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**Floral resource subsidies for the enhancement of the biological
control of aphids in oilseed rape crops**

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

at
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by
Yann-David Varennes

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Food production is achieved by the interaction of man-made infrastructures with natural ecosystems, the latter providing soil, light, and regulating services, including biological control. However, such natural capital has been put increasingly at risk by modern agricultural practices. For example, the use of insecticide compounds can be harmful to organisms in the soil, the water and the vegetation, including beneficial insects. This thesis investigated how the ecological management of a conventional oilseed rape (OSR) cropping system can enhance the biological control of insect pests by their natural enemies, which could alleviate the use of insecticides.

OSR hosts three aphid species, namely, *Brevicoryne brassicae* (L.), *Myzus persicae* (Sulzer), and *Lipaphis erysimi* (Kaltenbach). In New Zealand, these three species are attacked by the parasitic wasp *Diaeretiella rapae* (McIntosh) [Hymenoptera: Braconidae], which completes its larval development inside an aphid body, and is a free-living organism when adult. In that stage, the wasp only feeds on carbohydrate-rich fluids, e.g. floral nectars and honeydew.

Floral resource subsidies consist in the addition of nectar-providing vegetation in the habitat of parasitoids, to enhance their reproductive output, which in turn cascades into decreased pest density. This approach has known successes and failures, and its potential could be increased by a better understanding of its ecological functioning. In the introduction, this thesis lists current knowledge gaps in the ecology of floral subsidies targeted at enhancing the control of pests by parasitoids.

In the second chapter, this thesis reports how nectar feeding affects the behaviour of *D. rapae*. It was observed that feeding on buckwheat (*Fagopyrum esculentum* Moench) enhanced ca. 40-fold the time spent searching for hosts and greatly reduced the time spent stationary. The consequences of this for the reproduction of the parasitoid, and biocontrol, are discussed.

The third chapter addresses the potential competition between pollinators and parasitoids for nectar, when the latter is provided as a floral subsidies. This question is crucial because the potential effect of floral subsidies on biocontrol could be negated by if the provided nectar is consumed by pollinators. A manipulative field experiment indicated that this negative interaction is not existent or weak, although the power of the test was low.

A laboratory trial presented in the fourth chapter showed that the longevity of *D. rapae* fed on OSR or buckwheat nectar was enhanced ca. 3-fold compared to unfed conspecifics. Feeding on *M. persicae* honeydew and nectar from two candidate floral subsidies enhanced longevity ca. 2-fold, indicating a lower nutritional quality. Two other plants did not cause any longevity enhancement. The value of these results with regard to the understanding of the nutritional ecology of *D. rapae* is discussed.

The food-web of aphids, parasitoids and hyperparasitoids (fourth trophic level) living in OSR crops in New Zealand has not been documented. Understanding the composition and structure of the food-web is important to guide the implementation of floral subsidies. The fifth chapter presents a protocol for the reconstruction of food-webs, based on the molecular analysis of aphid mummies. The further use of this tool for the construction of aphid-based food-webs in general is discussed.

The thesis findings are discussed in the context of OSR as an ephemeral, multi-species, spatially complex and dynamic habitat. The concept of “foodscape” is adapted to parasitoids and biological control. In its last section, the discussion integrates ecological and agricultural considerations to suggest the intercropping of a flowering plant in OSR crops.

Keywords: parasitoid, *Diaeretiella rapae*, *Myzus persicae*, *Brevicoryne brassicae*, conservation biological control, companion plants, agriculture, ecosystem services, environmental DNA, *Fagopyrum esculentum*, search rate, foraging strategy, HIPV.

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Chapter 1

General Introduction

1.1 General introduction: ecosystem services, conservation biological control and habitat management

1.1.1 Agriculture and ecosystems

The concept of Ecosystem Services (ES) describes all ecological processes by which ecosystems contribute to sustaining and fulfilling human life (Costanza et al., 1997; Daily, 1997; Ehrlich and Mooney, 1983). These services range from supporting life (e.g. formation of soil, production of air and freshwater), providing goods (e.g. food, fuel, fibre), regulating dynamic phenomena (e.g. freshwater cycling, decomposition and nutrient cycling, carbon storage, pollination, pest control), to providing base elements for cultural developments (e.g. existence of iconic species and landscapes with aesthetic value). Alongside ES, some ecosystem phenomena negatively impact human well-being (e.g. infectious diseases, crop destruction by herbivores) and are referred to as ecosystem dis-services (EDS, Dunn, 2010). Many ES and EDS do not influence human well-being directly, but rather, interact with human structures (built capital, human knowledge and skills, social structures) to benefit (or not benefit, in the case of EDS) mankind (Fig. 1 in Costanza et al., 2014). An example of ES interacting with human capital is food production, as it is permitted by the existence of species, soils and favourable climate, but requires crop management, transformation, distribution and selling to consumers to fully benefit mankind.

ES contribute substantially to agriculture's productivity (Power, 2010; Zhang et al., 2007). For example, the activity of soil biota governs the biological, physical and chemical drivers of soil fertility (Barrios, 2007); about 35% of food production depends to some degree on animal pollination (Klein et al., 2007); the monetary value of the biological control of pests by natural enemies is estimated at 9% of total farm production in the USA (Losey and Vaughan, 2006), and in field trials, predation of aphids by arthropods has been reported to avoid a 23% yield loss in barley (Östman et al., 2003). On another hand, production is limited by EDS, including herbivory by agricultural pests, diseases to crops and livestock, competition with weeds for nutrients and light, extreme weather events (hails, droughts, floods, etc.).

Modern agriculture relies heavily on technical solutions to compensate for EDS and to maximise productivity (monocultures, machinery, mineral fertilisers, chemicals, high-yielding cultivars, etc.). However, alongside with their positive effects, some agricultural technologies have appeared to be

detrimental to human well-being. Among these, fertiliser use, landscape simplification, and pesticide use are recognised as important contributors to several global changes such as biodiversity loss, land degradation, emission of greenhouse gases, and to a general decline in several ES (Dale and Polasky, 2007; Foley et al., 2005). The predicted growth of global human population in the next 100 years would cause a significant increase in, and intensification of, cropped areas. This is likely to worsen the negative environmental impacts of agriculture. Multi-disciplinary efforts at all levels of the food production and distribution chain, including the design of productive and environmentally-sound agricultural practices, are essential to increase food availability while limiting environmental impacts (Foley et al., 2011; Struik et al., 2014; Tilman et al., 2002).

As a way forward, several authors suggest new agricultural practices harnessing existing ES, and alleviating agriculture's reliance on ES-degrading technologies. Altieri (1995) proposed Agroecology as a low-input farming based on ecological interactions and mimicking nature's functioning¹. Shennan (2008) and Médiène et al. (2011) listed several ecology-based solutions to enhance various ES in agriculture. Bommarco et al. (2013) presented the concept of Ecological Intensification, which consists in intensifying ES and ecological interactions on farmland to design sustainable cropping systems. These few examples illustrate a growing research field with numerous authors working at developing ES-based agriculture and agricultural sustainability.

1.1.2 Insect pest management: from chemicals only to integrated management

Among the various ways of alleviating agriculture's environmental impact, one possible option is to reduce insecticide use, by enhancing the naturally-occurring regulation of pests (Bale et al., 2008; Gurr et al., 2012; Lewis et al., 1997). Insecticides have a number of negative effects on human health and the environment: residues are left in foodstuffs, absorbed by consumers (Nougadère et al., 2012), and are suspected to cause health issues (Juhler et al., 1999; Rauch et al., 2012). Exposure to pesticides can be high among farmers, especially where social infrastructures do not guarantee a safe utilisation (Huang et al., 2000). Moreover, insecticides have a significant impact on the environment, as it has been evidenced for example with neonicotinoids, which are the most commonly used group of insecticides globally, in spray or seed coating (Task Force on Systemic Pesticides, see www.tfsp.info). These insecticides can be found in water bodies and soils, where they have lethal and sub-lethal effects on various aquatic species (Anderson et al., 2015) and vertebrates (Gibbons et al., 2014). Also, neonicotinoid uptake through nectar can affect pollinators (Feltham et al., 2014) and parasitoids (Krischik et al., 2007). Finally, because of their broad spectrum, and their persistence in

¹ Agroecology also goes beyond technical practices, advocating for the integration of societal and economic dimensions of farming (see Wezel et al., 2009).

soils and water, neonicotinoids can contribute to deteriorating several ES, including biological control (Chagnon et al., 2014).

Besides their detrimental effects on human health and the environment, insecticides are not a panacea against insect pests. In some agro-ecosystems, insecticides do not control pests more than natural enemies (Bommarco et al., 2011). Insecticide use has also caused cases of pest resurgence, i.e., a previously benign herbivorous insect developed to crop-damaging levels because its natural enemies had been killed by broad-spectrum pesticides (Settle et al., 1996 and examples therein). The repeated use of toxic molecules selects for resistant insects (Bass et al., 2014), decreasing pesticide efficiency. As of today, the use of insecticides appears to be unsustainable and at the same time, insecticide use degrades naturally-occurring biological control. In that sense, insecticide use can be viewed as a “trap” as defined by Folke et al. (2011), or as a driver of “wider sustainability gap” in the sense of Fischer et al (2007) and many countries fund pesticide-reduction programmes (e.g. PURE in the European Union, see www.pure-ipm.eu).

1.1.3 Natural control of pests through Conservation Biological Control

Conservation Biological Control (CBC) is the preservation of natural enemies of noxious organisms, through environmental management and adapted farming practices, aiming at maximising their activity as biocontrol agents. Metaphorically, if crop protection was a battle against pests, CBC consists in making the battleground as favourable as possible for our allies. Earliest reports of CBC approaches date back to the 10th-century China, where ant nests were moved into orange orchards to limit foliage consumers. Between 1950's and 1990's, conservation approaches were investigated (Letourneau and Altieri, 1999) but attracted limited practical interest (Ehler, 1998). The discipline was formally named in 1998 in the seminal work edited by P. Barbosa (1998), and since then, is increasingly represented in academia (Fig. 1.1). In addition to its fundamental aspects, CBC is also orientated towards application and solution-finding. As such, CBC research requires three key steps: the fundamental understanding of pest-enemy interactions, the suggestion of management actions, and the proofing that recommended measures benefit farmers and the society (either financially or by non-marketed retributions such as enhanced ES, see Cullen et al., 2008; Fiedler et al., 2008).

The concept of CBC can be applied to the control of various noxious organisms: arthropod pests, plant diseases and weeds. Natural enemies include predatory arthropods (insects, spiders and mites), parasitoid insects, microbial pathogens, antagonistic fungi. Yet, most attention has focused on the regulation of arthropod pests by their arthropod natural enemies, and on habitat management (Jonsson et al., 2008).

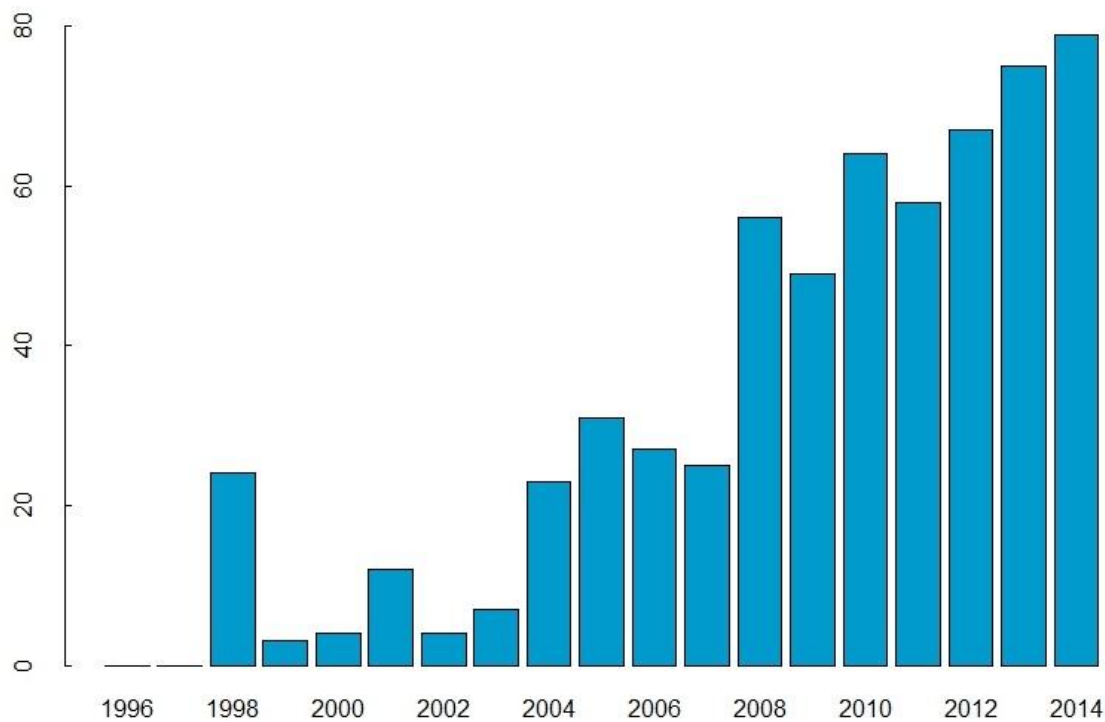


Figure 1.1 Number of 'hits' for « Conservation biological control » on ScienceDirect database referencing publications in peer-reviewed journals and books.

The foundation principle for habitat management is that providing shelter, nectar, alternative host/preys, pollen (summarised in the acronym SNAP, Gurr et al., 2012), or a combination of these, will increase natural enemies efficacy. In practical terms, habitat management includes for example: (i) the provision of flowering plants within or nearby crops, which provide nectar and pollen fed upon by natural enemies, (ii) the planting of perennial grassy strips within crops, that function as a refuge from disturbances and as an overwintering site, (iii) the provision of plants bearing alternative hosts/preys such as non-pest aphids, to sustain natural enemies when their host/preys densities are low, (iv) other physical alterations such as addition of mulch, grass cultivation or digging 10cm-deep holes to buffer micro-climatic conditions (see Landis et al., 2000 for a complete list). Successful habitat management depends on many ecological processes. For example, it requires that the provided habitat or resource are found by natural enemies; natural enemies must match in time with pests' vulnerable stages (Welch and Harwood, 2014); potential side-effects of habitat management must be avoided, etc. (Crowder and Harwood, 2014 listed the current challenges in biocontrol research).

1.1.4 Scope of this thesis

This thesis is intended to contribute to the implementation of floral subsidies in agricultural landscapes, by advancing our knowledge of the ecological processes at stake. The experimental work

was based on oilseed rape (canola, *Brassica napus* L. [Brassicaceae], abbreviated OSR) cropping in New Zealand. Floral subsidies have the potential to enhance biological control and many other ES including pollination (see Box 1.1), conservation of native plant and animal species (Tompkins, 2010), mitigation of erosion and mitigation of nitrate leaching (Fiedler et al., 2008). The present thesis focuses on the role of floral subsidies in enhancing biological control in OSR. The next section presents the OSR agroecosystem in which the work has been carried out. The third section covers the ecological principles behind floral subsidies in general, and identifies existing knowledge gaps. The fourth and last section presents the thesis outline.

Box 1.1 Importance of insect pollination for oilseed rape crops.

The importance of nectar and pollen for pollinators advocates for the provision of flower-rich habitats in agricultural landscapes (Decourtye et al., 2010). Besides their role in enhancing biological control, floral subsidies also have the potential to enhance pollination in OSR. Most OSR cultivars are self-fertile, i.e. pollen grains from a plant can fertilise ovaries of the same plant and allow fruit set (Williams, 1978). As a consequence, pollen-producing OSR cultivars are not heavily dependent on insect pollination, with plant movement in the wind causing enough fertilisation to reach near-normal yields. Insect pollination however gives a moderate increase in yield and seed market value, an earlier fruit set, and a more uniform seed maturation (Ali et al., 2011; Bommarco et al., 2012; Morandin and Winston, 2006). Some OSR cultivars, in particular those used for hybrid seed production, do not produce pollen (male sterility), and thus are not able to self-pollinate. Fruit set is thus dependent on the reception of pollen from other plants (cross-pollination), which is achieved much more efficiently by insects than by the wind (G. Ramsay, James Hutton Institute, pers. comm.). Supporting insect pollination in OSR is therefore (i) optional but advantageous for most commercial cultivars and (ii) necessary where male-sterile cultivars are used, such as in hybrid seed production.

1.2 The role of floral resource subsidies in oilseed rape crops

1.2.1 Biofuel feedstock crops in New Zealand

Biofuels, i.e. liquid fuels sourced from biomass, are a renewable and low-carbon alternative to fossil fuels (Pickett, 2008). Global biofuel production has dramatically increased in the last decade, mainly due to increasing fossil oil prices and government subsidies (Steenblik, 2007). Biofuels are classified in two groups: first generation biofuels, which are made of “*sugar, starch, oils bearing crops or animal fats that in most cases can also be used as food and feed or consist of food residues*” and represent most of biofuel production nowadays; and second generation biofuels, made of cellulose, hemicellulose and lignin (Eisentraut, 2010). The sustainability of first generation biofuels is often questioned because it requires arable land and water, which increases the pressure on these two

globally limited resources; and because it may negatively impact the environment, especially if the cropping system relies on the intensive use of chemical fertilizers and pesticides. Improving agronomic practices and considering ES are crucial in solving sustainability issues associated with first-generation biofuels (Gasparatos et al., 2011; Pickett, 2008).

A large-scale research programme was launched at Lincoln University (LINX 0802) in 2009, with funding from New Zealand companies (Biodiesel New Zealand, Chevron New Zealand) and the government (Ministry of Science and Innovation), to select suitable biodiesel feedstock crops for local conditions, and to develop appropriate production systems. Biodiesel is a biofuel made from the oil present in the seed of several crops such as OSR, the major feedstock crop in New Zealand. The above-mentioned research programme has also identified *Camelina sativa* L. [Brassicaceae] (camelina or false flax) as another potential oilseed crop.

1.2.2 Insect pests in oilseed rape crops.

In New Zealand, the prevalent insect pests of OSR are the cabbage aphid *Brevicoryne brassicae* (L.) [Hemiptera: Aphididae], the green peach-potato aphid *Myzus persicae* (Sulzer), and the turnip aphid *Lipaphis erysimi* (Kaltenbach) (Close and Lamb, 1961; Lamb, 1989) (a synthesis of the life history of these species is provided in Box 1.2). In addition to herbivory damage, *M. persicae* and, to a minor extent, *B. brassicae* and *L. erysimi*, transmit the Turnip yellows virus (TuYV, synonym to Beet western yellows virus) which can cause 30-50% yield losses in OSR (Jones et al., 2007; Kyriakou et al., 1983; Lammerink, 1968; Stevens et al., 2008). Aphids are also important pests of oilseed crops in Australia, the USA, and the Indian peninsula (Buntin and Raymerl, 1994; King et al., 2006; Patel et al., 2004; Saeed and Razaq, 2014). In contrast, aphid infestations do not ordinarily cause significant damage in Europe (Alford et al., 2003; Ellis et al., 1999), however virus transmission by aphids is an emergent problem in France and Germany (Desneux et al., 2006).

Box 1.2 Life history of *M. persicae*, *B. brassicae*, and *L. erysimi* (adapted from: Cottier, 1953; Hughes, 1963; van Emden and Harrington, 2007; van Emden et al., 1969).

These three species are not native to New Zealand and have probably been imported with plant material less than 200 years ago. They are common on exotic cultivated plant species, and may also develop on native species (from which they probably displace native aphids, see Teulon and Stufkens, 1998). The host plants of *M. persicae* range from annual species (e.g. beans, brassicas, potato) to trees (e.g. peach and citrus trees), making this species a remarkable generalist and an important crop pest worldwide. *B. brassicae* and *L. erysimi* also are major crop pests, but their host range is restricted to Brassicaceae. Apart from their host range, the life histories of these three species share a similar pattern. Under latitudes with a marked cold season, they overwinter as a sexually fertilised egg, deposited on the aerial parts of host plants available during winter (in winter, *M. persicae* has a preference for *Prunus* spp.). In spring, the hatching larvae develops into an alate (winged) adult which can disperse over long distances. Spring alate adults asexually produce clones (by parthenogenesis) and bear the conceived individuals in their body, in the form of larvae (viviparism). Once settled on a host plant, they establish a colony of clones. Most of these are wingless and do not disperse over long distances. In response to stress or other environmental signals, winged individuals are produced and these can disperse a second time. In autumn, winged sexual morphs are produced. These reproduce sexually, and the female will deposit the fertilised eggs, which have the ability to survive the winter, on a winter host plant. This life cycle is called holocyclic because a sexual phase alternates with an asexual one. In regions where the winter temperatures are not too cold (ca. 0 °C at minimum), adults and larvae can also survive to the winter, and the sexual phase is often omitted (the cycle is anholocyclic). New Zealand aphid populations are likely to be mainly anholocyclic in the North Island and probably also in the South Island, down to the Canterbury plains (ca. 43 ° latitude south).

These three aphid species, as all aphids, excrete honeydew from their anus. They have not been reported to be involved in a mutualism with ants.

The diamondback moth *Plutella xylostella* (L.) [Lepidoptera: Hyponomeutidae], and small white cabbage butterfly *Pieris rapae* L. [Lepidoptera: Pieridae] can also inflict damage to OSR in New Zealand (Lamb, 1989). Prevalent European insect pests (the pollen beetle *Meligethes aeneus* Fab., the seed weevil *Ceutorhynchus assimilis* Payk., the stem weevil *Ceutorhynchus quadridens* (Panz.), and the pod midge *Dasyneura brassicae* Winn.) have not been reported in New Zealand. Oilseed rape is thought to originate from southern or north-western Europe, or the Mediterranean area (Rakow, 2004), and has been cultivated in Europe for at least five centuries (Bunting, 1985), it is therefore an exotic plant in New Zealand.

New Zealand OSR cultivars are mostly winter cultivars, sown in mid- to late-April and harvested in January. Aphids can particularly impact OSR yield if infestation occurs in the pre-flowering stage (Ellis et al., 1999), and they are controlled by prophylactic insecticide spraying, starting from crop pre-

flowering stage in early October (S. Sim, PureOil New Zealand, personal communication). Estimating maximum acceptable pest levels, together with pest monitoring, could help reduce the use of pesticides by limiting spraying only to cases in which they are needed. Thresholds based on the economic loss due to aphid infestation have been investigated in Canada and the UK (Ellis et al., 1999; Gavolski, 2014) but not in New Zealand. Ellis et al. (1999) concluded that damaging levels of aphids in the UK are rarely attained; however this is likely to be different in the New Zealand context, because of a different climate, and perhaps different aphid life history traits and a different cohort of natural enemies.

Integrated Pest Management (IPM) and pesticide-use reduction programmes in New Zealand have been implemented in pip fruit and viticulture sectors (Walker et al., 2009), and more recently in wheat and vegetable brassica production (Horrocks et al., 2010; Walker et al., 2012), but not in OSR production. These projects resulted in a significant reduction of pest numbers, pesticide use, and a marked adoption of IPM practices by farmers, suggesting that a similar positive outcome could be obtained if IPM was to be tested in OSR.

1.2.3 *Diaeretiella rapae* – major potential biocontrol agent of aphids in OSR

The parasitic wasp *Diaeretiella rapae* (McIntosh) [Hymenoptera: Braconidae] attacks the three OSR aphid species (Pike et al., 1999). *D. rapae* was probably accidentally introduced to New Zealand from Australia, where it was introduced as a biocontrol agent of *B. brassicae* (Kant et al., 2012a). In New Zealand, ca. 30% of *B. brassicae* were parasitised by *D. rapae* on cabbage seedlings held in a shade house and naturally colonised by aphid and parasitoids (Kant et al., 2012b). *D. rapae* is a solitary koinobiont endoparasitoid, i.e. one parasitoid larvae grows within the aphid body, consuming host internal tissues without stopping host growth (aphids can still produce some offspring in the first days after parasitism, although much less than un-parasitised aphids, see Zhang and Hassan, 2003).

When larval growth is near to completion and most host tissue has been consumed, the *D. rapae* larva pupates inside its host, which turns brown and round-shaped, forming an aphid 'mummy' (Fig 1.2), from which the adult eventually emerges. As an adult, *D. rapae* does not feed on host tissue (unlike some parasitoid species), but it can feed on carbohydrate-rich fluids, such as floral and extra-floral nectar, honeydew, or synthetic sucrose solutions. After emergence, it can live 6-14 days when fed on nectar, which is about 2-5 times longer than if it has access to water only and no food (Araj and Wratten, 2015; Jamont et al., 2013; Tyljanakis et al., 2004). *D. rapae* is synovigenic, i.e. females are able to mature eggs during their adult life. In feeding trials without hosts, nectar-fed parasitoids matured 50%-200% more eggs during their lifetime than their initial egg load, whereas egg load of

parasitoids without access to food increased by 50% in the first 24h of adult life and then decreased because eggs were resorbed, probably to compensate for low nutrient intake (Araj and Wratten, 2015; Tylianakis et al., 2004). When presented aphids, fed parasitoids parasitised 2-4 times more aphids than unfed parasitoids (Araj and Wratten, 2015; Jamont et al., 2013). In semi-field experiments, the presence of *Vicia faba* L. plants providing extra-floral nectar increased *B. brassicae* parasitism by *D. rapae* and increased the proportion of females in the progeny (Jamont et al., 2014). Higher proportion of females is beneficial to biocontrol, as males do not parasitise aphids. The behaviour of parasitoids can also be affected by nectar feeding in various ways, e.g. increasing their propensity to search for hosts and to perform energy-demanding behaviours (Araj et al., 2011; Siekmann et al., 2004), however this has not been previously documented in *D. rapae*.

D. rapae, like most aphid and non-aphid parasitoids species, can be parasitised by hyperparasitoids. These represent a fourth trophic level living to the detriment of parasitoids. A large majority of hyperparasitoids belong to the order Hymenoptera, but a few species of Diptera and Coleoptera have developed this strategy too (Sullivan and Völkl, 1999). Their hosts include aphid parasitoids but also the parasitoids of insects in several other orders (Lepidoptera, Diptera, Coleoptera, other Homoptera such as whiteflies and psyllids, etc.). Hyperparasitoids of aphid parasitoids can be either endoparasitic or ectoparasitic - in the latter case, hyperparasitoid eggs are deposited on the surface of the parasitoid body. Fig. 1.2b illustrates the case of a metamorphosed *D. rapae* larva about to emerge, which has been hyperparasitised by *Asaphes vulgaris* Walker². As adults, most hyperparasitoids of aphid parasitoids are slightly shorter than parasitoids (ca. 1.5 mm body length). Their longevity and generation time is in general superior to that of parasitoids, but their fecundity is lower (Sullivan and Völkl, 1999). Hyperparasitism rate of aphids in OSR can reach ca. 50 % in late growing season, but is generally low earlier in the season (e.g. in Iran, see Nematollahi et al., 2014).

As explained in the present section and in 1.1.3, the addition of floral resources in OSR crops has the potential to ameliorate the control of aphids by their parasitoid natural enemies³. However, many points remain to be clarified before practical guidelines can be proposed for the implementation of floral subsidies in OSR. The next section will review floral resource subsidies research and identify key questions to be answered.

² This mummy was taken from a hyperparasitoid colony cultured in controlled conditions, see chapter 5 for details.

³ Floral resources can also benefit the parasitoids of the two other OSR pests: the diamondback moth *P. xylostella* (parasitised by the Ichneumonidae *Diadegma semiclausum* Hellen and *Diadegma insulare* (Cresson)) and the small cabbage white *P. rapae* (parasitised by the Braconidae *Cotesia glomerata* (L.) and *Cotesia rubecula* (Marshall)). The role of floral subsidies for these parasitoids has been substantially evaluated in cabbage crops (Lavandero Icaza, 2004; Lee and Heimpel, 2008, 2005; Winkler, 2005).



Figure 1.2a. Aphid colony on an OSR leaf, attacked by a parasitoid. The braconid wasp *Diaeretiella rapae* is ovipositing in the aphid *Brevicoryne brassicae*. When the host is located, the wasp bends its abdomen forwards and stings the aphid with its ovipositor. In this process, an egg is inserted in the host body. Photo credit: Yann-David Varennes.

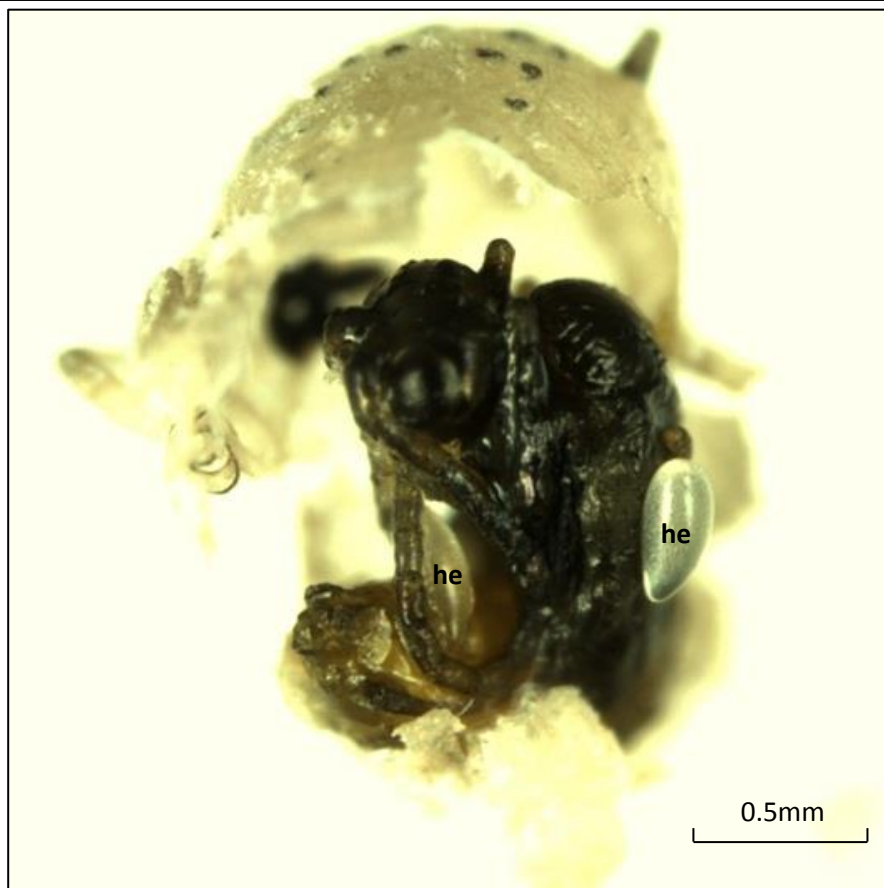


Figure 1.2b Up: Colony of aphids comprising winged and mummified aphids. Parasitoid larvae consume aphid internal tissues and pupates inside its host. The aphid turns into a 'mummy' (m), a light-brown and rounded pupal case containing the parasitoid.

