Use of floral resources by the lacewing *Micromus tasmaniae* and its parasitoid *Anacharis zealandica*, and the consequences for biological control by *M. tasmaniae*

A thesis
submitted in partial fulfilment
of the requirements for the Degree of Doctor of Philosophy

at
Lincoln University
by
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Lincoln University
2009
Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Ph.D.

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Arthropod species that have the potential to damage crops are food resources for communities of predators and parasitoids. From an agronomic perspective these species are pests and biocontrol agents respectively, and the relationships between them can be important determinants of crop yield and quality. The impact of biocontrol agents on pest populations may depend on the availability of other food resources in the agroecosystem. A scarcity of such resources may limit biological control and altering agroecosystem management to alleviate this limitation could contribute to pest management. This is a tactic of ‘conservation biological control’ and includes the provision of flowers for species that consume prey as larvae but require floral resources in their adult stage.

The use of flowers for pest management requires an understanding of the interactions between the flowers, pests, biocontrol agents and non-target species. Without this, attempts to enhance biological control might be ineffective or detrimental. This thesis develops our understanding in two areas which have received relatively little attention: the role of flowers in biological control by true omnivores, and the implications of flower use by fourth-trophic-level life-history omnivores. The species studied were the lacewing *Micromus tasmaniae* and its parasitoid *Anacharis zealandica*. Buckwheat flowers *Fagopyrum esculentum* provided floral resources and aphids *Acyrthosiphon pisum* served as prey.

Laboratory experiments with *M. tasmaniae* demonstrated that although prey were required for reproduction, providing flowers increased survival and oviposition when prey abundance was low. Flowers also decreased prey consumption by the adult lacewings. These experiments therefore revealed the potential for flowers to either enhance or disrupt biological control by *M. tasmaniae*. 
Adult *M. tasmaniae* were collected from a crop containing a strip of flowers. Analyses to determine the presence of prey and pollen in their digestive tracts suggested that predation was more frequent than foraging in flowers. It was concluded that the flower strip probably did not affect biological control by lacewings in that field, but flowers could be significant in other situations.

The lifetime fecundity of *A. zealandica* was greatly increased by the presence of flowers in the laboratory. Providing flowers therefore has the potential to increase parasitism of *M. tasmaniae* and so disrupt biological control. *A. zealandica* was also studied in a crop containing a flower strip. Rubidium-marking was used to investigate nectar-feeding and dispersal from the flowers. In addition, the parasitoids’ sugar compositions were determined by HPLC and used to infer feeding histories. Although further work is required to develop the use of these techniques in this system, the results suggested that *A. zealandica* did not exploit the flower strip. The sugar profiles suggested that honeydew had been consumed by many of the parasitoids.

A simulation model was developed to explore the dynamics of aphid, lacewing and parasitoid populations with and without flowers. This suggested that if *M. tasmaniae* and *A. zealandica* responded to flowers as in the laboratory, flowers would only have a small effect on biological control within a single period of a lucerne cutting cycle. When parasitoids were present, the direct beneficial effect of flowers on the lacewing population was outweighed by increased parasitism, reducing the potential for biological control in future crops.

The results presented in this thesis exemplify the complex interactions that may occur as a consequence of providing floral resources in agroecosystems and re-affirm the need for agroecology to inform the development of sustainable pest management techniques.

**Keywords**

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*Micromus tasmaniae* deciding whether or not to have nectar for dinner.
Chapter 1 Introduction

1.1 GENERAL INTRODUCTION

Agroecosystems, by definition, are dominated by one or more crops that have been established by people in place of natural vegetation. However, they potentially involve many interesting ecological interactions since the cropped area can be colonised by numerous non-crop species. With this colonisation the crops interact directly and indirectly with plants, animals and microorganisms in both the cropped and uncropped components of the landscape. Together these interacting species constitute the biotic component of a more-or-less tightly defined agroecosystem (Gliessman, 2007). Such systems have the production of food (or fuel or fibre) as their primary purpose and non-crop species many enhance or diminish their productivity. For example, pollinating insects and nitrogen-fixing bacteria may increase crop yield but pathogens and plants that compete with the crop for nutrients may reduce it. The beneficial processes such as pollination and nutrient-cycling have been referred to as ‘supporting ecosystem services’, drawing attention to indirect ways in which people benefit from nature (Daily, 1997; Cassman & Wood, 2005). (There is, however, no equivalent terminology for the negative interactions between the crop and non-crop species.)

In addition to increasing production of material goods, conversion of natural ecosystems to agroecosystems affects non-productive processes which impact upon society: the so-called regulating ecosystem services (e.g. regulation of water flows and climate) and cultural ecosystem services (e.g. support of species or landscapes of aesthetic value) (Daily, 1997; Cassman & Wood, 2005). Non-crop species influence the ability of the agroecosystem to provide these additional ‘services’. The value of many non-crop species in agroecosystems has become more apparent as new chemical and mechanical technologies to increase production have reduced their occurrence. Furthermore, the ability for agroecosystems to provide non-productive services has become more important as agricultural land use has expanded (Matson et al., 1997; Stoate et al., 2001; Tilman et al., 2002; Cassman & Wood, 2005).

The biotic and abiotic characteristics of a region impose constraints on the performance of an agroecosystem but the degree to which the productive and non-productive functions are fulfilled depends greatly on the way the agroecosystem is managed. Agroecology can help develop our understanding both of the roles of non-crop species in the overall performance of agroecosystems, and of how management practices might affect those roles. Together with other scientific disciplines, such understanding can help ensure agroecosystems perform the multiple functions desired by the farmer and wider society. Philosophies which aim for greater sustainability than ‘conventional’ agriculture, such as those of integrated pest management (IPM) and organic farming, are increasingly popular and draw on agroecology for their
development (Altieri, 1995; Glen et al., 1995; Thomas, 1999; Nicholls & Altieri, 2007; Zehnder et al., 2007). Failure to recognise the need for management to be informed by agroecology; treating the agro-ecosystem as an open-air factory where agri-chemicals are the dominant management tools, can lead to long-term losses for short-term gains and unintended consequences within and beyond the agroecosystem (Marco et al., 1987; Pimentel & Lehman, 1993; Pretty, 2005; Pedigo & Rice, 2006).

Although an ecological approach to management of agroecosystems requires a holistic perspective, it is necessary and appropriate for individual studies to consider components of the system in isolation. Here the focus is on the contribution that agroecology can make to control of arthropod pests. (Hereafter the word ‘pest’ is used to refer to arthropods without reference to other pests such as weeds, pathogens or non-arthropod animals.)

This thesis aims to contribute to the understanding that underpins one ecological approach to pest management: provision of floral resources to enhance pest suppression by indigenous (and previously-introduced) arthropod populations. At this time, providing floral resources is a minor component of pest management strategies in commercial agriculture (Pedigo & Rice, 2006; van Driesche et al., 2008). Where flowering plants are encouraged in agroecosystems, this may be for other primary purposes. For example, the agri-environment schemes of the European Union’s agricultural policy are designed to compensate farmers for management practices which otherwise would not be economically rational. Accordingly, in the U.K., the scheme ‘options’ that enhance flower abundance are primarily intended to conserve native flora and non-beneficial insects such as butterflies (Anon., 2005). There has, however, been much research interest in the use of flowers for pest management, mostly in the last few decades but with pioneering studies early last century (early references cited in Jervis et al. (1993) and Bernstein & Jervis (2008), later references cited in Fiedler et al. (2008) and below). Heimpel & Jervis (2005) provide an excellent evaluation of studies concerned with the role of floral resources in biological control by parasitoid wasps. Many further reviews discuss relevant research in the context of work on other ecology-based techniques for pest management and more general ecological principles (van den Bosch & Telford, 1964; van Emden, 1981; Way & Cammell, 1981; Altieri & Letourneau, 1982; Powell, 1986; van Emden, 1988, 1990; Wratten & van Emden, 1995; Jervis et al., 1996; Barbosa & Benrey, 1998; Barbosa & Wratten, 1998; Bugg & Pickett, 1998; Gurr et al., 1998; Wratten et al., 1998; Gurr et al., 2000; Landis et al., 2000; Gurr et al., 2003; Jervis et al., 2004; Pfiffner & Wyss, 2004; Gurr et al., 2005; Heimpel & Jervis, 2005; Wilkinson & Landis, 2005; Nicholls & Altieri, 2007; Orre et al., 2007; Wratten et al., 2007; Zehnder et al., 2007; Mills & Wajnberg, 2008).

An additional thorough review would not be of value at this time so that has not been attempted here. The existing reviews reveal a lack of field studies demonstrating substantial pest suppression through provision of floral resources in the field. However, many studies of the
processes which would contribute to such an outcome (e.g. effects of flowers on longevity of biocontrol agents or rates of parasitism) suggest that the technique has unrealised potential and their results invite further investigations of the ecological interactions involved in flower-provision for pest management (Gurr et al., 2000; Heimpel & Jervis, 2005).

This chapter provides the general context for the original research described in subsequent chapters. It explains the rationale for providing floral resources, introduces the themes of omnivory and use of floral resources by non-target species which are developed in later chapters, and provides an overview of the relationships between the species studied. Each research chapter is written in the style of a journal article and is self-contained to a similar degree. The chapters contain introduction and discussion sections which review pertinent literature and there is some repetition in the description of elements common to multiple chapters. To avoid further repetition, this introductory chapter does not provide a detailed discussion of previous studies.

1.2 PROVIDING RESOURCES TO ENHANCE BIOLOGICAL CONTROL

1.2.1 Alleviating resource limitation

Amongst the many herbivorous insects that colonise crops, a small proportion are given the status of pests. That is, the prevailing characteristics of the agroecosystem are such that the species can cause substantial reductions to crop yield or quality if management practices do not respond to this threat. Minimising crop losses due to arthropod pests is a key aspect of managing agroecosystems to maintain their productive value and there are numerous approaches which may be adopted to do so (Dent, 2000; Pedigo & Rice, 2006). Amongst those which are consistent with the ecological approach to management advocated above is provision of resources to increase the ability of indigenous (and previously-introduced) predators and parasitoids to suppress pest populations (Gurr et al., 2000; Landis et al., 2000; van Driesche et al., 2008). This is a tactic common to the two strategies ‘conservation biological control’ (which also includes reducing mortality through, for example, selective insecticide use and avoiding widescale simultaneous harvesting) and ‘habitat manipulation’ (which also includes management practices which have direct negative effects on the pest i.e. ‘bottom-up’ effects in contrast with ‘top-down’ effects) (Gurr et al., 2000). (In theory, removal of resources that reduce the efficacy of predators or parasitoids could also be practiced, but I am not aware of this tactic being studied and it is not discussed further here.)

When species (primarily arthropods) that consume a pest are present in an agroecosystem they can reduce the pest population relative to when they are absent, with a corresponding increase in crop yield. Depending on the strength of the ‘trophic cascade’, the effect on the crop may or may not be of agronomic significance (Polis et al., 2000). The phrase ‘biological
control’ is used here to refer to pest suppression by the predators and parasitoids, whether or not suppression occurs to a satisfactory degree. In performing this role, the predators and parasitoids are often referred to as biocontrol agents or natural enemies. The former term is used here.

The principle underlying this type of pest management is ensuring that the impact of biocontrol agents on the pest population is not limited by availability of resources. The development of an effective technique involves alleviating any resource limitations in such a way that there is a net gain in productive and/or non-productive value, either at the farm scale or a larger scale at which society measures value. This is a multi-layered, trans-disciplinary process but it must be founded on knowledge of the resources used by the biocontrol agents and how use of these resources affects their consumption of pests. In addition, as discussed in Section 1.4, there must be understanding of how availability of these resources affects other species in the agroecosystem and how they may consequently have direct or indirect effects on pest suppression (Gurr et al., 2000; Jervis et al., 2004; Gurr et al., 2005).

Potentially-limiting resources are diverse in nature. They include physical features of the agroecosystem such as places with microclimates suitable for overwintering. These have been the subject of pest management studies as, for example, in the development of grass strips in arable fields (‘beetle-banks’) for overwintering predatory beetles (Thomas et al., 1992; Collins et al., 2002). Potentially-limiting resources also include sources of nutrition i.e. food resources. As discussed in Section 1.3, biocontrol agents consume a wide range of foods in addition to pests, some of which might be sufficiently scarce in agroecosystems to limit biological control. Here we are particularly interested in nectar and pollen; that is, floral resources.

Nectar and pollen contain numerous minor organic and inorganic nutrients (Fægri & van der Pijl, 1979). However, nectar is primarily a source of sugars and provides energy for those arthropods which are first attracted to the flower and then can access the nectar (Wäckers, 2005; Bernstein & Jervis, 2008). In contrast, pollen is relatively rich in amino acids and lipids which may be required for development or reproduction (Wäckers, 2005). Since pollen (particularly that of anemophilous species) can be dispersed onto many surfaces in the habitat, it can be consumed by species that are not adapted for exploiting flowers e.g. some ladybirds (Coleoptera: Coccinellidae), predatory mites (Acarina: Phytoseiidae) and spiders (Arachnida: Araneidae) (Smith & Mommsen, 1984; McMurtry & Croft, 1997; Lundgren et al., 2005).

The extra-floral nectaries of some crops (e.g. some cotton cultivars) and weeds (e.g. some vetches Vicia spp.) provide a resource similar to floral nectar (Wäckers, 2005). This is not explicitly discussed here but, notwithstanding differing accessibility and apparenty, extra-floral nectar could often be substituted for floral nectar. Similarly, artificial sugar sprays could be used but are not discussed here (Wade et al., 2008). The significance of floral resources for biocontrol agents is explained in Section 1.3 below, following a brief discussion of how any limiting effect on biological control might be alleviated.
1.2.2 Providing floral resources

It can be argued that floral resources are likely to be limiting biological control in agroecosystems where their contribution to the productive and non-productive value of the system is not recognised. If other criteria dominate decision-making, as in most commercial agriculture today, the non-cropped area becomes a small proportion of the landscape and non-crop plants are not tolerated within the crop. Even where flowering plants are present, if they are not consciously managed for biocontrol agents they may be of little value due to inappropriate temporal or spatial availability in relation to pest populations. Observing an apparent scarcity of flowers is a reasonable premise from which to initiate research, but field studies are yet to establish whether availability of floral resources is commonly a limiting factor (Gurr et al., 2000; Heimpel & Jervis, 2005). It should be recognised that in situations where they are identified as limiting within a single field and single season, other factors may be limiting on broader spatial scale and longer time-frame. To optimise biological control it may be necessary to apply a suite of management practices across the landscape, which together ensure a population of biocontrol agents survives the disturbance and seasonality that characterises many agroecosystems, and is able to ‘track’ pests through the cropping cycles (Barbosa, 1998; Landis & Menalled, 1998; Tscharntke et al., 2008).

The crop itself may provide pollen and/or nectar at certain times in its development. For example, maize provides abundant pollen and oilseed rape provides pollen and nectar. Provision of floral resources could influence cultivar selection or the spatial arrangement of crops (e.g. intercropping species with and without such foods (Coll, 1998)). However, this is likely to be a minor influence on such decisions since other characteristics of the cultivars or cropping arrangement will have much greater impacts on the crop’s productive value (but see Brown & Mathews (2007)). Furthermore, there is little flexibility to optimise the temporal availability of the floral resources in relation to the pest populations. If floral resources are to be managed for their role in biological control, non-crop plants are therefore the most suitable targets of this management.

The abundance and distribution of non-crop flowering plants can be enhanced by altering management of adventive weeds or sowing species selected for their value in this pest management role (Altieri & Letourneau, 1982; Cowgill et al., 1993; Bugg & Waddington, 1994; Altieri & Nicholls, 2004; Pfiffner & Wyss, 2004). The former, involving modifying chemical or mechanical programmes of weed control within the crop or in non-cropped areas, is inherently variable and less amenable to study than the alternative approach. Furthermore, the positive effects on the crop arising from improved biological control under the altered weed management must outweigh any negative effects, such as competition, which were avoided with the previous methods of weed control. The ecological research concerning the role of floral
resources in pest management has therefore focused on aggregations of sown flowering plants (see reviews cited in Section 1.1).

Flowers may be sown as a single species or a mixture. When mixtures are used it is difficult to assess the relative significance of each species for biocontrol agents but a wider range of insects may benefit and diverse sowings may serve additional purposes such as recreating traditional plant communities of conservation or aesthetic value (Gurr et al., 2005; Pontin et al., 2006; Fiedler et al., 2008). Flower species vary greatly in their attractiveness to biocontrol agents and differ in the accessibility, quantity and quality of floral resources they provide (e.g. Idris & Grafius, 1995; Patt et al., 1997; Colley & Luna, 2000; Begum et al., 2004; Ambrosino et al., 2006; Irvin et al., 2007). Their value also depends on their temporal and spatial relationships to the target pest populations, on the landscape context of the provided flowers and, as described above, on how provision of floral resources fits into a broader pest management strategy (Gurr et al., 1998; Wilkinson & Landis, 2005; Kleijn & van Langevelde, 2006; Kohler et al., 2008). Given the idiosyncrasy of agroecosystems that contain different species or are in different regions, practical pest management is likely to require system-specific trials. However, these can be guided by general principles emerging from other research (Gurr et al., 2005).

1.3 OMNIVORY OF BIOCONTROL AGENTS - CONSUMPTION OF FLORAL RESOURCES IN ADDITION TO PREY

Few important biocontrol agents consume only a single pest species throughout their life-cycle (Riechert & Lockley, 1984; Gilbert, 1993; Alomar & Wiedenmann, 1996; Hodek & Honek, 1996; Jervis et al., 1996; McMurtry & Croft, 1997; Canard, 2001; Sunderland, 2002). A target pest should therefore be seen as one food amongst several which may be consumed by a particular predator or parasitoid species. If foods consumed as alternatives to the target pest are also potential pest species, there may be no overall agronomic significance to this feeding behaviour. However, often this is not the case. Non-pest foods consumed by biocontrol agents are diverse; ranging from other predators and parasitoids which might act as biocontrol agents (e.g. Prasad & Snyder, 2006), to plant-derived material which can include the crop itself (e.g. McGregor et al., 2000). As stated above and elaborated upon here, such non-pest foods also include floral resources.

Before discussing the particular effect of floral resources on the efficacy of biocontrol agents, it is useful to introduce a series of questions which should be addressed when determining the significance for biological control of consumption of non-pest foods. First, are the pest and non-pest foods consumed in the same life-stage of the biocontrol agent’s life-cycle? Second, if they can be consumed contemporaneously, is the non-pest food an essential component of the diet? Third, if the non-pest food is not essential, is it nutritionally superior,
equivalent or inferior to the pest in its ability to sustain survival and development or reproduction? In addition, the feeding behaviour of the biocontrol agent might also be considered: What is the biocontrol agent’s relative ability to consume alternative foods and what preferences does it exhibit as pest and non-pest foods change in availability? The responses to these questions have important implications both for how availability of non-pest foods affects the foraging behaviour of individual biocontrol agents, and for the persistence of their populations. In turn, these processes have implications for suppression of the target pest, with their relative significance depending on the time period being considered.

The terms which might be used to describe the relationship between pest and non-pest foods, and their value to the biocontrol agent, have not been used consistently (Bugg & Pickett, 1998; Landis et al., 2000; Coll & Guershon, 2002; Eubanks, 2005; van Rijn & Sabelis, 2005). To facilitate the discussion in this thesis, the following terminology has been adopted. Non-pest food resources that are essential for a biocontrol agent to complete its life-cycle are considered to be complementary to the pest resource. These resources may be consumed in the same or different life-stages. Non-pest foods that can be consumed contemporaneously and are not essential but are nutritionally superior or equivalent to the pest, are considered to be substitutable resources. Non-pest foods that can be consumed contemporaneously but do not provide adequate nutrition to sustain survival and reproduction or development, are referred to as partially or temporarily substitutable with respect to the target pest. In situations where non-essential non-pest foods enhance populations of biocontrol agents when consumed in addition to the pest, they may be described as supplementary. This could arise when the foods are fully or partially substitutable (as defined above), or when consumed facultatively by a life stage which does not consume the pest.

Pollen and/or nectar play all these roles, varying with the species of biocontrol agent and pest. The particular value of floral resources for two key groups of biocontrol agents, the hoverflies (Diptera: Syrphidae) and parasitoid wasps (Hymenoptera: Parasitica), has been widely recognised and species from these groups have been the subjects of many studies addressing different aspects of flower provision for pest management (e.g. Cowgill et al., 1993; White et al., 1995; Patt et al., 1997; Tylianakis et al., 2004; Ambrosino et al., 2006; Begum et al., 2006; Wanner et al., 2006a, 2006b; Irvin et al., 2007; Lee & Heimpel, 2008b; Scarratt et al., 2008). The Syrphini hoverflies exemplify the rationale for providing flowers to enhance biological control. They are life-history omnivores: larvae consume prey and the adults feed on pollen and nectar (Schneider, 1969). The larval consumption of prey makes them potentially valuable agents of biological control. For the species to be present in the agroecosystem, floral resources must be available for the adults to develop eggs, and so allow the population to persist. The pest and floral resources are therefore complementary and theory suggests that the consumption of pests will be greater in the presence of floral resources than in their absence.
(van Rijn & Sabelis, 2005). In practice, whether or not increasing the abundance of flowers has the desired effect on pest consumption will depend firstly on whether floral resources are limiting under the existing management practices, and secondly on numerous factors which determine whether the particular method of providing floral resources is appropriate (see Section 1.2.2). (Whether or not increased pest consumption by hoverflies is associated with reduced pest populations is considered in Section 1.4.)

Many parasitoid wasps are also life-history omnivores. Their eggs are laid in (or on) host arthropods that are subsequently consumed (and killed) by the larvae (Godfray, 1994). As adults, parasitoid species have varied feeding strategies, consuming fluids from the larval host species and/or sugar-rich foods such as nectar (Jervis et al., 1996). Although most parasitoids are not dependent on flowers since they do not require pollen, alternatives to floral nectar (such as homopteran honeydew) may be scarce or of inferior quality (Wäckers et al., 2008). Flowers may therefore serve as complementary or supplementary resources and increasing their abundance may benefit parasitoids in a similar manner to hoverflies. This is discussed further in Chapters 4, 5 and 6 (in the context of parasitoids which do not contribute to biological control but exhibit similar life-history omnivory to beneficial species).

In addition to life-history omnivores, the community of flower-feeding biocontrol agents includes true omnivores which feed on both prey and floral resources within a single life-stage (Coll & Guershon, 2002). These include species within the thrips (Thysanoptera), ladybirds (Coleoptera: Coccinellidae), lacewings (Neuroptera) and mites (Acarina: Phytoseiidae) (Hodek & Honek, 1996; McMurtry & Croft, 1997; Canard, 2001; Coll & Guershon, 2002). Because prey and floral resources are consumed contemporaneously, these resources could be substitutable. Several interesting questions therefore arise in relation to biological control by true omnivores: Given that the nutrient composition of plant and animal foods are often quite different, to what extent are floral resources and the target pest complementary, substitutable or supplementary resources for a foraging true omnivore? How does availability of floral resources affect *per capita* consumption of a target pest? How does availability of floral resources affect attraction, retention, survival and reproduction of the omnivore in the area colonised by the target pest?

Such questions have been addressed for several systems and the overall effect of flowers on pest suppression by particular omnivores has been studied (Eubanks & Styrsky, 2005). However, our understanding of the feeding ecology of true omnivores lags behind that of other types of biocontrol agent. Rectifying this situation could allow the development of techniques that enhance biological control by true omnivores. It could also help us predict how true omnivores might respond to management practices targeting other species. This is discussed more thoroughly in Chapters 2 and 3 where studies of a true omnivore, the adult stage of the brown lacewing *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae) are presented.
1.4 USE OF FLORAL RESOURCES BY NON-TARGET SPECIES – DISRUPTION OF BIOLOGICAL CONTROL

The discussion above considered three components of an agroecosystem: 1) a target pest 2) a biocontrol agent consuming that pest 3) a food resource provided to alleviate the limiting effect its scarcity had on pest consumption by the population of biocontrol agents. The focus was on the indirect effect of the resource on consumption of pests by the biocontrol agent. In order to determine whether any increase in pest consumption by the biocontrol agent would lead to suppression of the pest population, this focus should be broadened to consider direct effects of the food on the pest, and additional indirect effects (Gurr et al., 2000; Gurr et al., 2005). These interactions might arise through consumption of the resource, changes in the microclimate which result from its presence or changes to cues (visual or olfactory) perceived by other species. The interactions might further enhance biological control, negate the positive effects or disrupt biological control to a degree where presence of the food actually has a negative agronomic effect.

The need to consider multiple interactions when managing agricultural habitats for pest control has been widely acknowledged in reviews, but relatively few studies have focused on their occurrence when flowering plants are provided (see reviews cited in Section 1.1). Interactions that disrupt biological control are particularly interesting and the strongest effects might be expected when the pest interacts directly with the flowers. Many pests are known to consume floral resources (e.g. adult Lepidoptera whose caterpillars are pests) (Romeis et al., 2005; Wäckers et al., 2007) and several studies have investigated this in conjunction with use of the resources by biocontrol agents (Baggen & Gurr, 1998; Begum et al., 2006; Lavandero et al., 2006).

While indirect interactions involving non-target species may be subtle and take many forms, the effects of consumption of floral resources by species that also operate at the fourth trophic level should be clearly identifiable. Although hyper-parasitoids and parasitoids of biocontrol agents are common in some agroecosystems and are likely to exploit floral resources, they have rarely been studied in this context (Alrouechdi et al., 1984; Rosenheim, 1998; Sullivan & Volkl, 1999). Araj et al. (2008) studied the effects of flowers on the longevity and fecundity of an aphid parasitoid and one of its hyper-parasitoids. Irvin (1999) and Stephens et al. (1998) recorded a lacewing parasitoid (Anacharis zealandica) in orchard trials with and without flowering understoreys. Lee & Heimpel (2005) reported hyperparasitism of cabbage pests in plots with and without flowers. Banks et al. (2008) incorporated hyperparasitism in their model of the effects on biological control of spatial separation of floral resources and hosts. In studying fourth-trophic-level species, these studies are exceptional amongst those researching floral resources in the context of pest management.
If the species operating at the fourth trophic level gains relatively more than the biocontrol agent from the presence of floral resources, the population of the latter may be reduced with a subsequent loss of biological control activity. To my knowledge however, such an effect has not been demonstrated due to lack of studies in this area. Use of floral resources by antagonists of biological control is discussed further in Chapters 4, 5 and 6 where studies of the lacewing parasitoid *Anacharis zealandica* Ashmead (Hymenoptera: Figitidae) are presented.

### 1.5 INSIGHT FROM STUDIES OF A TRUE OMNIVORE AND ITS PARASITOID

As stated above, the work presented in this thesis is based on a true omnivore, the brown lacewing *M. tasmaniae*, and its parasitoid *A. zealandica*. *M. tasmaniae* is widespread in agroecosystems in Australasia (Wise, 1991; Horne et al., 2001). In New Zealand, it is the most abundant lacewing in such habitats and green lacewings (Neuroptera: Chrysopidae) are generally absent (Wise, 1991). The larvae and adults consume small soft-bodied arthropods and may be important biocontrol agents (Horne et al., 2001). The species was the subject of laboratory and field research when exotic aphids were causing substantial damage to lucerne crops soon after the aphids’ accidental introduction to New Zealand in the 1970s (Thomas, 1977; Samson & Blood, 1979, 1980; Cameron et al., 1979; Syrett & Penman, 1981; Cameron et al., 1983; Leathwick & Winterbourn, 1984; Rohitha et al., 1985; Bishop & Milne, 1986; Rohitha, 1986; Milne & Bishop, 1987; Leathwick, 1989). More recently its susceptibility to insecticides has been studied (e.g. Hodge & Longley, 2000; Booth et al., 2007; Walker et al., 2007). However, little is known about its use of floral resources. *M. tasmaniae* reproduces and develops in the laboratory on prey alone but pollen has been found in the guts of adults, and other brown lacewings are known to consume floral resources (Stelzl, 1991; Silberbauer et al., 2004). The opportunity for larvae to both exploit floral resources and contribute to biological control is likely to be limited by their inability to disperse by flight. They may, however, exploit non-prey foods within their foraging range, as do chrysopid larvae (Principi & Canard, 1984; Limburg & Rosenheim, 2001). This behaviour has not been studied in *M. tasmaniae* larvae, to my knowledge.

Leathwick (1989) collected more *M. tasmaniae* (presumably adults) in night-time sweep-net samples than during the day. In a video observation study of adult lacewings in Petri dishes, C. Gigot (unpublished) found activity was greater in red than white light. The adult of this species may therefore be nocturnal, like some other brown lacewings (New, 1975). Farrell (1988) cites unpublished observations of larvae also being active at night.

*A. zealandica* is also found in agroecosystems across Australasia and is a solitary koinobiont endoparasitoid of *M. tasmaniae* larvae (New, 1975, 1982) (i.e. eggs are laid in lacewing larvae but the hosts continue to develop until pupation and a single wasp emerges from each parasitised lacewing). Rates of parasitism have rarely been recorded but can be high
(a maximum rate of 86% was recorded by Leathwick (1989) but New (1984) reported a maximum rate of 15%). No studies of adult feeding behaviour or reproductive biology (egg maturation strategy) could be found for this species, or for other members of the genus which parasitise Micromus species elsewhere in the world. In studies comparing areas of orchards with and without flowering plants, it has sometimes (but not always) been more abundant in the presence of flowers (Stephens et al., 1998; Irvin, 1999).

In this thesis, M. tasmaniae and A. zealandica are the key species in a system which also contains the pea aphid Acyrthosiphon pisum Harris (Homoptera: Aphididae) as a prey species, buckwheat flowers Fagopyrum esculentum Moench (Polygonaceae) as a source of floral resources and lucerne Medicago sativa L. (Leguminosae) as a crop. These species were selected because they are amenable to experimentation and readily available. Buckwheat flowers have a shallow corolla and produce abundant nectar from exposed nectaries so the floral resources can be exploited by insects without specialised mouthparts (pers. obs.; Vattala et al., 2006).

The relationships between the species studied are depicted in Fig. 1.1 and the species are illustrated in Fig. 1.2. Crop yield depends on the relative strengths of the interactions indicated by arrows in Fig. 1.1, together with mortality, immigration and emigration which have been omitted for clarity. Because of the links between species, changing one process can have effects anywhere in the system. Here we are interested in the indirect effects of buckwheat on the aphids; that is, the effects of buckwheat on biological control by the lacewing in the presence of its parasitoid.

Neither nectar nor pollen is essential to adult lacewings but floral resources and aphids could be substitutable resources. If so, their presence could have a negative effect on biological control by decreasing per capita consumption by the lacewings. Alternatively, floral resources could be consumed as supplements to prey and have a positive effect on biological control by increasing the survival or fecundity of this omnivore. Chapter 2 presents the results of laboratory experiments which first verify that M. tasmaniae consumes floral resources from buckwheat and then studies how such consumption affects these ‘individual-level’ and ‘population-level’ processes.

While the laboratory studies revealed the potential for interactions to occur in the field, the effects observed when the lacewings are confined with flowers and prey may not be observed when the insects forage naturally. Chapter 3 describes a field study in which dissection of lacewings is used to determine consumption of pollen and prey, and patterns of consumption and lacewing abundance are analysed with respect to distance from a flowering buckwheat strip in a lucerne field. These results are used to refine conclusions drawn in Chapter 2 about the potential effects of flower strips on biological control by M. tasmaniae.
Fig. 1.1 Diagram depicting the relationships between the species studied in this thesis. Red arrows indicate consumption, blue arrows indicate development or reproduction. The species identities were as follows: lucerne = *Medicago sativa* (Leguminosae), buckwheat = *Fagopyrum esculentum* (Polygonaceae), aphid = pea aphid *Acyrthosiphon pisum* (Homoptera: Aphididae), lacewing = *Micromus tasmaniae* (Neuroptera: Hemerobiidae), parasitoid = *Anacharis zealandica* (Hymenoptera: Figitidae). Nectar, rather than pollen, is likely to be the key floral resource for these species but this has not been verified.

The aphid, as a herbivore feeding on lucerne, is a potential pest. The lacewing acts as a biocontrol agent, consuming aphids in its larval and adult stages. As a koinobiont parasitoid of lacewing larvae, the parasitoid is an antagonist to biological control. The adult lacewing is a true omnivore, operating at both the second and third trophic levels. The parasitoid is a life-history omnivore, operating at the fourth trophic level as a larva and the second trophic level as an adult.
In contrast with the lacewing, its parasitoid *A. zealandica* was expected to be a life-history omnivore, feeding on lacewing larvae in the larval stage and consuming floral nectar as an adult. In such a situation floral resources would be complementary or supplementary foods with respect to the pest. It would then be predicted that presence of flowers would increase parasitism of the lacewing and so reduce its efficacy as a biocontrol agent. Chapter 4 presents the results of laboratory experiments which study the degree to which presence of flowers affects parasitoid survival, egg load, rate of oviposition and the progeny sex ratio. These experiments demonstrated the potential for flowers to negatively affect biological control via their effect on this antagonist. It was then appropriate to consider whether the parasitoid exploits flower strips in the field. In the same field in which lacewings were studied, a rubidium-marking technique was used to assess buckwheat-feeding by *A. zealandica* and HPLC analysis was used to determine whether the parasitoids fed on other foods such as honeydew. These analyses are described in Chapters 5 and 6 respectively.

Having studied the effects of floral resources on the lacewing and its parasitoid separately, the question remained as to whether the presence of flowers in the system would have a net positive or negative effect on biological control of aphids. Evidently if flowers had a negative effect on consumption by *M. tasmaniae*, the detrimental effect on biocontrol would be
exacerbated by any increase in parasitism by *A. zealandica*. However, if flowers had a positive effect on consumption by *M. tasmaniae*, this may or may not be counteracted by an increase in parasitism. The results of the laboratory experiments were used to construct a model of aphid, lacewing and parasitoid population dynamics over the course of a period of re-growth in a lucerne cropping cycle. In Chapter 7 this model is used to explore the likelihood of flowers enhancing or diminishing crop damage by aphid populations.

The final chapter of the thesis reflects upon the conclusions drawn in Chapters 2–7 and discusses how they further our understanding of the role of floral resources in pest management.
Chapter 2 Laboratory studies revealing potential effects of floral resources on biological control by the omnivorous lacewing *Micromus tasmaniae*

2.1 SUMMARY

Understanding the relationship between a predator and its prey requires consideration of the other food resources used by the predator. In the case of true omnivores, these include plant-provided foods such as leaf tissue and nectar. The presence of plant resources can increase or decrease predation depending on the degree to which they substitute for prey or serve as a supplementary food. This has implications for the role of omnivores in biological control and some groups, notably heteropteran bugs and phytoseiid mites, have been studied in this context. However, few experiments have considered the effects of plant resources both on prey consumption by individual omnivores (which have an immediate effect on the prey population) and on attributes such as survival and fecundity that act over the longer term to affect predation at the population level. In this study, a model system comprising the omnivorous adult lacewing *Micromus tasmaniae*, buckwheat flowers *Fagopyrum esculentum* and aphid prey *Acyrthosiphon pisum* was used to investigate how floral resources affected per capita predation rate, survival and fecundity of the lacewing. Flowers reduced prey consumption. In the absence of prey, survival was higher for lacewings with flowers than those without. In an experiment where aphids were provided in abundance, lacewing fecundity was unaffected by flowers. However, when aphids were less abundant, providing flowers decreased the pre-oviposition period and increased the daily oviposition rate. The results demonstrate that floral resources can mediate omnivore–prey relationships and that, in the context of biological control, their effects may be either positive or negative.

2.2 INTRODUCTION

Debate continues over the degree to which predation drives the dynamics of herbivore populations (Polis & Strong, 1996; Rosenheim, 1998; Hawkins et al., 1999; Kindlmann & Dixon, 2001; Walker & Jones, 2001). However, it is clear that in some situations predators do suppress populations of their prey, and in agroecosystems this can prevent herbivores reaching
densities at which they cause economic damage (Gurr & Wratten, 2000). The strategy of conservation biological control is to understand how factors in the agricultural habitat interact to generate this top-down pressure, and then to manipulate the habitat to increase it (Gurr et al., 2000).

A prey population can be considered as one of several resources used by a predator to acquire the nutrients necessary for survival, development and reproduction. From this perspective, the relationship between a predator and any particular prey population can be understood by considering the predator’s use of, and requirements for, other resources. As a general simplification, the presence of complementary resources will lead to increased consumption of the prey while substitutable resources can enhance or diminish predation according to the time-scale of the interaction and the availability of the target prey relative to its potential substitutes (van Rijn & Sabelis, 2005).

In relation to biological control, complementary resources can be defined as those which, in addition to herbivore prey, are necessary for a predator to complete its life-cycle (van Rijn & Sabelis, 2005). For example, pollen and nectar are each complementary resources for hoverfly (Diptera: Syrphidae) species that are aphidophagous as larvae and feed on these floral resources as adults (Schneider, 1969). Nectar is also important for many hymenopteran parasitoids and providing flowers is commonly proposed as a technique for conservation biological control (Jervis et al., 1996; Landis et al., 2000; Gurr et al., 2004; Gurr et al., 2005).

Substitutable resources are those which can be consumed in place of the pest species under consideration. Many non-pest arthropods constitute substitutable resources for generalist carnivores such as spiders. The implications of such polyphagy for biological control have been widely discussed (e.g. Symondson et al., 2002; Harmon & Andow, 2004; van Rijn & Sabelis, 2005). If non-pest foods do not have the nutritional composition to be fully-substitutable for prey, they may nevertheless be temporary or partial substitutes.

True omnivores consume both plant material and animal prey within a single life-stage (Coll & Guershon, 2002). While they are likely to respond to multiple prey species in a similar way to generalist carnivores, predicting the effect of plant resources on consumption of prey requires special consideration of omnivore biology (Eubanks, 2005; Gillespie & Roitberg, 2006). In agroecosystems, omnivorous species are found within the true bugs (Heteroptera), lacewings (Neuroptera), thrips (Thysanoptera), mites (Acarina: Phytoseiidae) and ladybirds (Coleoptera: Coccinellidae), and to varying degrees they exploit leaf and fruit tissue, pollen, floral nectar and extra-floral secretions (Alomar & Wiedenmann, 1996; Hodek & Honek, 1996; McMurtry & Croft, 1997; Dixon, 2000; Coll & Guershon, 2002; Wäckers & van Rijn, 2005). Although these groups have been the subjects of many studies and their potential role in biological control has been recognised, few experiments have been designed to address the particular question of how plant resources affect pest suppression by omnivores (Eubanks &
This requires consideration of both the predatory behaviour of individual omnivores and the consequences of this for the omnivore population.

Over the short term, the effect of plant resources on biological control is determined largely by their effect on the daily number of pests killed by each omnivore (an ‘individual-level’ attribute). Over the longer term, the effects on omnivore longevity and fecundity (‘population-level’ attributes) are also important because they influence population size, and hence the overall mortality imposed on the pest population. Many studies have investigated the effect of plant resources on either individual-level or population-level attributes (Eubanks & Styrsky, 2005). Only a small number have considered both (McMurtry & Scriven, 1966; Nomikou et al., 2004), or have included field experiments which studied the combined effects (and could confidently attribute differences in prey populations to the effect of predation by omnivores) (Cottrell & Yeargan, 1998; Eubanks & Denno, 2000). These studies have shown that plant resources provided by the crop itself can have an important influence on omnivore–prey dynamics. It appears that the effect of non-crop plant resources, which might be independently managed for conservation biological control, has not been studied. Here I investigate how the presence of floral resources influences the predatory role of an omnivore by studying the adult lacewing Micromus tasmaniae Walker (Neuroptera: Hemerobiidae).

*M. tasmaniae* is widespread and abundant in Australasian agroecosystems (Horne et al., 2001). Both larval and adult stages consume a range of small soft-bodied arthropods and the species is considered to be a significant biocontrol agent, at least in lucerne where most published work has been conducted (Leathwick & Winterbourn, 1984; Milne & Bishop, 1987; Horne et al., 2001). Although omnivory is common in other hemerobiid adults (Stelzl, 1991) and pollen grains have been found in guts of adult *M. tasmaniae* (Silberbauer et al., 2004), research has focused on the lacewing’s role as a predator. Use of plant resources by this species has not been studied, to my knowledge. The species does not require floral resources to complete its life-cycle but they might be consumed as substitutable foods.

An initial experiment was conducted to confirm whether or not *M. tasmaniae* consumes floral resources as well as prey. The lacewing, buckwheat flowers *Fagopyrum esculentum* Moench ‘Katowase’ (Polygonaceae) and pea aphids *Acyrthosiphon pisum* Harris (Homoptera, Aphididae) were then used in a series of laboratory experiments investigating the effects of flowers on predation rate, longevity and fecundity of the omnivore.

### 2.3 Materials and Methods

#### 2.3.1 Rearing methods

Lacewing cultures were established from local field-caught insects and maintained for more than ten generations prior to the experiments. Larvae were raised on aphids. Adults were
provided with aphids, moist cotton wool and honey solution applied to the walls of their enclosure. Aphid cultures were maintained on broad bean *Vicia faba* L. ‘Coles Dwarf’ (Leguminosae). Buckwheat was grown in a glasshouse before use in the experiments. Buckwheat flowers were checked regularly during the experiments and since nectar droplets could always be seen it was assumed that this resource was continuously available in both cut and uncut flowers. Nectar quality was assumed not to have differed between these methods of provision (Wade & Wratten, 2007). To provide adult lacewings for the experiments, pupae were collected from the cultures and placed in pots without food or water. These were checked daily and adults were collected for use, unfed, within 24 h of emergence. Each experiment was run over several months. All insect rearing and experimental procedures took place in temperature-controlled rooms at a mean temperature of 20 °C (range ±1 °C). The proximity of enclosures to the lights in Experiments 1 and 2 exposed lacewings to a mean temperature of 22 °C. The light regime was 16 h light:8 h dark.

### 2.3.2 Experiment 1: consumption of floral resources

Newly-emerged lacewings were alternately allocated to two treatments: (1) with flowers and (2) without flowers. The lacewings were transferred to individual cylindrical clear plastic enclosures (height 18 cm, diameter 10 cm) with mesh over the open top. Each enclosure contained a water-filled vial with a cotton wool wick secured by a foam plug. For the ‘with flowers’ treatment, a freshly cut buckwheat inflorescence was also held in the water by the foam. ‘Without flowers’ replicates were provided with water only. The lacewings were exposed to the treatments for 24 h and then placed in a microcentrifuge tube and frozen.

The frozen lacewings were dissected in a drop of anthrone reagent to determine whether they had consumed nectar or pollen (van Handel, 1972; Heimpel *et al.*, 2004). At room temperature this reagent turns from yellow to blue-green in the presence of fructose. A positive reaction to the contents of the lacewing gut therefore indicates consumption of nectar (van Handel, 1972). The reagent also stains buckwheat pollen deep yellow. Working under a microscope the abdominal exoskeleton of each lacewing was pulled from the thorax to expose the gut to the anthrone reagent. The gut wall was pierced and, after ten minutes, a colour change or the presence of any pollen grains was recorded.

### 2.3.3 Experiment 2: effect of flowers on lacewing fecundity and prey consumption

Newly-emerged lacewings were sexed and systematically allocated to six treatments according to gender: 1) unpaired males with flowers, 2) unpaired males without flowers, 3) unpaired females with flowers, 4) unpaired females without flowers, 5) pairs with flowers and 6) pairs without flowers. The experimental enclosures were plastic Petri dishes (2 x 9 cm). Each dish contained moist cotton wool, a microcentrifuge tube and a small Petri dish (1 x 5 cm) rimmed
with polytetrafluoroethylene (Fluon). A piece of black cotton cloth (a favoured oviposition substrate) was attached to the lid using double-sided sticky tape. In all treatments, 30 aphids of edible sizes (second and third instars) were added to the small Petri dish. The aphids were contained by the Fluon but the lacewing could fly or walk over it. For ‘with flowers’ treatments, the microcentrifuge tube was filled with water and the cut stalk of a buckwheat inflorescence was inserted through a hole in the lid of the tube. The tube remained empty for treatments without flowers.

Each day for 10 days (unpaired lacewings) or 15 days (paired lacewings), the number of aphids remaining in the dish was counted under a microscope. Absent aphids and mis-shapen cadavers were assumed to have been eaten. Test dishes containing aphids without lacewings verified the accuracy of this method.

Oviposition only occurred in the paired treatments. For these treatments, when eggs were first seen in a dish the male was removed to allow prey consumption by a single laying female to be recorded. (Rates of oviposition do not differ between paired and separated females (Robinson K.A., unpublished).) The number of eggs laid in each enclosure was recorded daily.

The dish containing aphids was replaced daily, the inflorescence changed and the cotton wool moistened. If eggs were laid on the cloth it was replaced and eggs laid elsewhere were removed. The Petri dishes were replaced during the experiment as they became soiled.

2.3.4 Experiment 3: effect of flowers on lacewing survival and fecundity with or without prey

Pairs of newly-emerged lacewings were systematically allocated to four treatments: 1) with flowers and aphids, 2) with flowers, without aphids, 3) without flowers, with aphids and 4) without flowers or aphids. Each pair was transferred to a cylindrical clear plastic container (height 18 cm, diameter 10 cm) with mesh over the open top. The container was suspended from a rod positioned above a potted buckwheat plant and a buckwheat shoot was inserted through a hole (5 cm diameter) in the side of the container. A foodwrap-covered foam disc secured the shoot and plugged the hole. (Foodwrap prevented lacewing contact with the foam, the texture of which would have encouraged oviposition.) For ‘with flowers’ treatments, a buckwheat inflorescence and its subtending leaf were inserted. For ‘without flowers’ treatments, the inflorescence was removed and only a leaf provided. Each enclosure contained a small Petri dish (1 x 5 cm) rimmed with Fluon which, for ‘with aphids’ treatments contained approximately 0.05–0.10 g aphids. A mixture of aphid sizes was provided but this included sufficient nymphs to ensure there were usually live aphids of edible size in the dish the following day. Although not quantified, the aphid abundance was considered to be greater than that in Experiment 2. All enclosures also contained a piece of card folded lengthways to form a dark shelter, a piece of
black cotton oviposition cloth taped to the side and moist cotton wool in a foil dish (height 1.5 cm, diameter 4 cm).

