to treatment combinations (see Fig. 5.1)
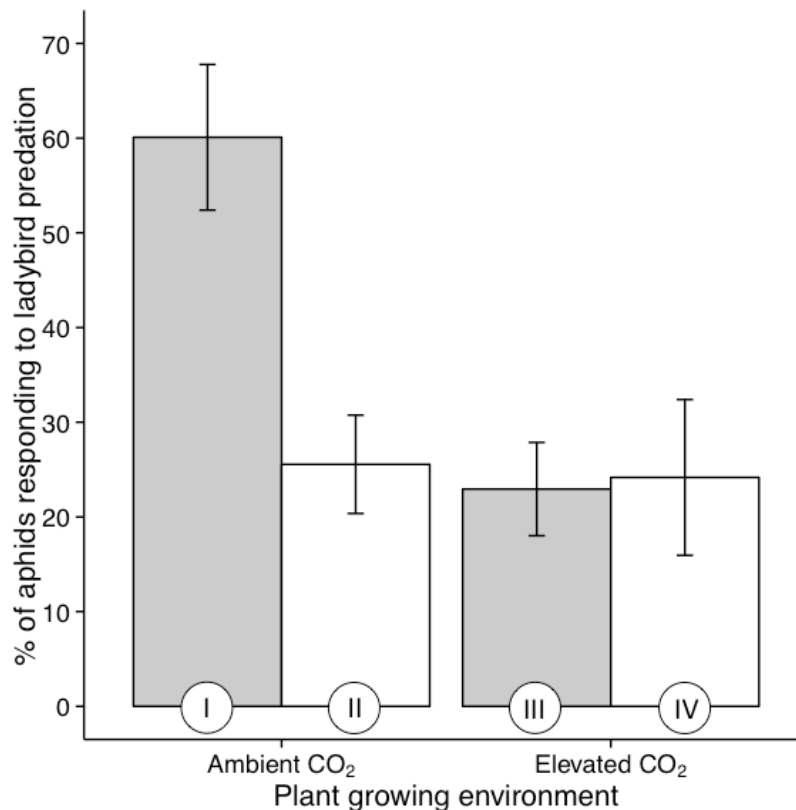
## 4.4 Results

Compared to aphids and plants under $aCO_2$ (Fig. 4.2-I), aphid escape responses to coccinellids were significantly reduced when they had been reared under $eCO_2$ (Fig. 4.2-II & IV, Table 4.1-b & c), even when transferred to plants grown under $aCO_2$ (Fig. 4.2-II). Moreover, aphids reared under $aCO_2$, but subsequently transferred to plants grown under $eCO_2$ for <24 h, showed the same reduction in escape responses (Fig. 4.2-III, Table 4.1-d) as seen in aphids reared under $eCO_2$ (Fig. 4.2-II & IV). Over twice as many aphids initiated escape responses when they fed under $aCO_2$ conditions (Fig. 4.2a-I). Aphids exposed to their alarm pheromone $E$βF, exhibited a reduction in escape behaviour, similar to when being attacked by the predator (Fig. 4.3a, Table 4.1-a & e). Aphids showed no behavioural response to the control solvent hexane (Fig. 4.3b).

**Figure 4.3** Mean (± S.E.) percentage of aphids reared at $aCO_2$ (grey bars) or $eCO_2$ (white bars) showing escape responses to a) $E$βF with hexane solvent, and b) hexane alone. Roman numerals refer to treatment combinations (see Fig. 4.1)
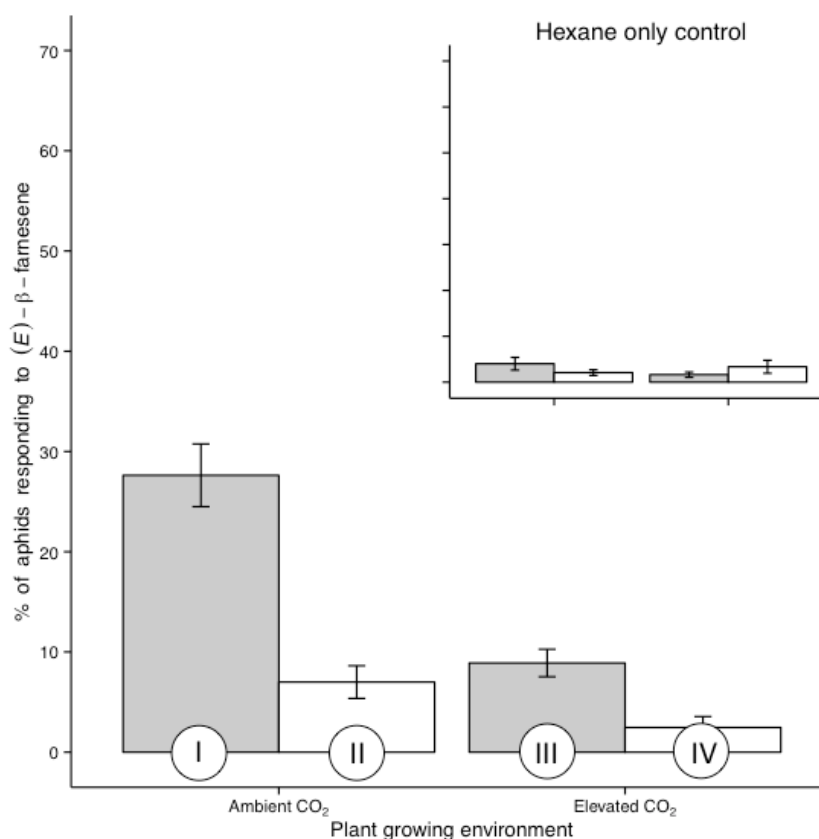
## 4.5 Discussion

Aphid escape responses to predator presence or exposure to the aphid alarm pheromone $E\beta F$ were rapidly (within 24 h) and hugely (>50%) impaired when feeding on a plant reared in $eCO_2$, which persisted even after individual aphids that were reared in $eCO_2$ subsequently fed on plants grown at $aCO_2$.

Aphid behaviour often differs when feeding on experimentally excised leaves compared with intact plants, because phloem hydraulics and chemistry are altered by excision (van Emden & Bashford, 1976; Douglas, 1993). This study, using whole plants, an insect predator, and a controlled dose of alarm pheromone $E\beta F$, rather than a mechanical disturbance of aphids to stimulate its release as performed by Awmack *et al*. (1997) and Mondor *et al*. (2004), in a reciprocal experimental design permitted us to conduct a more realistic test of $eCO_2$ impacts on this tri-trophic interaction.

Impairment of aphid escape responses by $eCO_2$ is likely to have been mediated via changes in the plant quality, which in turn impacted aphids; this is because the impairment always occurred when aphids had been, or were, feeding on plants grown at $eCO_2$. At least two possible mechanisms may underpin reduced escape responses. Firstly, aphids may 'hold their ground' rather than escape if feeding on a good quality host. In particular, *A. idaei* performance is known to be enhanced by $eCO_2$ induced changes in plant suitability (Martin & Johnson, 2011) and this species is known to continue feeding, even under threat, if the host plant is of good quality (Mitchell, 2007). Alternatively, if $eCO_2$ reduces host plant quality, then aphids can engage in more intense and sustained feeding activity (Sun *et al*., 2011b; Guo *et al*., 2013a; Guo *et al*., 2013b), akin to the compensatory feeding responses of chewing insect herbivores (*e.g.* Docherty *et al*., 1996; Johnson *et al*., 2014a). Ingesting more phloem will inevitably be energetically costly and may require deeper penetration of plant tissues, and possibly the manipulation of the plant's metabolism (Guo *et al*., 2013b). This investment may result in aphids being either physiologically less able, or behaviourally less inclined, to abandon a host plant. Either way, it is feasible that enhancement or deterioration in the nutritional quality of plants grown under $eCO_2$ is enough to make aphids continue feeding, even under the risk of predation.

Chapter 4: Elevated atmospheric $CO_2$ impairs aphid escape responses to predators and conspecific alarm signals.

59

## 4.5.1 Conclusions

Multi-trophic interactions must be accounted for to predict the net effect of $eCO_2$ on plants accurately (Harrington *et al*., 1999; Robinson *et al*., 2012; Facey *et al*., 2014). Crops may become more susceptible to insect pests, including aphids (Martin & Johnson, 2011; Johnson *et al*., 2014b), in an $eCO_2$ environment. Top-down control of aphids by natural enemies is a major factor in regulation of aphid populations (Dixon, 2000). Here we demonstrate that atmospheric change modified the behavioural response of a herbivore when a conspecific is being attacked by a natural enemy, which could increase the net impact of the predator. The lack of behavioural response from conspecifics will increase prey availability for the natural enemy, but also limit beneficial, non-consumptive effects, such as the herbivore dropping from the plant. Such modified predator–prey interactions clearly have implications for crop security in a changing world.

Together with previous evidence using other aphid species (Awmack *et al*., 1997; Mondor *et al*., 2004; Sun *et al*., 2010), it seems that the interference effect of $eCO_2$ on aphid escape responses is a general, and possibly widespread, reaction. Further work is, however, needed to establish the mechanistic basis of how atmospheric change mediates the chemical ecology of predator–prey interactions. Moreover, whether aphid populations will adapt to such atmospheric changes over time to re-establish responsiveness to predator-related conspecific alarm signals remains an unanswered question.

Chapter 4: Elevated atmospheric $CO_2$ impairs aphid escape responses to predators and conspecific alarm signals.

60

# Chapter 5: Antagonistic interactions between an invasive and native coccinellid species may promote coexistence

## 5.1 Abstract

Global change will facilitate the spread of certain species into new habitat. Endemic species sharing the same guild as the expanding species are often displaced or driven to extinction due to competition for resources. The indirect effects (*e.g.* altered prey choice or activity) of intraguild predation by invasive species on natives have received little or no attention.

The behavioural impact of intraguild predation by the invasive coccinellid beetle *Harmonia axyridis* on two other species of coccinellid, endemic to the UK, was determined using a series of arena experiments. To establish the extent of overlap in trophic niche the feeding preferences of each coccinellid species for different life stages of the aphid *Amphorophora idaei* was measured. The impact of an intra- or inter-specific competitor on consumption rate of aphids was then tested for each coccinellid species.

Both *A. bipunctata* and *H. axyridis* had a high degree of overlap in prey choice throughout development, however *C. septempunctata* showed no overlap during certain larval stages. During the final and most competitive coccinellid larval stage, the feeding preferences of all species converged on later instar aphid prey. The presence of inter- and intraspecific competitors had no impact on the feeding behaviour of *C. septempunctata* when in this artificial environment. In the wild, *C. septempunctata* occupies a different habitat niche and is therefore spatially separated from the other coccinellid species used here.

The intraguild predator *H. axyridis* and intraguild prey *A. bipunctata* demonstrated feeding behaviour consistent with predictions required for coexistence in models of intraguild dynamics. Unexpectedly, this behaviour was only expressed when these two coccinellid species were together. When not in the presence of each other, intraguild predation dynamics models suggest these two species will not coexist, with *A. bipunctata* potentially going extinct. The limitations of using ecological theory, such as predation dynamics models, to predict the impact of invasive species are discussed.

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

61

## 5.2 Introduction

Invasive alien species are one of the five major drivers of biodiversity change and decline (Hooper *et al*., 2005). The globalisation of trade and commerce has led to the worldwide human-mediated dispersal of organisms into novel environments (Mack *et al*., 2000). Global climate and land-use changes can aid the invasion of new territories by alien species (Hobbs & Mooney, 2000). Invaders can affect endemic species by competing for shared resources or niches, consuming them or by the concomitant introduction of novel pathogens (Mack *et al*., 2000; Snyder & Evans, 2006; Kenis *et al*., 2009; Vilcinskas *et al*., 2013). Invading species can occupy all trophic levels within the community, from plants (Vila *et al*., 2011) to top predators (Kenis *et al*., 2009). It has been recently questioned whether invasive species cause losses in biodiversity or are simply co-occurring with other drivers of biodiversity loss (Roy *et al*., 2012). For example, invasive species may take advantage of other changes to the ecosystem rather than causing the change themselves (Gurevitch & Padilla, 2004; Comont *et al*., 2014). Most studies to date have illustrated the correlation between invasive dominance and native decline, with little focus on the mechanisms (Didham *et al*., 2005; Light & Marchetti, 2007).

Once established, invasive predators may either outcompete endemic predator species for prey (Parker *et al*., 1999) or act as an intraguild predator (Polis *et al*., 1989). Both situations represent a disruption of community function, which can cause declines in the endemic population. Intraguild predation (IGP) involves the consumption by a predator of another predatory species that competes for a similar, often limited prey resource (Polis *et al*., 1989). Predatory arthropods are often associated with IGP due to their generalised feeding habits (Snyder & Evans, 2006). For example, the red fire ant, *Solenopsis invicta* Buren, invasive to North America, has halved the abundance of other predatory arthropods by indiscriminately consuming them in addition to prey species (Eubanks *et al*., 2002). As with predation generally, IGP can have a direct negative effect on the abundance of the intraguild prey, but also indirect effects where the intraguild prey avoid predation. These non-consumptive effects can include eliciting plasticity in prey choice, activity, morphology and life history strategy (Tollrian & Harvell, 1998; Relyea, 2001). Despite its contribution to shaping predator–prey interactions and food web structure, indirect effects of IGP have received little or no attention (Preisser *et al*., 2005). These more cryptic behavioural aspects of IGP, particularly those that may

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

62

alter individual fitness (Dixon & Baker, 1988) may be facilitating the success of invasive predatory species. Simple models that predict the outcome of intraguild predation take into account non-consumptive effects between predatory species such as attack rate of shared resource, digestion efficiency and death rates unrelated to predation (Polis *et al*., 1989; Holt & Polis, 1997).

**Figure 5.1** Diagrammatic representation adapted from Polis *et al*. (1989) of hypothesised intraguild predation occurring between three coccinellid species when competing for a shared aphid prey *Amphorophora idaei*. The direction of arrow indicates the predicted direction of the trophic interaction from prey to predator.
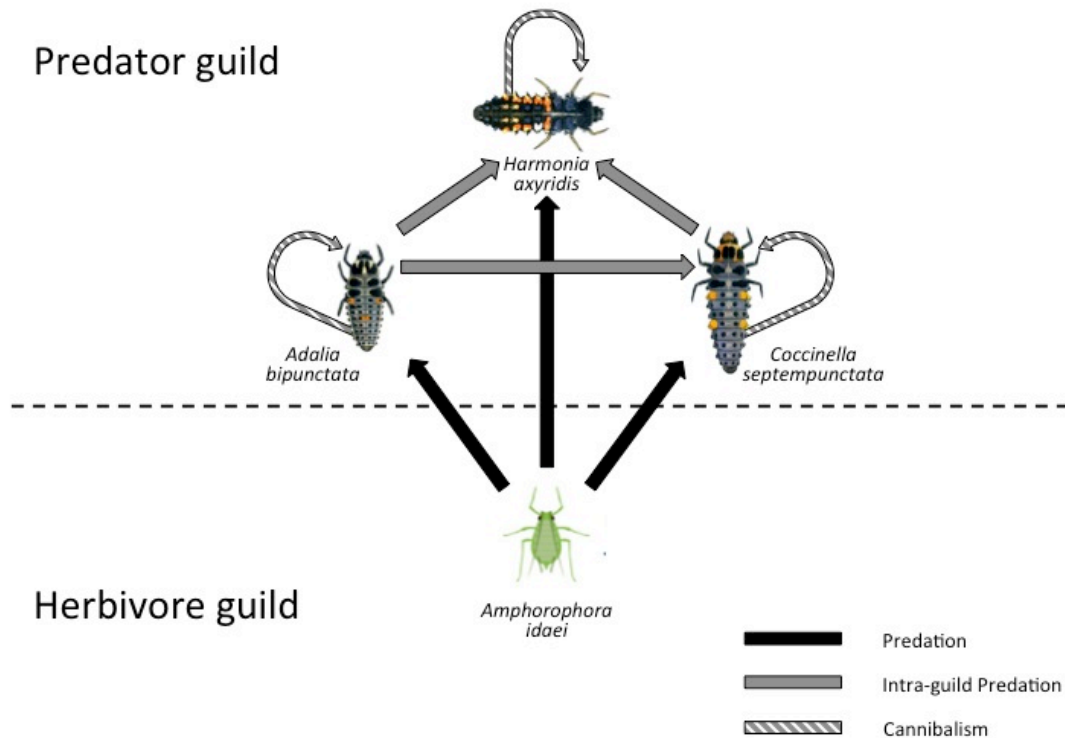


Recent species invasions provide an opportunity to understand impacts on native species and, for invasive predatory species, to establish the role of intraguild predation in interspecific interactions.  In most recent invasions, the date of first appearance is recorded, making it possible to monitor the impact on native species over time. One such example is the predatory coccinellid *Harmonia axyridis* (Pallas). This beetle spread from its native range in Asia, mostly as a result of being used as

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

63

a biological control agent, into continental Europe and North America (Brown *et al*., 2008a). Since 2004, *H. axyridis* has spread to the UK, either via accidental human transport or natural dispersal, to become the most abundant coccinellid species (Brown *et al*., 2008b). Field observations have shown that the increased abundance of *H. axyridis* was correlated with endemic coccinellid decline (Brown *et al*., 2011; Roy *et al*., 2012). Laboratory experiments support the view that *H. axyridis* is an intraguild predator as it readily consumed the eggs of endemic coccinellid species (Katsanis *et al*., 2013), although it does not actively consume eggs of endemic coccinellid species in the field (Smith & Gardiner, 2013). Moreover, *H. axyridis* possesses alkaloid chemical defences that make it inaccessible as prey for other coccinellid species (Fig. 5.1; Sato & Dixon, 2004; Sloggett & Davis, 2010).

Defensive behavioural traits against predation (*e.g.* feeding behaviour, oviposition) are much more plastic than others such as morphological or chemical defences (Relyea, 2001). The indirect effects of IGP on the behaviour of endemic coccinellids (*e.g.* switching prey choice) may therefore offer new insights into the success of *H. axyridis* as an invasive species.

This study aimed to investigate the indirect impacts of IGP on the behaviour of *H. axyridis* on two endemic coccinellid species (*Adalia bipunctata* L. and *Coccinella septempunctata* L.). It was hypothesised that *H. axyridis* would share a similar specificity for aphid life stages as the other coccinellid species, which would confirm the potential for competitive interspecific interactions.

For IGP to persist without either the intraguild predator or intraguild prey becoming extinct, the intraguild prey must be superior to the intraguild predator at exploitative competition of the shared prey resource (Polis & Holt, 1997). If both of these conditions are not fulfilled it would suggest that intraguild predation between coccinellid species is unsustainable and one species will therefore exclude the other (Holt & Polis, 1997). It was also hypothesised that *A. bipunctata* and *C. septempunctata*, the predicted intraguild prey*,* will have a higher consumption rate of aphid prey compared to *H. axyridis*, the potential intraguild predator. Furthermore, consumption of the shared prey resource by the intraguild predator (*H. axyridis*) should be reduced when sympatric with other coccinellids in favour of increased consumption of the intraguild prey due to the metabolic and competitive benefits gained (Holt & Polis, 1992). *Adalia bipunctata* has declined significantly in the UK, it was therefore predicted that this coccinellid species would not show the appropriate

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

64

feeding behaviour required for coexistence with the intraguild predator, *H axyridis*. In contrast, *C. septempunctata*, which has not declined since the appearance of *H. axyridis*, is predicted to show characteristic feeding behaviour of intraguild prey that would allow for co-existence.

# 5.3 Methods

Coccinellid predators used in the experiments were the invasive species, *H. axyridis* (harlequin ladybird), and two coccinellid species endemic to the UK, *C. septempunctata* (7-spot ladybird) and *A. bipunctata* (2-spot coccinellid). Both *A. bipunctata* and *H. axyridis* are arboreal (Roy *et al*., 2012), sharing a similar habitat niche, but *H. axyridis* is significantly larger (Pell *et al*., 2008; Roy *et al*., 2008). In contrast, *C. septempunctata* is mainly associated with herbaceous vegetation (Roy *et al*., 2012), but is a similar size to *H. axyridis*. Both these endemic coccinellid species have been in decline in the UK since the appearance of *H. axyridis* (Brown *et al*., 2011). Both *C. septempunctata* and *H. axyridis* were collected from the field in Oxfordshire, UK, during May 2013. Low numbers of *A. bipunctata* in the field required them to be sourced from a bio-control supplier (Adavalue S. P. R. L., Burdinne, Belgium). All coccinellid species were reared for at least one generation in a controlled environment (20 ± 1°C, 16 h photoperiod) with a diet of mixed age *Acyrthosiphon pisum* (Harris) (pea aphid). Coccinellid larvae were starved for 24 h prior to being used in trials.

## 5.3.1 Aphid prey

The European large raspberry aphid, *Amphorophora idaei* (Börner) is a specialist phloem-feeding herbivore of the European red raspberry, *Rubus idaeus* L. and was used as the focal prey species for the arena experiments. This aphid species was selected because it is a significant pest of raspberry, a small but high-value crop, that cannot be treated with pesticides. It is, however, a prime candidate crop for control with coccinellids as raspberry production largely takes place under cover (McMenemy *et al*., 2007). The aphid culture was initiated from field-collected aphids at the James Hutton Institute, Dundee, UK (JHI) and maintained (20 ± 1°C, 16 h photoperiod) for multiple generations before being moved to the Centre for Ecology and Hydrology (CEH), Wallingford, UK, where the experiments were carried out. *R. idaeus* (cv. Glen Ample) was grown from root-stock at JHI. When approximately 1

cm in height, the plants were transferred to CEH, where they were potted-out into 3 L pots filled with peat-based compost (M3 with no additional fertilizer, Levington, UK). At CEH, plants were grown in a controlled environment chamber (20 ± 1°C, 16 h photoperiod) and used as stock plants to support the aphid population utilised in the arena experiments.

## 5.3.2 Experiment 1: individual feeding preferences

Fifty *A. idaei*, ten apterous adults and ten of each aphid larval instars (I–IV) were placed into a circular metal arena (185 mm diam., 25 mm high) with a pane of glass acting as a lid. Fluon® was applied to the dish sides, preventing vertical movement of aphids and coccinellids. A first instar coccinellid larva was placed in the centre of the arena. Each bioassay ran for 1 h, after which the larva was removed and the remaining aphids counted. This was repeated ten times for each coccinellid larval life stage (1–4) and for each coccinellid species (x3), a total of 120 bioassays.

## 5.3.3 Experiment 2: interspecific coccinellid competition

Twenty-five *A. idaei*, five apterous adults and five of the four larval instars were placed at -20°C for 15 minutes prior to the experiment. This killed the aphids whilst ensuring their tissue remained intact and palatable to coccinellid larvae as prey (Roy *et al*., 2008). The aphid cadavers were randomly assigned to 25 points around the circumference of the circular arena. Only the voracious fourth instar (IV) larvae of each coccinellid species were used for the competition experiment. Two coccinellid larvae in pairwise hetero- and conspecific combinations were placed in the centre of the arena. One of the larvae was randomly marked with non-toxic paint to aid in species identification. Each bioassay ran for 1 h and was recorded using a high definition webcam (Logitech 920). Video footage was later viewed to determine the number of aphids and the life-stage consumed by each coccinellid larva. Bioassays were repeated for each species combination, including a control with no competing hetero- or conspecific individuals (n=10), giving a total of 90 arenas.