Down: Open aphid mummy containing a near-adult *D. rapae* and hyperparasitoid eggs. Eggs of the hyperparasitoid *Asaphes vulgaris* (he) were laid on a *D. rapae* parasitoid before it emerged from the aphid mummy. Photo credit: Yann-David Varennes.

1.3 Knowledge gaps in the ecology of floral resources for parasitoids

1.3.1 The parasitoid nectar provision hypothesis

Considering that (i) parasitoids are known to visit and feed on flowers, (ii) feeding on nectar benefits parasitoid longevity, fecundity and searching performance, and (iii) monocultures are generally devoid of sugar resources, floral subsidies are intuitively expected to augment parasitism rates and pest control (Heimpel and Jervis, 2005). However, field implementation of floral subsidies have shown successes and failures. In a 2005 review, only seven out of 19 field studies reported an increased parasitism rate in response to nectar provision (Heimpel and Jervis, 2005). A 2011 meta-analysis showed an overall positive effect of nectar provision on parasitism rate in the field, however it failed to record a significant effect on herbivore abundance (Letourneau et al., 2011). The “parasitoid nectar provision hypothesis” proposed by Heimpel and Jervis (2005) formalises the links between nectar provision, parasitoid nutrition, pest abundance and yield. The causal link between these mechanisms has received fragmented field-based empirical support, and most field studies leave room for equivocal interpretation (Heimpel and Jervis, 2005; Wäckers and van Rijn, 2012). The parasitoid nectar provision hypothesis can be divided in four consecutive steps (Fig 1.3), as follows:

Do parasitoids forage for nectar?

The parasitoid nectar provision hypothesis firstly relies on the assumptions that parasitoids are sugar-limited in the field and that they will visit the provided flowers. Most of modern agricultural landscapes are characterised by monocultures, leaving little room for nectar-providing plants, and in such environment, floral subsidies can allow a significant improvement of biocontrol. However, some agroecosystems occasionally comprise nectar-providing vegetation or other food sources. Certain weeds such as *Capsella bursa-pastoris* can provide nutritious nectar to parasitoids (Araj and Wratten, 2015). Also, parasitoids may exploit sugar present in honeydew; e.g., in citrus orchards, the parasitoid *Aphytis melinus*, whose host does not produce honeydew, feeds on honeydew of various phloem-feeding insects (Tena et al., 2013). It is necessary to know and list these sources, because if they provide enough, accessible and constantly-available carbohydrates, floral subsidies may not be visited by parasitoids.

Parasitoids are expected to be attracted to floral nectar, however, this may not be the case for all parasitoid species. Various parasitoids are attracted to visual and olfactory floral cues when starved, and attracted to cues of host presence when fed (including *Cotesia vestalis*, see Kugimiya et al., 2010; *Apanteles aristoteliae*, see Lightle et al., 2010; *Cotesia rubecula*, see Wäckers, 1994). This can be expected to be the case for parasitoids whose hosts are spatially separated from food sources. Indeed, those species for which finding hosts and food are two distinct tasks might have evolved innate attraction to both hosts and food (Wäckers and van Rijn, 2012; Wäckers et al., 2008). These

species can therefore be expected to be attracted to nectar supplied in the field. In contrast, parasitoids whose hosts are associated with sugar sources (e.g. parasitoids of aphids, as their hosts produce honeydew) would find hosts and potential food at the same location. This type of parasitoids might not be innately attracted to floral nectar. This point has been hypothesised by Wäckers and van Rijn (2012; see also Wäckers et al., 2008) but, to our knowledge, has not been empirically confirmed. It is therefore not known whether parasitoids of honeydew-producing hosts, when starved and given a choice between hosts and floral nectar, would orient towards flowers, or would neglect flowers for hosts.

Do flowers provide nutritional nectar in sufficient quantity?

The accessibility of nectar depends on the matching between floral architecture and parasitoid morphology. For example, the corolla of Sweet Alyssum (*Lobularia maritima*, Brassicaceae) flowers being narrower than the head of *Edovum puttleri*, it impeded parasitoid's access to nectaries (Patt et al., 1997). The time from sowing to flowering, duration of flowering stage, and daily patterns of nectar production must also be considered to timely match parasitoid's needs (Bowie et al., 1995; Herrera, 1990). The quantity of available nectar may also be modulated by consumption by other flower visitors. In particular, nectar stocks can be depleted by pollinators (Lee and Heimpel, 2002; Wäckers and van Rijn, 2012).

Environmental conditions such as low soil moisture can also impact nectar production (Carroll et al., 2001). Similarly, on flower with exposed nectaries, low humidity can cause the evaporation of water from nectar droplets, augmenting their viscosity (Cawoy and Kinet, 2009; Koptur, 2005), which can potentially hinder absorption by parasitoids. The fact that viscosity reduces food intake is illustrated by *Cotesia marginiventris* (Cresson) [Hymenoptera: Braconidae] which gained three times less weight when feeding on undiluted (therefore, more viscous) honeydew than on diluted (70% dilution in water, less viscous) honeydew (Faria et al., 2008).

Does metabolism of nectar translate into higher parasitism?

With regard to carbohydrate metabolism, parasitoid species span across a gradient from high dependency on sugar intake (income-breeders) and no dependency at all (capital-breeders, carbohydrates are sourced in their pre-imaginal reserves). Sugars contained in nectar can serve in general metabolism, enabling locomotion, host searching, maturation of eggs and prolonged life (reviewed by Jervis et al., 2008; for *D. rapae*, see section 1.2.2). In some instances, starved parasitoids can also mature eggs at a high rate, as it has been observed in *Macrocentrus grandii* (Goidanich); however, the mechanisms of this phenomenon are not known, and it was suggested to be a compensation of short life-expectancy linked with starvation (Jervis et al., 2008; Olson et al., 2000). Overall, a vast majority of parasitoids can derive benefits from nectar feeding including host-

feeding species (i.e. consuming host tissue) and pro-ovigenic species (i.e. emerging with fully mature egg load) (Casas et al., 2005; Jervis et al., 2008, 2001; Rusch et al., 2012). Although field studies are rare, one study has shown that the longevity and fecundity of *Diadegma insulare* (Cresson) foraging in field-plots was higher in plots with flowering buckwheat, than in plots devoid of nectar sources (Lee and Heimpel, 2008).

The composition and concentration of sugars in parasitoid meals can affect metabolism. For example, the presence of rhamnose or lactose in diet shortened the longevity of *Cotesia glomerata* compared to sucrose, fructose or glucose diets (Wäckers, 2001). Sugar concentrations of 50-70% enabled longer life span in *Aphidius ervi* Haliday [Hymenoptera: Braconidae] than lower (10-30%) concentrations (Azzouz et al., 2004). The sucrose:hexose ratio might condition the nutritive quality of nectar, as sucrose-rich nectar is expected to cause less osmotic stress than hexose-rich nectar, however it has been observed that *Dolichogenidea tasmanica* and *Diadegma semiclausum* have similar longevities when fed on solutions with a range of sucrose:hexose ratios (Tompkins et al., 2010). Other components of nectar may drive its nutritional quality. Nectar can contain fatty acids (Baker, 1977; Bender et al., 2012; Heil, 2011), and because parasitoids are not capable of lipogenesis (Jervis et al., 2008), lipid intake can be crucial to parasitoids. Nectar can also contain antioxidant compounds (Baker, 1977; Carter and Thornburg, 2004; Horner et al., 2007) which could improve insect health.

In the field, increased fitness traits do not necessarily translate into enhanced parasitism rate (Lee and Heimpel, 2008). Although the lack of impact in this study may be a false-negative result⁴, it may also indicate that parasitoids altered their foraging strategy when fed. For example, fed parasitoids may show lower residence times in host patches, and may have dispersed away from experimental plots to search for hosts elsewhere; alternatively, the time spent in flowering vegetation may have competed with time spent searching for and parasitising hosts (discussed by Lee and Heimpel, 2008).

Does higher parasitism cascade into reduced pest density and increased yield?

This last step of the parasitoid nectar provision hypothesis is equivalent to Root's (1973) enemies hypothesis, which states that pest densities can be driven down in plant-diverse habitats because predation and parasitism are more efficient. For example, concomitant increase in parasitism rate and decrease in pest density in nectar-subsidised systems have been reported on *Acyrtosiphon pisum* Harris aphids infesting lucerne (Araj et al., 2009) and *B. brassicae* infesting cabbage (Ponti et

⁴ Authors argued that a small and variable effect together with sample size of 8 or 16 plots may have impeded the detection of a significant effect

al., 2007). Out of the 19 studies of floral subsidies reviewed by Heimpel and Jervis (2005), only 10 quantified pest densities, and only one reported a significant decrease.

However, natural enemies do not always drive the density of their hosts and preys down, because in some cases hosts can reproduce faster than, or ahead of, their parasitoids (White, 2013). In laboratory conditions, *D. rapae* demographic parameters were estimated to be close to that of its hosts *Diuraphis noxia* and *Brevicoryne brassicae* (i.e. intrinsic population increase rate of *D. rapae* and *D. noxia* were 0.19 day^{-1} and 0.22 day^{-1} , respectively) suggesting its potential to be an efficient biocontrol agent (Tazerouni et al., 2012). However, in the field, the lack of synchrony between *D. rapae* and *B. brassicae* minimises the impact of the parasitoid on its host (Nematollahi et al., 2014).

Very few studies have quantified the impact of nectar provision on crop yield. Balmer et al. (2014) reported an 18% increase in yield of cabbage in the presence of nectar-providing cornflower in the 1st of the two years of their study. However, parasitism rate was only increased in the second year of the study. This study focussed on the pest *Mamestra brassicae*, but authors acknowledged that the presence of another un-monitored major pest, and/or predation of *M. brassicae* eggs may have confounded their results.

Floral subsidies can attract and provide nectar not only to parasitoids, but also to pests and hyperparasitoids (Araj et al., 2009; Lavandero et al., 2006; Winkler, 2005), and they can also act as a virus reservoir (Irvin et al., 2014). Therefore floral subsidies can potentially attract pest insects, increase the incidence of viral infections, or intensify parasitoid mortality due to hyperparasitism, counteracting the effects of nectar provision. A targeted selection of nectar-providing plants is necessary to avoid harmful side-effects (Wäckers and van Rijn, 2012). Certain nectar-providing species of plants selectively enhanced parasitoid fitness, but not pest fitness, such as sesame (*Sesamum indicum*) which nectar enhanced the longevity of *Anagrus nilaparvata*, a parasitoid of the rice planthopper, but did not enhance neither the longevity of this pest nor that of other rice pests (the Lepidoptera *Cnaphalocrocis medinalis* and *Marasmia patnalis*, see Zhu et al., 2014, 2013). To date, no plant species has been found to provide a nectar that would benefit parasitoids only and not hyperparasitoids (Araj et al., 2008).

The eventual role of hyperparasitoids in mitigating the effect of floral subsidies in OSR is difficult to evaluate, because hyperparasitoids have received little attention in previous investigations of OSR fauna in New Zealand. At least two species, *Asaphes vulgaris* Walker and *Alloxysta victrix* Westwood can be found in OSR crops in New Zealand (personal observation). Two endemic species, *Alloxysta thorpei* and *Alloxysta rubidus*, have been recently described (Ferrer-Suay et al., 2012), and could be found in OSR, but a reliable description of the aphid-parasitoid-hyperparasitoid food web is lacking.

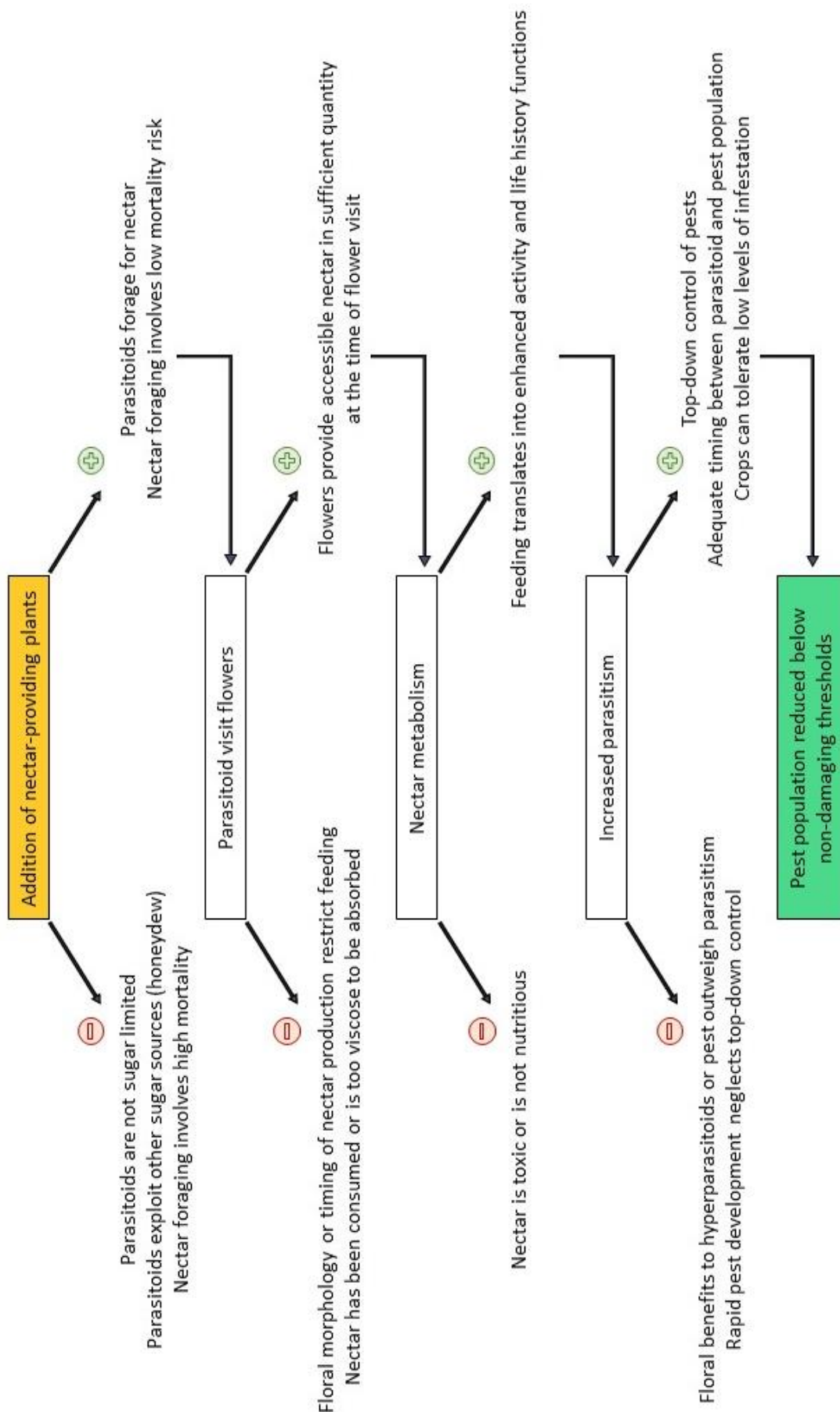


Figure 1.3 Consecutive chain of events linking floral resource subsidies to increased crop yield via nectar provision to parasitoid. *Modified from Heimpel and Jervis (2005) and Wäckers and van Rijn (2012).*

1.3.2 Other mechanisms by which flowering plants contribute to pest control

Besides the parasitoid nectar provision hypothesis, floral subsidies can also alleviate pest impact by at least two other non-exclusive mechanisms. Firstly, nectar and pollen can also benefit pest predators, which are likely to contribute to reducing pest densities, according to Root's enemies hypothesis; this has been observed in lettuce fields where the addition of floral resources increased the density of hoverfly (*Eupeodes fumipennis* (Thompson)) larvae and decreased aphid (*Nasonovia ribisnigri* (Mosley)) densities (Hogg et al., 2011); and in lucerne fields where floral resources increased lacewing (*Micromus tasmaniae* Walker) numbers and decreased aphid (*Acyrtosiphon pisum* Harris) numbers (Jacometti et al., 2010). Secondly, floral subsidies can disrupt herbivory merely by 'breaking' the monoculture. Root's "resource concentration hypothesis" states that herbivory is more intense in monocultures than in diversified vegetation. Thirdly, the presence of flowering vegetation may have other beneficial impacts on natural enemies, such as offering shelter, buffering micro-climatic conditions, and harbouring alternative hosts (Landis et al., 2000). These three mechanisms can be simultaneously at play, as suggested by the lower *Sitobion avenae* densities on wheat interplanted with flowering OSR (Wang et al., 2009). The coexistence of these effects makes difficult the task of disentangling the mechanisms of pest suppression; however, floral resources seem to be a key element in enhancing biocontrol by natural enemies (Ramsden et al., 2014).

1.3.3 From theory to thesis

"The nectar provision hypothesis contains a great deal of complexity despite its intuitive appeal" (Heimpel and Jervis, 2005); "This conceptually-simple approach often fails to reduce pest numbers" (Gontijo et al., 2013). The use of floral subsidies for enhancing biological control is, on one side, a hypothesis that is grounded on reliable data, and, on the other side, a cascade of ecological effects that are difficult to unequivocally link together on the other side. In general, the impact of floral subsidies in the field moderately meets expectations. Theoretical and empirical approaches to the question have to integrate the numerous interacting factors driving insect densities and crop yield; and in order to set practical guidelines for the deployment of floral subsidies, research must also consider the particularities of each agroecosystem studied. The present thesis investigates some of the several questions yet to be answered about floral resource subsidies and biological control using an OSR-aphid-parasitoid system.

1.4 Thesis outline

This section will introduce and detail the problematics addressed in the four experimental chapters of this thesis.

1.4.1 Does nectar feeding change wasp behaviour?

Increased reproductive output of *D. rapae* implies that more eggs are matured, but also that more hosts are found, i.e. nutrition also affects the behaviour of female parasitoids. Few studies documented how nectar feeding can affect the activities performed by some parasitoids, and the available knowledge on *D. rapae* behaviour does not cover the effect of metabolic state. To fully understand how nectar affects *D. rapae* fitness, behavioural observations of wasps fed with buckwheat nectar or water only, and then exposed to hosts, were conducted in controlled conditions, and are reported in Chapter 2.

1.4.2 Do pollinators impact parasitism rate by consuming nectar?

Feeding trials on parasitoids are performed with fresh flowers maintained in optimal conditions for nectar secretion, whereas in the field several factors such as climate, plant phenological stage, and nectar removal by other insects, mainly pollinators, can alter resource availability and biological control. Even though the impact of pollinators has been alluded to in the literature, it has not received experimental support prior to the work presented in Chapter 3. Nectar quantity provided by buckwheat, interplanted in OSR patches, were recorded in spring and summer. Pollinators were either allowed to or prevented to visit buckwheat flowers by using field cages, and parasitism rates were monitored to test whether pollinator visits could alter biological control.

1.4.3 Do OSR nectar and *M. persicae* honeydew enhance longevity as much as companion plants' nectar?

D. rapae can find in OSR fields two natural sources of carbohydrates, namely, its hosts honeydew, and OSR nectar when the crop is flowering. Companion plants can bring an additional resource when no other is present, and may serve as a complement if their nectar is comparatively more nutritious than OSR nectar or honeydew. The quality of carbohydrate-rich foods can indeed vary depending on its source (see section 1.3.1.3), and the nutritional quality of OSR nectar and *M. persicae* honeydew for *D. rapae* is not known. In Chapter 4 the longevity of female *D. rapae* fed with nectar from various plant sources, including OSR, and *M. persicae* honeydew, are measured, and results are discussed in the context of habitat management.

1.4.4 A protocol to quantify the structure and composition of aphid food webs in New Zealand.

As mentioned earlier, the structure and the composition of the food-web associated with OSR aphids in New Zealand is unknown. In addition to *D. rapae* (parasitoid), *Asaphes vulgaris* and *Alloxysta victrix* (hyperparasitoids), other species might be present, such as the parasitoid *Aphidius matricariae* Haliday (reported in Europe by Desneux et al., 2006), or the newly-described species *Alloxysta thorpei* and *Alloxysta rubidus* (Ferrer-Suay et al., 2012). It is crucial to have a thorough knowledge of the species present and their interactions if floral subsidies are to be implemented. With the aim of constructing the complete food web of parasitic wasps associated with OSR aphids in New Zealand, a sampling and identification method was designed, based on the molecular analysis of aphid mummies. Because of time limitations, the actual sampling and food-web construction could not be carried out in the timeframe of the present PhD, however, a significant effort was put into the development of a novel and efficient protocol, which is described in Chapter 5 and tested on laboratory-reared samples. The use of this protocol to understand the functioning of aphid-associated food webs in OSR crops, as well as its applicability in other food-web studies are discussed.

Chapter 2

The consequences of nectar feeding on the behaviour of the parasitoid *Diaeretiella rapae*.

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2.1 Abstract

Feeding on floral nectar has multiple positive effects on parasitic wasps, including increased longevity and fecundity, and in addition, nectar feeding also impacts parasitoid behaviour. To complete our understanding of the role of nectar feeding on *Diaeretiella rapae* performance, we observed the activities performed by 1 day-old female *D. rapae* with or without a prior buckwheat (*Fagopyrum esculentum*) nectar meal. Feeding on nectar increased the searching time of *D. rapae* by a factor of 40 compared with unfed conspecifics, and reduced time spent stationary. The number of attacks on aphids by nectar-fed *D. rapae* was only marginally higher than that of unfed ones ($P = 0.08$), suggesting that experimental conditions may have facilitated host finding by unfed parasitoids. The alteration of behaviour represents an additional mechanism through which nectar feeding impacts parasitoid activity. The discussion examines how behaviour and foraging strategy can impact parasitoid reproductive output, and suggests further research questions on the role of nutritional state in parasitoid's behaviour.

2.2 Introduction

In their adult stage, parasitoid wasps feed on carbohydrate-rich liquids, e.g. floral nectar or honeydew (Jervis, 1998; Jervis et al., 1993), which strongly increases parasitoid longevity, egg maturation and host parasitism rate in laboratory and field conditions (Chapter 1). In addition to increasing longevity and fecundity, nectar feeding can also affect other biological attributes of parasitoids, such as host searching, host perception, and flying abilities. Previous behavioural studies of parasitoids focussed either on parasitoid preferences in choice-tests, or on the pattern of activities performed by parasitoids in observational studies. Feeding changes the preferences of several parasitoid species when given a choice between hosts and food, i.e. fed individuals showed a preference for hosts over food, as opposed to starved ones which orientated towards food rather than hosts (Kugimiya et al., 2010; Lightle et al., 2010; Siekmann et al., 2004; Wäckers, 1994). Sugar feeding occasionally decreases parasitoid responsiveness to the presence of hosts in choice

experiments, suggesting that feeding can impede parasitoid movement. This could be a short-term effect due to the digestion of sugar meals (Lee and Heimpel, 2007; Lightle et al., 2010).

Observational studies showed that feeding also altered the suite of actions performed by parasitoids. For example, the aphid parasitoid *Aphidius ervi* Haliday allocated more time to walking (explorative behaviour) and attacking hosts when fed nectar than when starved, in which case it stayed stationary (Araj et al., 2011). Also, *Microplitis croceipes* (Cresson) [Hymenoptera: Braconidae] had longer flying bouts when fed on honey than when not fed (Takasu and Lewis, 1995). In *Cotesia rubecula*, honey-fed individuals initiated flights earlier and spent more time flying than unfed ones. However, a similar time budget was allocated to walking and being stationary in fed and starved *C. rubecula*, suggesting that feeding did not increase exploration by walking, and did not reduce times of apparent inactivity (Siekmann et al., 2004). These contrasting results suggest that there may be specific differences in nectar quality, parasitoid metabolism and their behavioural responses to feeding.

Diaeretiella rapae (McIntosh) is a solitary koinobiont endoparasitoid of about 60 aphid species, including major agricultural pests such as *Diuraphis noxia* (Kurdjumov) in cereals, *Brevicoryne brassicae* L. and *Myzus persicae* (Sulzer) in Brassicaceae (Desneux et al., 2006; Pike et al., 1999). *D. rapae* longevity, egg load, number of offspring, can be increased by nectar feeding (Jamont et al., 2014, 2013; Tyljanakis et al., 2004). Nectar, aphid honeydew, and other natural sources of carbohydrates, are the only food available to adult *D. rapae* as this species does not host-feed. The effect of feeding on *D. rapae* behaviour has not been studied but could be expected to be similar to that of *A. ervi*, because they are phylogenetically and morphologically close to each other. To advance our understanding of how nectar feeding affects the biological functions of *D. rapae*, the searching behaviour of nectar-fed and starved individuals was studied.

2.3 Materials and methods

We used buckwheat (*Fagopyrum esculentum* Moench) as a nectar source. This plant is commonly used as a nectar provider in studies of parasitoid nutrition and habitat management for conservation biological control (Fiedler et al., 2008). Plants were sown continuously to ensure a full provision of flowers during the experiment, and grown under a glasshouse at the Lincoln University nursery, with natural lighting and no heating.

D. rapae parasitoids were reared on colonies of green peach-potato aphid *M. persicae* feeding on OSR plants (cv. Ability), at 24±2 °C, 16h:8 h photoperiod, maintained at Lincoln University. These cultures were initially started in March 2012 from field-collected aphids and parasitoids identified as *M. persicae* and *D. rapae* following Cottier (1953) and Kavallieratos *et al.* (2001) respectively, and

also identified by molecular analyses (see Chapter 5). Specimens had initially been collected at the Biological Husbandry Unit (bhu.org.nz, Lincoln University) from a range of cultivated brassica plants.

Mummified aphids from the cultures were isolated in individual 1.5 mL microcentrifuge tubes with a cotton stopper until parasitoid emergence. Two to three hours after emergence, parasitoids were sexed visually (the ovipositor appears clearly as a black spike at the tip of female's abdomen) and groups composed of two female and three male parasitoids were directly placed in transparent plastic containers (6 cm * 6 cm * 12 cm) with a 3 cm * 5 cm mesh opening on one side for ventilation, and were given access to either (i) water only (starved parasitoids) or (ii) water and live buckwheat flowers from a potted plant (nectar-fed parasitoids). Visual checks ensured that flowers produced nectar on the day they were used. Parasitoid groups were left in the containers for 24 h, during which time behaviour was not recorded. Mating could not be ascertained for all females, but on several occasions, parasitoids were seen mating immediately after being placed in the container. Because female *D. rapae* mate with one male only, usually on the day of emergence (Kant et al., 2012b), and because there were three males for two females in the containers, it is highly probable that all females mated in the 24 h of conditioning.

After 24 h, only one female at a time was randomly selected and placed in an observation arena, i.e. a Petri dish containing an oilseed rape leaf bearing ca. 40 *M. persicae* (all instars). The leaf originated from a parasitoid-free culture, and was cut from the plant few minutes before starting the experiment. To minimise disturbance to aphids, no aphid was removed from the leaf. In consequence, the number of aphids on the leaf was not constant, and varied between 30 and 50. The transfer of the parasitoid female to the observation arena was done by enclosing the targeted insect in a 1.5mL microcentrifuge tube and shaking the opened tube above the Petri dish; then the lid of the Petri dish was put back on. This method was not always successful but it was preferred to others (paintbrush, buccal aspirator) because it did not involve touching the insect and avoided mechanical damage to wings or legs. Occasionally, wasps escaped during transfer from the container to the observation arena, and in this case the second female was used. If the first selected female was successfully transferred to the observation arena, the second female was discarded. Males' behaviour was not recorded, and they were also discarded.

In the first five minutes after their transfer to the observation arena, most wasps started moving, but a few stayed immobile for a short period. This was interpreted as a transient response to the transfer to a new environment, which could bias observations. Therefore the behaviour was not recorded in the first 5 minutes following transfer to the arena. This choice was based only on personal observation, as no relevant information was found in the literature. Wasp behaviour was then recorded using the software The Observer XT 7.0 (Noldus Information Technology, Wageningen, The

Netherlands) for 30 ± 2 min. A new Petri dish and a new OSR leaf were used for each behavioural observation. All observations were performed by the same observer (YDV), in a laboratory at ambient temperature (20 ± 2 °C) and ambient light intensity. The intensity of outside light entering the experimental area might have varied slightly from one day to another day. Observations were performed on ten different days, and the two treatments were tested on each day. Each date was thus a block of two observations, which prevented potential changes in light intensity from confounding the results.

Parasitoid activities were categorised as behaviours as listed in Table 2.1, developed following Araj et al. (2011) and Ayal (1987). ‘Stinging’ corresponded to the parasitoid touching an aphid with its ovipositor, while ‘Attacking’ represented the parasitoid holding its attack position, i.e. the abdomen was placed below the thorax and pointed forward. Two categories of locomotion were distinguished: ‘Checking’ and ‘Searching’. ‘Checking’ consisted of the parasitoid walking while palpating the substrate with antennal tips, whereas ‘Searching’ consisted of walking while holding antennae forward. Based on visual observation, wasps walked more slowly when ‘Checking’ than when ‘Searching’. ‘Checking, motionless’ was the same as ‘Checking’ but the parasitoid completely stopped walking. ‘Grooming’ corresponded to the parasitoid rubbing its body with its legs or licking its legs and antennae. ‘Stationary’ described periods of total immobility. The time spent displaying each behaviour was divided by the total observation time to homogenise the measures, and relative times spent displaying each behaviour were analysed. Also, the number of occurrences of each behaviour was divided by total observation time before analysis and expressed as number of occurrences per minute.

Table 2.1. Behavioural catalogue used for *D. rapae* observations.

Behaviour	Description
Sting	Touching an aphid with the tip of the abdomen (discrete event).
Attacking	Bending of the abdomen under the thorax and occasional forward extension of it. Associated with the preparation of an oviposition.
Searching	Quick walking on the leaf surface and intense movement of antennal tips.
Checking	Slow walking on leaf surface while poking the substrate with antennal tips.
Checking, motionless	Poking of the substrate with antennal tips without walking.
Grooming	Rubbing of body, antennae or wings with legs or rubbing of legs.
Stationary	Complete immobility: the insect neither walks, grooms nor moves antennae.