The containers were assessed daily and when eggs were first seen, the male lacewing was removed to minimise its influence on within-treatment variation. Survival and the number of eggs laid by each female lacewing were recorded for 20 days. The aphid dishes were replaced daily. Buckwheat inflorescences were changed if fewer than four flowers remained open and the cotton wool was kept moist. Cloth strips were changed each day if eggs had been laid on them and eggs laid elsewhere were removed.

Two supplementary studies were carried out in conjunction with this experiment. Firstly, on 18 dates during the experiment the egg-bearing cloth strips were collected. They were kept in Petri dishes for four days, after which time the eyes of the developing larvae could be seen through the chorion. The numbers of sterile and viable eggs on each cloth were then counted under microscope. Secondly, at the end of the 20-day experimental period, the treatments for half the lacewings were changed. For these lacewings, ‘with aphids’ treatments became ‘without aphids’ and *vice versa*, with provision of flowers continuing unchanged. Survival and oviposition were recorded daily for a further 10 days.

2.3.5 Statistical analysis

Mann-Whitney tests were used to analyse differences in the fecundity and aphid consumption of lacewings with and without flowers in Experiment 2. Kruskal-Wallis tests with *post hoc* comparisons were used to analyse the effects of food treatments on measures of fecundity in Experiment 3. A General Linear Model was used to determine the effect of flowers on the relationship between oviposition and aphid consumption. ‘Eggs laid per day of oviposition’ was the dependent variable, ‘flowers’ was a factor and ‘aphids eaten per day while ovipositing’ was a covariate. Analyses were conducted using Minitab 14.1.

Only lacewings that survived the duration of the experiment and laid at least one egg were included in the analyses of fecundity data. Daily rates of aphid consumption for paired lacewings were calculated for the period after females had started ovipositing and males had been removed. One lacewing that stopped laying eggs on day 11 of the 20-day study of fecundity was excluded from the analyses presented. The conclusions, however, were unaffected by exclusion of this individual.

Survival of lacewings in Experiment 3 was analysed using Chi-squared tests. Descriptive statistics are presented for consumption of floral resources and for the supplementary studies.
2.4 RESULTS

2.4.1 Experiment 1: consumption of floral resources

None of the control lacewings contained pollen or nectar in their gut. Of those provided with buckwheat flowers, nine of the ten females and six of the seven males contained both floral resources. Neither resource was found in the two remaining individuals. No green plant material was found in any of the lacewings.

2.4.2 Experiment 2: effect of flowers on lacewing fecundity and prey consumption

Fecundity

Providing flowers shortened the time from emergence until the first egg was laid ($W = 142.0$, $P < 0.001$). In the presence of flowers, most lacewings started laying after three or four days but the pre-oviposition period for those with aphids only ranged from four to thirteen days (Fig. 2.1i). One lacewing without flowers did not lay eggs. Flowers also increased the daily rate of oviposition ($W = 316.0$, $P < 0.001$) (Fig. 2.1ii). These effects combined to triple egg production over the 15 days of the experiment (median (range) without flowers: 68 (0–177); with flowers: 245 (140–296); $W = 323.0$, $P < 0.001$). In both treatments, eggs were laid daily once oviposition had started. All lacewings survived the duration of the experiment.

Prey consumption

Providing flowers as an alternative food source reduced consumption of prey (Fig. 2.2). This effect was apparent in all classes of lacewing: unpaired males ($W = 21.0$, $P = 0.008$); unpaired females ($W = 15.0$, $P = 0.012$); laying females ($W = 167.5$, $P = 0.013$).

Relationship between oviposition and prey consumption

Through both increasing the rate of oviposition and decreasing the rate of prey consumption, flowers had a strong effect on the number of eggs laid per aphid consumed (Fig. 2.3). Without flowers, lacewings laid $0.557 \pm 0.072$ (mean ± standard error) eggs per aphid consumed. This increased to $1.42 \pm 0.065$ when flowers were provided. Oviposition increased with aphid consumption but the gradient of the regression equations relating prey consumption and oviposition was not affected by provision of flowers (prey: $F_{1,25} = 31.6$, $P < 0.001$; flowers: $F_{1,25} = 7.71$, $P = 0.010$; prey x flowers: $F_{1,25} = 0.45$, $P = 0.509$; $R^2 = 79.7\%$).

2.4.3 Experiment 3: effect of flowers on lacewing survival and fecundity with or without prey

Survival

In the absence of prey, lacewings without buckwheat died within four days ($n = 12$). Within the 20 day experimental period, survival was high in lacewings with flowers and/or prey (flowers and prey = 12/13, flowers only = 11/12, prey only = 9/12). Survival of lacewings without either
Fig. 2.1 Effect of flowers on fecundity of lacewings with prey (30 aphids daily). Boxes indicating interquartile ranges and bold lines indicating medians are superimposed on the raw data. Sample sizes are given above each box and exceed the number of points due to overlapping data.

Fig. 2.2 Effect of flowers on prey consumption by lacewings. Boxes indicating interquartile ranges and bold lines indicating medians are superimposed on the raw data. Sample sizes are given above each box and sometimes exceed the number of points due to overlapping data.
Fig. 2.3 Effect of flowers on the relationship between fecundity and prey consumption. Regression lines were calculated using the least-squares method.

food resource was significantly lower than that of lacewings with food but the differences between survival with buckwheat and/or prey were not significant (all treatments: $X^2 = 30.95$, d.f. = 3, $P < 0.001$, with food treatments: $X^2 = 2.01$, d.f. = 2, $P = 0.367$).

**Fecundity**

Lacewings with neither flowers nor prey died without laying eggs. Lacewings feeding only on floral resources had very low rates of oviposition. Eggs were laid intermittently and on the few days when oviposition occurred, very few eggs were laid (Fig. 2.4). In contrast, those provided with prey in addition to flowers laid every day once oviposition had started (apart from one insect which stopped laying part way through the experiment), giving a significantly higher proportion of laying days ($Q = 3.77$, $P < 0.001$) (Fig. 2.4i). The daily rate of oviposition also increased dramatically with prey ($Q = 4.09$, $P < 0.001$) (Fig. 2.4ii). These effects combined so that over the 20 days of the experiment the median number of eggs laid by lacewings with only flowers was 9 (range 1–31), but lacewings with flowers and prey laid an average of 394 (274–572) eggs.

In contrast with Experiment 2, the effect on fecundity of providing prey-fed lacewings with flowers was not significant (Fig. 2.4) (proportion of laying days: $Q = -1.19$, $P > 0.500$; eggs laid per day of oviposition: $Q = 0.42$, $P > 0.500$; eggs laid in 20 d: $Q = 0.30$, $P > 0.500$). A power analysis was not performed because sample sizes were too low to assume the data were normally distributed. However, comparison of Figs. 2.1 and 2.4 indicates that any effect of flowers which did not reach statistical significance in Experiment 3 was much smaller than the
effect in Experiment 2. Sterile eggs usually comprised less than 15% of the daily total. Sample sizes were too low to permit statistical analysis but there was no indication that the rate of sterility varied between treatments.

Fig. 2.4 Effect of flowers on fecundity of lacewings with or without prey (abundant aphids). Lacewings without flowers or prey died without laying eggs. Boxes indicating interquartile ranges and bold lines indicating medians are superimposed on the raw data. Sample sizes are given above each box and exceed the number of points due to overlapping data. (The outlier in (i) ‘prey and flowers’ is a lacewing which had a long pre-oviposition period.)

Response to change in prey availability

Lacewings showed a gradual response over several days to the changes in prey availability that were imposed 20 days after emergence. For the four ovipositing lacewings with flowers and prey initially, removal of prey led to oviposition falling to a near-zero rate within five days. Within five days of receiving aphids, the four lacewings that had survived for 20 days on flowers with a low rate of oviposition were laying at a rate comparable to lacewings which had continually received prey. Four of the five lacewings without flowers that were not provided with prey after day 20 died within five days of food being removed. The rate of oviposition was maintained by nine of the ten lacewings that continued to receive prey (with or without flowers).
2.5 DISCUSSION

2.5.1 Implications of flowers for predation by individual lacewings

Providing buckwheat flowers reduced consumption of aphids, suggesting that lacewings exploited these resources according to their availability and that floral resources and aphids are at least partially or temporarily substitutable. Similar relationships between plant and animal resources have been found in other omnivores feeding on extra-floral nectar, pollen or fruits (e.g. Wei & Walde, 1997; Eubanks & Denno, 2000; Janssen et al., 2003; Nomikou et al., 2004; Spellman et al., 2006). However, the effects observed in the present study, and others, may depend on their design. Where experiments have allowed a choice of feeding on prey in areas with or without plant resources, the plant resources attracted and/or retained predators and so increased predation in those areas (Eubanks & Denno, 1999; Harmon et al., 2000). Many factors influence how omnivorous and other polyphagous species compose their diet, including the relative availability of resources and potential benefits of a mixed diet (Schoener, 1971; Waldbauer & Friedman, 1991; Sherratt & Harvey, 1993; Coll & Guershon, 2002; Singer & Bernays, 2003). These are yet to be studied in *M. tasmaniae*.

Through modelling, van Rijn & Sabelis (2005) showed how substitutable resources can decrease *per capita* prey consumption but nevertheless be associated with reduced pest populations. However, this effect occurred when the system reached equilibrium; a state that is unlikely to occur at a field scale in annual crops.

2.5.2 Implications of flowers for lacewing population size

Through their effect on the *per capita* rate of predation, flowers evidently have the potential to affect omnivore–prey dynamics directly. The presence of flowers also showed a potential to influence the future lacewing population size through its effects on survival and fecundity.

Flowers enhanced longevity well beyond the period survived with water alone. Since this was associated with consumption of nectar and pollen, it is concluded that the effect was due to the nutritional value of these resources rather than a microclimatic effect. Recent work determined that the longevity of lacewings with buckwheat flowers and water was 21–52 days, while that of lacewings with water only was 2–4 days (6 males and 6 females in each treatment, Robinson et al., 2008). Although the relative value of nectar and pollen as foods has not been studied, two observations suggest nectar is the floral resource which enhances longevity. Firstly, lacewings provided with sucrose solution show comparable longevity to those with buckwheat flowers, indicating the nutrients in pollen are not required (Tompkins, J.-M. & Sam, S.A., unpublished). Secondly, lacewings provided with phacelia flowers *Phacelia tanacetifolia* Bentham (Hydrophyllaceae) do not live longer than those with only water (Jonsson, M., unpublished). Phacelia flowers provide abundant pollen but the nectaries are at the base of a
deep corolla tube. This suggests that phacelia nectar is inaccessible to lacewings and pollen alone does not provide adequate nutrition.

Although flowers prolonged the life of the lacewings in the absence of aphids, they could not sustain the rate of oviposition achieved when aphids were provided. Prey should therefore be considered an essential component of the diet, with floral resources not being fully-substitutable. Despite low sample sizes in the supplementary component of Experiment 3, it was clear that lacewings responded to changing availability of prey. These observations suggest that floral resources could serve to maintain populations of *M. tasmaniae* when prey are scarce, with a resumption of oviposition when prey resources are sufficient. Such a pattern has been found in some true bugs (Heteroptera), ladybird beetles (Coleoptera: Coccinellidae) and green lacewings (Neuroptera: Chrysopidae). In these groups, while plant material and prey are substitutable as energy sources for predator survival, rates of development and/or reproduction are often only maximised when prey are available (e.g. Kiman & Yeargan, 1985; Wiedenmann *et al.*, 1996; Limburg & Rosenheim, 2001; Patt *et al.*, 2003; Lundgren & Wiedenmann, 2004).

Prey may act as an oviposition stimulus to the laying female, particularly for species for which the juvenile stage requires animal resources in order to develop. In such cases, a scarcity of prey would lead to a low rate of oviposition irrespective of the nutritional value of the prey to the adult. Adults of many hoverfly species, for example, feed solely on pollen and nectar but oviposit in response to cues from aphids (Scholz & Poehling, 2000). The current study did not investigate this aspect of lacewing biology. However, the response to changing aphid availability in Experiment 3 took place over several days. This timescale suggests a physiological rather than a behavioural response. An additional study in which lacewings did not oviposit when provided with aphids in a mesh-covered microcentrifuge tube, provides further support for this explanation (Robinson, K.A., unpublished).

When prey were present, adding flowers increased fecundity in one of the two experiments studying this variable. Several factors differed between the experiments (see Methods, Experiments 2 and 3) so the cause of the contrasting results cannot be conclusively determined. However, I suggest that the key difference was the quantity of aphid prey provided. It is well-established that the fecundity of many arthropods increases with food availability until a maximum is reached (Jervis & Copland, 1996). Thus, if the prey supply was limiting when 30 small aphids were available, providing additional food in the form of floral resources would increase fecundity. If fecundity was maximal when mixed-size aphids were provided at a greater (unquantified) rate, flowers would not have any effect. Pre-oviposition period and daily oviposition rate of lacewings with the combination of 30 aphids and flowers in Experiment 2 were similar to those in Experiment 3, supporting this interpretation. The similarity of the measured variables in the ‘with flowers and aphids’ and the ‘with aphids only’ treatments in Experiment 3 suggests that a diet of only aphids is not itself detrimental.
The results of this study indicate that floral resources can increase lacewing fecundity by both decreasing the pre-oviposition period and increasing the daily rate of oviposition, but that further work is required to ascertain the conditions under which this occurs. The importance of prey availability on the effect of flowers has recently been confirmed by Jonsson et al. (in press). They provided lacewings with 0, 5 or 50 aphids day\(^{-1}\) with or without buckwheat flowers. Flowers increased the rate of oviposition for lacewings with 5 aphids day\(^{-1}\), but not for lacewings with 50 aphids day\(^{-1}\).

2.5.3 Implications of flowers for the impact of lacewings on prey populations

This study provides empirical evidence supporting the expectation that floral resources can affect omnivore-prey dynamics (Gurr et al., 2005; van Rijn & Sabelis, 2005; Gillespie & Roitberg, 2006). The results suggest the effect of flowers on lacewing-prey dynamics will be influenced by both the prey density and the length of time over which the populations are observed.

The following scenario provides a simple framework for conceptualising the relationships in this system: at low densities, prey populations are suppressed by lacewings which survive only with supplementary nutrition from floral resources, but under these conditions reproduction is not maintained so predation pressure declines over time. At high prey densities floral resources have a negligible effect. At intermediate prey densities, floral resources decrease per capita predation but increase fecundity. Increased fecundity leads to a larger population of both adults and larvae (which are also predatory) in the future. Total prey consumption at any time in the future will be a function of both the increased lacewing population size and the decreased per capita prey consumption. The consequences for the prey population will depend on how predation varies with respect to the rate of prey population growth over time. Increasing the predator’s ‘conversion efficiency’ (eggs laid per prey item consumed) can have a strong influence on predator-prey dynamics (Kean et al., 2003). However, even if the predation rate is ultimately higher, a temporary decrease may allow the pest to exceed acceptable densities. When arthropod populations do not complete many generations during a crop’s development, it may be more important to consider these transient population dynamics than theoretical equilibria.

While the interactions between omnivores, plant resources and prey will be influenced by numerous factors in the field, the potential to increase pest populations inadvertently by providing flowers has been demonstrated here. The contrasting implications of the results of the different experiments also highlight the importance of studying both the behaviour of individual predators and attributes such as longevity and fecundity that have population-level effects. This will improve our ability to predict how alternative resources, with varying degrees of substitutability, affect the relationship between predators and their prey.
Chapter 3  Consumption of floral resources and prey by an omnivorous lacewing: Preliminary investigations of Micromus tasmaniae in the field

3.1  SUMMARY

The damage caused to a crop by a pest species depends on the size of the pest population, and this in turn depends on consumption of the pest by predators and parasitoids. Consumption of non-pest foods by these biocontrol agents can affect the degree to which they suppress the pest population. Understanding the use of different food resources by biocontrol agents can inform the development of pest management strategies that aim to enhance the efficacy of biological control by manipulating resource availability.

Laboratory studies have shown that presence of flowers could have positive, negative or neutral effects on biological control by the lacewing *Micromus tasmaniae*, depending on the abundance of the pest and the timescale of the interaction. These effects can arise because the adult stage is a true omnivore, consuming both floral resources and prey. Although the species is known to be a biocontrol agent, the relative frequency with which it consumes floral resources and prey in the field is unknown. In this study, adult *M. tasmaniae* were collected from a lucerne field containing a central strip of flowering buckwheat *Fagopyrum esculentum*. The abundance of lacewings did not vary over a distance of 1–100 m from the flower strip. Insects were either dissected to determine the presence of prey in the gut or were chemically digested to determine the presence of pollen. All dissected lacewings contained prey. Most digested lacewings contained pollen but the abundance of grains was generally low and probably resulted from incidental consumption of dispersed pollen rather than foraging within flowers for nectar or for pollen itself. Only 6% of the lacewings (three individuals) were considered to have foraged in flowers ‘recently’ and only one individual contained abundant buckwheat pollen. Although further work is required to understand the relationship between presence of pollen in the gut and foraging in flowers, it is concluded that the presence of the flower strip probably had little effect on biological control by the lacewings in this field. Flowers may, nevertheless, be significant in other situations.

3.2  INTRODUCTION

The food webs of temperate agroecosystems are simple compared with many others, but are nevertheless sufficiently complex for our understanding of them to be far from complete. Building up this understanding can aid the development of sustainable pest management strategies such as conservation biological control. The strategy of conservation biological control is one of farm management to enhance the efficacy of indigenous (and previously-
introduced) predators and parasitoids, in order to reduce losses caused by pests (Gurr et al., 2000). In practice it involves avoidance of activities which have negative effects, such as use of broad spectrum insecticides, and adoption of activities with positive effects. The latter includes managing habitats within the agroecosystem to enhance populations of biocontrol agents (Gurr et al., 1998; Gurr et al., 2000; Landis et al., 2000).

The first question for ecologists interested in conservation biological control to answer is ‘Which species eat or parasitise this pest?’. Once potential agents of biological control have been identified, further knowledge of their ecology is needed to determine how habitat management might enhance their populations. Amongst the many aspects which must be considered are: What other food resources do the biocontrol agents consume and how does availability of those resources affect their ability to suppress pest populations? Manipulation of these resources might then contribute to a conservation biological control programme. In temperate agroecosystems the key predators and parasitoids have long been identified and their basic food requirements are known. However, the practice of conservation biological control is constrained by our limited understanding of their use of particular non-pest food resources and how this use affects their role in biological control (Gurr et al., 2000; Gurr et al., 2005).

When considering the feeding ecology of potential agents of biological control, it is useful to distinguish between food resources which are complementary to pest prey, and those which are substitutable (van Rijn & Sabelis, 2005). Complementary resources are required in addition to pest prey for the consumer to complete its life-cycle. In the context of conservation biological control, complementarity is exemplified by the differing food requirements of the larval and adult stages of life-history omnivores such as hoverflies. Many hoverfly larvae are predatory while the adults require pollen and sources of sugar such as nectar (Schneider, 1969). Thus, the pest and floral resources are complementary and if either is not available in the agroecosystem, biological control by hoverflies cannot be sustained beyond a single generation.

Substitutable resources are those which can be consumed as alternatives to pest prey. For example, adult carabid beetles which contribute to biological control of aphids can consume non-pest arthropods in place of aphids (Sunderland, 2002). The presence of substitutable resources can enhance or diminish biological control; they may decrease per capita consumption of the pest species, but they may increase the predator population by reducing emigration or mortality, or increasing immigration or fecundity (Eubanks & Styrsky, 2005; van Rijn & Sabelis, 2005).

Conservation biological control has focused on availability of complementary rather than substitutable resources, with particular attention given to floral resources and their use by hoverflies and hymenopteran parasitoids (Hymenoptera: Parasitica). There is an attractively simple logic to follow: 1) flowering weeds are scarce in conventional industrialised agroecosystems (relative to organic or traditional systems), 2) availability of complementary
resources is therefore likely to be limiting biological control, so 3) increasing the abundance of flowers can alleviate this limitation. Ecological reality is complicated by factors such as consumption of other sugar sources like honeydew, and benefits conferred by the floral resources to the pests themselves, hyperparasitoids and parasitoids of predators. Furthermore, other factors such as phenology may prevent populations of biocontrol agents having an agronomically significant effect on the pest. As argued above, although the ecology may be challenging, research can help us predict how the technique could be deployed effectively (Gurr et al., 2005).

In addition to providing complementary resources for life-history omnivores, flowers offer substitutable resources for some true omnivores i.e. species which consume both plant and animal resources within a single life-stage. Amongst the potential agents of biological control, true omnivores are found within the groups Heteroptera, Thysanoptera, Neuroptera, Phytoseiidae (Acarina) and Coccinellidae (Coleoptera) (Alomar & Wiedenmann, 1996; Hodek & Honek, 1996; McMurtry & Croft, 1997; Dixon, 2000; Coll & Guershon, 2002; Wäckers & van Rijn, 2005). Substitutability may only be partial, since the plant and animal resources are often equivalent with respect to their effect of longevity, but prey may be required for optimal rates of reproduction or development (Chapter 2; Kiman & Yeargan, 1985; Wiedenmann et al., 1996; Limburg & Rosenheim, 2001; Patt et al., 2003; Lundgren & Wiedenmann, 2004). Nevertheless, agroecologists might also consider whether increasing availability of flowers could affect predation by omnivores that exploit nectar and/or pollen. The true omnivores could themselves be the target of conservation biological control, or practices targeted at other species could be more or less effective due to their effects on the true omnivores.

True omnivores have been the subject of many studies and their role in biological role has been recognised (Eubanks & Styrsky, 2005; Gillespie & Roitberg, 2006). However, most studies have investigated either the effect of floral resources (or other plant material) on per capita predation, or on longevity/fecundity, but not on the combination of processes which determines the impact on the pest population (Eubanks & Styrsky, 2005). The few studies which have addressed the question of how plant resources affect biological control suggest that they can have an important influence on omnivore-prey dynamics (McMurtry & Scriven, 1966; Cottrell & Yeargan, 1998; Eubanks & Denno, 2000; Nomikou et al., 2004). The brown lacewing *Micromus tasmaniae* Walker (Hemerobiidae: Neuroptera) was the subject of one such study (Chapter 2; Robinson et al., 2008).

*M. tasmaniae* may play an important role in biological control of small soft-bodied pests in Australasian agroecosystems (Horne et al., 2001). Both the larvae and adults are predatory. The consumption of alternative resources by the larvae has not been studied but the adults are known to consume floral resources in the laboratory (Chapter 2; Robinson et al., 2008). For the adults, flowers and prey are substitutable as foods to enhance longevity, but prey are essential
Laboratory experiments have found that presence of floral resources could enhance or diminish biological control by the adult lacewings: flowers decreased per capita prey consumption but increased longevity and fecundity when prey were scarce (Chapter 2; Robinson et al., 2008).

Although it is known that *M. tasmaniae* can exploit floral resources, the extent to which it does so in the field is unknown. It is possible that the lacewing feeds on pollen and/or nectar when confined with flowers in the laboratory but does not actively forage for these resources. If this were the case, lacewings might feed opportunistically on flowers encountered when foraging for prey in the field, but this would have a negligible effect on biological control if the distributions and abundances of prey and flowers were such that the predators rarely encountered flowers. Alternatively, the lacewings may be able to detect flowers from a distance and direct their movement towards them like parasitoids and key pollinators with which the flowers have co-evolved (Fægri & van der Pijl, 1979; Wäckers, 2004).

For conservation biological control, flower abundance might be increased across a field by modifying herbicide applications or other methods of weed control. Alternatively, strips of selected flower species may be sown adjacent to the crop or between occasional rows. The latter method minimises competition and crop contamination, and ensures the flowers are suitable as sources of floral resources for the target biocontrol agents. However, the resources are aggregated and spatially separated from the areas of crop where biological control is required, in contrast with randomly distributed weeds. The efficacy of flower strips as a way to provide floral resources depends on the distance over which biocontrol agents respond to the flowers and the spatial scale over which they forage to enter this ‘zone of detection’. For true omnivores such as *M. tasmaniae*, it is of particular interest to determine whether such flower strips are used by a significant proportion of the population in a field.

Here I present the results of a study of *M. tasmaniae* sampled 1–100 m from a strip of flowers sown in the centre of a lucerne field. The abundance of lacewing larvae and adults were assessed in relation to distance from the flower strip since the pattern of abundance may have direct implications for the pattern of predation. Decreasing adult abundance with increasing distance from the flower strip could also provide supporting evidence for use of flowers, with physiological and behavioural consequences and further implications for biological control. As a more direct indicator of flower-feeding, presence of pollen in the guts of adult lacewings was assessed. Lacewings were expected to ingest pollen when foraging in flowers for nectar or for pollen itself. Presence of arthropod material was used to indicate predation. To assist interpretation of the latter, a laboratory experiment provided an approximate time over which aphid material remains visible in gut dissections. These observations complement previous laboratory studies by allowing preliminary conclusions to be drawn about the relative significance of flower-feeding and prey consumption by *M. tasmaniae* in the field when
aggregated floral resources have been provided. These are discussed in the context of using floral resources as a technique in conservation biological control.

3.3 MATERIALS AND METHODS

3.3.1 Sampling of lacewings from the experimental field

The study took place through the summer of 2005–06 in a field (12.5 ha) of established lucerne *Medicago sativa* L. ‘Kaituna’ (Leguminosae) in a mixed farming area of Canterbury, New Zealand (Appendix 1, Fig. A1.1i). The crop management was typical for the region and not altered for the study. The lucerne was periodically grazed by sheep or cut for hay. This occurred before flowering and sampling took place when re-growth was sufficient for sweep-net sampling. The field was not sprayed with insecticide. Herbicide was not used and although the density of the lucerne stand varied across the field, the density of flowering weeds was considered to be low throughout. Irrigation was applied when necessary.

The field was divided by a 5 x 360 m strip of grass. The central 80 m of this strip was ploughed and sown with buckwheat *Fagopyrum esculentum* Moench ‘Katowase’ (Polygonaceae) (Appendix 1, Fig. A1.1ii). Rows of buckwheat were sown on ten dates during the study to provide continuous flowering through the sampling period. The strip was surrounded by an electric fence to protect it when sheep were grazing the lucerne. Flowering buckwheat rows were sprayed with rubidium chloride solution during the experiment. This was part of a rubidium-marking study described in Chapter 5.

The insect population was sampled by sweep-net sampling across a grid of 30 points each side of the buckwheat strips (Appendix 1, Fig. A1.2). In order to identify the points for sampling, the sampler aligned herself with canes positioned within the buckwheat and red flags on the boundary fences of the field. This avoided the need to remove and re-establish position markers when the lucerne was cut for hay and avoided any possible influence of markers on grazing by the sheep. On each sampling occasion, five samples were taken at each of six distances: 1, 5, 10, 25, 50 and 100 m from the buckwheat. Each sample comprised 14 sweeps made while walking parallel to the buckwheat strip from a starting position identified using the grid. The starting positions for the five samples at each distance were spaced 15 m apart and sweeping covered approximately 10 m.

When a 14-sweep sample had been collected in the net, any *M. tasmaniae* were sucked into a plastic specimen tube. This was kept in an insulated bag with ice-packs for a maximum of 2 hours until returning to the laboratory. The lacewing parasitoid *Anacharis zealandica* Ashmead (Hymenoptera: Figitidae) was also collected and other species were released. (See Chapters 5 and 6 for discussion of *A. zealandica* samples.) The specimen tubes were placed in a
domestic freezer to kill the insects. Adult lacewings were then put in individual 0.6 ml microcentrifuge tubes and re-frozen. Lacewing larvae were counted and discarded.

With a minimum time between sampling dates of two days, samples were taken as frequently as possible when there was adequate re-growth, the crop was dry and the wind was not too strong to affect sampling efficiency. Side 1 (the lucerne one side of the buckwheat strip) was sampled on 11 dates 18 November – 17 February. Side 2 was sampled on 12 dates 18 November – 24 February. On some dates both sides were sampled but on other dates insects were collected only from one side.

A total of 1658 lacewings were collected. The spatial distribution of the lacewings in relation to the buckwheat was analysed and subsamples of insects were subjected to various analyses to determine their feeding status, as described below.

### 3.3.2 Analysis of spatial distributions

Lacewing larvae were sufficiently abundant for analysis only on 18 November. Even on this date, many samples did not contain larvae so abundances were converted to presence-absence data. These were analysed using a generalised linear mixed model with a binomial distribution and logit link function. ‘Side’ (i.e. which half of the field) and ‘Position’ (i.e. which of the five sampling positions along the buckwheat strip) were included as random variables. The model was run with main effects only and with the interactions Side x Distance and Position x Distance. Distance was treated as a factor rather than a covariate because inspection of the data suggested there was not a linear relationship between number of lacewings and distance over the scale sampled, so it was appropriate to determine whether abundance differed between any of the sampling distances.

Abundance of adult lacewings was analysed for ten dates of sampling on each side of the buckwheat strip. On some dates lacewings were not collected from all distances because conditions became unsuitable for sampling. These dates were excluded from the analysis. The samples collected from the five positions along the length of the buckwheat strip were combined to give the total number of lacewings at a given distance on a given date at each site. One such total had a value of zero which disrupted the logratio link function so a value of one was added to all the totals. This formed the response variable for a generalised linear mixed model with a negative binomial distribution, logratio link function and aggregation factor of unity. ‘Date’ and ‘Side’ were included as random variables. The analysis was run with main effects only and with the interactions Side x Distance and Date x Distance. Distance was treated as a factor rather than a covariate, as for larvae.

On three dates the sex of the adult lacewings was determined. The variation in sex ratio with distance from the buckwheat strip was analysed with Chi-squared tests. Analyses were performed using SPSS Version 15 and Minitab 15.1.
3.3.3 Determining consumption of prey

In order to assist interpretation of dissections of the field-collected lacewings, a laboratory experiment was conducted to determine the gut residence time of prey. The experiment took place at a mean temperature of 22°C (range ± 2°C) with a light regime of 16 h light: 8 h dark. Vials containing *M. tasmaniae* pupae were obtained from a laboratory culture (see Chapter 2 for details). The vials were checked daily and within 24 hours of emergence, lacewings were placed in individual Petri dishes (2 x 9 cm) rimmed with polytetrafluorethylene. A damp cotton dental roll and pea aphids *Acyrthosiphonpisum* Harris (Homoptera: Aphididae) were provided *ad libitum*. The lacewings were kept in these enclosures for three days. The dental roll was kept damp and the aphids were replaced daily. After three days, male and female lacewings were systematically allocated to five treatments; they were either frozen immediately or kept in a clean Petri dish without food for 1, 2, 4 or 6 days. After the appropriate starvation time, all lacewings were frozen in a domestic freezer until dissection. There were five female and five male lacewings in each treatment, except the ‘freeze immediately’ and ‘1 day starvation’ treatments which contained four females and five males.

These laboratory lacewings were dissected under a binocular microscope (x20) in a drop of 70% ethanol. The abdominal exoskeleton was pulled away from the thorax revealing the crop and mid-gut (Appendix 1, Fig. A1.3). The exoskeleton of the thorax was pulled back to reveal the oesophagus. The presence of aphid material in the oesophagus, crop and mid-gut was recorded, as seen through the transparent gut wall. (The hind-gut was not assessed because it was difficult to dissect cleanly.) The presence of distinct parts of the aphid exoskeleton (e.g. cornicle tips and tarsal claws) were noted when they occurred in the absence of homogenous material.

An initial batch of field-collected lacewings was dissected in the same way as the laboratory lacewings. However, it was apparent that arthropod material in the mid-gut could often be seen through the translucent abdominal exoskeleton. Most lacewings were subsequently determined to contain prey without dissection and only when this assessment was uncertain, was the insect dissected. Presence of prey in the gut was assessed for 258 lacewings. These insects were collected from Side 1 on 17, 20 and 24 February, and from Side 2 on 12 February.

3.3.4 Determining consumption of pollen

In order to see clearly pollen grains in the gut of the lacewings, it is necessary to remove other gut contents such as prey. This can be achieved by chemically digesting the arthropod material using a process that leaves the pollen grains intact. The following procedure was adapted from Moore *et al.* (1991) (also see references in Silberbauer *et al.* (2004)). A pilot study with...
buckwheat-provisioned laboratory lacewings demonstrated that the process allowed pollen to be identified in the digested samples.

Forty eight lacewings collected from Side 2 on 21 December were digested to determine whether or not they contained pollen. The sex of each lacewing was determined. The insects were then rinsed in 70% ethanol to remove external pollen and dried at 40 °C for 24 h. Each insect was placed in a 15 ml centrifuge tube and 1 ml glacial acetic acid added. The sample was shaken and the liquid then pipetted off before 1 ml acetolysis mixture was added. (Acetolysis mixture was prepared daily by slowly adding concentrated sulphuric acid to acetic anhydride in a ratio of 1:9.) The samples were then placed in a 90 °C water bath for 15 min. After this time, most of the arthropod material had been digested but small wing fragments remained in some samples. The samples were centrifuged at 3000 rpm for 3 min and the supernatant was discarded. The samples were then rinsed and stained by successively adding the following liquids, centrifuging the samples and discarding the supernatant between each step: 1 ml glacial acetic acid, 1 ml deionised water, 1 ml deionised water containing 1 drop 5% (w/v) aqueous safranine solution, and 1 ml deionised water. 1 ml molten glycerine jelly was then added to each sample. The samples were stirred using a pipette tip and a 50 µl drop was placed on each of four microscope slides and covered with a cover slip. Any visible lacewing material that remained in the sample was used to prepare a fifth slide. (Glycerine jelly was prepared by mixing 7 g gelatin in 150 ml water to hydrate it and then heating without boiling until the gelatin dissolved. 200 ml glycerin was then stirred into the solution and the mixture poured into glass Petri dishes. They were left overnight for water to evaporate and then covered and stored in refrigerator until use.)

The slides were examined under a binocular microscope (x40) to determine the presence of pollen grains. Those containing pollen were then examined under a compound microscope (x400) and the presence of buckwheat pollen determined by comparison to a reference slide prepared from the digestion of a laboratory lacewing provided with buckwheat flowers. Presence of other pollen grains was recorded according to morphotypes. Abundance of each type was classified as < 10 grains or ≥ 10 grains. This crude classification was adequate since most slides contained only a few (< 5) or many (> 20) pollen grains.

A Chi-squared test was used to determine whether the frequency of presence of buckwheat pollen or other pollen types differed between lacewings collected at different distances from the buckwheat strip. Due to low expected frequencies, the results for distances ≥ 5 m were combined. The proportions of male and female lacewings containing pollen were also analysed using a Chi-squared test. Analyses were performed using Minitab 15.1.
3.4 **RESULTS**

3.4.1 **Population abundance and spatial distribution**

Lacewing larvae were found in negligible numbers except on 18 November. Fig. 3.1 shows that although the abundance was highest 1 m from the buckwheat strip, there was also a large number 100 m away in one half of the field. The frequency of larval presence in samples did not differ between distances (effect of distance with main effects only: Wald $X^2 = 9.79$, d.f. = 5, $P = 0.082$, with selected interactions: Wald $X^2 = 7.24$, d.f. = 5, $P = 0.203$).

The number of lacewing adults did not vary with distance from the buckwheat strip (Fig. 3.2, effect of distance with main effects only: $F = 0.19$, d.d.f. = 99.1, $P = 0.967$, with selected interactions: $F = 0.09$, d.d.f. = 5.9, $P = 0.991$). There was considerable variation between sampling dates but there were no clear patterns related to time in the season or time within each cropping cycle.

Adult lacewings collected on three dates were sexed. On 21 December the sex ratio overall was 0.56 (females/total) but differed significantly with distance ($X^2 = 12.098$, d.f. = 1, $P = 0.001$ (distances 5–100m combined to ensure expected values >5)). One metre from the buckwheat the ratio was strongly male-biased (0.15) but at other distances it was weakly male-biased or female-biased (0.4–0.83). The number of lacewings was also highest at 1 m on this date. However, on 20 and 24 February the overall sex ratio was 0.65 (range 0.46–0.76), there was no difference in sex ratio with distance from the buckwheat strip ($X^2 = 4.461$, d.f. = 5, $P = 0.485$) and the number of lacewings was not highest in the 1 m samples.

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**Fig. 3.1** Effect of distance from the buckwheat strip on the number of lacewing larvae collected. Each data point is the sum of five samples taken at a given distance. Larvae were collected on 18 November on both sides of a strip of flowering buckwheat which divided a lucerne field. A total of 14 larvae were collected on the other sampling dates (11 dates on Side 1 and 10 dates on Side 2). They were found at all distances. (Note the x-axis is not scaled.)
Fig. 3.2 Effect of distance from the buckwheat strip on the number of adult lacewings collected. Adult lacewings were collected both sides of a flowering buckwheat strip dividing a lucerne field: i) Side 1 and ii) Side 2. Each data point is the sum of five samples taken at a given distance on a given date. The lucerne re-growth period (1-4) and the chronological order of sampling within the cycle (i-iv) are given in parentheses. (Note the x-axes are not scaled.)
3.4.2 Consumption of prey

In laboratory lacewings, prey material was absent from the oesophagus in most lacewings after one day without food but all lacewings starved for six days still contained prey material in the mid-gut (Table 3.1). One of ten lacewings starved for four days did not contain detectable prey material. The material persisting in the oesophagus of one lacewing after a day without food comprised of distinct parts of the exoskeleton rather than homogenous material. Similarly, only ‘hard’ material was found in the crop of three lacewings starved for one day and four lacewings starved for two days.

All the field-collected lacewings contained prey material.

Table 3.1 Number of lacewings containing prey material in three, two or one sections of their gut. Groups of cultured lacewings were dissected after different numbers of days since prey were available and material persisted sequentially in the oesophagus (O), crop (C) and mid-gut (M). Results for males and females were similar and so have been combined.

<table>
<thead>
<tr>
<th>Days starvation</th>
<th>O-C-M</th>
<th>C-M</th>
<th>M</th>
<th>None</th>
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<tbody>
<tr>
<td>0</td>
<td>4</td>
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<td>1</td>
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</tr>
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<td>6</td>
<td>3</td>
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3.4.3 Consumption of pollen

Buckwheat pollen was found in 17% (8/48) of the field-collected lacewings. All but one of these insects also contained pollen from other species, and 69% (33/48) of the lacewings contained some type of pollen. Seven pollen morphotypes were distinguished. For most samples, pollen was evenly distributed across the slides, the exceptions being where grains were caught in remains of the lacewing. Almost all of slides contained fewer than ten grains. However, for three lacewings, all the slides prepared from their samples contained more than ten grains of a single type. One of these insects, a male collected 1m from the buckwheat strip, contained abundant buckwheat pollen.

The proportion of lacewings containing buckwheat pollen was higher 1m from the buckwheat strip than at greater distances (Fig. 3.3, $X^2 = 17.4$, d.f. = 1, $P < 0.001$). The proportion containing other pollen types was also higher at this distance ($X^2 = 5.28$, d.f. = 1, $P = 0.022$). There was no difference between male and female lacewings in the proportion containing pollen ($X^2 = 0.075$, d.f = 1, $P = 0.784$).
3.5 DISCUSSION

3.5.1 Population abundance and spatial distribution

It is notable that so few lacewing larvae were collected when adults were found throughout the season. This may be an artefact of the efficacy of the sweep-net for sampling larvae (Leathwick & Penman, 1989) or the time the samples were collected relative to the time the lucerne was last cut. Alternatively, it may indicate disruption of the lifecycle by the repeated cutting and grazing of the lucerne. This would have negative implications both for the contribution that larvae make to biological control by the species, and the dependence on immigration of adult lacewings to sustain the local population. Rosenheim et al., (1999) observed high rates of intraguild predation of green lacewing larvae. Such mortality could also have occurred in this study.

Distance from the buckwheat strip did not have a statistically significant effect on the number of lacewing larvae or adults. However, on a few dates many more adult lacewings were collected one metre from the buckwheat strip than at other distances, in one half of the field. A pilot study in a different field found significantly more lacewings in samples collected closest to one edge of the field. The field was bordered by a shelter-belt of poplar trees and there were few flowering weeds in the margins, suggesting a microclimatic effect on the distribution of the lacewings. The buckwheat in the experimental field grew taller than the surrounding lucerne so it could have altered the microclimate. Therefore, whether or not buckwheat actually affected the distribution of lacewings under certain conditions, this should not be taken as evidence of feeding on the flowers.
The growth of the lucerne itself appeared to be enhanced near the strip. This could be a result of microclimatic effects or reduced competition and may have increased the volume of foliage sampled, with a concomitant effect on the apparent abundance of lacewings but not the predator-to-prey ratio. It is also possible that enhanced lucerne growth affected the pest population, and that was reflected in the lacewing population, or that the enhanced lacewing abundance decreased pest populations to the point where lucerne was visibly affected. Because these latter scenarios depend on trophic cascades which are often difficult to detect, they are considered less probable than microclimatic or sampling effects. While it would be satisfying to confirm and explain any such effects, they are on such a small spatial scale that they are likely to be of no consequence for the efficacy of biological control across the field.

3.5.2 Predation and use of the flower strip by lacewings

Prey-feeding by lacewings was found to be detectable with a high degree of reliability for at least six days in the laboratory. While gut retention times would vary with temperature and detectability is likely to depend on the volume and nature of material consumed, this provides a provisional guide to aid interpretation of the presence/absence of prey in field-collected lacewings. In the current study, all field-collected lacewings contained prey. Assuming the prey were herbivores feeding on the lucerne, these lacewings were contributing to biological control, albeit with unknown agronomic significance.

Although not quantified, variation in the abundance of aphids was observed during the period over which lacewings were sampled from the field. Other small arthropods were also abundant but aphids (mostly blue-green lucerne aphids Acyrthosiphon kondoi Shinji) are thought to have been the dominant prey group for adult lacewings due to their relative ease of capture and consumption. The lacewings found to contain prey were collected in February when the aphid population was high. For logistical reasons, no lacewings from this time were assessed for pollen content so no evidence of flower-feeding in addition to predation is available.

In December, the aphid population was relatively low. Lacewings collected at this time could not be assessed for consumption of prey due to dehydration in the freezer. It is not known whether this was due to longer time in storage than the insects collected in February, or whether the feeding status of the lacewings affected their storability. These insects were, however, assessed to determine their pollen content as an indicator of flower-feeding.

Interpretation of the presence of pollen in the lacewings is constrained in two ways. Firstly, absence of pollen does not indicate that the lacewing never fed on floral resources. Since the gut retention time for pollen is unknown, absence of pollen indicates lack of flower-feeding for an undetermined period of time. Secondly, presence of pollen does not indicate active use of floral resources since dispersed pollen is prevalent in the environment and may be ingested incidentally (with or without nutritional benefit to the lacewings) (Jervis & Heimpel, 1996). It
could also have been consumed by herbivores that were subsequently eaten by lacewings. Feeding on flowers, either deliberately consuming pollen or ingesting pollen-contaminated nectar, would result in a greater abundance of pollen in the gut than incidental consumption (except when lacewings were foraging for prey within the flowers and this situation is likely to be rare). The difference in these abundances has not been quantified but a bimodal distribution was observed in this study. It is therefore suggested that most of the field-collected lacewings consumed pollen incidentally, and that active flower-feeding only occurred for the 6% (three individuals) containing much greater abundances of pollen. Since only one of these three lacewings contained abundant buckwheat pollen, use of the flower strip seems to have been rare. This conclusion must be corroborated by further work since bimodal abundances could also arise through other factors. For example, pollen may appear as abundant when still in the insect’s crop but be rapidly digested when passing into other parts of the gut, or the high abundances may be anomalous amongst a population that did frequently consume floral resources.

The abundance of flowering weeds (including grasses) was higher in the buckwheat strip than the surrounding lucerne and this is likely to account for the similar spatial patterns of prevalence of buckwheat and non-buckwheat pollen ingestion by the lacewings. Dispersal of weed pollen could also have been affected by differing effects of the buckwheat and lucerne on air-flow. Although weeds were present, the number of flowering buckwheat plants greatly exceeded the number of weed plants so the dominance of non-buckwheat pollen may reflect presence of weed species which produce larger quantities of pollen, or whose pollen is more readily dispersed. It is also possible that the lacewings actively foraged in the weed species in preference to the buckwheat. The pollen identified as buckwheat was quite distinct from the other pollen found and was indistinguishable from known buckwheat pollen at the magnification used. However, the possibility of misidentification must be acknowledged since pollen was not collected from weeds to confirm that the characteristics used to identify buckwheat pollen were unique to that species.

Whether pollen was consumed incidentally or actively, the differing prevalence of pollen content at 1m and other distances suggests that the lacewings were not dispersing on a scale of tens of metres within the gut retention time of the pollen. However, it cannot be inferred whether lacewings dispersed to the 1m sampling distance from the buckwheat strip itself, or whether the presence of pollen in lacewings reflects a gradient in abundance of dispersed pollen consumed by insects which moved smaller distances than this. *M. tasmaniae* is capable of long-distance dispersal flights, but the scale over which individuals move in the course of foraging is unknown (Leathwick, 1989).
3.5.3 Implications for biological control

In this study pest populations were not assessed so the actual impact of the buckwheat strip on biological control, either by lacewings or by other species affected directly or indirectly by the strip, could not be ascertained. Nevertheless, studying the population distributions and feeding status of the lacewing could have reinforced laboratory studies by demonstrating effects in the field which have the potential to enhance or disrupt biological control by these omnivores. Failure to observe such effects could be taken as evidence for an absence of direct effects of the buckwheat strip on biological control by *M. tasmaniae*, but the spatial and temporal scales of sampling might have been inappropriate.

This study provided preliminary evidence that adult *M. tasmaniae* do consume floral resources in the field, but that prey-feeding is much more prevalent in this lacewing. Further work is required to confirm flower-feeding and to determine how rates of predation and flower-feeding relate to prey density through the season. Consumption of alternative foods such as homopteran honeydew should also be considered. Honeydew can enhance survival under laboratory conditions (Robinson, K.A., unpublished) and could be a substitute for nectar. Its association with prey might mean it is more frequently encountered by lacewings and they may be attracted or arrested by semiochemicals within it, as are more specialised aphidophages (e.g. (Carter & Dixon, 1984; Budenberg & Powell, 1992; Shaltiel & Ayal, 1998; Mandour *et al.*, 2007).