Both experiments took place in a controlled environment room (20 ± 1°C, 16 h photoperiod); aphid and coccinellid individuals were used only once per arena.

## 5.3.4 Statistical analysis

Statistical analysis was performed within the R environment (R Core Team, 2014). To test the first hypothesis that coccinellid species share a preference for aphid life stages throughout development, a General Additive Mixed effect Model was used

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

66

(GAMM – gamm4 package, (Wood & Scheipl, 2013)). The GAMM included Poisson errors, where the response was the number of aphids consumed. On this response variable, we tested the effect of aphid life stage and coccinellid larval stage. Main effects and interactions were evaluated using log-likelihood ratio tests. Experimental arena was identified as a random term and included in the model.

The second hypothesis where native coccinellid species will have a different feeding rate to the invasive *H. axyridis* was tested using GAMM with binomial errors. The response variable, proportion of aphids consumed within an hour after accounting for number consumed by the competitor, was tested against the fixed effects: competing coccinellid species and aphid life stage. As above, main effects and interactions were evaluated using log-likelihood ratio tests. Experimental arena and presence of identifying paint were used as random terms in the model.

## 5.4 Results

### 5.4.1 Experiment 1: individual feeding preferences

All three coccinellid species share the same preference for aphid life stages at some point during their development (Fig. 5.2). In particular, the invasive *H. axyridis* and the declining native *A. bipunctata* shared the same prey preference throughout most of their larval stages (Fig. 5.2a,b). The aphid prey preference of the only stable native coccinellid, *C. septempunctata,* significantly differed from the other coccinellids during most of the larval stages (Fig. 5.2c, Table 5.1).

There was a positive linear relationship between aphid and *H. axyridis* larval stage, indicating that older *H. axyridis* larvae select larger aphid prey items (Fig. 5.2b). Generally *A. bipunctata* and *H. axyridis* selected similar life stages of aphid prey during their development (Fig. 5.2a & b, respectively). However, the *A. bipunctata*-prey size relationship was non-linear with later developmental stages of *A. bipunctata* (III and IV instar) preferring third instar aphids (Fig. 5.2a), compared to *H. axyridis'* preference for fourth instar aphids (Fig. 5.2b).

The prey selection of *C. septempunctata* was more complex over its larval development (Fig. 5.2c). Early larval stages (I and II instar) preferred early aphid instars; prey selection became bimodal as the coccinellid larvae developed with consumption of both early and late instar aphids, and finally a strong preference for adult aphids by the fourth instar (Fig. 5.2c).

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

67

**Table 5.1** GAMM results summary for the numbers of aphids consumed in relation to coccinellid species (CS), coccinellid larval stage (CL) and aphid prey life stage (AL). Main effects were evaluated using log-likelihood ratio tests. ΔAIC – difference in AIC from full model to reduced. Graphical output from GAMM presented in Figure 5.2.

| Test | Likelihood ratio test (full vs reduced) | ΔAIC (full – reduced) | $\chi^2$ | d.f. | p |
|---|---|---|---|---|---|
| Coccinellid species (CS) | AL + CL + CS *vs* AL + CL | -112.87 | 124.87 | 2 | |
| Coccinellid larval stage (CL) | AL + CL + CS *vs* AL + CS | -217.26 | 223.36 | 3 | all <0.0001 |
| Aphid life stage (AL) | AL + CL + CS *vs* CL + CS | -301.86 | 307.87 | 4 | |

## 5.4.2 Experiment 2: interspecific coccinellid competition

In the absence of competition, the fourth larval stage of all three coccinellid species had a preference for later aphid life stages (Fig. 5.3 a–c with no competitor), mirroring the results from Experiment 1.

There was no statistical evidence for an interaction between the presence of a competing coccinellid and aphid life-stage consumed (Table 5.2). This suggests that neither intraspecific nor interspecific competition significantly affected the species' preference for aphid life stages.

However, the number of aphids consumed by coccinellid larvae in the presence of a competitor differed between focal species (Table 5.2, Fig. 5.3). The presence of competing coccinellid significantly affected rate of aphid consumption in both the native *A. bipunctata* and invasive *H. axyridis* (Table 5.2).  In the absence of competition (*i.e.* feeding in isolation), the feeding rate of *A. bipunctata* on aphid prey was 63% lower than that of *H. axyridis* (Fig.  5.3 a vs. b). When these two species were competing, the feeding rate of *A. bipunctata* was 68% higher than *H. axyridis* (Fig. 5.3 a vs. b). Aphid consumption by *H. axyridis* was reduced when *A. bipunctata* was present compared to other treatments (no competition, conspecific and *C. septempunctata* competitor) (Fig. 5.3b). The aphid consumption rate of *A.*

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

68

*bipunctata* doubled in the presence of interspecific competition from either *C. septempunctata* or *H. axyridis* (Fig. 5.3a). In contrast, the feeding rate of *C. septempunctata* did not significantly change in the presence of competitors (Table 5.2, Fig. 5.3c). In the presence of *H. axyridis*, the feeding rate of *C. septempunctata* was 10% lower than when feeding alone, although this reduction was not statistically significant.

**Figure 5.2** Predicted output from GAMM with mean number of aphids consumed in response to coccinellid larval stage and aphid life stage for the coccinellid species a) *Adalia bipunctata*, b) *Harmonia axyridis* and c) *Coccinella septempunctata*. Contours and heat map indicate number of aphids consumed and are derived from GAMM output. Contour numbers refer to mean number of aphids consumed. Arrows are qualitative and used to illustrate the direction of feeding preference between coccinellid species. Coccinellid larval stage x aphid life stage = 10

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

69
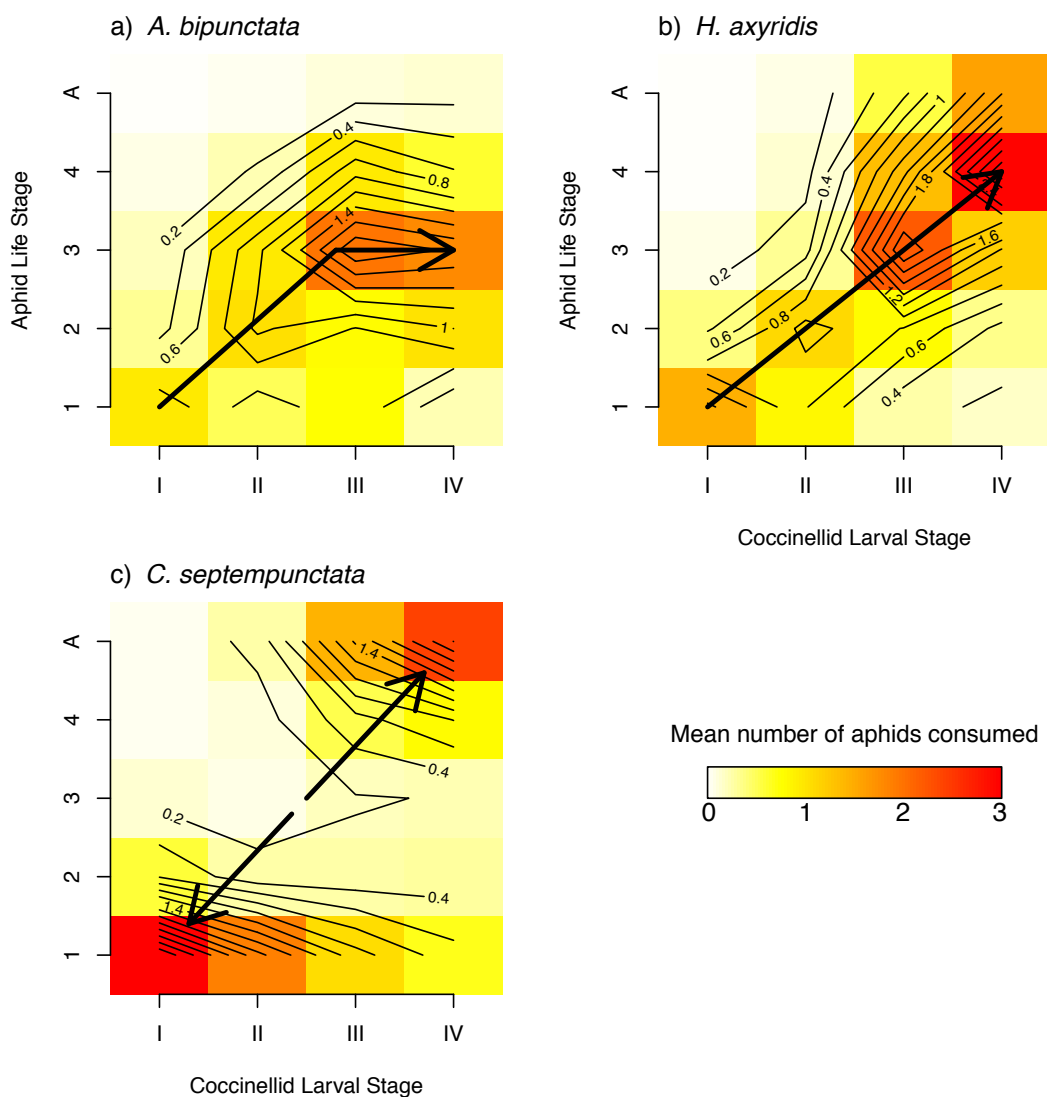
**Table 5.2** GAMM results summary for number of aphids consumed per focal coccinellid species in relation to competing coccinellid species and aphid prey life-stage. Interaction and main effects were evaluated using log-likelihood ratio tests. Entries in bold are statistically significant effect. AL = aphid life stage; CC = competing coccinellid.

| Focal species | Test | Likelihood ratio test (full *vs* reduced) | $\chi^2$ | d.f. | p |
|---|---|---|---|---|---|
| a) *H. axyridis* | Interaction | AL * CC *vs* AL + CC | 0.63 | 12 | 0.996 |
| | **Aphid life stage (AL)** | **AL + CC *vs* CC** | **6.67** | **2** | **0.0355** |
| | **Competing coccinellid (CC)** | **AL + CC *vs* AL** | **9.561** | **3** | **0.0226** |
| b) *C. septempunctata* | Interaction | AL * CC *vs* AL + CC | 2.402 | 12 | 0.879 |
| | **Aphid life stage (AL)** | **AL + CC *vs* CC** | **22.616** | **2** | **<0.0001** |
| | Competing coccinellid (CC) | AL + CC *vs* AL | 7.797 | 3 | 0.05 |
| c) *A. bipunctata* | Interaction | AL * CC *vs* AL + CC | 1.004 | 12 | 0.985 |
| | **Aphid life stage (AL)** | **AL + CC *vs* CC** | **10.64** | **2** | **0.005** |
| | **Competing coccinellid (CC)** | **AL + CC *vs* AL** | **50.42** | **3** | **<0.0001** |

**Figure 5.3** Predicted output from GAMM, proportion of aphids consumed (± S.E.) at each developmental state (instar 1–4, adult) for a) *Adalia bipunctata*, b) *Harmonia axyridis* and c) *Coccinella septempunctata* in response to the presence of other coccinellids.

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

71
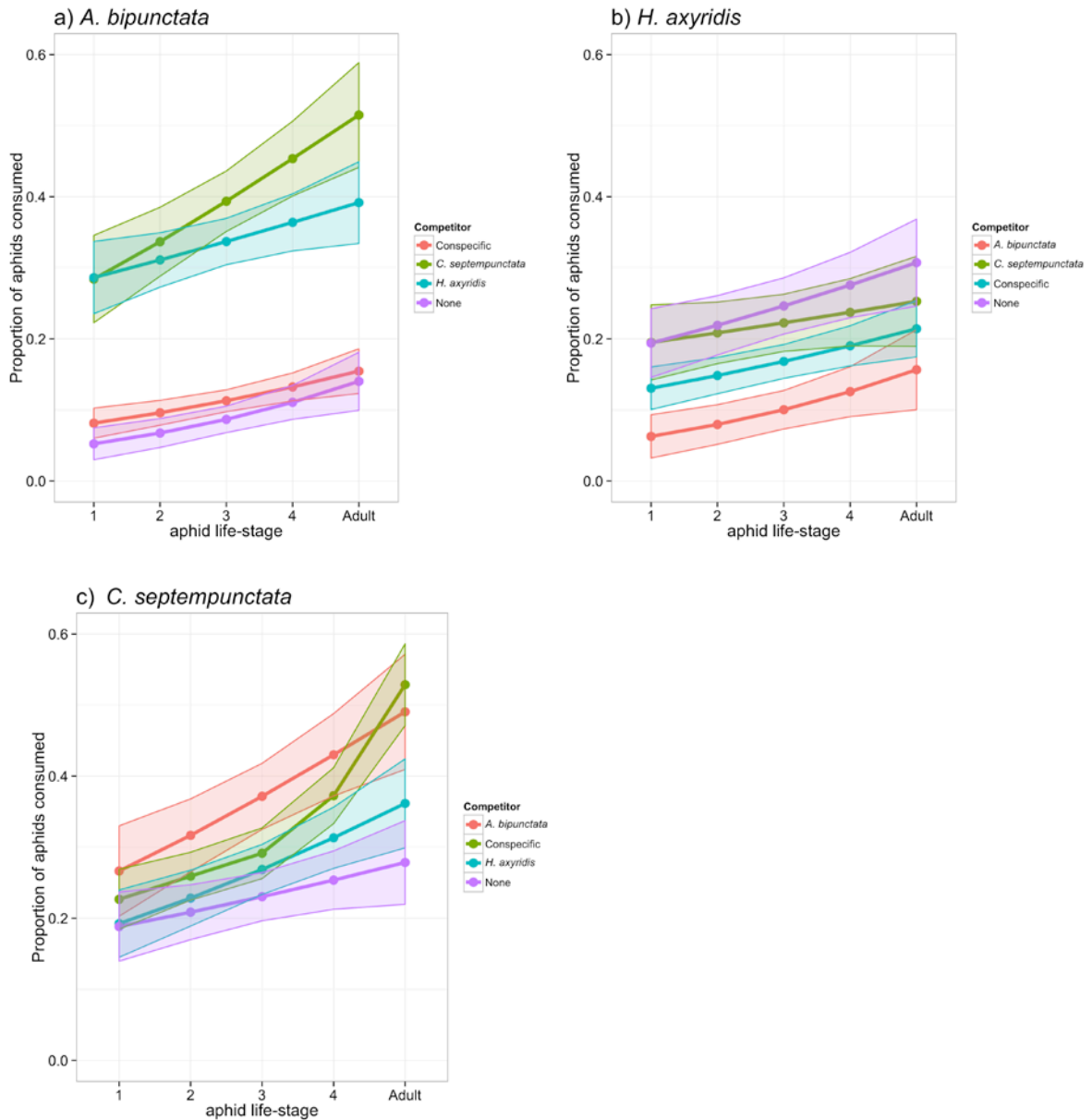
# 5.5 Discussion

## 5.5.1 Interspecific aphid consumption rates

As hypothesised, the results confirm that the invasive *H. axyridis* may be directly competing for aphid resources with at least one endemic and declining coccinellid species (*A. bipunctata*).

Prey choice throughout the development of coccinellids should converge regardless of which species is the intraguild predator or prey. Early instar coccinellid larvae will be limited to early instar aphids due to difficulty in handling larger prey, while later larval instars will be able to handle and prefer nutritionally beneficial larger, late instar aphid prey. This was true for *H. axyridis* and *A. bipunctata*; *C. septempunctata* however, did not, only sharing a similar specificity for aphid life-stages in the early and late larval stages. The feeding preferences of all three species converge at the fourth-larval stage by choosing later aphid life stages, despite the differences in prey choice at intermediate larval stages. Direct interspecific competition and intraguild predation are more likely at the fourth larval-instar due to the voracious nature of late instar coccinellids (Seko & Miura, 2008).

## 5.5.2 Congeneric interactions

Cannibalism is often reported in coccinellid species (Agarwala & Dixon, 1992; Hodek & Evans, 2012) and therefore a behavioural response to conspecific competitors was expected. There was, however, no evidence of this as aphid consumption rate and life stage preference were the same under intraspecific competition as it was for the no competition control (*i.e.* feeding in isolation). During interspecific interactions, *H. axyridis* demonstrated the characteristic feeding behaviour of an intraguild predator by reducing its consumption of aphid prey in the presence of potential intraguild prey *A. bipunctata* (Holt & Polis, 1997; Katsanis *et al.*, 2013). Similarly, consistent with its status as the intraguild prey (Polis & Holt, 1992), *A. bipunctata* had a much higher prey consumption rate in the presence of the invasive and other endemic coccinellid species, whereas, *C. septempunctata* did not display such behavioural plasticity in the presence of the intraguild predator, *H. axyridis*, potentially because it occupies a different ecological niche to *H. axyridis* or *A. bipunctata* (Roy *et al.*, 2012). The feeding rate of *A. bipunctata* was also increased when competing with *C. septempunctata*, confirming previous

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

72

observations that the former may be an intraguild prey for the latter (Sato & Dixon, 2004).

## 5.5.3 Intraguild predation

Intraguild predation between competing predators has the potential to stabilise food web structure (Polis *et al.*, 1989). The dynamics of intraguild predation models have been used to understand better the impact of invasive species (MacNeil *et al.*, 2003). Dynamics of these intraguild predation models suggest either the intraguild predator or intraguild prey becomes extinct, or stability (*i.e.* species coexistence) occurs (Morin, 2011). The precise outcome is dependent on the interplay between various traits, which include: the attack rates on shared prey and on intraguild prey; conversion efficiency of prey; and the mortality rate from sources other than intraguild predation or resource abundance (*e.g.* environmental stochasticity) (Holt & Polis, 1997).

Extinction of the intraguild predator would require density independent mortality of the intraguild predator to be higher than that of the intraguild prey. This is highly unlikely given *H. axyridis* is an invasive species. As an invasive species, *H. axyridis* is currently free from most forms of predation (Soares *et al.*, 2008; Sloggett *et al.*, 2011) and parasitism (Pell *et al.*, 2008) that can impact native species such as *A. bipunctata* and *C. septempunctata*. Additionally, body size, such as that of the larger *H. axyridis*, has been shown to reduce density independent mortality, increasing resilience to abiotic variability such as extreme climatic events (Parmesan *et al.*, 2000).

Extinction of intraguild prey, *A. bipunctata* also seems unlikely based on these results. For this to occur, the attack rate of the intraguild predator on the shared resource must be approximately 10% lower than that of the intraguild prey. In this study it was found the attack rate of *H. axyridis* was approximately 50% lower than that of *A. bipunctata*. In contrast, extinction of the intraguild prey *C. septempunctata* seems very likely given the feeding behaviour observed in this study. This, however, is clearly not the case, as *C. septempunctata* is one of the only native coccinellid species to have not declined since the appearance of *H. axyridis*. Finally, coexistence between intraguild predator and prey may occur, but only between *H. axyridis* and *A. bipunctata*. For coexistence to occur, attack rate of the shared resource by the intraguild predator should be approximately 50% lower compared to the intraguild prey, of which data from this study supports. Additionally, this scenario

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

73

requires the death rate of the intraguild prey to be higher than that of the intraguild predator, which is very likely in this study system.

The use of equilibrium models such as the intraguild predation model used here must be interpreted while considering the caveats of such models. Of the aforementioned parameter values used to predict the outcomes, only empirical data for the attack rate were collected in this study. Whereas the death rate attributed to factors other than intraguild predation could be estimated based on previous work (Hodek *et al*., 2012), other parameters such as the conversion efficiency of prey (*i.e.* reproductive success) can only be determined from long-term studies (Briggs & Borer, 2005). The *a priori* hypothesis, based on theory from which the intraguild predation model was conceived (Polis *et al*., 1989), was supported by this study for the interaction between *A. bipunctata* and *H. axyridis*, but not for *C. septempunctata*. Based on the intraguild predation model, *C. septempunctata* would go extinct when competing with *H. axyridis*, but field evidence suggests the contrary (Roy *et al*., 2012). Additionally, *A. bipunctata* and *C. septempunctata* should not be able to co-exist based on intraguild predation models, but these two species have coexisted for long periods of time (Hodek *et al*., 2012). The habitat occupied by the three coccinellid species used in this study may offer some explanation as to why *C. septempunctata* does not fit within the conceptual framework presented by the intraguild predation models. Both *A. bipunctata* and *H. axyridis* occupy the same arboreal habitat for most of the growing season, whereas *C. septempunctata* occupies more herbaceous habitats (Roy *et al*., 2012). Therefore using intraguild predation models to interpret the interaction between *C. septempunctata* and the other two coccinellids would be erroneous.

## 5.5.4 Conclusions

The study suggests that co-existence could occur between *H. axyridis* and *A. bipunctata* based on the predictions of intraguild predation dynamics; the parameter measured to determine the possibility of co-existence was prey consumption rate. The feeding rate of *H. axyridis* and *A. bipunctata* was, however, plastic, that is, when these two species were not in contact with each other, their feeding rate changed accordingly. When not in a competitive situation the feeding rate of *H. axyridis* increased while that of *A. bipunctata* decreased. Co-existence is only predicted by intraguild predation dynamics model between these two species because of the plastic response caused by the presence of the competitor species. If feeding rate

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

74

between these two species were considered when in isolation (*i.e.* the control treatment), an intraguild predation dynamics model would predict *A. bipunctata* to go extinct when competing against *H. axyridis*. Size of the individual has an impact on the outcome of IGP in coccinellids, the larger individuals being superior (Ware & Majerus, 2008). The results of this study do not reject the hypothesis that the coccinellid species were responding to competitor size rather than species. Heterospecific coccinellid faeces have, however, been shown to alter feeding and oviposition, suggesting olfactory species identification occurs (Agarwala *et al*., 2003). Future work with different larval stages (and therefore sizes) of coccinellids would be required to determine how the coccinellids identify competing species. Despite evidence of *H. axyridis* consuming *A. bipunctata* (Thomas *et al*., 2013), at no point prior to the experiment did any coccinellid species come in contact with each other; therefore the change in feeding rate observed in *H. axyridis* and *A. bipunctata* to other coccinellid species is evidence of behavioural plasticity. This is further supported by the fact that *A. bipunctata* feeding rate was not affected by the presence of a conspecific, highlighting the cost incurred by altering behaviour in response to potential predators. This type of plastic behavioural response to different predators is commonly seen in many organisms subject to predation (Relyea, 2001) and demonstrates the significant cost incurred by its occurrence.

Given the significant declines of *A. bipunctata* in Europe (Roy *et al*., 2012) and the lack of field evidence of direct predation (Smith & Gardiner, 2013), although gut analysis confirms it does occur (Thomas *et al*., 2013), changes to coccinellid feeding behaviour may be contributing to the declines in *A. bipunctata*.

This study provides some potential mechanistic insights from controlled laboratory based experiments to contribute to understanding native and invasive predator population dynamics. Biological invasions can be monitored by citizen science projects, creating large datasets, such as the invasion of *H. axyridis* to the UK (Roy *et al*., 2012), but the causal agents facilitating this invasion remain elusive. Theoretical models can be used to estimate the outcomes of biological invasions, but a much larger set of parameters is required to increase accuracy of such predictions. For the study system used here, long-term data spanning multiple generations for the conversion efficiency of prey by coccinellids is needed to complete the picture. This study is the first example of where a plastic behavioural

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

75

feeding response has been observed in both native and invasive species, which could potentially allow for their coexistence.