The effect of feeding condition on (i) relative time spent in each behaviour and (ii) number of occurrences per minute of each behaviour was analysed for each behaviour separately, with one-way-ANOVA with Date as a blocking factor. Data were log (x+1)-transformed to comply with the

ANOVA normality and homogeneity assumptions. All analyses were performed with R 3.1.1 (R Core Team, 2012).

2.4 Results

Grooming was the main activity of all wasps (43% of the observation period). Flying was not analysed as only four individuals initiated very brief flights. Date had no effect on time spent and number of occurrences of any behaviour (see Table 2.2 for a summary of statistical tests). Feeding altered the pattern of behaviours displayed by *D. rapae* females (Fig 2.1, Fig 2.2): fed females spent significantly more time searching (i.e. rapid walk) than unfed ones (d.f. = 1/9, $F = 7.13$, $P = 0.03$) and performed more searching bouts ($F = 15.8$, $P = 0.003$). Unfed parasitoids showed almost no searching over the leaf surface, thus the relatively modest time that fed parasitoids spent searching (ca. 4% of the observation period) was about 40 times greater than that achieved by unfed parasitoids. Fed females spent less time being stationary ($F = 12.7$, $P = 0.006$), or antennating motionlessly ($F = 8.26$, $P = 0.02$) than unfed ones. However, the number of motionless antennation bouts did not differ between fed and unfed insects ($F = 1.02$; $P = 0.34$). The length of time spent in attack position was not significantly different between fed and unfed females ($F = 2.19$, $P = 0.17$). The number of stings/minute was only marginally higher for fed females compared to that of unfed females (Fig 2.2; $F = 4.81$, $P = 0.06$). Fed and unfed insects spent similar lengths of time checking the leaf surface or grooming (respectively, $F = 0.55$, $P = 0.48$; $F = 0.38$, $P = 0.55$); however, fed females performed more leaf-checking and grooming bouts (respectively, $F = 6.42$, $P = 0.03$; $F = 20.39$, $P = 0.001$).

Table 2.2 Results of ANOVA testing the effect of diet (fed vs unfed) and date (random blocking factor) on time spent performing, or number of occurrences of, different behaviours. Non-significant results ($P > 0.05$) are italicised, significant results are highlighted in grey.

	Attack		Check		Chmotionless		Groom		Search		Stationary		Sting	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Time spent														
Date (df = 9)	<i>1.51</i>	<i>0.27</i>	<i>0.86</i>	<i>0.59</i>	<i>2.50</i>	<i>0.09</i>	<i>0.82</i>	<i>0.61</i>	<i>1.37</i>	<i>0.32</i>	<i>1.70</i>	<i>0.22</i>	NA	NA
Diet (df = 1)	<i>1.02</i>	<i>0.34</i>	<i>0.31</i>	<i>0.59</i>	5.68	0.04	<i>1.07</i>	<i>0.33</i>	13.5	0.005	17.6	0.002	NA	NA
Occurrences														
Date (df = 9)	<i>1.72</i>	<i>0.22</i>	<i>1.81</i>	<i>0.20</i>	5.09	0.01	<i>1.26</i>	<i>0.37</i>	<i>1.17</i>	<i>0.40</i>	<i>0.89</i>	<i>0.56</i>	<i>2.23</i>	<i>0.12</i>
Diet (df = 1)	<i>3.03</i>	<i>0.12</i>	5.43	0.04	<i>1.05</i>	<i>0.33</i>	21.1	0.001	10.6	0.01	10.5	0.01	3.93	0.08

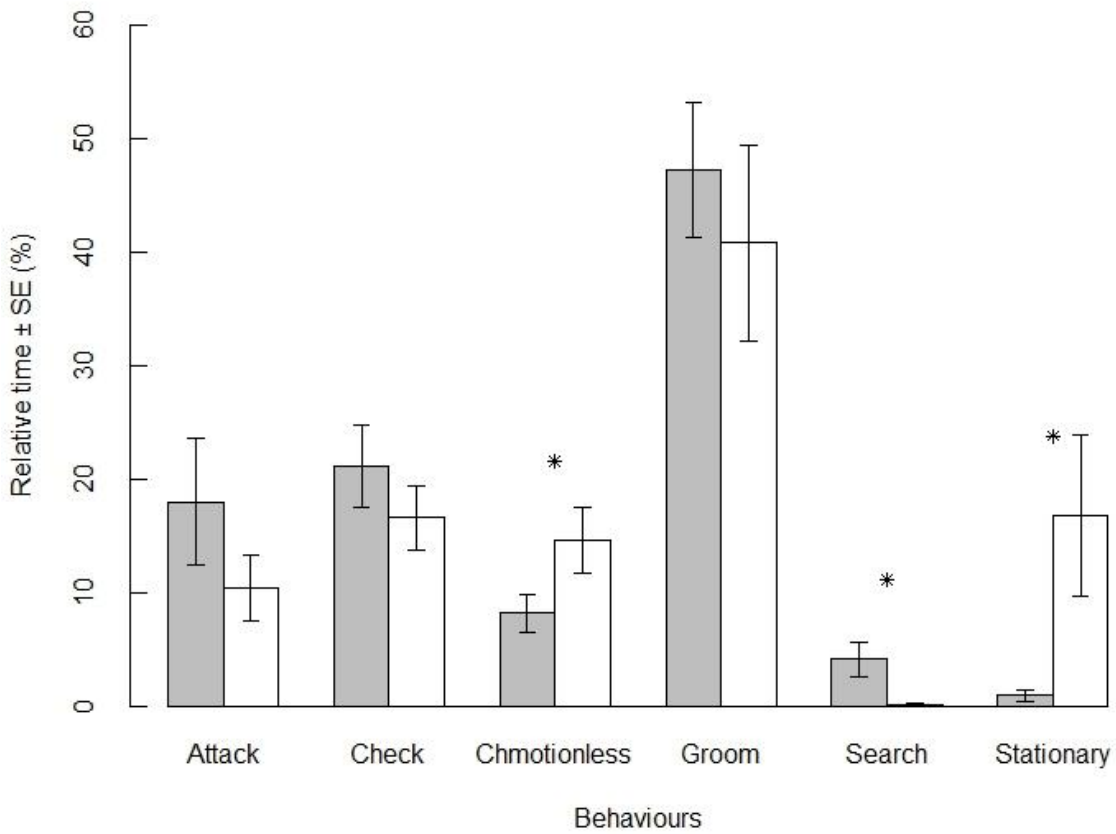


Figure 2.1 Percentage of time (relative times) spent performing different behaviours by nectar-fed (grey bars) and unfed (white bars) *D. rapae* females. N = 10 wasps of each condition were observed for 30 minutes. Behaviours are described in Table 2.1. Asterisks above bars indicate a significant difference between fed and unfed insects ($P < 0.05$). Each behaviour was tested independently.

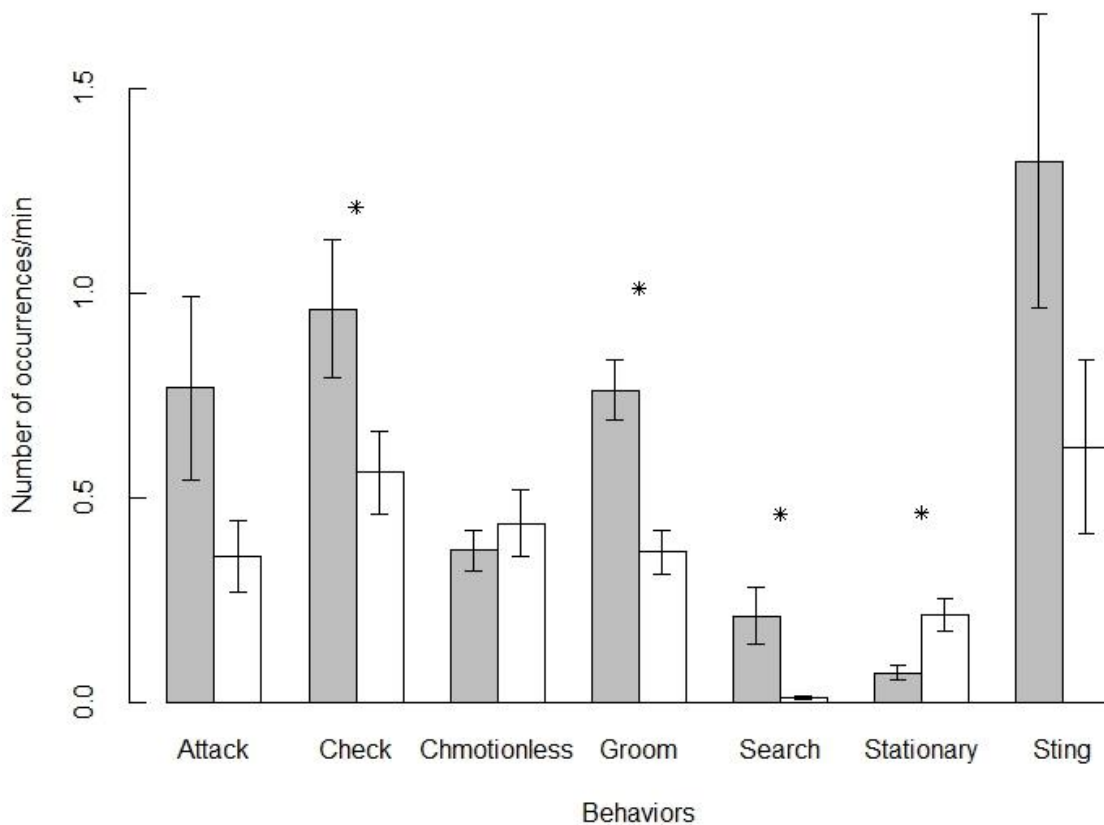


Figure 2.2 Number of occurrences per minute of different behaviours performed by nectar-fed (grey bars) and starved (white bars) *D. rapae* females. N = 10 wasps of each condition were observed for 30 minutes. Behaviours are described in Table 2.1. Asterisks above bars indicate a significant difference between fed and unfed insects ($P < 0.05$). Each behaviour was tested independently.

2.5 Discussion

The impact of buckwheat nectar feeding on *D. rapae* behavioural pattern while exposed to hosts on a leaf was tested. Feeding increased the time allocated to searching, and greatly decreased time spent immobile. Searching, defined as rapid walking, was almost absent in unfed parasitoids. Results may suggest that, in 30 min, fed parasitoids could explore an area that is 40 times larger than unfed parasitoids, and according to model studies, a 40-fold increase in search rate over a parasitoid's lifetime would divide pest equilibrium population by the similar rate of 40, which would represent a very significant improvement of biocontrol (Kean et al., 2003). However, as ingested sugars are consumed via metabolic activity (Jervis et al., 2008), one feeding event increases search rate for a limited time only. Therefore, a 40-fold increase in search rate probably is an over-estimate of the gains due to feeding, because fed insects were observed immediately after being taken out of the

feeding arena, and were compared to insects that had been starved for 24 h. *D. rapae* may maintain an intense searching behaviour if they feed frequently on nectar, which would also increase their longevity (Jamont et al., 2013; Tylianakis et al., 2004).

The present experiment compared wasps at 24 h after emergence, which represents about 50 % of the lifetime of *D. rapae* provided with water only (see Chapter 4) and is probably also the case of *D. rapae* in fields devoid of nectar source. In their first hours of life, wasps can fulfil their energetic needs by consuming the metabolic reserves accumulated during the larval stage (Jervis et al., 2008). Therefore, the importance of nectar feeding in the early life of *D. rapae* may be limited, and in the first hours after emergence, fed or unfed wasps may display the same searching abilities. Yet, by providing compounds that are absent from metabolic reserves, such as vitamins, lipids, and antioxidants (Baker, 1977; Carter and Thornburg, 2004; Horner et al., 2007), nectar might alter parasitoid behaviour immediately after its consumption, even before metabolic reserves are depleted. Further research could test whether nectar feeding can increase the searching activity of *D. rapae* during the first hours after emergence.

The number of aphid-stinging events was not significantly different between fed and unfed insects, in contrast with observations on *Aphidius ervi* (Araj et al., 2011). Results showed that fed *D. rapae* had on average twice more stings than unfed conspecifics; however, high individual variability in the number of stinging events led to no significant differences between fed and unfed parasitoids. This was somehow unexpected, as ten replicates were used here compared to six in the work of Araj et al. Stinging by unfed parasitoids might have been facilitated in the present experiment, because hosts were close to each other (c. 2 mm), and as a consequence they could be reached easily by unfed parasitoids, despite longer periods of immobility in these individuals. As a synovigenic parasitoid, *D. rapae* is able to mature eggs during adult life, and does so to a greater extent when nectar-fed than when starved; however, 24 h-old female *D. rapae*, either fed or starved, have 100-300 mature eggs in their ovaries and a difference in egg load only appears 48h after emergence (Araj and Wratten, 2015; Tylianakis et al., 2004). In our experiment, wasps performed between 0 and 90 aphid attacks (interquartile range: 6 – 48), less than the typical egg load of 24 h-old *D. rapae*, and were therefore not likely to be egg-limited, even if starved. In trials where *D. rapae* had access to an unlimited number of hosts during their lifetime, wasps performed more oviposition when fed on nectar than when starved (Jamont et al., 2013). Our experiment illustrates a case in which an increased search rate does not necessarily translate into a higher attack rate. In the field, parasitoids may not need to fly or walk long distances to find aphids if they emerge close to dense host colonies. Also, if suitable hosts are not available in sufficient numbers, or if dispersal is too costly (mortality due to predation or other external factors), parasitoids may not sting more aphids even with an increased search rate.

Foraging strategy can be a major factor influencing the efficiency of a biocontrol agent (Mackauer and Völkl, 1993), however it is not known how nectar feeding may impact parasitoid's foraging strategy. Starved arthropods tend to accept sub-optimal hosts (Javoiš and Tammaru, 2004; Vet and Dicke, 1992). Sub-optimal aphid patches can be characterised by unhealthy aphids, high density of conspecifics (van Alphen et al., 2003) and/or presence of hyper-parasitoids (Höller et al., 1994). Colony size also determines *D. rapae* preference, as aphids in small colonies are more parasitised than those in large ones (Lopez et al., 1990). Well-fed parasitoids may disperse away from colonies presenting all these characteristics, and as a consequence, nectar feeding may increase parasitism in "good" host patches but not in sub-optimal ones. Further research is needed to understand how behavioural changes would strengthen or mitigate the effect of feeding on biological control at field level.

Grooming was the activity most performed by insects from both treatments (43% of the observation time on average). Similar high prevalence of grooming was observed by Gentry and Barbosa (2006) on waxy leaf surfaces, due to the accumulation of wax on *D. rapae* tarsi. Heavy wax layer on OSR leaves has been showed to increase grooming time and decrease the attack rate of the parasitoid *Aphidius colemani* Viereck on its host *M. persicae* (Desneux and Ramirez-Romero, 2009). In our study, grooming time was not affected by feeding, despite fed individuals having walked more than unfed ones, suggesting that the plants used in this study did not deposit significant wax on insects' legs. It is also possible that feeding allowed wasps to groom more efficiently, compensating for a potentially higher wax load. Testing whether feeding can shorten parasitoid grooming times would require controlling for equal wax load on fed and starved insects.

Unfed parasitoids occasionally adopted the attack posture even though no aphid was within their ovipositor's reach, which resembled an exploratory behaviour performed with the ovipositor instead of the antennae. Further observation is however needed to confirm that fed parasitoids do not perform such behaviour. If this is confirmed, it would question whether this behaviour has some degree of fitness relevance or derives from altered perception abilities, triggering an oviposition-like movement. Starved *C. rubecula* and *M. croceipes* (1 and 2 days old respectively) displayed feeding-like behaviours (e.g. scraping the substrate with their mouthparts) on plastic or undamaged leaf surfaces where no food was apparently present (Siekmann et al., 2004; Takasu and Lewis, 1995). Together, these observations suggest that starvation may alter perception accuracy, neural computing of sensory information or behavioural response.

2.6 Conclusion

Alongside with other benefits of nectar feeding – namely, increased longevity and fecundity – the enhancement of searching activity is another mechanism that can favour the reproductive success of *D. rapae*, depending on the availability and proximity of hosts and mates. Experiments could be conducted with continuous parasitoid access to nectar to understand the consequences of feeding on search rate and reproductive output over the entire parasitoid lifetime. Also, the impact of nectar feeding on foraging strategy and plant-to-plant movement in field situations should to be investigated.

Chapter 3

Possible role of floral subsidies in the mediation of the interactions between pollinators and aphid parasitoids.

3.1 Abstract

Provision of ecosystem services (ES) such as biological control of pests can contribute substantially to reductions in pesticide use, with associated mitigation of harmful environmental effects. However, most research on insect-mediated ES addresses only one ecological guild, ignoring possible synergistic or negative interactions between different guilds and the ES they provide. In the current work, the impact of floral resource subsidies in the form of buckwheat nectar on the parasitism of aphids was quantified in relation to concurrent use of this resource by pollinators within an oilseed rape (canola) crop. Exclusion experiments were used in the field to allow or prevent pollinators' access to the buckwheat flowers on which parasitoids were foraging. The results indicate that there may not be a negative interaction between these two ES – biological control and pollination. Such interactions between invertebrate communities need to be better understood in the context of the provision of floral nectar to enhance pest biological control.

3.2 Introduction

In addition to potentially enhancing biological control, floral subsidies can be multi-functional, benefiting several other ecosystem services (ES; Carrié et al., 2012; Fiedler et al., 2008). Ecosystem dis-services (EDS) in agricultural landscapes (Zhang et al., 2007) can also be mitigated or intensified by the presence of floral subsidies. Although the mechanisms driving interactions between ES and EDS have received little attention in the literature, such knowledge is essential if successful ES management measures are to be implemented (Bennett et al., 2009).

Floral subsidies are an ideal model for the study of interactions between insect-mediated ES in agroecosystems, because nectar-providing flowers potentially benefit functional groups other than natural enemies. For example, flowers may also benefit pest species (Lavandero et al., 2006; Winkler et al., 2009a, 2009b) or hyperparasitoids (Araj et al., 2009). Floral subsidies can also benefit pollinators and enhance the pollination of crops (Wratten et al., 2012). However, the visit of pollinators to flowers might have a negative impact on the enhancement of biocontrol by floral subsidies (Wäckers and van Rijn, 2012). First, parasitoids may be deterred from flowers by either defensive behaviour of larger flower-visitors (Severinghaus et al., 1981) or the scent left after the

visit by other insects (George et al., 2013; Goulson et al., 2000; Yokoi and Fujisaki, 2007). Such direct or indirect interference competition might impact small parasitic wasps in particular, as they require more time for feeding than do large parasitoids (Jervis et al., 1993). Second, sugars remaining in nectaries after foraging by a large nectar-consumer may be in limited quantity; moreover, the small volume of remaining nectar could rapidly evaporate and become viscous or dry, rendering nectar difficult to exploit by parasitoids (Jervis, 1998). Such competition for nectar resources is documented among pollinators, e.g. between European honey bees (*Apis mellifera*) and native American bumble bees (*Bombus occidentalis* Greene) (Thomson, 2004). It seems, however, that potential competition between pollinators and parasitoids, via the intense consumption of nectar by pollinators has only been reported once (Lee and Heimpel, 2002). In that particular study, nectar availability was recorded on one date only, with no quantification of parasitism rates in relation to the presence of nectar-feeding insects.

Here, we hypothesise that flower visits by pollinators may be a significant factor limiting nectar availability for parasitoids, and that this might cascade into decreased parasitism rate of pests. The present study presents a first investigation of these hypotheses, using aphid parasitoids as a natural-enemy population subsidised with buckwheat nectar, and a locally-available community of large nectar-consumers (including honeybees). We designed a manipulative field experiment to test whether consumption of buckwheat nectar by large flower-visitors resulted in reduced parasitism rate of aphids.

3.3 Materials and methods

3.3.1 Biological material

The study system comprised oilseed rape (OSR) (cv. Ability) as the crop, and buckwheat (*Fagopyrum esculentum* L. cv. Katowase) as a floral subsidy. A strong local enhancement of aphid parasitism rate has been observed in wheat crops subsidised with this plant (Tylianakis et al., 2004). In New Zealand, OSR can be attacked by three aphid species: *Myzus persicae*, *Brevicoryne brassicae* and *Lipaphis erisimi* (Lamb, 1989), all being hosts of the parasitoid *Diaeretiella rapae* (Pike et al., 1999).

3.3.2 Field-trial design

14 experimental plots of 6 * 2 m² were established at Iversen Fields (Lincoln University, New Zealand, 43°38'56"S, 172°27'55"E). Each plot was sown with two patches (2 * 2 m²) of oilseed rape on the sides and a patch (same dimensions) of buckwheat planted at the centre of the plot (Fig 3.1). 2 m was the minimal width achievable with the machinery used for sowing. Plot size was minimised in order to allow a maximum number of plots on the experimental field, and was comparable to that of

plots or cages used in other semi-field studies involving parasitoids (Araj et al., 2009; Winkler et al., 2006). Plots were at least 22m apart, separated by bare ground. Previous studies (Tylianakis et al., 2004) showed that the effect of buckwheat on aphid parasitism rate was very localised (up to 10m). It was therefore assumed that the parasitism rate in each plot was independent of other plots located 22m away or further. Plots were sown with oilseed rape and buckwheat on October 15, 2013. Buckwheat started flowering on November 25 (six weeks after sowing), while OSR flowered on December 12 (eight weeks after sowing). No fertilisers had been applied as the previous crop in the rotation (lucerne, *Medicago sativa* L.) had enriched the soil. Irrigation and hand-weeding were done as needed during the establishment period. No insecticide was applied during the study. Two hives were installed approximately 500m from the experimental site to ensure the presence of honeybees on site, in addition to other naturally-occurring large pollinators (Fig 3.2).

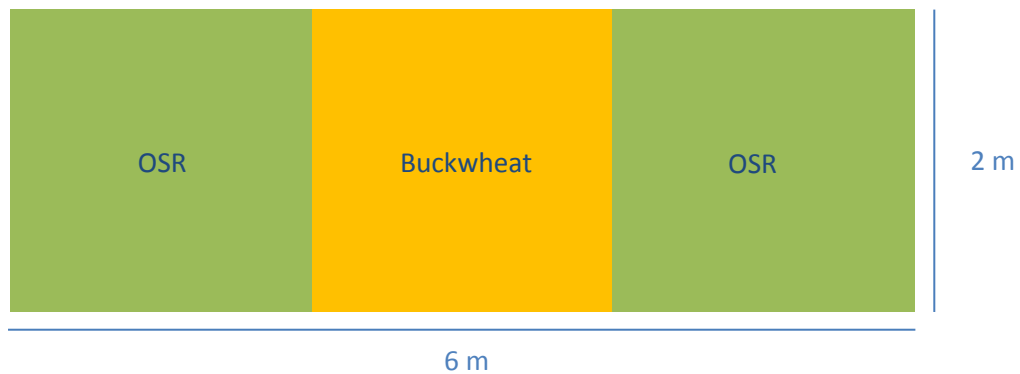


Figure 3.1 Layout of 14 identical experimental plots, before the addition of cages. All plots are composed of a patch of buckwheat in the middle of two patches of oilseed rape (OSR).

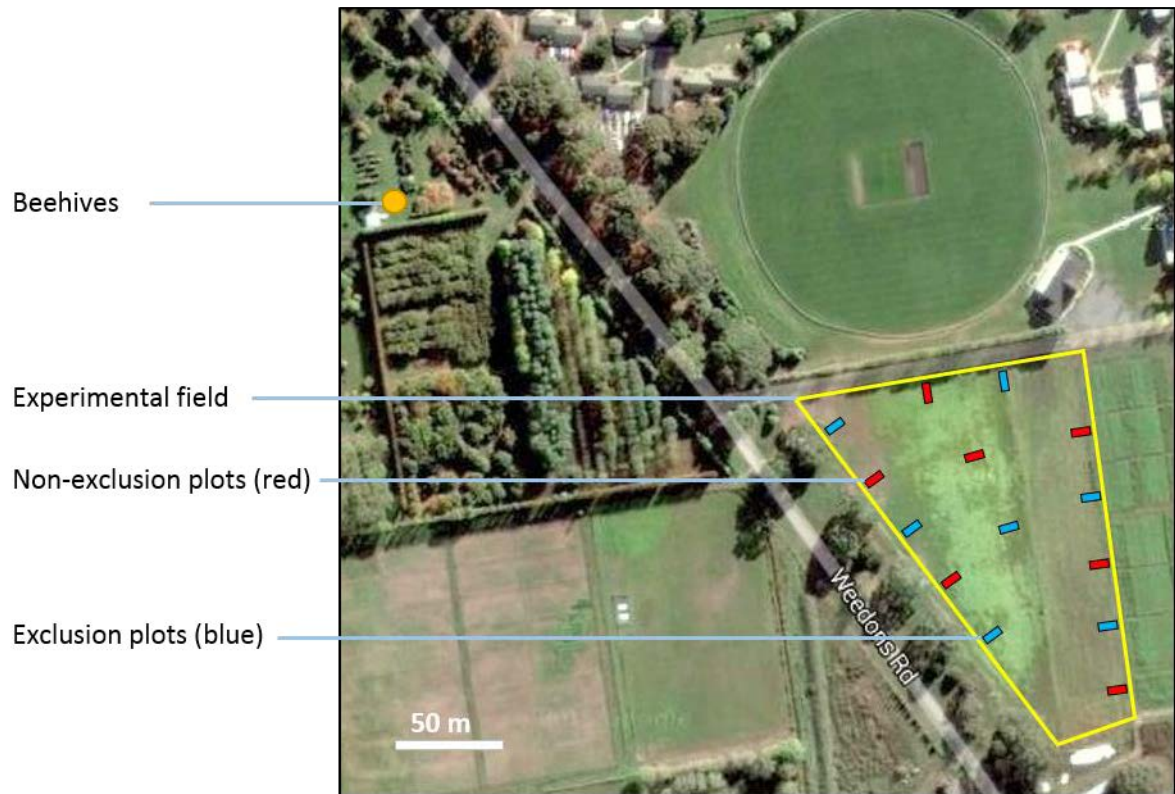


Figure 3.2 Layout and view of the experimental site. a) Red and blue rectangles represent, respectively, non-exclusion plots (open cages) and exclusion cages (closed cages). Plots are separated by at least 22 m. The orange circle marks the location of the two beehives. Image: © 2015 CNES / Astrium, DigitalGlobe (GoogleMaps). b) View on 20th December 2013, showing plots separated by bare ground.

Two treatments were applied to the experimental plots: pollinators were either allowed access to, or excluded from plots. To exclude pollinators, each of seven plots was covered with an exclusion cage, which consisted of a 1.5 m-tall mesh-cage with 3 * 6 mm² holes (Fig 3.3.a). Prior to the use of such mesh for the construction of cages, its permeability to honeybees, bumblebees and parasitoids was tested as follows: insects were individually trapped in 5 * 5 * 12 cm³ transparent plastic containers which top was tightly closed with the aforementioned mesh. Honeybees and bumblebees stayed trapped until death whereas parasitoids could all exit the plastic containers in a few hours. In the field, honeybees attempted to pass through the mesh, but did not succeed (see results). Seven other plots were covered with a non-exclusion cage, i.e., a structure of similar design to that above, but with a continuous 30cm-wide opening along all four sides of the cage, which allowed pollinators to enter and exit the cage (Fig 3.3.b). The two treatments are henceforth referred to as 'exclusion cage' and 'non-exclusion cage'.

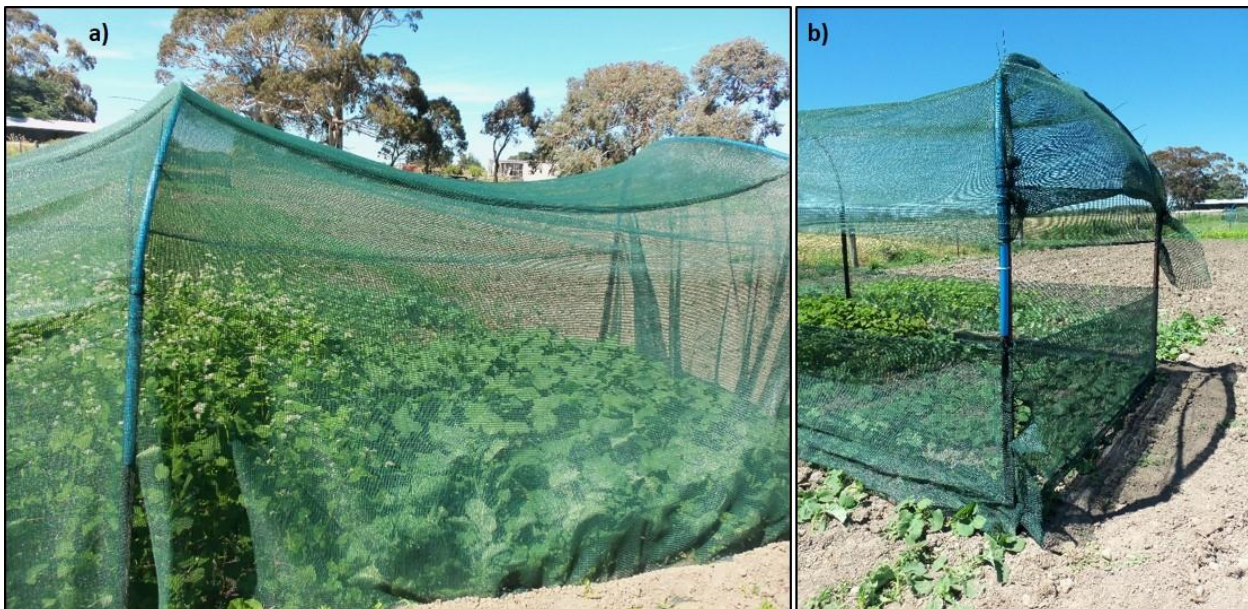


Figure 3.3 Experimental plots: a) Exclusion plots, covered by a completely closed cage excluding large insects (mesh size 3 mm * 6 mm); b) Non-exclusion plots, covered by a cage with a 30 cm opening along the sides, allowing access to all insects.