Laboratory studies with *M. tasmaniae* have shown that by providing resources that are partially substitutable for prey, buckwheat flowers have the potential to either disrupt biological control by reducing prey consumption, or to enhance it by increasing longevity and fecundity (Chapter 2). The results of this field study do not suggest that presence of flower strips would significantly affect biological control in the field in either of these ways, or by affecting the distribution of lacewings. However, the potential remains for flower strips to have a significant effect at certain critical times, such as when prey density is low.
Chapter 4  Effects of floral resources on the fecundity of *Anacharis zealandica*, a parasitoid with the potential to disrupt biological control

4.1 SUMMARY

Consumption of floral nectar increases the lifetime fecundity of many of the hymenopteran parasitoids that contribute to biological control. Increasing the abundance of flowers in agroecosystems has consequently been proposed as a technique to enhance the contribution of these species to pest management. However, the floral resources may be exploited by antagonists such as hyperparasitoids and parasitoids of predators. Depending on the relative benefits to the third and fourth trophic level species, providing flowers could therefore lead to disruption rather than enhancement of biological control. Although the potential for such effects to occur has been recognised, they have rarely been studied.

In the study presented here, the effects of floral resources on the fecundity of *Anacharis zealandica* were assessed in the laboratory. This species is a parasitoid of a biocontrol agent, the brown lacewing *Micromus tasmaniae*, so increasing rates of parasitism could be detrimental to biological control. Only 60% of parasitoids provided with buckwheat *Fagopyrum esculentum* leaves and water were alive after two days, while all those with buckwheat flowers and water survived. Evidently this effect on survival affected the number of progeny the parasitoids produced in this time. Over the two days, flowers also increased the number of lacewing larvae stung and the number of stung larvae that were successfully parasitised by surviving parasitoids. Egg load and progeny sex ratio did not differ between parasitoids with and without flowers. The species was observed to be strongly synovigenic and could continue to parasitise lacewings for fifteen days when flowers were provided. The study therefore demonstrated that availability of floral resources has the potential to significantly affect parasitism by *A. zealandica*, and hence biological control by *M. tasmaniae*.

In addition, the experiments involved presenting a succession of lacewing larvae to individual parasitoids so the fate of each larva could be analysed in relation to its position in the presentation sequence (i.e. whether it was the first presented, the second presented etc.). These analyses, together with the first assessments of survival and fecundity of this species, provide information about its basic biology that will be valuable in future studies.

4.2 INTRODUCTION

Within the Hymenoptera, the parasitoid wasps are defined by the feeding strategy of the larval stage of the species: the larvae are parasitic on a single animal host and kill that host in the course of their development (Godfray, 1994). Adult parasitoids exhibit a range of feeding
strategies and vary in how feeding relates to their fecundity and longevity. The key distinctions in the present context are synovigenic versus pro-ovigenic reproduction and host versus non-host feeding, as discussed by Olson et al. (2005) and Jervis & Kidd (1999). In synovigenic species, females mature eggs after eclosion and require, or benefit from, feeding to do this. Feeding may also reduce oosorption and prolong life so consuming food can increase the number of hosts parasitised before the wasp becomes egg- or time-limited (i.e. encountering hosts without eggs to lay or dying with unlaid eggs). In pro-ovigenic species, females eclose with all their eggs in a mature state so adult feeding does not affect egg maturation. However, feeding by these species may enhance longevity and fuel mate and host foraging activity, and so increase the probability of the parasitoid laying all its eggs.

The foods these parasitoids exploit are classified as host or non-host foods and species are characterised by whether or not they host-feed (Olson et al., 2005). Host-feeders may also exploit non-host resources in addition to body fluids obtained from their host species. Non-host foods are typically sugar-rich substances such as floral and extra-floral nectar and homopteran honeydew. Males do not require nutrients for egg maturation and, lacking an ovipositor may be unable to puncture hosts to obtain body fluids. They may, however, feed on sugar-rich foods that prolong life and so increase mating opportunities.

For parasitoids which require non-host foods to maximise their longevity and/or fecundity, the availability of these resources is likely to be a significant determinant of their success at a given time and location. When these species are agents of biological control, the degree to which food is limiting is of particular interest since, if such limits are identified, biological control might be improved by providing sources of these foods (Jervis et al., 2004; Heimpel & Jervis, 2005). One technique of the conservation biological control pest management strategy is to enhance indigenous (and previously-introduced) parasitoids in this way (Powell, 1986; Landis et al., 2000). For example, flowering ‘weeds’ can be managed with regard to their potential role as nectar sources or plants can be sown for this purpose (e.g. Cowgill et al., 1993; Horton et al., 2003; Pflifner & Wyss, 2004; Berndt et al., 2006; Irvin et al., 2006).

Managing floral resources does not have the proven track record held by other forms of pest management in modern commercial agriculture, but it is the subject of ongoing research (van Driesche et al., 2008). A number of field experiments have studied the effect of flowers on rates of parasitism in the field, albeit often at a small scale and/or with inoculated pest populations (Baggen & Gurr, 1998; Stephens et al., 1998; Irvin et al., 2000; Nicholls et al., 2000; Berndt et al., 2002; English-Loeb et al., 2003; Tylianakis et al., 2004; Lee & Heimpel, 2005; Begum et al., 2006; Bell et al., 2006; Berndt et al., 2006; Irvin et al., 2006; Pontí et al., 2007). Some of these found flowers increased rates of parasitism but this was not necessarily
associated with reduced pest populations. (Baggen & Gurr, 1998; Nicholls et al., 2000; English-Loeb et al., 2003; Berndt et al., 2006; Ponti et al., 2007).

Most studies have addressed a single link in the complex chain of processes which connect habitat management to pest control (see reviews by Jervis et al. (1996) and Heimpel & Jervis (2005)). Such research has demonstrated that many parasitoids of pests do show an increase in some measure of fecundity when sugar sources are available, and that these beneficial species do exploit flowers in the field (e.g. Idris & Grafius, 1995, 1997; Baggen & Gurr, 1998; Freeman-Long et al., 1998; Baggen et al., 1999; Jacob & Evans, 2000; Tylianakis et al., 2004; Zhang et al., 2004; Berndt & Wratten, 2005; Begum et al., 2006; Irvin & Hoddle, 2006; Lavandero et al., 2006; Winkler et al., 2006; Lee & Heimpel, 2008a). The role of flowers in biological control by hoverflies (Diptera: Syrphidae) (e.g. Cowgill et al., 1993; Hickman & Wratten, 1996) and brown lacewings (Chapters 2 and 3) have also been studied.

The results to date encourage further research in this field, studying both the overall effects of flowers on biological control and the individual ecological relationships involved in this process. These relationships should include those which result in non-target effects of the resources provided (Gurr et al., 2000). Such effects vary from benefiting pests (Baggen & Gurr, 1998; Begum et al., 2006; Lavandero et al., 2006) to competing with the crop for water (e.g. Brown & Glenn, 1999). Inadvertently benefiting hyperparasitoids of a target parasitoid is also possible (Araj et al., 2008). Similarly, enhancing parasitoids of beneficial predators could negate any positive effects on the predators themselves, or target parasitoids.

Anacharis zealandica Ashmead (Hymenoptera: Figitidae) is a parasitoid of Micromus tasmaniae (Neuroptera: Figitidae), a brown lacewing which may play an important role in biological control in Australasian agroecosystems (Horne et al., 2001). It is a solitary, koinobiont wasp which attacks the larval lacewing stage (i.e. eggs are laid in lacewing larvae but the hosts continue to develop until pupation and a single wasp emerges from each parasitised lacewing) (Appendix 2). While the species has been recorded in a number of published surveys sampling lucerne and orchard habitats (Leathwick, 1989; Stephens et al., 1998; Irvin et al., 2006), to my knowledge there are no published accounts of its ecology or reproductive biology. Since flower-feeding is common in other parasitoids, it was expected that this species would benefit from the presence of flowers with accessible nectar. Given the fourth-trophic-level status of A. zealandica, if this were the case the effects of flowers on biological control in a given agroecosystem could be positive or negative depending on the relative effects on beneficial insects, including M. tasmaniae, and antagonists, including A. zealandica.

Here I present the results of two laboratory experiments which were conducted in order to ascertain whether A. zealandica does benefit from buckwheat flowers Fagopyrum esculentum Moench ‘Katowase’ (Polygonaceae), and to provide a preliminary quantification of any such benefit in terms of survival and fecundity. The key measure of fecundity was the number of
lacewing larvae successfully parasitised. In order to help explain any variation in this variable, egg load (with or without opportunities to oviposit) and stinging behaviour were also recorded. Sex ratio of progeny was also recorded since only females determine the effect of that generation on the host population.

In pilot studies where parasitoids were held in individual containers with ten lacewing larvae for 24 hours, the rates of parasitism were extremely low. A technique was therefore developed in which successive larvae were presented to individual parasitoids. This elicited much higher daily rates of parasitism and although parasitoids had access to larvae for only a small proportion of each day, it was considered to be a better measure of fecundity. The method also allowed analysis of the fate of presented larvae with respect to their position in the presentation sequence (i.e. first presented, second presented etc.). This was therefore an opportunity to study the basic reproductive biology of the species.

The first experiment assessed the fecundity of parasitoids that were either provided with buckwheat flowers or just a buckwheat leaf and water. This experiment was restricted to a two-day period since beyond this time survival of parasitoids without flowers was low. The second experiment was conducted over fifteen days using parasitoids that were provided with buckwheat flowers. This was to determine whether the increased survival conferred by flowers was associated with a commensurate increase in fecundity over this period.

4.3 MATERIALS AND METHODS

All insects were raised in controlled-temperature rooms at a mean temperature of 20 °C (range ± 2 °C) with a light regime of 16 h light:8 h dark (on 06:00, off 22:00). Presentation of larvae took place in a laboratory with constant light intensity but variable temperature. Experiments 1 and 2 took place consecutively. Because the measurement of fecundity was time-intensive, only a small number of wasps could be assessed each day. Replicates for each treatment were therefore assessed in succession over four and one months for Experiments 1 and 2 respectively.

4.3.1 Experiment 1: The effect of flowers on parasitoid fecundity within two days of emergence

Rearing of parasitoid wasps

A culture of *A. zealandica* was established from local field-caught insects and maintained in 2.5-litre plastic jars for more than ten generations prior to the experiments. Adult wasps in the culture were provided with honey-soaked cotton wool and a water-filled vial with a cotton wool wick. To provide adults for experimentation, second instar lacewing larvae were added to the jars to allow parasitism. The larvae were provided with pea aphids (*Acyrthosiphon pisum* Harris, Homoptera: Aphididae) daily until pupation. Each jar contained a crumpled paper towel and the larvae usually pupated on this surface. When the larvae had pupated, the paper was removed
and cut to separate the pupae. Each pupa was put in a 5 cm plastic Petri dish with a 1 cm length of moist cotton dental roll. After 15 days, parasitised and unparasitised pupae could be distinguished and the latter were discarded. Shortly before emergence, as indicated by melanisation of the wasp pupae, wasps were alternately allocated to two treatments: 1) with flowers, 2) without flowers. In the ‘with flowers’ treatment a buckwheat inflorescence was added to the Petri dish. The cut stalk of the inflorescence was inserted through a hole in the lid of a water-filled 1.5 ml microcentrifuge tube to prevent wilting. The inflorescences were changed and the dental roll was moistened every two days until emergence.

The wasp pupae in dishes with and without flowers were checked at 8:30 and 17:30 each day and newly-emerged wasps were sexed (males have 14 antennal segments, females have 13). In order to ensure there were always male wasps available for pairing with newly-emerged females, males were kept for 2–6 days before use in the experiment. For newly-emerged males the buckwheat was removed, if present, and a ball of honey-soaked cotton wool was added to Petri dishes. Within the ‘with flowers’ and ‘without flowers’ treatments, female wasps were alternately allocated to the following additional treatments: 1) freeze immediately, 2) without larvae, 3) with larvae. Wasps in the first group were frozen at -80 °C for storage until dissection to determine egg load, as described in the section ‘Assessment of egg load’. The remaining wasps were transferred to individual clear plastic containers (height 18 cm x diameter 10 cm) with mesh over the open top and a honey-fed 2–6 day old male was added. Each container was suspended from a rod positioned above a potted buckwheat plant and a buckwheat shoot was inserted through a hole (5 cm diameter) in the side of the container. A foam disc secured the shoot and plugged the hole. For ‘with flowers’ treatments, a buckwheat inflorescence and its subtending leaf were inserted. For ‘without flowers’ treatments, the inflorescence was cut off and only a leaf provided. Each enclosure contained a ball of moist cotton wool in a foil dish (height 1.5 cm, diameter 4 cm).

The following day, females in the ‘with larvae’ treatment were presented with lacewing larvae to parasitise, as described in the section ‘Presentation of larvae’. At the same times, females in the ‘without larvae’ treatment were frozen for later dissection. ‘With larvae’ females were also presented with larvae on the second day after emergence, but no ‘without larvae’ insects were retained for a second day.

The death of any female during the experiment was recorded. If a paired male died on the first day, the replicate was discarded. Dead females were frozen at -80 °C and stored for later dissection.

Rearing of lacewings
A culture of *M. tasmaniae* was established from local field-caught insects and maintained in 5.8-litre plastic boxes for more than ten generations prior to the experiments. Adult lacewings in
the culture were provided with honey applied to the walls of their enclosure and a water-filled vial with a cotton wool wick, and pea aphids were added daily. A strip of cotton cloth was provided as an oviposition substrate and this was changed daily. To provide larvae for experimentation, the cloth bearing 0–24 hour old eggs was put in a plastic vial. After three days, pea aphids were added daily to feed the newly-emerged larvae. Larvae were raised in the vial until eight days after egg collection, when they were used in the experiment. Checking samples of larvae under a microscope indicated that most are second instars at this time. Larvae used in the experiment were therefore assumed to be second instars and any markedly small or large larvae were not used.

**Rearing of aphids and buckwheat**

Lacewing larvae and adults were fed pea aphids raised on broad bean *Vicia faba* L. ‘Evergreen’ (Leguminosae). Buckwheat was raised in a glasshouse prior to use in the experiments.

**Presentation of lacewing larvae to parasitoid wasps**

For female wasps which were newly-emerged at 08:30, lacewing larvae were first presented to wasps in the ‘with larvae’ treatment at 09:30 the following day (i.e. after 25 hours). Those which were newly-emerged at 17:30 first received larvae at 13:30 the following day (i.e. after 20 hours). Larvae were presented again at the same time on the second day after emergence (i.e. after 49 and 44 hours respectively). These times are approximate, with the actual time of presentation for each wasp depending on how long it took to present larvae to other wasps in the same batch. For practical reasons it was not possible to standardise the times more accurately.

A succession of second instar lacewing larvae were presented to each wasp in a small arena. The base of the arena was a 10 cm plastic Petri dish. The sides were formed from an acetate sheet rolled to create a tube (height 4.5 cm, diameter 2.5 cm). The top of arena was the base of a glass vial (height 5 cm, diameter 2.5 cm) pushed into the acetate tube. The wasp was transferred to the Petri dish base using a paintbrush and the tube placed over it. The vial was pulled up to maximise the volume of the arena and when the wasp was near the top (to minimise the probability of escape), a lacewing larva was placed on the Petri dish and the tube placed over it. The vial was then pushed down to a height of 1–2 cm to reduce the volume and so increase the probability of the wasp encountering the larva.

The wasp was observed until one of the following occurred: 1) the wasp stung the larva, 2) the wasp encountered the larva three times without stinging it and a minimum of five minutes had elapsed, 3) ten minutes had elapsed. A sting was taken to be the action of the wasp bringing its abdomen forwards and maintaining this position close to the larva for at least five seconds. An encounter was taken to be contact between the insects or a close approach in which the wasp clearly reacted to the presence of the larva. Pilot studies and a sample of larvae from this experiment which were ‘stung’ for less than five seconds showed that this method was
appropriate to determine the propensity of wasps to sting, and potentially parasitise, lacewing larvae (see Appendix 2).

If the larva was stung, when the wasp moved away from it the larva was removed using a paint brush and placed in a glass or plastic vial (height 5 cm, diameter 1.5 cm). Unstung larvae were discarded. Further larvae were presented until two consecutive larvae were rejected.

To minimise the risk of wasp behaviour being influenced by persistent pheromones, the tube and glass vial were changed between larvae and wiped with 70% ethanol. The position of the tube on the Petri base was moved between larvae and the dish changed after approximately ten larvae.

At the end of the first presentation session for each wasp, the wasp was returned to its enclosure with or without buckwheat as appropriate. At the end of the second session the wasp was frozen at -80 °C.

Assessment of parasitism

The larvae that were stung when presented to the wasps were kept in their individual vials and provided with aphids daily until pupation. The pupae were checked periodically during the experiment and on emergence or death they were recorded as one of the following: 1) male wasp, 2) female wasp, 3) wasp of unknown sex, 4) male lacewing, 5) female lacewing, 6) lacewing of unknown sex, 7) died as pupa or larva before species could be identified, 8) missing.

Assessment of egg load

Live female wasps were frozen either without having been presented with larvae (‘without larvae’), or after two presentation sessions (‘with larvae’). These insects were dissected in methylene blue under a binocular microscope (x40). The ovaries were gently pulled from the abdomen and the ovarioles separated to allow the eggs to be seen (Appendix 1, Fig A1.4). The number of large eggs was determined by counting the eggs approximately 300 µm long. These were elongated and often stained darkly, in contrast with smaller eggs that were usually paler and ovoid in shape. The large eggs were taken to constitute the mature egg load.

Statistical analysis

Survival was analysed using a Chi-squared test. The low number of larvae stung by wasps without flowers on Day 2 led to heterogeneous data that were best analysed using multiple one-way comparisons (within homogenous groups) rather than a two-way analysis of variance. Mann-Whitney tests were used to determine whether flowers affected the number of larvae stung, the number of wasps and lacewings emerging and the sex ratio of progeny on each day. For each variable, the number of wasps which showed an increase from Day 1 to Day 2 and the number which showed a decrease were calculated. These were analysed using a binomial test to determine whether flowers affected how individual wasp behaviours changed between days.
identify any effect of flowers on the success rate of stinging, the number of wasps which achieved 100% parasitism on Day 1 was analysed with a Chi-squared test.

Logistic regression was used to investigate whether the fate of a larva depended on its position in the presentation sequence (i.e. first larva presented, second larva etc.). The binomial dependent variables were 1) larva stung/rejected, 2) emerged as lacewing/wasp if stung and 3) male/female if wasp. (Larvae of unknown fate comprised 5% and were excluded.) Larva position was a covariate in the model, flower treatment and day were factors and parent wasp was included as a factor nested within flower treatment. In the analysis of the probability of a larva being stung, the final three larvae in the sequence were excluded since the protocol dictated that they were a stung larva followed by two rejected larvae. The analyses were repeated with various measures of position: 1) the actual position of the larva in the sequence, 2) the position of the larva considering only the subset of larvae being analysed e.g. just stung larvae or just larvae which emerged as wasps, 3) as (1) but scaled by dividing the position by the number of larvae presented and, if only one larva was presented, a value of zero was used. The analyses gave similar conclusions so results using method (2) are presented here.

The effects of wasp age and flower treatment on egg loads for wasps without larvae were analysed using two-way analysis of variance. A t-test was used to determine whether flowers affected egg load for wasps with larvae.

Sample sizes varied between analyses depending on the survival and behaviour of the wasps and can be determined from the raw data presented in the graphs. Analyses were performed using SPSS Version 15.

4.3.2 Experiment 2: Fecundity of parasitoids with flowers for fifteen days after emergence

Insects were reared as in Experiment 1 but only wasps which were first observed at the 08:30 check were used and they all emerged in Petri dishes with flowers. Females were systematically allocated to the following treatments: 1) 15 days with lacewing larvae, 2) 15 days without lacewing larvae. They were paired with males and kept in containers with a buckwheat inflorescence, as described for Experiment 1. Those in the ‘with larvae’ treatment were presented with a succession of lacewing larvae each day for fifteen days, as in Experiment 1. The buckwheat inflorescences were changed when fewer than four flowers were open and the cotton wool was moistened daily. After fifteen days, all wasps were frozen. There were five wasps in the ‘with larvae’ treatment and eight wasps without larvae.

The stung larvae were assessed for parasitism as in Experiment 1 and the wasps were similarly dissected to determine their egg load. Logistic regression was used to determine the effect of larva position in the presentation sequence on its fate. Larva position was a covariate in
the model, day and parent wasp were factors and the dependent variables were as in Experiment 1 i.e. 1) larva stung/rejected, 2) emerged as lacewing/wasp if stung and 3) male/female if wasp.

4.4 RESULTS

4.4.1 Experiment 1: The effect of flowers on parasitoid fecundity within two days of emergence

Wasps provided with flowers produced more progeny in the two daily opportunities (median number of wasps produced without flowers = 1, with flowers = 5; $W = 303.5; P = 0.0017$). As described below, this was a result of differences in survival, stinging behaviour and rate of successful parasitism, but not differences in egg load.

Survival

All wasps survived the first day of the experiment but on Day 2 survival of wasps without flowers was lower than that of wasps with flowers ($X^2 = 8.203$, d.f. = 1, $P = 0.004$). By Day 2, only 13 of the 21 wasps without flowers were still alive but all 17 wasps with flowers survived.

Stinging behaviour and rate of parasitism

On the first day, providing wasps with flowers did not affect the number of wasps emerging from the lacewing larvae they stung i.e. the number of successfully parasitised larvae (Table 4.1, Fig. 4.1i). However, the number of lacewings emerging from stung larvae was higher for wasps without flowers than those with flowers (Table 4.1, Fig. 4.1ii). This might suggest that wasps without flowers should have stung more larvae, but no such difference was found (Table 4.1, Fig. 4.1iii). Rather, it seems that wasps without flowers were more likely to either sting no larvae or to sting large numbers of larvae which emerged as lacewings, but sample sizes were too small to determine the statistical significance of this pattern. The relationship between the number of larvae of known fate and the number successfully parasitised is shown in Fig. 4.2. This indicates that amongst the wasps which stung large numbers of larvae, those without flowers produced fewer wasps than those with flowers. Irrespective of number of larvae stung, wasps without flowers were less likely than those with flowers to have a 100% rate of parasitism ($X^2 = 5.743$, d.f. = 1, $P = 0.017$) and the logistic regression (see below) showed that the probability of a stung larva emerging as a wasp rather than a lacewing was higher for parent wasps with flowers than without.

On the second day, wasps with flowers stung more larvae than surviving wasps without flowers (Table 4.1i, Fig. 4.1iii) and produced more wasps (Table 4.1i, Fig. 4.1i). Flowers did not affect the number of stung larvae emerging as lacewings (Table 4.1i, Fig. 4.1ii).
Fig. 4.1 Effect of flowers and days after emergence on parasitoid stinging behaviour and rate of successful parasitism. Day 0 was the day of emergence. Each point on each graph represents one parasitoid. i) The number of lacewing larvae successfully parasitised, ii) The number of lacewing larvae stung but not successfully parasitised, iii) The number of lacewing larvae stung.
Table 4.1 Effect of flowers on parasitoid stinging behaviour and rate of successful parasitism. i) Results of Mann-Whitney tests to determine whether there was an effect of flowers on behaviour and parasitism on each day of the experiment. Day 0 was the day of emergence. ii) Results of binomial tests to determine whether behaviour and parasitism of individual parasitoids differed between the two days of the experiment when the parasitoids were provided with flowers or not. ‘Inc.’ and ‘Dec.’ are the numbers of parasitoids which showed an increase or decrease respectively, from Day 1 to Day 2, in the variable indicated.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Number of larvae stung</th>
<th>Number of wasps*</th>
<th>Number of lacewings*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W</td>
<td>P</td>
<td>W</td>
</tr>
<tr>
<td>Day 1, without flowers v Day 1, with flowers</td>
<td>413.0</td>
<td>0.929</td>
<td>225.5</td>
</tr>
<tr>
<td>Day 2, without flowers v Day 2, with flowers</td>
<td>139.5</td>
<td>0.007</td>
<td>17.0</td>
</tr>
</tbody>
</table>

*insects emerging from stung larvae

<table>
<thead>
<tr>
<th></th>
<th>Number of larvae stung</th>
<th>Number of wasps*</th>
<th>Number of lacewings*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without flowers</td>
<td>1</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>With flowers</td>
<td>8</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>

*insects emerging from stung larvae
Fig. 4.2 Effect of flowers on rates of successful parasitism. The graph show the effect of flowers on the relationship between the number of larva successfully parasitised and the number of larva stung by parasitoids on Day 1 (excluding larva which could not be identified as successfully or unsuccessfully parasitised). Successful and unsuccessful parasitism resulted in emergence of wasps and lacewings respectively. Overlying points have been slightly offset.

While individual performance of wasps with flowers did not differ between Days 1 and 2, the wasps which survived to Day 2 without flowers stung fewer larva and produced fewer wasps (Table 4.1ii).

The sex ratio of the wasps produced on Day 1 was not affected by flowers (median without flowers = 0.500, with flowers = 0.450; $W = 172.0$, $P = 0.613$). The value on Day 2 was not analysed due to low sample size.

Flowers did not affect the probability of a larva being stung, but if stung, the larva was more likely to be successfully parasitised with flowers (Table 4.2, Fig. 4.3, Fig. 4.4). The probability of emerging as a female rather than male wasp was not affected (Table 4.2, Fig. 4.5). These probabilities did not differ between Day 1 and Day 2. However, within each day there were highly significant effects of larva position. Larvae presented earlier were more likely to be stung than later larva. If stung, they were more likely to be successfully parasitised and if successfully parasitised they were more likely to be female.
Fig. 4.3 Bar charts to show the effects of flowers, day and larva position on the frequency of larvae stung and rejected by parasitoids. Larva position 1 is the first larva presented, position 2 the second larva presented etc., Declining bar heights reflect the decreasing number of wasps that were presented with higher numbers of larvae (see Section 4.3.1). The logistic regression analysis (see text) included parent wasp as a nested factor which is not represented in the graphs.

Fig. 4.4 Bar charts to show the effects of flowers, day and larva position on the frequency of successful parasitism. Successfully and unsuccessfully parasitised larvae are stung larvae which emerged as parasitoids or lacewings respectively. See notes with Fig. 4.3.
Fig. 4.5 Bar charts to show the effects of flowers, day and larva position on the sex ratio of progeny. The graphs indicate the numbers of successfully parasitised larvae which emerged as male or female wasps. See notes with Fig. 4.3.

**Egg load**

Newly-emerged wasps (frozen 0–15 hours after emergence) did contain large eggs but the number of eggs increased over the following 24 hours. The number of eggs was not affected by providing flowers (Fig. 4.6, effect of age $F_{1,72} = 85.2, P < 0.001$; effect of flowers $F_{1,72} = 0.09, P = 0.764$; interaction $F = 0.59, P = 0.446$).

Wasps that had just been presented with a succession of lacewing larvae and then failed to sting two consecutive larvae, still contained large eggs. The number of eggs was not affected by flowers (Fig. 4.7, $t = 0.42$, d.f. = 28, $P = 0.680$).
Table 4.2 Effects of flowers, day and larva position on the fate of larvae in Experiment 1. Results of a logistic regression to determine whether the fate of host lacewing larvae presented to parasitoids varied with provision of flowers (to the parent parasitoids), day of presentation and the position of the larva in the sequence of larvae presented in each session. Parent wasp was included as a factor nested within flower treatment but is not reported here. Larvae were presented on Days 1 and 2 of the experiment. Day 0 was the day of emergence.

<table>
<thead>
<tr>
<th>Probability</th>
<th>Flowers</th>
<th></th>
<th></th>
<th></th>
<th>Day</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Larva position</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>S.E.</td>
<td>expβ</td>
<td>X^2</td>
<td>P</td>
<td>β</td>
<td>S.E.</td>
<td>expβ</td>
<td>X^2</td>
<td>P</td>
<td>β</td>
<td>S.E.</td>
<td>expβ</td>
<td>X^2</td>
<td>P</td>
</tr>
<tr>
<td>rejected v stung</td>
<td>0.933</td>
<td>1.01</td>
<td>2.54</td>
<td>0.851</td>
<td>0.356</td>
<td>-0.849</td>
<td>0.504</td>
<td>0.428</td>
<td>2.84</td>
<td>0.092</td>
<td>-0.215</td>
<td>0.061</td>
<td>0.806</td>
<td>12.4</td>
<td>0.000</td>
</tr>
<tr>
<td>wasp v lacewing</td>
<td>3.12</td>
<td>1.41</td>
<td>22.4</td>
<td>4.87</td>
<td>0.027</td>
<td>-0.926</td>
<td>0.619</td>
<td>0.396</td>
<td>2.24</td>
<td>0.135</td>
<td>0.424</td>
<td>0.107</td>
<td>1.53</td>
<td>15.6</td>
<td>0.000</td>
</tr>
<tr>
<td>female v male wasp</td>
<td>-2.28</td>
<td>1.60</td>
<td>0.103</td>
<td>2.04</td>
<td>0.153</td>
<td>-0.039</td>
<td>0.575</td>
<td>0.962</td>
<td>0.005</td>
<td>0.946</td>
<td>0.842</td>
<td>0.19</td>
<td>2.32</td>
<td>19.6</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 4.3 Effects of flowers, day and larva position on the fate of larvae in Experiment 2. Results of a logistic regression to determine whether the fate of host lacewing larvae presented to parasitoids varied with day of presentation and the position of the larva in the sequence of larvae presented in each session. Parent wasp was included as a factor nested within flower treatment but is not reported here. Larvae were presented each day for fifteen days after parasitoid emergence and the parasitoids were provided with flowers.

<table>
<thead>
<tr>
<th>Probability</th>
<th>Day</th>
<th></th>
<th></th>
<th></th>
<th>Larva position</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>S.E.</td>
<td>expβ</td>
<td>X^2</td>
<td>P</td>
<td>β</td>
<td>S.E.</td>
<td>expβ</td>
</tr>
<tr>
<td>stung v rejected</td>
<td></td>
<td>-0.023</td>
<td>0.028</td>
<td>0.977</td>
<td>0.679</td>
<td>0.410</td>
<td>-0.223</td>
<td>0.044</td>
<td>0.795</td>
</tr>
<tr>
<td>wasp v lacewing</td>
<td></td>
<td>0.067</td>
<td>0.030</td>
<td>1.069</td>
<td>4.90</td>
<td>0.027</td>
<td>0.123</td>
<td>0.059</td>
<td>1.131</td>
</tr>
<tr>
<td>female v male wasp</td>
<td></td>
<td>0.005</td>
<td>0.035</td>
<td>1.005</td>
<td>0.017</td>
<td>0.895</td>
<td>0.553</td>
<td>0.100</td>
<td>1.738</td>
</tr>
</tbody>
</table>
Fig. 4.6 Effect of flowers on the number of mature (large) eggs in newly-emerged parasitoids and day-old parasitoids that have not had access to host lacewing larvae. Each point on the graph represents one parasitoid.

Fig. 4.7 Effect of flowers on the number of mature (large) eggs in parasitoids that were presented with host lacewing larvae. Parasitoids were presented with larvae on Days 1 and 2 of the experiment and dissected following the session on Day 2. Day 0 was the day of emergence. Each point on the graph represents one parasitoid.
4.4.2 Experiment 2: Fecundity of parasitoids with flowers for fifteen days after emergence

Stinging behaviour and rate of parasitism

Wasps provided with flowers continued to sting a constant number of larvae per day for fifteen days (Table 4.3, Fig. 4.8) and did so with a consistent rate of larva rejection (Table 4.3, Fig. 4.9i). They produced a mean (± standard error) of 3.18 (± 0.357) wasp progeny per day and over this period the mean proportion of larvae successfully parasitised (i.e. stung larvae emerging as wasps rather than lacewings) was 0.781 (± 0.010). Although the probability of successful parasitism decreased over the 15 days, this effect was small (Table 4.3, Fig. 4.9ii). The mean proportion of female wasp progeny was 0.727 (± 0.023) and did not change significantly over time (Table 4.3, Fig. 4.9v).

As in Experiment 1, the position of a larva in the presentation sequence affected its fate (Table 4.3, Fig. 4.9ii, iv, vi). Earlier larvae were more likely to be stung than later larvae. If stung, they were more likely to be successfully parasitised and if successfully parasitised they were more likely to be female.

Egg load

As in Experiment 1, wasps that had opportunities to parasitise lacewing larvae immediately prior to freezing still contained large eggs. However, they contained fewer eggs that those which had not been presented with larvae ($W = 15.0, P = 0.004$, Fig. 4.10).

![Fig. 4.8 Cumulative number of lacewing larvae stung by five parasitoids provided with flowers for fifteen days. Each line represents one parasitoid.](image)
Fig. 4.9 Bar charts to show the effect of day (i, iii, v) and larva position (ii, iv, vi) on the fate of host lacewing larvae presented to parasitoids over fifteen days. Larva position 1 is the first larva presented, position 2 the second larva presented etc. In (ii), (iv) and (vi), declining bar heights reflect the decreasing number of wasps that were presented with higher numbers of larvae (see Section 4.3.1).
4.5 DISCUSSION

This study has shown that *A. zealandica* can gain substantial benefit from buckwheat flowers. Without flowers, survival over two days was approximately 60%. In contrast, survival of parasitoids with flowers was 100% (n = 5) for fifteen days. This is consistent with recent work which found male and female *A. zealandica* with buckwheat flowers (without hosts) lived for a mean of 16.81 days (standard error = 2.31), with a range of 6–35 days (Jonsson *et al.*, in press). Wasps with only water lived for a maximum of 3 days. Since the parasitoids have been seen ingesting the droplets of nectar which are visible in buckwheat, and wasps provided with buckwheat flowers have higher sugar levels than those without (Chapter 6), it can be inferred that the increase in longevity is due to the energy acquired from nectar-feeding. Although hymenopteran parasitoids can benefit from pollen, nectar is usually considered to be the primary resource (Leius, 1963; Jervis *et al.*, 1993).

Longevity itself does not determine the impact of *A. zealandica* on a population of *M. tasmaniae*. Rather, increased lifespan creates the possibility for a wasp to encounter additional lacewing larvae and so increases the probability of the wasp realising its potential fecundity. Previous studies have shown that in species which mature eggs after emergence i.e. synovigenic species (Jervis *et al.*, 2001), sugar-feeding can also have a substantial effect on egg maturation and daily rate of oviposition (e.g. Harvey *et al.*, 2001; Tylianakis *et al.*, 2004; Lavandero *et al.*, 2006; Sivinski *et al.*, 2006; Faria *et al.*, 2007; Kehrli & Bacher, in press). This study demonstrated that *A. zealandica* is strongly synovigenic. While newly-emerged ‘buckwheat’ wasps contained a mean of five mature eggs and an undetermined number of immature eggs, the number of mature eggs increased to 11 in the following 24 hours and over 15 days the wasps produced 40–68 progeny. Increased longevity can therefore provide *A. zealandica* with the opportunity to mature and oviposit eggs that are not present in the newly-

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**Fig. 4.10** Effect of providing host lacewing larvae on the number of mature (large) eggs in parasitoids provided with flowers for fifteen days. Each point on the graph represents one parasitoid.
emerged wasp. Thus, although parasitoids with and without flowers may initially reproduce at similar rates, as morbidity and mortality accrue in wasps without flowers the benefits of flowers become increasingly apparent and may lead to substantial differences in the effect of parasitoids on the lacewing population.

Since both sexes of A. zealandica are commonly produced in the laboratory and unmated A. zealandica produce only male progeny (Robinson, K.A., unpublished), it is assumed that this species is arrhenotokous. In such species, females have the ability to manipulate the sex ratio of their progeny by altering the ratio of fertilised (female) and unfertilised (male) eggs that they lay (King, 1987). Many factors affecting sex ratios have been studied in the context of evolutionary theory and production of parasitoids for classical and inundative biological control (e.g. King, 1987; Godfray, 1994; Ode & Hardy, 2008). However, the effect of food on the sex ratio of solitary non-host-feeding parasitoids has only rarely been reported and the confounded effects of food increasing longevity and maternal age affecting sex ratio have not always been distinguished (King, 1987; Leatemia et al., 1995). These few studies have found sugars to have either no effect on lifetime sex ratio (e.g. Zhang et al., 2004; Irvin & Hoddle, 2006), an increase in the proportion of females (e.g. Berndt & Wratten, 2005) or an increase in the proportion of males over the wasp lifetime (e.g. Leatemia et al., 1995; Onagbola et al., 2007; Witting-Bissinger et al., 2008). The latter can arise when the increased longevity of fed females is associated with a high proportion of male offspring being produced towards the end of their lifetime. In this study, no effect was detected for A. zealandica for the period when sufficient progeny were produced by wasps without flowers to enable analysis (and lifetime fecundity with flowers was not determined). Given the substantial effect of flowers on the total number of progeny, any effects on sex ratio would have to be extreme in order to be of ecological significance by comparison. This could arise if, for example, feeding affected the proportion of the population which remained unmated, and so produced only male progeny. No wasps were unmated in this study and further work suggested there is no effect of flower provision on the frequency of males mounting females in Petri dishes (Robinson, K.A., unpublished). Food availability could, however, affect the parasitoids’ ability to search for mates.

Previous studies have found sex ratio and daily oviposition rate can change over time (e.g. King, 1987; Godfray, 1994; Matos Neto et al., 2005; Fuchsberg et al., 2007) but such effects are not always apparent (e.g. Paine et al., 2004; Heinz, 1998). No change was found in A. zealandica but the period studied only comprised a fraction (unknown) of the reproductive life of the wasps and daily oviposition rate may have been constrained by the protocol. The sex ratio in the 15-day experiment was female-biased, in contrast with the equal ratio in the 2-day experiment. The reason for this difference is not known and since sex ratio can be affected by host quality, both values could be dependent on the size of larvae chosen for the study (Godfray,
1994; Heinz, 1998; Chow & Heinz, 2005). The values should therefore be used with caution. In a field study, 99 A. zealândica were sexed and only 7 were males (Chapters 5 and 6).

In contrast with most assessments of parasitoid fecundity, this study presented wasps with a succession of hosts rather than providing an excess number of hosts for a given period of time. This enabled both the behaviour of the wasp i.e. stinging or rejecting a host, and the outcome of that behaviour i.e. successful or unsuccessful parasitism, to be recorded. Although the data were not conclusive, they suggest that on the first day after emergence when no effect of flowers on the number of progeny could be detected, the wasps without flowers may have had more unsuccessful encounters with larvae. In a situation where larvae are encountered infrequently, a higher rate of unsuccessful encounters may lead to differences in numbers of progeny that were not apparent in this study where larvae were available until satiation. Studies of other species suggest that food-deprived wasps may be less inclined to forage for hosts, searching instead for food resources (Takasu & Lewis, 1993; Wäckers, 1994; Lewis et al., 1998; Jacob & Evans, 2001; Siekmann et al., 2004; Bernstein & Jervis, 2008). The effects of flowers on foraging behaviour and rate of successful host encounters might often be trivial relative to the effects on longevity. However, when comparing the extreme situations of no-food versus abundant food, at intermediate levels of availability they might be significant.

The method used here also allowed analysis of the effect of the larva’s position in the presentation sequence on the fate of the larva. This revealed changes within one day’s presentation sequence that were not carried over to the following day. These changes could have been adaptive behaviour, or the high rate at which the wasps encountered larvae could have led to physiological constraints which would not occur in the field. For example, the decreasing probability of stinging with successive larvae could arise from wasps becoming more discerning about host quality as their egg load is depleted (Minkenberg et al., 1992; Godfray, 1994; Heimpel et al., 1996). Alternatively it could be because after several eggs had been laid, the oviposition processes that precede stinging could not operate within the two five minute periods allowed in the protocol. Similarly, the change in sex ratio could reflect a changing preference for producing female offspring as the perception of host density changes (Ode & Hardy, 2008), or it could be due to an inability to fertilise eggs at a sufficient rate. Further work to make such distinctions is required before the ecological significance of these results can be inferred.

It is notable that the parasitoids held with flowers but without hosts for fifteen days did not contain substantially more eggs than those dissected after two days. There was no evidence of oosorption during the study, even in wasps that died naturally, but the process could have occurred without being identified. The numbers of large eggs seen in this study and pilot studies were similar to the number of ovarioles. This may indicate that wasps mature one egg per ovariole and do not mature additional eggs in the absence of hosts.
The presence of large eggs in wasps that had recently rejected larvae might indicate that the technique led to a more conservative measure of fecundity than intended, or that large eggs are not necessarily mature. In either case, by restricting oviposition to a small proportion of each day, these results only provide a minimum estimate of the potential daily fecundity of *A. zealandica*. Furthermore, an estimate of lifetime fecundity is yet to be obtained. However, since the value of any measure of potential fecundity of laboratory wasps is limited by the uncertain and complex relationship with fecundity realised in the field, and the latter is extremely difficult to assess, the results of this study provide useful estimates for modelling (Chapter 7) and future work.

In hundreds of wasp-larva interactions observed in the laboratory, *A. zealandica* has never been seen host-feeding. Sugar-rich non-host foods are therefore likely to be the principal nutritional resource exploited by adults of this species. Notwithstanding differences in availability and quality, parasitoids are usually able to exploit a wide range of sources, including honeydew and extra-floral nectar, in addition to floral nectar from species with accessible nectaries (Jervis et al., 1996; Wäckers, 2005). *A. zealandica* does feed on honeydew in some circumstances (Chapter 6) but the relative benefits of honeydew and nectar for this species are unknown.

As a number of reviews have discussed, to predict how varying floral resources in the field will affect the host-parasitoid population dynamics demands a thorough understanding of numerous aspects of a species’ ecology (Gurr et al., 1998; Landis & Menalled, 1998; Jervis et al., 2004; Gurr et al., 2005; Heimpel & Jervis, 2005). As a first step towards understanding the ecology of *A. zealandica*, this study has demonstrated that the species can realise a substantially higher lifetime fecundity when it has access to a sugar source such as nectar, in comparison with when it only has water. The relationship between *A. zealandica* and *M. tasmaniae* is therefore likely to be affected by availability of such resources and they could therefore indirectly affect biological control by *M. tasmaniae*. The study consequently demonstrates the potential for adverse effects on biological control when flowers are provided with the intention of enhancing beneficial predators and parasitoids.
Chapter 5  Does the lacewing parasitoid *Anacharis zealandica* exploit aggregated floral resources in farmland? Evidence from rubidium-marking

### 5.1 SUMMARY

The value of floral resources to many hymenopteran parasitoids in the laboratory is well known, and sowing strips of flowering plants in fields has been proposed as a technique to enhance parasitism of pests in agroecosystems where floral resources would otherwise be limiting. The value of such flower strips depends on, amongst other factors, how parasitoids forage for floral resources and hosts within the field. Rubidium-marking is a technique in which field plants are sprayed with rubidium chloride solution and insects collected from the field are analysed to determine their rubidium content. Insects that have fed on the sprayed plants have elevated rubidium levels. By assessing the distribution of such marked insects in relation to the sprayed plants, feeding and subsequent dispersal can be studied.

Rubidium-marking was employed to study the use of a flowering buckwheat *Fagopyrum esculentum* strip in a lucerne field by the parasitoid *Anacharis zealandica*. This species parasitises the lacewing *Micromus tasmaniae*, which is a biocontrol agent. Therefore, in contrast with use of the flower strip by beneficial parasitoids, use by *A. zealandica* could have a detrimental effect on biological control. Analysis of 90 field-collected *A. zealandica* found two individuals with very high rubidium levels and a further 12 had levels which were above the threshold used to distinguish marked and unmarked wasps. These were found at all sampled distances, 1–100 m from the flower strip.

Unlike previous studies using this technique, the efficacy of rubidium-marking as an indicator of nectar-feeding was assessed in complementary laboratory studies. The results of these experiments suggested a risk of false-positives due to acquisition of rubidium from leaves. They also suggested false-negatives could occur since parasitoids that did not die, and were therefore assumed to have consumed nectar from the flowers provided, did not have elevated rubidium levels. The results of the field experiment should therefore be interpreted with caution and further work should be conducted to allow the potential value of this technique to be realised.

### 5.2 INTRODUCTION

For female parasitoids, searching for insects in which to lay their eggs is of fundamental importance. However, in order to sustain this activity, many species consume sugars (Jervis *et al.*, 1996). Finding sources of exploitable sugar may therefore also be an important behaviour. The degree to which these activities conflict will depend on the abundance and distribution of
hosts and food resources. If parasitoids forego opportunities to parasitise hosts in order to find food, their impact on the host population may be affected (Bernstein & Jervis 2008). Furthermore, if searching for sugars is unsuccessful due to scarcity at the scale of parasitoid foraging, parasitoids may die of starvation without fulfilling their reproductive potential. Clearly, if this is a significant source of parasitoid mortality, it too would reduce their impact on host numbers.

In agroecosystems, many parasitoid species can play a valuable role in biological control of pests and it is in the interest of farmers to ensure that this service is not limited by lack of sugar resources (Powell, 1986). Parasitoids may derive sugars from a range of sources including floral nectar, extra-floral nectar, other plant exudates, honeydew and fruits (Jervis et al., 1996; Wäckers, 2005). It has been suggested that in agroecosystems where herbicides are applied widely and non-crop habitats form a small proportion of the landscape, sugar resources may be at a sub-optimal level so biological control could be enhanced if flowers were more abundant (Landis et al., 2000; Gurr et al., 2005).

If providing flowers does indeed enhance biological control, to be useful it must do so without undue costs in terms of labour demands, land usage, or competition between ‘flower’ plants and crop plants. Sowing strips of selected flower species, adjacent to a crop or between occasional rows, is often likely to be the most practical method. Such flower strips in a weed-free crop produce a more aggregated distribution of floral resources than randomly distributed flowering weeds. These differing patterns of resource distribution could affect the frequency of resource encounter for insects which forage randomly with respect to flowers, and the detectability and attractiveness of the resources for insects which can direct their movement towards flowers. Whether or not aggregated floral resources are an efficient means of provision will depend on the spatial scale over which parasitoids forage within the timescale in which they need to feed, and the distance over which they can detect the flowers.