Chapter 5: Evidence the antagonistic interactions between an invasive and native coccinellid species may result in coexistence.

76

# Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

## 6.1 Abstract

Drought episodes are predicted to increase under global climate change scenarios. Conditions of reduced water generally lead to lower aboveground dry mass and cause water stress within plant tissue. The response of invertebrate herbivores to drought conditions is guild-specific and generally known. The Plant Stress Hypothesis predicts that some herbivore guilds (*e.g.* aphids) will benefit from plants under drought stress due to increased availability of nitrogenous material in plant tissue. How their associated natural enemies will respond to drought conditions remains largely unknown.

Using a woody perennial, the European red raspberry, this study investigated the impact of drought on multiple trophic levels, specifically the colonisation and abundance of invertebrate herbivores and their associated natural enemies. Plants were exposed to normal or drought watering regimes where soil moisture was maintained at approximately 40% and 20%, respectively.

Aboveground plant dry mass was reduced in the drought treatments, while belowground dry mass remained unaffected. Colonisation and abundance of aphids, and damage caused by chewing herbivores was not significantly affected by drought treatment. Aphid colonisation was, however, higher on plants with the greatest aboveground dry mass; plants in the drought treatment, which had reduced dry mass, had lower aphid densities. Aphid natural enemy colonisation was generally unaffected by drought treatment but was more likely on plants with the greatest aboveground dry mass. Lacewings, a known predator of aphids, were an exception to this, where their colonisation was reduced on plants under drought conditions.

This study provides further evidence to reject the Plant Stress Hypothesis because herbivore abundance and colonisation were not impacted by drought treatment. Aboveground plant dry mass is, however, a good predictor of both aphid and natural enemy colonisation, which was negatively affected by drought treatment. This work suggests drought is impacting both herbivore and natural enemy colonisation through changes in plant size, rather than plant stress, which may affect the quality, quantity or availability of food resources.

Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

77

## 6.2 Introduction

Global change has the potential to alter significantly the composition of natural communities (Parmesan, 2006). Indeed, its impact is already being felt at a community level in terms of, for example, phenological changes (Menzel & Dose, 2005), mismatch (Visser & Both, 2005), and range shifts (Parmesan *et al*., 1999). In addition to the many direct effects of climate change on ecological communities, these changes in abiotic conditions can affect trophic groups indirectly, especially via plant-mediated mechanisms (Facey *et al*. 2014). Most aspects of global change will influence plants, the base of food webs. Global change can alter host plant quality by, for example, reducing nutritional value of plant tissue (Lincoln *et al*., 1986) and palatability (Stiling & Cornelissen, 2007; Ballare *et al*., 2011), which can have a knock-on effect higher up the food chain. There is a growing understanding of how global change will impact plant–herbivore interactions, with some generalisations becoming possible (Zavala *et al*., 2013). For example, increases in temperature are beneficial to most insect herbivores (Bale *et al*., 2002), whereas elevated $CO_2$ is generally (phloem-feeders being the exception) detrimental to insect herbivores (Robinson *et al*., 2012). However, there remains a dearth of research into higher trophic (*e.g.* predator–prey) and more specifically multi-trophic (*e.g.* tri-trophic) interactions (Van der Putten *et al*., 2010).

The complex nature of multi-trophic, plant–herbivore–predator, interactions has limited the number of studies undertaken. The Menge–Sutherland Hypothesis (Menge & Sutherland, 1987) suggests that abiotic stress will have a greater effect on higher tropic levels than lower ones. Abiotic stress caused by global change will therefore have significant impacts on higher trophic levels. Of the predicted changes to the abiotic environment, increased temperature and atmospheric carbon dioxide ($CO_2$) are probably understood best. Under elevated atmospheric $CO_2$, parasitism generally increases (Stiling *et al*., 1999; Sun *et al*., 2011a) or remains unaffected (Roth & Lindroth, 1995; Holton *et al*., 2003). Temperature increase shows a similar pattern to elevated $CO_2$ (although acting through different mechanisms) with parasitism rates increasing (Hance *et al*., 2007; Guay *et al*., 2009) or remaining unaffected (Klapwijk *et al*., 2010; Bannerman *et al*., 2011). Reduced precipitation can have the opposite effect on multi-trophic interactions, with drought conditions generally reducing parasitism (Calatayud *et al*., 2002; Aslam *et al*., 2013). This can occur through several mechanisms, including de-coupling of predator–prey

Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

78

populations, altered prey population demography (*e.g.* Aslam *et al.*, 2013) and altered prey quality (*e.g.* Thomson *et al.*, 2010; Johnson *et al.*, 2011).

The frequency of drought events is predicted to increase (IPCC, 2013). In northern Britain, for example, incidence of summer droughts is predicted to increase significantly over the next few decades (Christensen *et al.*, 2007). Increases in either the frequency or duration of droughts can alter the structure and composition of natural communities (Kallis, 2008; Dai, 2011). Water stress in plants as a result of drought conditions can increase herbivore abundance by not only increasing the availability of nutrients, and specifically nitrogenous compounds, in plant tissue (White, 1974, 1984), but also by reduced plant investment in secondary metabolites associated with plant defence (Rhoades, 1985). In terms of nutritional improvements, drought can lead to impairment of water uptake which causes degradation of mesophyll cells in the foliage, releasing nitrogen compounds into the phloem (Brodbeck & Strong, 1987), a process called the Plant Stress Hypothesis (White, 1974). While phloem-feeding insects were initially regarded as beneficiaries of these changes, mainly because of increased availability of amino acids (such as glutamic acid, proline, isoleucine) in the phloem (Mewis *et al.*, 2012), the Plant Stress Hypothesis has generally not received wide support from evidence from other feeding guilds (Larsson, 1989; Koricheva *et al.*, 1998). Moreover, meta-analysis has shown that sap-feeding insects are regarded as benefitting from plant stress only when plants are in a recovery phase and turgor has returned to plant tissue (Huberty & Denno, 2004).

Whether herbivore populations will increase in future drought scenarios will also depend on the response to altered precipitation patterns of higher trophic levels, which regulate insect herbivore populations. The impact of drought on natural enemies remains little understood (Calatayud *et al.*, 2002). Research has focused on the negative response of parasitoids to drought conditions (Johnson *et al.*, 2011; Aslam *et al.*, 2013); very little, if any, attention has been given to other natural enemy species such as coccinellids or Chrysopidae (lacewings). Natural enemies, particularly parasitoids, exploit the plant release of volatile organic compounds to find plants infested with potential prey, thereby defending the plant indirectly (Fatouros *et al.*, 2008; Dicke & Baldwin, 2010). The composition and volume of volatiles produced by a plant experiencing herbivory can be altered by drought stress (Holopainen & Gershenzon, 2010). This could have a significant impact on

Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

79

the ability of plants to attract natural enemies in field conditions. There is, however, little empirical field evidence to suggest that drought may alter recruitment of natural enemies. If the effectiveness of natural enemies is reduced, pest outbreaks may increase if drought events become more common under climate change (Thomson *et al*., 2010).

When woody plants experience drought stress, the response of resident herbivores depends on the feeding guild, with boring and sucking insects performing better, and galling and chewing invertebrates adversely affected (Koricheva *et al*., 1998). The highly variable response of insect herbivores on woody plants leaves the response of their natural enemies largely unknown. Moreover, most field studies apply summer drought (*e.g.* Johnson *et al*., 2011), whereas seasonal shifts in rainfall are also predicted (IPCC, 2013). Changes in rainfall pattern in autumn could have significant impacts on aphid populations. In particular, the "see-saw effect" suggests that when aphid populations are low in the autumn, competition is reduced during the following spring, resulting in greater aphid populations during the summer (Dixon, 1973).

This study investigates whether exposing a woody perennial, the European red raspberry (*Rubus idaeus* L.), to drought conditions, can alter the recruitment and abundance of insect herbivores and their associated natural enemies. It was hypothesised that drought will reduce 1) plant dry mass, and 2) the colonisation of certain herbivore feeding guilds such as aphids, but increase colonisation by others, such as chewing herbivores. It was also hypothesised that 3) recruitment of natural enemies will be reduced by drought treatment.

## 6.3 Methods

### 6.3.1 Host plant

The European red raspberry (*R. idaeus*) cultivar (Glen Ample) used in this experiment is a common, commercially available raspberry cultivar. Glen Ample possesses the resistance gene ($A_1$), which is largely ineffective against large raspberry aphid (*Amphorophora idaei* (Börner)) herbivory (Birch *et al*., 2004). Despite this, high yields have ensured both commercial and domestic growers are still using this cultivar. Plants were grown from root-stock at the James Hutton Institute (JHI), Dundee, UK. When approximately 1 cm in height, plants were

transported to the Centre for Ecology and Hydrology (CEH), Wallingford, UK. There, the plants were potted-out into 5 L pots using a 1:1 mix of peat-based compost (Levington M3) and fine sand. The plants were given three weeks prior to the experiment to adapt to new soil conditions and watered daily. A total of 26 plants were used throughout the experiment.

## 6.3.2 Aphids

The specialist phloem-feeding herbivore, the large raspberry aphid (*A. idaei*), is found only on the European red raspberry (McMenemy *et al*., 2009). To simulate an established field population that would have already been present on the plants, five adult large raspberry aphids were placed on the second unfurled leaf of each plant at the start of the drought experiment. The aphid culture was initiated from field-collected aphids at JHI and maintained in a CEH laboratory for multiple generations.

## 6.3.3 Experimental design

The experiment took place in a large modified Polytunnel (Fig. 6.1). The sides were removed creating a 1 m gap between the roof and floor, which allowed a constant flow of ambient air throughout the experimental area ensuring temperature under the shelter was consistent with ambient temperatures. The tunnel was fitted with anti-fog polythene with 95% light transmission (First Tunnels Ltd, Lancashire, UK). Individual plants were randomly assigned to a watering treatment and situated in a randomised blocked design. Watering was delivered by a dripper irrigation system. Soil moisture in control plants was maintained at approximately 40% and drought treatment plants were maintained at approximately 20% of field capacity.

Thirty-six plants were destructively harvested (18 drought, 18 control) every two weeks for 12 weeks. At each harvest interval the following were recorded: plant metrics (dry mass), the abundance (counts) of large raspberry aphid, the colonisation (%) by other herbivore species, the visible damage (score 0–5, 0 being no damage) caused by chewing herbivory and abundance (counts) of predatory invertebrates.

## 6.3.4 Statistical analysis

All data were analysed using generalised linear mixed models using PROC GLIMMIX, version 9.2 (SAS Institute, Cary, North Carolina). Above- and belowground plant dry mass were modelled using a normal (Gaussian) error distribution with identity-link function. Aphid abundance was modelled using a

Poisson error distribution and log-link function. Presence/absence of colonising aphids, evidence of chewing herbivory (score of 0–5) and natural enemies were modelled using a binomial error distribution with a logit-link function. Position within the rain shelter (experimental block) was used as a random term to account for spatial variance within the rain shelter. To control for over-dispersion in the data, an observation (plant replicate) level term was added to the random effects (Elston *et al.*, 2001).

**Figure 6.1** Image of modified Polytunnel used as the rain shelter before the start of the drought experiment. Dimensions of width, length and height of gap in plastic are indicated.



## 6.4 Results

Drought treatment significantly reduced soil moisture ($F_{1,160} = 305.22$, p<0.0001) throughout the study period. Aboveground plant dry mass was lower in the drought treatment compared to the control plants (Table 6.1a); irrespective of drought treatment, aboveground dry mass was highly variable over time (harvest day, Table 6.1a). Aboveground dry mass initially increased over the first four harvest days and then decreased for the final two harvests (Fig. 6.2a). Belowground, plant dry mass was not influenced by watering treatment or aphid abundance (Fig. 6.2b, Table 6.1b). Unlike the highly variable aboveground dry mass, belowground dry mass consistently increased over time (Fig. 6.2b, Table 6.1b). Above- and belowground dry masses were highly correlated (Spearmen's rho$_{(213)}$ = 0.291, P<0.0001).

**Table 6.1** GLMM results summary for plant response parameters. Entries in bold retained in final model. MPE, multiple parameter estimates.

| Response variable | Explanatory | Estimate | $F_{(ndf,\ ddf)}$ | $P$ |
|---|---|---|---|---|
| a) Aboveground dry mass | **Drought treatment** | | **4.53$_{(1,160)}$** | **0.0349** |
| *Random effect estimate (var):* | *Control, drought* | *0.730, 0* | | |
| *Experimental block = 6.297 ± 0.707* | **Harvest day** | **MPE** | **11.11$_{(5,47)}$** | **<0.0001** |
| | Aphid abundance | 0.0114 | 0.04$_{(1,207)}$ | 0.841 |
| b) Belowground dry mass | Drought treatment | | 1.02$_{(1,207)}$ | 0.313 |
| *Random effect estimate (var):* | *Control, drought* | *0.0967, 0* | | |
| *Experimental block = 0 ± 0* | **Harvest day** | **MPE** | **29.82$_{(5,47)}$** | **<0.0001** |
| | Aphid abundance | 0.0004 | 0.004$_{(1,207)}$ | 0.841 |

Large raspberry aphid abundance at harvest was not affected by drought treatment, aboveground dry mass or harvest day (Fig. 6.3a, Table 6.2a). In addition to large raspberry aphid populations, 24% of the plants were also colonised by other aphid species, mainly *Myzus persicae* (Sulzer). Watering treatment did not influence colonisation by these aphid species, but colonisation was more likely to happen on plants with the greatest aboveground dry mass (Fig. 6.3b, Table 6.2b). Similarly, the total abundance of all aphid species, was not impacted by drought treatment, but plants with greater aboveground dry mass carried a higher abundance of aphids (Fig. 6.3c, Table 6.2c). Presence of herbivore species other than aphids was very limited. Chewing herbivores were found on eight plants and while chewing damage was obvious on nearly all plants, the extent of the damage was not affected by drought treatment (Fig. 6.3d, Table 6.2d). Insect predators (coccinellids, lacewing and hoverfly larvae) and hymentopteran parasitoids were found on 57% of the plants. Similar to total aphid abundance, natural enemies were more common on plants with greater aboveground biomass (Fig. 6.3a, Table 6.3a).

Colonisation by natural enemies was higher (34%) on control plants than on plants receiving the drought treatment (20%), only just failing statistical significance at the 95% confidence interval (Fig. 6.4, Table 6.3a). Of the observed predatory species, lacewings were the most common, found on 53% of the plants where predation occurred. Identification at the larval stage was not possible during harvest, but adult common green lacewings (*Chrysoperla carnea* (Stephens)) were frequently

Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

83

observed within the rain shelter. Lacewing larvae were significantly more common on the control plants than on the drought treatment plants (Fig.6.4, Table 6.3b).

**Figure 6.2** Plant dry mass along a linear predictor scale a) aboveground and b) belowground predicted from a GLMM at different harvest intervals. Plants were exposed to either control (circles, dashed line) or drought (squares, solid line) watering treatments.



Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

84

**Table 6.2** GLMM results summary for herbivore response parameters. The large raspberry aphid (LRA) was experimentally introduced to all plants. Aphid colonisation refers to naturally colonising aphid species. Evidence of chewer refers to damage on plants by naturally colonising chewing herbivores, scored 0–5, 0 being no evidence. Entries in bold retained in final model. MPE, multiple parameter estimates.

| Response variable | Explanatory variables | Estimate | $F_{(ndf, ddf)}$ | $P$ |
|---|---|---|---|---|
| b) LRA abundance | Aboveground dry mass | 0.043 | $2.26_{(1,195)}$ | 0.1344 |
| *Random effect estimate (var):* | Drought treatment | | $0.20_{(1,150)}$ | 0.6563 |
| | *Control, drought* | *-0.0606, 0* | | |
| *Experimental block = 0.625 ± 0.181* | | | | |
| *Replicate = 0.732 ± 0.108* | Harvest day | MPE | $1.47_{(5,43)}$ | 0.2206 |
| | Initial aphid abundance | 0.008 | $0.5_{(1,161)}$ | 0.6163 |
| b) Aphid colonization | **Aboveground dry mass** | **0.278** | **$19.30_{(1,212)}$** | **<0.0001** |
| *Random effect estimate (var):* | Drought treatment | | $2.22_{(1,150)}$ | 0.1374 |
| | *Control, drought* | *0.517, 0* | | |
| *Experimental block = 0.291 ± 0.381* | | | | |
| | Harvest day | MPE | $1.49_{(5,51)}$ | 0.2088 |
| | Initial aphid abundance | 0.0445 | $1.43_{(1,211)}$ | 0.2335 |
| c) Total aphid abundance | **Aboveground dry mass** | **0.0821** | **$7.67_{(1,198)}$** | **0.0061** |
| *Random effect estimate (var):* | Drought treatment | | $0_{(1,150)}$ | 0.9936 |
| | *Control, drought* | *-0.001, 0* | | |
| *Experimental block = 0.527 ± 0.163* | | | | |
| *Replicate = 0.871 ± 0.123* | Harvest day | MPE | $1.05_{(5,47)}$ | 0.3976 |
| | Initial aphid abundance | 0.0291 | $1.67_{(1,165)}$ | 0.0964 |
| d) Evidence of chewer | Aboveground dry mass | -0.0127 | $0.16_{(1,212)}$ | 0.6889 |
| *Random effect estimate (var):* | Drought treatment | | $0.6_{(1,212)}$ | 0.4382 |
| | *Control, drought* | *0.128, 0* | | |
| *Experimental block = 0.106 ± 0.092* | | | | |
| | **Harvest day** | **MPE** | **$3,84_{(5,54)}$** | **0.0047** |
| | **Initial evidence of chewer** | **0.754** | **$4.37_{(1,207)}$** | **0.0377** |

Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

85

**Table 6.3** GLMM results summary for predator response parameters. Entries in bold retained in final model. MPE, multiple parameter estimates.
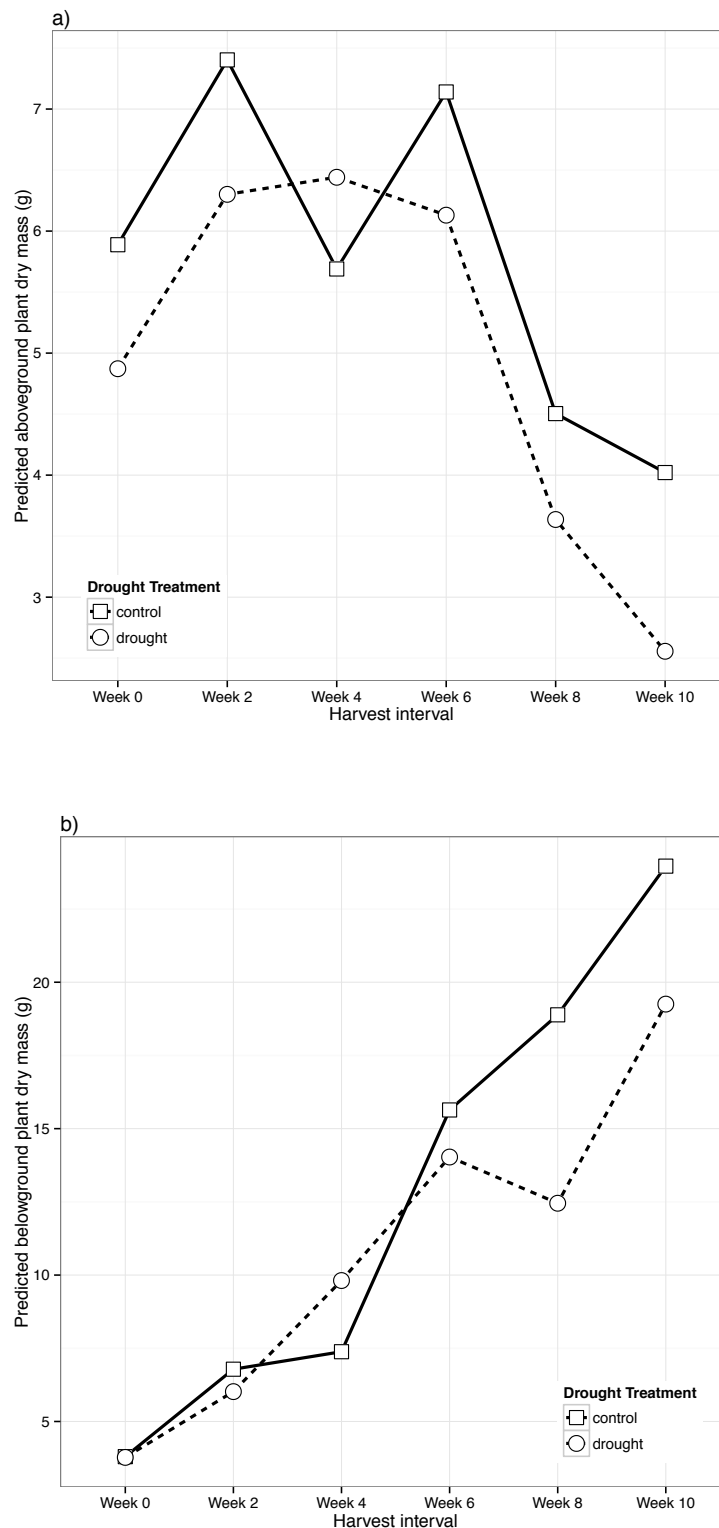
| Response variable | Explanatory variables | Estimate | $F_{(ndf, ddf)}$ | $P$ |
|---|---|---|---|---|
| a) Evidence of predation | **Aboveground dry mass** | **0.151** | **$7.6_{(1,212)}$** | **0.0063** |
| *Random effect estimate (var):* | Drought treatment | | $3.8_{(1,210)}$ | 0.0527 |
| *Experimental block = 0.0412 ±* | *Control, drought* | *0.636, 0* | | |
| *0.259* | Harvest day | MPE | $10.96_{(5,55)}$ | 0.4489 |
| | Aphid abundance | -0.0069 | $0.73_{(1,211)}$ | 0.3925 |
| | Pre–treatment predation | -0.755 | $2.09_{(1,211)}$ | 0.1502 |
| b) Lacewing | **Aboveground dry mass** | **0.2919** | **$12.81_{(1,211)}$** | **0.0004** |
| *Random effect estimate (var):* | **Drought treatment** | | **$6.63_{(1,211)}$** | **0.0107** |
| *Experimental block = 0.975 ±* | *Control, drought* | *1.248, 0* | | |
| *0.619* | Harvest day | MPE | $0.44_{(5,129)}$ | 0.8219 |
| | Aphid abundance | -0.013 | $0.88_{(5,210)}$ | 0.3487 |

Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

86

**Figure 6.3** Aphid responses based on GLMM predictions. a) Large raspberry aphid abundance between plants exposed to control and drought watering treatments. b) Logistic regression showing the probability of aphid colonisation with increasing aboveground plant dry mass. Dashed line indicates the upper and lower boundaries of 95% confidence intervals. c) Aphid abundance in response to plant aboveground dry mass. d) Damage caused by chewing herbivory on plants exposed to control or drought watering treatments.