To monitor the micro-climatic conditions inside the cages, temperature and humidity were each recorded twice hourly during the period of data collection (from November 29, 2013, to January 6, 2014) using data loggers (i-Buttons, Eclo Express Thermo 2007 Basic) attached to one of the holding poles inside each cage, at 50 cm over the ground. Data were available for five exclusion cages and three non-exclusion cages only, because there were not as many data loggers as cages, and because some loggers were lost or ceased functioning during the experiment. The number of development degree days for *D. rapae*, *M. persicae*, and *B. brassicae* in each plot were calculated using base

temperatures of, respectively, 2.83 °C (Bernal and González, 1997, 1993), 4.3 °C (van Emden et al., 1969) and 3.47 °C (Satar et al., 2005). This reflected the amount of time during which insects could be active.

3.3.3 Large flower-visitors

Because the mesh excluded pollinators and all flower-visiting insects with a body width larger than 3 mm, the cohort of insects excluded from cages were referred to as 'large flower-visitors'. In this experiment, seven groups of insects corresponded to the definition of 'large flower-visitors', namely: honeybees *Apis mellifera* L., bumblebees *Bombus* spp., butterflies (Lepidoptera, mostly the small cabbage white *Pieris rapae* L.), droneflies *Eristalis tenax* L. (Diptera: Syrphidae), other hoverflies (Syrphidae), blowflies (Calliphoridae) and ladybirds (Coccinellidae). All large flower-visitors are generally classified as pollinators (*P. rapae*: see Conner et al., 1995) except Coccinellidae; however we included the latter in our counts as they were observed feeding on nectar in non-exclusion cages. Large flower-visitors were counted on buckwheat patches on six sunny days with low wind conditions (Fig 3.4). On November 29, December 3, 9, and 20, and January 6, one observation was made per day, in all plots at 10:00. Observations were also made on patches of oilseed rape after it started flowering, i.e. on December 20 and January 6. To monitor the dynamics of large flower-visitors over 12 h, four observations were made on December 6, every three hours between 08:00 and 17:00 in six plots only (three exclusion plots and three non-exclusion plots). Each observation consisted in standing by the plot or inside the cage (in the case of exclusion cages) and counting each individual 'large flower-visitor' seen over the plots. The observation time was 1.5 min per plot, as with such a period it was possible to keep a visual memory of the insects already counted and avoid double-counting. This corresponded to 7.5 s.m⁻² per counting, which is comparable to that reported in other studies: 3 s.m⁻² in Bommarco et al. (2012) and 18 s.m⁻² in Woodcock et al. (2013). Plot observations were performed in a random sequence.

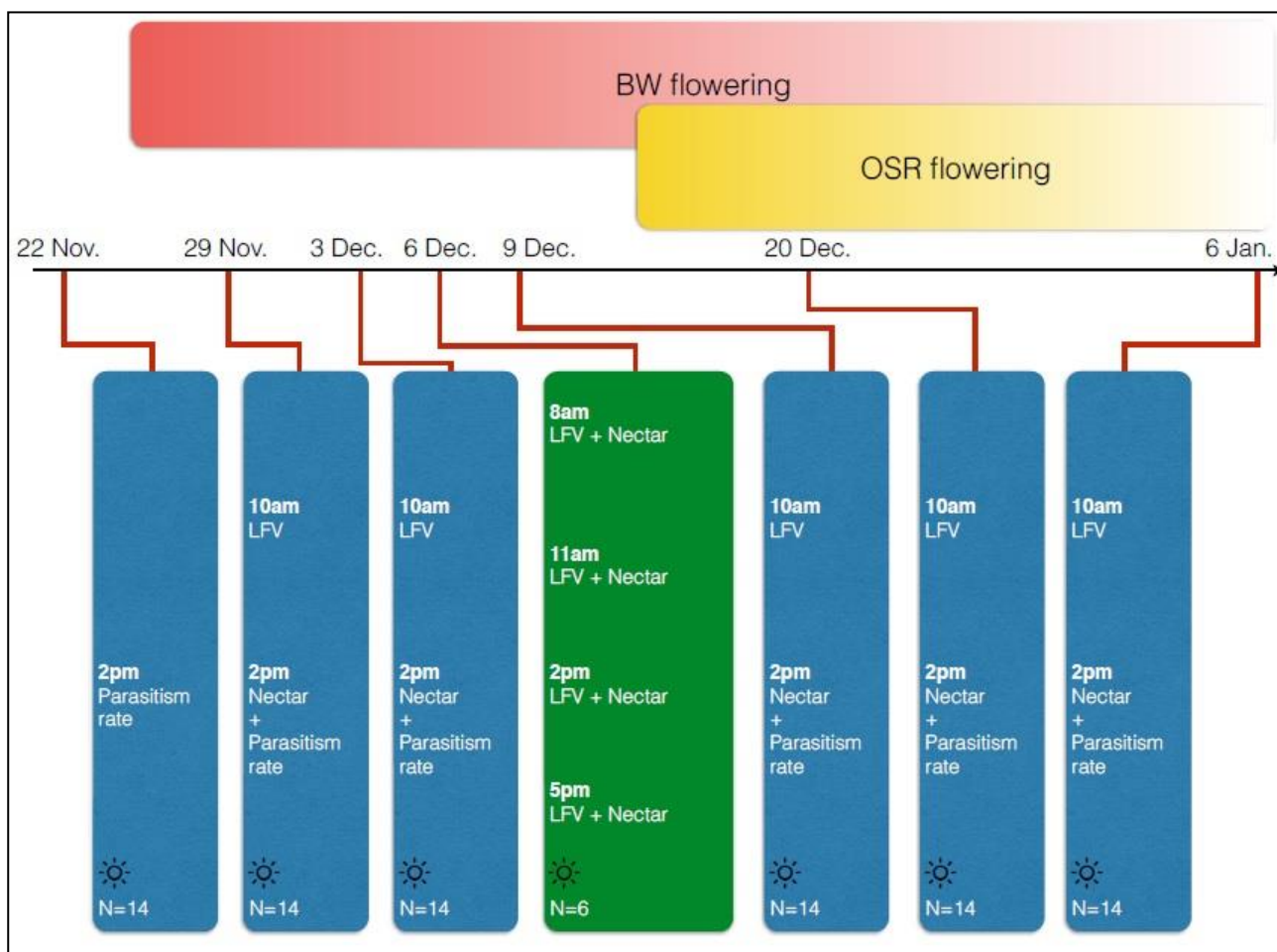


Figure 3.4 Timeline of the experimental season. Flowering periods of buckwheat (BW) and oilseed rape (OSR) are indicated above the axis. Counts of large flower-visitors (LFV), quantification of nectar sugars in flowers (Nectar) and aphid parasitism rates (Parasitism rate) are indicated below the time axis. Diurnal variation of LFV and Nectar was monitored on December 6 (green box). Other sampling dates reflected seasonal variation (blue boxes).

3.3.4 Nectar availability

To monitor the effect of large flower-visitors on the availability of nectar in flowers, nectar was extracted from buckwheat on the same dates as indicated above (Fig 3.4). On December 6, samples were taken on four occasions, every 3 h between 08:00 and 17:00, on six plots only (three of each treatment). On other dates, samples were taken only once per day on all plots, between 14:00 and 15:00. Nectar was extracted from oilseed rape flowers after the plant started flowering, i.e. on December 20 and January 6 only. In each sampling event, ten flowers were collected, each from ten different plants. The amount of sugars (in μg) present as nectar in individual flowers was measured.

This measure was preferred over nectar volume or sugar concentration in the nectar because it reflected the quantity of nutritive resource available for insects.

The first step of the protocol was performed in the field directly after flower collection, and consisted in placing each single flower in 100 μL of Milli-Q[®] water in a 600 μL microcentrifuge tube, and shaking manually for 1 min before removing the flower from the tube. The tube was then placed on ice, transferred within 1-2 h to a -20 °C freezer and stored until laboratory analysis (adapted from Marrant et al., 2009). Total sugars in the nectar extract were quantified by the hot anthrone test (adapted from Lee et al., 2006; Olson et al., 2000; and Van Handel, 1985) as follows: 100 μL of water were added to the nectar extract to dilute the sample, then 100 μL of the diluted sample were transferred to a 1.5 mL microcentrifuge tube. 950 μL of anthrone reagent (prepared according to the protocol indicated in Van Handel, 1985) were added and left to react at ambient temperature for 1.5 h. The tube was then heated at 90 °C for 15min and placed on ice. 200 μL of this preparation were transferred onto a microplate well and light absorbance was read on a Thermo Scientific Multiskan[™] Go spectrophotometer at 625 nm in accordance with the protocol initially developed by Van Handel. Solutions with known concentrations of glucose (from 0 to 0.5 $\text{g}\cdot\text{L}^{-1}$) were also prepared and processed in each series of analysis, which allowed to infer the total sugar concentrations in the nectar samples (Van Handel, 1985) and ensured that the protocol was performed correctly.

Based on the concentration values, the total amount of nectar sugars per flower was calculated and expressed in μg .

3.3.5 Parasitism rate

To ensure the establishment of aphids in the plots, OSR plants were inoculated at the start of stem elongation with two species of aphids, *M. persicae* and *B. brassicae* L. [Homoptera: Aphididae], obtained from parasitoid-free laboratory cultures (Varennes et al., 2014), by placing 3-5 leaves covered with aphids (200-500 individuals in total) in the OSR patches in the first week of November. Aphids coming from the surrounding environment also probably established in the plots. The aphid *L. erisimi* was not observed. Parasitoids present at the study site came exclusively from naturally existing populations. Although other parasitoid species may occur on this site, 35 specimens captured on December 20 and December 31 were all morphologically identified as *D. rapae* (based on Kavallieratos et al., 2001). Aphids were counted by the same observer throughout the experiment, starting on November 22, then on the same dates and times as nectar sampling except December 6 (Fig 3.4). Living aphids as well as dead parasitised ones (aphid mummies) were counted on 10 randomly selected oilseed rape plants per plot, on all plots. The number of samples was set to

ten because parasitism rate and aphid infestation was homogeneous within each OSR plot. Aphid parasitism rates were calculated as the total number of mummies per plot divided by the number of aphids (living aphids plus mummies) per plot, a proxy for accurate measurement of parasitism rate (Hughes, 1963).

3.3.6 Parasitoid nutrition in the field

To test whether parasitoids had fed, individuals collected in the field for identification were tested for fructose. The detection of this sugar generally indicates that the insect has fed on plant nectar or honeydew not long before being captured (Lee et al., 2004). It is however possible that some newly emerged parasitoids contain traces of fructose such as *Binodoxys communis* (Gahan)[Hymenoptera: Braconidae] (Wyckhuys et al., 2008). Therefore, *D. rapae* mummies were also collected to obtain newly emerged *D. rapae* and measure their “base” level of fructose upon emergence.

To measure the level of fructose in field-collected *D. rapae*, live parasitoids were captured with a buccal aspirator, at 4pm on December 20 and December 31, on exclusion plots (n = 18) and non-exclusion plots (n = 17). The number of captures was limited by time constraints inherent to the sampling of live insects. Insects were put into a 1.5 mL microcentrifuge tube, placed on ice and transferred to a -20 °C freezer (identification was completed in less than 2 min per specimen before transfer to the freezer). To measure the base level of fructose in newly emerged parasitoids, mummies were collected in the field on December 20 (n = 13), and kept in controlled-temperature rooms at 20 ± 4 °C and 16 h light:8 h dark photoperiod, in 1.5 mL microcentrifuge tubes until emergence. Newly emerged parasitoids were not given access to water or food, and were frozen (-20 °C) within 4 h after emergence, until further analysis.

Fructose in insects was quantified with the cold anthrone test, which is similar to the hot anthrone test described above, except that the reaction of the anthrone reagent with the sample occurs at ambient temperature. At this temperature, only fructose reacts with the anthrone reagent, while the other sugars do not (Lee et al., 2004; Olson et al., 2000; Van Handel, 1985).

Parasitoids were individually weighed with a 6-digits microbalance (courtesy of K. Trought, Landcare research), individually placed into a 1.5 mL centrifuge tube with 50 µL of Na₂SO₄ (2 %) and 450 µL of chloro-methanol (1:2) and crushed with a pestle. The tube was centrifuged at 16 000 g for 2 min. The Na₂SO₄ absorbed insect tissue and precipitated, leaving a clear supernatant. 200 µL of supernatant were transferred to a 1.5 mL centrifuge tube and heated at 90 °C until only 50 µL remained. This facilitated detection and did not alter the quantity of fructose (as in Lee et al., 2004). The remaining solution was cooled to ambient temperature and 950 µL of anthrone reagent were added. The tube was left to react at ambient temperature during 1:30 hours. 200 µL of this preparation were

transferred onto a microplate well and light absorbance was read on a spectrophotometer at 625 nm as previously. The concentration of fructose in the original sample was inferred from an adapted calibration curve (from 0 to 0.05 g.L⁻¹ of fructose). Necessary calculations were made to obtain the total weight of fructose in each insect, which was divided by insect body mass to obtain ratios of fructose : body mass (no units).

3.3.7 Data analysis

The effect of cage type on the micro-climate inside the enclosures was tested. Temperature and humidity in each cage were averaged over the sampling period (from November 29 to January 6). The effect of cage type on mean temperature, mean humidity, and number of development-degree days for *D. rapae*, *M. persicae* and *B. brassicae*, was tested using the Welch *t*-test, which is an equivalent of the Student *t*-test adapted for unequal sample sizes (here, $n = 3$ for non-exclusion cages and $n = 5$ for exclusion cages).

The effect of cage type on the number of large flower-visitors, mean quantity of nectar per flower, total nectar per plot, aphid numbers and parasitism rate was tested using *t*-tests on their mean over the entire period (adapted from Saville, 1990). The mean over the entire period is a summary statistic that yielded, for each response variable, a single value per plot ($n = 14$ plots in total). This summary statistic reflected the cumulative effect of treatment on the response variables, and avoided biases inherent to other repeated-measure analysis procedures. For the analysis of nectar quantities per flower, plant species (buckwheat and OSR) were distinguished. The total nectar per plot was the sum of all values without distinction of flower species, which reflected the fact that there were only buckwheat flowers prior to Dec 12, date at which OSR started flowering and producing nectar. None of the response variables were transformed for these analyses. These summary statistics were also used to investigate the relationship between the response variables. Four simple linear regressions were performed: (i) sugar quantity against the number of large flower-visitors, (ii) parasitism rate against sugar quantities per plot, (iii) parasitism rate against aphid density, and (iv) parasitism rate against the number of large flower-visitors. A multiple linear regression of parasitism rate against nectar per plot plus the number of aphids was also performed. The coefficient estimate (slope) for each explaining variable was tested against 0 using a *t*-test. In complement to the analysis of summary statistics and regression slopes, the evolution of the number of large flower-visitors, the amount of nectar per flower, aphid numbers and parasitism rate over the period from November 29 to January 6 are presented in section 3.4.3.

As explained earlier, the number of large flower-visitors and nectar quantities were also measured at four moments on December 6, to capture the dynamics of these variables at the scale of a day. This was intended to be only informative, and therefore, was done on one day only. As above, these values were averaged over the entire day, which yielded one single value per plot (here, $n = 6$ in total). The effect of the treatment was then tested using a *t*-test. All statistical analyses were performed in R 3.1.1 (R Core Team, 2014).

3.4 Results

3.4.1 Micro-climatic conditions in cages

Temperature in cages ranged from 5.1 °C to 35 °C, humidity from 22 % to 100 %. Temperature was higher and humidity lower in non-exclusion cages on most days of the sampling period from 8am to ca. 4pm, the average difference around 12am being ca. 2 °C and 5 % humidity (Fig 3.5). On sampling days, during a period ranging from ca. 8am to ca. 2pm, temperature was higher in non-exclusion cages than in exclusion cages (Fig 3.6.a). On sampling days, humidity was similar in both cage types (Fig 3.6.b). Despite these differences, mean temperature, mean humidity and number of development degree days were not influenced by cage type (see Table 3.1).

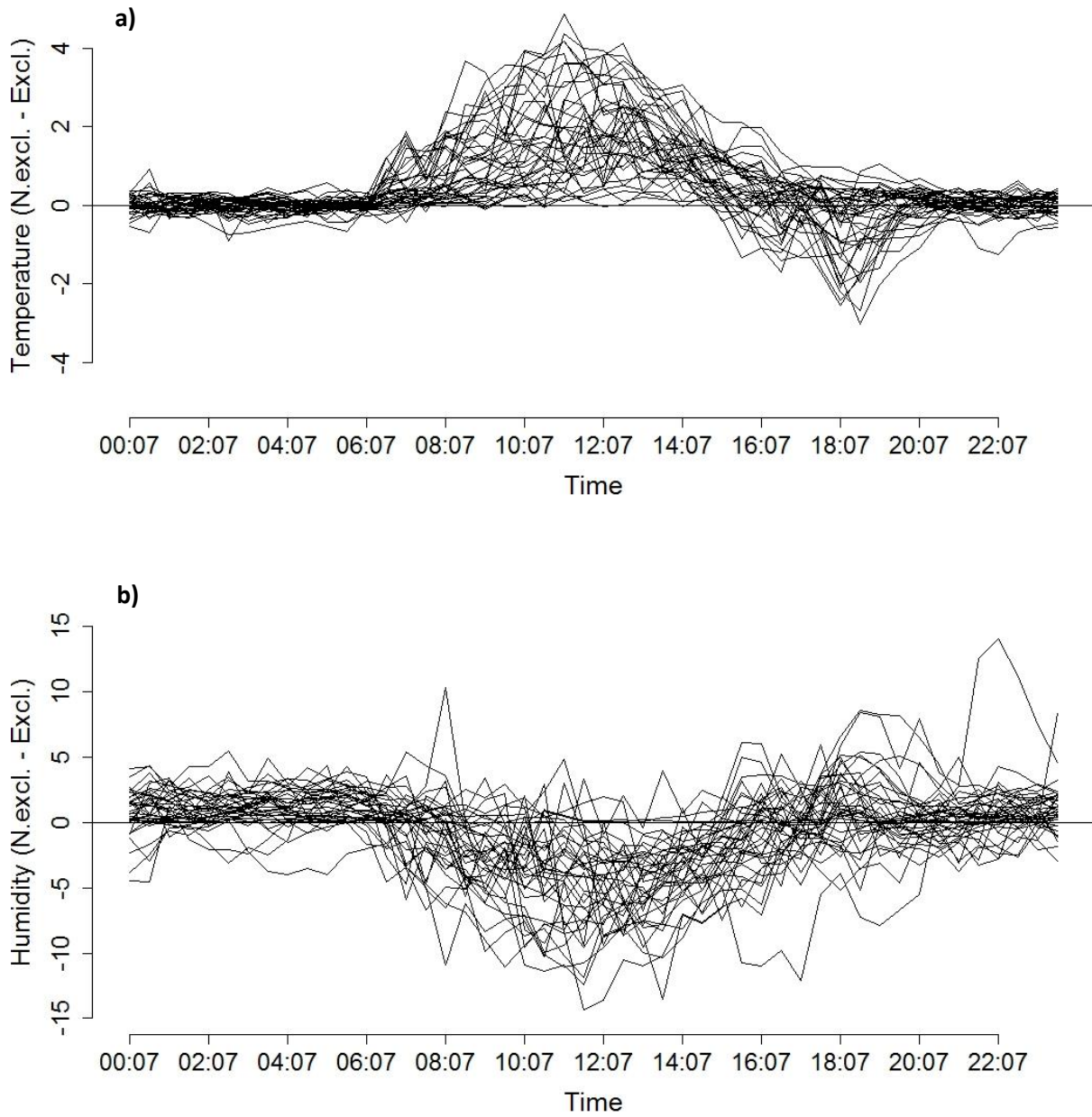
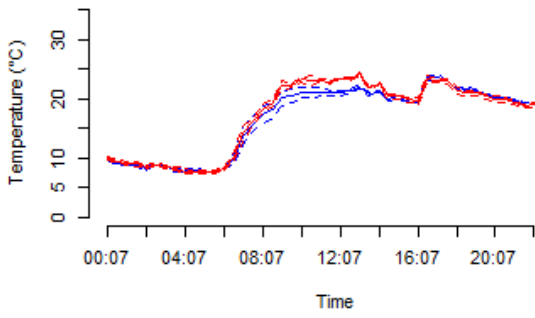


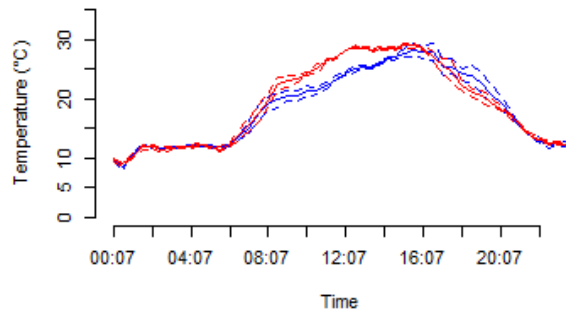
Figure 3.5 Difference between (a) mean temperature and (b) mean humidity in non-exclusion and exclusion cages between November 29 and January 6 (39 days). Each line corresponds to a day; all days are plotted simultaneously on the x-axis from 00:07 to 23:57. Positive values indicate that non-exclusion cages have a higher temperature or humidity.

a)

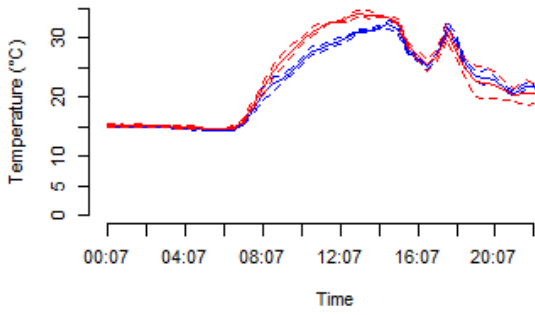
November 29



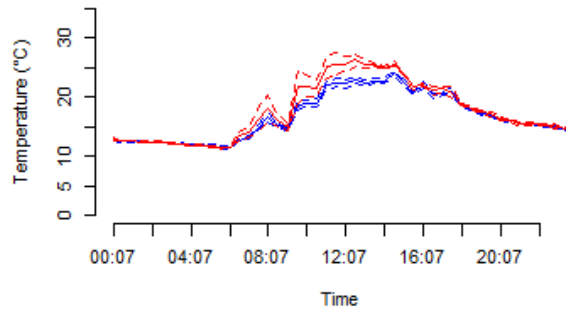
December 3



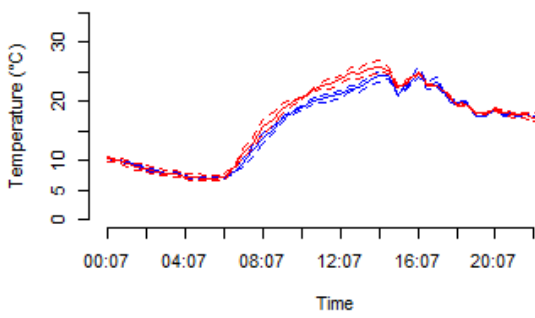
December 6



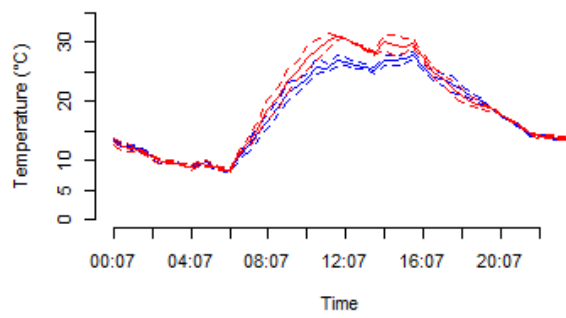
December 9



December 20



January 6



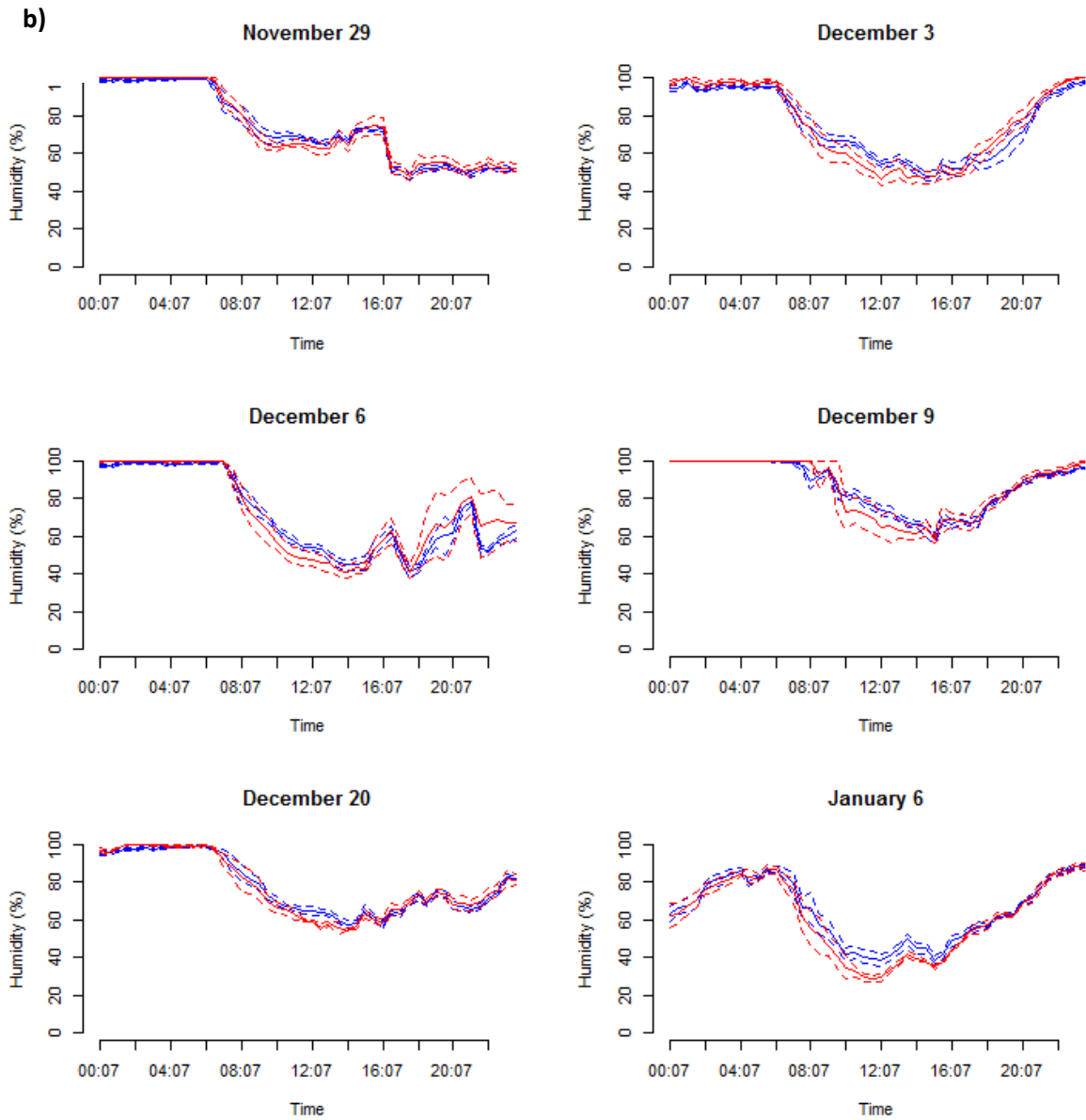


Figure 3.6 Mean temperature (a) and humidity (b) in exclusion and non-exclusion cages. The blue solid line is the mean of $n = 5$ exclusion plots, the red solid line is the mean of $n = 3$ non-exclusion plots. The dashed lines represent the mean \pm standard error.

Table 3.1 Mean climatic conditions inside field cages over the period from November 29 to January 6.

	Mean \pm s.e.		Welch <i>t</i> -test	
	Exclusion cages (n = 5)	Non-exclusion cages (n = 3)	<i>t</i>	<i>P</i>
Temperature	16.9 \pm 0.14	17.3 \pm 0.26	-1.46	0.24
Humidity	78.1 \pm 1.3	77.5 \pm 2.3	0.22	0.83
Accumulated degree days for <i>D. rapae</i> ¹	563 \pm 5.6	581 \pm 12	-1.40	0.26
Accumulated degree days for <i>M. persicae</i> ²	506 \pm 5.6	542 \pm 12	-1.40	0.26
Accumulated degree days for <i>B. brassicae</i> ³	538 \pm 5.6	556 \pm 12	-1.40	0.26

¹ base 2.83 °C (mean of the values reported in Bernal and González 1993, 1997)

² base 4.3 °C (van Emden et al, 1969)

³ base 3.47 °C (mean of the values reported in Satar et al. 2005)

3.4.2 Pollinators, nectar and parasitoids.

In total, 336 flower-visitors were observed, 6314 aphids and 578 mummies were counted. Also, 784 buckwheat flowers and 200 OSR flowers were collected. The caging type had a significant effect on the number of large flower-visitors, the total amount of nectar per plot and the amount of nectar per flower (for both plant species), but not on aphid numbers or parasitism rate (see Table 3.2).

Table 3.2 Effect of cage type on the number of large flower-visitors, nectar per plot, nectar per flower, aphid numbers and parasitism rate. Each variable was averaged over all sampling events. The effect of cage type on each variable was tested with a *t*-test.

Mean November 29 to January 6 (n = 14)

	Exclusion cages	Non-exclusion cages	<i>t</i> ₁₂	<i>P</i> -value
Large flower-visitors	1.22	5.63	-9.48	<0.001
Nectar per plot ($\mu\text{g}/\text{plot}$)	2030.5	617.9	5.67	<0.001
Nectar per buckwheat flower ($\mu\text{g}/\text{flower}$)	168.1	22.3	5.04	0.02
Nectar per OSR flower ($\mu\text{g}/\text{flower}$)	168.4	119.2	10.5	0.02
Aphids	58.1	60.8	-0.11	0.91
Parasitism rate (%)	18.6	16.2	0.68	0.51

Five times more large flower-visitors were observed in non-exclusion cages than in exclusion ones, and the most common insects were *E. tenax*, honeybees and Calliphoridae flies (Table 3.4). Some large flower-visitors were observed in exclusion plots, most of them (23 in total) being *P. rapae* adults, which were probably present at the larval stage on oilseed rape plants during caging, and completed their life cycle within the cages. 20 calliphoridae flies were also observed in exclusion

cages, they may have been present on plants as larvae before the cage was erected, but may also have succeeded to go through the mesh as adults, as some individuals had a body section smaller than 3 mm.