The net effect of flower strips on crop yield (or profit) is the ultimate concern of pest management. However, in order to better predict the circumstances under which desirable effects might be achieved, it is necessary to understand the processes that contribute to the overall impact. It is therefore valuable to know whether parasitoids actually feed on aggregated floral resources provided by sown flower strips, and how the insects subsequently disperse into the crop where they may contribute to biological control. Our knowledge of these processes is limited to a few species in a few flower-crop situations. Lavandero et al. (2005) working in a broccoli crop, and Scarratt et al. (2008) working in vines, found that some parasitoid wasps did feed on provided flowers and dispersed to the maximum distances sampled (80 m and 30 m respectively) within the duration of the experiments (4 days and 7 days respectively). However, there were no clear relationships between the frequency of marked parasitoids and distance, and less than one third of the parasitoids could be identified as having fed on the flower strips.
Freeman-Long *et al.* (1998) studied dispersal from mixed ‘insectary plantings’ into vegetable crops and an almond orchard and recorded dispersal up to 75 m. The proportion of wasps at this distance which were known to have been in the ‘insectary plantings’ varied from 0–40% in the three genera studied. Observing parasitoids in the field is challenging and these three studies used rubidium-marking to avoid some of the limitations of direct observation or using external markers (Hagler & Jackson, 2001).

Rubidium-marking is a technique that allows both parasitoids’ use of a flower strip, and their subsequent dispersal to be studied (Berry *et al.*, 1972; van Steenwyk, 1991; Hagler & Jackson, 2001). An aqueous solution of rubidium chloride is applied to the flowering plants. The rubidium is absorbed and incorporated into the plant material, elevating its rubidium concentration above that found in untreated plants. This ‘mark’ of elevated rubidium is transferred to insects feeding on the plant. The rubidium concentration of sampled insects can then be measured with atomic absorption spectrophotometry and insects that have fed on treated plants can be distinguished from those which have not. Although rubidium marks have not been verified in floral nectar, elevated rubidium has been recorded in extra-floral nectar so nectar-feeding insects are likely to acquire rubidium marks in the same way as leaf-feeders (Berry *et al.*, 1972; Gu *et al.*, 2001). In this study, by sampling parasitoids throughout a crop containing a rubidium-sprayed flower strip and identifying those which were marked, the proportion which had fed on the flowers could be estimated and the dispersal of the fed insects could be tracked.

The study focused on the parasitoid *Anacharis zealandica* Ashmead (Hymenoptera: Figitidae). This species parasitises larvae of the brown lacewing *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae). Both species are reported to be common in Australasian agroecosystems and the lacewing may play a significant role in biological control of small soft-bodied pests such as aphids (Horne *et al.*, 2001). *A. zealandica* is therefore antagonistic to biological control, in contrast with the beneficial parasitoids of pests discussed above. Where this species occurs, sowing flower strips with the intention of enhancing biological control may in practice have an adverse effect. As well as providing insight regarding use of aggregated floral resources by parasitoids, the study therefore also improves understanding of how floral resources might affect interactions in a model system with four trophic levels. The direct effects of flower strips on hyperparasitoids or parasitoids of predators have rarely been studied (but see Stephens *et al.*, 1998; Lee & Heimpel, 2005; Irvin *et al.*, 2006; Araj *et al.*, 2008). The results presented here form part of a wider study which also investigated use of the flower strip by the lacewing *M. tasmaniae* (see Chapter 3). In addition, sugar profiles were used to investigate feeding by *A. zealandica* (see Chapter 6).

In order to interpret the results from rubidium-marking in the field, it is necessary to have some indication of the efficacy of the marking technique. In particular: 1) how many nectar ‘meals’ do the parasitoids have to take from marked plants in order acquire a detectable
rubidium mark? 2) how long does this mark remain detectable? 3) can the parasitoids acquire rubidium from the plant other than by nectar-feeding?. Although previous studies have investigated uptake and elimination of rubidium for the purposes of marking leaf-feeders (e.g. Frazer & Raworth, 1974; van Steenwyk et al., 1978; Johnson & Reeves, 1995), parasitoids emerging from marked hosts (e.g. Fleischer et al., 1986) and parasitoids feeding on artificial foods (Gu et al., 2001), use of nectar to mark parasitoids has not been studied, to my knowledge. Furthermore, since detectability will depend on rates of application to the food source, as well as the method of feeding and insect species, existing data are of limited value to the current study. As a first step towards answering these questions for my model system, a laboratory experiment was conducted to compare rubidium concentrations in A. zealandica that had been exposed to flowers or foliage of sprayed or unsprayed plants for varying lengths of time, with or without subsequent opportunity to feed on unmarked food. In order to assess the comparability of the sprayed plants used in this laboratory experiment with the plants in the field experiment, an additional experiment exposed cultured parasitoids to these plants in identical enclosures set up in both the laboratory and the field. In all experiments, buckwheat Fagopyrum esculentum Moench ‘Katowase’ (Polygonaceae) was used as a source of floral resources. A. zealandica is known to exploit this species and does not host-feed (Chapter 4).

The results presented here thus arise from three experiments with A. zealandica: one with cultured parasitoids in the laboratory, one with cultured parasitoids in the laboratory and field, and one entirely in the field sampling naturally-occurring insects, with the laboratory components serving to assist interpretation of the rubidium-marking technique used in the field.

5.3 MATERIALS AND METHODS

5.3.1 Experiment 1: Effect of duration of rubidium exposure and post-exposure access to unmarked food on rubidium content of cultured parasitoids

Rearing of parasitoids and exposure to rubidium-marked buckwheat

This experiment was conducted in a controlled-temperature room at a mean temperature of 21 °C (range ± 2 °C) with a light regime of 16 h light: 8 h dark. It was run over six weeks as parasitoids and buckwheat became available.

A culture of A. zealandica was established from local field-caught insects and maintained in 2.5-litre plastic jars for more than ten generations prior to the experiments. Adult wasps in the culture were provided with honey-soaked cotton wool and a water-filled vial with a cotton wool wick. To provide adults for experimentation, second instar lacewing larvae (M. tasmaniae) were added to the jars to allow parasitism. The larvae were provided with pea aphids (Acyrtosiphon pisum Harris, Homoptera: Aphididae) daily until pupation. Each jar contained a crumpled paper towel and the larvae usually pupated on this surface. On pupation, the paper was removed and
placed in a plastic vial. Vials containing pupae were checked daily and wasps emerging on the same day were transferred to a clear plastic container (height 18 cm, diameter 10 cm) with mesh over the open top. They were provided with a ball of honey-soaked cotton wool in a 5 cm Petri dish and a ball of water-soaked cotton wool in a foil dish (height 1.5 cm, diameter 4 cm). The parasitoids were kept in this enclosure for 6 days and, being in mixed-sex groups, may have mated in this time. Honey and water were replenished daily.

Buckwheat plants were grown in a glasshouse. Two days before the wasps were exposed to them, half the plants were sprayed with 2000 ppm rubidium chloride solution using a backpack sprayer (2 gL⁻¹ RbCl 99.5% purity, Aldrick-APL). They were sprayed using the same equipment and at a similar rate to the field buckwheat strip, as described in Experiment 3 below. The plants were transferred to a controlled-temperature room for use in the experiment.

Six days after emergence, the parasitoids were systematically allocated to five treatments: 1) Rb-marked buckwheat inflorescence for 24 h, 2) unmarked buckwheat inflorescence for 24 h, 3) Rb-marked buckwheat leaf for 24 h, 4) Rb-marked buckwheat inflorescence for 5 days, 5) Rb-marked buckwheat inflorescence for 24 h followed by honey for 4 days. The parasitoids were placed in individual clear plastic pots (height 18 cm, diameter 10 cm) with mesh covering the open top. A foil dish (height 1.5 cm, diameter 4 cm) containing moist cotton wool was placed in each enclosure. According to treatment, a buckwheat inflorescence or leaf was inserted through a hole in the side of the pot (diameter 5 cm) and secured with a foam disc plugging the hole. A loop of string was taped to each pot and a wire hook attached to the string to enable the pot to be hung vertically. The pots were suspended from a rod positioned above the buckwheat plants in a controlled-temperature room. For Treatment 5, after 24 hours the buckwheat inflorescence was removed and a honey-soaked ball of cotton wool in a 5 cm Petri dish was added to the pot. For the 5-day treatments water was replenished daily, together with the honey if present.

There were six females and six males in each treatment with two exceptions: only five females were included in Treatment 3 and nine males were included in Treatment 2. After 24 h or 5 days depending on the treatment, each parasitoid was placed in a 0.6 ml microcentrifuge tube and frozen in a domestic freezer.

**Analysis of parasitoid rubidium content**

Each parasitoid was sexed and the length of one hind tibia was measured under a binocular microscope (x40). The wasp was rinsed with doubly-deionised water, checked under the microscope for contamination by external pollen grains and re-rinsed if necessary. It was then placed in an open 1.5 ml microcentrifuge tube and dried in an oven at 60 °C for 24 hours.

After drying, each parasitoid was chemically digested in its individual microcentrifuge tube to produce a solution suitable for rubidium analysis. A two-step wet oxidation method was
adapted from Corbett et al. (1996). 50 µl concentrated hydrogen peroxide (H$_2$O$_2$ 30%, Merck) was added to each tube, the lids were closed and the tubes were incubated at 30°C for 12 hours and 12 °C for a further 12 hours. 50 µl concentrated nitric acid (Tracepur HNO$_3$ 69%, Merck) was then added and the incubation stage was repeated. At the end of this process there were no visible remains of the parasitoids. Finally, 1.2 ml doubly-deionised water was added and the tubes inverted three times to ensure mixing. The solutions were then transferred to sample cups suitable for the spectrophotometer and refrigerated until analysis. Eight tubes without wasps were prepared in a similar way to check for contamination.

Rubidium concentrations of the parasitoid digests were determined by graphite furnace spectrophotometry using a GBC Scientific GF 3000 with PAL 3000 autosampler and a rubidium lamp of wavelength 780 nm, programmed for 20 s 700 °C for charring and 1 s 2500 °C for atomisation. Two 20 µl sub-samples of each sample were analysed and a mean calculated. If the coefficient of variation exceeded 10%, the samples were reanalysed.

The rubidium concentration of each sample was divided by the hind tibia length of the wasp, giving a rubidium level expressed as parts per billion in the parasitoid digest per millimetre of hind tibia length (ppb Rb mm$^{-1}$ hind tibia). This provided a correction for wasp size given that mass could not be determined accurately. (Correcting for insect size is standard practice although van Steenwyk et al. (1978) found increased insect mass decreased rubidium measurements due to matrix interference.) The blank samples indicated there was no contamination.

Previous studies using rubidium-marking have used a variety of methods to distinguish marked and unmarked insects. The most common method (attributed to Stimmann (1974)) is to identify as marked those test insects with levels exceeding three standard deviations above the control mean; the control insects being from an unmarked source. Assuming the control concentrations follow a normal distribution, there is ≤ 0.13% probability that the test insects identified in this way were actually unmarked. This approach is based on the idea that a highly conservative Type I error rate is appropriate in this context and it does not take account of knowledge of the distribution of the concentrations in test insects. Furthermore, the assumption that concentrations in the control insects are normally distributed may be unjustified, either because sample sizes are too small to ascertain this, or because it can be seen that they are not (e.g. positively skewed distributions are evident in Hopper & Woolson (1991), Corbett & Rosenheim (1996) and Pickett et al. (2004)). A simple alternative is to use the maximum control value as the threshold for marking (e.g. Hopper & Woolson, 1991) but this may be unsatisfactory, giving an arbitrary classification if the control and test distributions overlap. Corbett & Rosenheim (1996) used re-sampling of cumulative distributions to estimate the proportion of a test population that had originated from a marked source, rather than classifying
individual insects as marked or not. Their method requires larger sample sizes than available in this study.

In this study there were insufficient control parasitoids to either confirm that the concentrations in these insects did follow a normal distribution, or generate a reliable estimate of the standard deviation if this was assumed. Nevertheless, in the absence of a satisfactory alternative, the ‘3 S.D. method’ was used i.e. test insects with rubidium concentrations exceeding the control mean plus three standard deviations were classified as marked. However, because the distributions of concentrations of the test insects were such that classification was sensitive to the value of these thresholds, raw values are presented and proximity to the thresholds are discussed.

5.3.2 Experiment 2: Comparing rubidium content of cultured parasitoids exposed in the laboratory and field

Rearing of parasitoids and exposure to rubidium-marked buckwheat

A. zealandica was reared as in Experiment 1. Vials containing pupae were checked daily for newly-emerged parasitoids. Due to limited availability of wasps, only males were used and wasps emerging on two consecutive days were combined. The wasps were transferred to a pot containing honey and water as in Experiment 1 and were kept in this enclosure for 5 or 6 days (depending on which day they had emerged). Honey and water were replenished daily.

Five or six days after emergence, the parasitoids were alternately allocated to two treatments: 1) laboratory exposure and 2) field exposure. There were five male parasitoids in each treatment. In both treatments, the parasitoids were placed in individual clear plastic pots (height 18 cm, diameter 10 cm) with mesh covering the open top. A foil dish (height 1.5 cm, diameter 4 cm) containing moist cotton wool was placed in each enclosure. A buckwheat inflorescence whose subtending leaf had been removed was inserted through a hole in the side of the pot (diameter 5 cm) and secured with a foam disc plugging the hole. A loop of string was taped to each pot and a wire hook attached to the string to enable the pot to be hung vertically.

For the ‘laboratory exposure’ parasitoids, the pots were suspended from a rod positioned above buckwheat plants in a controlled-temperature room. These plants had been grown and sprayed with rubidium chloride solution as in Experiment 1. They were sprayed 2 or 5 days prior to use. For the ‘field exposure’ treatment, the buckwheat plants were growing within a strip sown in a lucerne paddock. The buckwheat strip is described in Experiment 3 below and had been sprayed both 19 and 6 days before this experiment took place. Plants were selected arbitrarily along the length of the strip. Each pot was hung on the hook of an electric fence post placed close to a plant and prevented from moving in the wind by tying it to the post with a strip of cloth. Any insects on the inflorescence were removed before it was inserted into the pot. When the pots were checked mid-morning, condensation had formed so the top of each pot was
covered with a piece of cloth to reduce direct insolation. The mean temperature in a field pot over the period the wasps were exposed was 19 °C (minimum 8 °C, maximum 32 °C). The mean temperature in a laboratory pot was 22 °C (minimum 20 °C, maximum 23 °C).

After 24 hours exposure, the parasitoids were sucked into individual plastic vials and frozen in a domestic freezer. After a further 24 hours, the wasps were transferred to 0.6 ml microcentrifuge tubes and then stored at -80 °C until analysis.

**Collection of leaf samples**

Logistical constraints prevented analysis of nectar samples so in order to compare the rubidium concentrations of the plants on which the parasitoids were feeding, two leaves were collected from each laboratory and field plant used in the experiment: the oldest non-senescent leaf and a young fully-expanded leaf subtending an inflorescence. The laboratory plants had been sprayed 2 or 5 days before collection (see above) and the field plants (in row A) had been sprayed 19 and 6 days before collection.

Leaves were also collected from the field buckwheat strip fourteen days before Experiment 2. Four leaves were sampled from plants near each of the six markers which divided the buckwheat strip into sections for sweep-net sampling in the adjacent lucerne. The four leaves were taken from two rows at different stages of growth and with different spraying histories. In each row a young leaf and an old leaf were collected. At the time of collection, row B had been sprayed 20 and 11 days previously, and row C had been sprayed 11 days previously. Collection occurred just before re-spraying.

Each leaf was placed in a plastic zip-seal bag and stored in a domestic freezer until analysis.

**Analysis of parasitoid rubidium content**

Parasitoids were analysed to determine their rubidium content as described in Experiment 1. The significance of difference between levels in laboratory and field parasitoids was tested using a t-test not assuming equal variances.

**Analysis of leaf rubidium content**

Each leaf was placed in a paper bag and dried at approximately 65 °C for 48 hours. The leaves were weighed and then chemically digested using a method developed by L. Clucas (Lincoln University, pers. comm.). Each dried leaf was placed in a 100 ml glass tube and 5 ml concentrated nitric acid was added. The tubes were placed in a heating block and heated for 30 min at 40 °C, 2 hours at 80 °C, 2 hours at 125 °C and 2 hours at 140 °C. The tubes were agitated occasionally during this process to help break up the leaf and ensure all the leaf material was covered by the liquid. The solutions were then transferred to volumetric flasks and doubly-deionised water added to bring the solution to 10 ml. The diluted solutions were...
transferred to 30 ml plastic vials and refrigerated until analysis. A tube was prepared without a leaf to check for contamination.

Rubidium concentrations of the leaf digests were determined by atomic absorption spectrophotometry (using a GBC Avanta FAAS). Three 3 ml sub-samples of each digest were analysed and the mean automatically calculated. The sample was reanalysed if the coefficient of variation exceeded 10%.

The sample concentrations were corrected for the leaf mass to determine micrograms of rubidium per gram of dry leaf. The significance of difference between concentrations in the leaves were tested using analysis of variance with post hoc Tukey HSD comparisons. Concentrations were log_{10}-transformed before statistical analysis. Rubidium concentrations of laboratory leaves sprayed two days before wasp exposure were not higher than those sprayed five days previously so these leaves are treated as a single group.

5.3.3 Experiment 3: Use of rubidium-marked buckwheat by field-collected parasitoids

Sampling of parasitoids

The study took place through the summer of 2005–06 in a 12.5 ha field of established lucerne Medicago sativa L. ‘Kaituna’ (Leguminosae) in a mixed farming area of Canterbury, New Zealand (Appendix 1, Fig. A1.1i). The crop management was typical for the region and not altered for the study. The lucerne was periodically grazed by sheep or cut for hay. This occurred before flowering and sampling took place when re-growth was sufficient for sweep-net sampling. The field was not sprayed with insecticide. Herbicide was not used and although the density of the lucerne stand varied across the field, the density of flowering weeds was considered to be low throughout. Irrigation was applied when necessary.

The field was divided by a 5 x 360 m strip of grass. The central 80 m of this strip was ploughed and on ten dates during the study, a row of buckwheat was drilled along its length (Appendix 1, Fig. A1.1ii). This provided continuous flowering through the sampling period. The area of buckwheat was surrounded by an electric fence to protect it when sheep were grazing the lucerne.

On 12 December the flowering buckwheat rows were sprayed with a 2000 ppm solution of rubidium chloride (2 gL^{-1} RbCl 99.5% purity, Aldrick-APL) using a hand-pumped backpack sprayer. Flowering rows were then re-sprayed every 9–14 days, except the final spray was 20 days after the previous application. Spraying took place when there was minimal risk of drift onto adjacent lucerne. After spraying, the leaves were visibly wet but the run-off point was not reached.

The insect population was sampled by sweep-net sampling across a grid of 30 points each side of the buckwheat strip (Appendix 1, Fig. A1.2). In order to identify the points for sampling, the sampler lined herself up with canes positioned within the buckwheat and red flags on the
boundary fences of the field. This avoided the need to remove and re-establish position markers when the lucerne was cut for hay and avoided any possible influence of markers on grazing by the sheep. On each sampling occasion, five samples were taken at each of six distances: 1, 5, 10, 25, 50 and 100 m from the buckwheat. Each sample comprised 14 sweeps made while walking parallel to the buckwheat strip from a starting position identified using the grid. The starting positions for the five samples at each distance were spaced 15 m apart and sweeping covered approximately 10 m. When a 14-sweep sample had been collected in the net, any *A. zealandica* or *M. tasmaniae* were sucked into a plastic specimen tube. This was kept in an insulated bag with ice-packs for a maximum of two hours until returning to the laboratory. Species other than *A. zealandica* and *M. tasmaniae* were released. In the laboratory, insects were frozen in a domestic freezer. Within two weeks of initial freezing, parasitoids were transferred to individual 0.6 ml microcentrifuge tubes and placed in a -80 °C freezer until analysis several months later. (See Chapter 3 for further discussion of *M. tasmaniae* samples.)

With a minimum time between sampling dates of two days, samples were taken as frequently as possible when there was adequate re-growth, the crop was dry and the wind was not too strong to affect sampling efficiency. On some dates both sides were sampled but on other dates insects were only collected from one side.

A total of 167 parasitoids were collected. Ninety wasps were analysed to determine their rubidium content. They were collected in ten sampling sessions on 15, 17, 19 and 20 January and 12, 15 and 17 February. There were five sampling sessions in each half of the field, with both sides being sampled on the same day on three occasions. Thirty seven wasps collected 20 and 24 February were analysed using HPLC to determine their sugar composition and the results of those analyses are presented in Chapter 6. Remaining wasps were not analysed due to small sample sizes on those collection dates.

**Analysis of parasitoid rubidium content**

Parasitoids were analysed to determine their rubidium content as described in Experiment 1. They were analysed in two batches with separate blanks and control wasps. In the first batch there were 27 parasitoids collected from Side 2 of the field on 15 February. Five controls and four blanks were also analysed. In the second batch there were 62 parasitoids collected over five dates on both sides, nine controls and five blanks. Due to differences between the rubidium levels in the control wasps for these two batches, the results are presented separately. The blank samples indicated there was no contamination.

Due to difficulty obtaining control parasitoids from comparable field sites without rubidium spraying, wasps were taken from the laboratory culture. Background rubidium levels are likely to differ between the field and laboratory but this variation is likely to be much less
than differences between control and marked insects. Nevertheless, this is considered when interpreting the results.

5.4 RESULTS AND DISCUSSION

5.4.1 Experiment 1: Effect of duration of rubidium exposure and post-exposure access to unmarked food on rubidium content of cultured parasitoids

The rubidium levels of parasitoids (ppb mm\(^{-1}\) hind tibia) varied greatly within each treatment, including the ‘unsprayed buckwheat’ treatment where one parasitoid had a much higher rubidium level than the remaining fourteen (Fig. 5.1). Previous studies have also found occasional high background levels (e.g. Graham et al., 1978; Corbett & Rosenheim, 1996; Pickett et al., 2004) so further work would be required to determine whether this measurement is likely to be an error or if it does reflect a high level of natural variability. Since it was a large outlier, it was excluded from the calculation of the 3 S.D. threshold (see methods). (The threshold calculated including the outlier was 5.62 ppb Rb mm\(^{-1}\) hind tibia and the outlier exceeded this.)

Six males that had been exposed to sprayed leaves rather than flowers had rubidium levels exceeding the 3 S.D. threshold and four of these were more than ten times the threshold value. These observations indicate that elevated rubidium levels are not necessarily an indication of nectar feeding. Rubidium could have been absorbed via their exoskeleton when in contact with the leaf, particularly if high humidity in the enclosures led to small water droplets forming on the leaf surface. Alternatively, the parasitoids might have absorbed it orally. Other species have been observed scraping vegetative material in the absence of typical food sources (Sisterson & Averill, 2002). The reason for the higher levels in males compared with females is unknown. The sexes may have behaved differently but in a study with *M. tasmaniae* using a similar protocol, distinct layers were evident in the sample digests and it was hypothesised that the high fat content of gravid females might reduce the detectability of rubidium (Robinson, K.A., unpublished).

In the remaining treatments, only parasitoids that had been exposed to a sprayed buckwheat inflorescence for five days had consistently higher levels than parasitoids with an unsprayed inflorescence. Of these twelve wasps, eight exceeded the 3 S.D. threshold but only four had distinctly elevated levels. Buckwheat nectar is known to be a suitable food source for *A. zealandica*, extending longevity from a few days to several weeks (Chapter 4; Jonsson et al., in press). Since none of the parasitoids in this treatment group died during the five days, it is likely that they all fed during the experiment. Since many of the parasitoids were not clearly marked, it can be concluded that the method used did not produce a reliable indicator of nectar feeding i.e. rubidium-marked parasitoids are likely to be an underestimate of nectar-fed
parasitoids. However, those parasitoids with elevated levels may have acquired rubidium from petals via the same unknown mechanism as those in the leaf treatment group, rather than by nectar feeding. If these parasitoids did not also nectar-feed, there is also a risk of overestimating consumption of this floral resource.

Three of twelve wasps that were exposed to a sprayed inflorescence for 24 hours had rubidium levels which exceeded the 3 S.D. threshold. The low frequency of clearly-marked parasitoids in this treatment could indicate that wasps did not feed on the available nectar in this time period. Alternatively, they may have fed on the nectar but their energy requirements, or the rubidium spray dosage, was too low for the parasitoids to imbibe sufficient volume to raise their rubidium levels above the background level. It should also be noted that these parasitoids did not acquire rubidium from petals in the same way that others acquired it from leaves.

Providing parasitoids with honey for four days after 24 hours with a sprayed inflorescence did not reduce rubidium levels relative to those without honey. However, since the rubidium levels in most wasps without honey did not exceed background levels, no change would be expected.

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**Fig. 5.1 Rubidium levels in parasitoids with different exposure treatments in the laboratory.** Rubidium levels are expressed as parts per billion in the parasitoid digest divided by the hind tibia length (mm) to account for wasp size.
5.4.2 Experiment 2: Comparing rubidium content of cultured parasitoids exposed in the laboratory and field

Rubidium levels for the parasitoids exposed to the inflorescence of a sprayed buckwheat plant in the laboratory or the field are compared in Fig. 5.2. There was no difference between the treatments, whether or not one laboratory parasitoid which had an exceptionally high level was excluded (including outlier, $t = 1.043, P = 0.355$).

Although Experiments 1 and 2 were conducted separately, the conditions under which the parasitoids were reared and exposed were very similar. It is therefore reasonable to compare the rubidium levels measured in the two experiments and doing so suggests that all but one of the parasitoids were not effectively marked in Experiment 2.

Although there was considerable overlap in the ranges of leaf rubidium concentrations from the laboratory and field, those from the field were significantly lower. There was no difference between young and old leaves (Fig. 5.3i, location $F_{1,16} = 5.42, P = 0.033$; age $F_{1,16} = 1.08, P = 0.315$; location x age $F_{1,16} = 0.64, P = 0.435$). There was also no difference in the concentrations of the old and young field leaves collected six days before the wasps’ exposure. These leaves were not significantly different from the field leaves to which the wasps were exposed, but the concentration in row C was lower than row B (Fig. 5.3ii, ANOVA: row $F_{2,28} = 3.833, P = 0.032$; age $F_{1,28} = 2.262, P = 0.144$; row x age $F_{2,28} = 0.162, P = 0.851$. Tukey HSD: row A v B $P = 0.325$; row A v C $P = 0.519$; row B v C $P = 0.031$).

The focus of this study was the variation within sprayed plants so no unsprayed plants were analysed. In a separate experiment (Robinson, K.A., unpublished), unsprayed laboratory leaves contained 22–49 µg Rb g$^{-1}$ leaf, indicating that although the plants in this study often did not lead to effective marking of parasitoids exposed to them, the plants did contain greatly elevated levels of rubidium as a result of spraying. Since young leaves are likely to have developed since spraying, particularly in the field, the similarity of old and young leaves indicates that the rubidium was effectively translocated within the plants. This supports the assumption that rubidium would have been present in the nectar so parasitoids could become marked by feeding on it.

Although the behaviour of contained and free-flying field wasps will differ greatly, the levels of rubidium in parasitoids in this experiment suggest that the risk identified in Experiment 1 of underestimating use of buckwheat by parasitoids, could also occur in the field study (Experiment 3). The relationship between rubidium levels in leaves and nectar is unknown, but if the large variation in leaf concentrations is reflected in nectar concentrations, this risk may have been particularly great for parasitoids feeding on the buckwheat rows with lower levels of rubidium and those feeding shortly before re-spraying.
Fig. 5.2 Comparison of rubidium levels in cultured parasitoids exposed to marked buckwheat flowers in the laboratory and field. Rubidium level is expressed as parts per billion in the parasitoid digest divided by the hind tibia length (mm) to account for body size.

Fig. 5.3 Rubidium levels in buckwheat leaves. i) Young and old leaves sampled from the field and laboratory buckwheat plants to which parasitoids were exposed in Experiment 2, collected on the day of wasp exposure, ii) Young and old leaves sampled from other plants in the field buckwheat strip, collected 6 days before Experiment 2 wasp exposure. (Unsprayed laboratory leaves from a separate experiment contained 22–49 µg Rb per g leaf.)
5.4.3 Experiment 3: Use of rubidium-marked buckwheat by field-collected parasitoids

Rubidium levels of field-collected parasitoids analysed in the two batches are presented in Fig. 5.4, alongside levels in cultured parasitoids which had not been exposed to rubidium. Two of the 90 parasitoids had rubidium levels more than ten times the 3 S.D. threshold for their batches. They were collected at 1 m and 10 m from the buckwheat strip. A further twelve parasitoids had levels which exceeded the 3 S.D. thresholds but for several of these, identifying them as marked would be arbitrary as other parasitoids had levels just below the threshold. These parasitoids were collected at all distances (i.e. 1, 5, 10, 25, 50 and 100 m) from the sprayed buckwheat strip, and throughout the season. It must also be recognised that the 3 S.D. threshold may have been calculated from laboratory wasps with higher or lower background levels than field wasps so only the extreme outliers could confidently be interpreted as marked.

In this experiment the cultured parasitoids used as controls in Batch 2 showed a large difference between males and females. This was not evident in the ‘unsprayed inflorescence’ treatment in Experiment 1 but males in the leaf exposure treatment had higher levels than females. (All controls were female in Batch 1 of Experiment 3.) Only seven of the 55 field-collected parasitoids of known sex were male but they were not over-represented amongst the parasitoids with elevated rubidium levels. As discussed in Section 5.4.1, further work is required to determine whether there are genuine sex differences, and if so, whether these are due to differential feeding patterns or differences in the way rubidium is metabolised and then detected.

5.5 General Discussion

The principal aim of this study was to investigate the use of aggregated floral resources by the lacewing-parasitoid A. zealandica in an agricultural setting. To enable this, a strip of flowering buckwheat was marked with rubidium. Such a flower strip might be sown as part of a conservation biological control programme for pest management. Only a small percentage (2.2%) of field-collected parasitoids was clearly marked with rubidium. The majority had rubidium levels indistinguishable from cultured parasitoids that had not been exposed to rubidium. The remainder (approx. 15%) had levels that, although significantly higher than the ‘control’ parasitoids, were not clearly distinct from the majority of field-collected insects. Taken alone, these results might suggest that most parasitoids in the field did not feed on the buckwheat nectar but a few individuals did exploit this resource. However, when interpreted alongside the results of the laboratory experiments, the results are inconclusive.

The results of the laboratory experiments suggest that the rubidium-marking in the field experiment could either overestimate or underestimate the extent of nectar feeding by A. zealandica. It was shown that parasitoids can become marked when only leaves, not nectar,
Fig. 5.4 Rubidium levels in field-collected parasitoids collected at six distances from the rubidium-sprayed buckwheat strip. The parasitoids were analysed in two batches, Batch 1 (i) and Batch 2 (ii). Rubidium levels are expressed as parts per billion in the parasitoid digest divided by the hind tibia length (mm) to account for body size.
are available. Marked parasitoids collected in the field could therefore have been on the buckwheat plants without using them as a food source. This is a source of misinterpretation additional to the risk of direct spraying identified by previous authors who have used rubidium-marking (Scarratt et al., 2008). It is also possible that the parasitoids consumed honeydew produced by aphids feeding on the marked plants. The likelihood of parasitoids being in the flower strip without feeding on nectar depends, in part, on the presence of hosts there. This was not determined so no conclusion is drawn about the risk of overestimating nectar-feeding. The laboratory experiments also suggested that the rubidium spraying of the buckwheat plants might not have effectively marked all parasitoids that fed on the plants’ nectar. Since the rubidium concentrations in field plant leaves were lower than those in the laboratory and the differences in feeding behaviour of the wasps are unknown, it must also be considered possible that feeding in the field experiment was undetected.

Low frequencies of buckwheat-feeding, if they occurred, would be consistent with the sugar profiles of A. zealandica that were determined for a sub-sample of parasitoids collected as part of this study, later in the season (Chapter 6). Few wasps (up to 5 of 37 depending on interpretation) had profiles indicative of recent nectar-feeding. Although the feeding history of most parasitoids could not be determined with confidence, many were considered to have fed on honeydew due their high glucose/(glucose + fructose) value. While not exclusive of nectar-feeding, use of honeydew might indicate parasitoids did not exploit the aggregated floral resources because an alternative sugar source was dispersed across the field.

This study was unable to provide satisfactory answers to the questions asked about A. zealandica’s use of and dispersal from aggregated floral resources. Future studies using higher rates of rubidium application to improve marking efficacy, and with larger sample sizes of control and test insects to obtain more accurate distributions of rubidium concentrations, might provide these answers. Rubidium-marking of field insects is a potentially powerful technique for agroecologists and has provided insight into movement of parasitoids from overwintering sites (e.g. Corbett & Rosenheim, 1996; Pickett et al., 2004) and dispersal of predators between crops (e.g. Prasifka et al., 2004), as well as use of flower strips by parasitoids (Freeman-Long et al., 1998; Lavandero et al., 2005; Scarratt et al., 2008). However, this study shows that for its potential to be realised it is necessary to conduct complementary laboratory studies of uptake and retention of the rubidium mark. These can help ensure that the results of field studies are interpreted appropriately.
Chapter 6  Feeding histories of the lacewing-parasitoid *Anacharis zealandica* in the field: Assessing the potential for flower strips to disrupt biological control

6.1 SUMMARY

Many biocontrol agents of arthropod pests are life-history omnivores and, as such, require food resources other than the pest to maximise their impact on the pest population. One approach to ecology-based pest management is managing agroecosystems to ensure the availability of these complementary foods does not limit the efficacy of biological control. Sowing flower strips increases the abundance of floral resources for biocontrol agents such as parasitoids that consume nectar as adults. However, the value of doing so depends on whether floral resources are limiting and whether the biocontrol agents exploit the flowers provided. The nutrient composition of parasitoids reflects their feeding history so, to improve understanding of the value of flower provision, sugar profiles have been used to study the feeding history of parasitoids collected from the field. Consumption of nectar, and alternative foods such as honeydew, can be determined using this method.

Whether or not biological control is enhanced by flower provision depends not only on the value of flowers to beneficial parasitoids, but also on their use by non-target species such as hyperparasitoids and parasitoids of predators. In this study, High Performance Liquid Chromatography was used to study feeding histories of *Anacharis zealandica*. This species parasitises the lacewing *Micromus tasmaniae*, a biocontrol agent, and so has the potential to disrupt biological control. Parasitoids were collected 1–50 m from a flowering buckwheat *Fagopyrum esculentum* strip in the centre of a lucerne field and their sugar profiles were determined. The total sugar content and the profile composition of the field-collected parasitoids were compared with laboratory-raised wasps subjected to various feeding treatments.

The profiles of most field-collected parasitoids did not closely match those of any of the laboratory parasitoids. Five of the 37 parasitoids analysed had profiles consistent with recent nectar-feeding. The glucose/(glucose + fructose) value and presence of an unidentified carbohydrate suggested that a larger proportion may have fed on honeydew. Total sugar levels of most field-collected parasitoids were lower than those fed in the laboratory. Sugar profiles did not vary with distance from the buckwheat strip. Although further work is required to interpret fully sugar profiles as indicators of feeding history, these results suggest that *A. zealandica* consumed honeydew more frequently than it exploited the flower strip and may have been sugar-limited despite the presence of both these resources.
6.2 INTRODUCTION

Sown flower strips have been proposed as a conservation biological control technique to enhance the efficacy of indigenous (and previously-introduced) hoverflies (Diptera: Syrphidae) and parasitoid wasps (Hymenoptera: Parasitica) (Powell, 1986; Wratten & van Emden, 1995; Gurr et al., 1998; Landis et al., 2000). These beneficial insects are life-history omnivores (Coll & Guershon, 2002): their larvae are predatory or parasitic respectively, and the adults feed on pollen and/or nectar (Schneider, 1969; Jervis et al., 1996). In order for the larvae to have maximum impact on a pest population, floral resources must be readily available to maximise the fecundity and longevity of the adults. The concept of sown flower strips arose as a way to ameliorate the resource-limitation that these insects might experience in agroecosystems where flowering ‘weeds’ are minimal (Heimpel & Jervis, 2005). Biocontrol agents may also be attracted to flower strips, or be ‘retained’ by them. The strips could therefore affect the spatial distribution of the predators and parasitoids and this too may affect biological control (Macleod, 1999; Wäckers, 2004; Bianchi & Wäckers, 2008).

In addition to beneficial hoverflies and parasitoids, antagonists such as hyperparasitoids and parasitoids of predators may benefit from flower strips. Such effects have the potential to disrupt biological control so life-history omnivores which operate at the fourth trophic level must be considered alongside the ‘third-level’ target species to develop an understanding of the role this technique can play in pest management. Although inadvertent effects on pest species have been studied in a few systems (Baggen & Gurr, 1998; Begum et al., 2006; Lavandero et al., 2006), the question of whether higher level antagonists gain disproportionate benefits has rarely been addressed (but see Lee & Heimpel (2005) and Araj et al. (2008)). To investigate the potential for such disruption, I studied the effects of flower strips on Anacharis zealandica Ashmead (Hymenoptera: Figitidae), a non-host-feeding parasitoid of the biocontrol agent Micromus tasmaniae Walker (Neuroptera: Hemerobiidae). The flower strip contained buckwheat Fagopyrum esculentum Moench ‘Katowase’ (Polygonaceae), a species which produces abundant sucrose-rich nectar (Vattala et al., 2006). A. zealandica is known to benefit from these flowers in the laboratory (Chapter 4; Jonsson et al., in press) and has been found in experimental strips of flowering buckwheat in orchards (Stephens et al., 1998; Irvin et al., 2006).

The overall impact of flower strips on flower-feeding species is a result of a number of processes: attraction of insects to the strip, their dispersal from it, their ability to exploit the floral resources and the effect of feeding on their longevity and fecundity. These, in turn, determine the impact of the flower-feeder on their prey or host species. The effect of a flower strip will also depend on the availability of alternative resources in the agroecosystem. These may be flowers, or when sugars rather than pollen nutrients are required, other sources such as
extra-floral nectar and homopteran honeydew may be exploited (Jervis et al., 1996). Studies in various crop systems have looked at the effects of flower strips on yield or, more commonly, proxies such as pest populations, levels of pest parasitism or populations of biocontrol agents (e.g. Kloen & Altieri, 1990; White et al., 1995; Hickman & Wratten, 1996; Baggen & Gurr, 1998; Hook et al., 1998; Platt et al., 1999; Berndt et al., 2002; English-Loeb et al., 2003; Tylianakis et al., 2004; Lavandero et al., 2005; Lee & Heimpel, 2005; Begum et al., 2006; Irvin et al., 2006; Lee et al., 2006; Wanner et al., 2006b; Ponti et al., 2007). These studies drew varied conclusions about the efficacy of flower strips for conservation biological control and do not provide convincing evidence to argue either for or against wider use of this technique at this time.

Although the net effect of flower strips on crop yield (or profit) is the ultimate concern for pest management, focusing on specific insect behaviours and physiological effects which contribute to this can help us understand why flower strips are beneficial or not in different situations. With this approach, key questions to address are whether biocontrol agents do experience sugar-limitation in the absence of flower strips, and whether this is alleviated when they are present. Heimpel & Jervis (2005) discuss the types of evidence that may be used to identify sugar-limited populations, once it is known that the species can benefit from sugar-feeding: 1) observing the abundance of sugar sources in the habitat, 2) analysing the nutritional state of the insects and 3) measuring a predicted response to appropriate manipulation of the habitat.

In recent years, several authors have used analyses of lipid, glycogen and sugar content to study the nutritional state of biocontrol agents in agroecosystems, sometimes in combination with manipulation of flower abundance in the habitat (see references below; Ellers, 1996). Sugar profiles are particularly useful because they can be used not only to infer sugar-limitation, but also to determine what sources of sugar the insects have exploited. For example, the proportion of fructose differs between most nectars and honeydews, and these differences can be reflected in the sugar profile of the biocontrol agents which consume them (Lee et al., 2006). In addition, insects which have never fed may have similar total sugar levels to those which fed some time ago, but these feeding histories may be distinguished by the composition of the sugar profiles (Steppuhn & Wäckers, 2004). The profiles can be determined from either a series of biochemical assays to measure fructose and total sugar content (Olson et al., 2000), or using High Performance Liquid Chromatography (HPLC) to obtain a complete sugar profile (Wäckers & Steppuhn, 2003; Steppuhn & Wäckers, 2004). The latter has the advantage of revealing the presence of minor sugars such as melezitose and erlose, which are further indicators of honeydew feeding in some species (Wäckers & Steppuhn, 2003; Hogervorst et al., 2007a).

Although analysing sugar profiles is a valuable technique, a number of limitations must be acknowledged. Profiles are affected by insect age and time since feeding, and it may not be
appropriate to extrapolate patterns from one species to another (Olson et al., 2000; Fadamiro & Heimpel, 2001; Lee et al., 2004; Steppuhn & Wäckers, 2004; Vattala et al., 2005; Hogervorst et al., 2007a). When complementary laboratory studies are limited, feeding histories of field-collected insects may therefore have to be inferred with a variable level of certainty. Similarly, when insects in the field exploit more than one type of sugar resource, this may not be detectable when a small number of single-source feeding histories are available for comparison. Inferring sugar-limitation from total sugar levels is restricted by lack of knowledge about the levels at which behaviour and mortality are affected. Furthermore, when interpreting proportions of ‘fed’ and ‘unfed’ insects it must be recognised that an unknown proportion of any sampled cohort may already have died of starvation. Notwithstanding these limitations, comparing the sugar profiles of parasitoids collected in different habitats has provided insight regarding sugar-feeding by biocontrol agents in the field.

Olson & Wäckers (2007) suggest that parasitoids (Hymenoptera: Braconidae) may be sugar-limited in field margins that were designed to conserve quail without considering the potential benefits of promoting nectar-producing species. Most parasitoids collected in the quail margin had much lower sugar levels than those collected from a patch of vetch where sugar sources (floral and extra-floral nectar, and honeydew) were abundant. Wäckers & Steppuhn (2003) found a higher proportion of parasitoids (Hymenoptera: Braconidae) were ‘fed’ in samples collected adjacent to a sown flower margin than those collected adjacent to a grass margin, but this difference did not extend from the edge of the cabbage fields to the centre. Studies in broccoli (Lavandero et al., 2005) and cabbage (Lee et al., 2006) found no effect, or inconsistent effects, of presence of buckwheat on the proportion of parasitoids (Hymenoptera: Ichneumonidae) identified as ‘fed’ rather than ‘unfed’. In some trials these studies could have been affected by movement of parasitoids between treatments, and in others honeydew feeding was found to be prevalent. Hogervorst et al. (2007a) found a substantial proportion of parasitoids (Hymenoptera: Braconidae), hoverflies and green lacewings (Neuroptera: Chrysopidae) in wheat fields were ‘fed’. The majority of parasitoids and hoverflies were identified as having consumed honeydew, but differences in sugar metabolism meant this could not be determined for the green lacewings.

Given the small number of studies addressing the questions, general conclusions cannot be drawn about the degree of sugar-limitation in biocontrol agents or their use of flowers. It is apparent however, that when homopterans such as aphids are present in a crop, the value of floral nectar might be reduced. It should be noted nevertheless, that abundance of aphid honeydew is a function of aphid population and it is not clear whether the pests in these studies reached levels which would be unacceptable in a commercial crop. Further work with a range of species, in a range of agroecosystems, will allow better-informed decisions to be made in conservation biological control. The results presented here contribute to that goal, and through
studying the lacewing-parasitoid *A. zealandica* in particular, we improve understanding of the potential effects of flower strips on biological control where both beneficial and antagonistic species may exploit floral resources.

*A. zealandica* was sampled 1–100 m each side of a flowering buckwheat strip sown in the centre of a lucerne field. If the flowers had an effect on the feeding behaviour of the parasitoids, this was expected to be seen as changes in the sugar profiles with increasing distance from the strip. In addition, by comparing the sugar profiles of the field-collected *A. zealandica* with those of parasitoids raised in the laboratory with various feeding histories, preliminary conclusions could be drawn about sugar-limitation and use of floral nectar or alternatives such as honeydew.

6.3 MATERIALS AND METHODS

6.3.1 Sampling of parasitoids from the experimental field

The study took place through the summer of 2005–06 in a field (12.5 ha) of established lucerne *Medicago sativa* L. ‘Kaituna’ (Leguminosae) in a mixed farming area of Canterbury, New Zealand (Appendix 1, Fig. A1.1i). The crop management was typical for the region and not altered for the study. The lucerne was periodically grazed by sheep or cut for hay. This occurred before flowering and sampling took place when regrowth was sufficient for sweep-net sampling. The field was not sprayed with insecticide. Herbicide was not used and although the density of the lucerne stand varied across the field, the density of flowering weeds was considered to be low throughout. Irrigation was applied when necessary.

The field was divided by a 5 x 360 m strip of grass. The central 80 m of this strip was ploughed and sown with buckwheat. Rows of buckwheat were sown on ten dates during the study to provide continuous flowering through the sampling period (Appendix 1, Fig. A1.1ii). The strip was surrounded by an electric fence to protect it when sheep were grazing the lucerne. Flowering buckwheat rows were sprayed with rubidium chloride solution during the experiment. This was part of a rubidium-marking study described in Chapter 5.