Chapter 6: The effect of late season drought on colonisation of *Rubus idaeus* by herbivorous invertebrates and their natural enemies

87
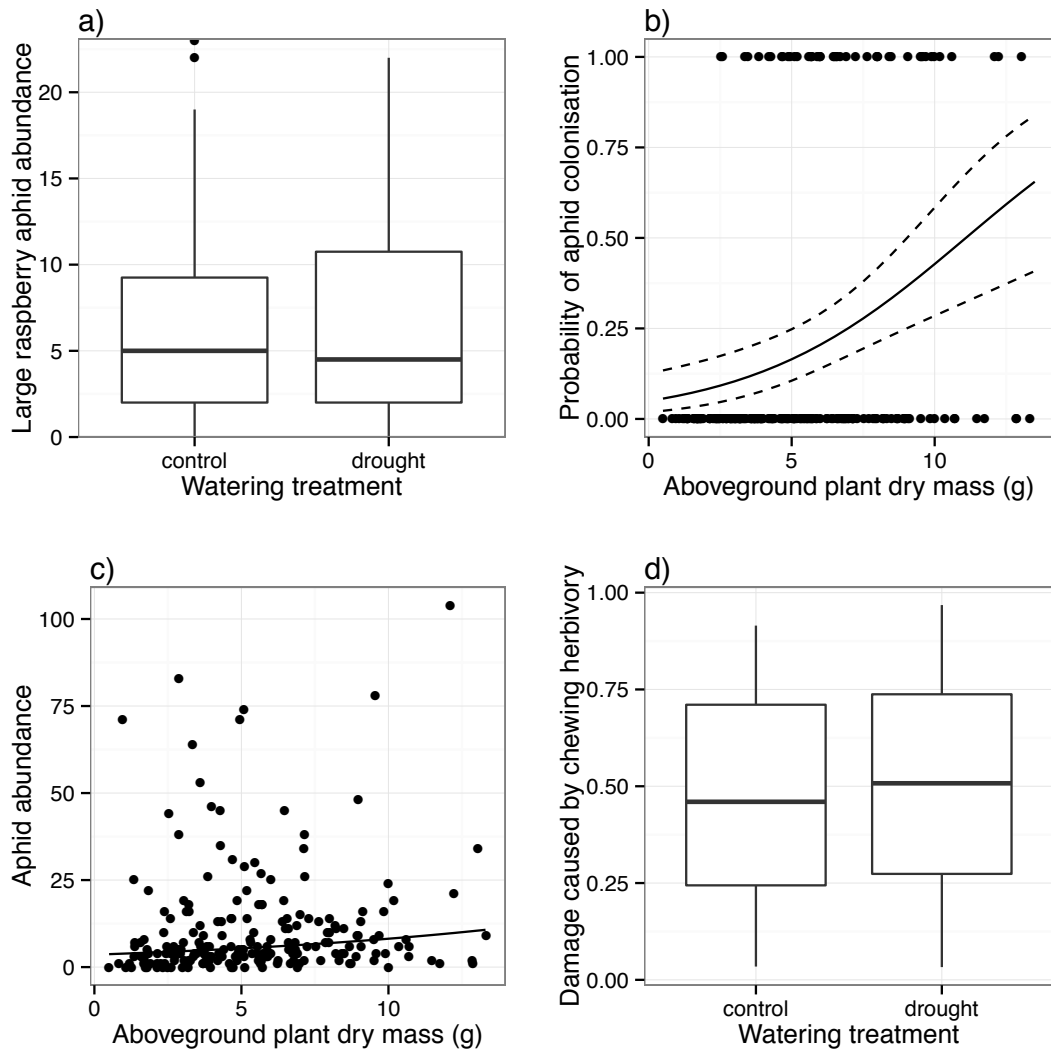
**Figure 6.4** The probability of colonisation by a) predatory invertebrates and b) lacewing predators in response to aboveground plant dry mass and watering treatment. Red, control treatment, green, drought treatment. Shaded area denotes 95% confidence intervals derived from the GLMM.
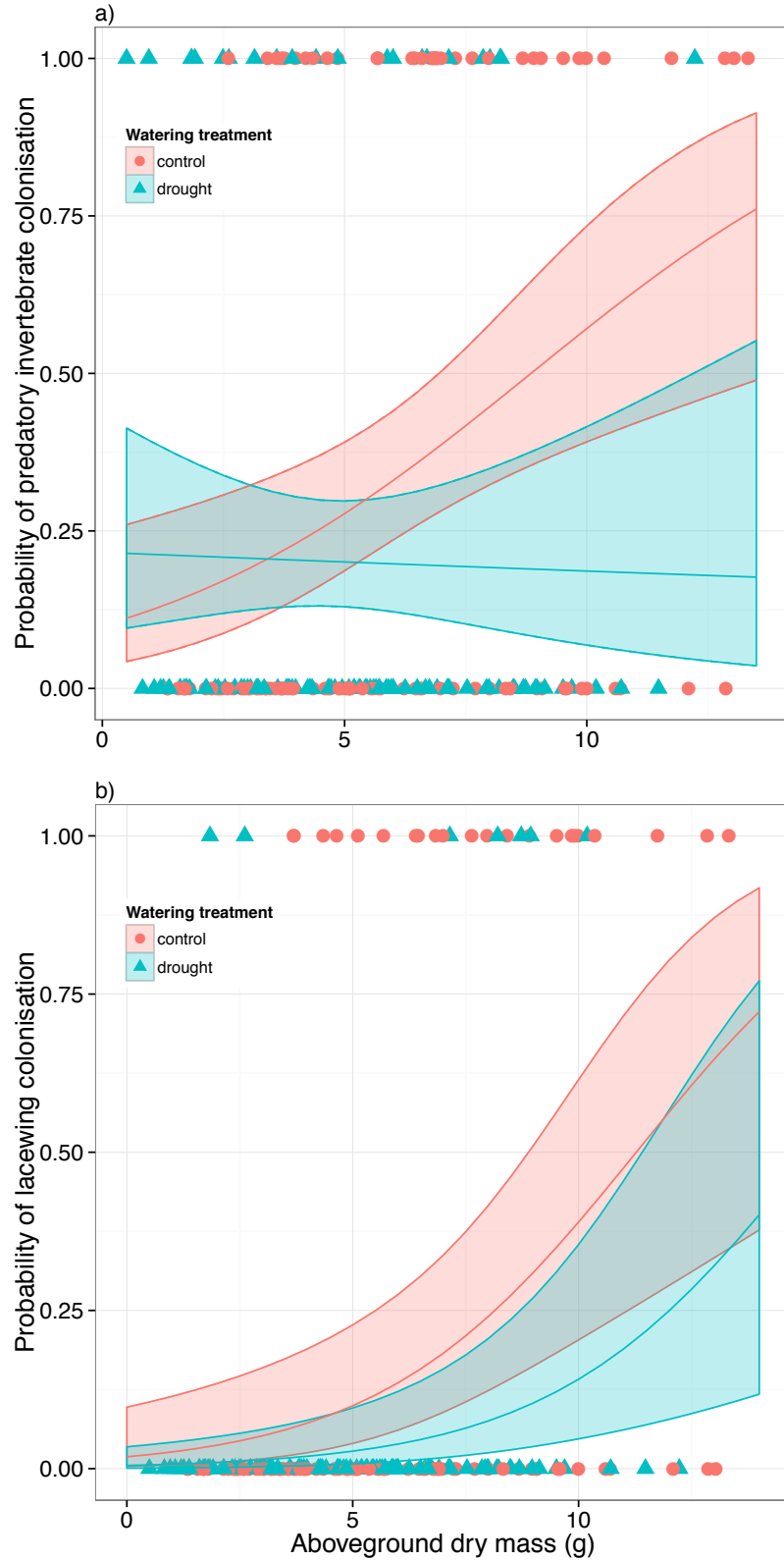
# 6.5 Discussion

Plants experiencing drought conditions (<20% soil moisture) are predicted to have reduced growth rate and dry mass compared to plants experiencing a normal watering regime (Dai, 2011). This is supported by the current study, raspberry plants had significantly lower dry mass when experiencing drought conditions. Root dry mass, however, was not affected by drought treatment; this is contrary to previous studies where root mass generally increases under drought conditions due to increased exploration for moisture (Hoogenboom *et al*., 1987). These other studies, however, were conducted with annual cereals, which have limited capacity for extra root growth to access a lower water table in the short term. The present study used a woody perennial crop which probably has a much greater capacity to maintain root growth as it attempts to access limited amounts of water (Johnson *et al*., 2011; Aslam *et al*., 2013).

Large raspberry aphid abundance was not affected by drought treatment and colonisation by herbivores was not influenced by drought treatment, contrary to Hypothesis 2. Previous studies have found the opposite, where aphid abundance decreases when host plants are exposed to drought conditions (Gregory, 2006). Plants experiencing drought conditions were less likely to have natural enemies present than control plants, albeit this was marginally non-significant. This drought effect on presence of natural enemies was, however, mainly driven by a reduction in lacewing abundance, suggesting that drought may have been impairing recruitment of this predator. Drought treatment may have altered morphological features (*i.e.* plant size) used as cues by natural enemies and they are responding to these changes, rather than altered recruitment through chemical changes to the plant. Future work must aim to determine changes to plant chemistry under drought conditions and the importance this change may have on the orientation behaviour of natural enemies.

The variation in dry mass over time (harvest interval) was not consistently linear. Aboveground plant dry mass initially increased, then declined towards the end of the experimental period. Despite this variability over time, plants with a normal watering regime had, as predicted, a greater aboveground dry mass. This reduction in dry mass over time could be explained by the season in which the experiment took place. The drought experiment started in peak summer and ran until mid-autumn; therefore towards the end of the experimental period, plant growth will have stopped

in preparation for winter dormancy (Dixon, 1973). This, however, was not mirrored belowground where root dry mass was unaffected by drought and accrued biomass linearly over time. Host plants were experiencing seasonal changes in addition to an altered watering regime during this study. The recruitment of herbivores and their natural enemies at the end of the growing season has rarely been investigated, but may have a significant impact on the next year's productivity (Menzel & Fabian, 1999). It was demonstrated, however, that drought is unlikely to impact colonisation by herbivores at the end of the growing season, suggesting herbivore populations at the beginning of the following season will not be altered by the previous season's drought experience.

Despite the effect of time on plant biomass, harvest interval had little or no impact on aphids or their natural enemies, suggesting these populations were static and not increasing over time. Once colonisation took place, aphids reached a certain density and population growth stopped, regardless of drought treatment. This was not the case for chewing herbivores where damage to plants increased over the harvest intervals. Chewing herbivores were observed directly on only 12% of the plants, but damage caused by chewing herbivory was observed on most plants. This may indicate many of the chewing herbivores are nocturnal (*e.g.* weevil species.), or are highly mobile and capable of consuming material from multiple plants. In addition, damage caused by chewing herbivory will not disappear over time; it was not possible to date the damage caused by chewing herbivores therefore the increase in damage from chewing herbivory over time reflects the additive effect, possibly by multiple herbivores, over time.

Natural enemies were found on 57% of plants, a low percentage given that all plants had aphid infestations. Given the high mobility and efficacy in locating prey of many invertebrate natural enemies, such as parasitoids, a higher percentage of plants might have been expected to support natural enemies. Plants that were experiencing normal watering conditions (*i.e.* 40% soil moisture) appeared more likely to have natural enemies present. Aphid abundance was not influenced by drought treatment; this suggests predatory invertebrates such as lacewings were responding to cues other than prey density. Both aphid and natural enemy colonisation was more likely on plants with the greatest aboveground biomass. Larger plants may have offered a larger "target" for colonising invertebrates; alternatively, smaller plants may be the result of high levels of plant stress, possibly

from drought, resulting in biochemical changes within the plant. Additionally, colonisation by natural enemies was reduced by drought treatment, albeit marginally. Herbivore-induced plant volatiles, which attract natural enemies (Crawley, 1989), can change composition under drought conditions, altering the foraging behaviour of natural enemies (Gouinguené & Turlings, 2002). Although it was not possible to measure plant volatile emission in this study, the combined effect of aboveground dry mass and drought treatment on the colonisation of natural enemies, suggest plant chemistry may have been altered by drought treatment. Further work is required to determine the interactive effect between drought and aboveground dry mass on plant volatile emission.

## 6.5.1 Conclusions

The insensitivity of herbivore abundance and colonisation to drought conditions provides further empirical evidence to reject the plant stress hypothesis. However, this study confirms that the recruitment of herbivore natural enemies by plants is impaired by drought conditions. In particular, predatory lacewings appear to be the most drought-susceptible natural enemy and this may have considerable ramifications given its importance in biological control (James, 2003; Heil, 2008). Further research is required to understand the mechanisms behind this pattern. Research into the impact of global change on the colonisation of natural enemies is rare, particularly on perennial plant species such as raspberries. These perennial species will normally have a baseline population of aphid herbivores (Daane *et al*., 1996); changes to the host plant will therefore have minimal impact on herbivore populations. Natural enemies, however, which do not remain on the plant throughout the year, will rely on host plant cues to locate their prey, so any changes to the host plant will disproportionately impact natural enemies.

# Chapter 7: Discussion

## 7.1 Major findings

There are a range of raspberry genotypes along a gradient of resistance under ambient climatic conditions: no resistance, $A_1$, multigenic, $A_{10}$, $A_{10+k4a}$ (cv. Octavia). These resistant genotypes have been developed over the last 50 years, ranging from multigenic and $A_1$ in the 1960s, $A_{10}$ in 1980s and then more recently $A_{10+k4a}$ in the early 2000s (Briggs, 1965; Hall *et al*., 2009; McMenemy *et al*., 2009). It is only the most recent and also most resistant plant genotype ($A_{10+k4a}$ or Octavia) that currently proves effective against aphid herbivory (Chapter 2). The short generation time of aphid herbivores allows for rapid adaptation (McMenemy *et al*., 2009), which has left only this most recently discovered genotype completely resistant to aphid herbivory.

When grown under conditions of elevated atmospheric $CO_2$ (e$CO_2$) (550 ppm as projected for 2050 (IPCC, 2013)), plant resistance to *Amphorophora idaei* (Börner) was generally reduced, particularly in the most resistant genotype. While overall aphid abundance still remained very low on the resistant genotype, colonisation significantly increased (Chapter 3). Such small populations under strong, plant mediated, selection pressure may create an opportunity for rapid adaptation, which could result in even this most resistant genotype becoming ineffective against *A. idaei* within this century. Furthermore, even the occurrence of small aphid populations can have a profound influence on plant disease incidence, for instance, virus transmission from an aphid vector to the host plant can occur within two minutes of the onset of feeding (Stace-Smith, 1955). Plant genotype specific responses have been seen under e$CO_2$ in other systems, such as aphids feeding on different lucerne (*Medicago sativa* L.) and tall fescue (*Schedonorus arundinaceus* Schreb.) genotypes (Johnson *et al*., 2014; Ryan *et al*., 2014 respectively). In particular, some lucerne genotypes were found to increase in aphid resistance whereas other genotypes decreased in resistance when grown in an e$CO_2$ environment (Johnson *et al*., 2014). Unlike previous studies that have looked at *Rubus* plant genotype and aphids herbivory in a pairwise interaction, in this thesis it was found the addition of a coccinellid predator removed any advantage that aphids had, due to reduced plant resistance maintaining aphid densities at ambient levels (Chapter 3).

These results provide further evidence that future enhanced levels of atmospheric $CO_2$ have the potential to modify top-down processes via plant-mediated change in aphid behaviour. The escape responses of individual aphids to the alarm signals from conspecifics under predation were significantly reduced under $eCO_2$ (Chapter 4). This suggests that although aphid abundance on susceptible plant genotypes may remain the same under $eCO_2$, the effectiveness of top-down processes (predation by natural enemies such as coccinellids) will increase due to the reduced level of escape response by aphids.

Among many other factors, climate change is likely to increase the occurrence and distribution of invasive species (Walther *et al*., 2009). For the system being studied, *Harmonia axyridis* (Pallas), an invasive predatory coccinellid, was found to have a significant impact on both aphid prey but also the behaviour of native coccinellid species. In this study, *H. axyridis* is a dominant intraguild predator and has the potential to reduce the effectiveness of top-down processes controlling aphid abundance, by consuming other coccinellid species (Chapter 1.1.2). Despite reports of *H. axyridis* threatening many native coccinellids with extinction, one native species, *Coccinella septempunctata* L., has not declined (Roy *et al*., 2012). *Coccinella septempunctata* by occupying a different feeding habitat for most of the year may create a refuge from competitive interactions with *H. axyridis* (Chapter 5). Another native coccinellid, *Adalia bipunctata* L.*,* has, however, significantly declined since the appearance of *H. axyridis* in the UK (Roy *et al*., 2012). The work presented here suggests *A. bipunctata* alters its feeding behaviour in response to *H. axyridis* (Chapter 5). Based on this observation this shift in feeding behaviour could result in these two species coexisting.

Invasive predatory species can disrupt natural enemy efficiency (Snyder & Evans, 2006). Abiotic challenges, such as increased drought frequency as predicted under future climate scenarios may also affect top-down processes influencing herbivory. This is particularly true for phloem-feeding and other sucking pests, which can under certain circumstances (*e.g.* pulse drought) benefit from drought-induced changes to their host plants (Huberty & Denno, 2004). Using a field experiment (Chapter 6) to explore the effect of drought on colonisation of raspberry plants by herbivore and natural enemies, while aboveground raspberry dry mass was markedly reduced, herbivore abundance was not. Natural colonisation of natural enemies occurred on

over 50% of the plants, but only lacewing colonisation was significantly lower on raspberry plants experiencing drought.

## 7.2 The value of multi-trophic interaction studies

Interspecific interactions both within and between species of different trophic levels play major roles in ecosystem functioning (Hunter *et al*., 1997). Exploring pairwise interactions, particularly within a global change context, helps to determine the mechanisms underpinning the response of individual species to perturbations. However, the limitations of such an approach are highlighted by this study. For example, in a pairwise interaction, $eCO_2$ can reduce plant resistance to aphid herbivory (Martin & Johnson, 2011; Guo *et al*., 2014; Chapter 3), suggesting aphid herbivory and abundance will increase (Robinson 2012). However, when this pairwise interaction is placed within a tri-trophic framework, the inclusion of an aphid natural enemy removes the advantage aphids had in $eCO_2$, therefore suggesting that $CO_2$ has no net effect on the aphid population (Chapter 3). This pattern has been seen in large-scale climate change experiments in the field. Free Air Carbon dioxide Enrichment (FACE) experiments, for instance, show community level responses to $eCO_2$ are much less profound than pairwise species experiments may suggest (Ainsworth & Long, 2005). In the present study, aphid responses to conspecific alarm pheromone were impaired under conditions of $eCO_2$ (Chapter 4). Reduced predator avoidance will increase the availability of prey to natural enemies, even if the total aphid abundance does not increase, altering further the plant–aphid interaction.

## 7.3 Consequences for future populations of large raspberry aphids

In the present study, cultivars with $A_1$ and multi-genic resistance genes were no more effective at limiting *A. idaei* abundance than non-resistant cultivars under ambient climatic conditions. This contrasts with work published only three years ago, where cultivars with $A_1$ resistance were more resistant than susceptible cultivars under ambient climatic conditions (Martin & Johnson, 2011). There were, however, differences between the $A_1$ cultivars used, Martin and Johnson used the cultivar Glen Lyon while the present study used Glen Ample (Chapter 2). The observed

differences could be attributed either to variation in minor, undocumented resistance genes between cultivars (Hall *et al*., 2009; McMenemy *et al*., 2009) or that the cultivar Glen Ample is one of the most common, commercially available raspberry cultivars used (personal observation) and its ubiquity may have led to *A. idaei* developing resistance mechanisms specific to Glen Ample.

Under $eCO_2$ conditions, *A. idaei* abundance increased on Glen Lyon (Martin & Johnson, 2011) but remained constant on Glen Ample (Chapter 3). Adult biomass of *A. idaei* also increased in $eCO_2$ when feeding on Glen Lyon (Martin & Johnson, 2011), but no difference was observed on Glen Ample (Chapter 3). The increase in adult mass observed on Glen Lyon implies host plant quality was increased under $eCO_2$. The behaviour of *A. idaei* has also been shown to change under $eCO_2$ (Chapter 4). After feeding on Glen Ample grown under $eCO_2$, the escape response of *A. idaei* to natural enemies is significantly reduced; previous studies indicate that this is more likely to happen on higher quality host plants when aphids tend to "stand their ground" against predators (Dill *et al*., 1990; Losey & Denno, 1998; Mitchell *et al*., 2010). The reduction in escape response occurred within 24 hours of feeding on Glen Ample under $eCO_2$. Removing *A. idaei* from feeding on Glen Ample under $eCO_2$ to ambient conditions resulted in reduced escape responses being maintained (Chapter 4). This would suggest that an, as yet unidentified, physiological change took place within the aphid.

This thesis highlights the importance of natural enemies to complement bottom-up plant resistance. The reduction in escape response seen under $eCO_2$ increased the effectiveness of natural enemies, reducing aphid abundance. It is unknown if a similar reduction in escape response was observed in the previous study involving Glen Lyon (Martin & Johnson, 2011). Martin and Johnson did not include a natural enemy, but if *A. idaei* escape responses were reduced when feeding on Glen Lyon as seen in this work (Chapter 3), the increased aphid abundance observed may have been diminished by the presence of a natural enemy. The importance of natural enemies at complementing plant resistance to achieve biocontrol was emphasised in this study by the greater susceptibility of a resistant cultivar (Octavia) to *A. idaei* under $eCO_2$ being offset by top-down predation pressure.

Discussion

# 7.4 Constraints and caveats of the research

Most studies that set out to investigate the response of species interactions to climate change focus on pairwise interactions. This method is key for elucidating the mechanisms by which species interact and therefore how climate change will impact species interactions. The work presented here highlights the importance of considering multi-species interactions.

Climate change is a multi-factorial perturbation to the abiotic environment, but most research has focused on one or two abiotic perturbations. The work presented here has only focused on one aspect of climate change. Logistical and theoretical challenges limit the manipulation of the biotic environment. Experimentally altering the concentration of atmospheric $CO_2$ has been a technical and logistical challenge, requiring specialised facilities, often with limited size chambers, however recent technological advances are making this more achievable. Adding further changes to the abiotic environment, such as increased $O_3$ and temperature, is technically possible, but logistically challenging given the large number of replicate chambers required to ensure high replication. In this thesis, using multiple runs, moving replicates between chambers and statistical methods, mitigated to an extent the lack of high replication. This however, came at a cost of extending the duration of experiments that limited the number of questions that can be answered within a given timeframe.

Furthermore, the scale of the experiment can have a significant impact on the ecological questions that can be answered. Controlled microcosm work has many advantages by being able to carefully control all biotic and abiotic conditions but limited by the number of variables that can be included in an artificial environment. For example, $eCO_2$ research can vary in scale, from small chambers to large-scale FACE facilities open to the natural world. Both approaches have their limitations. The community within a chamber will be limited by the size of the chamber and the duration of the experiment. A criticism of all such experiments in which plants and animals are exposed to modified environmental conditions in closed chambers is that although they may lead to considerable advances in understanding underlying mechanisms of responses, they do not necessarily permit predictions of ecological changes in the field situation, where expected responses may be buffered by additional species interactions and other factors (Whittaker, 2001). Unlike chamber

experiments, FACE experiments allow for natural variation in temperature and daylight, which can significantly alter biotic interactions (Ainsworth *et al*., 2008). Changes to community composition and species abundance have been observed under $eCO_2$ in FACE (Ainsworth *et al*., 2008), but the actual mechanism underlying these changes remains unknown.