Table 3.3 Total number of large flower-visitors in each taxonomic group observed over the season¹ in exclusion and non-exclusion cages.

<i>Taxonomic group</i>	<i>Cage type</i>		<i>Total</i>
	<i>Exclusion cages</i>	<i>Non-exclusion cages</i>	
Visits to buckwheat flowers			
<i>Apis mellifera</i> L.	0	54	54
<i>Bombus</i> spp.	0	8	8
<i>Pieris rapae</i> L. and other large Lepidoptera	15	0	15
<i>Eristalis tenax</i> L.	2	93	95
Syrphidae other than <i>E. tenax</i>	5	15	20
Calliphoridae	20	46	66
Coccinellidae	8	11	19
Total buckwheat	50	227	277
Visits to OSR flowers			
<i>Apis mellifera</i> L.	0	21	21
<i>Bombus</i> spp.	0	12	12
<i>Pieris rapae</i> L. and other large Lepidoptera	8	4	12
<i>Eristalis tenax</i> L.	1	9	10
Syrphidae other than <i>E. tenax</i>	1	1	2
Calliphoridae	0	2	2
Coccinellidae	0	0	0
Total OSR	10	49	59
Total both flowers	60	276	336

¹: Counts of December 6 were not included here as four counts per plot were performed on that day.

The slope of the linear regression of mean nectar per plot against the number of flower visitors was negative and significantly different from zero (table 3.5 and figure 3.7). The slopes of the regressions of parasitism rate against nectar per plot or aphid numbers, or both, were not significantly different from zero (table 3.5 and figure 3.7).

Table 3.4 Simple or multiple linear regressions of some response variables against other response variables. The significance of the regression coefficient estimates is tested with a *t*-test.

$$\text{Nectar per plot} = a \times (\text{number of large flower-visitors}) + b$$

Coefficient	Estimate	t_{12}	<i>P</i>
<i>a</i>	-188.58	-2.96	0.01
<i>b</i>	2201.9	5.94	<0.001

$$\text{Parasitism rate} = a \times (\text{aphid numbers}) + b$$

Coefficient	Estimate	t_{12}	<i>P</i>
<i>a</i>	-8.10^{-5}	-0.46	0.65
<i>b</i>	0.18	7.45	<0.001

$$\text{Parasitism rate} = a \times (\text{nectar per plot}) + b$$

Coefficient	Estimate	t_{12}	<i>P</i>
<i>a</i>	2.10^{-5}	1.31	0.21
<i>b</i>	0.14	5.05	<0.001

$$\text{Parasitism rate} = a \times (\text{number of large flower-visitors}) + b$$

Coefficient	Estimate	t_{12}	<i>P</i>
<i>a</i>	$-3.9.10^{-4}$	-0.07	0.94
<i>b</i>	0.17	5.50	<0.001

$$\text{Parasitism rate} = a \times (\text{nectar per plot}) + b \times (\text{number of aphids}) + c$$

Coefficient	Estimate	t_{12}	<i>P</i>
<i>a</i>	$2.9.10^{-5}$	1.56	0.15
<i>b</i>	$-1.9.10^{-4}$	-0.98	0.34
<i>c</i>	0.15	5.11	<0.001

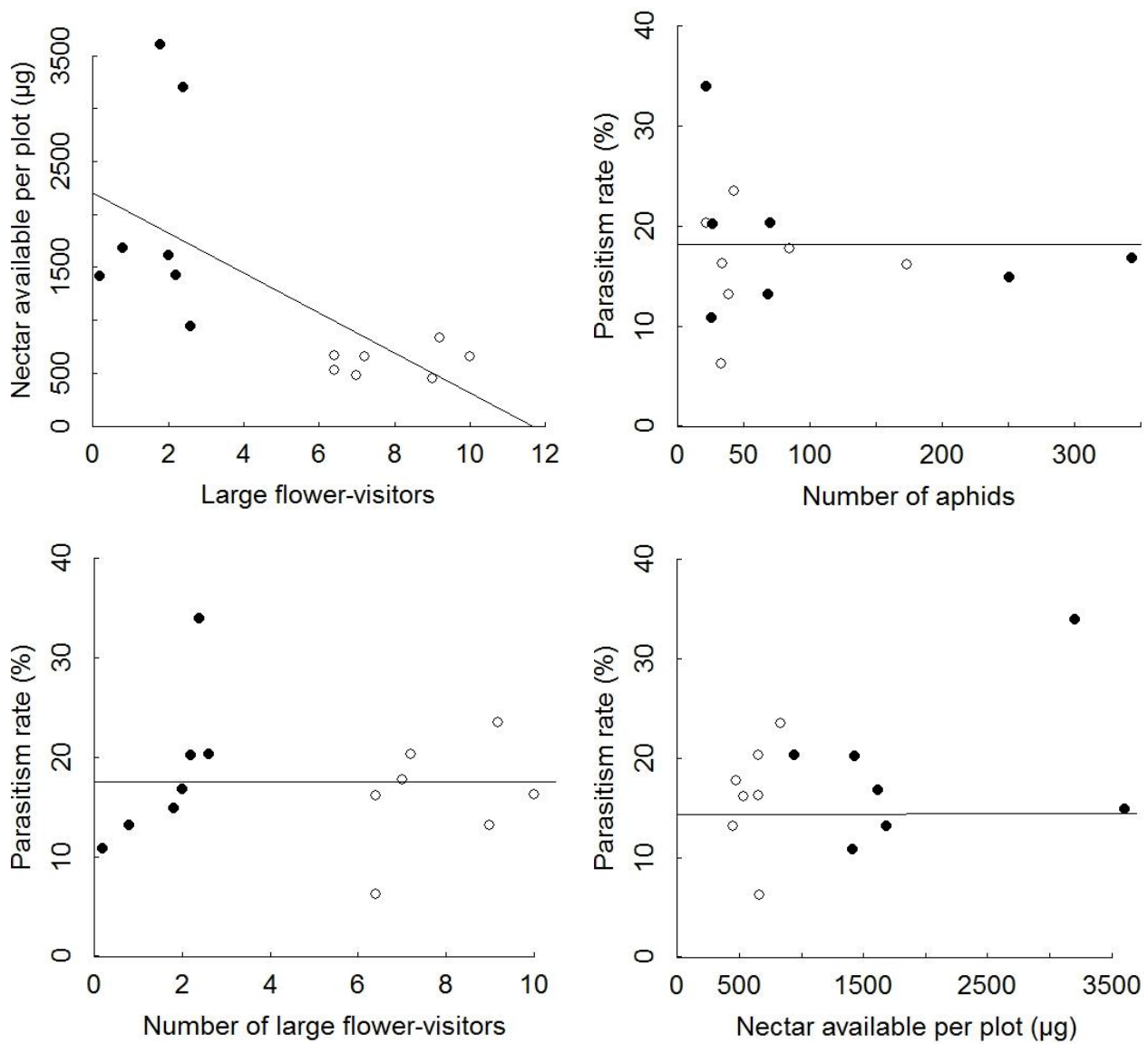


Figure 3.7. Linear regression of nectar per plot or parasitism rate against other response variables. Closed circles represent exclusion plots, open circles represent non-exclusion plots.

3.4.3 Temporal dynamics over the season

In exclusion cages, the number of large flower visitors was always inferior to 4 per observation, for both buckwheat and OSR flowers, during the entire experimental period (Fig 3.8.a). In non-exclusion cages, the number of large flower visitors increased from 6 to 12 per observation over the three first observation dates. On the fourth observation date, more than 5 visits were observed on OSR but only one on buckwheat, indicating a switch in visitors' preference from one flower species to the other. On the last observation date, less than 1 visit was observed in all plots, on both buckwheat and OSR.

In exclusion plots, nectar sugars in buckwheat flowers were at their highest level with more than 400 $\mu\text{g}/\text{flower}$ on the first sampling date (4 days after flowering started), and declined dramatically over time, to reach ca. 20 μg of nectar sugars per flower 25 days after flowering (Fig 3.8.b). 8 days after buckwheat started flowering (on Dec 3), the mean amount of nectar sugar was 261 $\mu\text{g}/\text{flower}$, whereas in OSR, 8 days after flowering started (on Dec 20), 120 μg of sugar was found per flower. This difference is significant (t -test, $t_{69} = 2.25$, $P = 0.03$), indicating that at comparable times after the start of the flowering stage, buckwheat had more sugars available in the form of nectar than OSR.

In non-exclusion plots, nectar sugar quantities in buckwheat stayed between 13 and 30 $\mu\text{g}/\text{flower}$ from the start to the end of the experiment. This represents a ca. 90 % reduction compared to the level of nectar in buckwheat flowers in exclusion plots on the first sampling (419 $\mu\text{g}/\text{flower}$). OSR flowers contained ca. 100 μg of sugar, which represents a reduction of only 20 % compared to the amount of nectar found in OSR flowers in exclusion plots.

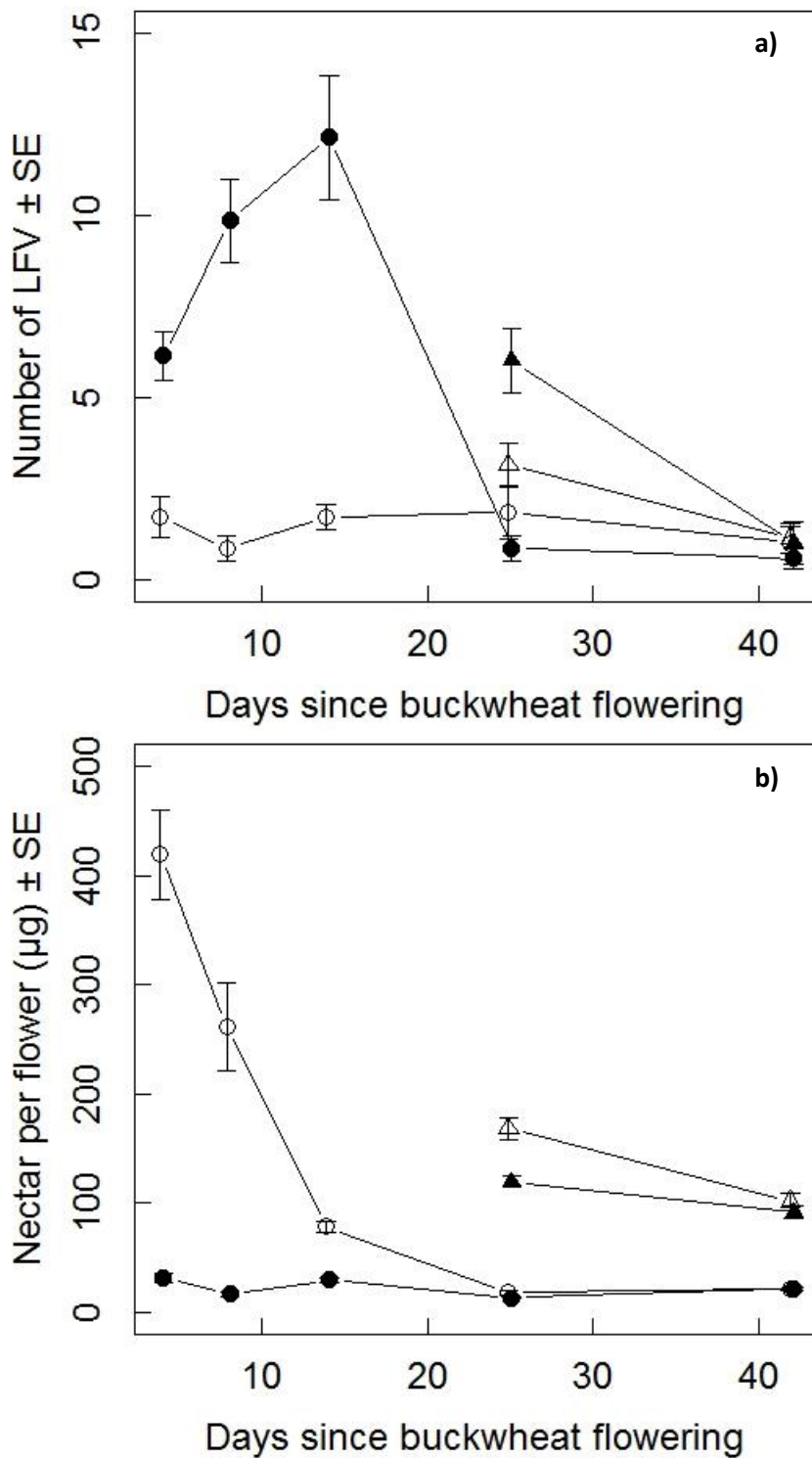


Figure 3.8 Number of large flower-visitors (a) and nectar per flower (b) in relation with the number of days since buckwheat started flowering. Circles correspond to visits of (a), or nectar in (b), buckwheat flowers. Triangles correspond to visits of (a), or nectar in (b), OSR flowers. Empty symbols indicate exclusion cages, full symbols indicate non-exclusion cages.

On November 22, no aphid mummies were observed on any of the plots. Four days after buckwheat started flowering, ca. 10 % parasitism was recorded in both exclusion and non-exclusion cages. In both cage types, parasitism rate increased up to 30-40 %, with a slight drop on the third sampling date. Parasitism rate dramatically decreased between Dec 20 and Jan 6, to reach almost 0 % (Fig 3.9.a).

In exclusion as in non-exclusion cages, aphid numbers slowly and steadily increased from 10 to ca. 100. Only on the last sampling date, the number of aphids in exclusion cages reached ca. 400 aphids per plot, whereas it stayed around 100 aphids per plot in non-exclusion cages (Fig 3.9.b). This difference, however, was not significant (t -test, $t_7 = 1.53$, $P = 0.17$), and the standard error associated with the mean number of aphids in exclusion cages on the last sampling date was markedly larger than on earlier measures, indicating a high variability among plots on that date.

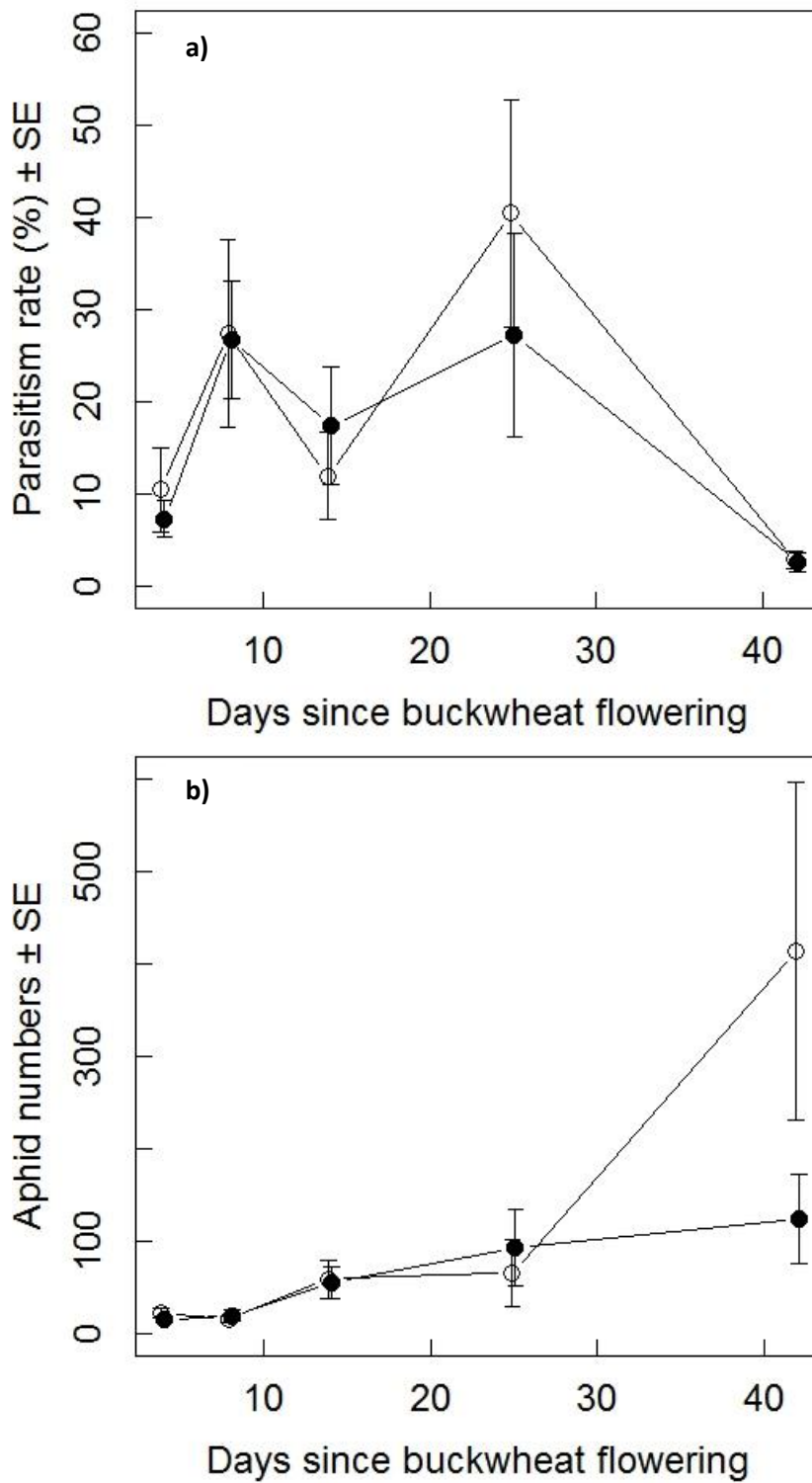


Figure 3.9. Parasitism rate (a) and number of aphids (b) in relation with the number of days since buckwheat started flowering. Empty circles refer to exclusion cages, full circles refer to non-exclusion cages.

3.4.4 Daily dynamics

On December 6, BW had started flowering for 11 days and OSR was at the stem elongation stage.

Cage type had an effect on the number of large flower visitors, the amount of nectar per plot, and the amount of nectar per flower (Table 3.6).

Table 3.5 Effect of cage type on the number of large flower-visitors, nectar per plot and nectar per flower. Each variable was averaged over all sampling times. The effect of cage type on each variable was tested with a *t*-test.

Mean December 6 (n = 6)

	<u>Exclusion cages</u>	<u>Non-exclusion cages</u>	<u><i>t</i>₄</u>	<u><i>P</i>-value</u>
Large flower-visitors	0.66	5.17	-4.30	0.01
Nectar per plot (µg/plot)	627.9	68.89	8.52	0.001
Nectar per buckwheat flower (µg/flower)	133.6	13.9	6.96	0.02

No more than two large flower-visitors were observed at a time in exclusion cages (Fig 3.10). In non-exclusion cages, most visits occurred in the morning. At 08:00, sugar levels were low in both exclusion and non-exclusion cages. Sugar values only increased in exclusion cages, but not in non-exclusion cages. The maximal amount of sugar in buckwheat flowers (221 µg/flower) was attained at 17:00.

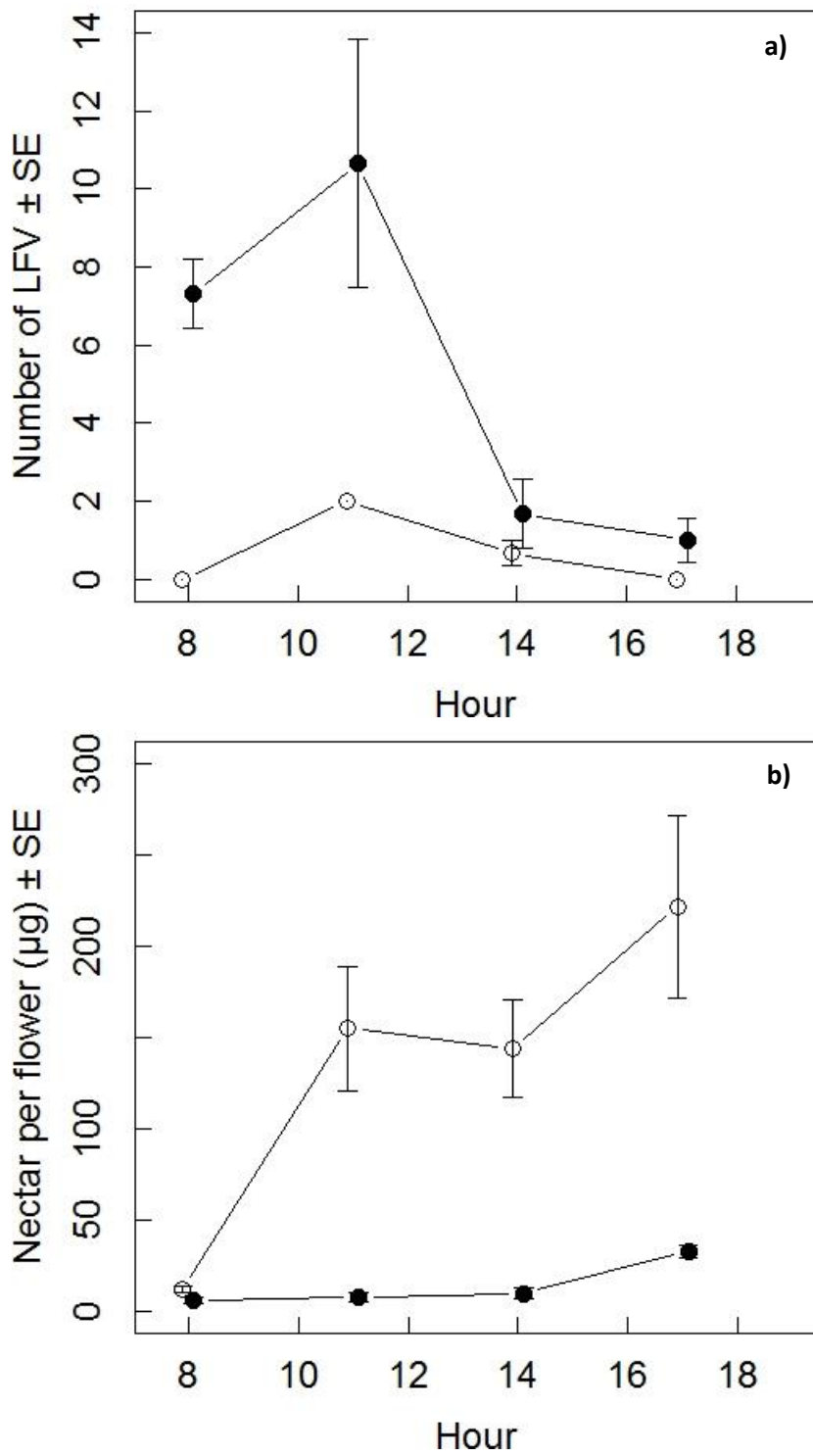


Figure 3.10 Diurnal variation of the number of large flower-visitors (a) and sugar amount in nectar (b) measured over the course of December 6, 2014. Empty circles correspond to exclusion cages, full circles to non-exclusion cages.

3.4.5 Nectar consumption

The mean ratio of fructose to body mass in wasps emerged from mummies collected in the field was 0.13 ± 0.04 % ($n = 13$). Based on this, it was decided to consider 0.21 % (equal to $0.13 + 2$ times the standard error) as a conservative base level of fructose. Out of 35 adult *D. rapae* collected in the field, 30 were above this base level (Fig 3.11). The fructose ratio in wasps collected in exclusion plots (1.3 ± 0.3 %) was not significantly different from that of wasps collected in non-exclusion plots (1.6 ± 0.3 %) ($t_{33} = -0.53$, $P = 0.6$).

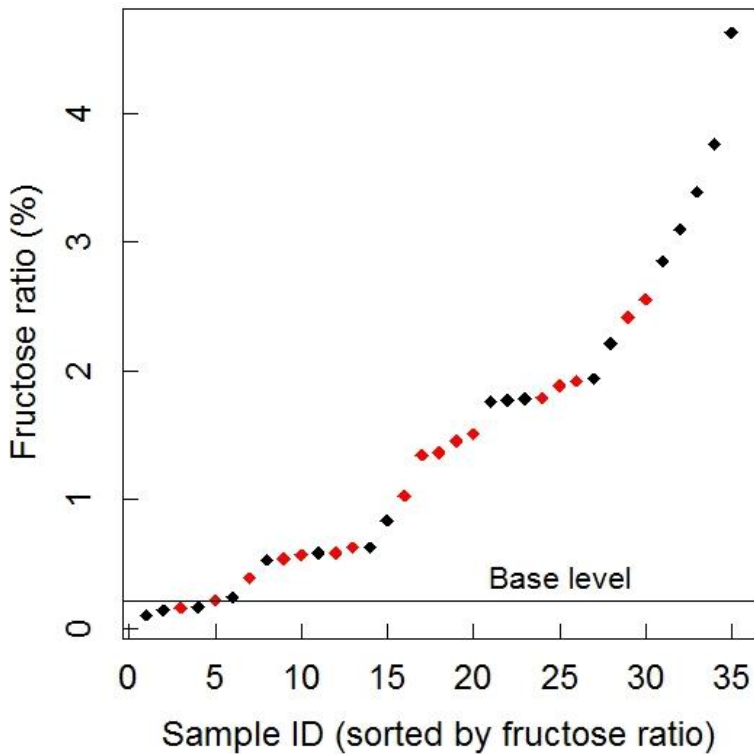


Figure 3.11 Ratio of fructose to body mass in 35 field-collected *D. rapae*. Black symbols represent insects collected in exclusion cages, red symbols indicates those collected in non-exclusion cages. The “base level” was defined as the ratio of fructose to body mass (+ 2 standard errors) in newly emerged *D. rapae* that were collected as mummies in the field and frozen within 4 h after emergence.

3.5 Discussion

To allow a sound interpretation of the results, it was important to be sure that the parasitism rate and the quantity of nectar in flowers responded only to the treatment (i.e. exclusion of pollinators) and not to a difference in climatic conditions in the experimental setup. The non-exclusion cages were often warmer and less humid than the exclusion ones around mid-day (Fig 3.5), probably due to more sunlight entering through the 30 cm-wide openings along the four sides of the cage. However, the fact that the differences in mean temperature, humidity and number of degree days between cage types were not significant (Table 3.1) suggests that the occasional differences recorded were negligible when considered at the scale of the whole sampling period. This suggests that the production of nectar, and the dynamics of aphid and parasitoid populations were comparable in both cage types. This, in turn, suggests that the potential effect of pollinators on parasitism rate and nectar quantities in flowers was not confounded by the experimental setup

The foraging of large nectar-feeders on flowering buckwheat reduced substantially the amount of sugar present in flowers. This effect was particularly strong in buckwheat in the 25 days following the start of flowering (late November to mid-December), with up to 90% reduction compared to non-visited flowers. During this period, nectar consumption by large flower-visitors occurred mostly in the morning. In the current work, nectar consumers had depleted flowers earlier in the day than in that of Lee and Heimpel (2002). This could be related to a different foraging behaviour of local pollinator faunas (the Lee and Heimpel study took place in Minnesota, USA). 25 days after the start of buckwheat flowering, sugar amounts in exclusion cages dropped to amounts similar to those in non-exclusion cages; however, buckwheat plants continued developing new flowers. This is consistent with observations in controlled conditions, which reported that nectar production per flower in buckwheat is at its highest during flowering peak (Cawoy et al., 2008) and then decreases. This indicates that the presence of fresh flowers on buckwheat does not necessarily translate into high amounts of nectar.

Large nectar-consumers did not significantly impact aphid parasitism, which indicates that there is no strong and short-term negative interaction between pollinators (large flower-visitors) and aphid parasitoids. The lack of correlation between mean parasitism rate and amount of sugar in flowers corroborates this conclusion. Several scenarios may explain the lack of negative interaction. First, the potential negative effect of nectar depletion on parasitoids may be cumulative. Fecundity of parasitoids may have been slightly reduced due to a partial nectar depletion, which may not have cascaded into a noticeable reduction of parasitism rates during the first weeks of buckwheat flowering (Lee and Heimpel, 2008; Lee et al., 2006). This fecundity gap could have accumulated during parasitoid generations, especially if the experiment had lasted longer. Between November 22

and December 20, the mean temperature was 18°C, which theoretically allows for the development of two generations of *D. rapae* since egg-to-adult development time is about 15 days at this temperature (Saleh et al., 2009). If more generations had passed, a potential small effect of nectar depletion may have translated into a noticeable reduction in parasitism.

Second, parasitism rates in exclusion cages may have been limited by the caging system itself. Under laboratory conditions parasitoids were able to cross the mesh used for caging (personal observation); however the exclusion cages may have limited plot colonisation by parasitoids in the field. *D. rapae* uses both visual and olfactory cues to find host-plants (Ayal, 1987), therefore cage walls could have altered the detection of oilseed rape by parasitoids in exclusion plots. This potential bias towards lower parasitism rate could have at least partially negated an effect of higher nectar availability. On January 6, parasitism rates in all plots had fallen to less than 5%, probably responding to other factors than food availability (e.g., unattractive senescing mature plants, hyperparasitism, etc.).

Third and last scenario: parasitoids may accommodate to low amounts of nectar. Parasitoids in this study seem to have frequently fed on sugars, as 30 of 35 individuals, collected across all treatments in late December, tested positive for fructose, indicating a very high incidence of sugar feeding. Parasitoids may have (i) found enough remaining nectar to satisfy their needs even after bee visits (as is thought to be the case for the small solitary bees *Pereirapis* sp., see discussion in Goulson et al., 2001); (ii) collected a sufficient quantity of nectar by visiting numerous flowers; (iii) displayed behaviours that enable them to avoid interference with bees, such as shifting the timing of their foraging periods, as has been documented in *Bombus pascuorum* Scopoli and *Anthidium manicatum* (L.) (Comba et al., 1999) and in the sub-species *Apis mellifera carnica* and *Apis mellifera ligustica* (Walther-Hellwig et al., 2006). The fructose found in parasitoids could originate from either nectar or aphid honeydew, suggesting that parasitoids possibly relied mostly on aphid honeydew as a carbohydrate source (Chapter 4, Wäckers et al., 2008), or shifted their diet from nectar to honeydew when large nectar-consumers were present.