The insect population was sampled by sweep-net sampling across a grid of 30 points each side of the buckwheat strips (Appendix 1, Fig. A1.2). In order to identify the points for sampling, the sampler aligned herself with canes positioned within the buckwheat and red flags on the boundary fences of the field. This avoided the need to remove and re-establish position markers when the lucerne was cut for hay and avoided any possible influence of markers on grazing by the sheep. On each sampling occasion, five samples were taken at each of six distances: 1, 5, 10, 25, 50 and 100 m from the buckwheat. Each sample comprised 14 sweeps made while walking parallel to the buckwheat strip from a starting position identified using the grid. The starting positions of the five samples at each distance were spaced 15 m apart and sweeping covered approximately 10 m.
When a 14-sweep sample had been collected in the net, any A. zealandica or M. tasmaniae were sucked into a plastic specimen tube. This was kept in an insulated bag with ice-packs for a maximum of two hours until returning to the laboratory. Species other than A. zealandica and M. tasmaniae were released. The specimen tubes were placed in a domestic freezer to kill the insects. Parasitoids were then put in individual 0.6 ml microcentrifuge tubes and re-frozen. Within two weeks of initial freezing, they were transferred to a -80 °C freezer until HPLC analysis nine months later. (See Chapter 3 for further discussion of M. tasmaniae samples.)

With a minimum time between sampling dates of two days, samples were taken as frequently as possible when there was adequate re-growth, the crop was dry and the wind was not too strong to affect sampling efficiency. On some dates both sides were sampled but on other dates insects were only collected from one side.

A total of 167 parasitoids was collected. Subsamples of insects were analysed to determine their feeding history. Thirty seven wasps, collected 20 and 24 February from Side 1, were analysed using HPLC to determine their sugar profiles. In addition, 90 wasps were analysed to determine whether they were marked with rubidium, indicating they had fed on the sprayed buckwheat. The results of the rubidium analyses are presented in Chapter 5.

6.3.2 Rearing laboratory parasitoids with known feeding history

In order to infer the feeding history of the field-collected parasitoids from their sugar profiles, parasitoids were reared in the laboratory on known diets and their sugar profiles determined for comparison. This part of the study took place in controlled-temperature rooms at temperatures of approximately 20 °C with a light regime of 16 h light: 8 h dark.

Vials containing pupae of A. zealandica were obtained from a laboratory culture (see Chapter 4 for details). The vials were checked daily and newly-emerged wasps were allocated to different feeding treatments. The feeding treatments were applied in three sequential batches (A-C). (Treatments A1 and C1 were the same (see below) to check whether there were differences between the batches. Due to limited availability of parasitoids, Batch B did not contain a treatment in common with the other batches so their similarity cannot be verified. It is nevertheless assumed that any differences between the wasps are due to the treatments not confounded factors.) Apart from the insects that were frozen immediately, the parasitoids were kept in clear plastic containers (height 18 cm, diameter 10 cm) with mesh over the open top. A foam-plugged hole (5 cm diameter) in the side of the container allowed insertion of plant shoots as appropriate and parasitoids were provided with a water-soaked cotton wool ball in a foil dish (height 1.5 cm, diameter 4 cm). Each enclosure contained 2–4 wasps (single or mixed sexes) and 1–4 enclosures were set up on different days for each treatment. On each day within each batch, parasitoids were allocated systematically to treatments. The treatments were as follows, with sample sizes of females (f) and males (m) given in parentheses:
A1) freeze immediately - frozen within 24 h of emergence without access to food or water (5f, 7m),
A2) 48 h sucrose - 1M sucrose solution (in tap water) provided in a plastic vial (height 7 cm, diameter 2.5 cm) with a cotton dental roll wick (5f, 9m),
B1) 48 h buckwheat flowers - inserted buckwheat inflorescence and its subtending leaf from a potted plant (5f, 6m),
C1) freeze immediately (2f, 1m)
C2) 48 h water only (3f, 2m)
C3) 48 h blue-green lucerne aphid honeydew - inserted aphid-infested shoots from potted lucerne plants (6f, 4m),
C4) 48 h pea aphid honeydew - inserted aphid-infested shoots from potted lucerne plants (3f, 4m),

Buckwheat and lucerne were grown in a glasshouse prior to use in the experiment. Blue-green lucerne aphids *Acyrthosiphon kondoi* Shinji (Homoptera: Aphididae) were sourced from a lucerne field. Pea aphids *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) were sourced from a long-term colony cultured on broad beans *Vicia faba* L. ‘Evergreen’ (Leguminosae). The aphids were cultured on lucerne for several weeks prior to use in the experiment and honeydew was visible on the shoots provided to the parasitoids. High humidity in the enclosures (indicated by condensation) minimised the probability that this sugar source was made inaccessible to the wasps by evaporation increasing its viscosity.

After exposure to their feeding treatment, parasitoids were frozen in a domestic freezer for several days, transferred to individual 0.6 ml microcentrifuge tubes and then stored in a -80 °C freezer until analysis up to a year later.

### 6.3.3 Determining sugar profiles of laboratory and field-collected parasitoids

Thirty seven field-collected parasitoids were sexed and a hind tibia from each wasp was measured under a binocular microscope (x40) as an indicator of body size. Approximately 400 µl 70% ethanol was added to each microcentrifuge tube and the wasps were air-freighted to Lancaster Environment Centre (Lancaster University, U.K.) for High Performance Liquid Chromatography (HPLC) analysis. The volume of ethanol was made up to 1 ml and the parasitoids were homogenised using a pestle. They were then centrifuged at 13,000 rpm for 10 min. The supernatant was collected and diluted 10-fold with Milli-Q water. 10 µl aliquots of each sample were injected into a Dionex DX 500 HPLC-system (Dionex Corp., Sunnyvale, CA, USA). The system was equipped with a GP 40 gradient pump, and Carbopac PA1 guard (4 x 50 mm²), an analytical column (4 x 250 mm²) and an ED 40 electrochemical detector for pulsed amperimetric detection (Dionex, Leeds, UK). The column was eluted with 1M NaOH and Milli-Q water (10:90%, 1ml min⁻¹) and kept at 20 °C. Reference curves were obtained for
sorbitol, mannitol, trehalose, galactose, glucose, sucrose, mannose, fructose, melibiose, melezitose, raffinose, stachyose and erlose. The concentrations of each sugar were analysed using PEAKNET Software Release 5.1 (DX-LAN module) (Dionex, Leeds, UK). These were converted to micrograms of sugar per sample and then divided by the hind tibia length of the parasitoid to correct for wasp size.

6.3.4 Statistical analysis of sugar profiles

The profiles of laboratory parasitoids were analysed to identify differences between the food treatments and between male and female parasitoids. Two newly-emerged parasitoids (identified in Fig. 6.2i and Fig. 6.3i) were excluded from all analyses because their anomalous profiles suggested contamination had occurred. The two batches of newly-emerged parasitoids were combined to form a single food treatment group.

Total sugar content (excluding sugar alcohols) of the laboratory parasitoids was log_{10}-transformed and analysed using analysis of variance with post hoc Tukey’s HSD comparisons. Further statistical analysis of the sugar profiles was restricted to proportions of the major sugars, with the proportion denominators being selected to allow comparison with previous studies. Glucose/(glucose + fructose) was log_{10}-transformed and analysed using analysis of variance with post hoc Tukey’s HSD comparisons. All parasitoids in the ‘water only’ treatment had a glucose/(glucose + fructose) value of one so this treatment was excluded from the analysis. The proportion of sucrose could not be analysed in this way because a large number of parasitoids had zero values for this variable. Two analyses were therefore performed. First, a generalised linear model with a binomial distribution and logit link function was used, with LSDs for predicted values, to determine which food treatments differed in the proportion of parasitoids containing sucrose. The second analysis was restricted to those food treatments where all parasitoids contained sucrose. In this test, the sucrose content, expressed as a proportion of the three principal sugars (sucrose/(sucrose + glucose + fructose)), was log_{10}-transformed and analysed using analysis of variance with post hoc Tukey’s HSD comparisons. In all analyses sex was included in the initial model but it was not significant and so was excluded from post hoc comparisons.

Profiles of the field-collected parasitoids were compared with those of the laboratory parasitoids using graphical analysis. The distinctive profiles of many field-collected parasitoids suggested that a statistical approach to inferring feeding histories, such as discriminant function analysis, would not be appropriate.

The effects of distance from the flower strip on the total sugar content (log_{10}-transformed) and glucose/(glucose + fructose) values (untransformed) of field-collected parasitoids were analysed using analysis of variance as above. Since no trend with distance was apparent in these variables, distance was treated as a factor rather than a continuous variable, enabling differences
between any two distances to be detected. All parasitoids of known sex were female so sex was not included as a variable in the analyses. All analyses were performed using SPSS Version 15 and Minitab 15.1.

6.4 RESULTS

6.4.1 Characterising the sugar profiles resulting from the food treatments

Total sugar content
Laboratory parasitoids emerged with a mean sugar content of 7.4 µg per wasp. As expected, sugar content fell when parasitoids were provided only with water, and increased when food was available (Fig. 6.1i). There was considerable variation within each feeding treatment even when adjusted for wasp size but, on average, those fed buckwheat nectar had higher levels than those fed blue-green aphid honeydew. Other fed parasitoids had intermediate sugar contents which did not differ significantly from the higher values of nectar-fed wasps or the lower values of those fed blue-green aphid honeydew. There was no difference between male and female parasitoids (Table 6.1i).

![Graph showing total sugar content](image)

**Fig. 6.1 Effect of feeding history on total sugar content of parasitoids.** i) Effect of sex and feeding treatment of laboratory parasitoids (see methods for sample sizes), ii) Field-collected parasitoids (n = 37). The horizontal line marks the median, the box marks the 25th and 75th percentiles, the bars mark the 10th and 90th percentiles and outliers are shown as individual values.
Table 6.1 Effect of feeding treatment and parasitoid sex on total sugar content. Total sugar content excluded sugar alcohols and was log_{10} transformed. i) Results of analysis of variance, \( R^2 = 78\% \). ii) Significance groups based on Tukey’s HSDs. Means are untransformed values.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d.f.</th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>5</td>
<td>32.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>1.834</td>
<td>0.182</td>
</tr>
<tr>
<td>Treatment x sex</td>
<td>5</td>
<td>0.430</td>
<td>0.826</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n female</th>
<th>n male</th>
<th>Mean ( \mu g ) per mm hind tibia (S.E.)</th>
<th>Significance group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly-emerged</td>
<td>7</td>
<td>6</td>
<td>9.74 (1.52)</td>
<td>b</td>
</tr>
<tr>
<td>Water-only</td>
<td>3</td>
<td>2</td>
<td>1.76 (0.28)</td>
<td>a</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>9</td>
<td>40.04 (8.19)</td>
<td>c, d</td>
</tr>
<tr>
<td>Buckwheat nectar</td>
<td>5</td>
<td>6</td>
<td>43.65 (3.76)</td>
<td>d</td>
</tr>
<tr>
<td>Pea aphid honeydew</td>
<td>3</td>
<td>4</td>
<td>29.91 (4.67)</td>
<td>c, d</td>
</tr>
<tr>
<td>Blue-green aphid honeydew</td>
<td>6</td>
<td>4</td>
<td>16.61 (2.43)</td>
<td>b, c</td>
</tr>
</tbody>
</table>

Profile composition

The sugars glucose, fructose and sucrose comprised more than 99.5% of the sugar content of newly-emerged laboratory wasps and their profiles were strongly dominated by glucose (Fig. 6.2i). Starvation (only water provided) led to an absence of fructose (i.e. a glucose/(glucose + fructose) value of one). Feeding led to a decrease in the proportion of glucose and an increase in the proportion of fructose but the degree to which this occurred differed between feeding treatments. The glucose/(glucose + fructose) values were lower in the sucrose and nectar treatments than the honeydew treatments, with male and female wasps containing similar proportions (Table 6.2).

Honeydew-fed wasps were more likely than sucrose-fed and nectar-fed wasps to contain sucrose (Table 6.3i, ii). Parasitoids provided only with water contained substantially higher proportions of sucrose than other wasps (Fig. 6.2i, Table 6.3iii, iv).

In most fed parasitoids, glucose, fructose and sucrose still comprised more than 95% of the sugar content. However, some wasps fed blue-green aphid honeydew contained substantial proportions of melezitose (Table 6.4). This sugar was also present in sucrose-fed wasps but in much lower amounts. Erllose and trehalose were also present as small constituents in fed wasps.
These minor sugars were not present in newly-emerged or starved parasitoids, except for a trace of trehalose in one newly-emerged wasp.

An unidentified carbohydrate (retention time 33–35 minutes) comprised 5–45% of the HPLC chromatogram for seven of the ten parasitoids provided with blue-green aphid honeydew. For an eighth parasitoid, the value was 1%. This compound was only present in three parasitoids from other treatments, and then had a relative area of less than 1.5%.

Table 6.2 Effect of feeding treatment and parasitoid sex on glucose/(glucose + fructose). Glucose/(glucose + fructose) was analysed as $(\log_{10}(\text{glucose}/(\text{glucose} + \text{fructose}))$. All parasitoids in the 'water only' treatment had ratios of 1 (i.e. they did not contain fructose). This treatment was excluded from the analysis. i) Results of analysis of variance, $R^2 = 87\%$, ii) Significance groups based on Tukey's HSDs. Means are untransformed values.

i)

<table>
<thead>
<tr>
<th>Variable</th>
<th>d.f.</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>75.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>3.290</td>
<td>0.076</td>
</tr>
<tr>
<td>Treatment x sex</td>
<td>4</td>
<td>1.515</td>
<td>0.214</td>
</tr>
<tr>
<td>Residual</td>
<td>45</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

ii)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n female</th>
<th>n male</th>
<th>Mean (S.E.)</th>
<th>Significance group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly-emerged</td>
<td>7</td>
<td>6</td>
<td>0.963 (0.017)</td>
<td>a</td>
</tr>
<tr>
<td>Water-only</td>
<td>3</td>
<td>2</td>
<td>1.000 (0.00)</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>9</td>
<td>0.457 (0.019)</td>
<td>b</td>
</tr>
<tr>
<td>Buckwheat nectar</td>
<td>5</td>
<td>6</td>
<td>0.516 (0.0054)</td>
<td>b</td>
</tr>
<tr>
<td>Pea aphid honeydew</td>
<td>3</td>
<td>4</td>
<td>0.621 (0.047)</td>
<td>c</td>
</tr>
<tr>
<td>Blue-green aphid honeydew</td>
<td>6</td>
<td>4</td>
<td>0.722 (0.036)</td>
<td>d</td>
</tr>
</tbody>
</table>
Fig. 6.2 Effect of feeding history on the proportions of glucose, fructose and sucrose in parasitoids. i) Laboratory parasitoids exposed to different food treatments. Data points surrounded by squares are newly-emerged wasps which were excluded from the analyses due to suspected contamination (one is obscured by overlying points). The arrows indicate the direction to follow to read the axis values. For example, the proportions in the lower boxed newly-emerged wasp were glucose = 0.62, fructose = 0.037, sucrose = 0.34. ii) Comparison of sugar profiles of field-collected parasitoids and laboratory parasitoids with known feeding histories. Lines are minimum-perimeter polygons for the laboratory parasitoids shown in (i), excluding points which lie more than two standard deviations from the treatment means on any axis.
Table 6.3 Effect of feeding treatment and parasitoid sex on sucrose content.

i) Results of a logistic regression of the probability of sucrose being present.

<table>
<thead>
<tr>
<th>Variable</th>
<th>d.f.</th>
<th>Deviance ratio</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment + sex</td>
<td>6</td>
<td>5.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ii) Significance groups for the logistic regression based on LSDs of predicted values in the model without sex. Dropping sex and its interaction with treatment did not significantly affect the model fit.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( n ) female</th>
<th>( n ) male</th>
<th>Proportion females cont. sucrose</th>
<th>Proportion males cont. sucrose</th>
<th>Predicted value (S.E.)</th>
<th>Significance group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly-emerged</td>
<td>7</td>
<td>6</td>
<td>0.57</td>
<td>0.5</td>
<td>0.539 (0.14)</td>
<td>a</td>
</tr>
<tr>
<td>Water only</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1.000 (0.0016)</td>
<td>b</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>9</td>
<td>0.4</td>
<td>0.33</td>
<td>0.357 (0.13)</td>
<td>a</td>
</tr>
<tr>
<td>Buckwheat nectar</td>
<td>5</td>
<td>6</td>
<td>0.4</td>
<td>0.17</td>
<td>0.273 (0.13)</td>
<td>a</td>
</tr>
<tr>
<td>Pea aphid honeydew</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1.000 (0.0014)</td>
<td>b</td>
</tr>
<tr>
<td>Blue-green aphid honeydew</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1.000 (0.0012)</td>
<td>b</td>
</tr>
</tbody>
</table>
Table 6.3 continued.

iii) Results of analysis of variance of the proportion of sucrose ($\log_{10}(\text{sucrose/(glucose + fructose + sucrose)})$. Only feeding treatments where all wasps contained this sugar were analysed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>52.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.094</td>
<td>0.763</td>
</tr>
<tr>
<td>Treatment x sex</td>
<td>2</td>
<td>0.819</td>
<td>0.459</td>
</tr>
<tr>
<td>Residual</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

iv) Significance groups for analysis of variance based on Tukey's HSDs. Means are untransformed values.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$ female</th>
<th>$n$ male</th>
<th>Mean (S.E.)</th>
<th>Significance group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-only</td>
<td>3</td>
<td>2</td>
<td>0.452 (0.064)</td>
<td>a</td>
</tr>
<tr>
<td>Pea aphid honeydew</td>
<td>3</td>
<td>4</td>
<td>0.0598 (0.0088)</td>
<td>b</td>
</tr>
<tr>
<td>Blue-green aphid honeydew</td>
<td>6</td>
<td>4</td>
<td>0.0545 (0.0053)</td>
<td>b</td>
</tr>
</tbody>
</table>
Table 6.4 Effect of parasitoid feeding treatment on the presence of minor sugars and sugar alcohols. Table cells contain the percentage of parasitoids with different feeding histories containing various minor sugars/sugar alcohols, and the minimum and maximum percentages those sugars contributed to the parasitoids' total sugar contents. A dash indicates no wasps in the treatment contained the sugar. No wasps contained mannose, melibiose, raffinose, lactose or stachyose.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample size</th>
<th>Melezitose</th>
<th>Erlose</th>
<th>Rhamnose</th>
<th>Galactose</th>
<th>Trehalose</th>
<th>Sorbitol</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly-emerged</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8%</td>
<td>31%</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%parasitoids</td>
<td></td>
<td>%sugar</td>
<td>(min - max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water only</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%parasitoids</td>
<td></td>
<td>%sugar</td>
<td>(min - max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>14</td>
<td>71%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50%</td>
<td>93%</td>
<td>57%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%parasitoids</td>
<td></td>
<td>%sugar</td>
<td>(min - max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buckwheat nectar</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>36%</td>
<td>45%</td>
<td>36%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%parasitoids</td>
<td></td>
<td>%sugar</td>
<td>(min - max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pea-aphid honeydew</td>
<td>7</td>
<td>29%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>43%</td>
<td>100%</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%parasitoids</td>
<td></td>
<td>%sugar</td>
<td>(min - max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue-green aphid honeydew</td>
<td>10</td>
<td>40%</td>
<td>10%</td>
<td>-</td>
<td>-</td>
<td>30%</td>
<td>80%</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%parasitoids</td>
<td></td>
<td>%sugar</td>
<td>(min - max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field-collected</td>
<td>37</td>
<td>5%</td>
<td>-</td>
<td>3%</td>
<td>8%</td>
<td>38%</td>
<td>65%</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%parasitoids</td>
<td></td>
<td>%sugar</td>
<td>(min - max)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

%sugar (min - max): 0.21 - 0.21, 0.64 - 2.64, 0.16 - 0.16, 0.55 - 12.3, 0.05 - 12.3, 0.2 - 4.85, 0.07 - 5.20, 2.85 - 5.20, 0.14 - 2.45, 0.16 - 0.41, 0.08 - 2.45, 0.07 - 0.56, 0.14 - 0.27, 0.06 - 0.94, 0.01 - 0.19, 0.14 - 0.27, 0.61 - 4.56, 0.09 - 1.42, 0.75 - 3.67, 0.29 - 1.17, 0.14 - 2.21, 0.14 - 2.21, 0.26 - 6.21, 0.21 - 1.93.
**Fig. 6.3 Effect of feeding history on the sugar profile of parasitoids.** i) Laboratory parasitoids exposed to different food treatments. Data points surrounded by squares are newly-emerged wasps which were excluded from the analyses due to suspected contamination (also see Fig. 6.2i). ii) Comparison of sugar profiles of field-collected parasitoids and laboratory parasitoids with known feeding histories. Lines are minimum-perimeter polygons for the laboratory parasitoids shown in (i), excluding points which lie more than two standard deviations from the treatment mean on either axis.
Overall sugar profile

Although the average total sugar contents and sugar compositions of the parasitoids from some feeding treatments differed, there was overlap between the groups. Assessment of both the glucose/(glucose + fructose) value and total sugar content improved the between-group differentiation (Fig. 6.3i). Nectar and sucrose-fed wasps tended to have high total sugar content and low glucose/(glucose + fructose) values and newly-emerged and ‘water only’ wasps had low total sugar content and high glucose/(glucose + fructose) values. Honeydew-fed wasps had intermediate levels for both variables. The proportion of melezitose could be used to further differentiate the nectar-fed from the sucrose-fed wasps, and the pea aphid honeydew-fed and blue-green aphid honeydew-fed wasps (Table 6.4). In addition, the proportion of sucrose could be used to differentiate newly-emerged wasps from older wasps which had not fed (Fig. 6.2i). Thus, the six feeding treatments each produced a characteristic sugar profile as summarised in Table 6.5. The unidentified carbohydrate with a retention time of 33–35 minutes served as an additional indicator of blue-green aphid honeydew feeding but was also not completely reliable.

In order to quantify the profiles in Table 6.5 and to assess their statistical validity, multivariate analyses (canonical variates analysis and non-metric multidimensional scaling) of these variables, and of logged glucose, sucrose, fructose and melezitose contents, were performed. These analyses did not indicate that parasitoids from the different feeding treatments could be better distinguished by considering four variables (results not presented) rather than two (as in Fig. 6.3). The sugar profiles in Table 6.5 could therefore only be used as a qualitative guide to feeding history and because there is overlap between the groups, a probabilistic approach would have to be used to infer unknown feeding histories.

Previous studies with other parasitoids species have found differences between male and female wasps (Steppuhn & Wäckers, 2004), and have found different minor sugars to those in this study (Wäckers et al., 2006; Hogervorst et al., 2007a; Wyckhuys et al., 2008). The results presented here support the argument that conclusions about sugar metabolism in one species should not be uncritically applied to other species (Wäckers et al., 2006).

6.4.2 Sugar profiles of field-collected parasitoids - inferring feeding history

Fig. 6.1ii, Fig. 6.2ii and Fig. 6.3ii allow the sugar profiles of field-collected parasitoids to be compared with those of the parasitoids of known feeding history. Most field-collected parasitoids had low sugar levels (Fig. 6.1ii). 59% had levels lower than the minimum found in fed laboratory wasps. No fed laboratory wasps and only one of fourteen newly-emerged laboratory wasps (7.1%) had sugar levels within the range of the ‘water only’ laboratory wasps, but 32% of field-collected wasps had sugar contents this low. Although most field-collected parasitoids had total sugar levels comparable to unfed laboratory wasps, considering this
Table 6.5 Qualitative sugar profiles which differentiate laboratory wasps from different feeding treatments. Total sugars excluded sugar alcohols, though these were only present in small quantities. The proportion of sucrose could be calculated either as a proportion of total sugars, or of sucrose plus glucose plus fructose since these were strongly correlated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total sugars</th>
<th>Glucose/(Glucose + fructose)</th>
<th>Sucrose/Total</th>
<th>Melezitose/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>newly-emerged</td>
<td>low</td>
<td>high</td>
<td>low</td>
<td>zero</td>
</tr>
<tr>
<td>water only</td>
<td>low</td>
<td>high</td>
<td>high</td>
<td>zero</td>
</tr>
<tr>
<td>sucrose</td>
<td>high</td>
<td>low</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>buckwheat nectar</td>
<td>high</td>
<td>low</td>
<td>low</td>
<td>zero</td>
</tr>
<tr>
<td>pea aphid honeydew</td>
<td>intermediate</td>
<td>intermediate</td>
<td>low</td>
<td>zero</td>
</tr>
<tr>
<td>blue-green aphid honeydew</td>
<td>intermediate</td>
<td>intermediate</td>
<td>low</td>
<td>high</td>
</tr>
</tbody>
</table>

Table 6.6 Inferring feeding histories of field-collected parasitoids. The table cells contain the number of field-collected parasitoids at each sampling distance whose sugar profiles were consistent with none, one or more of the profiles generated from laboratory parasitoids subjected to different food treatments. Sugar profiles were classified as consistent with a treatment if the values of the four variables 'total sugars excluding sugar alcohols', 'sucrose/(sucrose + glucose + fructose)', 'melezitose/total sugars' and 'glucose/(glucose + fructose)' were within the range of the laboratory parasitoids subjected to the food treatment. Distances are measured from a buckwheat strip in the centre of a lucerne field.

<table>
<thead>
<tr>
<th>Food treatment</th>
<th>1m</th>
<th>5m</th>
<th>10m</th>
<th>25m</th>
<th>50m</th>
</tr>
</thead>
<tbody>
<tr>
<td>newly-emerged</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sucrose (but not nectar)</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>sucrose or nectar</td>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>pea-aphid honeydew</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>blue-green aphid honeydew</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>none of the tested treatments</td>
<td>3</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
variable in combination with glucose/(glucose + fructose) (Fig. 6.3ii), indicated that these
groups had different feeding histories; the field-collected parasitoids had lower glucose/(glucose
+ fructose) values than the unfed wasps.

The proportion of sucrose in three field-collected parasitoids (different to the three
containing minor sugars) was higher than that in any of the newly-emerged or fed laboratory
wasps, and was within the range of the ‘water only’ wasps in two individuals (Fig. 6.2ii). These
wasps had very low total sugar levels (< 1 µg mm$^{-1}$ hind tibia) but all contained a substantial
proportion of fructose, unlike the laboratory ‘water only’ wasps. Amongst the majority of field-
collected parasitoids that had a proportion of sucrose within the range of the non-starved
laboratory wasps, many had no sucrose and a low glucose/(glucose + fructose) value. This
combination differentiated them from any of the feeding treatment groups (Fig. 6.2ii).

For all but three of the field-collected parasitoids, glucose, fructose and sucrose
comprised more than 98% of their sugar content. The three exceptions were due to presence of
melezitose and rhamnose, with the former sugar constituting 20.5% of the total sugar content in
one wasp (Table 6.4). The two field-collected parasitoids containing melezitose had high
glucose/(glucose + fructose) values (0.601, 0.758) so their profile was similar to that of the
melezitose-containing blue-green aphid honeydew-fed laboratory wasps, rather than those fed
sucrose. A trace of galactose was also found in one field-collected parasitoid. Rhamnose and
galactose were present only in field-collected wasps, not in laboratory wasps.

Although the sugar profiles of the majority of field-collected parasitoids suggested their
feeding history differed from all laboratory treatments, some did have profiles consistent with
one or more of the known histories, as indicated by their values for the four variables in Table
6.5 falling within the ranges of those measured in laboratory wasps. Using this method, five
parasitoids could have fed on blue-green aphid honeydew, two on sucrose, three on sucrose or
nectar, and five could have been newly-emerged. These were found across the five distances
from which analysed parasitoids were sampled (Table 6.6).

The unidentified carbohydrate (retention time 33–35 minutes) which differentiated the
laboratory wasps fed blue-green aphid honeydew from those in the other food treatments was
present in 18 of the 37 field-collected wasps. In 14 of these it comprised more than 5% of the
chromatogram, a level which distinguished them from the few laboratory wasps fed sucrose or
pea aphid honeydew which contained traces of this compound. Parasitoids containing the ‘33–
35 minute’ carbohydrate were found at all distances. The proportions of sucrose, fructose and
glucose in the wasps containing this carbohydrate were consistent with feeding on blue-green
aphid honeydew, except in two cases. The exceptional wasps (with 5.7% and 2.5% ‘33–35
minute’ carbohydrate) had high proportions of fructose (0.64 and 0.68 respectively, calculated
as a proportion of total fructose, glucose and sucrose as in Fig. 6.2).
Inferring the feeding history of the field-collected parasitoids is limited by the food treatments for which sugar profiles were determined in the laboratory. In particular, no parasitoids were fed and then starved. Previous studies have found other species of parasitoids with such a feeding history have total sugar levels comparable to unfed wasps but have glucose/(glucose + fructose) values comparable to fed wasps (Steppuhn & Wäckers, 2004; Vattala, 2005). (Vattala (2005) suggests that in *Microtonus hyperodae*, fructose as a proportion of total sugars is a more reliable indicator of past feeding than glucose/(glucose + fructose) because the difference between unfed and starved wasps persists for longer. In this study the two variables were strongly correlated.) These fed-then-starved parasitoids thus fall in the quadrant of Fig. 6.3 in which many of the field-collected parasitoids in this study lie. If, for a given food source, the glucose/(glucose + fructose) value in fed-then-starved field-collected wasps was the same as that for fed wasps (i.e. approximately 0.5 for nectar-fed and greater than 0.5 for honeydew-fed), many of the field-collected parasitoids could have fed on honeydew. However, sugar ratios can change with age and time since feeding (Olson *et al.*, 2000; Vattala, 2005; Wäckers *et al.*., 2006; Hogervorst *et al.*, 2007a). It is therefore not possible to conclude that the parasitoids had exploited honeydew without further work, but the presence of the ‘33–35 minute’ carbohydrate supports this conclusion.

### 6.4.3 Sugar profiles of field-collected parasitoids - effect of distance from the flower strip

Whether or not nectar-feeding by the parasitoids could be inferred, a change in sugar profiles with increasing distance from the flower strip would suggest the parasitoids were affected by this feature. There was no such effect on two key components of the sugar profile: total sugar content (log$_{10}$(total sugars excluding sugar alcohols), $F_{4,32} = 0.603, P = 0.663$) or glucose/(glucose + fructose), $F_{4,32} = 0.859, P = 0.499$). No difference was apparent in the proportion of sucrose but this was not analysed due to inadequate sample sizes for the generalised linear model analyses required for this variable.

### 6.5 DISCUSSION

HPLC analyses did not provide evidence that *A. zealandica* exploited the flower strip as a source of nectar. A number of parasitoids had sugar profiles consistent with nectar-feeding but since the proportion was small and there was no relationship between possible nectar-feeding and distance from the flower strip, it is not possible to conclude that these parasitoids fed on buckwheat rather than flowering weeds. The low prevalence of possible buckwheat use is consistent with the results of the rubidium-labelling component of this study (see Chapter 5), but those results do not provide strong corroborating evidence since false-negatives may have occurred.
Some parasitoids had profiles consistent with feeding on sucrose solution, but since sources of pure sucrose would not be available in the field, this should be interpreted as feeding on a sucrose-rich resource, probably nectar of that composition. It is notable that in the laboratory-raised parasitoids, the variation in sugar profiles was much greater for sucrose-fed than nectar-fed parasitoids although the nectar treatment, which was subject to biological variation between the buckwheat plants, would have been less well standardised. Understanding the causes of such a high level of variation might help ensure feeding histories of field-collected parasitoids, which will be subject to many more potential sources of variation, are not inferred with undue confidence. Separate analysis of body and gut sugars may assist this understanding.

Drawing on the results of other studies (Steppuhn & Wäckers, 2004; Vattala, 2005), the low total sugar level of field-collected parasitoids, in combination with a low glucose/(glucose + fructose) value (and presence of minor sugars), suggests that most parasitoids had fed, but not recently enough or in sufficient quantity, to raise total sugar levels to those of the fed laboratory parasitoids. It is not known whether at such low sugar levels the activity of the parasitoids would be compromised. In searching for hosts and/or mates, the field-collected parasitoids may deplete their sugar levels below those of confined laboratory parasitoids with food ad libitum, but provided food was sufficiently available for sugar levels to kept above some minimum level, that might not be biologically significant. That minimum would depend not only on the level at which movement is impaired, but also on the level at which foraging for hosts is disrupted by a stronger motivation to forage for food (Takasu & Lewis, 1993; Wäckers, 1994; Stapel et al., 1997; Lewis et al., 1998; Jacob & Evans, 2001; Siekmann et al., 2004; Desouhant et al., 2005; Bernstein & Jervis, 2008). Further work is required to link the behaviour of parasitoids to their physiology. It must also be recognised that this study only analysed living parasitoids, so an unknown proportion of each cohort may have already died of starvation, with or without having achieved its potential fecundity.

In this study, the buckwheat strip was growing in a lucerne field infested with aphids (predominantly blue-green lucerne aphids). Honeydew was therefore a potential alternative to buckwheat (or weed) nectar as a food source. In foraging for host lacewing larvae which feed on aphids, *A. zealandica* would be likely to encounter this food. The composition of nectars and aphid honeydews varies with flower plant species, aphid species and aphid host-plant species, but honeydews can often be differentiated from nectars by higher glucose/(glucose + fructose) values and presence of a number of minor sugars (Lee et al., 2006; Wäckers et al., 2006; Hogervorst et al., 2007a, 2007b; Olson & Wäckers, 2007; Wyckhuys et al., 2008).

Honeydew can increase longevity and fecundity compared with water alone and previous field studies have found honeydew-feeding to be prevalent (Lee et al., 2006; Hogervorst et al., 2007a; Wäckers et al., 2008). However, honeydew may be a less suitable food source than nectar. Longevity of some species of parasitoids is shorter when they are fed some honeydew-
specific oligosaccharides, than when they are fed glucose, fructose or sucrose (Wäckers, 2001; Winkler et al., 2005; Williams III & Roane, 2007). For some parasitoids, longevity when fed honeydew itself is lower than when fed sucrose solution (Wäckers et al., 2008), and there is some evidence that this effect is not due to differing proportions of glucose, fructose or sucrose (Hogervorst et al., 2007b). Recent work has suggested that the suitability of this resource may be determined by viscosity rather than composition per se (Faria et al., 2007) so under conditions where the honeydew is of low enough viscosity to be imbibed, it may be exploited by the parasitoids. The laboratory parasitoids fed honeydew in the study presented here had lower total sugar levels than those provided with sucrose solution or nectar, as they were in a study by Wyckhuys et al. (2008). This could indicate that the honeydew was an inferior food source. However, the difference could be an artefact of the different ways the food resources were presented and further work would be required to determine the relative value of typical nectars and honeydews for this species.

Even if potential longevity when feeding on honeydew is shorter than when feeding on nectar, if this resource is present where A. zealandica are foraging for hosts, the parasitoid may not be motivated to search elsewhere for nectar. The ecological significance of the extended longevity will also depend on the degree to which the parasitoids are time- or egg-limited (Heimpel & Rosenheim, 1998) and whether other causes of mortality prevail over starvation (Heimpel et al., 1997).

Although the weather was generally hot and windy during the study, honeydew could have been frequently available if freshly excreted by abundant aphids or liquefied by dew. The analyses of field-collected parasitoids suggest that some did feed on honeydew. With more appropriate laboratory-generated sugar profiles the extent of honeydew-feeding might be better determined. Such profiles should indicate the variation arising from food deprivation and wasp age, and the variation associated with the changes in honeydew composition. The latter results from varying factors such as aphid age and plant quality.

Previous studies have discussed the suitability of melezitose and erlose as indicators of honeydew feeding (Hogervorst et al., 2007a; Wyckhuys et al., 2008). The results of this study show that A. zealandica can synthesise melezitose when fed sucrose solution and that erlose is not consistently present when honeydew from pea aphids or blue-green lucerne aphids on lucerne is available. Neither are therefore reliable indicators of honeydew-feeding in this system. However, an unidentified carbohydrate (retention time 33–35 minutes) may be an indicator of feeding on honeydew produced by blue-green aphids on lucerne and further work should consider this compound.

The sugar profiles of some field-collected parasitoids fell within the ranges of newly-emerged laboratory parasitoids. The variation in content of the newly-emerged laboratory parasitoids may reflect consumption of honeydew that had contaminated the lacewing cocoons.
through which some wasps emerged. The status of the field-collected parasitoids is therefore questionable; although cocoons may also be honeydew-contaminated in the field, the wasps may actually have fed on small quantities of honeydew in their wider environment. The probability of sampling newly-emerged wasps from the field is likely to be low since at any one time, unless mortality is high, they would constitute a small proportion of the population.

The potential of flower strips to disrupt biological control by lacewings was previously highlighted when the longevity and lifetime fecundity of lacewing parasitoids were found to increase with provision of buckwheat flowers in the laboratory (Chapter 4). It seems however, that this potential is not necessarily realised in the field. Only a small proportion of field-collected parasitoids had sugar profiles that suggested they might recently have fed on nectar (of buckwheat or other species) and sugar profiles did not vary with distance from the buckwheat strip. Therefore, while adverse effects of flower strips may occur in other situations, in this study the flowers are unlikely to have increased rates of lacewing parasitism. Rather, the results reinforce those of previous studies which suggest honeydew feeding can be common despite the apparent inferiority of this food (Wäckers & Steppuhn, 2003; Lee et al., 2006; Hogervorst et al., 2007a; Wäckers et al., 2008). On the other hand, despite the presence of flowers and apparent consumption of honeydew, few insects could be considered ‘well-fed’. Further interpretation would be greatly assisted by work to establish whether the relatively low total sugar levels in many field-collected insects were low enough to indicate parasitoid efficacy was sugar-limited.
Chapter 7  The effects of floral resources on biological control in a system with four trophic levels: Insights from a simulation model

7.1  SUMMARY

To date, most research concerned with the provision of flowers as a pest management technique has focused on the use of floral resources by life-history omnivores that contribute to biological control. In order to ensure this technique is effective in the context of complex agroecosystems, we need to increase our understanding of the effects of flowers on biological control by true omnivores. Similarly, greater understanding of the effects of flower use by species acting at the fourth trophic level, such as hyperparasitoids and parasitoids of predators, would be valuable. In this study, the effects of floral resources on biological control in a system with a true omnivore and its parasitoid were explored using a simulation model.

The modelled biocontrol agent was the brown lacewing *Micromus tasmaniae*. As an adult this species is a true omnivore, consuming floral resources and aphids. It consumes aphids in the larval stage. Lacewing larvae are parasitised by *Anacharis zealandica*, a life-history omnivore which benefits from floral resources as an adult. The dynamics of aphid, lacewing and parasitoid populations were simulated for a period equivalent to a single re-growth period in the cutting cycle of a lucerne crop. The model was parameterised using data from my own laboratory experiments and published sources, and was run using values ‘with flowers’ and ‘without flowers’. Simulations with and without flowers were compared to determine the indirect effects of flowers on the aphid populations; indirect effects which occurred as a result of the direct effects of flowers on aphid consumption by the lacewings. In addition, the effects of flowers on the number of lacewing pupae and adults at the end of the simulation were studied, with and without parasitoids. These populations indicated the potential for biological control in future crops. All simulations were run for a range of initial aphid and lacewing populations and sensitivity to the parameter values was investigated.

Flowers almost always improved biological control by lacewings, as measured by reductions in the aphid-days accumulated during the simulations. However, for most initial populations the reductions were small and did not affect whether the aphid-days variable exceeded the threshold at which crop damage was taken to occur. Parasitoids benefited more from flowers than did lacewings so the number of lacewing pupae at the end of the simulation was lower with flowers than without, when parasitoids were present. The model did not include immigration or emigration of insects and cannot be used to make quantitative predictions of field populations. However, it demonstrated how the substantial effects of flowers on a true omnivore that were measured in the laboratory may be of little significance in the overall
dynamics of biological control. In this system, the effects of flowers on the life-history omnivore acting at the fourth trophic level were of greater ecological significance, serving as a reminder of the need to consider such species when providing flowers for pest management.

7.2 INTRODUCTION

Pest management in agroecosystems is a process of suppressing the populations of one or more potentially-damaging species, within a web of interacting species, in order to reduce the target species’ effect on crop yield (or value) to an acceptable level (Dent, 2000). Understanding the interactions between the pest species and the other species in the agroecosystem can help guide the development of practices which satisfy short-term local goals, and do not have undesirable consequences in the longer-term or over a wider area. While such understanding is universally desirable in pest management, its value is most clearly evident in the development of techniques which aim to control pests through management of their habitat. Such techniques may act on the pest from the ‘bottom up’ or the ‘top down’ (Gurr et al., 2004). The latter comprise the strategy of conservation biological control: increasing the efficacy of indigenous (and previously-introduced) predators and parasitoids (Gurr et al., 2000).

One way in which the efficacy of biological control may be enhanced is through increasing the abundance of flowers where use of herbicides or other management practices would otherwise mean biocontrol agents are limited by the availability of floral resources (Gurr et al., 1998; Landis et al., 2000; Gurr et al., 2005). The species which are most likely to be limited in this way are life-history omnivores such as beneficial species of hoverflies (Diptera: Syrphidae) and parasitoid wasps (Hymenoptera: Parasitica). The larvae of these species consume pests but many of the adults require nectar and/or pollen in order to maximise their longevity and fecundity (Schneider, 1969; Jervis et al., 1996). True omnivores may also exploit flowers; some brown lacewings (Neuroptera: Hemerobiidae), ladybirds (Coleoptera: Coccinellidae), mites (Acarina: Phytoseiidae) and thrips (Thysanoptera) consume nectar and/or pollen and prey as partially or fully substitutable resources (Chapter 2; Stelzl, 1991; Alomar & Wiedenmann, 1996; Hodek & Honek, 1996; McMurtry & Croft, 1997; Dixon, 2000; Coll & Guershon, 2002; Wäckers & van Rijn, 2005).

The contribution of flower-provision to pest management in commercial agriculture is currently minor (van Driesche et al., 2008). However, building the agroecological foundation for this technique is an ongoing challenge and as this progresses, unrealised potential might become apparent. Progress is likely to be made through the combination of complementary approaches: reductionist and holistic, experimental and theoretical. To date, almost all pertinent research has been experimental. Numerous laboratory experiments have been conducted and field studies have been undertaken in several agroecosystems (e.g. Foster & Ruesink, 1984; Cowgill et al., 1993; Hickman & Wratten, 1996; Baggen & Gurr, 1998; Baggen et al., 1999;
Macleod, 1999; Platt et al., 1999; Harmon et al., 2000; Nicholls et al., 2000; Berndt et al., 2002; Tylianakis et al., 2004; Wäckers, 2004; Zhang et al., 2004; Berndt & Wratten, 2005; Ellis et al., 2005; Lee & Heimpel, 2005; Rahat et al., 2005; Begum et al., 2006; Berndt et al., 2006; Irvin & Hoddle, 2006; Irvin et al., 2006; Lavandero et al., 2006; Rebek et al., 2006; Vattala et al., 2006; Wanner et al., 2006b; Winkler et al., 2006; Ponti et al., 2007; Lee & Heimpel, 2008b; Kehrli & Bacher, in press). Only a small number of studies have used population modelling to investigate how the effects of floral resources on predators or parasitoids might affect biological control (van Rijn et al., 2002; Kean et al., 2003; Banks et al., 2008; Bianchi & Wäckers, 2008). A few more have modelled non-specific plant-provided foods (van Rijn & Sabelis, 2005; Gillespie & Roitberg, 2006; Daugherty et al., 2007). The study presented here developed such a model to generate additional insight from previous empirical work in this area.

A series of experiments has been conducted with the aim of understanding the role flowers might play in a system that includes biological control by a true omnivore and parasitism of that biocontrol agent by a fourth-trophic-level life-history omnivore. The system incorporated the following insects: 1) the pea aphid, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), which is a herbivorous pest, 2) a lacewing, *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae), which consumes only the pest as a larva but both the pest and floral resources as an adult, and 3) a lacewing-parasitoid, *Anacharis zealandica* Ashmead (Hymenoptera: Figitidae), which consumes the lacewing as a larva and floral resources as an adult (Chapters 2 and 4; Robinson et al., 2008; Jonsson et al., in press). In contrast with beneficial parasitoid species such as those which parasitise aphids, the lacewing-parasitoid (hereafter referred to as ‘the parasitoid’) is potentially detrimental to biological control of aphids. (Lacewing larvae might consume floral resources in addition to prey, but because their dispersal ability is limited they were assumed not to gain substantial benefit from floral resources that are aggregated and occupy a small proportion of the land area, as with a strip of flowers sown in a field for pest management purposes.)

This system is intended to exemplify the multiplicity of ecological interactions which must be considered if conservation biological control is to be deployed effectively (Gurr et al., 2005). Although each species would be involved in further interactions in the field, this simplified system was chosen to balance the need for tractability with the potential for flowers to generate an interesting mixture of positive and negative effects.

Laboratory experiments demonstrated that presence of buckwheat flowers *Fagopyrum esculentum* Moench ‘Katowase’ (Polygonaceae) can decrease predation by the lacewing and can increase this species’ fecundity and longevity when aphid density is low (Chapter 2; Robinson et al., 2008; Jonsson et al., in press). The results suggested that the effects of flowers on biological control would be negligible if aphid density is high, but when it is low flowers could have a detrimental effect in the short-term and a beneficial effect in the long-term, depending on
the balance of effects on *per capita* predation rate and the number of lacewings. Laboratory
experiments also showed that flowers can increase the longevity and fecundity of the parasitoid
(Chapter 4; Jonsson *et al.*, in press). Therefore, if parasitoids were present, any beneficial direct
effect of flowers on lacewings might be countered by the detrimental effect of flowers
increasing the rate of parasitism.

Following the laboratory work, experiments in field cages studied how the dynamics of
aphid, lacewing and lacewing-parasitoid populations varied over approximately 10–12 weeks
with or without flowers, and whether presence of flowers affected the lucerne crop (Jonsson *et al.*
in press; Jacometti *et al.* unpublished) (see Section 7.5.3). The modelling study presented
here is intended to further understanding of the effect of floral resources on biological control in
this four-trophic-level system. It integrates the positive and negative effects which flowers had
on the individual processes studied in the laboratory, and so allows us to see how the different
effects might combine to determine the efficacy of biological control with and without flowers.
When these overall effects of flowers are observed in the field cages, the observations are
inevitably made within a narrow range of initial populations due to logistical constraints. In the
open field, observations would be influenced by numerous interactions with other species so it
is difficult to elucidate the mechanisms through which flowers indirectly affect the aphid
population. The modelling approach adopted here complements the empirical studies; it allows
effects to be studied across a wide range of initial populations and the contributions of
individual processes to the overall effect can be clearly identified.