This work does have its limitations in terms of direct applicability to the wider community, but this is outweighed by the important insights this work provides into the processes that underpin species interactions. An ideal future approach is to combine both mechanistic experiments with field observations.

# 7.5 Future work

It has been the focus of this work to understand the interactions taking place between three trophic levels under climate change. Future work would develop this further by both increasing the scale of the experiments to determine community level changes, but also develop finer scale experiments to understand the cause of the changes. This research has therefore raised several questions; future work must gain greater insight into the mechanism of species interactions and extend this to natural communities.

## 7.5.1 Does aphid feeding behaviour change under $eCO_2$?

Determining the mechanism for the altered behaviour of *A. idaei* under $eCO_2$ requires work at a much finer scale. What has, for example, changed within the aphid to result in a prolonged change in behaviour? Analysis of the feeding behaviour of *A. idaei* using Electro Penetration Graph (EPG) could determine if feeding behaviour (*e.g.* intensity or duration) is altered under $eCO_2$. Pectinase is injected into the intracellular spaces to break down intracellular material, the middle lamina, during aphid probing, to allow the stylet to pass between cells (Dreyer & Campbell, 1987). If, for example, the composition of the pectin based middle lamina is altered under $eCO_2$, the effectiveness of aphid-derived pectinases may be significantly altered. In addition chemical analysis of the phloem sap would give insight into resource quality (*i.e.* dilution of C:N or concentration of defence compounds).

## 7.5.2 What causes aphid behaviour to change under $eCO_2$?

Molecular analysis could be used to determine the functioning of symbiotic bacteria (*e.g. Buchnera aphidicola* (Baumann, 2005)) that aid amino acid acquisition. Differences in host plant quality can reduce production of amino acids by *Buchnera* spp. which then reduces aphid larval mass (Wilkinson *et al*., 2001). Plant mediated interference with the nutritional function of the symbiotic bacteria under $eCO_2$ may be responsible for the reduced escape response to predation. The carbon : nitrogen ratio in plant material is increased under $eCO_2$ (Bezemer *et al*., 1998). Such a reduction in nitrogen based compounds in plant material, including phloem sap, results in poor food quality for aphid herbivores. A shift in resource partitioning within the aphid may occur, altering the symbiotic relationship with beneficial gut bacteria. It could therefore be hypothesised that 1) host plant quality increases under $eCO_2$ and aphids 'hold their ground'; or 2) host plant quality deteriorates and causes compensatory feeding that makes aphids physiologically unable, or behaviourally disinclined, to abandon a host after considerable investment in feeding.

In order to extrapolate this work to the wider ecological community, larger-scale $eCO_2$ experiments are required to determine how altered aphid behaviour can alter the effectiveness of top-down processes at modulating aphid abundance. In the field, both predation and parasitism interact to control aphid herbivory (Snyder *et al*., 2006), but the response of this top-down process to climate change remains unknown.

## 7.5.3 Can population dynamic models be used to predict the outcome of competing natural enemies? Will climate change alter the predictions?

This work has demonstrated that the feeding behaviour of predators can be predicted to some extent using intraguild predation dynamics models. However, many of the parameters of these models were assumed (*e.g.* prey and intraguild-prey conversion efficiency, predator death rate) and therefore more work is required to improve the accuracy of this work. There is also a substantial gap between studies such as this where individual behaviour was ascertained and results from the landscape scale (*i.e.* 1–10 km). Controlled field studies, where competition between predators can be observed at the community level, will provide valuable information to help link patterns seen at the landscape scale to species interactions. Furthermore, as demonstrated here (Chapter 5), climate change is capable of

modifying prey behaviour and abundance. How such changes can alter the interaction between natural enemies remains to be extensively tested.

## 7.5.4 Will altered indirect plant defences under eCO$_2$ alter herbivore suppression by multiple natural enemies?

Previous work suggests the ability of parasitoids to find their aphid host is diminished under eCO$_2$ due to changes to the composition of plant VOCs (Zavala *et al*., 2013). Generalist predators such as coccinellids do not rely as strongly on plant VOCs, but rather use other cues such as aphid semio-chemicals (Pettersson *et al*., 2008). The ability of natural enemies to find their prey may be differentially impacted by eCO$_2$. Therefore in natural communities where a combination of predator feeding guilds are responsible for suppressing herbivore populations (Snyder *et al*., 2006), the loss of one guild may have a significant knock-on effect to others. Furthermore, if reduced diet quality from feeding on plants under eCO$_2$ alters the relationship aphids share with their symbionts, changes to escape responses (Chapter 4) and immune function (Oliver *et al*., 2009) could significantly alter the outcome.

Plant resistance in a future climate is likely to be modified significantly. Elevated CO$_2$ reduced plant resistance to aphid herbivory, but this was counterbalanced by top-down control, highlighting the future importance of natural enemies to control aphid abundance. Modification of chemical signals governing trophic interactions are likely to modify the response of natural enemies, by either increasing prey availability (*e.g.* reduced behavioural response by herbivores) or limiting colonisation (*e.g.* location of prey). Niche partitioning and coexistence among native and invasive predatory species may further contribute to the effectiveness of natural enemies, but how this will be maintained in a future environment is not known. Further mechanistic research is required to understand multi-trophic interactions in dynamic environments.

Discussion

# References

Abe, T., Wada, K., Kato, Y., Makino, S. & Okochi, I. (2011) Alien pollinator promotes invasive mutualism in an insular pollination system. *Biological Invasions*, **13**, 957-967.

Agarwala, B.K. & Dixon, A.F.G. (1992) Laboratory study of cannibalism and interspecific predation in ladybirds. *Ecological Entomology*, **17**, 303-309.

Agarwala, B.K., Yasuda, H. & Kajita, Y. (2003) Effect of conspecific and heterospecific feces on foraging and oviposition of two predatory ladybirds: role of fecal cues in predator avoidance. *Journal of Chemical Ecology*, **29**, 357-376.

Agrawal, A.A. & Rutter, M.T. (1998) Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos*, **83**, 227-236.

Ainsworth, E.A. & Long, S.P. (2005) What have we learned from 15 years of free-air $CO_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising $CO_2$. *New Phytologist*, **165**, 351-371.

Ainsworth, E.A., Leakey, A.D.B., Ort, D.R. & Long, S.P. (2008) FACE-ing the facts: inconsistencies and interdependence among field, chamber and modeling studies of elevated [$CO_2$] impacts on crop yield and food supply. *New Phytologist*, **179**, 5-9.

Aqueel, M.A. & Leather, S.R. (2012) Nitrogen fertiliser affects the functional response and prey consumption of *Harmonia axyridis* (Coleoptera: Coccinellidae) feeding on cereal aphids. *Annals of Applied Biology*, **160**, 6-15.

Aslam, T.J., Johnson, S.N. & Karley, A.J. (2013) Plant-mediated effects of drought on aphid population structure and parasitoid attack. *Journal of Applied Entomology*, **137**, 136-145.

Awmack, C., Woodcock, C. & Harrington, R. (1997) Climate change may increase vulnerability of aphids to natural enemies. *Ecological Entomology*, **22**, 366-368.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Harrington, R., Hartley, S., Jones, T.H., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A.D. & Whittaker, J.B. (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, **8**, 1-16.

Ballare, C.L., Caldwell, M.M., Flint, S.D., Robinson, A. & Bornman, J.F. (2011) Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. *Photochemical & Photobiological Sciences*, **10**, 226-241.

Bannerman, J.A., Gillespie, D.R. & Roitberg, B.D. (2011) The impacts of extreme and fluctuating temperatures on trait-mediated indirect aphid–parasitoid interactions. *Ecological Entomology*, **36**, 490-498.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2014) *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7, http://CRAN.R-project.org/package=lme4.

Baumann, P. (2005) Biology of bacteriocyte-associated endosymbionts of plant sap–sucking insects. *Annual Review of Microbiology*, **59**, 155-189.

Bezemer, T.M. & Jones, T.H. (1998) Plant–insect herbivore interactions in elevated atmospheric $CO_2$: quantitative analyses and guild effects. *Oikos*, **82**, 212-222.

Bezemer, T.M., Jones, T.H. & Knight, K.J. (1998) Long-term effects of elevated $CO_2$ and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. *Oecologia*, **116**, 128-135.

Bilu, E. & Coll, M. (2007) The importance of intraguild interactions to the combined effect of a parasitoid and a predator on aphid population suppression. *BioControl*, **52**, 753-763.

Birch, A.N.E., Gordon, S.C., Fenton, B., Malloch, G., Mitchell, C., Jones, A.T., Griffiths, D.W., Brennan, R., Graham, J. & Woodford, J.A.T. (2004) Developing a sustainable IPM system for high value *Rubus* crops (raspberry, blackberry) for Europe. In Proceedings of the Euro Berry Symposium – Cost 836 Final Worskhop (ed. by D.W. Simpson), pp. 289-292. International Society for Horticultural Science, Leuven 1.

Blackman, R.L. (1967) The effects of different aphid foods on *Adalia bipunctata* L. and *Coccinella septempunctata* L. *Annals of Applied Biology*, **59**, 207-219.

Blum, M.S. (1969) Alarm pheromones. *Annual Review of Entomology*, **14**, 57-80.

Bolker, B. (2014) Convergence error for lme4, http://comments.gmane.org/gmane.comp.lang.r.lme4.devel/11674.

Bond, D.A. & Lowe, H.J.B. (1975) Tests for resistance to *Aphis fabae* in field beans (*Vicia fabia*). *Annals of Applied Biology*, **81**, 21-32.

Borer, E.T., Briggs, C.F. & Holt, R.D. (2007) Predators, parasitoids, and pathogens: a cross-cutting examination of intraguild predation theory. *Ecology*, **88**, 2681-2688.

Bowers, W.S., Nault, L.R., Webb, R.E. & Dutky, S.R. (1972) Aphid alarm pheromone: isolation, identification, synthesis. *Science*, **177**, 1121-1122.

Bradford, M.M. (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing principle of protein–dye binding. *Analytical Biochemistry*, **72**, 248-254.

References

Briggs, C.J. & Borer, E.T. (2005) Why short-term experiments may not allow long-term predictions about intraguild predation. *Ecological Applications*, **15**, 1111-1117.

Briggs, J.B. (1965) Distribution abundance and genetic relationships of 4 strains of *Rubus* aphid (*Amphorophora rubi* (Kalt.) in relation to raspberry breeding. *Journal of Horticultural Science & Biotechnology*, **40**, 109-117.

Brodbeck, B. & Strong, D. (1987) Amino acid nutrition of herbivorous insects and stress to host plants. In Insect Outbreaks (ed. by P. Barbosa & J.C. Schultz), pp. 347-364. Academic Press, London.

Brown, P.M.J., Adriaens, T., Bathon, H., Cuppen, J., Goldarazena, A., Hägg, T., Kenis, M., Klausnitzer, B.E.M., Kovář, I., Loomans, A.J.M., Majerus, M.E.N., Nedved, O., Pedersen, J., Rabitsch, W., Roy, H.E., Ternois, V., Zakharov, I.A. & Roy, D.B. (2008a) *Harmonia axyridis* in Europe: spread and distribution of a non–native coccinellid. *BioControl*, **53**, 5-21.

Brown, P.M.J., Roy, H.E., Rothery, P., Roy, D.B., Ware, R.L. & Majerus, M.E.N. (2008b) *Harmonia axyridis* in Great Britain: analysis of the spread and distribution of a non-native coccinellid. *BioControl*, **53**, 55-67.

Brown, P.M.J., Frost, R., Doberski, J., Sparks, T.I.M., Harrington, R. & Roy, H.E. (2011) Decline in native ladybirds in response to the arrival of *Harmonia axyridis*: early evidence from England. *Ecological Entomology*, **36**, 231-240.

Buffington, D.E., Bucklin, R.A., Henley, R.W. & McConnell, D.B. (2013) Greenhouse Ventilation. A.B.E. Department, University of Florida, Institute of Food and Agricultural Sciences.

Calatayud, P.A., Polania, M.A., Seligmann, C.D. & Bellotti, A.C. (2002) Influence of water–stressed cassava on *Phenacoccus herreni* and three associated parasitoids. *Entomologia Experimentalis et Applicata*, **102**, 163-175.

Cardinale, B.J., Harvey, C.T., Gross, K. & Ives, A.R. (2003) Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters*, **6**, 857-865.

Cardoso, M.Z. (2008) Herbivore handling of a Plant trichome: the case of *Heliconius charithonia* (L.) (Lepidoptera: Nymphalidae) and *Passiflora lobata* (Killip) Hutch. (Passifloraceae). *Neotropical Entomology*, **37**, 247-252.

Chacon, J.M., Landis, D.A. & Heimpel, G.E. (2008) Potential for biotic interference of a classical biological control agent of the soybean aphid. *Biological Control*, **46**, 216-225.

Chandler, D., Bailey, A.S., Tatchell, G.M., Davidson, G., Greaves, J. & Grant, W.P. (2011) The development, regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **366**, 1987-1998.

Chen, F., Ge, F. & Parajulee, M.N. (2005a) Impact of elevated $CO_2$ on tri-trophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. *Environmental Entomology*, **34**, 37-46.

Chen, F., Wu, G., Ge, F., Parajulee, M.N. & Shrestha, R.B. (2005b) Effects of elevated $CO_2$ and transgenic Bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm. *Entomologia Experimentalis et Applicata*, **115**, 341-350.

Chen, F., Wu, G., Parajulee, M.N. & Ge, F. (2007) Long-term impacts of elevated carbon dioxide and transgenic Bt cotton on performance and feeding of three generations of cotton bollworm. *Entomologia Experimentalis et Applicata*, **124**, 27-35.

Chen, M. (2008) Inducible direct plant defense against insect herbivores: A review. *Insect Science*, **15**, 101-114.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, R., Jones, R., Kolli, R.K., Kwon, W. & Laprise, R. (2007) Regional climate projections. *Climate Change, 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change,* University Press, Cambridge, Chapter 11, 847-940.

Clavijo McCormick, A., Unsicker, S.B. & Gershenzon, J. (2012) The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends in Plant Science*, **17**, 303-310.

Coley, P.D., Bryant, J.P. & Chapin, F.S. (1985) Resource availability and plant antiherbivore defense. *Science*, **230**, 895-899.

Colfer, R.G. & Rosenheim, J.A. (2001) Predation on immature parasitoids and its impact on aphid suppression. *Oecologia*, **126**, 292-304.

Colunga-Garcia, M. & Gage, S.H. (1998) Arrival, establishment, and habitat use of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Michigan landscape. *Environmental Entomology*, **27**, 1574-1580.

Comont, R.F., Roy, H.E., Harrington, R., Shortall, C.R. & Purse, B.V. (2014) Ecological correlates of local extinction and colonisation in the British ladybird beetles (Coleoptera: Coccinellidae). *Biological Invasions*, **16**, 1805-1817.

Couture, J.J., Servi, J.S. & Lindroth, R.L. (2010) Increased nitrogen availability influences predator–prey interactions by altering host-plant quality. *Chemoecology*, **20**, 277-284.

Crane, M., Norton, A., Leaman, J., Chalak, A., Bailey, A., Yoxon, M., Smith, J. & Fenlon, J. (2006) Acceptability of pesticide impacts on the environment: what do United Kingdom stakeholders and the public value? *Pest Management Science*, **62**, 5-19.

Crawley, M.J. (1989) Insect herbivores and plant–population dynamics. *Annual Review of Entomology*, **34**, 531-564.

Crawley, M.J. (2002) Statistical Computing: An Introduction to Data Analysis Using S-PLUS. Wiley & Sons Ltd, England.

Curtis, P.S. & Wang, X. (1998) A meta-analysis of elevated $CO_2$ effects on woody plant mass, form, and physiology. *Oecologia*, **113**, 299-313.

Daane, K.M., Yokota, G.Y., Zheng, Y. & Hagen, K.S. (1996) Inundative release of common green lacewings (Neuroptera: Chrysopidae) to suppress *Erythroneura variabilis* and *E. elegantula* (Homoptera: Cicadellidae) in vineyards. *Environmental Entomology*, **25**, 1224-1234.

Dai, A. (2011) Drought under global warming: a review. *Wiley Interdisciplinary Reviews–Climate Change*, **2**, 45-65.

Daoust, S.P., Mader, B.J., Bauce, E., Despland, E., Dussutour, A. & Albert, P.J. (2010) Influence of epicuticular-wax composition on the feeding pattern of a phytophagous insect: implications for host resistance. *The Canadian Entomologist*, **142**, 261-270.

De Moraes, C.M., Lewis, W.J., Paré, P.W., Alborn, H.T. & Tumlinson, J.H. (1998) Herbivore-infested plants selectively attract parasitoids. *Nature*, **393**, 570–573.

De Moraes, C.M., Mescher, M.C. & Tumlinson, J.H. (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature*, **410**, 577-580.

Dicke, M. & Baldwin, I.T. (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science*, **15**, 167-175.

Dickson, A.T. (1979) The population dynamics of raspberry aphids in eastern Scotland. Unpublished PhD Thesis, University of Dundee.

Didham, R.K., Tylianakis, J.M., Hutchison, M.A., Ewers, R.M. & Gemmell, N.J. (2005) Are invasive species the drivers of ecological change? *Trends in Ecology & Evolution*, **20**, 470-474.

Dill, L.M., Fraser, A.H.G. & Roitberg, B.D. (1990) The economics of escape behaviour in the pea aphid, *Acyrthosiphon pisum*. *Oecologia*, **83**, 473-478.

Dixon, A.F.G. (1973) Biology of Aphids. Edward Arnold Limited, London.

Dixon, A.F.G. (1985) Structure of aphid populations. *Annual Review of Entomology*, **30**, 155-174.

Dixon, A.F.G. (1986) The distribution of aphid infestation in relation to the demography of plant parts – a reply. *American Naturalist*, **127**, 410-413.

Dixon, A.F.G. (2000) Insect Predator – Prey Dynamics. Cambridge University Press, Cambridge, UK.

Dixon, S.M. & Baker, R.L. (1988) Effects of size on predation risk, behavioural response to fish, and cost of reduced feeding in larval *Ischnura verticalis* (Coenagrionidae: Odonata). *Oecologia*, **76**, 200-205.

Docherty, M., Hurst, D.K., Holopainen, J.K., Whittaker, J.B., Lea, P.J. & Watt, A.D. (1996) Carbon dioxide-induced changes in beech foliage cause female beech weevil larvae to feed in a compensatory manner. *Global Change Biology*, **2**, 335-341.

Douglas, A.E. (1993) The nutritional quality of phloem sap utilized by natural aphid populations. *Ecological Entomology*, **18**, 31-38.

Douglas, A.E. (2006) Phloem-sap feeding by animals: problems and solutions. *Journal of Experimental Botany*, **57**, 747-754.

Down, R.E., Gatehouse, A.M.R., Hamilton, W.D.O. & Gatehouse, J.A. (1996) Snowdrop lectin inhibits development and decreases fecundity of the glasshouse potato aphid (*Aulacorthum solani*) when administered in vitro and via transgenic plants both in laboratory and glasshouse trials. *Journal of Insect Physiology*, **42**, 1035-1045.

Dray, M.W., Crowther, T.W., Thomas, S.M., A'Bear, A.D., Godbold, D.L., Ormerod, S.J., Hartley, S.E. & Jones, T.H. (2014) Effects of elevated $CO_2$ on litter chemistry and subsequent invertebrate detritivore feeding responses. , **9**, e86246.

Dreyer, D.L. & Campbell, B.C. (1987) Chemical basis of host-plant resistance to aphids. *Plant Cell & Environment*, **10**, 353-361.

Dungan, R.J., Turnbull, M.H. & Kelly, D. (2007) The carbon costs for host trees of a phloem-feeding herbivore. *Journal of Ecology*, **95**, 603-613.

Ehrlich, P.R. & Raven, P.H. (1964) Butterflies and plants: a study in coevolution. *Evolution*, **18**, 586-608.

Elston, D.A., Moss, R., Boulinier, T., Arrowsmith, C. & Lambin, X. (2001) Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology*, **122**, 563-569.

Eubanks, M.D., Blackwell, S.A., Parrish, C.J., Delamar, Z.D. & Hull-Sanders, H. (2002) Intraguild predation of beneficial arthropods by red imported fire ants in cotton. *Environmental Entomology*, **31**, 1168-1174.

Facey, S.L., Ellsworth, D.S., Staley, J.T., Wright, D.J. & Johnson, S.N. (2014) Upsetting the order: how atmospheric and climate change affects predator–prey interactions. *Current Opinion in Insect Science*, **5**, 66-74.

FAOSTAT (2014) Food and Agriculture Organization of the United Nations – Production Data Archives.

Fatouros, N.E., Dicke, M., Mumm, R., Meiners, T. & Hilker, M. (2008) Foraging behavior of egg parasitoids exploiting chemical information. *Behavioral Ecology*, **19**, 677-689.

Ferrer, A., Dixon, A.F.G. & Hemptinne, J. (2008) Prey preference of ladybird larvae and its impact on larval mortality, some life-history traits of adults and female fitness. *Bulletin of Insectology*, **61**, 5-10.

References

Fordyce, J.A. & Agrawal, A.A. (2001) The role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail *Battus philenor*. *Journal of Animal Ecology*, **70**, 997-1005.

Frago, E. & Godfray, H.C.J. (2014) Avoidance of intraguild predation leads to a long-term positive trait-mediated indirect effect in an insect community. *Oecologia*, **174**, 943-952.

Fritz, R.S. & Simms, E.L. (1992) Plant Resistance to Herbivores and Pathogens: ecology, evolution, and genetics. University of Chicago Press.

Gao, F., Chen, F. & Ge, F. (2010) Elevated $CO_2$ lessens predation of *Chrysopa sinica* on *Aphis gossypii*. *Entomologia Experimentalis et Applicata*, **135**, 135-140.

Gibson, R.W. & Pickett, J.A. (1983) Wild potato repels aphids by release of aphid alarm pheromone. *Nature*, **302**, 608-609.

Gordon, S.C., Woodford, J.A.T. & Birch, A.N.E. (1997) Arthropod pests of *Rubus* in Europe: Pest status, current and future control strategies. *Journal of Horticultural Science & Biotechnology*, **72**, 831-862.

Gotthard, K., S. Nylin and C. Wiklund (1994) Adaptive variation in growth-rate-life-history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oecologia*, **99** (3-4), 281-289.

Gouinguené, S.P. & Turlings, T.C.J. (2002) The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, **129**, 1296-1307.

Gregory, P.J. (2006) Plant Roots: Growth, activity and interactions with soils Blackwell Publishing Ltd, Oxford, UK.

Gregory, P.J., Johnson, S.N., Newton, A.C. & Ingram, J.S.I. (2009) Integrating pests and pathogens into the climate change/food security debate. *Journal of Experimental Botany*, **60**, 2827-2838.