Further work is needed to confirm whether a limited and delayed negative interaction between parasitoids and large nectar-consumers is frequent. Such an investigation could be performed on a system allowing a longer aphid and parasitoid population development period, such as winter-sown or perennial crops. Also, it would be useful to monitor parasitoid fitness throughout the course of the experiment (Lee and Heimpel, 2008; Vattala et al., 2005); however, the small size of *D. rapae* (ca. 3mm) makes field collection a difficult task when densities are low. In this study, mummies were observed as early as November 29, but adult *D. rapae* were observed in sufficient numbers for standard sampling (>5 per treatment) in late December only. Furthermore, knowledge of parasitoid nutritional ecology could benefit from further data on the physical state and the temporal availability

of sugar sources in field conditions. Together with more information on the ability of parasitoids to exploit highly viscous or crystalline sugars (Bartlett, 1962; Faria et al., 2008), this could lead to a further understanding of parasitoids' 'nutritional landscape'.

Although the results indicate that nectar consumption by pollinators impacts parasitoids to a minor extent, it may be different for other nectar-feeding natural enemies, such as predators. Predatory arthropods also benefit from floral subsidies (Hogg et al., 2011; Robinson et al., 2008), but no work has been done on their interaction with other large nectar-feeders. In particular, syrphids rely heavily on pollen for reproduction (Laubertie et al., 2012), but it is not known whether pollen consumption by honeybees can reduce syrphids' fecundity or other fitness traits in the field.

Chapter 4

The consequences of feeding on oilseed rape nectar and aphid-host honeydew on the longevity of *Diaeretiella rapae*.

4.1 Abstract

Many adult parasitic Hymenoptera consume floral nectar and as a result live longer, spend more time searching for hosts and attack more hosts individuals. However, plant species vary in their nutritional value to parasitoids, which is probably due to their floral morphology, phenology and nectar composition. Aphid honeydew can also be exploited by some parasitoids (including non-aphid parasitoids), although it is in most cases a sub-optimal food source compared to floral nectars. Parasitoids of aphids, however, may be well-adapted to consuming honeydew when it is produced by their hosts. The nutritional value of honeydew for this group of parasitoids has often been tested against that of synthetic feeding solutions, but rarely against floral nectar, and never against the nectar from the aphids' host plant. In the present chapter, the relative nutritional values of honeydew from the aphid *Myzus persicae* (Sulzer), nectar from two cultivars of oilseed rape (OSR) and nectars from four 'companion' plants were assessed by testing their effect on the longevity of *Diaeretiella rapae* (McIntosh) [Hymenoptera: Braconidae]. Parasitoids fed on buckwheat (*Fagopyrum esculentum*) nectar or OSR nectar lived longer than insects fed on nectars from other companion plants or water only. This indicates that during its flowering, OSR provides parasitoids with a highly nutritive resource. *D. rapae* also lived longer when fed OSR nectar than when fed host honeydew, therefore the latter may be seen as a sub-optimal food. The applied implications of this study are discussed, particularly with regards to the availability and quality of food sources for *D. rapae* in OSR crops. Together with two other recent studies, these results also advance the debate as to whether aphid parasitoids may be able to metabolise honeydew as well as nectar.

4.2 Introduction

Many parasitoids can absorb carbohydrates from floral nectar, extra-floral nectar or homopteran honeydew; however, they do not benefit equally from these sugar sources. For example, nectars are not all equally nutritive to parasitoids: some plant species, such as buckwheat (*Fagopyrum esculentum* Moench. [Polygonaceae]), are known to produce a nectar that strongly enhances longevity and fecundity of several parasitoid species, to a consistently greater extent than other nectariferous species (Fiedler et al., 2008). Furthermore, nectar is for most parasitoids a better food source than honeydew, as revealed by laboratory feeding trials comparing the longevity of parasitoids when fed with this honeydew or other sugar sources such as nectar (reviewed by

Wäckers et al. 2008). However, it has been hypothesised that parasitoids whose hosts produce honeydew may have evolved the capacity to metabolise honeydew sugars as efficiently as nectar. Indeed, the ability to digest honeydew efficiently would allow parasitoids to find hosts and food at the same place, which would reduce food-foraging costs and associated risks, and has probably been selected by evolution (Wäckers, 2005, 2000; Wäckers et al., 2008). Until recently, honeydew diets had been experimentally compared to synthetic sugar diets, and no study comparing nectar and honeydew diets had been performed on parasitoids whose host produces honeydew (Wäckers et al., 2008).

Two recent studies provide such diet comparison in aphid parasitoids, however their results are contradictory; *Lysiphlebus testaceipes* (Cresson) [Hymenoptera: Braconidae] lived as long on buckwheat nectar as on host (*Aphis gossypii* (Glover)) honeydew diets (Hopkinson et al., 2013), but the longevity of *Diaeretiella rapae* (McIntosh) [Hymenoptera: Braconidae] was 3.5 times shorter when fed *Brevicoryne brassicae* L. honeydew than *Vicia faba* L. extra-floral nectar (Jamont et al., 2013). In that case, *D. rapae* longevity on honeydew was not significantly different from control wasps given access to water only, suggesting that *B. brassicae* honeydew has no nutritional value to this parasitoid.

Oilseed rape (*Brassica napus* L., OSR) hosts several guilds of pest insects (Williams, 2010), including three aphid species (*Myzus persicae* (Sulzer), *Brevicoryne brassicae* L. and *Lipaphis erisimi* (Kalt.)), which are the main pests of this crop in Australasia (Lamb, 1989). *D. rapae* is the most common parasitoid of the above three aphid species worldwide (Desneux et al., 2006; Pike et al., 1999). Because these three aphid host species produce honeydew, and because OSR produces nectar during flowering, aphid parasitoids can find various sugar sources in OSR crops: crop floral nectar, aphid honeydew, and, if provided, nectar from floral subsidies.

To evaluate the usefulness of deploying floral subsidies in OSR crops, this study compared the value of various floral nectars, OSR nectar, and *M. persicae* honeydew, for the aphid parasitoid *D. rapae*. Also, to investigate a potential interactive effect of honeydew and nectar, an additional diet treatment combining buckwheat nectar and aphid honeydew was performed (inspired by van Rijn et al., 2013). Changes in longevity of adult *D. rapae* when provided with the various diets mentioned above were measured in the laboratory.

4.3 Materials and methods

4.3.1 Insect and plant rearing

D. rapae cultures were started in March 2012 by collecting aphid (*M. persicae*) mummies from brassica plots at the Biological Husbandry Unit, Lincoln University (bhu.org.nz). Parasitoids were maintained on *M. persicae* feeding on OSR plants (cv. Ability) in controlled-temperature rooms set at 24°C (with a 4 °C range) and a 16h-light:8h-dark photoperiod. Before experiments began, *M. persicae* mummies were individually placed in a 1.5mL microcentrifuge tube with a cotton stopper. Within four hours after emergence, parasitoids were sexed and used in bioassays.

OSR plants and four companion plant species were grown under a glasshouse at the Lincoln University nursery, with natural lighting and no heating. They were sown continually over a period from September 2013 to February 2014 to ensure a full provision of flowers during the experiment. The four species of flowering plants used here have been commonly used as floral subsidies (Fiedler et al., 2008): alyssum (*Lobularia maritima* L. [Brassicaceae], cv. Benthamii), buckwheat (*Fagopyrum esculentum*, cv. Katowase), coriander (*Coriandrum sativum* L. [Apiaceae], cv. Slowbolt), and phacelia (*Phacelia tanacetifolia* Benth. [Borraginaceae], cv. Balo). Also, two cultivars of OSR were used, these were Ability and Flash. The latter is a winter cultivar requiring vernalisation to initiate flowering. Vernalisation was triggered by placing 30-day old plantlets under 4°C, 8 hours of light per day for 40 days. Camelina (*Camelina sativa* L. [Brassicaceae]) was also included in our tests, to evaluate its potential as nectar source. It is a recently developed low-input biofuel feedstock crop which oil-rich seeds can serve in biodiesel production (Shonnard et al., 2010). Two camelina cultivars were tested: CS3 and Suneson.

4.3.2 Nectar in living inflorescences

One male and one female *D. rapae* were placed in a transparent plastic container (11 cm * 6.5 cm * 6.5 cm) through a 2 cm wide hole on one side. The hole was sealed with a foam plug. A 3 cm * 6 cm mesh-covered aperture on another side ensured proper aeration of the container. A 1.5 mL microcentrifuge tube containing a damp piece of cotton was pasted to the inner wall of the container and was kept moist to ensure *ad libitum* access to water for parasitoids. One flowering stem of the tested plant was inserted through a 2 cm wide foam-plugged hole at the base of the container, while the stem was still attached to the plant (Fig 4.1.a). The container was tied to a wooden stake to maintain it above the stem. Visual checks at the start of each experimental unit confirmed that the stems bore no aphids and that nectar was present in more than five fresh flowers. The only exception was camelina, which did not show nectar droplets on flowers. Plants were watered throughout the experiment. It is assumed that the total amount of nectar present in the treatments (except camelina) represented an *ad libitum* provision of floral nectar. The experiment took place in a controlled-

temperature room, using the same conditions as for plant growing (24 ± 4 °C, and a 16h-light:8h-dark photoperiod). There were eight nectar treatments (Fig 4.2), originating from the eight plant types mentioned above as well as a control, where no flowers were inserted in the containers. There were eight experimental blocks, each containing one replicate of each treatment and the control. All replicates within one block were started on the same day. Containers were checked daily to assess the longevity of each insect.

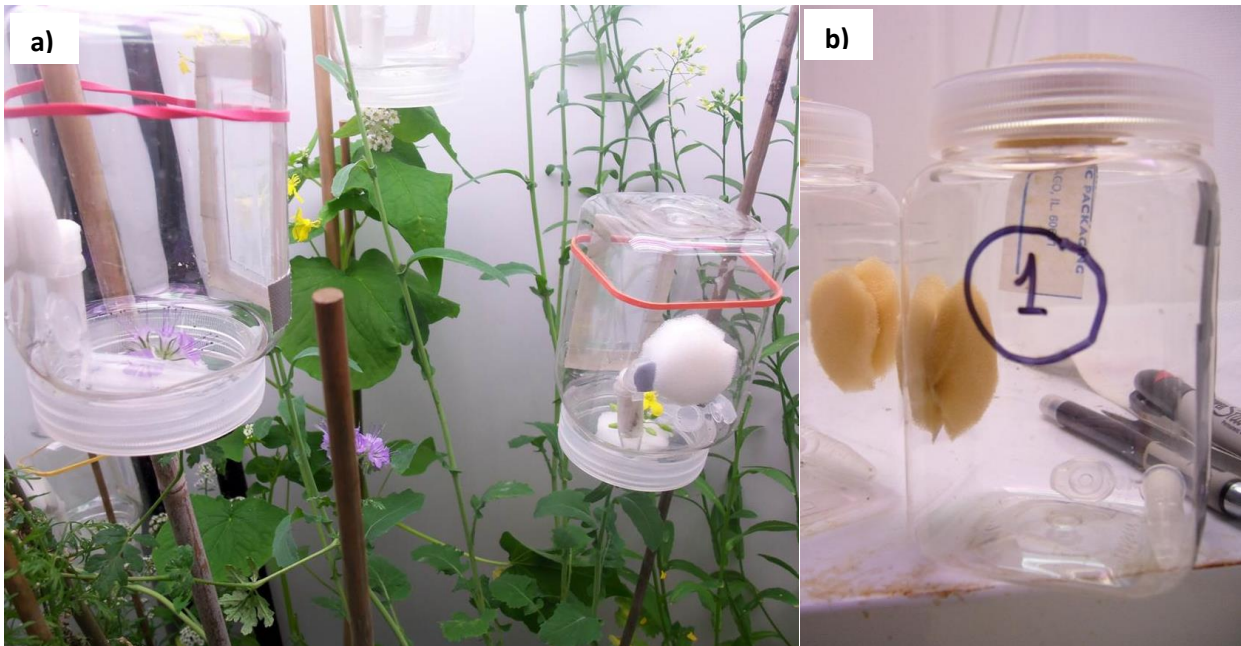


Figure 4.1 Experimental setup for the bioassays involving nectar in living inflorescences (a) and floral nectars and honeydew on Parafilm bands (b). In (b), the Parafilm band was maintained hanging from the top of the container with a foam plug. The treatments are detailed in section 4.3.2 and 4.3.3 respectively.

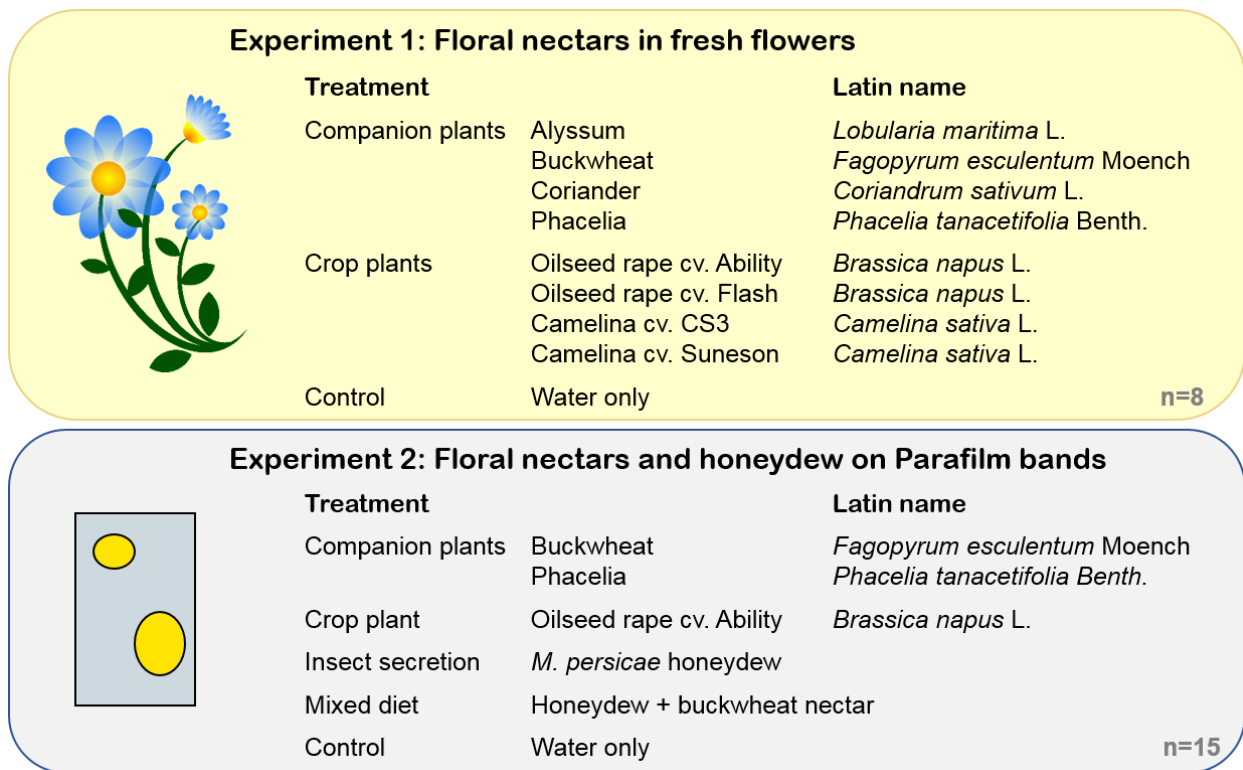


Figure 4.2 Treatments used in a longevity trial on adult *Diaeretiella rapae* fed with sugars from various sources.

4.3.3 Nectar and honeydew droplets on Parafilm

One male and one female *D. rapae* were placed in a transparent plastic container, with ventilation and access to water as above. *M. persicae* honeydew and various floral nectars were presented to parasitoids as individual treatments as droplets on a 2 * 4 cm band of Parafilm (Figure 4.1.b). All treatments were provided on Parafilm bands to avoid biases potentially induced by differing substrates (which was not the case in Hopkinson et al., 2013; Jamont et al., 2013). Nectar was taken from fresh buckwheat, phacelia, and OSR flowers, by carefully dissecting the flowers and depositing droplets of nectar on the Parafilm band. Bands with nectar were provided to parasitoids immediately after nectar was deposited. Honeydew was collected by placing the Parafilm bands under oilseed rape leaves heavily infested by *M. persicae* (culturing conditions as described above), for 24 h, and directly provided to parasitoids. It was not technically possible to measure the actual viscosity of droplets, but crystallised honeydew and nectar droplets did appear white, whereas liquid droplets looked transparent. On each instance that diets were provided, there were liquid droplets on the Parafilm band. Occasionally, some droplets of honeydew were crystallised, probably those that had been deposited at the beginning of the collection period. A treatment combining buckwheat nectar and honeydew was made by placing buckwheat nectar droplets on Parafilm bands covered with honeydew (as above). The band was provided to parasitoids immediately after buckwheat nectar

deposition. Parafilm bands in all treatments were refreshed daily, and were loaded with a similar volume of honeydew or nectar, that was visually estimated to represent the volume of the abdomen of *D. rapae*. Assuming that the daily consumption of nectar or honeydew by parasitoids is generally lower than the volume of their abdomen, it was considered that parasitoids could feed *ad libitum* in all treatments.

There were 15 blocks, each containing one replicate of the five treatments, plus a control consisting of a band of Parafilm alone (Fig 4.2). All containers within one block were started on the same day, and were checked daily to record the longevity of each insect.

4.3.4 Statistical analysis

The effects of sex, diet, and their interaction on longevity were analysed by analysis of variance, modelling a split-plot design with diet as a plot-level factor and sex as within-plot factor. Longevity data were log-transformed to achieve ANOVA assumptions of normality and homogeneity of residuals. Post-hoc least-square difference (LSD) tests were performed to examine differences between diet treatments and sexes. All analyses were performed with GenStat.

4.4 Results

4.4.1 Nectars in living inflorescences

The effect of floral diet and sex on parasitoid longevity were both significant and there was no significant interaction (Table 4.1). Males lived an average of 2.9 days, shorter than females (3.8 days). Access to buckwheat and OSR cv. Ability allowed parasitoids to reach the highest longevities (respectively, 5.9 and 5.7 days), three times longer than that of unfed parasitoids which lived only for a mean of 1.6 days (Fig 4.3; $LSD_{5\%} = 1.5$ days). Access to phacelia or camelina did not enhance parasitoid longevity significantly compared to the control (water only). The two OSR cultivars did not increase parasitoid longevity to the same extent: the winter cultivar Flash allowed parasitoids to reach 3.5 days only, i.e. significantly less than 5.9 days on cultivar Ability. Parasitoids provided with coriander or alyssum lived significantly longer than those on water only; however their longevities (respectively, 3.7 and 3.9 days) were significantly lower than on buckwheat.

Table 4.1 Analysis of variance testing the effect of various diets and sex on parasitoid longevity, in two different experimental conditions.

Experiment one: nectar in living inflorescences

Source of variation	d.f.	F	P-value
Block	7	0.69	
Diet	8	10.72	<.001
Residual	56	1.52	
Sex	1	6.70	0.012
Diet:Sex interaction	8	1.73	0.108
Residual	63		

Experiment two: nectar and honeydew on Parafilm bands

Source of variation	d.f.	F	P-value
Block	14	1.28	
Diet	5	13.63	<.001
Residual	69	1.34	
Sex	1	0.78	0.380
Diet:Sex interaction	5	0.56	0.727
Residual	79		

4.4.2 Nectar and honeydew droplets on Parafilm

There was a significant effect of diet on parasitoid longevity, however there was no significant effect of sex, nor was there an interaction between sex and diet (Table 4.1). All diets significantly enhanced parasitoid longevity compared to unfed parasitoids (Fig 4.4; $LSD_{5\%} = 1.3$ days). When fed on OSR cv. Ability nectar, parasitoids lived approximately 4.4 days, significantly longer than those fed on *M. persicae* honeydew (2.9 days). The effect of *M. persicae* honeydew, nectars of buckwheat and phacelia, and the mixed-diet (honeydew + buckwheat nectar) were not significantly different from one another. The mixed honeydew-nectar diet did not enhance parasitoid longevity more than the nectar-alone diet, which indicates that there was no positive synergistic effect of co-occurring honeydew and nectar feeding.

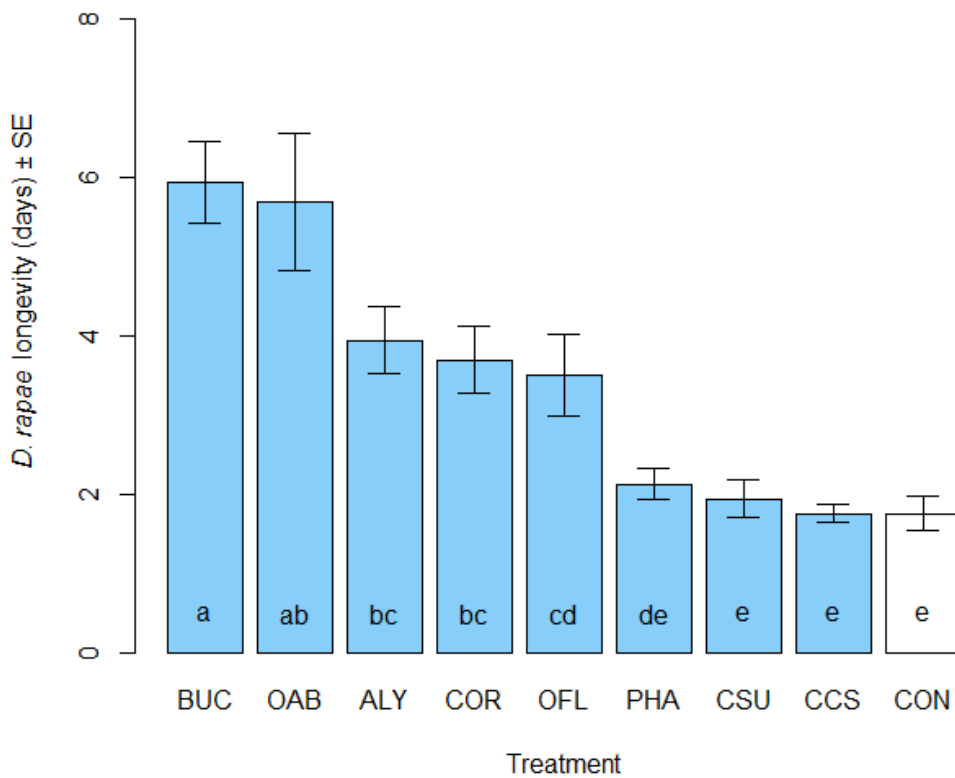


Figure 4.3 Longevity of *D. rapae* when fed on floral nectars from live inflorescences. Bars with the same letter are not significantly different ($LSD_{5\%} = 1.5$ days). Treatments, in order of effect size: BUC: buckwheat; OAB: oilseed rape cv. Ability; ALY: alyssum; COR: coriander; OFL: oilseed rape cv. Flash; PHA: phacelia; CSU: Camelina cv. Suneson; CCS: Camelina cv. CS3; CON: control (water only). N = 8 replicates.

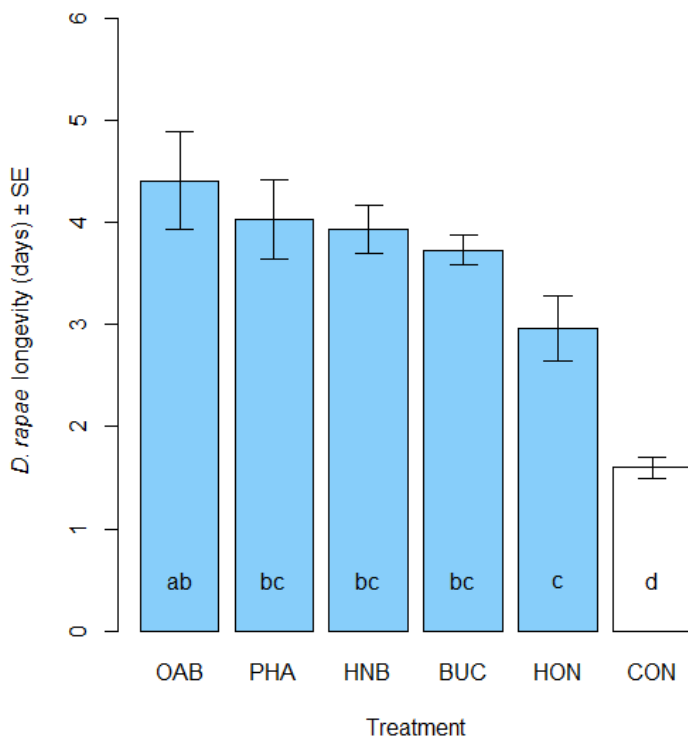


Figure 4.4 longevity of *D. rapae* when fed on various diets provided on Parafilm bands. Bars with the same letter are not significantly different ($LSD_{5\%} = 1.3$ days). Treatments, in order of effect size: OAB: oilseed rape cv. Ability; PHA: phacelia; HNB: honeydew + buckwheat mixed diet; BUC: buckwheat; HON: *M. persicae* honeydew; CON: control (water only). N = 15 replicates.

4.5 Discussion

4.5.1 Comparison of nectar diets

Nectars enhanced parasitoid longevity compared to water-only controls, except for phacelia and camelina flowers. Access to phacelia flowers did not enhance parasitoid longevity (Fig 4.3); however when phacelia nectar was provided as droplets on Parafilm, it increased parasitoid longevity more than two-fold (Fig 4.4). This discrepancy is probably due to flower morphology because phacelia nectaries are located at the bottom of a deep (10-15mm) corolla which entrance is blocked by small lid-like structures on petals, which may have impeded parasitoid exploration. Other parasitoids have also been shown to be unable to consume phacelia nectar (Vattala, 2005), which illustrates the importance of floral morphology for nectar exploitation by parasitoids (Patt et al., 1997). Camelina flowers did not seem to produce noticeable amounts of nectar. In field conditions, pollinators do visit camelina; however the extent to which this plant provides pollen or nectar has not been studied (Groeneveld and Klein, 2014). This plant is mainly self-pollinated and receives little benefit from insect pollination (Groeneveld and Klein, 2014); therefore production of nectar is not crucial for its reproduction.

Buckwheat nectar confers high longevities to many parasitoid species (Fiedler et al., 2008; Russell, 2015), and this was also the case for *D. rapae*, especially when nectar was provided on intact shoots. Buckwheat nectar increased the longevity of *D. rapae* to a ca. 50 % larger extent than alyssum and coriander. A similar difference in effect size has been observed on the aphid parasitoid *Aphidius ervi* (Araj and Wratten, 2013). The longevity of *Microctonus hyperodae* Loan [Hymenoptera: Braconidae], a parasitoid of the Argentine stem weevil, was increased twice more by feeding on buckwheat nectar than by feeding on alyssum (Vattala et al., 2006). These results on three parasitoids species point out buckwheat nectar as a superior food source for parasitoids.

Results also suggest differing nutritional quality between the nectars of the two OSR cultivars tested here, which could be explained by genetic differences between the two cultivars (Wang et al., 2011) and plant life history.

4.5.2 The value of honeydew

Honeydew enhanced *D. rapae* longevity to a certain extent but not as much as floral nectar, particularly OSR nectar. Feeding on *M. persicae* honeydew increased *D. rapae* longevity to 3 days on average, which represents an increase one third smaller than the increase caused by feeding on OSR nectar (on OSR cv. Ability nectar, mean longevity was ca. 4.5 days). Therefore, *D. rapae* oviposition

period was extended from 3 to 4.5 days, which is a period of particularly high fecundity. Indeed, *D. rapae* is a synovigenic parasitoid, i.e. it can mature eggs during its adult stage (Jamont et al., 2013), and the number of mature eggs in ovaries of *D. rapae* is at its highest between the second and fourth day after emergence (Araj and Wratten, 2015; Kant et al., 2013; Tylianakis et al., 2004). Feeding on OSR nectar rather than on honeydew could therefore significantly improve the fitness of *D. rapae*. This result has to be taken with caution, however, because honeydew droplets were noticeably smaller than OSR droplets, and as a result, they may have crystallised at a faster rate than OSR, which may have impeded their uptake by parasitoids (Faria et al., 2008). However, when parafilm bands were placed in the experimental containers, at least 50 % of the honeydew droplets appeared liquid, therefore they were available to parasitoids for at least the first hours after the Parafilm band was refreshed.

Longevity of *D. rapae* was not significantly different when fed on honeydew or buckwheat droplets. Vollhardt et al. (2010) observed that honeydew-fed *Aphidius ervi* tended to subsequently feed on buckwheat nectar, whereas nectar-fed parasitoids did not feed on honeydew. This suggests a gustatory mechanism that signals honeydew as being a food source of lower quality than nectar.

In contrast to honeydew from *M. persicae*, that from *B. brassicae* had no effect on the longevity of *D. rapae* (Jamont et al., 2013). Similarly, the longevity of the parasitoid *Aphidius colemani* Viereck was about three times higher when fed honeydew from *M. persicae* than when fed on that from *B. brassicae* (see Table 2 in Wäckers et al. 2008). Also, the solitary bee *Osmia bicornis* L. was observed feeding on *M. persicae* honeydew but avoided that from *B. brassicae* (Konrad et al., 2009). One potential explanation is that the latter is coated with wax (Lamb, 1959; Wäckers, 2005), which may limit its exploitation by parasitoids. The predatory syrphid *Episyrphus balteatus* (De Geer) seemed relatively unaffected by this wax and had increased longevity after consumption of *B. brassicae* honeydew (van Rijn et al., 2013).

4.5.3 Food sources in OSR fields

OSR fields are an agroecosystem that is not devoid of food sources for *D. rapae*: they may feed on OSR nectar and honeydew from *M. persicae*. In laboratory conditions, honeydew from *M. persicae* is not as valuable as OSR nectar, although this may have been confounded by its faster crystallisation. In the field, crystallisation might occur faster than in the laboratory, so honeydew quality might be lower than that measured in this experiment. In cases of heavy aphid infestation, honeydew can occasionally accumulate between the stem and the base of a leaf. This could prevent crystallisation and represent a food source for *D. rapae*; however this has not been studied to date. Therefore, even in periods of high honeydew availability, nectar provision may allow for a better enhancement of the longevity of *D. rapae*, which in turn may increase the biocontrol of pests by this parasitoid. Although

not as valuable as nectar, honeydew could potentially complement floral nectar when aphid densities are high and nectar not available. The presence of honeydew might mitigate potential exploitative competition for nectar, by providing a sugar source when nectar is intensely consumed by other insects such as pollinators (Chapter 3; Lee and Heimpel, 2002).