In modelling biological control there is a long tradition of studying the dynamics of
systems with few species (and minimalistic representation of environmental variation) over
many generations, often with a focus on how model structure affects occurrence of states such
as stable equilibria, unstable equilibria, limit cycles and chaos (e.g. Murdoch *et al.*, 1985; May
& Hassell, 1988; Barlow & Wratten, 1996; Mills & Getz, 1996; Murdoch & Briggs, 1996). Many of these models have been developed from Nicholson-Bailey-type and Lotka-Volterra-
type equations and represent quite abstract systems that can provide insight for biological
control at a strategic level (May & Hassell, 1988). If the simplest of these models form one
extreme of a continuum, at the other extreme are complex simulation models that incorporate
many interacting species and precise environmental variables in order to simulate accurately
short-term dynamics of one or more populations. They have a strong connection with particular
‘real world’ food-webs, being parameterised using a considerable body of empirical data and
validated with field population data. This approach has been adopted less frequently but is
exemplified by the models of Frazer & Gilbert (1976), Carter *et al.* (1982), Gosselke *et al.*

The model described here is not a direct development of such preceding parasitoid-host or
predator-prey models because its purpose differed from theirs. Unlike most analytical models,
the aim was to study the dynamics of greatest relevance to pest management in crops which are harvested frequently with respect to the generation time of the insect populations; that is the field-scale, short-term dynamics rather than landscape-level, long-term equilibrium states. In contrast with previous complex simulation models, the intention was to act as an intermediate step between existing, limited empirical data and further empirical studies, not to predict field populations. Furthermore, it was important that the model was sufficiently simple for its behaviour to be readily interpreted with respect to the question of interest i.e. the role of floral resources in biological control within the system. A model of intermediate complexity, realism and specificity was therefore developed (Barlow, 1999).

Once the model of the aphid, lacewing and parasitoid populations had been constructed, simulations were performed to provide a thorough understanding of the behaviour of the system. The duration of each simulation was equivalent to one period of re-growth in a perennial lucerne crop that is harvested several times in a season. The efficacy of biological control in the simulated re-growth period was indicated by the number of aphid-days accumulated during the simulation. The potential for biological control in the future was indicated by the final populations of adult and pupa lacewings, assuming these stages survived harvest and could recolonise future crops while eggs and larvae were destroyed.

In the first stage of the study the model was run with standard parameter sets and this output was interpreted to determine the following characteristics, across a range of initial aphid and lacewing populations: 1) whether aphid populations were damaging in the absence of biological control by lacewings, 2) the efficacy of biological control in the simulated re-growth period in the absence of flowers, 3) the potential for biological control in the future in the absence of flowers, 4) the effect of flowers on biological control in the simulated re-growth period, 5) the effect of flowers on the potential for biological control in the future in the absence of parasitoids and 6) the effect of flowers on the potential for biological control in the future in the presence of parasitoids.

Although parasitoids could affect the potential for biological control in the future, the model structure dictated that biological control within the simulated re-growth period was the same in the presence and absence of parasitoids. The effect of flowers on this process was therefore not studied. (The simulations were not extended for multiple periods of re-growth because modelling the response of the populations to harvesting, and subsequent recolonisation, was beyond the scope of this study.)

In the second stage of the study, these simulations were repeated with alternative parameter sets to determine the sensitivity of the model system to the parameter values. Finally, the implications of the behaviour of the model system for the real world were considered.
7.3 THE MODEL

7.3.1 Model structure

The populations and processes incorporated into the model are depicted in Fig. 7.1. Consumption of the floral resources provided by flowers was not modelled explicitly. Instead, other processes that were known to be affected by consuming floral resources were modelled with alternative parameter values: a value ‘with flowers’ and a value ‘without flowers’. The output from simulations with these alternative values was compared to determine the effect of flowers (see Section 7.3.3). The processes were modelled in an Excel workbook (Microsoft Corporation) using deterministic difference equations that calculated the number of individuals passing through each process in each time step. For most simulations the calculations were iterated for 420 time steps of 0.05 days (see Section 7.3.3). The model equations are presented in Appendix 3 and a brief description of the model structure is given here.

Lacewings predated aphid nymphs but not adult aphids, reflecting the poor ability of lacewings to capture the large mobile adult aphids (Leathwick, 1989). Predation by adult lacewings was modelled as a Type II functional response (Holling, 1959; Gotelli, 2001):

\[ c = \frac{La \cdot (k \cdot An)}{h + An} \]

where \( c \) is the number of aphid nymphs consumed in the time step, \( La \) is the density of adult lacewings, \( An \) is the density of aphid nymphs, \( k \) is the maximum rate of consumption and \( h \) is the aphid density at which consumption is half the maximum rate. The parameter \( h \) was affected by flowers so that at low aphid densities, consumption of aphids was lower when flowers were present than when they were absent. At high aphid densities the effect of flowers was negligible because lacewings were consuming aphids at a near-maximum rate with or without flowers. Oviposition was also modelled by a Type II functional response, reflecting the contribution that aphid food makes to egg production, and the likelihood that adult lacewings oviposit in environments where there are aphids for their larvae to eat. Presence of flowers increased the rate of oviposition at any given aphid density but as for aphid consumption, the effect was negligible at high aphid densities. In the absence of flowers, mortality was inversely proportional to consumption of aphids but when flowers were present the adult lacewings were only subject to a constant rate of background mortality. With and without flowers adult lacewings also had a maximum longevity.

The eggs produced by adult lacewings in each time step formed daily cohorts that were subject to a constant rate of mortality. Surviving eggs became lacewing larvae after their development time had elapsed (based on their cohort rather than the exact time step within which they were produced). As for adult lacewings, predation of aphid nymphs by lacewing larvae was modelled as a Type II functional response but this was unaffected by flowers. Lacewing larvae were also similarly subject to background mortality and mortality that was
Fig. 7.1 Relational diagram of the four-trophic-level system as modelled. Populations and processes incorporated in the model are depicted in black and red. Those depicted in grey were not modelled explicitly. The effects of consumption of buckwheat nectar and/or pollen were modelled using alternative parameter values for the processes depicted in red, so simulations were ‘with flowers’ or ‘without flowers’. The output variable aphid-days was used an indication of the damage caused by the aphids’ consumption of lucerne.
dependent on the aphid nymph density, but these processes were unaffected by flowers. In addition to mortality, lacewing larvae were subject to parasitism when adult parasitoids were present. Stinging of larvae by the adult parasitoids was modelled as a Type II functional response and the maximum rate of stinging was higher when flowers were present than when they were absent. The parasitised lacewing larvae predated aphid nymphs and died in the same way as unparasitised larvae. After their development time had elapsed, the cohorts of surviving unparasitised and parasitised lacewing larvae pupated and were then subject to background mortality until they developed into adult lacewings or parasitoids, respectively.

The adult parasitoids reproduced by stinging lacewing larvae, as described above. They had a maximum longevity that was greater when flowers were present and were also subject to a constant background rate of mortality.

Adult aphids had a maximum longevity and were subject to a constant rate of mortality, neither of which was affected by flowers. They produced aphid nymphs at a constant rate in each time step and these formed daily cohorts. The aphid nymphs were subject to constant background mortality and consumption by adult lacewings, and unparasitised and parasitised lacewing larvae as described above. The cohorts of aphid nymphs became adults when their development time had elapsed. It was assumed that aphid mortality, development and reproduction would not be substantially affected by density dependence until the population had already reached an unacceptable level (as defined in Section 7.3.3) so this phenomenon was not modelled.

7.3.2 Initial populations and parameterisation

The population densities of aphids, lacewings and parasitoids in lucerne vary greatly through the season, affected by temperature and the periodic cutting or grazing of the crop, as well as ecological interactions and other abiotic conditions (Rohitha et al., 1985; Milne & Bishop, 1987; Leathwick, 1989). The initial populations used in this study were chosen to represent populations early in the season, in the early stages of lucerne re-growth after a harvest. At this time there would be small numbers of adults that had immigrated or overwintered, and there would have been minimal reproduction of the aphids and no reproduction of lacewings or parasitoids. The initial populations values used were based on previous field studies and the population structure was simplified both to conform to the constraints of modelling in Excel and also to ensure the resulting dynamics could be easily interpreted (Chapter 3; Robinson, K.A., unpublished; Rohitha et al., 1985; Milne & Bishop, 1987; Leathwick, 1989). Because of the natural variability in these populations and uncertainty as to what values might be ‘representative’, a range of initial populations was used. Thirty values of the initial population of aphid nymphs (300–1025 An m\(^{-2}\)) were combined with 19 ratios of aphid nymphs to adult lacewings (20–200), to give 570 initial population scenarios. In each of these scenarios the
initial population of adult aphids was half the population of aphid nymphs and the populations of lacewing eggs, larvae and pupae were zero. These scenarios were run with and without two adult parasitoids per square metre.

The equations describing the population dynamics included 36 parameters (Appendix 3, Table A3.2). Where possible, values for these were based on my own work with *M. tasmaniae* (Chapter 1, Appendix 2) and *A. zealandica* (Chapter 4, Appendix 2), or published studies of *A. zealandica* (Jonsson *et al.*, in press) and *A. pisum* (Campbell *et al.*, 1977). In particular, studies of insects able to consume floral resources and insects not provided with flowers were used to determine the relative values of parameters for the simulations with and without flowers. The empirical data used to determine the parameter values were all recorded at approximately 20 °C. Where there were no data available, ‘reasonable’ values were chosen. These values together formed the parameter set for the ‘standard parameters’ scenario.

The sensitivity of the dynamics to the chosen values was investigated by running the model with alternative parameter sets. Given the high number of parameters, it was not possible to run all permutations of multiple values of each parameter. A selection of 27 scenarios was therefore constructed, in which one or more parameters were altered compared with the ‘standard’ scenario. Since there was a high degree of uncertainty associated with many of the parameter values and they may vary greatly in reality, the sensitivity analysis usually involved doubling and halving these values. These alternative parameter sets are described in Appendix 3, Table A3.3.

In addition to the alternative scenarios based on systematically altering parameter values according to their uncertainty, two scenarios were constructed using different data to determine the values (Appendix 3, Table A3.3). First, in a scenario with an aphid resistant lucerne cultivar, the fecundity of adult aphids and their longevity were both halved (based on data in Sandmeyer *et al.*, (1971)). Second, the aphid and lacewing parameter values were adjusted by temperature correction factors to model the dynamics at 15 °C. The temperature correction factors were determined using published data for the development times for lacewings and aphids (Campbell & Mackauer, 1975; Campbell *et al.*, 1977; Leathwick, 1989) and applied to all parameters except the functional response parameters which defined the aphid densities at which half the maximum rates occurred. The differing temperature responses of lacewings and aphids meant that lacewing processes slowed down more than aphid processes. The 15 °C simulations were run for longer than the 20 °C simulations to model an equivalent degree-day period (see below) and were only run without parasitoids. The results of this sensitivity analysis are summarised here and presented in detail in Appendix 3.
7.3.3 Running the model and interpreting the output

The duration of the 20 °C simulations was 21 days (420 time steps). This was the approximate degree-day equivalent of a single period of lucerne re-growth in one of the studies used to determine the initial populations (Chapters 3, 5 and 6; Robinson, K.A., unpublished), based on average temperatures in that region for the previous five years (Lincoln University weather station). Simulations using the 15 °C parameter set were run for an equal number of degree-days, equivalent to 28 days (assuming a lucerne growth threshold of zero degrees). The model determined the size of each population at each time step during this period and then summarised the dynamics as three key variables: aphid-days accumulated during the simulation and the populations of pupa and adult lacewings at the end of the simulation. The latter were expressed as actual final populations or as rates of survival as appropriate. These key variables were chosen to allow the efficacy of biological control in different simulations to be compared, and to provide indicators of the potential for biological control in the future.

For each simulation the aphid-days variable was calculated as the sum of the aphid populations in each time step, divided by the number of time steps per day. This provided a measure of the combined nymph and adult populations over the course of the simulation. The variable did not take account of the varying sensitivity to aphids of the lucerne as it developed. However, in the absence of sufficient data to correct for this, it provided a simple means of comparing the potential for aphid damage across a large number of simulations. Comparisons of the aphid-days variable for simulations with and without lacewings allowed the efficacy of biological control by the lacewings to be determined for a range of initial aphid and lacewing populations (see Section 7.3.2). Comparisons of simulations with lacewings in the presence and absence of flowers then allowed the effect of flowers on biological control to be assessed. The effect of flowers was calculated as the aphid-days in simulations with flowers divided by the value in simulations without flowers. A value of less than one therefore indicated a beneficial effect of flowers on biological control. (As explained above, the parameter values and simulation duration did not allow parasitoids to affect the aphid-days variable so comparisons with and without parasitoids were not made.)

In addition to considering these relative values, the absolute values of the aphid-days variable were compared with an injury level of 50,000 aphid-days m⁻². Economic injury levels are pest populations at which the potential loss of crop yield justifies the cost of intervention to control the pest (Pedigo & Rice, 2006). These vary with the potential pest population dynamics, the crop sensitivity to given pest populations and the economic costs of yield loss and intervention. Pest management guidelines use action thresholds which are lower than economic injury levels to ensure the injury level is not exceeded while the control measure takes effect. Economic injury levels for pea aphids in New Zealand lucerne could not be found. Action
thresholds for other aphid species were available but were expressed as individuals per sweep-net sample rather than per unit area (Anon., 2003). The injury level of 50,000 aphid-days m$^{-2}$ was therefore derived from a stem-density injury level for a pea aphids in Minnesota, USA (Cuperus et al., 1982), calibrated for the stem densities found locally (Robinson, K.A., unpublished). The economic components were not adjusted for local conditions. This proposed injury level was used as a guide to whether biological control was satisfactory i.e. whether aphid populations were reduced to a level at which they would be unlikely to cause significant crop damage, rather than just the degree by which they were reduced. Since the value of the model output is ecological rather than agronomic, this method was considered satisfactory.

At the end of a re-growth period, when the lucerne is grazed or cut, the majority of the crop foliage is removed. This was assumed to destroy any lacewing eggs or larvae present at that time. Lacewings often pupate at the base of plant stems or in the soil (Hilson, 1964; Leathwick, 1989) so it was assumed that this life-stage could survive. With their ability to fly, adult lacewings could also survive by temporary or permanent emigration. The populations of lacewing pupae and adults at the end of the simulations were therefore used as measures of the potential for biological control in the future. The relative values of these variables were compared across simulations with and without flowers and with and without parasitoids for the range of initial populations. The effects of flowers in the presence and absence of parasitoids were calculated as the values with flowers divided by the values without flowers. For these variables, values greater than one indicated beneficial effects of flowers.

In order to provide a guide as to what final populations constituted ‘high’ or ‘low’ potential for biological control, the pupa and adult populations were combined and used as new initial populations for additional simulations. The initial aphid population that could be kept below the injury level was then determined. Since the initial aphid population had to be determined by trial-and-error, only selected populations were subjected to this process. The calculations assumed that all the lacewing pupae emerged and that original and new adults recolonised the crop without mortality. This is clearly unrealistic but the additional simulations are nevertheless useful in interpreting the significance of the final population variables within the model system.

### 7.4 RESULTS AND DISCUSSION

As described in Section 7.3.3, simulations were run with and without lacewings, parasitoids and flowers in various combinations, and the key output variables were the aphid-days accumulated and the populations of lacewing adults and pupae at the end of each simulation. Taking aphid-days as an indicator of the efficacy of biological control within the simulated period of regrowth and the lacewing populations as indicators of the potential for biological control in the future, the results are presented below in five sections: 1) aphid populations in the absence of
lacewings, 2) efficacy of biological control by lacewings within the simulated re-growth period in the absence of flowers, 3) potential for biological control in the future in the absence of flowers, 4) effect of flowers on biological control by lacewings in the absence of parasitoids, within the simulated re-growth period and in the future, 5) effect of flowers on biological control by lacewings in the presence of parasitoids, within the simulated re-growth period and in the future. In these sections the results are discussed within the context of the model system. Section 7.5 discusses the model system itself, in relation to the real world.

7.4.1 Aphid populations in the absence of lacewings

With the standard parameter set in the absence of lacewings, the number of aphid-days accumulated was 630 times the initial number of aphids, a range of approximately 300,000–1,000,000 aphid-days m\(^{-2}\) for the range of initial populations used (Fig. 7.2). In all cases therefore, the populations greatly exceeded the injury level of 50,000 aphid-days m\(^{-2}\), proposed here as a guide to the level where significant damage to the lucerne crop might occur. For the first nine days, growth was relatively slow because only the founding adults were reproductive. After that time an increasing number of aphid nymphs became reproductive and the population increased rapidly.

Although the model allowed unrealistically high aphid populations to be reached (because aphid mortality, development and reproduction were not density dependent), in practice intervention to prevent crop damage would probably occur prior to density dependence becoming important. The simulations were not intended to predict aphid populations under these conditions, but to indicate whether or not unacceptable aphid populations developed in the absence of predation. The populations clearly did reach unacceptable levels with the standard parameter set across the range of initial populations simulated, so it is interesting to explore the conditions under which predation by lacewings can prevent such high populations arising.

7.4.2 Efficacy of biological control by lacewings within the simulated re-growth period in the absence of flowers

When lacewings were included in the simulations with the standard parameter set, the aphid-days accumulated were reduced, but the degree to which this occurred was dependent both on the initial ratio of aphid nymphs to adult lacewings and on the absolute size of the initial populations (Fig. 7.3). Decreasing the initial populations or the initial ratio (fewer aphids per lacewing) always increased the efficacy of biological control (i.e. decreased the aphid-days accumulated relative to the situation without lacewings), but two distinct sets of initial conditions could be distinguished according to their pattern of sensitivity. With initial An:La > 65 (depending slightly on initial An), the aphid-days variable was relatively sensitive.
Fig. 7.2 Aphid populations in the absence of lacewings. The aphid populations are measured as aphid-days accumulated during the simulations, for a range of initial populations in the absence of lacewings, flowers or parasitoids. The dashed line indicates the injury level of 50,000 aphid-days m\(^{-2}\). (An = aphid nymphs.)

Fig. 7.3 Efficacy of biological control by lacewings within the simulated re-growth period. The graph shows the aphid-days accumulated with lacewings as a proportion of the aphid-days accumulated without lacewings, for a range of initial populations (without flowers or parasitoids). Values < 1 indicate the lacewings reduced the aphid populations, with smaller values indicating more effective biological control. (An = aphid nymphs, La = lacewing adults.)
to initial An:La and also varied slightly with initial An at low values. With initial An:La less than this ‘critical ratio’, aphid-days was markedly less sensitive to the initial populations.

The pattern of varying sensitivity to initial populations can be understood by considering the population dynamics over the duration of the simulation. In doing so it is useful to distinguish four phases within the simulation, characterised by the key processes affecting the aphid nymph population. These are identified in Fig. 7.4i and described below. For most of the simulation period the adult aphid populations constituted only a small proportion of the total aphid populations so their dynamics had a negligible affect on the aphid-days accumulated and are not considered here.

1) Days 0–6: Adult lacewings were consuming aphid nymphs but at a lower rate than the founding adult aphids were reproducing, so the total aphid population increased.

2) Days 6–10: On Day 6 lacewing larvae started to appear, as determined by the parameter value for the development time of lacewing eggs. They consumed aphids so total consumption of aphids (by adults and larvae) increased. The aphid growth rate declined and usually became negative as consumption exceeded reproduction by the founding adults. As the aphid population fell, the lacewings were sometimes unable to consume their maximum intake of aphid nymphs so they suffered starvation-induced mortality and the fecundity of the lacewing adults was reduced. Under conditions where mortality occurred, it immediately reduced consumption of aphid nymphs.

3) Days 10–21: From Day 10 aphid nymphs started becoming adults and so started producing additional aphid nymphs. From Day 13 the lacewing larvae started pupating, reducing consumption of aphid nymphs. With some initial conditions, starvation-induced mortality and reduced fecundity continued, and lower larval populations resulting from previous reduced fecundity led to consumption being further reduced. In this phase, the balance of reproduction and consumption varied with the initial conditions. At high initial An:La ratios the aphid population started to grow again and continued to grow rapidly until the end of the simulation. At low initial An:La ratios, the aphid population continued to decline and sometimes reached very low levels, with extinction only being avoided because lacewings could not consume adult aphids. The ratio at which the dynamics switched between population decline and growth varied slightly with the absolute initial population (Fig. 7.4ii–v). This corresponds to the ‘critical ratio’ referred to above. Higher initial populations sometimes led to lower populations in Phase 3 due to the greater consumption by the greater number of lacewing larvae produced by the greater fecundity of lacewing adults when the nymph population was high.
Fig. 7.4 Effect of varying initial populations on the dynamics of the aphid nymph populations. (Continued overleaf.) Simulations were run with lacewings and without flowers or parasitoids. (An = aphid nymphs, La = lacewing adults.) i) Varying initial An:La with constant initial An = 600. See Section 7.4.2 for a description of the phases marked on the graph. In Phase 4, with low initial An:La the aphid population starts to increase but the increase is too small to be apparent at this scale.

4) As the lacewing larvae pupated and died but were not replaced due to previously-reduced lacewing fecundity, consumption of aphids was reduced and the aphid population sometimes started to grow again near the end of the simulation, but did not have time to reach high levels.

The variation in sensitivity to initial conditions of aphid-days apparent in Fig. 7.3 was a result of the divergent dynamics at low and high initial An:La ratios in Phase 3. At high ratios the prolonged period of rapid growth in Phase 3 meant most of the aphid-days were accumulated in this phase. With this growth, small differences in the initial conditions that led to small differences at the start of Phase 3 were exaggerated so that by the end of the simulation there were large differences in aphid-days (Fig. 7.4v). At low An:La ratios, most of the aphid-days were accumulated in Phases 1 and 2 because the aphid population was kept at a low level in Phase 3 (Fig. 7.4ii). Without the rapid divergence of populations at different ratios in Phase 3, there was lower sensitivity to initial conditions for these scenarios.

Although lacewings ‘controlled’ the aphid population below approximately An:La = 65, in the sense that the aphids did not achieve a prolonged period of rapid growth as in the absence of predation, the level of aphid-days accumulated was only below the injury level for those scenarios where the initial population was also sufficiently small (Fig. 7.5). In only 19.5% of the
Varying initial An with constant initial An:La:

- ii) An:La = 50,
- iii) An:La = 65,
- iv) An:La = 70
- v) An:La = 80.

Initial population scenarios were aphid populations kept below damaging levels (50,000 aphid-days m$^{-2}$). In this model system therefore, with the standard parameter set lacewings always reduced the aphid population but only led to effective biological control when initial population sizes and ratios were at the low end of the ranges simulated.

### 7.4.3 Potential for biological control in the future in the absence of flowers

With the standard parameter set, the duration of the simulation did not allow time for a new generation of lacewing adults to appear, or for the adult lacewings to reach their maximum longevity. The final population was therefore a result of background and food-dependent mortalities acting on the initial population. In the absence of flowers, the food-dependent mortality was a function of the aphid nymph population. The different dynamics associated with low and high An:La ratios (discussed in Section 7.4.2) are apparent in the pattern of adult lacewing survival (Fig. 7.6). With An:La > 65 (depending slightly on initial An) the survival of
**Fig. 7.5 Aphid populations in the presence of lacewings.** The graph shows the aphid-days accumulated during the simulations for a range of initial populations when lacewings were present (without flowers or parasitoids). The thick dashed line indicates the injury level of 50,000 aphid-days m$^{-2}$. (An = aphid nymphs, La = lacewing adults.)

**Fig. 7.6 Proportion of adult lacewings surviving to the end of the simulation.** The results are shown for a range of initial populations without flowers or parasitoids. (An = aphid nymphs, La = lacewing adults.)
adult lacewings was almost constant (at the background level) across the range of initial populations simulated; only at low initial An was survival slightly reduced, due to food-dependent mortality at the start of the simulation. At lower initial ratios, consumption by adult and larval lacewings reduced the aphid nymph population to levels where food-dependent mortality of adult lacewings was significant. With these initial conditions, survival decreased slightly as initial An increased because the higher aphid population early in the simulation led to more lacewing larvae later in the simulation and they subsequently reduced the aphid population to lower levels than were reached with lower initial An (as in Fig. 7.4ii). Over the duration of the simulation this led to a slight overall reduction in survival of the lacewings at low initial An:La and high initial An.

The way in which survival varied with initial populations meant that the population of adults available for recolonisation, and hence the potential for future biological control, was greatly reduced when the aphid population had been kept below the proposed injury level in the existing crop, in comparison to those scenarios where the aphid populations had been able to grow rapidly due to slightly different initial conditions. Counter-intuitively, a higher initial density could be associated with a lower final density of adult lacewings.

Whether or not the aphid population underwent sustained growth in Phase 3 also had a strong influence on the final number of lacewing pupae (Fig. 7.7). Above An:La = 65, the population increased as initial An increased and as initial An:La decreased, in direct proportion to the initial populations of lacewing adults. Below this ratio, the final number of lacewing pupae first decreased then increased again as the An:La ratio decreased.

Only those lacewing larvae hatching before Day 14 pupated within the simulation so the processes acting on these larvae must be considered to explain this pattern. The mortality of lacewing larvae is a function of the number of aphid nymphs and at An:La < 65, the excess of nymph consumption over production led to the nymph populations reaching levels where larval mortality was significant. This mortality was highest at the lowest An:La ratio. However, there were initially most lacewing larvae at this ratio. The number of larvae which pupated resulted from the balance between the rate of mortality and the size of the population this was acting upon. With the standard parameter set, this balance varied with An:La such that when 40 > An:La > 60, the final number of lacewing pupae decreased as the initial number of lacewing adults (and hence larvae) increased. At lower ratios, although the number of pupae produced per initial adult was lower, the overall number of pupae again increased.

In contrast with the situation for adult lacewings where aphid control was always at the expense of the adult lacewings available for recolonisation, high populations of pupae for recolonisation were obtained in some situations where control of aphids had been effective (high initial An, low initial An:La). Although both lacewing larvae (which became pupae) and adults suffered high mortality when the aphid populations were ‘depleted’, for the population of
**Fig. 7.7 Number of lacewing pupae at the end of simulation.** Results are shown for a range of initial populations without flowers or parasitoids. Higher values indicate greater potential for biological control in future crops. (An = aphid nymphs, La = lacewing adults.)

**Fig. 7.8 Effect of flowers on biological control by lacewings within the simulated re-growth period.** The effect of flowers is calculated as the aphid-days accumulated in simulations with flowers divided by the aphid-days accumulated in simulations without flowers. The results are shown for a range of initial populations without parasitoids. Values < 1 indicate flowers improved biological control. (An = aphid nymphs, La = lacewing adults.)
pupae this could be counter-balanced by additional recruitment that was not possible for the adults within the duration of the simulation. Nevertheless, for a narrow range of ratios aphid control was at the expense of lacewing pupae, and therefore potential for biological control in the future.

The minimum combined final adult and pupa lacewing populations was 19.33 m$^2$ (from initial $A_n = 300$ m$^2$, $A_n:La = 200$, the scenario with the smallest initial number of lacewing adults). If these insects were all to recolonise a crop as adults, the initial aphid population could be approximately $A_n = 850$ m$^2$ before the proposed injury level of 50,000 aphid-days m$^2$ was exceeded. The maximum density for recolonisation was 230.85 m$^2$ (from initial $A_n = 1025$ m$^2$, $A_n:La = 20$, the scenario with the largest initial number of lacewing adults); a population which could achieve control with approximately $A_n = 2825$ m$^2$. These values do not take account of mortality during pupa development or during any adult dispersal. However, they serve as a guide to what level of final population would be necessary for effective biological control in the future. They suggest that, within the model system, even when the lacewing density was at a level where it could not control the lowest aphid density in the current re-growth period, sufficient lacewings could be produced to control high aphid densities in future crops.

### 7.4.4 Effect of flowers on biological control by lacewings in the absence of parasitoids

#### Effects within the simulated re-growth period

In the model system, flowers could not affect the aphid population directly but did so via their effect on predation by lacewings. Their effect, measured as the aphid-days accumulated with flowers divided by that without flowers, was always beneficial ($< 1$) (Fig. 7.8). Flowers reduced aphid-days by increasing the fecundity of lacewing adults when aphid populations were low, which subsequently increased the population of lacewing larvae and increased predation of aphid nymphs. However, this effect was negligible ($> 0.95$) for the majority of the initial conditions simulated. Section 7.4.2 discussed how below a critical initial $A_n:La$ ratio the aphid population declined in Phase 3, and above this ratio the aphid population increased. Comparison of Fig. 7.8 and Fig. 7.5 reveals that the effect of flowers was greatest close to this ratio and this observation can be similarly explained by considering the processes active in the four phases of the simulation.

In Phase 1, the growth of the aphid population was higher in the presence of flowers because they reduced nymph consumption by the adult lacewings (Fig. 7.9). However, the effects on aphids of consumption by the adults alone were small, and the effects of flowers on this were negligible. During this time, flowers also increased the fecundity of the lacewing adults. With the appearance of lacewing larvae in Phase 2, the increased fecundity was associated with increased consumption of aphid nymphs by the greater numbers of larvae and the aphid population declined more rapidly. The aphid population was then lower when flowers
Fig. 7.9 Effect of flowers on the dynamics of aphid nymph populations in the presence of lacewings. Simulations were run with initial An=300, without parasitoids. Smaller values indicate flowers had greater beneficial effects on biological control. i) Initial An:La = 30, effect of flowers = 0.98, ii) Initial An:La = 60, effect of flowers = 0.82, iii) Initial An:La = 90, effect of flowers = 0.91. The effect of flowers is calculated as the aphid-days accumulated in the simulation with flowers divided by that in the simulation without flowers. (An = aphid nymphs, La = lacewing adults.)
were present. At low ratios (Fig. 7.9i) the aphid population fell to low levels with and without flowers. Since most aphid-days were accumulated in Phases 1 and 2, when small positive and negative effects of flowers counteracted each other, the effect of flowers on total aphid-days was small. At intermediate ratios (Fig. 7.9ii), flowers had a greater effect on fecundity, but more importantly, any effect on fecundity could lead to strongly diverging aphid populations in Phase 3 because it changed the dynamics from aphid population growth to decline. At high An:La ratios (Fig. 7.9iii), the proportional difference between the aphid populations with and without flowers was small because lacewing fecundity was only affected by low nymph populations for a short time, and there were consequently only small differences in the numbers of lacewing larvae.

With some initial populations, flowers prevented resurgence of the aphid population near the end of the simulations (Fig. 7.9i). This had a negligible effect on aphid-days in the 21-day simulations and in practice when the crop was at this growth stage, relatively large aphid populations could be supported without the damage that would be incurred by smaller plants early in the season. However, were the re-growth period to be extended, this suppression could become significant.

It is evident that within the model system with the standard parameter set, flowers could have a fundamental effect on the dynamics of the aphid and lacewing populations by changing them between states of decline and growth in the latter half of the re-growth period. This switch only occurred under a narrow range of initial An:La ratios and was only associated with substantial changes in the aphid population at low initial An. Nevertheless, in these situations the aphid-days accumulated during the simulation were in the region of the proposed injury level (for effects < 0.90 aphid-days were 32,000–84,000 m⁻²), suggesting that the effect on biological control could be significant. (If aphid populations were much lower or much higher than the injury level both in situations with and without flowers, their relative effects would not be of agronomic relevance.) In general however, across the range of initial conditions simulated, flowers had a negligible effect on biological control. The percentage of initial population scenarios where aphid populations were below the injury level only increased from 19.5 to 20.1%.

**Effects on the potential for biological control in the future**

The model was constructed so that in the presence of flowers, lacewings died at a constant background rate, and in the absence of flowers they died at a higher rate which tended towards the background rate as aphid density increased. Flowers therefore had a beneficial effect under all conditions, but running the model revealed that the magnitude of this effect varied considerably with initial conditions (Fig. 7.10). At ratios greater than An:La = 65, survival was near maximal and the increase in survival with flowers was relatively small (1.03–1.16). The
**Fig. 7.10 Effect of flowers on the survival of adult lacewings.** The effect of flowers is calculated as the survival in simulations with flowers divided by the survival in simulations without flowers. The results are shown for a range of initial populations without parasitoids. Values > 1 indicate a positive effect of flowers on the potential for biological control in the future. (An = aphid nymphs, La = lacewing adults.)

**Fig. 7.11 Effect of flowers on the number of lacewing pupae at the end of the simulation.** The effect of flowers is calculated as the final number of pupae in simulations with flowers divided by the number in the absence of flowers. Values are shown for a range of initial populations, without parasitoids. Values > 1 indicate a positive effect of flowers on the potential for biological control in the future. (An = aphid nymphs, La = lacewing adults.)
effect decreased consistently as the ratio increased and initial An increased. At lower ratios (An:La < 65), the effect of flowers increased as ratio decreased but showed a variable response to initial An. The greater and lesser effects of flowers mirrored the survival in the absence of flowers; where decreasing An:La (higher initial La) was sometimes associated with increased food-dependent mortality and consequently with counter-intuitive lower final populations of lacewings (Section 7.4.3), the effect of flowers was slightly greater.

It should be noted that the effect of flowers (measured as the survival of lacewings with flowers divided by the survival without flowers) was sensitive when the survival of lacewings without flowers was very low. For example, a change in initial populations which increased the proportion of lacewings surviving from 0.05 to 0.06 resulted in the effect of flowers changing from 7.00 to 5.83 (survival with flowers being 0.35 in both cases).

When the initial number of lacewings was low, with flowers the final number available for recolonisation was still low (min = 0.52 m$^{-2}$ with initial An = 300 m$^{-2}$, initial An:La = 200). However, the presence of flowers removed the trade-off between current and future capacity for biological control, with high initial populations being followed by high final populations (with initial An = 1025 m$^{-2}$ and initial An:La = 20, final La with flowers = 17.8m$^{-2}$ and without flowers = 2.38m$^{-2}$).

Flowers had mixed effects on the final number of lacewing pupae (Fig. 7.11). Above a ratio of approximately An:La = 65 the effect was always beneficial, though small (< 1.08). Below this ratio, the effect was negative or positive but again the effects were small (0.97–1.05). The effects were more positive or less negative at lower initial An, except at with An:La = c50, where decreasing An was associated with a more negative effect of flowers.

As explained in Section 7.3.1, flowers increased the fecundity of adult lacewings when aphid populations were low, increasing the subsequent larval population relative to that without flowers. Above An:La = 65, there was no food-dependent mortality of the larvae so the small increases in fecundity early in the simulation resulted in small increases in the final number of lacewing pupae. The effect of flowers was slightly lower at higher initial An and higher ratios as the fecundities with and without flowers converged on the maximum value with increasing aphid density. Below An:La = 65, the final number of pupae depended on the balance between increased populations of larvae arising from higher fecundity and the higher rates of food-dependent mortality associated with these populations. In the same way that lower initial An:La could result in fewer lacewing pupae (Section 7.4.3), flowers could similarly have a negative effect on the final populations of pupae through their effect on increased fecundity. This only occurred over a narrow range of initial An:La and at the lowest ratios the balance of additional mortality and additional production of larvae was again positive. At these low ratios (An:La < 65) the effect of flowers increased as initial An:La decreased at low initial An, but the relationship was reversed with high initial An. However, this complex pattern resulting from the
interaction between the many processes in the model is only of theoretical interest since the final number of lacewing pupae only varied by a few percent over this range of initial populations.

The effect of flowers on the combined adult and pupa populations was small (0.99–1.12). The relationship between the effect of flowers and the initial conditions followed a pattern of positive and negative effects similar to that of the effect of flowers on the pupa population. However, the large positive effect of flowers on the adult population at low An:La ratios ensured that the overall effect was negative only at ratios 50 < An:La < 60. The effects on the minimum and maximum combined populations were small (1.085 and 1.046 respectively) so the potential for future biological control within the model system was unaffected by presence of flowers.

7.4.5 Effect of flowers on biological control by lacewings in the presence of parasitoids

Effects within the simulated re-growth period
The model was constructed so that parasitised and unparasitised larvae behaved in exactly the same way. Parasitism could therefore not affect biological control of aphids until the adults emerged. (The adult lacewing population would then be reduced as a proportion of pupae emerged as parasitoids.) Since the re-growth period was too short to allow this to occur, the effects of flowers on the aphid populations was the same when parasitoids were present as when they were absent.

Effects on the potential for biological control in the future
Without flowers the longevity of adult parasitoids was only two days. This meant that they had all died by Day 6 when lacewing larvae became available for them to parasitise. Parasitoids therefore had no effect on the number of lacewing pupae in the absence of flowers.

The parameter values were set so that flowers increased the longevity of adult parasitoids to 20 days. Within this time they were subject to a background rate of mortality so approximately 75% of the population were alive on Day 6 and these parasitoids then parasitised lacewing larvae at a rate dependent on the larval density. In Section 7.4.4 it was shown that in the absence of parasitoids, flowers could have positive or negative effects on the lacewing populations available for future biological control. In the presence of parasitoids, by increasing parasitoid longevity, flowers enabled an additional process by which lacewing pupa populations could be reduced.

The relationship between initial populations and the proportion of lacewing larvae parasitised reflected the way in which parasitism was modelled as a Type II functional response and the variable density of lacewing larvae (Fig. 7.12). The proportion of parasitised pupae increased as the initial number of adult lacewings decreased (lower initial An, higher initial
**Fig. 7.12 Parasitism of lacewing pupae in simulations with flowers.** The graph shows the proportion of lacewing pupae that were parasitised in simulations with parasitoids and flowers, for a range of initial populations. The simulations started with a density of 2 adult parasitoids m$^{-2}$. (An = aphid nymphs, La = lacewing adults.)

**Fig. 7.13 Effect of flowers and parasitoids (in combination) on the final number of unparasitised lacewing pupae.** The graph shows the final number of unparasitised lacewing pupae in simulations with flowers and parasitoids divided by the number without flowers or parasitoids, for a range of initial aphid and adult lacewing populations. The simulations started with a density of 2 adult parasitoids m$^{-2}$. Values < 1 indicate loss of potential for biological control in future crops. (An = aphid nymphs, La = lacewing adults.)
An:La) but with a non-linearity at ratios where higher initial numbers of lacewings led to larval mortality and consequently to lower larval populations than expected (Section 7.4.3). The proportion of parasitised pupae ranged from 0.065 to 0.84. Since the total number of pupae was unaffected, the proportion of parasitised pupae was directly proportional to the reduction in the final number of lacewing pupae. A parasitised proportion of 0.84 corresponded to a reduction in the combined final adult and pupa populations from 20.9 to 3.79 m$^{-2}$ and a reduction from 875 to 275 m$^{-2}$ in the approximate initial density of aphid nymphs which could be kept at the injury level of 50,000 aphid-days m$^{-2}$ (once the pupae became adults and without further mortality). This indicates the loss of biological control potential caused by the presence of parasitoids when flowers are present.

These figures indicate that within the model system with the standard parameter set, flowers enabled parasitoids to have a significant negative effect on the potential for biological control in the future under some initial conditions. However in the present context, interest principally lies in how the negative effects of parasitism relate to the direct positive and negative effects of flowers on lacewing pupae within the model system. In particular, were the positive effects of flowers that were observed in the absence of parasitoids still seen in the presence of parasitoids, albeit to a smaller degree, or did parasitoids have an overriding effect so that flowers inevitably reduced the potential for biological control when parasitoids were present?

Fig. 7.13 shows that the negative effects of parasitoids do more than outweigh the positive effects of flowers: the final number of lacewing pupae with flowers and parasitoids is always lower than that without flower or parasitoids (values < 1 in Fig. 7.13). Because the effects of parasitism dominated the direct effects of flowers on lacewing pupae, the way in which the former varied with initial populations was apparent in their combined effects. The effect of parasitoids with flowers (comparing simulations with both to those with neither) varied from 0.17 at the lowest initial An and An:La (lowest initial La) to 0.91 at the highest initial An and An:La, with a slight non-linearity as discussed above.

The negative effect of parasitism which contributed to the effect of 0.17 is cited above as contributing to a reduction from 875 to 275 m$^{-2}$ in the approximate initial density of aphid nymphs which could be kept at the aphid injury level in the presence of flowers by the combined pupa and adult populations. With the direct positive effect of flowers on the populations of lacewing pupae and adults removed, the density which could be controlled in the absence of parasitoids was approximately 825 m$^{-2}$. Therefore, in the presence of parasitoids, adding flowers to the system would result in a loss of biological control potential (by both pupae and adults) from 825 to 275 aphid nymphs m$^{-2}$ in this worst-case amongst the initial conditions tested. In the best case, the effect was negligible; the effect of 0.91 on lacewing pupae was partly counteracted by a positive effect on the lacewing adults so that the combined
total populations were 226.7 and 230.85 m$^{-2}$ with and without flowers respectively. These correspond to non-damaging initial densities of aphid nymphs of approximately 2750 and 2825 m$^{-2}$, both of which are very high.

In the absence of flowers and parasitoids, the final populations of lacewing adults and pupae were high enough to provide effective biological control for initial aphid nymph densities of up to 850 m$^{-2}$. This could still be achieved in approximately 85% of the initial population scenarios tested when flowers and parasitoids were present, with substantial loss of biological control potential in just a few scenarios.

7.4.6 Comparison of effects of flowers with effects of initial populations

In Sections 7.4.1–7.4.5 the effects of flowers on aphid populations were presented as aphid-days in simulations with flowers divided by aphid-days in simulations without flowers. In order that the biological significance of these changes could be more readily appreciated, particular magnitudes of effect were converted into increases or decreases in the controllable aphid populations which occurred when flowers were present. It is also useful to compare the effects of flowers with the changes in aphid-days that occur when other components of the system change. Fig. 7.14 and Fig. 7.15 show the effects of changing the initial populations relative to a moderate initial population of An = 650 m$^{-2}$, An:La = 100 for the aphid-days accumulated and the final number of lacewing pupae respectively. Comparing these figures to those illustrating the effects of flowers (Fig. 7.8, Fig. 7.11), it can be seen that small changes in the initial populations were equivalent to the largest effects of flowers. If the model system comprised multiple crops with a range of initial populations similar to those modelled here, the variation in aphid-days or pupa populations due to presence or absence of flowers would be small compared with that arising due to the varying initial populations. Since initial populations would vary in practice, this supports the interpretation of the effects of flowers as being ‘small’.

7.4.7 Sensitivity analysis

Sections 7.4.1–7.4.5 described and discussed the model output when a standard parameter set was used in simulations run for a range of initial populations, with and without flowers. In Appendix 3, Section A3.3, the results of running the model with the 27 alternative parameter sets are presented. These show that the conclusions drawn from the standard output were generally robust to the parameter values used. The important exceptions were substantial changes in the proportion of initial populations with effective biological control when the fecundity of the aphids was changed relative to the lacewing parameter values. This occurred with the ‘resistant lucerne’ and ‘15 °C’ parameter sets. In the absence of lacewings, the aphid-days accumulated in these scenarios were substantially lower and higher respectively, than with
Fig. 7.14 Effect of varying initial populations on the aphid populations, in the presence of lacewings. The graph shows the aphid-days accumulated during simulations for a range of initial populations as a proportion of those accumulated with initial An = 650, initial An:La = 110 (without flowers or parasitoids). The actual aphid-days accumulated for each set of initial conditions are shown in Fig. 7.5. The wide range of values in this graph, compared with the range in Fig. 7.8 showing the effects of flowers, reveals the aphid populations are more sensitive to varying the initial populations across the range simulated than the presence of flowers. (An = aphid nymphs, La = adult lacewings.)

Fig. 7.15 Effect of varying initial populations on the number of lacewing pupae at the end of the simulation. The graph shows the final number of lacewing pupae for a range of initial populations as a proportion of the number with initial An = 650, initial An:La =110 (without flowers or parasitoids). The actual numbers of pupae for each set of initial conditions are shown in Fig. 7.7. The wide range of values in this graph, compared with the range in Fig. 7.11 showing the effects of flowers, reveals the lacewing pupa populations are more sensitive to varying the initial populations across the range simulated than to the presence of flowers. (An = aphid nymphs, La = adult lacewings.)
the standard parameter set. When lacewings were present, there was effective biological control across many more initial populations in the ‘resistant lucerne’ scenario but biological control was never effective in the ‘15 °C’ scenario.

The degree of reduction in biological control potential caused by parasitism of lacewings was sensitive to the functional response of the parasitoids. For some parameter values at high initial An:La and low initial An (i.e. low initial La), parasitism effectively led to loss of a lacewing population for recolonisation when flowers were present.

With the standard parameter set, the effect of flowers on the aphid-days variable was always beneficial. However, it was detrimental at low initial An:La ratios for some alternative parameter sets. This effect was of greatest magnitude and occurred in the highest proportion of initial populations in the ‘resistant lucerne’ scenario. Although interesting to note, this effect is unlikely to be of agronomic significance since the largest detrimental effect was 1.11 and this occurred when aphid populations were well below the proposed injury level with or without flowers (see Appendix 3, Section A3.3).

7.5 General Discussion

7.5.1 Effects of flowers within the model aphid-lacewing-parasitoid system

Some of the observed effects of flowers and their relationships with initial populations were inevitable consequences of the way the model was constructed, e.g. the increased rate of parasitism with flowers and the decreasing effect of flowers on biological control by lacewings with higher initial aphid populations. However, running the model revealed how positive and negative effects on individual processes combined to produce an overall positive or negative effect. It also showed the importance of the initial ratio of aphid nymphs to lacewing adults, in addition to the actual initial aphid population, and demonstrated that counter-intuitive effects could occur due to the mortality suffered by lacewings when they ‘over-consumed’ aphids as a result of flowers increasing lacewing fecundity.

Across the initial populations simulated, flowers generally had a minor beneficial effect on the ability of lacewings to provide satisfactory control of aphid populations within the simulated period of lucerne re-growth. The potential for detrimental effects of flowers was apparent, but these were also small. Although flowers could provide agronomically significant improvements in biological control for a small proportion of initial populations, if parameter values and initial populations were uncertain these situations would be difficult to identify a priori.