Guay, J.F., Boudreault, S., Michaud, D. & Cloutier, C. (2009) Impact of environmental stress on aphid clonal resistance to parasitoids: Role of *Hamiltonella defensa* bacterial symbiosis in association with a new facultative symbiont of the pea aphid. *Journal of Insect Physiology*, **55**, 919-926.

Guo, H., Sun, Y., Li, Y., Liu, X., Zhang, W. & Ge, F. (2013a) Elevated $CO_2$ decreases the response of the ethylene signaling pathway in *Medicago truncatula* and increases the abundance of the pea aphid. *New Phytologist*, **201**, 279-291.

Guo, H., Sun, Y., Li, Y., Tong, B., Harris, M., Zhu-Salzman, K. & Ge, F. (2013b) Pea aphid promotes amino acid metabolism both in *Medicago truncatula* and bacteriocytes to favor aphid population growth under elevated $CO_2$. *Global Change Biology*, **19**, 3210-3223.

Guo, H.J., Sun, Y.C., Li, Y.F., Liu, X.H., Zhang, W.H. & Ge, F. (2014) Elevated $CO_2$ decreases the response of the ethylene signaling pathway in *Medicago truncatula* and increases the abundance of the pea aphid. *New Phytologist*, **201**, 279-291.

References

Gurevitch, J. & Padilla, D.K. (2004) Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, **19**, 470-474.

Hackett, S.C., Karley, A.J. & Bennett, A.E. (2013) Unpredicted impacts of insect endosymbionts on interactions between soil organisms, plants and aphids. *Proceedings of the Royal Society B-Biological Sciences*, **280**, 20131275.

Hairston, N.G., Smith, F.E. & Slobodkin, L.B. (1960) Community structure, population control, and competition. *American Naturalist*, **94**, 421-425.

Hall, H.K., Hummer, K.E., Jamieson, A.R., Jennings, S.N. & Weber, C.A. (2009) Raspberry Breeding and Genetics. In Plant Breeding Reviews, pp. 39–353. John Wiley & Sons, Inc., Hoboken, USA.

Hamilton, W.D. (1971) Geometry for the selfish herd. *Journal of Theoretical Biology*, **31**, 295-311.

Hance, T., van Baaren, J., Vernon, P. & Boivin, G. (2007) Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology*, **52**, 107-126.

Hanley, M.E., Lamont, B.B., Fairbanks, M.M. & Rafferty, C.M. (2007) Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology Evolution and Systematics*, **8**, 157-178.

Harper, J.L. (1989) The value of a leaf. *Oecologia*, **80**, 53-58.

Harrington, R., Woiwod, I. & Sparks, T. (1999) Climate change and trophic interactions. *Trends in Ecology & Evolution*, **14**, 146-150.

Harvey, C.T. & Eubanks, M.D. (2005) Intraguild predation of parasitoids by *Solenopsis invicta*: a non-disruptive interaction. *Entomologia Experimentalis et Applicata*, **114**, 127-135.

Hatano, E., Kunert, G., Michaud, J.P. & Weisser, W.W. (2008) Chemical cues mediating aphid location by natural enemies. *European Journal of Entomology*, **105**, 797-806.

Heil, M. (2008) Indirect defence via tritrophic interactions. *New Phytologist*, **178**, 41-61.

Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants: To grow or defend. *The Quarterly Review of Biology*, **67**, 283-335.

Hobbs, R.J. & Mooney, H.A. (2000) Invasive Species in a Changing World. Island Press, Washington D.C., USA.

Hodek, I. & Evans, E.W. (2012). Food relationships. In: Ecology and Behaviour of the Ladybird Beetles (Coccinellidae), pp. 141-274. John Wiley & Sons, Ltd, West Sussex, UK.

Hodek, I., Honek, A. & van Emden, H.F. (2012) Ecology and Behaviour of the Ladybird Beetles (Coccinellidae) Wiley-Blackwell, Sussex, UK.

References

Holopainen, J.K. & Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, **15**, 176-184.

Holt, R.D. & Polis, G.A. (1997) A theoretical framework for intraguild predation. *American Naturalist*, **149**, 745-764.

Holton, M.K., Lindroth, R.L. & Nordheim, E.V. (2003) Foliar quality influences tree–herbivore–parasitoid interactions: effects of elevated $CO_2$, $O_3$, and plant genotype. *Oecologia*, **137**, 233-244.

Hoogenboom, G., Huck, M.G. & Peterson, C.M. (1987) Root growth rate of soybean as affected by drought stress. *Agronomy Journal*, **79**, 607-614.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J. & Wardle, D.A. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3-35.

Howe, G.A. & Schaller, A. (2008) Direct defences in plants and their induction by wounding and insect herbivores. In: Induced Plant Resistance to Herbivory (ed. by A. Schaller), pp. 7-29. Springer, Dordrecht, Netherlands.

Huberty, A.F. & Denno, R.F. (2004) Plant water stress and its consequences for herbivorous insects: A new synthesis. *Ecology*, **85**, 1383-1398.

Hughes, L. & Bazzaz, F.A. (2001) Effects of elevated $CO_2$ on five plant–aphid interactions. *Entomologia Experimentalis et Applicata*, **99**, 87-96.

Hull, R. (2002) Matthews' Plant Virology. Academic Press, Florida, USA.

Hunter, M.D., Varley, G.C. & Gradwell, G.R. (1997) Estimating the relative roles of top-down and bottom-up forces on insect herbivore populations: a classic study revisited. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 9176-9181.

Huntly, N. (1991) Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics*, **22**, 477-503.

IPCC (2013) Summary for Policymakers. In Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (ed. by T.F. Stocker, D. Qin, G.K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex & P.M. Midgley). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jackson, D. & Dixon, A. (1996) Factors determining the distribution of the green spruce aphid, *Elatobium abietinum*, on young and mature needles of spruce. *Ecological Entomology*, **21**, 358-364.

James, D.G. (2003) Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *Journal of Chemical Ecology*, **29**, 1601-1609.

References

Jamieson, M.A., Trowbridge, A.M., Raffa, K.F. & Lindroth, R.L. (2012) Consequences of climate warming and altered precipitation patterns for plant–insect and multitrophic interactions. *Plant Physiology*, **160**, 1719-1727.

Johnson, M.T.J. (2011) Evolutionary ecology of plant defences against herbivores. *Functional Ecology*, **25**, 305-311.

Johnson, S.N. & McNicol, J.W. (2010) Elevated $CO_2$ and aboveground–belowground herbivory by the clover root weevil. *Oecologia*, **162**, 209-216.

Johnson, S.N., Elston, D.A. & Hartley, S.E. (2003) Influence of host plant heterogeneity on the distribution of a birch aphid. *Ecological Entomology*, **28**, 533-541.

Johnson, S.N., Petitjean, S., Clark, K.E. & Mitchell, C. (2010) Protected raspberry production accelerates onset of oviposition by vine weevils (*Otiorhynchus sulcatus*). *Agricultural and Forest Entomology*, **12**, 277-283.

Johnson, S.N., Staley, J.T., McLeod, F.A.L. & Hartley, S.E. (2011) Plant-mediated effects of soil invertebrates and summer drought on above-ground multitrophic interactions. *Journal of Ecology*, **99**, 57-65.

Johnson, S.N., Young, M.W. & Karley, A.J. (2012) Protected raspberry production alters aphid–plant interactions but not aphid population size. *Agricultural and Forest Entomology*, **14**, 217-224.

Johnson, S.N., Mitchell, C., McNicol, J.W., Thompson, J. & Karley, A.J. (2013) Downstairs drivers – root herbivores shape communities of above-ground herbivores and natural enemies via changes in plant nutrients. *Journal of Animal Ecology*, **82**, 1021-1030.

Johnson, S.N., Lopaticki, G. & Hartley, S.E. (2014a) Elevated atmospheric $CO_2$ triggers compensatory feeding by root herbivores on a $C_3$ but not a $C_4$ grass. *PLOS ONE*, **9**, e90251.

Johnson, S.N., Ryalls, J.M.W. & Karley, A.J. (2014b) Global climate change and crop resistance to aphids: contrasting responses of lucerne genotypes to elevated atmospheric carbon dioxide. *Annals of Applied Biology*, **165**, 62-72.

Jones, A.T., McGavin, W.J. & Birch, A.N.E. (2000) Effectiveness of resistance genes to the large raspberry aphid, *Amphorophora idaei* Börner, in different raspberry (*Rubus idaeus* L.) genotypes and under different environmental conditions. *Annals of Applied Biology*, **136**, 107-113.

Kallis, G. (2008). Droughts. *Annual Review of Environment and Resources*, **33**, 85-118.

Katsanis, A., Babendreier, D., Nentwig, W. & Kenis, M. (2013) Intraguild predation between the invasive ladybird *Harmonia axyridis* and non-target European coccinellid species. *BioControl*, **58**, 73-83.

Kenis, M., Auger-Rozenberg, M., Roques, A., Timms, L., Pere, C., Cock, M.J.W., Settele, J., Augustin, S. & Lopez-Vaamonde, C. (2009) Ecological effects of invasive alien insects. *Biological Invasions*, **11**, 21-45.

References

Kim, J.H. & Jander, G. (2007) *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. *Plant Journal*, **49**, 1008-1019.

Klapwijk, M.J., Gröbler, B.C., Ward, K., Wheeler, D. & Lewis, O.T. (2010) Influence of experimental warming and shading on host–parasitoid synchrony. *Global Change Biology*, **16**, 102-112.

Knight, V.H. & Fernández-Fernández, F. (2008) New summer fruiting red raspberry cultivars from East Malling Research. *Acta Horticulture* **777**, 173-176.

Koppmann, R. (2007) Volatile Organic Compounds in the Atmosphere. Blackwell Publishing, Oxford, UK.

Koricheva, J., Larsson, S. & Haukioja, E. (1998) Insect performance on experimentally stressed woody plants: A meta-analysis. *Annual Review of Entomology*, **43**, 195-216.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2014) lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-20. http://CRAN.R-project.org/package=lmerTest

Langellotto, G.A. & Denno, R.F. (2004) Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia*, **139**, 1-10.

Larsson, S. (1989) Stressful times for the plant stress–insect performance hypothesis. *Oikos*, **56**, 277-283.

Leszczynski, B., Warchol, J. & Niraz, S. (1985) The influence of phenolic compounds on the preference of winter wheat cultivars by cereal aphids. *Insect Science and Its Application*, **6**, 157-158.

Levin, D.A. (1973) The role of trichomes in plant defense. *The Quarterly Review of Biology*, **48**, 3-15.

Li, G.Q. & Ishikawa, Y. (2006) Leaf epicuticular wax chemicals of the Japanese knotweed *Fallopia japonica* as oviposition stimulants for *Ostrinia latipennis*. *Journal of Chemical Ecology*, **32**, 595-604.

Light, T. & Marchetti, M.P. (2007) Distinguishing between invasions and habitat changes as drivers of diversity loss among California's freshwater fishes. *Conservation Biology*, **21**, 434-446.

Lincoln, D.E., Couvet, D. & Sionit, N. (1986) Response of an insect herbivore to host plants grown in carbon-dioxide enriched atmospheres. *Oecologia*, **69**, 556-560.

Lincoln, D.E., Fajer, E.D. & Johnson, R.H. (1993) Plant–insect herbivore interactions in elevated $CO_2$ environments. *Trends in Ecology & Evolution*, **8**, 64-68.

References

Long, S.P., Ainsworth, E.A., Rogers, A. & Ort, D.R. (2004) Rising atmospheric carbon dioxide: Plants face the future. *Annual Review of Plant Biology*, **55**, 591-628.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D.A. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*, **294**, 804-808.

Losey, J.E. & Denno, R.F. (1998) The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. *Ecological Entomology*, **23**, 53-61.

Losey, J.E. & Vaughan, M. (2006) The economic value of ecological services provided by insects. *Bioscience*, **56**, 311-323.

Loucks, O.L. (1977) Emergence of research on agro-ecosystems. *Annual Review of Ecology and Systematics*, **8**, 173-192.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: causes, epidemiology, global consequences and control. *Ecological Applications*, **10**, 689-710.

MacNeil, C., Bigsby, E., Dick, J.T., Hatcher, M.J. & Dunn, A.M. (2003) Differential physico-chemical tolerances and intraguild predation among native and invasive amphipods (Crustacea); a field study. *Archiv für Hydrobiologie / Fundamental and Applied Limnology*, **156**, 165-179.

Martin, P. & Johnson, S.N. (2011) Evidence that elevated $CO_2$ reduces resistance to the European large raspberry aphid in some raspberry cultivars. *Journal of Applied Entomology*, **135**, 237-240.

Masters, G.J., Brown, V.K., Clarke, I.P. & Whittaker, J.B. (1998) Direct and indirect effects of climate change on insect herbivores: Auchenorrhyncha (Homoptera). *Ecological Entomology*, **23**, 45-52.

Matis, J.H., Kiffe, T.R., van der Werf, W., Costamagna, A.C., Matis, T.I. & Grant, W.E. (2009) Population dynamics models based on cumulative density dependent feedback: A link to the logistic growth curve and a test for symmetry using aphid data. *Ecological Modelling*, **220**, 1745-1751.

McCall, A.C. & Fordyce, J.A. (2010) Can optimal defence theory be used to predict the distribution of plant chemical defences? *Journal of Ecology*, **98**, 985-992.

McKey, D. (1974) Adaptive patterns in alkaloid physiology. *American Naturalist*, **108**, 305-320.

McMenemy, L.S., Mitchell, C. & Johnson, S.N. (2009) Biology of the European large raspberry aphid (*Amphorophora idaei*): its role in virus transmission and resistance breakdown in red raspberry. *Agricultural and Forest Entomology*, **11**, 61-71.

References

Menge, B.A. & Sutherland, J.P. (1987) Community regulation – variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *American Naturalist*, **130**, 730-757.

Menzel, A. & Dose, V. (2005) Analysis of long-term time series of the beginning of flowering by Bayesian function estimation. *Meteorologische Zeitschrift*, **14**, 429-434.

Menzel, A. & Fabian, P. (1999) Growing season extended in Europe. *Nature*, **397**, 659-659.

Mewis, I., Khan, M.A.M., Glawischnig, E., Schreiner, M. & Ulrichs, C. (2012) Water stress and aphid feeding differentially influence metabolite composition in *Arabidopsis thaliana* (L.). *PLOS ONE*, **7**, e48661.

Miles, P.W. (1999) Aphid saliva. *Biological Reviews*, **74**, 41-85.

Mitchell, C. (2007) Novel approaches to the development of intregrated pest management in UK raspberry production. Unpublished PhD Thesis, Dundee University.

Mitchell, C., Johnson, S.N., Gordon, S.C., Birch, A.N.E. & Hubbard, S.F. (2010) Combining plant resistance and a natural enemy to control *Amphorophora idaei*. *BioControl*, **55**, 321-327.

Mondor, E.B., Tremblay, M.N., Awmack, C.S. & Lindroth, R.L. (2004) Divergent pheromone-mediated insect behaviour under global atmospheric change. *Global Change Biology*, **10**, 1820-1824.

Moran, P.J. & Thompson, G.A. (2001) Molecular responses to aphid feeding in Arabidopsis in relation to plant defense pathways. *Plant Physiology*, **125**, 1074-1085.

Morin, P.J. (2011) Community Ecology. John Wiley & Sons, West Sussex, UK.

Mumm, R. & Dicke, M. (2010) Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **88**, 628-667.

Musetti, L. & Neal, J.J. (1997) Resistance to the pink potato aphid, *Macrosiphum euphorbiae*, in two accessions of *Lycopersicon hirsutum* f. *glabratum*. *Entomologia Experimentalis et Applicata*, **84**, 137-146.

Nedved, O., Fois, X., Ungerova, D. & Kalushkov, P. (2013) Alien vs. Predator – the native lacewing *Chrysoperla carnea* is the superior intraguild predator in trials against the invasive ladybird *Harmonia axyridis*. *Bulletin of Insectology*, **66**, 73-78.

Newton, A.C., Johnson, S.N. & Gregory, P.J. (2011) Implications of climate change for diseases, crop yields and food security. *Euphytica*, **179**, 3-18.

Ode, P.J. (2006) Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. *Annual Review of Entomology*, **51**, 163-185.

Oliver, K.M., Degnan, P.H., Hunter, M.S. & Moran, N.A. (2009). Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science*, **325**, 992-994.

Parker, I.M., Simberloff, D., Lonsdale, W.M., Goodell, K., Wonham, M., Kareiva, P.M., Williamson, M.H., Von Holle, B., Moyle, P.B., Byers, J.E. & Goldwasser, L. (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, **1**, 3-19.

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637-669.

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W.J., Thomas, J.A. & Warren, M. (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, **399**, 579-583.

Passioura, J.B. (2006) The perils of pot experiments. *Functional Plant Biology*, **33**, 1075-1079.

Pell, J.K., Baverstock, J., Roy, H.E., Ware, R.L. & Majerus, M.E.N. (2008) Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspectives. *BioControl*, **53**, 147-168.

Pettersson, J., Ninkovic, V., Glinwood, R., Abassi, S.A., Birkett, M., Pickett, J. & Wadhams, L. (2008) Chemical stimuli supporting foraging behaviour of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae): volatiles and allelobiosis. *Applied Entomology and Zoology*, **43**, 315-321.

Pickett, J.A., Wadhams, L.J., Woodcock, C.M. & Hardie, J. (1992) The chemical ecology of aphids. *Annual Review of Entomology*, **37**, 67-90.

Pinto, D.M., Himanen, S.J., Nissinen, A., Nerg, A.M. & Holopainen, J.K. (2008) Host location behavior of *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) in ambient and moderately elevated ozone in field conditions. *Environmental Pollution*, **156**, 227-231.

Polis, G.A. & Holt, R.D. (1992) Intraguild predation: The dynamics of complex trophic interactions. *Trends in Ecology & Evolution*, **7**, 151-154.

Polis, G.A. & Strong, D.R. (1996) Food web complexity and community dynamics. *The American Naturalist*, **147**, 813-846.

Polis, G.A., Myers, C.A. & Holt, R.D. (1989) The ecology and evolution of intraguild predation – Potential competitors that eat each other. *Annual Review of Ecology and Systematics*, **20**, 297-330.

Powell, G., Tosh, C.R. & Hardie, J. (2006) Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, **51**, 309-330.

Powell, M.J. (2009) The BOBYQA algorithm for bound constrained optimization without derivatives. Cambridge Report NA2009/06, University of Cambridge, Cambridge.

References

Preisser, E.L. & Strong, D.R. (2004) Climate affects predator control of an herbivore outbreak. *American Naturalist*, **163**, 754-762.

Preisser, E.L., Bolnick, D.I. & Benard, M.F. (2005) Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology*, **86**, 501-509.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. (1980) Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41-65.

Purandare, S.R. & Tenhumberg, B. (2012) Influence of aphid honeydew on the foraging behaviour of *Hippodamia convergens* larvae. *Ecological Entomology*, **37**, 184-192.

R Core Team. (2014) *R: A language Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rahbé, Y., Deraison, C., Bonadé-Bottino, M., Girard, C., Nardon, C. & Jouanin, L. (2003) Effects of the cysteine protease inhibitor oryzacystatin (OC-I) on different aphids and reduced performance of *Myzus persicae* on OC-I expressing transgenic oilseed rape. *Plant Science*, **164**, 441-450.

Raven, J.A. (1983) Phytophages of Xylem and Phloem – a comparison of animal and plant sap-feeders. *Advances in Ecological Research*, **13**, 135-234.

Relyea, R.A. (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*, **82**, 523-540.

Rhoades, D.F. (1985) Offensive – defensive interactions between herbivores and plants: Their relevance in herbivore population dynamics and ecological theory. *The American Naturalist*, **125**, 205-238.

Riddick, E.W. & Wu, Z. (2011) Lima bean–lady beetle interactions: hooked trichomes affect survival of *Stethorus punctillum* larvae. *BioControl*, **56**, 55-63.

Robinson, E.A., Ryan, G.D. & Newman, J.A. (2012) A meta-analytical review of the effects of elevated $CO_2$ on plant–arthropod interactions highlights the importance of interacting environmental and biological variables. *New Phytologist*, **194**, 321-336.

Root, R.B. (1973) Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95-124.

Roth, S.K. & Lindroth, R.L. (1995) Elevated atmospheric $CO_2$ effects on phytochemistry, insect performance and insect parasitoid interactions. *Global Change Biology*, **1**, 173-182.

Roy, H.E., Baverstock, J., Ware, R.L., Clark, S.J., Majerus, M.E.N., Baverstock, K.E. & Pell, J.K. (2008) Intraguild predation of the aphid

pathogenic fungus *Pandora neoaphidis* by the invasive coccinellid *Harmonia axyridis*. *Ecological Entomology*, **33**, 175-182.

Roy, H.E., Adriaens, T., Isaac, N.J.B., Kenis, M., Onkelinx, T., Martin, G.S., Brown, P.M.J., Hautier, L., Poland, R., Roy, D.B., Comont, R., Eschen, R., Frost, R., Zindel, R., Van Vlaenderen, J., Nedvĕd, O., Ravn, H.P., Grégoire, J., de Biseau, J.C. & Maes, D. (2012) Invasive alien predator causes rapid declines of native European ladybirds. *Diversity and Distributions*, **18**, 717-725.

Rutledge, C.E., Robinson, A.P. & Eigenbrode, S.D. (2003) Effects of a simple plant morphological mutation on the arthropod community and the impacts of predators on a principal insect herbivore. *Oecologia*, **135**, 39-50.

Ruxton, G.D., Sherratt, T.N. & Speed, M.P. (2004) Avoiding Attack – The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry. Oxford University Press, New York, USA.

Ryan, G.D., Emiljanowicz, L., Härri, S.A. & Newman, J.A. (2014) Aphid and host-plant genotype × genotype interactions under elevated $CO_2$. *Ecological Entomology*, **39**, 309-315.

Salt, D.T., Brooks, G.L. & Whittaker, J.B. (1995) Elevated carbon dioxide affects leaf-miner performance and plant growth in docks (*Rumex* spp.). *Global Change Biology*, **1**, 153-156.

Salyk, R.P. & Sullivan, D.J. (1982) Comparative feeding behavior of two aphid species – Bean aphid (*Aphis fabae* Scopoli) and Pea aphid (*Acyrthosiphon pisum* (Harris)) (Homoptera, Aphididae). *Journal of the New York Entomological Society*, **90**, 87-93.

Sato, S. & Dixon, A.F.G. (2004) Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds: consequences for invasive species. *Agricultural and Forest Entomology*, **6**, 21-24.

Satterthwaite, F.E. (1946). An approximate distribution of estimates of variance components. *Biometrics*, **2**, 110-114.

Schaller, A. (2008) Induced Plant Resistance to Herbivory. Springer, The Netherlands.

Schaller, A., Jongsma, M.A. & Beekwilder, J. (2008). Plant protease inhibitors: Functional evolution for defense. In: Induced Plant Resistance to Herbivory, pp. 235–251. Springer, The Netherlands.

Schmidt, M.H., Lauer, A., Purtauf, T., Thies, C., Schaefer, M. & Tscharntke, T. (2003) Relative importance of predators and parasitoids for cereal aphid control. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 1905–1909.