In the mutualistic association between ants and aphids, ant-tended aphid species provide honeydew to ants, and these in return protect aphids against predatory or parasitic arthropods (Shik et al., 2014). Following this observation, it could be hypothesised that *D. rapae* and parasitoids in general may mimic ant behaviour to obtain honeydew directly from the anuses of their aphid hosts, from which honeydew is excreted. This, however, has never been reported, and was not observed during the work presented here and in other chapters. It is probable that providing honeydew directly to parasitoids would be very costly (in terms of fitness) to aphids, because fitter parasitoids would attack a higher number of aphids.

The nectar of OSR cv. Ability conferred a higher longevity to *D. rapae* than that from cv. Flash, but nevertheless, parasitoids are not likely to be limited by food availability during the flowering of this crop. The value of floral subsidies would therefore be more marked outside the period of crop flowering, in particular during pre-flowering, when the crop is highly sensitive to aphid infestation (Ellis et al., 1999). Furthermore, because OSR nectar is highly nutritive to *D. rapae*, and perhaps also to other natural enemies, it could be used itself as a floral subsidy for other crops. When flowering, OSR fields may also enhance the longevity of natural enemies that may subsequently disperse in surrounding crops (as hypothesised in Bowie et al., 1999). Alternatively, OSR can be used in floral strips or intercropped with other crops (as in Wang et al., 2009) to provide nutritional resources to natural enemies, and simultaneously yield a marketable seed.

Chapter 5

Un-nesting DNA Russian dolls – The potential for constructing food webs using residual DNA in empty aphid mummies

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5.1 Abstract

Constructing food web assemblages comprising parasitoid wasps involves large field collections of hosts followed by labour-intensive rearing of the insects to evaluate rates of parasitism along with morphological or molecular identification of the parasitoid species. This chapter presents a new molecular method for the practical and accurate construction of aphid-based food webs. It was hypothesised that parasitoid and hyperparasitoid DNA left inside aphid mummies after emergence of these third- and fourth-trophic level guilds can be simultaneously detected using universal polymerase chain reaction (PCR) primers for non-specific DNA amplification in combination with single-stranded conformation polymorphism (SSCP) analysis. Such a protocol theoretically allows food web construction to be performed with no *a priori* knowledge of the species present. Moreover, the use of empty mummies circumvents rearing and minimises labour and time in the field and laboratory.

To test this hypothesis, DNA analyses were conducted on laboratory-produced parasitised aphids (mummies) from *Myzus persicae* and *Brevicoryne brassicae* after exposure to the parasitoid *Diaeretiella rapae* and the hyperparasitoid *Asaphes vulgaris*.

DNA was amplified in empty aphid mummies for as long as three weeks after parasitoid emergence. However the simultaneous identification of several species in a single mummy sample was rare, which hinders the accurate inference of trophic links. DNA quality and relative quantity, together with preferential amplification, are potential explanations of current results. Technical refinements are needed to ensure full reliability and detection of complex trophic links. The use of PCR-SSCP for food web construction is novel and its potential to include an important number of different species is yet to be fully explored.

5.2 Introduction

Biological control of arthropods by arthropods has a history of at least 2,000 years (van den Bosch and Messenger, 1973). The most frequently-used type of biological control is often called “classical”; in this approach, a natural enemy of an introduced pest is sourced from the pest’s region of origin. However, this method has remained at a 10% success rate since 1880 (Gurr and Wratten, 2000), the main cause of this low rate being the failure of the agent to establish in its new environment (Gurr et al., 2012). Most cases of biological control release have concentrated only on the agent and its prey/host and have ignored the fact that the released agent may become part of a food web comprising species native to the region of introduction and others which had been previously introduced. This lack of awareness of the importance of other trophic levels impacting on the agent has been a major gap in the history of biological control research. Interactions in food webs, comprising up to five trophic levels composed of potential new hosts, new enemies and new competitors, can clearly influence biocontrol success.

Constructing parasitoid-host food web assemblages and attempting to analyse their dynamics is historically difficult and usually involves large field collections of hosts followed by labour-intensive rearing or dissecting of the insects to evaluate rates of parasitism, along with morphological identification of the parasitoid species (Alhmedi et al., 2011; Gagic et al., 2012; Höller et al., 1993; Lohaus et al., 2013; Müller et al., 1999; Tylianakis et al., 2007; van Emden and Harrington, 2007). Differential death of parasitised and healthy individuals during rearing, or the presence of non-viable parasitoids in dissected insects (Day, 1994) can lead to an incorrect quantification of trophic links and bias food web studies (Garipey and Messing, 2012). Moreover, with declining numbers of specialist invertebrate taxonomists worldwide (Kim and Byrne, 2006), it has become increasingly difficult to rely solely on the use of morphology for the identification of complicated parasitoid faunas, potentially comprised of cryptic species (Desneux et al., 2009; Müller et al., 1999). Although mummy morphology can allow species-level identification of aphids (Höller et al., 1993; Müller et al., 1999), it restricts parasitoid identification to the genus level (Gagic et al., 2012; Lohaus et al., 2013). In recent years, molecular ecology has been proposed and utilised as a tool of choice to overcome difficulties of trophic interaction studies (e.g. Andrew et al., 2013; Pompanon et al., 2012; Valentini et al., 2009), and was recently used in the analysis of aphid-parasitoid food webs. It has been shown that parasitoid DNA can be retrieved from living aphids a few days after parasitoid egg deposition (Derocles et al., 2012), or from mummified aphids in which the parasitoid or hyperparasitoid has developed (Garipey and Messing, 2012; Traugott et al., 2008). However, assessing persistence times of aphid and parasitoid DNA after, respectively, parasitism and hyperparasitism has never been attempted (Traugott et al., 2008).

Molecular analysis of food webs based on living aphids versus mummified aphids can yield dramatic differences due to biological processes occurring in the host in the interval between egg deposition and mummification (Garipey and Messing, 2012). These include host resistance (Oliver et al., 2005), parasitoid competition (Sampaio et al., 2006) and hyperparasitism (Sullivan and Völkl, 1999). Similarly, a significant proportion of parasitoids within already-formed mummies do not actually emerge, possibly because of estivation which can delay emergence for several months, accidental death due to environmental conditions or other reasons (Höller et al., 1993; Jervis and Kidd, 1986). Using post-emergence mummies for food web construction has not been attempted previously, but this approach has the potential to provide an accurate picture of the cohorts of Hymenoptera that have successfully exited from the mummy, thus enabling a representation of the hymenopteran community which has a potential action on subsequent generations of aphids.

The approach proposed here is to identify parasitic Hymenoptera through the DNA remaining in aphid mummies. This includes not only DNA in intact mummies (i.e. containing a parasitic wasp larva) but also and perhaps more importantly, residual DNA remaining within the empty mummified aphid following parasitoid emergence. Mummies are easy to collect and to preserve in large numbers, especially when empty and dried. Moreover, as only aphids killed by parasitic Hymenoptera are considered, this method minimizes costs by avoiding the analysis of “information-poor” samples (i.e. non-parasitised). Recent studies have demonstrated the potential of environmental DNA such as insect exuviae or faeces for invertebrate specimen identification (Lefort et al., 2012) and the investigation of trophic interactions (Boyer et al., 2011). We hypothesise that aphid DNA can be amplified from both full and empty mummies along with parasitoid and hyperparasitoid DNA; and that PCR followed by single-stranded conformation polymorphism (PCR-SSCP) analysis will enable species-level identification of aphid, parasitoid, and hyperparasitoid from both pre- and post-emergence mummies.

The methods proposed in existing molecular-based literature can retrace aphid-parasitoid and parasitoid-hyperparasitoid trophic links; however they are based on DNA amplification using species- or group-specific primers. The design of such primers requires prior knowledge of the species present (Garipey and Messing, 2012; Traugott et al., 2008); and even group-specific primers are often limited to some parasitoid (Derocles et al., 2012) or hyperparasitoid species (Traugott et al., 2008) due to their non-clustered phylogeny. Although such an approach is very valuable when the fauna of interest is well known, a more flexible approach would be to use so-called 'universal' primers which allow DNA amplification from species belonging to different groups in a single analysis. The use of

such primers would provide an ideal tool for discovering unexpected trophic links or detecting new species.

PCR-SSCP has been extensively used in medical research and population biology mainly to detect allelic variation (Hayashi, 1991; Lessa and Applebaum, 1993; Orita et al., 1989), to identify species (Ridgway et al., 2011), or analyse community composition (Schwieger and Tebbe, 1998). For a wider view of its applications in ecology, see Sunnucks *et al.* (2000). SSCP electrophoresis separates DNA strands depending on their sequence, thus differing from common post-PCR electrophoresis which is based on fragment length. Here, we expect that the DNA of the different species supposedly present in aphid mummies can be amplified simultaneously by PCR, yielding a mix of fragments differing in sequence, but of equal length. The SSCP electrophoresis is then intended to separate the various DNA strands amplified, yielding banding patterns specific to the species present in the analysed mummy. Species identification would be possible by merely reading the banding patterns and comparing them to known standards, which limits the need for sequencing and therefore is expected to minimise costs (Lee et al., 1996; Sunnucks et al., 2000).

The aim of this study is to test a methodology that combines PCR and SSCP for the retrospective construction and analysis of quantitative food webs, thus making the first steps towards providing a new molecular tool that has the potential to be used to gain insight into aphid food web structure. This in turn could help interpret the role of trophic interactions in enhancing or limiting biological control efficacy.

5.3 Methods

5.3.1 Insect cultures

To test our hypotheses, we conducted DNA analyses of laboratory-produced mummies of the aphids *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* L. These two globally distributed species are major pests of a wide range of plant families and many species of the family Brassicaceae (van Emden and Harrington, 2007). The parasitoid used for the study was *Diaeretiella rapae* (McIntosh), a koinobiont endoparasitoid (i.e. the parasitoid oviposits into the host body, and the host continues to develop after oviposition). This parasitoid is common worldwide, and attacks many aphid species (Pike et al., 1999). The study also included the hyperparasitoid *Asaphes vulgaris* Walker [Hymenoptera: Pteromalidae], a generalist idiobiont ecto-hyperparasitoid (i.e. the hyperparasitoid oviposits on the surface of the host body, and kills the host in the process of oviposition, Sullivan and Völkl, 1999).

Aphids and parasitoids were morphologically identified to species level following Cottier (1953) and Kavallieratos *et al.* (2001) respectively (as in Chapter 2 and 4, but for this experiment *B. brassicae* were also collected and cultured). Amplification and sequencing of the COI barcoding region confirmed aphid and parasitoid identity and allowed identification of the hyperparasitoid species (see protocol below). Insects were cultured in controlled temperature rooms (24°C with a 2°C range and a 16h photoperiod), under dome cages (BugDorm insect tent, MegaView Science Co., Ltd). Aphids were maintained on Brussels sprout plants replaced every three weeks. Parasitoids were supplied with plants bearing aphids and with a solution of 40% sucrose. Hyperparasitoids were supplied with plants bearing parasitised aphids and with a similar solution of sucrose. Cultures were maintained in separate rooms to ensure aphid cultures were parasitoid-free and that parasitoid cultures were hyperparasitoid-free.

5.3.2 Aphid mummy production

To obtain parasitised mummies, aphids of each species were exposed to parasitoids in separate culture cages. As soon as mummies were visible they were collected and individually isolated in 200 µL micro-tubes. A first cohort of mummies was frozen at this stage (-20 °C), and designated as “full mummies”. All other tubes were kept in controlled temperature rooms (as above) and sealed with a piece of cotton wool which was kept moist. Mummies were checked daily until parasitoid emergence, and were then frozen either seven or 21 days after emergence. In the first case, mummies were referred to as “young mummies” (YM), otherwise they were “old mummies” (OM).

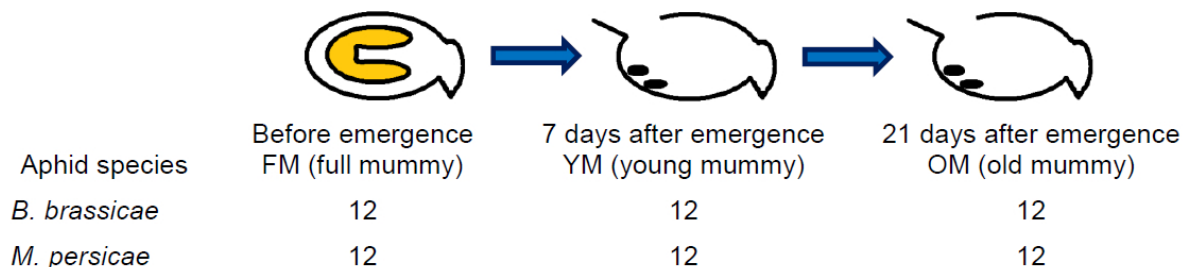
To obtain hyperparasitoids, mummies (one or two days after mummification) were exposed to hyperparasitoids for two days and individually isolated in micro-tubes. For *M. persicae*, a first cohort of mummies was frozen at this stage and considered as “full hyperparasitised mummies” (FHM) even though hyperparasitoid larvae presence could not be ascertained. For both aphid species, a cohort of mummies was frozen seven days after hyperparasitoid emergence, and designated as “young hyperparasitised mummies” (YHM).

5.3.3 DNA extraction

The DNA extraction protocol followed the ZR Tissue and Insect DNA MicroPrep extraction kit (Zymo Research) with the following modifications: i) 500 µL of Lysis Solution were added to the BashingBead Lysis tubes; ii) after crushing in the bead beater, samples were left to incubate overnight at 55°C; iii) 10 µL of elution buffer were added to a Zymo-Spin IC column and left for 30 minutes at room temperature before centrifugation. This step was then repeated once, resulting in a 20 µL final eluted DNA solution.

DNA was extracted from 12 mummies of each type (FM, YM, OM, YHM) for each aphid species and a further 12 *M. persicae* mummies of the type FHM (i.e. 108 extractions in total; see Fig. 5.1).

Parasitised mummies (*D. rapae*)



Hyperparasitised mummies (*A. vulgaris* on *D. rapae*)

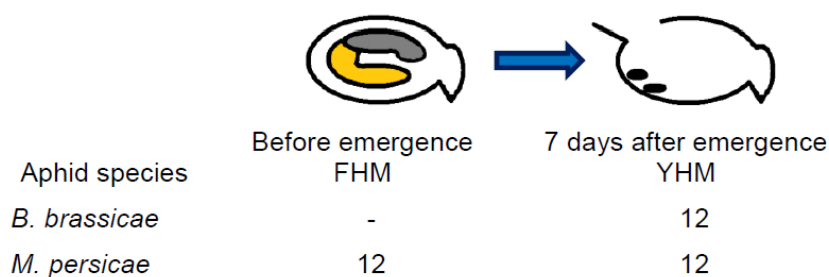


Figure 5.1. Mummy types and number of samples tested for each aphid species.

5.3.4 DNA amplification

Because DNA was likely to have been degraded into short fragments to some extent in empty mummies, a short region of DNA was targeted for amplification. However, the identification power of the selected region needs to ensure all amplified DNA could be accurately identified at the species level. Therefore, an internal region of the COI gene was targeted with the polymerase chain reaction (PCR) using the primers MLepF1 (forward GCTTTCCACGAATAAATAATA) and LepR1 (reverse TAAACTTCTGGATGTCCAAAAATCA) (Hajibabaei et al., 2006). These primers amplify a 450 base pair region within the COI gene, which is suitable for species identification in most animal groups, particularly invertebrates (Hajibabaei et al., 2006). The considerable advantage of using primers that are internal to the COI barcoding region is that sequences can be directly compared to the Barcode of Life Data System database (BOLD, see Ratnasingham and Hebert, 2007) to identify unsuspected species where DNA sequencing is required (beyond PCR-SSCP) for species separation. The R package SPIDER (Brown et al., 2012) was used to verify the suitability of the amplified fragment to identify most of the aphid, parasitoid and hyperparasitoid species potentially found in aphid systems. We

downloaded sequences for all species of Aphididae, Braconidae, Pteromalidae and Figitidae available on BOLD and extracted those with valid species names and for which the internal COI region described above was available. Based on this selection of 764 species, a sliding window analysis (Boyer et al., 2012) indicated that the identification success of the chosen COI region was 96%.

1.5 µL of DNA extract was used as a template in 10 µL PCRs comprising the following reagents: 5µL of KAPA2G™ Robust HotStart DNA Polymerase (Kapabiosystems); 0.4 µL of each primer MLep and Lep [10 µM]; 0.1 µL of MgCl₂ [25 µM]; 0.5µL of Bovine Serum Albumin (BSA); 2.1 µL of ultrapure water.

The thermocycler protocol consisted of an initial denaturation at 95°C for 3 min, followed by 38 cycles of denaturation at 95°C for 15 s, annealing at 48°C for 40 s, and elongation at 72°C for 60 s, followed by a 7 min final elongation at 72°C. PCR products were processed through electrophoresis on agarose gels, using SYBR Safe DNA gel stain (Invitrogen) to confirm DNA amplification.

5.3.5 Species identification

PCR products were processed in a SSCP electrophoresis (Schwieger and Tebbe, 1998; Sunnucks et al., 2000). The separation of PCR amplicons was performed with an SSCP electrophoresis using the same protocol as in Ridgway *et al.* (2011) but with the following modifications, i) 4 µL of PCR product were mixed with the loading dye; ii) heat denaturation of the DNA lasted 7 min at 99°C; iii) electrophoresis was run at 20°C for 16h. Bands were revealed according to the gel silver staining protocol given in Bassam and Gresshof (2007).

Adults of the insects involved in this work were also processed as described so their banding pattern (on SSCP gels) was known and could be used as a standard against which other samples could be compared. Species identification in mummy samples was then inferred by comparing banding patterns to control insects (un-parasitised aphids, parasitoids, hyper-parasitoids). To confirm identification of banding patterns, visible bands on the SSCP gels were excised and DNA extracted according to Schweiger & Tebbe (1998) with the following modification: the final ethanol evaporation step was extended to an overnight evaporation at 37°C, after which DNA was resuspended in 20 µL Tris-HCl and left for 1 h at 55 °C to allow for complete DNA solubilisation. PCR products extracted from SSCP gels were sequenced in both directions using the same primers as in the original PCR, with the BigDye Terminator Cycle Sequencing Kit according to the manufacturer's protocol. Sequences have been submitted to Genbank under the following accession numbers: KF802810-KF802815.

5.4 Results

The PCR-SSCP provided unique banding patterns for the four species included in the study (Fig. 5.2), allowing species identification by direct observation and scoring of the SSCP gel.

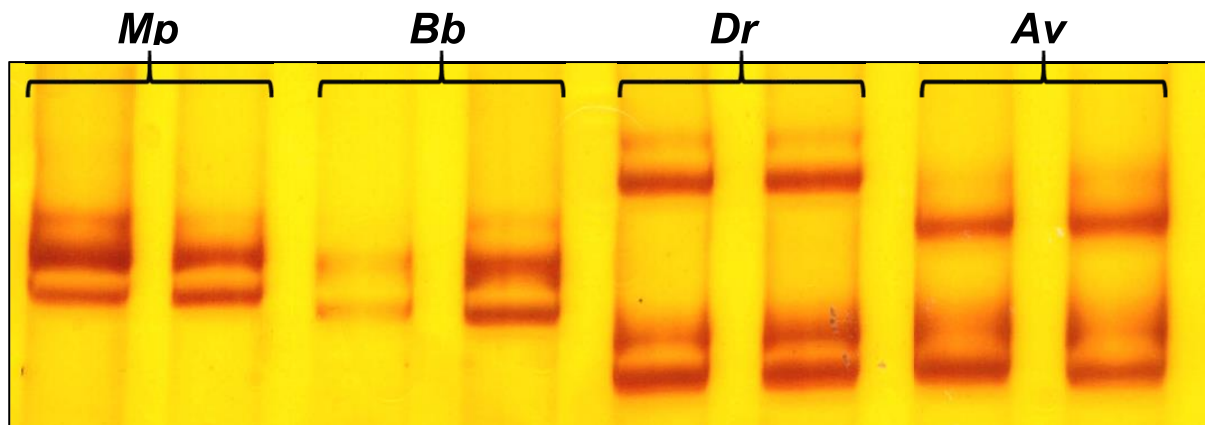


Figure 5.2 Banding patterns obtained after PCR-SSCP for the four species included in this study. *Mp*: *Myzus persicae*, *Bb*: *Brevicoryne brassicae*, *Dr*: *Diaeretiella rapae*, *Av*: *Asaphes vulgaris*. Banding patterns do not overlap, allowing for species identification.

The success of DNA amplification from *M. persicae* mummies varied with the type of mummy analysed (Pearson's Chi-squared test, $\chi^2 = 11.58$, $df = 4$, $p = 0.021$). Fresh material (full mummies, FM-type) always yielded successful amplification, whereas old mummies (OM-type) had a markedly lower success rate of about 0.66 (8/12). In contrast, there was no significant difference in amplification success between the different type of mummies for *B. brassicae* ($\chi^2 = 4.39$, $df = 3$, $p = 0.22$), with success rate averaging 0.83 (10/12).

A total of 17 samples out of 108 (0.16) which were positive after DNA amplification could not be identified after SSCP electrophoresis because no band was visible on the SSCP gel, so these have been qualified as "unidentified after performing PCR-SSCP". Those samples were mostly *B. brassicae* YHM (6/17) and *M. persicae* OM (5/17). In contrast, 6 samples out of 108 (0.06) for which no DNA amplification was detectable on post-PCR agarose gel electrophoresis actually displayed parasitoid DNA on SSCP gel electrophoresis.

Table 5.1 DNA identification success

Mummy type	Aphid species		Total
	<i>B. brassicae</i>	<i>M. persicae</i>	
<i>Parasitised mummies (D. rapae)</i>			
Before emergence – FM	12/12	12/12	24/24
7 days after emergence – YM	10/12	8/12	18/24
21 days after emergence – OM	8/12	5/12	13/24
<i>Hyperparasitised mummies (A. vulgaris)</i>			
Before emergence – FHM	-	12/12	12/12
7 days after emergence – YHM	1/12	8/12	9/24
Total	31/48	45/60	108

For both aphid species there was a clear relationship between the mummy type and the success of DNA identification (*B. brassicae* mummies: $\chi^2 = 25.04$, $df = 3$, $p < 0.001$; *M. persicae* mummies, $\chi^2 = 16$, $df = 4$, $p = 0.003$). DNA was successfully identified for all mummies containing a parasitoid or a hyperparasitoid larva (FM and FHM, see Table 5.1). Success rate was slightly lower for empty mummies seven days after emergence (about 0.75 of YM, 0.38 of YHM) and lower still for empty mummies 21 days after emergence (0.54 of OM).

In most samples, only parasitoid DNA was detected (Fig. 5.3). When parasitoid and aphid DNA could be detected simultaneously, this occurred more often for *M. persicae* samples (11/60, 0.18) than for *B. brassicae* (2/48, 0.04) and mostly in intact mummies. Hyperparasitoid DNA was found only in one YHM *B. brassicae* mummy. It was also found in two FHM mummies of *M. persicae*, simultaneously with parasitoid DNA.

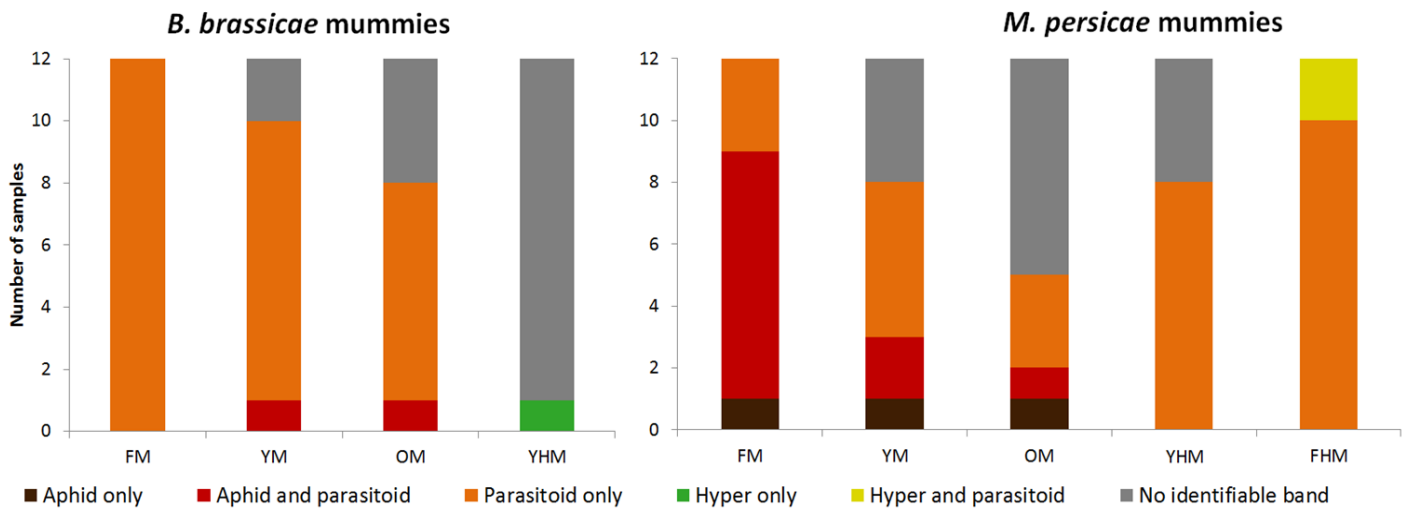


Figure 5.3 Identification of DNA amplified from different types of aphid mummies. Bars represent the different types of mummies analysed, coloured sections represent the types of DNA identified from each sample. Mummy types are coded as follows: FM: full mummies (before emergence); YM: young mummies (7 days after parasitoid emergence); OM: old mummies (21 days after parasitoid emergence); YHM: young hyperparasitised mummies (7 days after hyperparasitoid emergence); FHM: full hyperparasitised mummies (parasitised mummies exposed to parasitoids).

5.5 Discussion

DNA left inside aphid mummies was successfully amplified by PCR and the species identified by SSCP electrophoresis. However, it is only occasionally that more than one type of DNA was detected simultaneously in the same mummy (16/108, 0.14). Parasitoid DNA was found in most of the samples, even in hyperparasitised mummies. Amplification and identification of parasitoids were very successful for mummies still containing hymenopteran larvae and to a lesser extent for empty mummies (i.e. after emergence). Parasitoid DNA persisted at least 7 days after hyperparasitoid emergence which suggests that hyperparasitoids do not fully digest parasitoid tissues, probably leaving undigested remnants such as parasitoid exuviae and/or faeces (meconium). Though rarely, *A. vulgaris* DNA was detected in this study (3/36, 0.08). To our knowledge, the detection of hyperparasitoid DNA within empty aphid mummies has never been reported, and our results illustrate the possibility of using generalist primers to circumvent the difficulties of designing a specific primer (as is the case for *A. vulgaris*; see Traugott et al., 2008).

5.5.1 Lack of simultaneous amplification

Although it was expected that mummies would contain DNA from aphid, parasitoid and hyperparasitoid, hyperparasitoid and aphid DNA have been seldom detected. Simultaneous

detection of any species was rare in older mummies of *M. persicae* and almost nonexistent in any of the *B. brassicae* mummies. This simultaneous detection and identification is required to attempt food web construction; therefore the method proposed here needs further development before being used for field-collected mummies. In particular, the lack of multiple species detection is likely to under-estimate hyperparasitism because most of the DNA retrieved from hyperparasitised mummies was parasitoid DNA. Potential explanations for the low detection of aphid and hyperparasitoid DNA include: i) poor quality or very low quantity of aphid and hyperparasitoid DNA, ii) preferential amplification of parasitoid DNA.

Although *A. vulgaris* also expels a meconium before pupation (Haviland, 1922), the quantity of DNA left after emergence is likely to be limited due to the small size of this insect. Additionally, DNA in hyperparasitised mummies may undergo higher levels of degradation than in parasitised mummies. Indeed, the former are inherently older than parasitised ones, since the period of hyperparasitoid development (at 24°C) in the mummy is about three times longer than that of simply parasitised mummies (about 15-20 days compared to 5-6 days for parasitoids, pers. obs.). This degradation time is likely to be particularly detrimental to aphid DNA preservation, which undergoes the added actions of natural decay and parasitoid digestion. Aphid species can often be identified by morphological characters of the collected mummy (Höller et al., 1993; Müller et al., 1999). Morphology can thus compensate the failure of aphid DNA amplification. In field conditions, it is also likely that mummies will be encountered along with live aphid specimens which can be easily identified.

PCR amplification of mixed DNA samples can produce biased PCR products where the most abundant DNA in the sample dominates the PCR process (Deagle and Tollit, 2007) and masks the presence of other species. This phenomenon, referred to as preferential amplification, could explain the lack of simultaneous amplification in the current work. It would suggest that parasitoid DNA is present in higher quantities than that of other species in the mummy, which can explain parasitoid dominance over hyperparasitoid, given their respective body sizes (*A. vulgaris* is approximately half the size of *D. rapae*). Furthermore, DNA polymerase may have a higher binding affinity for parasitoid DNA than for hyperparasitoid DNA. Preferential amplification could be avoided by using threalose and betaine, plus additional NaCl in the PCR (Weissensteiner and Lanchbury, 1996). Chiang et al. (1998) observed that stepdown PCR can avoid preferential amplification of certain alleles over others, and this could be considered in further refinements of the proposed PCR-SSCP method. The use of blocking primers could be an option to mitigate preferential parasitoid DNA amplification. However, blocking primer design would have to be targeted to a certain species, so the method as proposed would lose the advantage of being applicable to any set of species. Lastly, another option would be to use more than one pair of generalist primers, which could avoid preferential amplification because of different

primers' affinities towards species DNA. However, to our knowledge no data exist on primer affinities towards the species studied here.

Simultaneous amplification of aphid and parasitoid DNA most readily occurred with intact *M. persicae* mummies, compared to *B. brassicae* mummies. This could be attributed to differential host exploitation by the parasitoid or differences in aphid species physiology leading to a differential preservation of DNA in host remnants. This suggests that DNA amplification partly depends on the aphid species involved, which could introduce a bias in food web studies. In the context of diet analysis, Deagle *et al.* (2013) observed that DNA from different prey species can be differentially degraded through digestion, and may therefore display different amplification success. A better understanding of DNA persistence after digestion would be required to assess the accuracy of the method we propose.