Although the effects of flowers on aphid populations were generally negligible even when key parameter values were halved or doubled, the aphid populations themselves were sensitive to the parameter values. This affected whether predation by lacewings led to effective
biological control. Of particular significance was the ability of lacewings to provide satisfactory control across a majority of initial populations with resistant lucerne, and the lack of control for all initial populations at 15 °C. Together with the sensitivity to initial populations, this reinforces the conclusion that the key influences on the population dynamics were the relationships between lacewings and aphids that were not affected by flowers: it was only at low aphid densities that flowers could affect rates of oviposition and predation by adult lacewings and their rate of mortality, but the effects of lacewings on the aphids were dominated by 1) the high-aphid-density rates of these processes, 2) the processes that could not be affected by flowers and 3) the initial populations.

Although flowers could substantially increase the survival of adult lacewings, in the absence of parasitoids the effects on the populations of pupae were negligible. Since pupae were much more numerous than adults, there were negligible overall effects of flowers on the numbers of lacewings which might be available to recolonise and provide biological control in the future. However, when parasitoids were present, flowers enabled them to survive to parasitise lacewings and the parasitoids reduced the populations of lacewing pupae. Although this effect often resulted in a relatively small reduction in a large population of pupae, when the initial populations of lacewing were small or the parameter values were adjusted to make the parasitoids more effective at parasitising larvae, the populations were reduced to levels where only small future populations of aphids could be controlled.

Within the model system therefore, in the absence of parasitoids any costs of providing flowers would be unlikely to be recovered and in the presence of parasitoids, flowers would reduce the potential for biological control in the future. It follows that providing flowers would not be an appropriate technique for conservation biological control within the system.

7.5.2 Limitations of the model

Although there was a high degree of uncertainty about many of the parameter values used in the simulations, the general conclusions regarding the role of flowers in mediating biological control were found to be robust to these values. In particular, the parameter values defining the slopes of the various functional responses were often found to be less influential than those defining the maximum rates, suggesting that the assumed Type II nature of the functional responses was not critical to the conclusions. It is acknowledged that in the absence of supporting field data, the aphid densities over which the functional responses occurred could be under- or over-estimated by a greater degree than was tested with the alternative parameter sets. The rates of background mortality could have been similarly inappropriate. Nevertheless, the key limitations of this model as a means of investigating the effect of flowers in an aphid-lacewing-parasitoid system seem to lie in the simplifying assumptions on which it was based rather than the uncertainty of the parameter values. Of the numerous inevitable simplifications,
the two deemed to be most significant are the initial population structure and the absence of dispersal.

The initial populations were simplified to adult and nymph aphids in a ratio of 1:2, and adult lacewings and parasitoids, all of which were at the start of their development period or adult lifespan. Although a range of aphid and lacewing populations was simulated, a single parasitoid density was used. The model output was found to be sensitive to the initial populations within this simplified population structure so it is likely that it would be similarly sensitive to changes which made the structure more realistic.

The model was intended to simulate populations in one period of a lucerne cropping cycle, from early in crop re-growth to the time of harvest. In practice, initial populations would therefore not only vary in structure from those in the model, but would also be supplemented by ongoing immigration. This would be most significant for the parasitoids in the absence of flowers: whereas no parasitism occurred in the simulations without flowers because the parasitoids died before lacewing larvae were present, immigration of parasitoids once larvae had appeared would allow parasitism to occur. This would reduce the apparent detrimental effect of flowers on the potential for future biological control.

If flowers affected the rate of dispersal, for example by attracting (and/or retaining) lacewings when aphids were at low density across the landscape, this could greatly increase their impact on the aphid population. Similar effects of flowers on the spatial distribution of parasitoids could also be important (in addition to their effects on longevity and fecundity), and other models have focused on this particular aspect of flower use in conservation biological control (Bianchi & Wäckers, 2008).

Later in the re-growth period, as crop quality declined, alate adult aphids might emigrate. As the aphid population declined (due to predation as well as emigration), lacewing adults might emigrate. Parasitoids might similarly disperse in response to the changing density of lacewing larvae. Production of alate pea aphids is dependent on, amongst other factors, population density and crop quality (Sutherland, 1969a, b; Muller et al., 2001). Omitting this process in the model was not considered to be a significant limitation because under the conditions where dispersal is significant, biological control would probably already have been deemed ineffective. In contrast, the inclusion of lacewing dispersal could have had a profound influence on the conclusions drawn. Lacewings are known to respond to aphid density (Leathwick, 1989) and density-dependent dispersal could have added to the effects of density-dependent fecundity and mortality which were included in the model.

Dispersal was not included in the model because the data required to inform how the process should be modelled were not available. Field-work to provide such data, while difficult, could greatly increase our understanding of the relationships between flowers, aphids, lacewings and lacewing-parasitoids. Further investigation of alternative initial population structures was
not undertaken simply due to the limited time available to develop a model structure that could simulate more complex populations. Developing the model to allow this could enable greater confidence in the conclusions currently drawn, but would be of limited value without the field work to determine how the crops are actually colonised.

In a small proportion of simulations, populations reached densities at which stochasticity could have led to extinction. However, these unrealistic densities would only affect the conclusions drawn from the model if the populations subsequently recovered and influenced the output variables. This did not occur.

7.5.3 From the model system to the real world

The model system cannot be used to make quantitative predictions of field populations. However, the results of the simulations do suggest that we can refine the conclusions that were drawn from the laboratory experiments which informed the development of the model.

Having measured the effects of flowers on the longevity and fecundity of adult lacewings and their rate of aphid consumption in the laboratory, it was concluded that flowers might have positive or negative effects on biological control and that these effects would depend on the timescale under consideration and the density of aphids. While these possibilities remain, it now seems that these effects are unlikely to be agronomically significant because the effects which were substantial when observed in isolation, did not have a commensurate impact on the overall population dynamics.

In addition, the fecundity and longevity of the parasitoid had been found to increase in the presence of flowers, but it was not known how this might balance against any positive effects of flowers on the lacewings. The model indicated that the net effects of flowers is likely to be detrimental to biological control. This suggests that future work on the role of flowers in conservation biological control might best be focused on life-history omnivores rather than true omnivores. Thus, the negative effects on lacewings which arise through the effects on the lacewing-parasitoids could be considered in relation to the positive effects on beneficial parasitoids and hoverflies rather than the lacewings themselves. A significant role for flowers in particular circumstances is not, however, precluded.

Jonsson et al. (in press) and Jacometti et al. (unpublished) studied the populations of aphids and lacewings in field-cages with and without flowers. Parasitoids were present in all cages in the study by Jonsson. They were introduced to half the cages by Jacometti but the introductions were deemed unsuccessful. These studies were not conducted in such a way that they can be used as tests of this model’s validity, but it is appropriate to consider whether the conclusions drawn are consistent with each other.

In both field-cage experiments there was considerable variation between cages within a treatment, demonstrating the importance of a stochastic component of population dynamics that
was not present in the model system. At the time of the first sampling (several weeks after insects had been introduced to the cages), aphid populations in Jonsson’s study exceeded 10,000 m\(^{-2}\) but were approximately 1000 m\(^{-2}\) in Jacometti’s study, and the ratio of aphids to lacewings were approximately 10,000:1 and 100:1 respectively. Presence of flowers reduced the aphid populations over the sampling period in the latter study but not the former. This might indicate that the modelling study underestimated the potential for flowers to enhance biological control by lacewings on this timescale (i.e. before parasitoids can impact upon the lacewing population). It should be noted however, that the dynamics of the aphid populations differed in the two studies and the effect of flowers was observed when the aphid populations fell rapidly to very low densities (often < 10 per 0.08 m\(^{-2}\) sample) from the start of the sampling period.

In relation to the presence of *A. zealandica*, Jonsson et al. (in press) concluded that when the aphid density was high, flowers reduced the lacewing population due to increased parasitism. This is consistent with the findings from the model.

Open field studies would be required to determine whether the dynamics observed in the field cages are common or if they were an artefact of the caged populations, in the same way that the modelled populations were potentially unrealistic. Field studies failed to find evidence of widespread use of a flower strip in a lucerne field by either *M. tasmaniae* or *A. zealandica* (Chapters 3, 5 and 6). Consequently, the value of results of the previous laboratory and field-cage experiments, and the modelling study presented here, should be considered contingent on future evidence that these insects do exploit floral resources under natural conditions. Furthermore, in the absence of flowers, alternative sugar sources such as honeydew may be exploited.

Although the choice of species studied here was driven primarily by their suitability to explore the potential role of floral resources in mediating interactions between beneficial and antagonistic omnivores, the lacewing *M. tasmaniae* may be an important agent of biological control in Australasian agroecosystems (Horne *et al.*, 2001). The process of developing the model has highlighted important gaps in our knowledge of this species’ ecology. Improving knowledge in these areas might assist identification of ways in which the agroecosystem may be managed to enhance the species’ contribution to pest suppression.

The first such gap is our lack of understanding of the within- and between-field dispersal processes which influence the density of lacewings, and how they depend on the prey density and crop growth and harvest. The role of flowers in this process cannot be inferred because, to my knowledge, the attraction of brown lacewings to floral stimuli has not been studied. The dispersal of this species between crops and non-crop habitats that are used as short-term refuges, or for overwintering, is also unstudied.

The second notable gap is our ignorance of the key factors affecting the lacewing population over the course of a year. What are the rates of mortality at each life stage and to
what degree are these rates density-dependent? In particular, what are the consequences for future lacewing populations of any given rate of parasitism? In sweep-net samples of a lucerne field collected over three summer months, adult *A. zealandica* were rare until the second half of this period but adult *M. tasmaniae* did not show a seasonal trend in abundance (Chapter 3; Robinson, K.A., unpublished). Identifying such temporal patterns could be crucial for appropriate implementation of conservation biological control techniques.

The population model developed here has complemented previous empirical studies in two areas: firstly, the role of floral resources in biological control by true omnivores (in contrast to life-history omnivores); and secondly, the effects of flower use by fourth-trophic-level parasitoids that have the potential to disrupt biological control. It has reminded us of the value of extending research beyond studying the effects of flowers on the beneficial life-history omnivores that are typically the target of flower-provision as a pest management technique. The model itself, however, has studied isolated components of an agroecosystem. The challenge of integrating such studies to the benefit of practical pest management remains to be met.
Chapter 8  General Discussion

8.1  ROLE OF FLORAL RESOURCES IN MEDIATING PEST SUPPRESSION BY A TRUE OMNIVORE

The ecology of indigenous and previously-introduced biocontrol agents has received much attention in recent decades and many studies have investigated the role of floral resources in the suppression of pest populations by life-history omnivores such as hoverflies (Diptera: Syrphidae) and parasitoid wasps (Hymenoptera: Parasitica). Reflecting this development, while early reviews identified many of the factors likely to affect these agroecological interactions, recently it has been possible to develop these discussions by drawing on a greater body of empirical evidence (van den Bosch & Telford, 1964; van Emden, 1981; Way & Cammell, 1981; Altieri & Letourneau, 1982; Powell, 1986; van Emden, 1988, 1990; Wratten & van Emden, 1995; Jervis et al., 1996; Barbosa & Benrey, 1998; Barbosa & Wratten, 1998; Bugg & Pickett, 1998; Gurr et al., 1998; Wratten et al., 1998; Gurr et al., 2000; Landis et al., 2000; Gurr et al., 2003; Jervis et al., 2004; Pfiffner & Wyss, 2004; Gurr et al., 2005; Heimpel & Jervis, 2005; Wilkinson & Landis, 2005; Nicholls & Altieri, 2007; Orre et al., 2007; Wratten et al., 2007; Zehnder et al., 2007; Mills & Wajnberg, 2008).

The relationships between pests, flowers and true omnivores are poorly understood relative to those between the lower trophic levels and beneficial life-history omnivores. While the latter are often likely to be of greatest relevance to pest management, interactions involving true omnivores may also be of significance. As described in Chapters 2 and 3, true omnivores consume both plant and animal material in a single life-stage and include species that should be considered if we are to understand the actual and potential functioning of agroecosystems e.g. some ladybirds (Coleoptera: Coccinellidae), true bugs (Heteroptera), lacewings (Neuroptera), thrips (Thysanoptera) and mites (Acarina: Phytoseiidae) (Alomar & Wiedenmann, 1996; Hodek & Honek, 1996; McMurtry & Croft, 1997; Dixon, 2000; Canard, 2001; Coll & Guershon, 2002; Wäckers & van Rijn, 2005).

The effect of floral resources and other plant-derived foods on biological control by true omnivores has rarely been investigated although theory predicts an interesting balance of positive and negative effects could occur (Eubanks, 2005; Eubanks & Styrsky, 2005; van Rijn & Sabelis, 2005). Much can be learned from discussion of use of substitutable resources by generalist predators (e.g. Chang & Kareiva, 1999; Symondson et al., 2002; Harmon & Andow, 2004; van Rijn & Sabelis, 2005) but empirical studies are required to determine the degree to which floral resources and pest prey are substitutable for different biocontrol agents, and how those foods are exploited in the field. This thesis has contributed to our understanding in this area by studying a brown lacewing, Micromus tasmaniae Walker (Neuroptera: Hemerobiidae),
which may contribute to biological control in Australasian agroecosystems (Horne et al., 2001). Consumption of plant-derived material by the larvae has not been studied but the adult stage was found to consume floral resources in addition to prey, confirming its status as a true omnivore (Chapter 2).

The work presented here (Chapter 2) showed that for *M. tasmaniae*, oviposition did not occur when flowers were the only source of food i.e. aphids (prey) were an essential food. However, floral resources could serve as supplementary foods, increasing adult survival and rate of oviposition when there were few aphids available. When aphids were abundant, flowers no longer had any such effects. The varying significance of flowers according to aphid abundance has since been confirmed by further work (Jonsson et al., in press). These effects demonstrated the potential for flowers to enhance biological control by this species when prey densities are low through increasing the size of the lacewing population. However, flowers were also found to decrease *per capita* consumption of aphids by adult lacewings, demonstrating a contrasting potential for flowers to disrupt biological control. Although similar effects have been observed with other true omnivores, this study was unusual in its consideration of both the ‘individual-level’ and ‘population-level’ effects of plant-derived food (Coll & Guershon, 2002; Eubanks, 2005; Eubanks & Styrsky, 2005). This dual perspective is of value since pest suppression is influenced by individual-level effects in the short-term and both types of effect in the long-term.

At the start of this study, foraging behaviour of *M. tasmaniae* was largely unknown so it could not be predicted whether the effects of flowers on *M. tasmaniae* observed in the laboratory would occur in the field. A field study was conducted to help fill this gap by investigating the relative prevalence of prey consumption and flower-feeding (Chapter 3). Lacewings were collected from a lucerne field at distances up to 100 m from a central strip of flowering buckwheat *Fagopyrum esculentum* Moench ‘Katowase’ (Polygonaceae). All dissected lacewings contained prey while only a small proportion of those analysed to determine the presence of pollen in their gut contained sufficient pollen to suggest flower-feeding rather than incidental consumption of dispersed pollen. No lacewings collected more than five metres from the buckwheat contained pollen from that species. Further work is required in this area, both on the methodology that allows inference of flower-feeding (deliberate consumption of nectar and/or pollen) from gut pollen content, and on the use of flowers by lacewings under situations with varying prey densities. Behavioural studies to investigate firstly whether any consumption of floral resources is a result of opportunistic foraging or directed movement towards flowers, and secondly whether such consumption alters subsequent foraging behaviour, would be valuable in helping ascertain the extent to which prey consumption is mediated by the floral resources in this species. Such studies with other true omnivores feeding on pollen and fruits have proved insightful (Eubanks & Denno, 1999; Harmon et al., 2000).
The laboratory and field studies were complemented by use of a simulation model to explore the effect of flowers on aphid and lacewing populations during one of the re-growth periods in a lucerne crop subject to repeated harvesting (Chapter 7). The aphid-days accumulated during the simulation (an indicator of crop damage) and the number of lacewings at the end of the simulation (an indicator of the potential for biological control beyond the simulated period) were determined across a range of initial populations and parameter values. The model output suggested that even if the effects observed in the laboratory did occur in the field, they may not have a substantial impact on the efficacy of biological control. The model incorporated reductions in aphid consumption and increases in longevity and fecundity when flowers were present at low aphid density. When combined with the parameters which were unaffected by flowers, these effects had a relatively small influence on the overall dynamics of the system. Flowers were nevertheless generally beneficial to biological control when lacewing-parasitoids were absent.

Just as studies in the laboratory and field are inevitably limited in the scope of their conclusions, so the output of the simulations must not be over-interpreted. In assessing the limitations of the model, attention was drawn to key processes which were omitted due to lack of data to guide parameterisation, or for which parameter values had to be based on intuition. Little is known about the dispersal of the adult lacewings in response to the regular disturbance of harvesting that characterises many agroecosystems, or their between-field movement in relation to flowers or prey. There is similar degree of ignorance concerning the relationship between food-dependent mortality and other causes of death. Lack of understanding about such processes for this species (and others in the agroecosystem) is presumably due to the difficulty of conducting studies that could provide insight in this area. They would nevertheless be valuable, particularly when trying to elucidate the mechanisms through which resource provision generates the overall effects on pest populations that are observed in field studies.

In combination, the laboratory, field and modelling studies presented in this thesis have demonstrated that the role of floral resources in pest suppression by the true omnivore *M. tasmaniae* is more complex than that for the life-history omnivores which are typically the subject of such studies. The work adds *M. tasmaniae* to a relatively small number of true omnivores which have been studied using multiple approaches, with the aim of understanding the interaction between plant and animal foods in biological control (Eubanks & Styrsky, 2005; van Rijn & Sabelis, 2005). The results invite further work on the role of flowers at key moments of low prey density, such as early in the season when small differences in the relative population sizes of a pest and biocontrol agent can have substantial impacts on subsequent dynamics. If the practical challenges can be surmounted, such work should focus on the foraging of lacewings at the within- and between-field scales.
8.2 ROLE OF FLORAL RESOURCES IN MEDIATING PEST SUPPRESSION IN THE PRESENCE OF A FOURTH-TROPHIC-LEVEL ANTAGONIST

The work evaluated above has furthered our understanding of the implications of true omnivory for pest management by studying the interactions between a true omnivore, a pest and a flower species. However, in an agroecosystem such interactions do not occur in isolation. The importance of studying pest suppression from a food-web perspective rather than linear tri-trophic cascades has been widely recognised (Sunderland et al., 1997; Snyder et al., 2005; Gurr et al., 2007). Nevertheless, few studies have expanded studies of the effects of floral resources on biocontrol agents (third trophic level) to consider their effects on species operating at the fourth trophic level. Any benefit to hyper-parasitoids or parasitoids of predators could counteract the benefits accrued to the biocontrol agent itself and indirectly increase the pest population. Although their significance for pest populations is variable, such fourth-trophic-level antagonists can be abundant in agroecosystems and where they are not at present, they could become more abundant with habitat management to increase biodiversity (Alrouechdi et al., 1984; Rosenheim, 1998; Sullivan & Volkl, 1999).

In this thesis I have presented results which demonstrated that like many other parasitoids, *Anacharis zealandica* Ashmead (Hymenoptera: Figitidae) does not host-feed but can benefit from floral resources, experiencing increased survival and fecundity when flowers are available (Chapter 4). It was found that the species can reproduce without feeding but is synovigenic so increased longevity has the potential to be associated with greatly increased lifetime fecundity. Since *A. zealandica* is a parasitoid of the biocontrol agent *M. tasmaniae*, increasing abundance of flowers in its presence could disrupt biological control; an ironic effect if flowers were being managed to enhance beneficial parasitoids or hoverflies. (This would occur through reduction in the size of the lacewing population since it was found that parasitism of lacewing larvae did not affect their consumption of prey (Appendix 2)). In the field, any disruption to biological control occurring via this mechanism would depend on whether the food provided by flowers was limiting the effect of *A. zealandica* on the *M. tasmaniae* population (Heimpel & Jervis, 2005). If flowering weeds were sufficiently abundant, or alternative foods such as honeydew were available, additional floral resources might be of no consequence.

A field study of the parasitoid in a lucerne field containing a strip of flowering buckwheat did not suggest that these flowers were a valuable resource in this situation. Rubidium-marking did not indicate that a substantial proportion of parasitoids had fed on buckwheat nectar (Chapter 5) and HPLC analysis suggested that consumption of honeydew might have been more frequent (Chapter 6). However, these results did not provide strong evidence for absence of buckwheat-feeding since complementary laboratory studies on rubidium-marking suggested a
substantial risk of false-negatives (as well as possible false-positives), and the sugar profiles generated by HPLC for field-collected parasitoids did not closely match any of those from parasitoids of known diet. This outcome highlights the importance of thorough methodological studies to ensure results of field studies are interpreted appropriately. The results also serve as a reminder that the value of flower strips to parasitoids cannot be assumed even when flowering weeds are apparently scarce.

Other authors have studied the relative value of honeydew and nectar as food sources for laboratory-reared parasitoids (usually in terms of their effects on longevity) and concluded that while honeydew is better than water alone, it is often inferior to nectar (Wäckers, 2005; Wäckers et al., 2008). Further work on whether these differences are ecologically significant in the field and how parasitoids are attracted to the alternative resources would greatly enhance our understanding of the role of floral resources in host-parasitoid relationships. For parasitoids such as A. zealandica whose hosts are not necessarily associated with aphids, whether or not honeydew can serve as an adequate food source when aphids are at low densities must also be considered. This also applies to all honeydew-feeders early in the season when aphids are scarce.

This study of A. zealandica found sugar levels in most field-collected parasitoids were low compared with those provided with abundant food in the laboratory (Chapter 6) but it is not known whether their rates of parasitism were compromised by lack of energy to forage or develop eggs, or excessive time spent foraging for food rather than hosts. It has been demonstrated that parasitoids show an increasing tendency to forage for non-host foods rather than hosts as time since feeding increases (e.g. Takasu & Lewis, 1993; Wäckers, 1994; Lewis et al., 1998; Jacob & Evans, 2001; Siekmann et al., 2004) but the sugar levels which are associated with this behaviour have not been measured. Such studies would be valuable, enabling the levels measured in field-collected wasps to be interpreted as indicative of sugar limitation or not.

In addition to studying the effect of flowers on biological control in a system comprising aphids and lacewings, the simulation model described above was run with the lacewing-parasitoid incorporated (assuming it benefited from flowers as in the laboratory studies) (Chapter 7). The indirect effect of parasitoids on aphids could not occur within the duration of the simulations but the wasps could affect the number of lacewing pupae which would be available for biological control in the future. When flowers were present, adding parasitoids always reduced the population of lacewing pupae. In other words, the negative effects of parasitism outweighed the direct positive effects of the flowers on the lacewing population. This finding illustrates the importance of considering effects of floral resources on both third- and fourth-trophic-level species and complements a field-cage study which suggested that at high aphid densities, the presence of buckwheat had a detrimental effect on lacewings (Jonsson et al.,
in press). However, as discussed above, our poor knowledge of the ecology of the modelled species both restricted the incorporation of processes into the model and limited interpretation of the model output. As with the lacewings, we need to know more about processes such as the dispersal of *A. zealandica* between fields and its within-field foraging behaviour in relation to flowers and host lacewing larvae. Furthermore, the ecological significance of parasitism for the lacewing population is also currently unknown because other causes of mortality have not been studied. Empirical studies and subsequent developments in modelling could build on the understanding of this system that has begun here.

## 8.3 Final Reflections

As revealed through this thesis, studying a lacewing and its parasitoid has exemplified how flowers can indirectly affect lacewing prey via consumption of floral resources by the adult stages of these two species. The effects on prey occurred via the effects of flowers on *per capita* prey consumption by the adult lacewings, their effects on adult lacewing survival and fecundity, and their effect on parasitism of lacewing larvae. These processes could balance each other in such a way as to result in either positive or negative effects on the prey population. The work thus uses interactions between these species to demonstrate how true omnivory and occurrence of effects across four trophic levels could affect the value of floral resource provision as a technique to enhance biological control.

The discussion above, and in preceding chapters, acknowledges that our understanding of interactions between the species studied here is greatly limited by an absence of behavioural and landscape-level studies. It is also recognised that this prey-flower-lacewing-parasitoid system does not occur in isolation. In the series of studies presented here, no attempt was made to study prey populations in the field, where interactions between the focal species and numerous others would influence the overall effect of flowers on the prey population. For example, other biocontrol agents and antagonistic species would exploit the flowers, and phenomena such as intraguild predation, synergistic interactions between biocontrol agents, apparent competition between prey and interactions with ‘bottom-up’ effects on the pest would occur (e.g. Evans & England, 1996; Heimpel *et al.*, 1997; Sunderland *et al.*, 1997; Sih *et al.*, 1998; Rosenheim *et al.*, 1999; Cardinale *et al.*, 2003; Dyer & Stireman, 2003; Snyder *et al.*, 2005; van Veen *et al.*, 2006; Snyder & Ives, 2008; Straub *et al.*, 2008). In addition, the lethal effects of the predator and parasitoid on their prey/host(s) could be supplemented by non-lethal effects such as disruption of feeding through avoidance behaviour by the prey (Preisser *et al.*, 2005; Thaler & Griffin, 2008). The work presented here does not, therefore, lead to specific predictions about the relationship between flowers and pests in the field. As proposed in the introductory chapter, manipulating the availability of floral resources as a technique for pest management depends on net effects in the agroecosystem, but studying sub-systems can contribute to our understanding of why such
effects might occur and therefore improve our ability to extrapolate beyond the particular circumstances studied. This work was motivated by the hope it would make such a contribution, rather than be applied directly.

Providing floral resources is one technique amongst many which might be employed to enhance biological control, or to reduce pest populations from the ‘bottom-up’ (Landis et al., 2000; Zehnder et al., 2007). As studies of the ecological interactions underlying these techniques accumulate, they provide a foundation for pest management strategies which optimise ecological processes. Such ecology-based pest management should allow the productive value of agroecosystems (i.e. crop yield) to be maintained or increased, while avoiding loss of non-productive values (e.g. their aesthetic value and so-called ‘regulating ecosystem services’), and minimising costs imposed on other ecosystems (Nicholls & Altieri, 2007). Paradoxically, these studies also reveal the complexities of agroecosystems and hence the difficulties inherent in the ecological approach to pest management. However, the limitations of alternative approaches, particularly those which use insecticides as their primary tools, are also evident and widely documented (Marco et al., 1987; Pimentel & Lehman, 1993; Pretty, 2005; Pedigo & Rice, 2006). Ecologists should therefore persist in their attempts to understand the composition and functioning of agroecosystems.

Perhaps an equally important role of agroecology over the long term will be contributing to the ability of ecology as a whole to foster a culture with a brighter future: a culture with greater recognition of society’s dependence on nature and whose activities are amended to show all ecosystems due appreciation and respect.
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Acknowledgements

For the ecologist, agroecosystems can satisfy two important appetites: they are a source of both food and intellectual challenges. With pleasing circularity, the knowledge acquired by those seeking to understand the ecology of the agroecosystem can be applied to improve the methods of food production, ensuring they optimise ecological processes rather than naively fight against them. In this thesis I have presented the outcomes of my efforts in this field. The shortcomings of the work are a reflection of my own, persisting despite the support I had over the last three (and a half) years. I would like to acknowledge that the thesis would not exist without the help of many other people. The following thanks may not be effusive, but they are sincere:

My supervisor, Steve Wratten, offered me the opportunity to undertake this Ph.D. when very few other people would have done so. I am extremely grateful to him for changing my life in that very positive way. Subsequently he has been a great supervisor. I thank him for, in particular, invariably focusing on the solutions not the problems, and never saying “no”.

I would also like to thank my associate supervisor, Roddy Hale, for his advice over the last three years; advice which was always sound, even if I did not follow it.

I would not have considered undertaking the modelling aspect of this Ph.D. without the support of John Kean. The presence of the modelling chapter is entirely due to his ongoing help, for which I would like to thank him.

Chapter 1 is based on a paper co-authored with Mattias Jonsson, Steve Wratten, Mark Wade and Hannah Buckley. I would like to acknowledge their contributions, and the contributions of two anonymous referees to that work.

The HPLC analyses were undertaken by Felix Wäckers. Chapter 6 benefited greatly from his help in the study design and his subsequent assistance interpreting the results. I was also able to enjoy a visit to Lancaster to see the HPLC in action and would like to express my thanks for his assistance in those ways.

The rubidium analyses were conducted by Lynne Clucas. I am very grateful to her for that, and for the advice she and Roger Cresswell gave in how to prepare the insects for analysis.

I would like to thank Alison Lister, Chris Frampton, James Ross and Richard Sedcole for their assistance with statistical analyses and for answering my numerous queries about what to do with messy data.

I have used a lot of broad bean and buckwheat plants in the last three years. I would like to thank Brent Richards and Leona Meachen for providing the facilities to grow these and watering them for me. Many of the plants were sown by Shona Sam, who also helped with my culture maintenance and some laboratory experiments. I am grateful to Shona for always helping enthusiastically.
Don Heffer, Dave Jack, Merv Spurway, Dean Judson and Tony Whatman made the field studies possible by allowing use of the field sites and preparing them for me. I appreciate their assistance.

Throughout my Ph.D. I have depended on equipment borrowed from, or maintained by, various people. I would particularly like to thank Margaret Auger, Mike Bowie, Sandy Hammond, Myles Mackintosh and John Marris in this respect.

Nothing gets done without the help of people who deal with the administration. Thanks to Linda Forbes, Dianne Fyfe, Chrissy Gibson, Bernadette Mani, Dianne O’Connor and Julie Ward for helping in this way.

Finally, but to no lesser degree, I would like to thank Mattias Jonsson, Salah Araj, Annie Barnes, Mark Gillespie, Marco Jacometti, Nina Jorenson, Sofia Orre, Samantha Scarratt, Katrin Schmidt, Jean Tompkins and Don Vattala for their support in all sorts of ways; from tending insect cultures so I could go away for more than two days consecutively, to providing stimulating and entertaining conversation.

Thanks to Steve Wratten, I was generously funded by the New Zealand Foundation for Research, Science and Technology (LINX 0303: Biodiversity, Ecosystem Services and Sustainable Agriculture).

If I have forgotten to include anyone here, please accept it is due to lack of sleep at the time of writing, not lack of appreciation.

22nd August 2008
Appendix 1 Illustrations

A1.1 Illustrations of the field experiment described in Chapters 3, 5 and 6

i) Photographs of the lucerne field in which the field study was conducted.
   i) Overall view, ii) Flowering buckwheat strip in the centre of the field. Rows were sown in succession to provide continual flowering through the sampling period.

Fig. A1.2 Plan of the lucerne field where the field study was conducted.
(Plan not to scale, with approximate field dimensions. Field area is 12.5 ha.). Sweep-net samples were collected 1, 5, 10, 25, 50 and 100 m each side of the flowering buckwheat strip. See main text for the sampling procedure.
A1.2 ADDITIONAL ILLUSTRATIONS FOR CHAPTERS 3 AND 4

Fig. A1.3 Dissection of the adult lacewing *Micromus tasmaniae* to determine the presence of arthropod material in the gut.

Fig. A1.4 Slide preparation of eggs dissected from the lacewing-parasitoid *Anacharis zealandica*. Four mature eggs have been released from the cluster of ovarioles which comprise one ovary.
Appendix 2  Sub-lethal effects on *Micromus tasmaniae* larvae of parasitism by *Anacharis zealandica*

A2.1 INTRODUCTION

*Anacharis zealandica* Ashmead (Hymenoptera: Figitidae) is a parasitoid of larval *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae) (New, 1982). It has been observed stinging first, second and third instars, though successful parasitism has only been confirmed in second instar larvae (pers. obs.). *M. tasmaniae* is reported to be a significant agent of biological control in Australasian agroecosystems (Horne *et al.*, 2001). Both the adults and larvae are predators of small soft-bodied arthropods such as aphids. Parasitism could affect biological control by the lacewings through one or more of the following mechanisms: 1) reducing the population in subsequent generations because lacewings are replaced by parasitoids, 2) affecting the development time of the larvae, 3) affecting per capita daily predation rates of the larvae. In order to establish whether mechanisms (2) or (3) could occur in this species, an experiment was conducted to determine the effect of parasitism on the development time of lacewing larvae, and their daily rate of aphid consumption. In addition to the effects of parasitism, presence of adult parasitoids could affect adult or larval lacewings by causing them to modify their behaviour. This effect is not considered here.

A2.2 MATERIALS AND METHODS

A culture of the parasitoid *A. zealandica* was established from local field-caught insects and maintained in 2.5-litre plastic jars for more than ten generations prior to the experiments. Lacewing larvae were added periodically to provide hosts for the parasitoid larvae. The lacewing larvae were fed pea aphids *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) from cultures maintained on broad bean *Vicia faba* L. ‘Coles Dwarf’ (Leguminosae). Adult wasps in the culture were provided with honey-soaked cotton wool and a water-filled vial with a cotton wool wick. Parasitoids were taken directly from the culture to parasitise larvae in the experiment.

A culture of the lacewing *M. tasmaniae* was established from local field-caught insects and had been maintained in 5.8-litre plastic boxes for more than ten generations prior to the experiments. Adult lacewings in the culture were provided with honey applied to the walls of their enclosure and a water-filled vial with a cotton wool wick, and pea aphids were added daily. A strip of cotton cloth was placed in the lacewing culture as an oviposition substrate and this was changed daily.

To provide larvae for experimentation, pieces of cloth bearing 0–24 hour old eggs were put in plastic Petri dishes (height 1 cm, diameter 5 cm). After three days, aphids were added to feed newly-emerging larvae and once the eggs had hatched, all but one larva were removed
from each dish. The larvae were checked daily to record the day they entered the second instar, as indicated by loss of the trumpet-shaped empodia (Monserrat et al., 2001). The day after larvae became second instars they were alternately allocated to two treatments: 1) exposed and, 2) not exposed. The ‘exposed’ larvae were presented to a parasitoid to allow parasitisation. Until this time, aphids were added ad libitum each day.

The larvae were exposed to a single parasitoid in a small arena. The base of the arena was a 10 cm plastic Petri dish. The sides were formed from an acetate sheet rolled to create a tube (height 4.5 cm, diameter 2.5 cm). The top of arena was the base of a glass vial (height 5 cm, diameter 2.5 cm) pushed into the acetate tube. A parasitoid was removed from the culture using a suction device (pooter). The parasitoid was then transferred from the pooter pot to the Petri dish base using a paintbrush and the arena tube placed over it. The vial was pulled up to maximise the volume of the arena and when the wasp was near the top (to minimise the probability of escape), a lacewing larva was placed on the Petri dish and the tube placed over it. The vial was then pushed down to a height of 1–2 cm to reduce the volume and so increase the probability of the wasp encountering the larva.

Ideally a different female parasitoid would have been used for each lacewing larva and a standardised protocol would have been developed to determine when to remove the larva from the arena. However, this was not the case for two reasons. Firstly, many parasitoids did not sting larvae presented to them within a practical time period (approx. 15 min) so there were insufficient active parasitoids to use one per larva. Since, at the time of the experiment, it was not known how to determine the sex of the parasitoids, the inactive wasps could have been males, or they could have been female wasps that had depleted their egg load while in the culture. Secondly, this experiment formed part of an observational study on the oviposition behaviour of the parasitoids so some larvae were allowed to be stung repeatedly if the stings were considered to be of short duration. Since the larva often moves while the parasitoid is attempting to sting it (as indicated by the wasp abdomen being curled under the body), attempts to sting may not be associated with oviposition. Timing the duration of the stings and subsequently determining whether parasitism was successful or not, allowed a more rigorous protocol to be developed for future experiments.

The lacewing larvae were removed from the arena after a variable amount of time, and were sometimes consecutively exposed to more than one wasp if they were not stung by the first wasp. If the larva had not been stung it was discarded. If it had been stung it was returned to its individual Petri dish. All larvae (‘exposed’ and ‘not exposed’) were then provided with 40 medium-sized aphids (second or third instar).

Each day until pupation, the number of aphids consumed was counted. The aphids were observed under a binocular microscope (x20–x40) and misshapen cadavers (as formed by the suction-feeding action of the larvae) were recorded as consumed. Forty aphids was usually
sufficient to provide an ‘excess’ food supply i.e. live aphids remained in the dish while allowing for natural aphid mortality. Each day, survival and pupation were also recorded and the aphids were refreshed. After pupation the pupae were checked daily for survival and emergence, and emerging insects were recorded as male lacewings, female lacewings or parasitoids (undetermined sex).

Exposure of lacewing larvae to parasitoids and feeding of larvae took place in a laboratory at 16–20 °C. At other times the insects were in a controlled temperature room at approximately 20 °C (range ± 2 °C) and a light regime of 16 h light:8 h dark. The experiment was run using four batches of eggs collected four days apart.

Rates of aphid consumption and development times were analysed for a total of 13 exposed larvae that emerged as parasitoids and 26 ‘not exposed’ larvae. Hereafter these groups are referred to as ‘parasitised’ and ‘unparasitised’ respectively. Lacewing larvae which were exposed to parasitoids but emerged as lacewings were excluded from the analyses, as were larvae that did not emerge as live adults. Aphid consumption and development time were assumed to be unaffected by the identity of the parent wasp and the number of times the larva was stung.

The difference between daily aphid consumption for parasitised and unparasitised larvae was analysed using a t-test. The time to pupation was not subject to statistical analysis because almost all data were the same value and the number of data with alternative values were too low for Chi-squared frequency analysis.

A2.3 RESULTS AND DISCUSSION

All ten of the exposed lacewing larvae which were stung by a parasitoid for less than 26 seconds emerged as lacewings. Only two larvae of the fifteen stung for longer than this were not successfully parasitised, so this time can be used as a guide to distinguishing attempts at oviposition from actual oviposition in future behavioural work. The mean duration of successful stings was 69.08 seconds (standard error = 9.62, range = 26–146 s).

The rates of mortality were 25.0% and 17.6% for larvae stung for more than 26 seconds and ‘not exposed’ larvae respectively. Mortality varied between batches and may have been related to the quality of aphid food, itself dependent on the varying quality of plants in the aphid culture. Mortality occurred primarily at the pupal stage, which rarely occurred in the lacewing and parasitoid cultures when aphid cultures were in good health.

The time to pupation of parasitised and unparasitised lacewing larvae did not differ: 12/13 parasitised larvae and 21/26 unparasitised larvae pupated four days after the day of exposure. One unparasitised larva pupated on the fifth day and the remainder pupated on the third day. This indicates that A. zealandica is a koinobiont parasitoid. It should also be noted that a single parasitoid emerged from each larva so the species is solitary rather than gregarious.
The duration of each life stage of *M. tasmaniae* has been recorded across a range of temperatures and with various prey species (Samson & Blood, 1979; Syrett & Penman, 1981; New, 1984; Leathwick, 1989; Islam & Chapman, 2001). The times observed in this experiment are consistent with previous results (modal duration: egg stage: 5 days; first instar stage: 3 days; total larva stage: 8 days; pupa stage: 11 days). The modal duration of the pupa stage in the parasitoids was 21 days (range 21–23 days).

Amongst the unparasitised larvae, all four which pupated on the third day were males and since males are smaller (as indicated by shorter hind tibia length and mass (Robinson, K.A., unpublished)), further work may reveal a difference in development time between male and female *M. tasmaniae*. The overall proportion of females was 0.48.

The daily number of aphids consumed by parasitised and unparasitised lacewing larvae did not differ (mean ± standard error parasitised: 11.15 ± 0.53; unparasitised: 10.94 ± 0.40, \( t = 0.31, \) d.f. = 37, \( P = 0.760 \)). Fig. A2.1 shows the total number of aphids consumed by each larva according to development time. Since the majority of aphids are consumed in the third instar and very few are consumed by first instar larvae (this experiment; Leathwick, 1989; Islam & Chapman, 2001), the number of aphids counted during the experiment are indicative of the overall consumption of the lacewings during the larval stage. This, however, can only be taken as a very approximate indication of consumption in the field since it was measured under the artificial foraging conditions of the Petri dish and with aphids of potentially sub-optimal quality.

![Fig. A2.1 Effect of parasitism and time to pupation on the number of aphids consumed by lacewing larvae.](image-url)

The days to pupation are recorded from the day after the larvae entered the second instar. Aphid consumption was recorded daily from this time.
The rates of aphid consumption were highly variable, resulting in low power for the statistical analysis. However, the similarity of the means together with the lack of difference in development times, suggests that biological control by lacewing larvae would be unaffected by parasitism. Notwithstanding any behavioural effects of the presence of parasitoids, the principal effects of *A. zealandica* would therefore only be apparent through their effect on the lacewing population in the subsequent generation.
Appendix 3 Supplementary material for Chapter 7

A3.1 Introduction

This appendix provides a full description of the model discussed in Chapter 7. The notation used to represent each population in the model is given in Table A3.1, together with other key notation used in the equations below. The processes which link the populations are depicted in Fig. A3.1. Table A3.2 presents the notation, descriptions, sources and values of the parameters used to model these processes.

Fig. A3.1 Relational diagram of the four-trophic-level system as modelled. (See also Fig. 7.1 in the main text.) See Table A3.1 for notation. Populations at time $t$ are depicted in boxes and the number of individuals leaving or being recruited to those populations in each time step are depicted by arrows. Each population was divided into daily cohorts which are not depicted. The effects of consumption of buckwheat nectar and/or pollen by adult lacewings and parasitoids were modelled using alternative parameter values for the processes depicted in red, so simulations were ‘with flowers’ or ‘without flowers’. Single asterisks indicate the value was a function of aphid nymph density and double asterisks indicate the value was a function of lacewing larva density. Key output variables were aphid-days and the populations of adult and pupa lacewings at the end of the simulation.
Each population comprised daily cohorts $i=0$ to $i=21$ for the 21-day simulations. The time step $(t)$ was 0.05 days but $i$ increased in increments of 1 day. All the lacewing eggs ($Le$) laid in the period $t$ to $t+20$ (where $t$ is an integer and $t$ to $t+20$ is 1 day) formed a single cohort $Le_i$ and developed into lacewing larvae ($Ll_i$) in the same time step $((t+20)/20 + d_{Le}$, where $d_{Le}$ is the development time). In addition to the cohorts of lacewing eggs which developed into later lacewing stages and potentially became parasitoids, there were daily cohorts of aphid nymphs ($An_i$). These were produced in the period $t$ to $t+20$ and developed into aphid adults at step $(t+20)/20 + d_{An}$ in a similar way.

At the start of the simulation, the values for $i=0$ (‘initial populations’) varied and are described in Section 7.3.2. The value of cohorts $i=1$ to $i=21$ for all populations were zero. All populations had a minimum value of zero.

Most simulations were run for 420 time steps (21 days) with 21 cohorts. One set of simulations used parameter values adjusted to simulate populations at 15 °C. These simulations were run for 28-days (the degree-day equivalent of the 20 °C 21-day simulations) with cohorts $i=0$ to $i=28$. The populations which did not develop or reach their maximum longevity within the 21-day simulations (see below), were also unable to do so in the 28 day simulations.

**Table A3.1 Notation used in the model equations.**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>An</td>
<td>aphid nymphs</td>
</tr>
<tr>
<td>Aa</td>
<td>aphid adults</td>
</tr>
<tr>
<td>Le</td>
<td>lacewing eggs</td>
</tr>
<tr>
<td>Ll</td>
<td>lacewing larvae (unparasitised)</td>
</tr>
<tr>
<td>Lp</td>
<td>lacewing pupae</td>
</tr>
<tr>
<td>La</td>
<td>lacewing adults</td>
</tr>
<tr>
<td>Pl</td>
<td>parasitised lacewing larvae</td>
</tr>
<tr>
<td>Pp</td>
<td>parasitoid pupae</td>
</tr>
<tr>
<td>Pa</td>
<td>parasitoid adults</td>
</tr>
<tr>
<td>i</td>
<td>cohort</td>
</tr>
<tr>
<td>t</td>
<td>time step</td>
</tr>
<tr>
<td>M</td>
<td>number of insects subject to background mortality</td>
</tr>
<tr>
<td>C</td>
<td>number of insects being consumed</td>
</tr>
<tr>
<td>D</td>
<td>number of insects developing out of the population</td>
</tr>
<tr>
<td>R</td>
<td>number of insects recruited through reproduction</td>
</tr>
<tr>
<td>S</td>
<td>number of lacewing larvae stung (parasitised)</td>
</tr>
</tbody>
</table>
Table A3.2 Descriptions of parameters used in the model. (Continued overleaf.) Values are those used in the standard parameter set with and without flowers. Values which differed between simulations with and without flowers are highlighted in bold. Sources are references to empirical data which guided selection of the parameter values. Where no source is given, values were based on informed judgment with an emphasis on ensuring they related to each other in biologically plausible but parsimonious ways. All values are measured at approximately 20 °C. For references, see Reference section in main text.

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>Symbol</th>
<th>Without flowers</th>
<th>With flowers</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of aphid nymphs dying due to background mortality per unit time</td>
<td>( m_{An} )</td>
<td>0.1</td>
<td>0.1</td>
<td>day(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Maximum number of aphid nymphs consumed by lacewing larvae per unit time</td>
<td>( kc_{LJ} )</td>
<td>8</td>
<td>8</td>
<td>An ( \text{L}^1 \text{day}^{-1} )</td>
<td>Appendix 2</td>
</tr>
<tr>
<td>Density of aphid nymphs at which consumption by lacewing larvae is half the maximum rate</td>
<td>( hc_{LJ} )</td>
<td>100</td>
<td>100</td>
<td>An ( \text{m}^2 )</td>
<td></td>
</tr>
<tr>
<td>Maximum number of aphid nymphs consumed by parasitised larvae per unit time</td>
<td>( kc_{PL} )</td>
<td>8</td>
<td>8</td>
<td>An ( \text{P}^1 \text{day}^{-1} )</td>
<td></td>
</tr>
<tr>
<td>Density of aphid nymphs at which consumption by parasitised larvae is half the maximum rate</td>
<td>( hc_{PL} )</td>
<td>100</td>
<td>100</td>
<td>An ( \text{m}^2 )</td>
<td></td>
</tr>
<tr>
<td>Maximum number of aphid nymphs consumed by lacewing adults per unit time</td>
<td>( kc_{La} )</td>
<td>25</td>
<td>25</td>
<td>An ( \text{L}^1 \text{day}^{-1} )</td>
<td>Chapter 2</td>
</tr>
<tr>
<td>Density of aphid nymphs at which consumption by lacewing adults is half the maximum rate</td>
<td>( hc_{La} )</td>
<td>100</td>
<td>250</td>
<td>An ( \text{m}^2 )</td>
<td>Relative values derived from Chapter 2</td>
</tr>
<tr>
<td>Proportion of aphid nymphs developing into aphid adults per unit time</td>
<td>( d_{An} )</td>
<td>9</td>
<td>9</td>
<td>days</td>
<td>Campbell et al. (1977)</td>
</tr>
<tr>
<td>Number of aphid nymphs produced per aphid adult per unit time</td>
<td>( r_{A} )</td>
<td>4.79</td>
<td>4.79</td>
<td>An ( \text{A}^1 \text{day}^{-1} )</td>
<td>Campbell et al. (1977)</td>
</tr>
<tr>
<td>Maximum longevity of adult aphids</td>
<td>( l_{Aa} )</td>
<td>30</td>
<td>30</td>
<td>days</td>
<td>Campbell et al. (1977)</td>
</tr>
<tr>
<td>Proportion of aphid adults dying due to background mortality per unit time</td>
<td>( m_{Aa} )</td>
<td>0.05</td>
<td>0.05</td>
<td>day(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Proportion of lacewing eggs dying due to background mortality per unit time</td>
<td>( m_{Le} )</td>
<td>0.1</td>
<td>0.1</td>
<td>day(^{-1})</td>
<td></td>
</tr>
</tbody>
</table>
### Table A3.2 continued.