Schoonhoven, L.M., Loon, J.J.A. & Van Dicke, M. (2005) Insect–Plant Biology. Oxford University Press, Oxford.

References

Seko, T. & Miura, K. (2008) Functional response of the lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) on the aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Applied Entomology and Zoology*, **43**, 341-345.

Sentis, A., Hemptinne, J.L. & Brodeur, J. (2014) Towards a mechanistic understanding of temperature and enrichment effects on species interaction strength, omnivory and food-web structure. *Ecology Letters*, **17**, 785-793.

Shepherd, T., Robertson, G.W., Griffiths, D.W. & Birch, A.N.E. (1999) Epicuticular wax composition in relation to aphid infestation and resistance in red raspberry (*Rubus idaeus* L.). *Phytochemistry*, **52**, 1239-1254.

Sloggett, J.J. & Davis, A.J. (2010) Eating chemically defended prey: alkaloid metabolism in an invasive ladybird predator of other ladybirds (Coleoptera: Coccinellidae). *Journal of Experimental Biology*, **213**, 237-241.

Sloggett, J., Magro, A., Verheggen, F., Hemptinne, J.L., Hutchison, W. & Riddick, E. (2011) The chemical ecology of *Harmonia axyridis*. *BioControl*, **56**, 643-661.

Smith, C.A. & Gardiner, M.M. (2013) Biodiversity loss following the introduction of exotic competitors: does intraguild predation explain the decline of native lady beetles? *PLOS ONE*, **8**, e84448.

Smith, C.M. (2005) Plant Resistance to Arthropods. Springer Press, Dordrecht, The Netherlands.

Snyder, W.E. & Evans, E.W. (2006) Ecological effects of invasive arthropod generalist predators. *Annual Review of Ecology Evolution and Systematics*, **37**, 95-122.

Snyder, W.E. & Ives, A.R. (2001) Generalist predators disrupt biological control by a specialist parasitoid. *Ecology* **82**, 705-716.

Snyder, W.E., Ballard, S.N., Yang, S., Clevenger, G.M., Miller, T.D., Ahn, J.J., Hatten, T.D. & Berryman, A.A. (2004) Complementary biocontrol of aphids by the ladybird beetle *Harmonia axyridis* and the parasitoid *Aphelinus asychis* on greenhouse roses. *Biological Control*, **30**, 229-235.

Snyder, W.E., Snyder, G.B., Finke, D.L. & Straub, C.S. (2006) Predator biodiversity strengthens herbivore suppression. *Ecology Letters*, **9**, 789-796.

Soares, A., Borges, I., Borges, P.V., Labrie, G. & Lucas, É. (2008). *Harmonia axyridis*: What will stop the invader? In: From Biological Control to Invasion: the Ladybird *Harmonia axyridis* as a Model Species (ed. by H. Roy & E. Wajnberg), pp. 127-145. Springer, The Netherlands.

Stace-Smith, R. (1955) Studies on *Rubus* virus diseases in British Columbia. I. *Rubus* yellow-net. *Canadian Journal of Botany*, **33**, 269-274.

Stacey, D.A. & Fellowes, M.D.E. (2002a) Influence of temperature on pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae) resistance to natural enemy attack. *Bulletin of Entomological Research*, **92**, 351-357.

Stacey, D.A. & Fellowes, M.D.E. (2002b) Influence of elevated $CO_2$ on interspecific interactions at higher trophic levels. *Global Change Biology*, **8**, 668-678.

Staudt, M., Jackson, B., El-aouni, H., Buatois, B., Lacroze, J.-P., Poëssel, J.L. & Sauge, M.-H. (2010) Volatile organic compound emissions induced by the aphid *Myzus persicae* differ among resistant and susceptible peach cultivars and a wild relative. *Tree Physiology*, **30**, 1320-1334.

Steflen, W., Andreae, M.O., Bolin, B., Cox, P.M., Crutzen, P.J., Cubasch, U., Held, H., Nakidenovic, N., Scholes, R.J. & Talaue-McManus, L. (2004) Abrupt changes: the Achilles' heels of the Earth System. *Environment: Science and Policy for Sustainable Development*, **46**, 8-20.

Stiling, P. & Cornelissen, T. (2005) What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control*, **34**, 236-246.

Stiling, P. & Cornelissen, T. (2007) How does elevated carbon dioxide ($CO_2$) affect plant–herbivore interactions? A field experiment and meta-analysis of $CO_2$-mediated changes on plant chemistry and herbivore performance. *Global Change Biology*, **13**, 1823-1842.

Stiling, P., Rossi, A.M., Hungate, B., Dijkstra, P., Hinkle, C.R., Knott, W.M. & Drake, B. (1999) Decreased leaf-miner abundance in elevated $CO_2$: Reduced leaf quality and increased parasitoid attack. *Ecological Applications*, **9**, 240-244.

Stireman, J.O., Dyer, L.A., Janzen, D.H., Singer, M.S., Lill, J.T., Marquis, R.J., Ricklefs, R.E., Gentry, G.L., Hallwachs, W., Coley, P.D., Barone, J.A., Greeney, H.F., Connahs, H., Barbosa, P., Morais, H.C., and Diniz, I.R., 2005. Climatic unpredictability and parasitism of caterpillars: Implications of global warming. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 17384-17387.

Sun, Y.C., Su, J. & Ge, F. (2010) Elevated $CO_2$ reduces the response of *Sitobion avenae* (Homoptera: Aphididae) to alarm pheromone. *Agriculture, Ecosystems & Environment*, **135**, 140-147.

Sun, Y.C., Yin, J., Cao, H., Li, C., Kang, L. & Ge, F. (2011a) Elevated $CO_2$ influences nematode-induced defense responses of tomato genotypes differing in the JA pathway. *PLOS ONE*, **6**, e19751.

Sun, Y.C., Feng, L., Gao, F. & Ge, F. (2011b) Effects of elevated $CO_2$ and plant genotype on interactions among cotton, aphids and parasitoids. *Insect Science*, **18**, 451-461.

Sun, Y.C., Guo, H., Zhu-Salzman, K. & Ge, F. (2013) Elevated $CO_2$ increases the abundance of the peach aphid on *Arabidopsis* by reducing jasmonic acid defenses. *Plant Science*, **210**, 128-140.

Tans, P. (2014) Concentrations of $CO_2$ in the Earth's atmosphere derived from in situ air measurements at the Mauna Loa Observatory. National Oceanic and Atmospheric Administration.

References

Tedders, W.L. & Schaefer, P.W. (1994) Release and establishment of *Harmonia axyridis* (Coleoptera, Coccinellidae) in the southeastern United States. *Entomological News*, **105**, 228-243.

The Royal Society (2009). Reaping the benefits: science and the sustainable intensification of global agriculture, The Royal Society, London, UK.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Rose, U.S.R. & Schnitzler, J.P. (2006) Practical approaches to plant volatile analysis. *Plant Journal*, **45**, 540-560.

Thomas, A.P., Trotman, J., Wheatley, A., Aebi, A., Zindel, R. & Brown, P.M.J. (2013) Predation of native coccinellids by the invasive alien *Harmonia axyridis* (Coleoptera: Coccinellidae): detection in Britain by PCR-based gut analysis. *Insect Conservation and Diversity*, **6**, 20-27.

Thomson, L.J., Macfadyen, S. & Hoffmann, A.A. (2010) Predicting the effects of climate change on natural enemies of agricultural pests. *Biological Control*, **52**, 296-306.

Tollrian, R. & Harvell, C.D. (1998) The Ecology and Evolution of Inducible Defenses. Princeton University Press.

Ton, J., D'Alessandro, M., Jourdie, V., Jakab, G., Karlen, D., Held, M., Mauch-Mani, B. & Turlings, T.C.J. (2007) Priming by airborne signals boosts direct and indirect resistance in maize. *Plant Journal*, **49**, 16-26.

Traugott, M. & Symondson, W.O.C. (2008) Molecular analysis of predation on parasitized hosts. *Bulletin of Entomological Research*, **98**, 223-231.

Trotter, R.T., Cobb, N.S. & Whitham, T.G. (2008) Arthropod community diversity and trophic structure: a comparison between extremes of plant stress. *Ecological Entomology*, **33**, 1-11.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, **250**, 1251-1253.

Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, **11**, 1351-1363.

Udayagiri, S. & Mason, C.E. (1997) Epicuticular wax chemicals in *Zea mays* influence oviposition in *Ostrinia nubilalis*. *Journal of Chemical Ecology*, **23**, 1675-1687.

United Nations (2013) World population prospects: the 2012 Revision. P.D. Department of Economic and Social Affairs, United Nations.

Valkama, E., Koricheva, J. & Oksanen, E. (2007) Effects of elevated $O_3$, alone and in combination with elevated $CO_2$, on tree leaf chemistry and insect herbivore performance: a meta-analysis. *Global Change Biology*, **13**, 184-201.

Van der Putten, W.H., Macel, M. & Visser, M.E. (2010) Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 2025-2034.

Van Driesche, R.G. (2008) Control of Pests and Weeds by Natural Enemies – an introduction to biological control, Blackwell Publishing, Oxford, UK.

Van Emden, H.F. & Bashford, M.A. (1976) The effect of leaf excision on the performance of *Myzus persicae* and *Brevicoryne brassicae* in relation to the nutrient treatment of the plants. *Physiological Entomology*, **1**, 67-71.

Vandermoten, S., Mescher, M.C., Francis, F., Haubruge, E. & Verheggen, F.J. (2012) Aphid alarm pheromone: An overview of current knowledge on biosynthesis and functions. *Insect Biochemistry and Molecular Biology*, **42**, 155-163.

Vannette, R.L. & Hunter, M.D. (2011) Genetic variation in expression of defense phenotype may mediate evolutionary adaptation of *Asclepias syriaca* to elevated $CO_2$. *Global Change Biology*, **17**, 1277-1288.

Vila, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarosik, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y. & Pysek, P. (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, **14**, 702-708.

Vilcinskas, A., Stoecker, K., Schmidtberg, H., Röhrich, C.R. & Vogel, H. (2013) Invasive harlequin ladybird carries biological weapons against native competitors. *Science*, **340**, 862-863.

Visser, M.E. & Both, C. (2005) Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 2561-2569.

Völkl, W. (1989) Resource partitioning in a guild of aphid species associated with creeping thistle *Cirsium arvense*. *Entomologia Experimentalis et Applicata*, **51**, 41-47.

Vuorinen, T., Nerg, A.M., Ibrahim, M.A., Reddy, G.V.P. & Holopainen, J.K. (2004) Emission of *Plutella xylostella*-induced compounds from cabbages grown at elevated $CO_2$ and orientation behavior of the natural enemies. *Plant Physiology*, **135**, 1984-1992.

Walker, M. & Jones, T.H. (2001) Relative roles of top-down and bottom-up forces in terrestrial tritrophic plant–insect herbivore–natural enemy systems. *Oikos*, **93**, 177-187.

Walling, L.L. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, **19**, 195-216.

Walther, G.R., Roques, A., Hulme, P.E., Sykes, M.T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S., Botta-Dukát, Z., Bugmann, H., Czúcz, B., Dauber, J., Hickler, T., Jarošík, V., Kenis, M., Klotz, S., Minchin, D., Moora, M., Nentwig, W., Ott, J., Panov, V.E., Reineking, B., Robinet, C., Semenchenko, V.,

Solarz, W., Thuiller, W., Vilà, M., Vohland, K. & Settele, J. (2009) Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution*, **24**, 686-693.

Ware, R.L. & Majerus, M.E.N. (2008) Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. *BioControl*, **53**, 169-188.

Watt, A.D., Whittaker, J.B., Docherty, M., Brookes, G. & Salt, D.T. (1995) The impact of elevated $CO_2$ on insect herbivores. In: Insects in a Changing Environment (ed. by R. Harrington & M.E. Stork), pp. 197-217. Academic Press, San Diego, California.

Way, M.J. & Cammell, M. (1970) Aggregation behaviour in relation to food utilization by aphids. In: Animal populations in relation to their food resources. A symposium of the British Ecological Society, Aberdeen 24-28 March 1969., pp. 229-247. Blackwell, Oxford and Edinburgh.

White, T.C.R. (1974) A hypothesis to explain outbreaks of looper caterpillars, with special reference to populations of *Selidosema suavis* in a plantation of *Pinus radiata* in New Zealand. *Oecologia*, **16**, 279-301.

White, T.C.R. (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, **63**, 90-105.

Whittaker, J.B. (2001) Insects and plants in a changing atmosphere. *Journal of Ecology*, **89**, 507-518.

Whittingham, M.J., Stephens, P.A., Bradbury, R.B. & Freckleton, R.P. (2006) Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology*, **75**, 1182-1189.

Wilkinson, T.L., Adams, D., Minto, L.B. & Douglas, A.E. (2001) The impact of host plant on the abundance and function of symbiotic bacteria in an aphid. *Journal of Experimental Biology*, **204**, 3027-3038.

Will, T., Kornemann, S.R., Furch, A.C.U., Tjallingii, W.F. & van Bel, A.J.E. (2009) Aphid watery saliva counteracts sieve-tube occlusion: a universal phenomenon? *Journal of Experimental Biology*, **212**, 3305-3312.

Wittwer, S.H. & Castilla, N. (1995) Protected cultivation of horticultural crops worldwide. *HortTechnology*, **5**, 6-23.

Wood, S. & Scheipl, F. (2013) *gamm4: Generalized additive mixed models using mgcv and lme4*. http://cran.r-project.org/web/packages/ gamm4

Yuan, J.S., Himanen, S.J., Holopainen, J.K., Chen, F.J. & Stewart, C.N. (2009) Smelling global climate change: mitigation of function for plant volatile organic compounds. *Trends in Ecology & Evolution*, **24**, 323-331.

Zavala, J.A., Casteel, C.L., DeLucia, E.H. & Berenbaum, M.R. (2008) Anthropogenic increase in carbon dioxide compromises plant defense against invasive insects. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 5129-5133.

References

Zavala, J.A., Nabity, P.D. & DeLucia, E.H. (2013) An emerging understanding of mechanisms governing insect herbivory under elevated $CO_2$. *Annual Review of Entomology*, **58**, 79-97.

Zuberbuehler, K. (2009) Survivor signals: the biology and psychology of animal alarm calling. In: Advances in the Study of Behavior, Vol 40 (ed. by M. Naguib, K. Zuberbuehler, N.S. Clayton & V.M. Janik), 277-322. Elsevier, Oxford, UK.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009) Mixed Effects Models and Extensions in Ecology with R. Springer, New York, USA.

Zvereva, E.L., Lanta, V. & Kozlov, M.V. (2010) Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecologia*, **163**, 949-960.

References

# Appendix: Paper published to Frontiers in Plant Science October 2013: Global climate change and above – belowground insect herbivore interactions

## Global climate change and above – belowground insect herbivore interactions.

SCOTT W. McKENZIE, WILLIAM T. HENTLEY, ROSEMARY S. HAILS[1], T. HEFIN JONES[4], ADAM J. VANBERGEN[3] & SCOTT N. JOHNSON[5]

**Abstract.**

Predicted changes to the Earth's climate are likely to affect above–belowground interactions. Our understanding is limited, however, by past focus on two–species aboveground interactions mostly ignoring belowground influences. Despite their importance to ecosystem processes, there remains a dearth of empirical evidence showing how climate change will affect above–belowground interactions. The responses of above– and belowground organisms to climate change are likely to differ given the fundamentally different niches they inhabit. Yet there are few studies that address the biological and ecological reactions of belowground herbivores to environmental conditions in current and future climates. Even fewer studies investigate the consequences of climate change for above–belowground interactions between herbivores and other organisms; those that do provide no evidence of a directed response. This paper highlights the importance of considering the belowground fauna when making predictions on the effects of climate change on plant–mediated interspecific interactions.

## 1. Introduction

Trophic interactions are likely to be crucial in shaping net effects of global climate change on ecosystems (*e.g.* Harrington *et al.*, 1999; Tylianakis *et al.*, 2008). Modified interactions between trophic groups (*e.g.* spatial or phenological decoupling of herbivore and predator populations) could have far reaching consequences across a range of natural and managed ecosystems with implications for food security (Gregory *et al.*, 2009). In particular, the plant–mediated interactions between above– and belowground herbivores (Blossey & Hunt–Joshi, 2003; Johnson *et al.,* 2012) may be important in the structuring of herbivore and multi–trophic communities (Bardgett & Wardle, 2010; Johnson *et al.*, 2013; Megías & Müller, 2010; Soler *et al.*, 2012). Surprisingly, investigating the potential impacts of climate change on above–belowground interactions, has received little attention (Schroter *et al.*, 2004). Given that root and shoot herbivores affect plants in dramatically different ways, but also interact with each other (Meyer *et al.*, 2009), the conclusions drawn from studies of climate change impacts limited to only aboveground herbivores may be misleading.

This perspectives paper uses empirical examples to illustrate how belowground herbivores influence aboveground plant–insect interactions. It draws on studies concerning above–belowground interactions as well as studies showing how climate change can alter soil herbivore communities. Finally, it considers the few examples that exist where above–belowground interactions have been studied under climate change scenarios to show how such plant–mediated interactions are, or may be, modified. Thus, this paper will highlight the potential for incomplete or inaccurate predictions of climate change impacts on plant–insect relationships, because of lack of consideration of belowground interactions.

## 2. Above–belowground interactions in the current climate

Studies of plant–mediated interactions between spatially–separated herbivores have revealed contrasting ecological patterns (van Dam & Heil, 2011) that have evolved and built upon two major hypotheses: the Stress Response Hypothesis (Masters,

Appendix

1993; Bezemer *et al.*, 2004) and the Defence Induction Hypothesis (Bezemer *et al.,* 2002). The Stress Response Hypothesis suggests root herbivory impairs the plants capacity for water and nutrient uptake, which can lead to the accumulation of nitrogen compounds in foliage (White, 1984) to increase palatability to aboveground herbivores. In contrast, the Defence Induction Hypothesis, suggests that belowground herbivores will induce a systemic increase in plant–defence chemicals, making it more difficult for herbivore colonisation to occur aboveground (Bezemer & van Dam, 2005; Kaplan *et al.*, 2008). These plant–mediated mechanisms arise through a complex path of communication between root and shoot tissues involving primary (*e.g.* Johnson *et al.*, 2009) and secondary (Bezemer & van Dam, 2005) chemicals. The nature and mode of signalling between roots and leaves is a rapidly expanding area of research (Rasmann & Agrawal, 2008). Some hypotheses suggest that interactions between phytohormonal pathways regulate interspecific herbivore interactions (Soler *et al.*, 2013). Different feeding guilds elicit different phytohormonal pathways. For example, jasmonic acid (induced by root–chewers) reduces a plant's salycilic acid defence response against aphids (Soler *et al.*, 2013). Given that above– and belowground herbivores can systemically alter the defensive phenotype of plants, future models of plant defence allocation would benefit greatly from a systemic–plant approach (Rasmann *et al.*, 2009).

The consequences of interactions between spatially–segregated organisms are more far–reaching than simple pair–wise herbivore–herbivore interactions, with effects cascading across species networks spanning trophic levels and the above– and belowground sub–systems (Scheu, 2001; Wardle *et al.*, 2004). The effects of root herbivory can, for instance, affect tertiary trophic levels. Root herbivores such as the cabbage root fly (*Delia radicum*) have been observed to affect, via the host plant, an aboveground herbivore (*Pieris brassicae*), its parasitoid (*Cotesia glomerata)* and hyper–parasitoid (*Lysibia nana*) (Soler *et al.*, 2005). In this instance, *D. radicum* increased the development time of *P. brassicae* and *C. glomerata*, and the body size of both parasitoid and hyper–parasitoid were reduced. These effects were attributed to an alteration in the blend of phytotoxins (glucosinolates) emitted post–herbivory (Soler *et al.*, 2005). Conversely, aboveground herbivory can have a negative effect on belowground herbivores and associated natural enemies (Jones & Finch, 1987;

124

Soler *et al*., 2007). For instance, the presence of butterfly larvae (*P. brassicae*) reduced the abundance of the belowground herbivore (*D. radicum*) and its parasitoid (*Trybliographa rapae*) by up to 50% and decreased the body size of emerging parasitoid and root herbivore adults (Soler *et al*., 2007). If these broader interactions between organisms inhabiting the plant rhizosphere and canopy are typical, they could scale–up to play important roles in governing ecosystem function.

## 3. Climate change and belowground herbivores

Many studies and comprehensive reviews address the effects of global climate change on aboveground insect herbivores (*e.g.* Bale *et al*., 2002; Cornelissen, 2011), whereas there are substantially fewer studies of the impacts on belowground organisms (Staley & Johnson, 2008). Soil fauna are, at least to some extent, buffered from the direct impacts of climate change (Bale *et al*., 2002). Carbon dioxide concentrations are already high within the soil due to root respiration and microbial processes (Haimi *et al*., 2005), and therefore soil fauna are less likely to be affected by increased atmospheric $CO_2$ directly. Soil fauna may, however, be affected indirectly by increased growth of root resources caused by increased atmospheric $CO_2$ (Norby, 1994). While higher soil temperature may also increase root growth, temperature increase may directly affect soil herbivore development and insect phenology (van Asch *et al*., 2007). Reduced soil moisture, potentially a consequence of increased temperature, can also impact many soil insect life–history traits, such as survival and abundance (Pacchioli & Hower, 2004). Predicted increases in climatic extremes under a future climate (*e.g.* increased flooding and drought events) may also drown or desiccate soil biota and herbivores, thus reducing their prevalence in the soil (Parmesan *et al*., 2000).

Soil–dwelling insect herbivores feed on the roots and therefore have very different effects on plant traits than their aboveground counterparts. These effects may alter the predicted consequences of global climate change on shoot herbivores (Robinson *et al*., 2012; Zavala *et al*., 2013). For instance, most plants increase biomass accumulation and rates of photosynthesis in response to elevated $CO_2$ (Ainsworth & Long, 2005); this depends on plants maximising water and nitrogen use efficiency. To facilitate this, many plants increase their root:shoot biomass ratio in response to

elevated $CO_2$, but this may be compromised by root herbivores, which remove root mass, therefore impairing water and nutrient uptake (Johnson & Murray, 2008). A recent meta–analysis by Zvereva and Kozlov (2012) showed that root herbivores reduced rates of photosynthesis in host plants; this contrasts with many aboveground herbivores that actually stimulate it (Thomson *et al*., 2003). Empirical evidence also suggests that root herbivory can effectively reverse the effects of elevated $CO_2$ on eucalypt chemistry (*e.g.* increased foliar C:N ratio) and biomass, potentially altering the outcomes for aboveground herbivores (Johnson,*pers. obs*.).