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>Symbol</th>
<th>Without flowers</th>
<th>With flowers</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of lacewing eggs developing into lacewing larvae per unit time</td>
<td>(d_{Le})</td>
<td>5</td>
<td>5</td>
<td>days</td>
<td>Appendix 2</td>
</tr>
<tr>
<td>Maximum number of eggs laid per lacewing adult per unit time (assuming a sex ratio of 0.5)</td>
<td>(kf_{La})</td>
<td>12</td>
<td>12</td>
<td>Le La(^{-1}) day(^{-1})</td>
<td>Chapter 2</td>
</tr>
<tr>
<td>Density of aphid nymphs at which oviposition by lacewing adults is half the maximum rate</td>
<td>(hf_{La})</td>
<td>100</td>
<td>50</td>
<td>An m(^{-2})</td>
<td>Relative values derived from Chapter 2</td>
</tr>
<tr>
<td>Minimum proportion of lacewing larvae dying per unit time (background mortality)</td>
<td>(km_{Ll})</td>
<td>0.1</td>
<td>0.1</td>
<td>day(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Density of aphid nymphs at which the proportion of lacewing larvae dying per unit time is half the maximum proportion</td>
<td>(hm_{Ll})</td>
<td>100</td>
<td>100</td>
<td>An m(^{-2})</td>
<td></td>
</tr>
<tr>
<td>Maximum proportion of lacewing larvae dying per unit time (background mortality plus starvation)</td>
<td>(im_{Ll})</td>
<td>0.33</td>
<td>0.33</td>
<td>day(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Maximum number of lacewing larvae stung by parasitoid adults per unit time</td>
<td>(kf_{Pa})</td>
<td>9</td>
<td>11</td>
<td>Ll Pa(^{-1}) day(^{-1})</td>
<td>Chapter 4</td>
</tr>
<tr>
<td>Density of lacewing larvae at which the rate of parasitism by parasitoid adults is half the maximum rate</td>
<td>(hf_{Pa})</td>
<td>5</td>
<td>5</td>
<td>Pl m(^{-2})</td>
<td></td>
</tr>
<tr>
<td>Proportion of parasitoid adults which are female</td>
<td>(s_{Pa})</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of lacewing larvae developing into lacewing pupae per unit time</td>
<td>(d_{Ll})</td>
<td>7</td>
<td>7</td>
<td>days</td>
<td>Appendix 2</td>
</tr>
<tr>
<td>Proportion of lacewing pupae dying due to background mortality per unit time</td>
<td>(m_{Lp})</td>
<td>0.1</td>
<td>0.1</td>
<td>day(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Proportion of lacewing pupae developing into lacewing adults per unit time</td>
<td>(d_{lp})</td>
<td>11</td>
<td>11</td>
<td>days</td>
<td>Appendix 2</td>
</tr>
<tr>
<td>Maximum longevity of adult lacewings</td>
<td>(l_{La})</td>
<td>35</td>
<td>35</td>
<td>days</td>
<td>Robinson et al. (2008)</td>
</tr>
<tr>
<td>Minimum proportion of lacewing adults dying per unit time (background mortality)</td>
<td>(km_{La})</td>
<td>0.05</td>
<td>0.05</td>
<td>day(^{-1})</td>
<td></td>
</tr>
</tbody>
</table>
Table A3.2 continued.

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>Symbol</th>
<th>Without flowers</th>
<th>With flowers</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density of aphid nymphs at which the proportion of lacewing adults dying per unit time is half the maximum proportion</td>
<td>$h_{m_{La}}$</td>
<td>50</td>
<td>50</td>
<td>$\text{An m}^{-2}$</td>
<td>Robinson et al. (2008)</td>
</tr>
<tr>
<td>Maximum proportion of lacewing adults dying per unit time (background mortality plus starvation)</td>
<td>$i_{m_{La}}$</td>
<td><strong>0.35</strong></td>
<td><strong>0.05</strong></td>
<td>$\text{day}^{-1}$</td>
<td>Robinson et al. (2008)</td>
</tr>
<tr>
<td>Minimum proportion of parasitised larvae dying per unit time (background mortality)</td>
<td>$k_{mPl}$</td>
<td>0.1</td>
<td>0.1</td>
<td>$\text{day}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Density of aphid nymphs at which the proportion of parasitised larvae dying per unit time is half the maximum proportion</td>
<td>$h_{m_{Pl}}$</td>
<td>100</td>
<td>100</td>
<td>$\text{An m}^{-2}$</td>
<td>Robinson et al. (2008)</td>
</tr>
<tr>
<td>Maximum proportion of parasitised larvae dying per unit time (background mortality plus starvation)</td>
<td>$i_{m_{Pl}}$</td>
<td>0.33</td>
<td>0.33</td>
<td>$\text{day}^{-1}$</td>
<td>Robinson et al. (2008)</td>
</tr>
<tr>
<td>Proportion of parasitised larvae developing into parasitoid pupae per unit time</td>
<td>$d_{Pl}$</td>
<td>7</td>
<td>7</td>
<td>$\text{days}$</td>
<td>Appendix 2</td>
</tr>
<tr>
<td>Proportion of parasitoid pupae dying due to background mortality per unit time</td>
<td>$m_{Pp}$</td>
<td>0.1</td>
<td>0.1</td>
<td>$\text{day}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Proportion of parasitoid pupae developing into parasitoid adults per unit time</td>
<td>$d_{Pa}$</td>
<td>22</td>
<td>22</td>
<td>$\text{days}$</td>
<td>Appendix 2</td>
</tr>
<tr>
<td>Maximum longevity of adult parasitoids</td>
<td>$l_{Pa}$</td>
<td>2</td>
<td>20</td>
<td>$\text{days}$</td>
<td>Jonsson et al. (in press)</td>
</tr>
<tr>
<td>Proportion of parasitoid adults dying due to background mortality per unit time</td>
<td>$m_{Pa}$</td>
<td>0.05</td>
<td>0.05</td>
<td>$\text{day}^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>
A3.2 Model equations

Aphid nymphs

At any given time step, the total number of aphid nymphs was the sum of nymphs in each cohort at that time:

$$A_{n(t)} = \sum_{i=0}^{i=21} A_{n_i(t)}$$  \hspace{1cm} (1)

At the start of the simulation \((t = 0)\), \(A_{n_i(0)} = x\) for \(i = 0\), where \(x\) was an initial population in the range 300–1025 nymphs m\(^{-2}\).

In subsequent time steps the number of aphid nymphs in each cohort in each time step was given by:

$$A_{n_i(t+1)} = A_{n_i(t)} - M_{n_{i(t+1)}} - C_L A_{n_i(t+1)} - C_P A_{n_i(t+1)} - C_{La} A_{n_i(t+1)} - D A_{n_i(t+1)} + R A_{n_i(t+1)}$$  \hspace{1cm} (2)

where \(M_{n_{i(t+1)}}\) was the number of aphid nymphs dying as a result of background mortality

$$M_{n_{i(t+1)}} = A_{n_i(t)} \cdot M_{An}$$  \hspace{1cm} (3)

\(C_L A_{n_i(t+1)}\) was the number of aphid nymphs consumed by unparasitised lacewing larvae

$$C_L A_{n_i(t+1)} = L_{lt} \cdot \frac{k c_{lt} \cdot (A_{n_i(t)} - M_{n_{i(t+1)}})}{h c_{lt} + (A_{n_i(t)} - M_{n_{i(t+1)}})} \cdot \frac{A_{n_i(t)} - M_{n_{i(t+1)}}}{A_{n_i(t)} - M_{n_{i(t+1)}}}$$  \hspace{1cm} (4)

\(C_P A_{n_i(t+1)}\) was the number of aphid nymphs consumed by parasitised lacewing larvae

$$C_P A_{n_i(t+1)} = W_k \cdot \frac{k c_{pl} \cdot (A_{n_i(t)} - M_{n_{i(t+1)}})}{h c_{pl} + (A_{n_i(t)} - M_{n_{i(t+1)}})} \cdot \frac{A_{n_i(t)} - M_{n_{i(t+1)}}}{A_{n_i(t)} - M_{n_{i(t+1)}}}$$  \hspace{1cm} (5)

\(C_{La} A_{n_i(t+1)}\) was the number of aphid nymphs consumed by lacewing adults

$$C_{La} A_{n_i(t+1)} = L_{at} \cdot \frac{k c_{la} \cdot (A_{n_i(t)} - M_{n_{i(t+1)}})}{h c_{la} + (A_{n_i(t)} - M_{n_{i(t+1)}})} \cdot \frac{A_{n_i(t)} - M_{n_{i(t+1)}}}{A_{n_i(t)} - M_{n_{i(t+1)}}}$$  \hspace{1cm} (6)

\(D A_{n_i(t+1)}\) was the number of aphid nymphs developing into adults

If \(\frac{t}{20} < i < d_{An}\)

then \(D A_{n_i(t+1)} = A_{n_i(t)} - M_{n_{i(t+1)}} - C_L A_{n_i(t+1)} - C_P A_{n_i(t+1)} - C_{La} A_{n_i(t+1)}\)

otherwise \(D A_{n_i(t+1)} = 0\)

and \(R A_{n_i(t+1)}\) was the number of aphid nymphs recruited through parthenogenic reproduction of adult aphids

If \(\frac{t}{20} < i < \frac{t+20}{20}\)

then \(R A_{n_i(t+1)} = A a(t) \cdot r_{Aa}\)

otherwise \(R A_{n_i(t+1)} = 0\).
The conditionalities for $RAn_{i(t+1)}$ and $DAn_{i(t+1)}$ ensured that the nymphs produced on each day generated distinct cohorts and all the nymphs in each cohort developed in a single time step, as described in Section 7.3.1 in the main text.

The equations for consumption of aphid nymphs allowed for the reductions in population due to background mortality, and the equation for development allowed for the reductions due to mortality and consumption. However, consumption by each predator population (L1, P1, L2) did not allow for consumption by the other populations within a time step. This meant that at low aphid nymph populations, the combined predator populations could consume more aphid nymphs than were available. In such cases, the aphid population fell to zero and consumption was zero at the next time step. In this time step aphid nymphs were produced and in the third time step these could be consumed. This artefactual behaviour at low aphid densities has a minimal effect because the time steps were small but it was taken into consideration when interpreting the model output.

**Aphid adults**

At any given time step, the total number of aphid adults was the sum of adults in each cohort at that time:

$$Aa_i(t) = \sum_{i=0}^{i=21} Aa_i(t)$$  \hspace{1cm} (9)

At the start of the simulation ($t = 0$), $Aa_i(t) = \frac{An_i(t)}{2}$ for $i = 0$ (i.e. half the initial population of aphid nymphs).

If the maximum longevity of the aphid adults had not been exceeded i.e.

$$\text{if } \frac{t}{20} - i < l_{Aa}$$

then in subsequent time steps the number of aphid adults in each cohort in each time step was given by:

$$Aa_i(t+1) = Aa_i(t) - MAa_i(t+1) + DAn_i(t+1)$$  \hspace{1cm} (10)

otherwise $Aa_i(t+1) = 0$.

$MAa_i(t+1)$ was the number of adult aphids dying as a result of background mortality

$$MAa_i(t+1) = Aa_i(t) \cdot m_{Aa}$$  \hspace{1cm} (11)

and $DAn_i(t+1)$ was the number of aphid adults being recruited through development of aphid nymphs (Equation 7).

The adult aphids formed cohorts following on from the cohorts of aphid nymphs but only exceeded their longevity within the simulations when the ‘resistant lucerne’ alternative parameter set was used.

**Lacewing eggs**

At any given time step, the total number of lacewing eggs was the sum of eggs in each cohort at that time:

$$Le_i(t) = \sum_{i=0}^{i=21} Le_i(t)$$  \hspace{1cm} (12)

At the start of the simulation ($t = 0$), $Le_i(t) = 0$ for $i = 0$. 

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In subsequent time steps the number of lacewing eggs in each cohort in each time step was given by:

\[ \text{Le}_{i(t+1)} = \text{Le}_{i(t)} - \text{MLE}_{i(t+1)} - \text{DLe}_{i(t+1)} + \text{RLe}_{i(t+1)} \]  \hspace{1cm} (13)

where \( \text{MLE}_{i(t+1)} \) was the number of lacewing eggs dying as a result of background mortality

\[ \text{MLE}_{i(t+1)} = \text{Le}_{i(t)} \cdot m_{Le} \]  \hspace{1cm} (14)

and \( \text{DLe}_{i(t+1)} \) was the number of lacewing eggs developing into lacewing larvae

\[
\begin{align*}
&\text{if } \frac{t}{20} - i > d_{Le} \\
&\text{then } \text{DLe}_{i(t+1)} = \text{Le}_{i(t)} - \text{MLE}_{i(t+1)} \\
&\text{otherwise } \text{DLe}_{i(t+1)} = 0
\end{align*}
\]  \hspace{1cm} (15)

and \( \text{RLe}_{i(t+1)} \) was the number of lacewing eggs recruited through oviposition by adult lacewings as a function of the density of aphid nympha

\[
\begin{align*}
&\text{if } \frac{t}{20} < i < \frac{t+20}{20} \\
&\text{then } \text{RLe}_{i(t+1)} = \frac{k_{fLa} \cdot (\text{An}(t) - \text{MAN}(t+1)) \cdot \text{La}_t}{h_{fLa} \cdot (\text{An}(t) - \text{MAN}(t+1))} \\
&\text{otherwise } \text{RLe}_{i(t+1)} = 0
\end{align*}
\]  \hspace{1cm} (16)

The conditionalities for \( \text{DLe}_{i(t+1)} \) and \( \text{RLe}_{i(t+1)} \) ensured that the eggs produced on each day produced distinct cohorts and all the eggs in each cohort developed in a single time step, as described in Section 7.3.1 in the main text. The value of \( k_{fLa} \) was adjusted to allow for an adult lacewing sex ratio of 0.5.

**Unparasitised lacewing larvae**

At any given time step, the total number of lacewing larvae was the sum of larvae in each cohort at that time:

\[ \text{LL}_{i(t)} = \sum_{i=0}^{i=21} \text{LL}_{i(t)} \]  \hspace{1cm} (17)

At the start of the simulation \( (t = 0) \), \( \text{LL}_{i(0)} = 0 \) for \( i = 0 \).

In subsequent time steps the number of lacewing larvae in each cohort in each time step was given by:

\[ \text{LL}_{i(t+1)} = \text{LL}_{i(t)} - \text{MLL}_{i(t+1)} - \text{SLL}_{i(t+1)} - \text{DLL}_{i(t+1)} + \text{DLe}_{i(t+1)} \]  \hspace{1cm} (18)

where \( \text{MLL}_{i(t+1)} \) was the number of lacewing larvae dying as a function of aphid density

\[ \text{MLL}_{i(t+1)} = \text{LL}_{i(t)} \cdot \left( \frac{im_{LL} - (km_{LL})(\text{An}(t) - \text{MAN}(t+1))}{hm_{LL} + (\text{An}(t) - \text{MAN}(t+1))} \right) \]  \hspace{1cm} (19)

\( \text{SLL}_{i(t+1)} \) was the number of lacewing larvae being stung by parasitoids (i.e. being parasitised) as a function of the density of lacewing larvae

\[ \text{SLL}_{i(t+1)} = \frac{k_{fPa} \cdot s_{Pa} \cdot (\text{LL}_{i(t)} - \text{MLL}_{i(t+1)}) \cdot \text{Wa}_{t} \cdot \text{LL}_{i(t)} - \text{MLL}_{i(t+1)}}{k_{fPa} + (\text{LL}_{i(t)} - \text{MLL}_{i(t+1)}) \cdot \text{LL}_{i(t)} - \text{MLL}_{i(t+1)}} \]  \hspace{1cm} (20)

\( \text{DLL}_{i(t+1)} \) was the number of lacewing larvae developing into lacewing pupae

\[
\begin{align*}
&\text{if } \frac{t}{20} - i > d_{Le} + d_{LL}
\end{align*}
\]
\[ \text{then } DLl_{l(t+1)} = Ll(t) - MLl_{l(t+1)} - SLLl_{l(t+1)} \]  
otherwise \( DLl_{l(t+1)} = 0 \)

and \( DLLe_{l(t+1)} \) was the number of lacewing larvae being recruited through the development of lacewing eggs (Equation 15).

The conditionalities of \( DLLe_{l(t+1)} \) and \( DLl_{l(t+1)} \) ensured that the larvae formed cohorts which followed on from the cohorts of eggs.

**Lacewing pupae**

At any given time step, the total number of lacewing pupae was the sum of pupae in each cohort at that time:

\[ LP_{p(t)} = \sum_{i=0}^{i=21} LP_{p(t)} \]  
(22)

At the start of the simulation \( t = 0 \), \( LP_{p(t)} = 0 \) for \( i = 0 \).

In subsequent time steps the number of pupae in each cohort in each time step was given by:

\[ LP_{p(t+1)} = LP_{p(t)} - MLP_{p(t+1)} - DLp_{p(t+1)} + DLl_{l(t+1)} \]  
(23)

where \( MLP_{p(t+1)} \) was the number of lacewing pupae dying as a result of background mortality

\[ MLP_{p(t+1)} = LP_{p(t)} \cdot m_{LP} \]  
(24)

\( DLp_{p(t+1)} \) was the number of lacewing pupae developing into lacewing adults

\[ \text{if } \frac{t}{20} - i > d_{Le} + d_{Ll} + d_{LP} \]  
then \( DLp_{p(t+1)} = LP_{p(t)} - MLP_{p(t+1)} \)  
otherwise \( DLp_{p(t+1)} = 0 \)  
(25)

and \( DLl_{l(t+1)} \) was the number of lacewing pupae being recruited by development of lacewing larvae (Equation 21).

The conditionalities of \( DLl_{l(t+1)} \) ensured that the pupae formed cohorts which followed on from the cohorts of larvae. With the parameter values and simulation durations used here, lacewing pupae were unable to reach their development time and the value of \( DLp_{p(t+1)} \) was always zero.

**Lacewing adults**

At any given time step, the total number of lacewing adults was the sum of adults in each cohort at that time:

\[ LA_{a(t)} = \sum_{i=0}^{i=21} LA_{a(t)} \]  
(26)

With the parameter values and simulation duration used here lacewing pupae were unable to become adults and the number of adults in cohorts \( i = 1 \) to \( i = 21 \) was always zero.

At the start of the simulation \( t = 0 \), \( LA_{a(t)} = v \) for \( i = 0 \), where \( v \) was an initial population in the range 1.5–51.25 lacewings \( m^{-2} \), or zero.

If the maximum longevity of the lacewing adults had not been exceeded i.e.

\[ \text{if } \frac{t}{20} - i < l_{La} \]
then in subsequent time steps the number of lacewing adults in each cohort in each time step was given by:

\[ La_{i(t+1)} = La_{i(t)} - MLA_{i(t+1)} + DL_{p(i(t+1)}} \quad (27) \]

otherwise \( La_{i(t+1)} = 0. \)

MLA\(_{i(t+1)}\) was the number of lacewing adults dying as a function of aphid density

\[ MLA_{i(t+1)} = La_{i(t)} \cdot \left( \frac{im_{La} - km_{La}(An_{t(t)} - MAN_{t(t+1)})}{hm_{La} + (An_{t(t)} - MAN_{t(t+1)})} \right) \quad (28) \]

and DL\(_{p(i(t+1))}\) was the number of lacewing larvae developing into pupae (Equation 25), which always had a value of zero with the parameter values and simulation duration used here. The longevity of the lacewing adults was greater than the simulation durations so in the simulations presented, the population of adults was a single cohort that persisted to the end of the simulation and was subject to mortality as a function of aphid density.

**Parasitised lacewing larvae**

At any given time step, the total number of parasitised larvae was the sum of larvae in each cohort at that time:

\[ PL_{i(t)} = \sum_{i=0}^{i=21} PL_{i(t)} \quad (29) \]

At the start of the simulation \( t = 0 \), \( PL_{i(0)} = 0 \) for \( i = 0 \).

In subsequent time steps the number of parasitised larvae in each cohort in each time step was given by:

\[ PL_{i(t+1)} = PL_{i(t)} - MPL_{i(t+1)} - DPL_{i(t+1)} + SL_{l(i(t+1)}} \quad (30) \]

where MPL\(_{i(t+1)}\) was the number of parasitised larvae dying as a function of aphid density

\[ MPL_{i(t+1)} = PL_{i(t)} \cdot \left( \frac{im_{pl} - km_{pl}(An_{t(t)} - MAN_{t(t+1)})}{hm_{pl} + (An_{t(t)} - MAN_{t(t+1)})} \right) \quad (31) \]

DPL\(_{i(t+1)}\) was the number of parasitised larvae developing into parasitoid pupae (with a correction factor of 0.05 to prevent lacewing larvae being stung and developing into parasitoid pupae in the same time step)

\[ \text{if } \frac{t}{20} - i > d_{la} + d_{pl} + 0.05 \]

\[ \text{then } DPL_{i(t+1)} = PL_{i(t)} - MPL_{i(t+1)} \quad (32) \]

otherwise \( DPL_{i(t+1)} = 0 \)

and SL\(_{l(i(t+1))}\) was the number of parasitised larvae being recruited through stinging of lacewing larvae (Equation 20).

**Parasitoid pupae**

At any given time step, the total number of parasitoid pupae was the sum of pupae in each cohort at that time:

\[ PP_{(t)} = \sum_{i=0}^{i=21} PP_{i(t)} \quad (33) \]

At the start of the simulation \( t = 0 \), \( PP_{i(0)} = 0 \) for \( i = 0 \).
In subsequent time steps the number of parasitoid pupae in each cohort in each time step was given by:
\[
P_{p_i(t+1)} = P_{p_i(t)} - MP_{p_i(t+1)} - DP_{p_i(t+1)} + DP_{i(t+1)}
\]  
(34)

where \(MP_{p_i(t+1)}\) was the number of parasitoid pupae dying as a result of background mortality
\[
MP_{p_i(t+1)} = P_{p_i(t)} \cdot m_{pp}
\]  
(35)

\(DP_{p_i(t+1)}\) was the number of parasitoid pupae developing into adults (with a correction factor of 0.05 for consistency with Equation 32).

\[
\text{if } \frac{t}{20} - i > d_{Le} + d_{Lp} + 0.05 \\
\text{then } DP_{p_i(t+1)} = P_{p_i(t)} - MP_{p_i(t+1)}
\]  
(36)

otherwise \(DP_{p_i(t+1)} = 0\)

and \(DP_{i(t+1)}\) was the number of parasitised pupae recruited through the development of parasitoid larvae (Equation 32).

The conditionalities of \(DP_{i(t+1)}\) ensured that the pupae formed cohorts which followed on from the cohorts of larvae. With the parameter values and simulation durations used, parasitoid pupae were unable to reach their development time and the value of \(DP_{p_i(t+1)}\) was always zero.

**Parasitoid adults**

At any given time step, the total number of parasitoid adults was the sum of adults in each cohort at that time:
\[
Pa_{i(t)} = \sum_{i=0}^{i=21} Pa_{i(t)}
\]  
(37)

With the parameter values and simulation durations used, parasitoid pupae were unable to become adults and the number of adults in cohorts \(i=1\) to \(i=21\) was always zero.

At the start of the simulation (\(t = 0\)), \(Pa_{i(0)} = z\) for \(i = 0\), where \(z = 0\) or \(z = 2\) parasitoids \(m^{-2}\).

If the maximum longevity of the parasitoid adults had not been exceeded i.e.
\[
\text{if } \frac{t}{20} - i < l_{Pa}
\]

then in subsequent time steps the number of parasitoid adults in each cohort in each time step was given by:
\[
Pa_{i(t+1)} = Pa_{i(t)} - MPa_{i(t+1)} + DP_{pa_{i(t+1)}}
\]  
(38)

otherwise \(Pa_{i(t+1)} = 0\)

\(MPa_{i(t+1)}\) was the number of parasitoid pupae dying as a result of background mortality
\[
MPa_{i(t+1)} = Pa_{i(t)} \cdot m_{pa}
\]  
(39)

and \(DP_{pa_{i(t+1)}}\) was the recruitment of parasitoid adults through development of parasitoid pupae (Equation 36). This always had a value of zero for the parameter values and simulation durations used.
**Key output variables**

The potential for crop damage in the simulated crop cycle was indicated by the variable aphid-days m$^{-2}$. The correction factor converts the value from time steps to days.

$$\text{aphid days} = \left( \frac{1}{0.05} \right) \cdot \left( \sum_{t=0}^{t=21} \text{An}_t + \sum_{t=0}^{t=21} \text{Aa}_t \right)$$  \hspace{1cm} (40)

The potential for biological control in the future was indicated by the final populations of adult and pupa lacewings.

$$\text{final lacewing adults} = \text{La}_{(t=21)}$$  \hspace{1cm} (41)

$$\text{final lacewing pupae} = \text{Lp}_{(t=21)}$$  \hspace{1cm} (42)
A3.3 SENSITIVITY ANALYSIS

As described in Chapter 7, Section 7.3.2, the model was first run with a standard parameter set and then with 27 alternative parameter sets to determine the sensitivity of the output to the parameter values. The results of simulations with the standard parameter set are presented in detail in Chapter 7. Here the six key results of those simulations are summarised, the degree to which they differed when alternative parameter sets were used is described, and the implications of any differences for interpreting the model output are discussed. For each key result these are described in sections titled ‘standard result’, ‘sensitivity’ and ‘conclusions’ respectively. The alternative parameter sets are described in Table A3.3 (page 201).

1 Aphid populations in the absence of lacewings

1.1 Standard result
In the absence of lacewings, the aphid populations reached damaging levels even with the lowest initial populations.

1.2 Sensitivity
Aphid populations varied greatly when the parameter values affecting aphid processes were altered. Relative to the populations with the standard parameter set, the populations in these scenarios were: high adult mortality: 0.76; high nymph mortality: 0.38; resistant lucerne: 0.30; 15 °C: 1.82. However, the aphid-days accumulated always exceeded the 50,000 aphid-days $m^{-2}$ injury level, reaching 83,769 aphid-days with the lowest initial population and the resistant lucerne parameter set.

1.3 Conclusion
In the model system there was always potential for the aphid populations to damage the lucerne crop so there was a need for biological control by lacewings.

2 The efficacy of biological control in the simulated re-growth period in the absence of flowers

2.1 Standard result
In the absence of flowers, lacewings could suppress potentially-damaging aphid populations to a significant degree. Suppression was associated with a ratio of initial An:La = 65 at which there was a fundamental shift in the population dynamics between ‘control’ of the aphid population and its sustained growth. (This ratio is hereafter referred to as the ‘critical ratio’.) The aphid population was only kept at a non-damaging level when both the initial population of aphid nymphs and the initial ratio of aphid nymphs to adult lacewings were relatively low.

2.2 Sensitivity
Fig. A3.2i shows that the alternative parameter sets had very different effects on the percentage of initial population scenarios which were kept at non-damaging aphid populations. The
parameter sets that resulted in large changes in this summary statistic caused notable changes in the relationship between the aphid population dynamics and the initial populations.

The ‘critical ratio’ was sensitive to the maximum rate of consumption by lacewing larvae (kcLl) and the maximum rate of eggs production by lacewing adults (kfLa). Doubling these parameter values reduced the ratio from An:La = 65 to approximately An:La = 40 and halving them increased it to An:La = 120. This respectively reduced and increased the percentage of scenarios with non-damaging aphid populations (Fig. A3.2).

When selecting the parameter values, there was particular uncertainty about the parameters in the functional responses which quantified the aphid nymph densities at which the rates were half their maximum value (hcLa, hcLl, hfLa, hmLa, hmLl). The dynamics were robust to doubling and halving these values and this is apparent as changes in the degree of effective aphid control which are negligible compared with those caused by changing the maxima of the functional responses (kcLa, kcLl, kfLa, kmLl, kmLa).

The accumulation of aphid-days was altered substantially with the ‘resistant lucerne’ parameter set. Aphid populations were non-damaging in 77% of the initial population scenarios. Although the proposed injury level was greatly exceeded (>100,000 aphid-days m\(^{-2}\)) with the highest initial An and An:La, the aphid population did not undergo sustained growth as with the standard parameter set. A second peak was reached on Day 15 and the aphid population then declined to the end of the simulation. Day 15 is the day the founding adult aphids reach their maximum longevity and die; an event which did not occur with the other parameter sets because the longevity was longer than the duration of the simulation.

When the model was run to simulate 15 °C, damaging aphid populations were reached with all initial populations. The minimum aphid-days accumulated was 77,446 m\(^{-2}\) (for initial An = 300 m\(^{-2}\) and An:La = 20). The aphid populations were never brought to low levels as with the standard parameter set but at low initial An and initial An:La the aphid population fell very slightly after Phase 1, rose to a second peak and then declined to the end of the simulation.

Doubling the rate of aphid nymph mortality (mAn) only had a small effect on the percentage of scenarios with satisfactory control but greatly reduced the growth of the aphid population in Phase 3 when the initial ratio was high (An:La > 65).

### 2.3 Conclusion

For most parameter sets, lacewings in the model system were able to keep aphids at non-damaging levels in some initial population scenarios but these scenarios were a minority of those simulated. However, this was not the case with the ‘resistant lucerne’ parameter set or the ‘15 °C’ parameter set. In these cases the fecundity of aphids was changed and the situation in which initial populations determined whether the aphid population was controlled or not,
Fig. A3.2 Summary of simulations to assess how the efficacy of biological control varied with alternative parameter values. i) The percentage of initial population scenarios with effective biological control for simulations using the standard parameter set and a range of alternative parameter sets, with and without flowers. Effective biological control was defined as accumulation of < 50,000 aphid-days m\(^{-2}\) during the simulation. The percentage is calculated from 570 simulations with 300 \(\leq\) An \(\leq\) 1025 and 20 \(\leq\) An:La \(\leq\) 200. (An = aphid nymphs, La = lacewing adults.) The parameter sets are ordered by the proportional effect of flowers. ii) Brief parameter set descriptions for the codes used in (i). See Table A3.3 for full descriptions.
changed to situations respectively dominated by control of aphids and uncontrolled aphid growth.

The maximum rates of lacewing egg production and consumption of aphid nymphs by the lacewing larvae had greater influence on the population dynamics than the functional responses or rates of mortality. The values of these parameters could have a substantial effect on the outcome of simulations with particular initial populations, but did not lead to fundamental changes across the range of initial population scenarios as with the ‘resistant lucerne’ and ‘15 °C’ parameter sets.

3 The potential for biological control in the future in the absence of flowers

3.1 Standard result

In the absence of flowers, few adult lacewings survived in situations where biological control was effective, but across the range of initial populations sufficient lacewing pupae were produced for biological control to be effective for most future initial aphid populations. If the pupae emerged and formed new initial populations with the surviving adults without further mortality, future initial populations of 650 aphid nymphs m\(^{-2}\) would be kept at non-damaging levels by 100% of the current final populations.

3.2 Sensitivity

The alternative parameter sets which had the greatest effects on the final number of lacewing pupae generally also had the greatest effect on survival of lacewing adults. The exceptions were those sets with more efficient or less efficient foraging by lacewing adults when the relationship between consumption and mortality was maintained, and the parameter set ‘high adult mortality’. In these cases the parameter value for kmLa itself was changed. In all cases however, the final number of adults was small relative to the final number of pupae so the latter dominated recolonisation potential. Only pupa populations are therefore discussed here.

The sensitivity of the ‘critical ratio’ to the maximum consumption by lacewing larvae (kcLl) and the maximum rate of eggs production by lacewing adults (kfLa) which affected accumulation of aphid days, also affected the final populations of lacewing pupae. When the fecundity of lacewings was halved, the peak population fell and moved to the new ‘critical ratio’ of An:La = 40, with a second peak at An:La = 20. Above An:La = 65 the populations were approximately half the standard populations but below this ratio the effect was less severe and for some initial conditions the populations were increased, indicating a shift in the pattern rather than a general decrease in values. Conversely, when fecundity doubled the peak increased and moved to An:La = 120, with the second peak remaining at An:La = 20. Above An:La = 120 the final lacewing populations were approximately double those with the standard parameter set but below this ratio the populations could be higher or lower than the standard values. Doubling and halving consumption by lacewing larvae had the same effects on the ‘critical ratios’ as changing the lacewing fecundity. However, the final populations of lacewing pupae with increased kcLl
were half those with increased kfLa, and those with decreased kcLl were twice those with decreased kfLa.

Despite substantial changes in the combined final populations of adults and pupae, the effects on the measure of biological control potential were minor: the percentage of initial populations which could satisfactorily control (< 50,000 aphid-days m$^{-2}$) a moderate future initial aphid population (An = 650) was 91% when the maximum rate of lacewing egg production was halved. Control was still achieved for 100% of initial populations when the maximum rate of consumption by lacewing larvae decreased.

Increasing mortality of lacewing larvae (mLl) also had substantial effects on the final number of lacewing pupae. Above the critical ratio of An:La = 65, doubling the mortality of lacewing larvae approximately halved the final number of lacewing pupae. Below this ratio the effect was reduced, and the range of ratios over which there was an inverse relationship between the initial number of lacewing adults and the final number of lacewing pupae was reduced. However, this only reduced the measure of biological control potential from 100% to 99%.

The final number of lacewing pupae was greatly reduced with the ‘resistant lucerne’ parameter set. The range of An:La ratios over which there was an inverse relationship between the initial number of lacewings and the final number of lacewing pupae extended from 80 to 140 (compared with 40 to 60 for the standard parameter set) and the final populations were 35-96% of the standard values. The reduction was relatively small when there were fewest lacewing pupae but was sometimes substantial with moderate numbers of pupae. Nevertheless, because the fecundity of aphids was low with this parameter set, the measure of biological control potential remained at 100%.

With the 15 °C parameter set, the number of lacewing pupae was reduced to approximately 31% of the standard value above An:La = 65. As the ratio fell below this value the population was also reduced but to a decreasing degree. In contrast with the effects of resistant lucerne therefore, the minimum number of lacewing pupae was much lower than the standard value (31%) and the maximum value was similar to the standard (91%). Furthermore, the aphid fecundity was increased relative to that of the lacewings. As a consequence, the measure of biological control potential fell to 27%.

With the remaining alternative parameter sets, the final populations of lacewing adults and pupae produced by the range of initial aphid and lacewing adult populations simulated were all able to keep moderate future aphid populations below the injury level. The maximum aphid populations that could be controlled however, would vary.

3.3 Conclusion

Relatively large populations of lacewing pupae were produced during the simulations so even when altering the parameter values led to reductions in the populations of adults and/or pupae, they were sufficient to provide satisfactory biological control of moderate future aphid
populations. The only exception to this was in the ‘15 °C’ scenario where altering the relative rates of processes for aphids and lacewings reduced the population of adults and pupae to a level where control would only be achieved if the current initial aphid nymph population was high and the ratio of aphid nymphs to lacewings was low (i.e. initial populations of lacewing adults were high).

4 The effect of flowers on biological control in the simulated re-growth period

4.1 Standard result

Adding flowers to the system with aphids and lacewings generally had a positive but small effect on biological control by the lacewings. For a small proportion of initial conditions, flowers could substantially reduce aphid populations and these reductions might be agronomically significant because they occurred at aphid densities near the injury level.

4.2 Sensitivity

Figure A3.2i shows that across the range of alternative parameter sets, the flowers had positive but small effects on the percentage of initial population scenarios that maintained aphid populations at non-damaging levels (< 50,000 aphid-days m⁻²). Only with the 15 °C parameter set did no effect occur, when aphids always exceeded the injury level, with or without flowers. The differences between the parameter sets in this measure of biological control were trivial, amounting to a few of the simulated initial population scenarios falling either side of the injury level. Parameter sets which had a smaller difference between the ‘with flowers’ percentage and the ‘without flowers’ percentage in Fig A3.2i sometimes had larger effects of flowers on the aphid-days variable for each initial population scenario. These discrepancies arose because the effect of flowers on the percentage of non-damaging scenarios depended on the relationship between the injury level and the aphid-days variable for the particular sample of initial population scenarios simulated, and this varied between parameter sets.

For most of the initial populations with alternative parameter sets, the effect of flowers remained small and positive with the biggest effect being restricted to a small proportion of initial population conditions. Changing the maximum rates of lacewing egg production (kFLa) and consumption of aphid nymphs by lacewing larvae (kCLl) changed the ratio at which the maximum effects of flowers occurred, in correspondence with the change in ‘critical ratio’ described above. The magnitude of the most beneficial effect of flowers varied from 0.70–0.97 (minimum standard effect = 0.82). The effect of 0.70 occurred in the parameter set where lacewing adults foraged more efficiently and the relationship between consumption and mortality and fecundity was maintained. The effect of 0.97 occurred with the ‘15 °C’ parameter set; in this case flowers had negligible effects with all initial populations.

Although the effect of flowers was usually small and positive, a notable difference between some of the alternative parameter sets and the standard set was the occurrence of initial populations where the effect of flowers on the aphid-days variable was negative. This occurred
at low initial An:La ratios and was particularly frequent, and of greatest magnitude, with the parameter set where there was a greater effect of flowers on aphid consumption by adult lacewings, and with the ‘resistant lucerne’ parameter set. In Phase 1, adult lacewings with flowers ate fewer aphids than those without flowers, leading to higher aphid populations. With most initial populations and with most parameter sets, this negative effect was outweighed by the higher fecundity that led to lower aphid populations in Phase 3. In the parameter set where the effect of flowers on aphid consumption was increased by halving the value of hcLa with flowers, the higher aphid population with flowers in Phase 1 outweighed the lower population in Phase 3, for some initial populations, leading to a negative effect of flowers on the total aphid-days accumulated. When the fecundity of the aphids was reduced with the resistant lucerne parameter set, consumption by adult lacewings had a greater impact on the aphid population dynamics and the negative effect of flowers in Phase 1 contributed much more to the total-aphid days than the small differences in Phase 3 when the aphid population was very low.

Similar negative effects occurred to a lesser degree with other parameter sets. Although theoretically interesting, the effects were of little agronomic significance, being small and infrequent. The most negative effect was 1.11, occurring for ‘resistant lucerne’ with initial An:La = 20 and initial An < 850 m$^{-2}$. Under these conditions the aphid population was well below the proposed injury level with or without flowers (For initial An:La = 20 and initial An = 850 m$^{2}$, with flowers = 11,187 aphid-days m$^{-2}$, without flowers = 10,121 aphid-days m$^{-2}$).

4.3 Conclusion

Adding flowers to the system with aphid and lacewings could have positive or negative effects on biological control by the lacewings. Although there could be agronomically significant biological control in some situations, in general the effects would be negligible.

5 The effect of flowers on the potential for biological control in the future in the absence of parasitoids

5.1 Standard result

Adding flowers to the system with aphids and lacewings could have positive or negative effects on the populations of lacewings available for recolonisation (adults and pupae combined). However, these effects were small so the populations were always at levels that could satisfactorily control most future aphid populations (within the range simulated).

5.2 Sensitivity

With the alternative parameter sets, providing flowers had little effect on the percentage of initial population scenarios which resulted in sufficient final populations of adult and pupa lacewings for satisfactory control (< 50,000 aphid-days m$^{2}$) of moderate future initial aphid populations (initial An < 650). For most parameter sets this percentage was 100% with and without flowers. When lacewings had a lower maximum rate of oviposition, the percentage
increased from 91 to 94% with flowers. The values for increased the larval mortality and 15 °C parameter sets were 98 to 99% and 27 to 28% respectively.

The effect of flowers on the final populations of lacewing adults varied considerably between the alternative parameter sets but this variation was due to the sensitivity of the measure to low populations (Section 7.4.4). In all situations the population of adults was small relative to the population of pupae so only the latter are discussed.

With the alternative parameter sets, the complex relationships between initial An:La, initial An and the effect of flowers on the final number of lacewing pupae were similar to the standard, except with the parameter sets where the ‘critical ratio’ shifted (see 3.2 above). In these situations there was a corresponding shift in the pattern of effects of flowers on final populations of lacewing pupae. The magnitude of the effects remained small. Negative effects never resulted in a reduction of more than 5% (< 0.95). Positive effects were greatest for the resistant lucerne parameter set (up to 1.25) and when adult lacewings foraged less efficiently but aphid consumption had a constant relationship with lacewing mortality and fecundity (up to 1.17). The corresponding changes in the aphid populations that could be controlled were proportionally smaller. For example, if the final population of pupae emerged and combined with the final populations of lacewing without further mortality, the effect of flowers of 1.25 with the resistant lucerne parameter set would correspond to an increase in the initial population of aphid nymphs which could be kept at non-damaging levels from 1133 m$^{-2}$ to 1210 m$^{-2}$ (current initial An = 300 m$^{-2}$, initial An:La = 20, final La+Lp without flowers = 34.16 m$^{-2}$, with flowers = 47.67 m$^{-2}$, future simulations assume absence of flowers). This is an increase of 13% in a high aphid population and the equivalent percentage for the effect of 1.17 was approximately 7%.

5.3 Conclusion

Across the alternative parameter sets, adding flowers to the lacewing-aphid system generally had small positive or negative effects on the potential of biological control in the future. Even the largest effects were unlikely to be agronomically significant. In the few situations where future control would be unsatisfactory without flowers, it would not be significantly improved by the presence of flowers.

6 The effect of flowers on the potential for biological control in the future in the presence of parasitoids

6.1 Standard results

Adding parasitoids to the system with aphids and lacewings only led to parasitism when flowers were present. Biological control in the current cropping cycle could not be affected but there were invariably negative effects on the potential for biological control in the future. The effects were generally small but with low initial number of lacewings (low An and high An:La), the
reduction in final numbers of combined lacewing adults and pupae led to substantial reductions in the densities of aphids that might be controlled satisfactorily in the future.

6.2 Sensitivity
Fig A3.3i shows that for the initial population scenarios where there were large negative effects of flowers and parasitoids, altering the parameter values for the parasitoid’s functional response could substantially worsen or improve these effects. Increasing the mortality of adults of all species also significantly reduced the final number of pupae when flowers and parasitoids were present, compared with when they were both absent. When initial An was high and initial An:La was low, parasitoids with flowers had relatively little impact on the final number of lacewing pupae irrespective of the parameter values.

The effect of doubling the maximum rate of parasitoid oviposition reduced the final number of lacewing pupae to 0.018 m\(^{-2}\). This was lower than the final population of lacewing adults (0.53 m\(^{-2}\)) and the combined populations for recolonisation would only be able to keep a future initial population of 76 aphid nymphs m\(^{-2}\) at non-damaging levels (in the absence of flowers), compared with 275 m\(^{-2}\) with the standard parameter set. The effect of halving the maximum rate of oviposition was to increase this value to 620 aphid nymphs m\(^{-2}\). These compare with the value without flowers or parasitoids of 825 m\(^{-2}\).

6.3 Conclusion
Flowers invariably had a negative effect on lacewing populations when parasitoids were present. Although these effects often had only minor consequences for biological control potential, with low initial numbers of lacewings the effects could be significant. The degree of reduction in biological control potential was sensitive to the functional response of the parasitoids and in some cases effectively led to loss of a lacewing population for recolonisation.
Fig. A3.3. Summary of simulations to assess how the potential for biological control in the future varied with alternative parameter values. i) The final number of lacewing pupae in simulations with flowers and parasitoids as a proportion of the number in simulations without flowers or parasitoids. Simulations used the standard parameter set or an alternative parameter set. The values plotted are the maximum and minimum values from 570 simulations run with a range of initial populations for each parameter set. Values < 1 indicate flowers reduce the potential for biological control in the future, in the presence of parasitoids. ii) Brief parameter set descriptions for the codes used in (i). See Table A3.3 for full descriptions.
Table A3.3 Alternative parameter sets used to test the sensitivity of the model to the parameter values. i) Verbal descriptions of each alternative parameter set, described relative to the standard parameter set. ii) Numerical descriptions of each alternative parameter set. The table contents are the factors by which the standard parameter values were multiplied. Column codes refer to the alternative parameter set codes (Table A3.3i) and row codes refer to the parameters (Table A3.2). Italicised values were only applied in simulations with flowers. Parasitoids were not present in simulations with the 15 °C parameter set so the parasitoid parameters were not adjusted.

Table A3.3i

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<th>Code</th>
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<td>A</td>
<td>aphid resistant lucerne variety (values based on Sandmeyer et al. 1971)</td>
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<td>B</td>
<td>15°C (values based on Leathwick (1989), Campbell &amp; Mackauer (1975), Campbell et al. (1977))</td>
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<td>C</td>
<td>adult lacewings foraged less efficiently but rates of oviposition and mortality were maintained</td>
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<td>D</td>
<td>adult lacewings foraged more efficiently but rates of oviposition and mortality were maintained</td>
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