**4. Climate change and above–belowground interactions: empirical evidence**

To our knowledge, there are only two peer–reviewed published examples describing how an elevated $CO_2$ environment affects the interaction between above– and belowground herbivores. The first focused on the interaction between the root–feeding (*Pemphigus populitransversus*) and shoot–feeding (*Aphis fabae fabae*) aphids*,* on *Cardamine pratensis* (Salt *et al*., 1996). The study concluded the interaction between these spatially–separated aphids was unaffected by $CO_2$, because root herbivore populations were always smaller in the presence of an aboveground herbivore regardless of the $CO_2$ environment. The second study investigated the conspecific interaction between aboveground adults and belowground larvae of the clover root weevil (*Sitona lepidus*) (Johnson & McNicol, 2010). Elevated $CO_2$ increased leaf consumption by adult weevils but resulted in lower rates of oviposition. These patterns were interpreted by the authors to be a compensatory feeding response to reduced leaf nitrogen and lower reproductive output due to inadequate nutrition. Despite reduced rates of oviposition, larval survival was much greater at elevated than at ambient $CO_2$ –levels potentially due to increased nodulation (increased food source) of the host plant (*Trifolium repens*) under elevated $CO_2$ conditions (Johnson & McNicol, 2010).

Enrichment with $CO_2$ is not only expected to increase plant biomass both above– and belowground, but also to reduce plant tissue quality through increases in the C:N ratio and secondary metabolite concentrations (Bezemer & Jones, 1998). Compensatory feeding by phytophagous insects in an elevated $CO_2$ environment may thus increase exposure to defensive chemicals present in plant tissue. This is likely,

however, to be contingent on plant taxonomic identity, as concentrations of defensive chemicals may increase (*e.g.* glucosinolates in *Aradopsis thaliana* (Bidart–Bouzat *et al.*, 2005)), or remain unchanged (*e.g.* tannins in *Quercus myrtifolia* (Rossi *et al.*, 2004)) in response to $CO_2$ enrichment.

Temperature changes may alter above–belowground interactions either by affecting invertebrate phenology directly (Gordo & Sanz, 2005; Harrington *et al.*, 2007) or indirectly through changes in the plant (Bale *et al.*, 2002; Harrington *et al.*, 1999; Singer & Parmesan, 2010), although this remains to be tested empirically. A predicted increase in global mean temperatures may also result in an increased water stress response in plants (Huberty & Denno, 2004), making them more susceptible to herbivory both above– and belowground.

Summer drought is another factor associated with climate change that has been shown to influence above–belowground interactions. Typically, root–chewing *Agriotes sp.* larvae reduced the abundance and performance of leaf–mining *Stephensia brunnichella* larvae and its associated parasitoid (Staley *et al.*, 2007). This effect was, however, negated under drought conditions. Changes to summer rainfall may, therefore, reduce the occurrence or alter the outcome of plant–mediated interactions between insect herbivores.

Above–belowground interactions may also be influenced by variation in soil moisture. Experimentally elevated rainfall increased the suppression of an outbreak of the herbivorous moth larvae *Hepialus californicus* by an entomopathogenic nematode (*Heterorhabditis marelatus*), thereby indirectly protecting the host plant – bush lupine (*Lupinus arboreus*) (Preisser & Strong, 2004). Thus climate change, by altering patterns of precipitation, has the potential to modify herbivore–natural enemy interactions to reduce herbivore pressure.

Few studies have integrated the multiple abiotic factors associated with climate change (*i.e.* water supply, temperature, $CO_2$ etc) to investigate their combined effects on above–belowground interactions. One such study (Stevnbak *et al.*, 2012) manipulated $CO_2$ concentration, air and soil temperature and precipitation to show that soil microbial biomass was altered by aboveground herbivory (*Chorthippus brunneus*). The combination of multiple climate change treatments with aboveground

herbivory increased microbivorous protist abundance in the soil, emphasising the importance of considering climate change in above–belowground interactions.

## 5. The future of above–belowground interactions and climate change research

Johnson *et al*. (2012) conducted a meta–analysis on two–species above–belowground herbivore interactions. Although restricted by not including other trophic groups, the meta–analysis did identify several factors that determine the outcomes of interactions between spatially–separated herbivores. From these outcomes it is possible to develop hypotheses of how specific interactions are likely to be affected by climate change. The chronological sequence in which herbivores fed on shared plants was a major determinant of interaction outcome. In particular, aboveground herbivores negatively affected belowground herbivores when they fed first, but not when feeding synchronously or following belowground herbivores. Conversely, belowground herbivores typically had positive effects on aboveground herbivores only when synchronously feeding, otherwise they had a negative impact (Johnson *et al*., 2012). Many of the data on aboveground species are from aphids; we know that elevated $CO_2$ and temperature results in earlier and longer seasonal occurrences of many pest species, including aphids (Harrington *et al*., 2007). Therefore in the future it might be reasonable to expect that some aphids may initiate feeding on the plant prior to belowground herbivores. Under such circumstances, aphids may negatively affect the belowground herbivore while remaining unaffected themselves, the reverse of the interaction under current conditions. Likewise, if drought conditions delayed root herbivore development this change could become even more pronounced.

Feeding guild identity (*e.g.* chewers, suckers, gallers) can affect the outcome of above–belowground interactions. Johnson *et al*. (2012) showed that the effects on aboveground herbivores depended on belowground herbivore guild. Individual feeding guilds and trophic levels respond differently to climate change (Voigt *et al*., 2003), but how this translates into changes in above– belowground trophic interactions remains unexplored. The increased level of defence compounds in plant tissue, predicted to occur under climate change scenarios (Robinson *et al*., 2012), are likely to have a disproportionate effect between (a) herbivores feeding above– or belowground: defence compounds may be concentrated in either leaf or root tissue,

128

and (b) different feeding guilds: chewing insects being more susceptible to defensive compounds than phloem–feeders. There is, however, a strong bias in the literature, with certain herbivore guilds and orders (e.g Lepidoptera) having been represented disproportionately within empirical studies (Robinson *et al*., 2012). Conclusions extrapolated regarding general herbivore–responses to climate change should, therefore, be treated with appropriate caution.

There are few long–term above–belowground interaction studies. Some Arctic long–term manipulative field studies (*e.g.* Ruess *et al*., 1999) that illustrate the effects of climate warming on soil fauna provide essential information on legacy effects in natural ecosystems. These indicate that above–belowground interactions may be separated temporally (Kostenko *et al*., 2012) as well as spatially. Long–term field experiments may also yield different results to laboratory experiments conducted over a smaller timescale (Johnson *et al*., 2012).

## 6. Conclusions and Research Agenda

Our understanding of how individual species respond to climate change has increased dramatically over the past 25 years. We have a relatively well–informed understanding of how aboveground herbivores may react to different aspects of climate change (*e.g.* Bale *et al.,* 2002) but our knowledge of belowground species responses remains lacking. Johnson and Murray (2008) illustrate how this area of research is a "hot topic" for multidisciplinary research while others (Soler *et al*., 2005; van Dam & Heil, 2011) underline the importance of a more integrated understanding of climate change impacts on ecosystems that incorporates above– and belowground trophic linkages.

Based on current knowledge of above–belowground interactions we are able to formulate hypotheses that could be tested empirically in future research. For example:

1. Root herbivory is likely to change fundamentally plant responses to an elevated $CO_2$ environment, since root function usually underpins the plants ability to respond to environmental changes. We hypothesise that inclusion of root herbivores will reverse

the effects of elevated $CO_2$ on certain aboveground herbivores, particularly those negatively affects by higher C:N ratios (*e.g.* leaf-miners).

2. Plant functional identity may shape how above–belowground interactions respond to climate change. For instance, plants with $C_3$ and $C_4$ photosynthetic pathways will respond differently to climate change, and notably elevated $CO_2$ (Barbehenn *et al.*, 2004a). In particular, $C_3$ plants potentially show a greater decline in nutritional quality than $C_4$ plants, which are often inherently less favourable hosts to insect herbivores (see the $C_3$–$C_4$ hypothesis of Schiers *et al.*, 2001). This might lead to compensatory feeding on $C_3$, but not $C_4$, plants in future climates (Barbehann *et al.*, 2004b). We hypothesise that above–belowground interactions are likely to be more affected on $C_3$ than $C_4$ plants.

3. Belowground herbivory induces a water stress on the plant, similar to drought. Experiments investigating drought effects on aboveground plant–herbivore interactions may, therefore, be analogous to above–belowground herbivore interactions generally. We hypothesise that the combination of a drought treatment and a belowground herbivore may have additive negative effects on the plant and consequently on aboveground herbivores (through increased susceptibility to herbivory).

Increasing trophic complexity in empirical climate change research will strengthen the ability to make more accurate predictions of trophic interactions in future environments (Robinson *et al.*, 2012). Making predictions based on simple plant–herbivore interactions compared to wider communities may be misleading and interaction outcomes may be altered with the inclusion of higher trophic levels. As seen aboveground, climate change may not directly affect the abundance of a herbivore, however, if the abundance or impact of an associated antagonist is reduced then climate change may increase herbivore abundance indirectly. Disrupted phenological synchrony between predator and prey (Hance *et al.*, 2007) may be one mechanism, another may be a reduction in plant production of chemical attractants (synomones) that recruit natural enemies, which then regulate herbivore numbers (Yuan *et al.*, 2009). Alternatively, climate change may benefit the prey and antagonist equally, with any increase in herbivore abundance merely supporting greater numbers of natural enemies and thus leading to no net change in populations

(*e.g.* Chen *et al*., 2005). An integrated approach considering trophic interactions as an integral part of an ecosystem comprising above– and belowground components will provide a more accurate estimation of climate change impacts. For example, a positive effect of root herbivores on folivores at higher temperatures may, if climate change positively affected antagonist efficacy (*e.g.* Bezemer *et al*., 1998; Hance *et al*., 2007), be cancelled–out with the inclusion of an above– or belowground antagonist. For the most part this remains to be tested empirically. Moreover, with more empirical data it may be possible that – as has been observed with other areas of climate change research (Robinson *et al*., 2012) – apparent idiosyncratic outcomes of climate change impacts on plant–herbivore interactions give way to reveal generalities. Trends have become apparent in some aspects of insect herbivory in elevated $CO_2$ (Zavala *et al*., 2013), for example, phloem feeders increase in abundance under elevated $CO_2$, whereas leaf-miners decrease (Robinson *et al*., 2012). Alternatively, further research may simply reveal a lack of general responses of above–belowground interactions to climate change. For instance, despite the large body of research on aphid–plant interactions under climate change, aphid responses to $CO_2$ enrichment still appear to be highly species–specific (see Sun & Ge, 2011 and references therein). The challenge for ecologists therefore is to utilise current knowledge of individual species responses to climate change and develop our understanding into general hypotheses for functional guilds, networks of species and ecosystem processes.

Appendix

References

Ainsworth, E. A. & Long, S. P. (2005). What have we learned from 15 years of free–air $CO_2$ enrichment (FACE)? A meta–analytic review of the responses of photosynthesis, canopy properties and plant production to rising $CO_2$. *New Phytol*, 165, 351–372.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., & Whittaker, J. B. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biol*, 8, 1–16.

Barbehenn, R.V., Chen, Z., Karowe, D. N., & Spickard, A. (2004a). $C_3$ grasses have higher nutritional quality than C4 grasses under ambient and elevated atmospheric $CO_2$. *Global Change Biol*, 10, 1565–1575.

Barbehenn, R., Karowe, D., & Chen, Z. (2004b). Performance of a generalist grasshopper on a $C_3$ and a $C_4$ grass: compensation for the effects of elevated $CO_2$ on plant nutritional quality. *Oecologia*, 140, 96–103.

Bardgett, R. D. & Wardle, D. A. (2003). Herbivore–mediated linkages between aboveground and belowground communities. *Ecology*, 84, 2258–2268.

Bardgett, R. D. & Wardle, D. A. (2010). *Aboveground–Belowground Linkages; Biotic Interactions, Ecosystem Processes and Global Change*. Oxford University Press, Oxford.

Bezemer, T. M. & Jones, T. H. (1998). Plant–insect herbivore interactions in elevated atmospheric $CO_2$: quantitative analyses and guild effects. *Oikos*, 82, 212–222.

Bezemer, T. M., Jones, T. H. & Knight, K. J. (1998). Long–term effects of elevated $CO_2$ and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. *Oecologia*, 116, 128–135.

Bezemer, T., Wagenaar, R., van Dam, N. M., & Wäckers, F. L. (2002). Interactions between root and shoot feeding insects are mediated by primary and secondary plant compounds. *Proc Neth Soc Entomol Meet*, 13, 117–121.

Appendix

Bezemer, T. M., Wagenaar, R., van Dam, N. M., van der Putten, W. H., & Wäckers, F. L. (2004). Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. *J Chem Ecol*, 30, 53–67.

Bezemer, T. M. & van Dam, N. M. (2005). Linking aboveground and belowground interactions via induced plant defenses. *Trends Ecol Evol*, 20, 617–624.

Bidart–Bouzat, M., Mithen, R., & Berenbaum, M. (2005). Elevated $CO_2$; influences herbivory–induced defense responses of *Arabidopsis thaliana*. *Oecologia*, 145, 415–424.

Blossey, B. & Hunt–Joshi, T. R. (2003). Belowground herbivory by insects: Influence on plants and aboveground herbivores. *Annu Rev Entomol*, 48, 521–547.

Chen, F., Ge, F., & Parajulee, M. N. (2005). Impact of elevated $CO_2$ on tri–trophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. *Environ Entomol*, 34, 37–46.

Cornelissen, T. (2011). Climate change and its effects on terrestrial insects and herbivory patterns. *Neotrop Entomol*, 40, 155–163.

Gange, A. C. & Brown, V. K. (1989). Effects of root herbivory by an insect on a foliar–feeding species, mediated through changes in the host plant. *Oecologia*, 81, 38–42.

Gordo, O. & Sanz, J. (2005). Phenology and climate change: a long–term study in a Mediterranean locality. *Oecologia*, 146, 484–495.

Gregory, P. J., Johnson, S. N., Newton, A. C., & Ingram, J. S. I. (2009). Integrating pests and pathogens into the climate change/food security debate. *J Exp Bot*, 60, 2827–2838.

Haimi, J., Laamanen, J., Penttinen, R., Räty, M., Koponen, S., Kellomäki, S., & Niemelä, P. (2005). Impacts of elevated $CO_2$ and temperature on the soil fauna of boreal forests. *Appl Soil Ecol*, 30, 104–112.

Hance, T., van Baaren, J., Vernon, P., & Boivin, G. (2007). Impact of extreme temperatures on parasitoids in a climate change perspective. *Annu Rev Entomol*, 52, 107–126.

Appendix

Harrington, R., Woiwod, I., & Sparks, T. (1999). Climate change and trophic interactions. *Trends Ecol Evol*, 14, 146–150.

Harrington, R., Clark, S. J., Welham, S. J., Verrier, P. J., Denholm, C. H., Hullé, M., Maurice, D., Rounsevell, M. D., Cocu, N., & European Union Examine Consortium. (2007). Environmental change and the phenology of European aphids. *Global Change Biol*, 13, 1550–1564.

Huberty, A. F. & Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, 85, 1383–1398.

Johnson, S. N. & Murray, P. J. (2008). *Root Feeders: an ecosystem perspective.* CABI, Wallingford, U.K.

Johnson, S. N., Hawes, C., & Karley, A. J. (2009). Reappraising the role of plant nutrients as mediators of interactions between root– and foliar–feeding insects. *Funct Ecol*, 23, 699–706.

Johnson, S. N. & McNicol, J. (2010). Elevated $CO_2$ and aboveground–belowground herbivory by the clover root weevil. *Oecologia*, 162, 209–216.

Johnson, S. N., Clark, K. E., Hartley, S. E., Jones, T. H., McKenzie, S. W., & Koricheva, J. (2012). Aboveground–belowground herbivore interactions: a meta–analysis. *Ecology*, 93, 2208–2215.

Johnson, S. N., Mitchell, C., Thompson, J., & Karley, A. J. (2013). Downstairs drivers – root herbivores shape communities of aboveground herbivores and natural enemies via plant nutrients. *J Anim Ecol*, doi: 10.1111/1365–2656.12070.

Jones, T. H. & Finch, S. (1987). The effect of a chemical deterrent, released from the frass of caterpillars of the garden pebble moth, on root fly oviposition. *Entomol Exp Appl,* 45, 283–288.

Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, S., Denno, R. F. (2008). Constitutive and induced defenses to herbivory in above– and belowground plant tissues. *Ecology*, 89, 392–406.

Kostenko, O., van de Voorde, T. F. J., Mulder, P. P. J., van der Putten, W. H., & Bezemer, T. M. (2012). Legacy effects of aboveground–belowground interactions. *Ecol Lett*, 15, 813–821.

Appendix

Masters, G. J., Brown, V. K., & Gange, A. C. (1993). Plant mediated interactions between above– and below–ground insect herbivores. *Oikos*, 66, 148–151.

Megías, A. G. & Müller, C. (2010). Root herbivores and detritivores shape above–ground multitrophic assemblage through plant–mediated effects. *J Anim Ecol*, 79, 923–931.

Meyer, K. M., Vos, M., Mooij, W. M., Hol, W. H. G., Termorshuizen, A. J., Vet, L. E. M., van der Putten, W. H. (2009). Quantifying the impact of above– and belowground higher trophic levels on plant and herbivore performance by modeling. *Oikos*, 118, 981–990.

Norby, R. J. (1994). Issues and perspectives for investigating root responses to elevated atmospheric carbon–dioxide. *Plant Soil*, 165, 9–20.

Pacchioli, M. A. & Hower, A. A. (2004). Soil and moisture effects on the dynamics of early instar clover root *Curculio* (Coleoptera: Curculionidae) and biomass of alfalfa root nodules. *Environ Entomol*, 33, 119–127.

Parmesan, C., Root, T. L., & Willig, M. R. (2000). Impacts of extreme weather and climate on terrestrial biota. *B Am Meteorol Soc*, 81, 443–450.

Preisser, E. L. & Strong, D. R. (2004). Climate affects predator control of an herbivore outbreak. *Am Nat*, 163, 754–762.

Rasmann, S. & Agrawal, A. A. (2008). In defense of roots: A research agenda for studying plant resistance to belowground herbivory. *Plant Physiol*, 146, 875–880.

Rasmann, S., Agrawal, A. A., Cook, S. C., & Erwin, A. C. (2009). Cardenolides, induced responses, and interactions between above– and belowground herbivores of milkweed (*Asclepias* spp.). *Ecology*, 90, 2393–2404.

Robinson, E. A., Ryan, G. D., & Newman, J. A. (2012). A meta–analytical review of the effects of elevated $CO_2$ on plant–arthropod interactions highlights the importance of interacting environmental and biological variables. *New Phytol*, 194, 321–336.

Rossi, A., Stiling, P., Moon, D., Cattell, M., & Drake, B. (2004). Induced defensive response of Myrtle Oak to foliar insect herbivory in ambient and elevated $CO_2$. *J Chem Ecol*, 30, 1143–1152.

Appendix

Ruess, L., Michelsen, A., Schmidt, I., & Jonasson, S. (1999). Simulated climate change affecting microorganisms, nematode density and biodiversity in subarctic soils. *Plant Soil*, 212, 63–73.

Salt, D. T., Fenwick, P., & Whittaker, J. B. (1996). Interspecific herbivore interactions in a high $CO_2$ environment: root and shoot aphids feeding on *Cardamine. Oikos*, 77, 326–330.

Scheirs, J., Bruyn, L.D., & Verhagen, R. (2001). A test of the $C_3$–$C_4$ hypothesis with two grass miners. *Ecology*, 82, 410–421.

Scheu, S. (2001). Plants and generalist predators as links between the below–ground and above–ground system. *Basic Appl Ecol*, 2, 3–13.

Schroter, D., Brussaard, L., De Deyn, G., Poveda, K., Brown, V. K., Berg, M. P., Wardle, D. A., Moore, J., & Wall, D. H. (2004). Trophic interactions in a changing world: modelling aboveground–belowground interactions. *Basic Appl Ecol*, 5, 515–528.

Singer, M. C. & Parmesan, C. (2010). Phenological asynchrony between herbivorous insects and their hosts: signal of climate change or pre–existing adaptive strategy? *Philos T R Soc B*, 365, 3161–3176.

Soler, R., Bezemer, T. M., van der Putten, W. H., Vet, L. E. M., & Harvey, J. A. (2005). Root herbivore effects on above–ground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality. *J Anim Ecol*, 74, 1121–1130.

Soler, R., Bezemer, T., Cortesero, A., van der Putten, W., Vet, L., & Harvey, J. (2007). Impact of foliar herbivory on the development of a root–feeding insect and its parasitoid. *Oecologia*, 152, 257–264.

Soler, R., van der Putten, W. H., Harvey, J. A., Vet, L. E. M., Dicke, M., Bezemer, T. M. (2012). Root herbivore effects on aboveground multitrophic interactions: patterns, processes and mechanisms. *J Chem Ecol*, 38, 755–767.

Soler, R., Erb, M., & Kaplan, I. (2013). Long distance root–shoot signalling in plant–insect community interactions. *Trends Plant Sci*, 18, 149–156.

Staley, J. T., Mortimer, S. R., Morecroft, M. D., Brown, V. K., & Masters, G. J. (2007). Summer drought alters plant–mediated competition between foliar– and root–feeding insects. *Global Change Biol*, 13, 866–877.

Staley, J.T. & Johnson, S.N. (2008). "Climate change impacts on root herbivores" in *Root Feeders: An Ecosystem Perspective*, ed. S. N. Johnson & P. J. Murray (CABI, Wallingford), 192–215.

Stevnbak, K., Scherber, C., Gladbach, D. J., Beier, C., Mikkelsen, T. N., & Christensen, S. (2012). Interactions between above– and belowground organisms modified in climate change experiments. *Nature Clim Change*, 2, 805–808.

Sun, Y. & Ge, F. (2011). How do aphids respond to elevated $CO_2$? *J Asia Pacific Entomol*, 14, 217–220.

Thomson, V., Cunningham, S., Ball, M., & Nicotra, A. (2003). Compensation for herbivory by *Cucumis sativus* through increased photosynthetic capacity and efficiency. *Oecologia*, 134, 167–175.

Tylianakis, J. M., Didham, R. K., Bascompte, J., & Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecol Lett*, 11, 1351–1363.

van Asch, M., van Tienderen, P. H., Holleman, L. J. M., & Visser, M. E. (2007). Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Global Change Biol*, 13, 1596–1604.

van Dam, N. M. & Heil, M. (2011). Multitrophic interactions below and above ground: en route to the next level. *J Ecol*, 99, 77–88.

Voigt, W., Perner, J., Davis, A. J., Eggers, T., Schumacher, J., Bährmann, R., Fabian, B., Heinrich, W., Köhler, G., Lichter, D., Marstaller, R., & Sander, F. W. (2003). Trophic levels are differentially sensitive to climate. *Ecology*, 84, 2444–2453.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setala, H., van der Putten, W. H., & Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, 304, 1629–1633.

White, T. C. R. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia,* 64, 90–105.

Yuan, J. S., Himanen, S. J., Holopainen, J. K., Chen, F., & Stewart, C. N. (2009). Smelling global climate change: mitigation of function for plant volatile organic compounds. *Trends Ecol Evol*, 24, 323–331.

Zavala, J. A., Nabity, P. D., & DeLucia, E. H. (2013). An emerging understanding of mechanisms governing insect herbivory under elevated $CO_2$. *Annu Rev Entomol*, 58, 79–97.

Zvereva, E. & Kozlov, M. (2012). Sources of variation in plant responses to belowground insect herbivory: a meta–analysis. *Oecologia*, 169, 441–452.

Appendix