5.5.2 Potential for aphid food web analysis from field collections

Aphid mummies typically are papery and brown or black in colour and can be easily distinguished from living aphids in the same colony. They can be easily collected from the field whether they are intact (i.e. a parasitoid larva or pupa is developing in the dead insect) or when there is an obvious emergence hole in the mummy. The identification of DNA in mummies three weeks after emergence is compatible with one-off field collections where the age of the mummies is unknown. Due to predation (Brodeur and Rosenheim, 2000), wind, rain and other environmental factors, it is likely that empty mummies older than three weeks will have disappeared from the plant. Thus, most of the mummies available for collection in a field are likely to be younger than three weeks old and should therefore contain some informative DNA that would permit the identification of one or more members of a parasitic trophic interaction. Results from the current experiment must however be considered with caution since mummies were kept under controlled conditions and it is likely that field conditions would have accelerated the degradation of DNA.

Despite the limitations mentioned above, our method had a success rate comparable to that of Hrcek *et al.* (2011) who reported a 50% DNA amplification and identification from Lepidoptera host remnants analysed after parasitoid rearing and emergence. In that case, host remnants were mostly pieces of caterpillar epidermis or head. Our success rate is also higher than the 25% success of the approach explored by Rougerie *et al.* (2011) to detect host (Lepidoptera) DNA from the gut of recently emerged parasitoids. Identification by SSCP electrophoresis does not require systematic sequencing of all samples and therefore would be cost-effective compared to previous works, (Derocles *et al.*, 2012; Traugott *et al.*, 2008). Thus, PCR-SSCP would allow the testing of a large number of samples for a limited cost, since species identification can be performed directly by reading gel banding patterns.

Such economies of scale are of great value for the construction and comparison of quantitative food webs, which usually require large sample sizes.

Unlike other post-PCR methods such as high resolution melting (Šimenc and Potočnik, 2011), PCR-SSCP allows for the detection and sequencing of unexpected species whether they have been barcoded before or not. The latter could include native species new to science (as found by Hrcek et al., 2011) as well as newly-introduced species. The method investigated here would therefore be relevant not only to biological control but also to biological conservation and biosecurity.

In our study PCR-SSCP was used to segregate four species, but the potential for it to separate a vast number of species is still to be investigated. In a very diverse system, many more species may need to be distinguished, leading to a higher risk of having cryptic bands, i.e. two species yielding the same banding pattern even though sequences are different, a phenomenon also referred to as comigration of amplicons (Schwieger and Tebbe, 1998). This potential drawback of the method cannot be predicted with existing knowledge, and will require empirical proofing. However, the method presented here yields at least two bands per species, therefore two species yielding the same banding pattern is unlikely. PCR-SSCP should be able to separate many species, provided that the amplicons differ by at least one base pair (89% separation probability, see Hayashi, 1991) and that the gel is long enough for the species to be separated by migration (Lee et al., 1996; Schwieger and Tebbe, 1998). Lee et al. (1996) and Schweiger & Tebbe (1998) used PCR-SSCP to characterize bacterial community composition. Their analysis of environmental samples displayed enough distinct bands to separate 25 taxonomic entities. Among existing field studies of aphid-parasitoid-hyperparasitoid food webs, molecular approaches have included about 10-15 species (Garipey and Messing, 2012; Traugott et al., 2008), whereas morphological methods generally have included 20-30 species (Gagic et al., 2012; Höller et al., 1993; see also the noticeably extensive study by Müller et al., 1999 with 71 species). In highly diverse systems, a triage of samples prior to PCR-SSCP at the aphid level (based on mummy morphology), or at the plant level (host-plant from which samples are collected) can limit the number of species to separate per analysis. Assessing the resolution power of PCR-SSCP is essential before its application to field settings, especially if it is to be employed in ecosystems of high species richness.

5.6 Conclusion

Understanding invertebrate parasitoid food web assemblages and functional links can help understand successes and failures in biological control as well as detect and interpret new invasions of pests or beneficial insects. However, to date most such analyses have involved the substantial

collection of potential and actual hosts of parasitoids, technically difficult rearing and morphological identification of parasitoid and hyperparasitoid adults. The present work examines the prospects for overcoming these problems by evaluating the potential of DNA detection in intact aphid mummies or in those from which parasitoids or hyperparasitoids have emerged. Results based on laboratory cultures of aphids (two species), parasitoids (1 species) and hyperparasitoids (1 species) indicate that the DNA can be detected in aphid mummies up to 21 days after parasitic wasp emergence. However, the lack of simultaneous detection of species from one single mummy indicates the need for further research to technically improve the method before its application to field collected samples. Further areas of research include mitigation of preferential amplification, species-specific bias in DNA preservation and amplification, and the potential of the proposed PCR-SSCP approach to separate a large number of species.

Chapter 6 General Discussion

Ecosystems offer services that can benefit mankind and its activities, especially agriculture. Harnessing and enhancing ecosystem services (ES) is an appealing development for a more sustainable and productive agriculture. This could be based on thorough knowledge of ecological processes in agroecosystems. The addition of flowering vegetation in or around fields is a key strategy to provide natural enemies of pests with resources generally lacking in ‘conventional’ monocultural agricultural landscapes. This thesis examined the case of oilseed rape (OSR), an arable crop whose major pests in New Zealand are the three aphid species *Brevicoryne brassicae* (L.), *Myzus persicae* (Sulzer), and *Lipaphis erysimi* Kalténbach. These are currently managed by prophylactic and repeated insecticide applications. This thesis investigated the potential addition of flowering vegetation in OSR crops, targeted at enhancing the biological control of aphids by their parasitoid *Diaeretiella rapae* (McIntosh). Previously undocumented aspects of this parasitoid’s nutrition, behaviour, and interaction with pollinators are reported in this thesis, and a molecular tool to study food webs has been developed. This chapter summarises the main findings, and discusses their impact on the future of floral subsidies ecology and practice. Based on this work, further agronomical and fundamental research points are suggested.

6.1 *D. rapae* nutrition and foraging

6.1.1 Longevity trials

D. rapae can live up to three times longer when fed on nectar than when starved, depending on the type of nectar (Chapter 4). Buckwheat and OSR cv. Ability provided the most ‘nutritious’ nectar, i.e. the nectar that enhanced longevity the most. 3-fold longevity increases in *D. rapae* had been previously reported when it was fed *Vicia faba* extra-floral nectar (Jamont et al., 2013) and buckwheat nectar (Araj and Wratten, 2015). In contrast, wasp longevity was increased only 2-fold when fed *Myzus persicae* honeydew, or nectar in alyssum or coriander inflorescences. Phacelia nectar enhanced *D. rapae* longevity only when provided as nectar droplets, but not when it was inside intact flowers, indicating that floral morphology impeded the parasitoid’s reaching the nectar (as hypothesised in Vattala et al., 2006). Wasp longevity differed between two different cultivars of OSR. This could be due to the genetics of the two cultivars and their growing conditions (cv. Flash required vernalisation to initiate flowering, whereas cv. Ability did not). Similarly, nectars from various alyssum cultivars were observed to differ in their impact on parasitoid (*Trichogramma carverae* Oatman and Pinto) fitness, due to their different petal colours (Begum et al., 2004). This

indicates that attention should be paid to cultivar type and growing conditions when plants are being assessed in trials aiming at selecting nectar-providing plants.

D. rapae lived ca. 2 days when provided water only, ca. 6 days when fed on buckwheat nectar (Chapter 4), in contrast, some parasitoids such as *Diadegma semiclausum* (Helen) lived 2 days when provided water only, and 28 days when provided buckwheat nectar (Lavandero et al., 2006), or such as *Cotesia glomerata* (L.) that lived ca. 2 days when provided water only, and ca. 17 days when fed on buckwheat nectar (Winkler et al., 2009b). Can nectar feeding enhance the longevity of *D. rapae* as much as that of these two other parasitoids? Jervis et al. (2008) suggest that parasitoids can be classified into four types of species with differing lifetime reproductive strategies and life history traits. Type 1 includes pro-ovigenic species, which possess their total egg load at emergence and do not mature additional eggs during adult life, and Type 2 includes weakly-synovigenic species which also emerge with an initial load of mature eggs but can mature additional ones. Type 3 and Type 4 include species which do not possess mature eggs at emergence, and mature a substantial part, or all, of their eggs during adult life. Typically, Type 1 and 2 have high fecundities, short lives and concentrate ovipositions at the beginning of their life, whereas Type 3 and 4 have low fecundities, long lives, and oviposit all along their life. *D. rapae* is weakly synovigenic and lays the majority of its eggs in the first four days of life (Jamont et al., 2013), and it lives a short time compared to other parasitoid species. This suggests that *D. rapae* is a Type 2 species. It is therefore possible that *D. rapae* longevity cannot be enhanced more than 3 times that of starved wasps. However, as most ovipositions are performed during the first four days of *D. rapae* adult life, enhancing lifespan more than four days may not significantly enhance *D. rapae* impact on aphid populations.

A recent meta-analysis compared the effects of nectars from various plants on the longevity of parasitoids, quantifying effect size as the ratio of the longevity of nectar-fed wasps to that of wasps provided with water only. The two plants which effect on parasitoid longevity was strongest were cow parsley, *Anthriscus sylvestris* (L.) [Apiaceae], which had a 42% stronger effect on parasitoid longevity than buckwheat nectar, and bittercress, *Barbarea vulgaris* R. Br. [Brassicaceae], which had a 27% stronger effect than buckwheat nectar (Russell, 2015). However, these results have to be taken with caution, as the meta-analysis did not take into account the reproductive type (*sensu* Jervis et al., 2008) of parasitoids, thus the nectar from a plant species that was tested only on Type 3 and Type 4 parasitoids will be ranked among nectars having a strong effect on longevity, although this effect might be limited on for Type 1 or Type 2 parasitoids, which have typically shorter lifespans.

6.1.2 *D. rapae* 'foodscape'

OSR nectar and *M. persicae* honeydew can be fed upon by *D. rapae*, implying that this parasitoid could find food sources in its habitat, even if no floral subsidies have been planted. *M. persicae*

colonies can be present on OSR at all plant growth stages, at all times of the year (Desneux et al., 2006; Kant et al., 2012b). These aphids are generally located on the apical meristem or on the underside of leaves (personal observation on OSR; Kant et al., 2012b made similar observations on cabbage). Honeydew sources are probably rare in the early season, however they become more common subsequently, along with aphid density. In contrast with that of *M. persicae*, *Brevicoryne brassicae* honeydew did not significantly increase *D. rapae* longevity in laboratory trials (Jamont et al., 2013), suggesting that it cannot be exploited in the field. Parasitoids may find nectar in OSR flowers during crop flowering, and also in flowers of shepherd's purse (*Capsella bursa-pastoris* L.) or white rocket (*Diplotaxis eruroides* L.), the nectar of which were shown to enhance *D. rapae* longevity (Araj and Wratten, 2015). The latter plant is not reported in New Zealand (<http://www.nzflora.info>); however *C. bursa-pastoris* is naturalised there and may occasionally be present in field margins or as a weed within fields, especially when OSR plants do not form a dense canopy.

The above description gives a picture of *D. rapae*'s 'foodscape' (Fig 6.1). The concept of 'foodscape' has been used in mammal herbivore ecology (Searle et al., 2007) and is here defined as a delineation of what type of nutritive resources are available, their respective nutritional quality and their spatio-temporal distribution. Foodscapes can help understanding the nutritional quality of an agroecosystem for a given species. This specificity implies that a single agroecosystem might represent different foodscapes to different species, depending on their nutritional needs and their abilities to exploit food sources. For example, the foodscape of hoverflies (e.g. *Episyrphus balteatus* (De Geer)) should include pollen sources as well as nectar and honeydew sources (Laubertie et al., 2012; van Rijn et al., 2013), whereas the foodscape of *D. rapae* should not comprise pollen sources, as the wasp does not feed on pollen (as observed in a feeding trial by Tylianakis et al., 2004). Environmental conditions may also alter the foodscape, for example high temperatures and low humidity can reduce nectar production, and trigger water loss in honeydew droplets, decreasing the honeydew's nutritional value for parasitoids (Chapter 1).

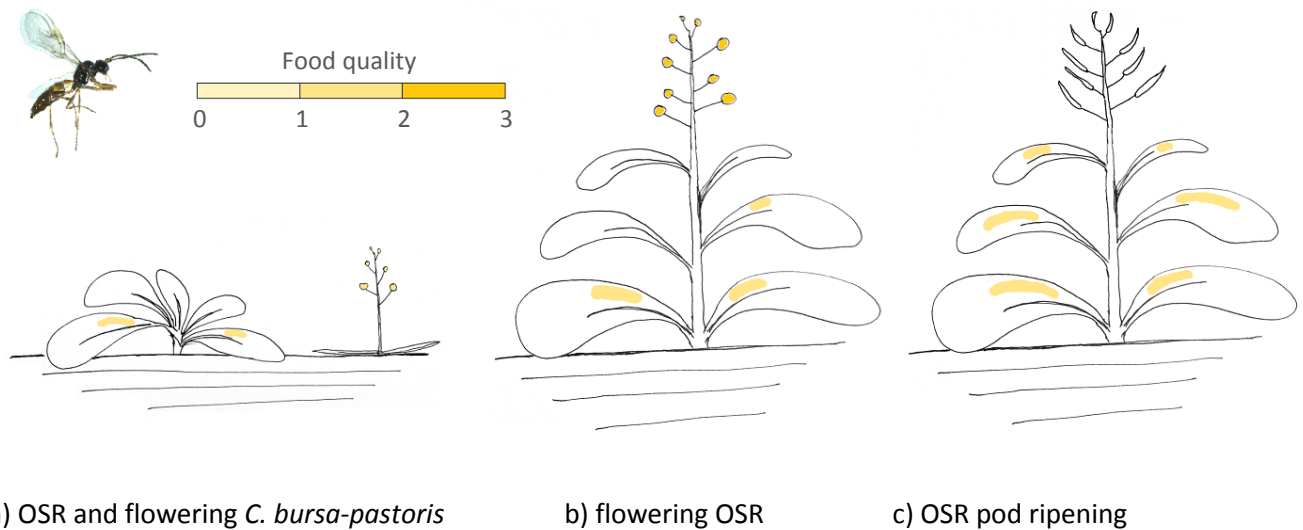


Figure 6.1. Theoretical 'foodscape' of *Diaeretiella rapae* in OSR fields in New Zealand, at three different crop growth stages. Potential foods include: *M. persicae* honeydew (on OSR leaves, accumulating under *M. persicae* colonies, a, b, c); *C. bursa-pastoris* nectar (in flowers, a); and OSR nectar (in OSR flowers, b). Foods are represented by a spot of colour according to their nutritional quality to *D. rapae*, which is derived from feeding trials in controlled conditions, and expressed as the ratio of the longevity of wasps fed on a given item to the longevity of starved wasps.

6.1.3 Search rate and reproductive output.

In addition to increasing the longevity and fecundity of *D. rapae*, feeding on buckwheat nectar also extended the proportion of time spent searching on leaves, and greatly decreased the time spent stationary, as revealed by 30-minute observations of individual parasitoids presented with ca. 40 aphids on a single OSR leaf (Chapter 2). The number of attacks per minute tended to be higher for fed than unfed wasps, but this effect was only marginally significant ($P = 0.08$). This lack of significant difference is probably linked to the aphid density on the observed leaf being high enough to allow host finding with low energy expenditure, which could have masked the difference of activity between wasps in high or low nutritional state.

In the field, aphids are distributed in patches, or colonies, which parasitoids search for and exploit. The optimal foraging theory predicts that parasitoids optimise the time spent in each patch to maximise their reproductive output (van Alphen et al., 2003). This theory is supported by studies showing that patch residence time can be affected by various parameters such as the perception of other patches, host density, number of hosts attacked, and various environmental conditions (Pierre, 2011; van Alphen et al., 2003), and also parasitoid's energy reserves, however this last parameter has

only rarely been included in empirical studies (van Alphen et al., 2003). Lucchetta et al. (2007) observed that *Venturia canescens* (Gravenhorst) (a parasitoid of various pyralid moths) starved for 48 hours spent less time in host patches than honey-fed conspecifics. Starved *D. rapae* spent more time stationary than well-fed wasps (Chapter 2), which suggests that, under starvation, they would remain for longer time in a given aphid colony, not performing any activity, in contrast with Lucchetta's observations.

Ayal (1987) observed that after alighting on a Brussels sprout plant, female *D. rapae* tended to walk up the stem, stopping at leaf-nodes and walking up petioles to explore leaves, and eventually reached the top of the plant which was checked intensively for hosts, before the parasitoid flew off. Because nectar-fed *D. rapae* spent more time in rapid walk than did starved individuals, they might be faster in their journey from leaf to leaf. As feeding on honey solutions increased the propensity to initiate flights in other parasitoids (Siekmann et al., 2004; Takasu and Lewis, 1995), *D. rapae* could be expected to show a higher tendency to leave plants, and perhaps visit more plants.

6.2 Management of interacting ES: pollination and biocontrol

Pollinators may compete with parasitoids as both guilds exploit floral nectar. Competition may be exploitative (i.e. parasitoids cannot consume nectar because it is entirely consumed by pollinators) or by interference (i.e. pollinators deter parasitoids from feeding on floral nectar, despite nectar being present in flower), or both. This question has not, to date, received experimental support; however it is crucial because a negative interaction could minimise or negate the benefits targeted by the addition of floral subsidies. The field experiment presented in Chapter 3 investigated the dynamics of nectar availability on flowers of buckwheat and OSR, either visited by pollinators or not. Pollinators' visits were managed by using either (i) exclusion cages that prevented pollinators' ingress, or (ii) cages of similar design that established the same micro-climate as in the exclusion cages, but were designed to allow pollinators to enter and exit the cage freely (non-exclusion cages). In non-exclusion plots, there were approximately five times more large flower-visitors and approximately three times less nectar than in exclusion plots. However, the mean parasitism rate and aphid numbers were not significantly different, and parasitism rate was not correlated to the amount of available sugar per plot. The low number of replicates (n=7 per treatment) may have been insufficient to compensate for the biological variability in nectar production and pollinators visits.

This experiment is therefore rather in-conclusive as to whether pollinators impact parasitism rate. However it indicates that if pollinators had any impact, this was neither very strong nor immediate, which, in applied terms, could be beneficial. This adds experimental support to the claim that floral

subsidies can enhance biological control and pollination at the same time (Wratten et al., 2012). Perhaps resource consumption by pollinators does not negate the effect of floral subsidies on natural enemies, but restricts its full potential. The combination of plant species highly attractive to pollinators, spatially separated from plant species selectively attractive to natural enemies (but less so to pollinators) might separate resources and avoid conflicting consumption. For example, large flower-visitors of the genus *Apis* and *Eristalis* have been observed to displace predatory hoverflies from coriander to other flowering plants (phacelia, alyssum, buckwheat), which were located in nearby plots (Ambrosino et al., 2006).

6.3 Construction of food webs to inform the deployment of companion plants and to maximise the delivery of ES

In order to construct a food web including aphids, parasitoids and hyperparasitoids on OSR, a molecular method has been developed and presented in Chapter 5. A precise knowledge of the hyperparasitic fauna is particularly relevant to habitat management, because floral subsidies can also benefit hyperparasitoids, whose longevity (Araj and Wratten, 2013; Araj et al., 2006), fecundity (Araj et al., 2009) and search rate (Araj et al., 2011) can be enhanced by feeding on nectar. Furthermore, the food web of OSR-associated insects may be quite different in New Zealand compared to Europe, where it has been cultivated for more than five centuries (Bunting, 1985). The presence of two *Alloxysta* spp. endemic to New Zealand (Ferrer-Suay et al., 2012) leaves room for a parasitoid-hyperparasitoid association that does not exist elsewhere; however, it is not known whether these *Alloxysta* spp. can be found in agricultural landscapes. These hyperparasitoid species might prevent *D. rapae* from reaching high population densities, thus impeding biological control from achieving its full pest-suppressive potential in New Zealand.

The PCR-SSCP method developed in this thesis was successful in amplifying aphid, parasitoid and hyperparasitoid DNA left in aphid mummies. The main pitfall was that hyperparasitoid DNA was sometimes not retrieved from mummies in which a hyperparasitoid had pupated and had emerged. The excretory activities of parasitoid and hyperparasitoid larvae prior to emergence (in particular, the production of a meconium, see Haviland, 1922) suggest that DNA of these organisms should persist in aphid mummies, and that the inconsistent amplification of hyperparasitoid DNA may not be due to an absence of DNA, but rather to the method lacking the sensitivity to detect DNA below a certain threshold. Preferential amplification of parasitoid DNA over hyperparasitoid DNA may occur during the initial PCR step, possibly due to relatively more parasitoid than hyperparasitoid DNA being present in mummies, or to a higher affinity of PCR enzymes toward parasitoid DNA (Weissensteiner and Lanchbury, 1996). This would leave hyperparasitoid DNA poorly amplified and consequently not

detectable on SSCP gels. This could be solved by some methodological adaptations avoiding preferential amplification (see discussion in Chapter 5), or by using high-throughput sequencing (next-generation sequencing). In the PCR-SSCP method, each species will produce a specific set of bands, therefore, once the observed bands have been identified (by re-amplification and sequencing or by comparison with control bands of known species), subsequent species identification can be done by reading the SSCP banding pattern, thereby allowing for a rapid and low-cost sample processing.

6.4 Key research needs for pest control innovations in OSR

6.4.1 Target phase

OSR is an annual crop, and in most systems, the first action is the preparation of the sowing bed, which clears all vegetation from the ground, leaving no habitat for insects. Thus, each year pest and natural enemies are initially absent from OSR fields. Typically, crop colonisation is started by relatively few insects immigrating from outside sources, which multiply gradually until they reach an epidemic growth phase (Wiedenmann and Smith, 1997). For example, in Iranian OSR crops, populations of the aphid *B. brassicae* showed a latent phase from autumn to early spring (October to March) and peaked in spring (April) (Nematollahi et al., 2014). In the same study, aphid population growth was affected by *D. rapae* only 2-3 weeks after it started increasing in spring, and this lag in parasitoid numerical response may have limited biocontrol. Several authors attribute the lack of control of *B. brassicae* by *D. rapae* to a delay in parasitoid response to aphid population increase (discussed by Kant et al., 2012b; and Nematollahi et al., 2014). Similarly, in citrus orchards infested by aphids, mummies only appear after the aphid population had reached high levels, and parasitism did not show a major impact on aphid populations later on (Kavallieratos et al., 2002). Mortality inflicted to aphid populations in the early phase of population growth can delay the onset of epidemic growth and have a major suppressive impact in subsequent pest population growth (Legrand et al., 2004; Wiedenmann and Smith, 1997). Further field experiments should therefore not only aim at enhancing *D. rapae* parasitism rate per se, but rather at enhancing parasitism rates during the latent phase of pest population increase.

A strategy preventing pest outbreaks may be supported by providing floral nectar as early as possible after OSR emergence. Because aphid densities during that phase are likely to be low, it is the moment when parasitoids are most likely to benefit from an enhanced searching behaviour, as caused by nectar feeding (Chapter 2). Moreover, the early stages of OSR growth are probably the moment at which food sources for parasitoids are the scarcest, as *M. persicae* edible honeydew

patches would be at low densities, and only few weeds would have flowered (see Section 6.1.2). Therefore it is at that time that floral subsidies are likely to be the most needed.

6.4.2 Floral intercropping in OSR

Floral subsidies can be planted either as a floral strips around fields, within-field floral strips, or intercropped, i.e. as single flowering plants sown uniformly across the field, mixed with crop plants. Providing floral resources in field margins implies that natural enemies visit the flowers and disperse within the field, to reach pest population within the range of their dispersal. Parasitism of the aphid *Metopolophium dirhodum* Walker by *Aphidius rhopalosiphi* De Stefani-Perez was increased up to 10m away from a patch (5x5m) of flowering buckwheat (Tylianakis et al., 2004); and as the body size of *D. rapae* is close to that of *A. rhopalosiphi* (ca. 2-3mm), parasitism by *D. rapae* can be expected to be enhanced up to 10m from a nectar-rich habitat. Given that the area of arable crop fields in Canterbury typically covers several hectares, providing floral resources only on field margins would have, at the field scale, a minor effect.

Sowing within-crop flowering strips would require an agricultural operation in supplement to crop sowing. The additional labour potentially involved, together with the loss of productive area, may hinder the adoption of this solution by farmers, unless its benefits are proved to balance the extra costs involved with its implementation. In contrast, the intercropping of OSR with a floral subsidy may fit simultaneously the ecological conditions of successful biocontrol enhancement, and the agronomical constraints of conventional systems, because the intercropped plant could be sown at the same time as OSR. To comply with other agronomical constraints, careful selection of a flowering plant must ensure that the chosen species neither competes with OSR for light and nutrients, nor produces seeds that contaminate harvest or become a weed in subsequent years. Interplanting trials could be performed (as in Balmer et al., 2014) to test whether OSR is sensitive to competition at the rosette stage. After stem elongation stage, OSR forms a tall and dense canopy, absorbing incident light and limiting full growth of plants underneath, which makes it a strong competitor. Current harvesting techniques cut OSR plants to ca. 30cm from the ground; therefore any plant that is shorter than 30cm should not contaminate harvest with its seeds. Finally, it would be necessary to test the potential for any intercropped plant to become a weed, by testing whether its seeds can persist in soils, and germinate in years following cultivation.

To facilitate parasitism of aphids as early as possible in the growing season, (see Section 6.4.1), the intercropped plant should be sown at the same time as OSR, and should flower as soon as possible. In the case of autumn-sowings, the intercropped plant should produce nectar during autumn and, ideally, be frost-tolerant and flower in early spring also. Parasitoids can indeed be active in autumn, e.g. sentinel aphids were parasitised by *D. rapae* in late October in France (Desneux et al., 2006).

With intercropping, the potential nectar source is brought close to aphids, which maximises the chances of parasitoids detecting it, and minimises the cost of travel between hosts and food. No data are available on *D. rapae* movement in the field, and on distances this insect may travel to reach nectar sources; however such information would be crucial in choosing a sowing density for the potential flowering plant. As discussed above, a buckwheat patch enhanced aphid (*M. dirhodum*) parasitism up to 10m around, suggesting that the parasitoid (*A. rhopalosiphi*) dispersed 10m away from the flowers after feeding on nectar. If this was also the case for *D. rapae*, flowering plants sown on a regular pattern at a density of 100 plants per hectare might enhance aphid parasitism.

6.4.3 Candidates

Findings from this thesis point to buckwheat being a strong candidate for use as flower subsidy in OSR crops. Buckwheat flowered in 5-6 weeks after sowing in Canterbury conditions, more than two weeks ahead of spring OSR (Chapter 3) and enhanced *D. rapae* longevity ca. 3-fold (Chapter 4). Buckwheat nectar also enhanced the longevity of the pests *Plutella xylostella* and *Pieris rapae*, (Lepidoptera which are able to feed on nectar in the adult stage) and the longevity of their parasitoids, but the longevity-enhancing effect was stronger on parasitoids than on pests (a 230% and 180% increase in longevity, respectively), thus the outcome of nectar provision on the control of this species can be expected to be positive overall (Winkler et al., 2009b). However, buckwheat nectar increased 10-fold the longevity of the hyperparasitoid *Alloxysta victrix* (Araj and Wratten, 2013), which attacks *D. rapae* (personal observation). This may affect *D. rapae* populations; however, hyperparasitoids are typically most abundant late in the season (Nematollahi et al., 2014), and thus may not impact *D. rapae* during the early phases of crop growth. Buckwheat is frost-sensitive, and therefore might be used only on spring OSR. Buckwheat can grow to about 50cm tall (personal observation), and therefore may require harvesting material to be fitted to cut higher than usual to avoid harvest contamination. The size of buckwheat seeds (tetrahedral, approx. 7mm) might impede its simultaneous sowing with OSR seeds (spherical, approx. 3mm diameter) depending on the machinery used for sowing.

Of the plants tested, alyssum could be the more agronomically acceptable as an interplanted floral subsidy because of its small size preventing harvest contamination (10-20 cm) and the small size of its seeds (spherical, ca. 1mm) allowing its simultaneous sowing with OSR. Alyssum plants were able to survive the 2013 winter conditions of Canterbury, NZ (personal observation), but not those of the Netherlands (Geiger et al., 2005), indicating it is sensitive to frost to some degree. However its nectar nutritional value is somehow lower than that of buckwheat: it increased the longevity of *D. rapae* twofold, and the longevity of *Alloxysta victrix* 4.5-fold (Araj and Wratten, 2013). It enhanced neither *P. xylostella* longevity nor that of its parasitoid *D. semiclausum* (Lavandero et al., 2006). Also, time

from sowing to flowering is estimated to be 7 weeks (Kelly McCone, horticulturalist, personal communication), which is about that of OSR, meaning that alyssum may only flower at the same time than OSR if both plants are sown simultaneously. Field sowing trials (as in Bowie et al., 1995) should be performed to confirm this point.

As discussed in Section 6.1.1, nectar from *Anthriscus sylvestris* (L.) or *Barbarea vulgaris* R. Br. might enhance *D. rapae* longevity more than, or as much as, buckwheat. These plants are fully naturalised in New Zealand, and could be considered in further habitat management studies. Moreover, *B. vulgaris* can be used as a trap crop for *P. xylostella*, as it is an attractive oviposition site that inhibits the feeding of diamondback-moth larvae after eclosion (Shelton and Nault, 2004). This plant is biennial, and therefore will only flower in the second year of growth, for example *B. vulgaris* could be sown simultaneously with autumn-OSR and may flower early in the following spring.

Beyond conventional systems, floral subsidies could be implemented in more audacious designs, such as cropping systems with perennial shelterbelts (Littlejohn et al., 2014; Porter et al., 2009). Perennial shelterbelts could be interplanted with various species that would provide a constant source of nectar. Floral subsidies can also be used concomitantly with various other agroecological techniques, such as delayed sowing date, resistant cultivars, or selective pesticide products.

6.5 The un-answered Food vs. Hosts dilemma in aphid parasitoids

As exposed in Chapter 1, the attraction of starved parasitoids towards food (nectar sources, floral cues) instead of hosts has been demonstrated empirically only for parasitoids whose hosts do not produce honeydew (Kugimiya et al., 2010; Takasu and Lewis, 1995; Wäckers and van Rijn, 2012; Wäckers, 1994). In contrast, parasitoids whose hosts produce edible honeydew will find hosts and food in close vicinity. Therefore, the presence of their aphids generally ensures the presence of some food, and by visiting only host patches, parasitoids may avoid the potential costs (metabolic cost of flight, predation or other risks) associated with nectar foraging. As a consequence, it is hypothesised that aphid parasitoids may not be attracted to nectar sources if host patches are present; however this has not been tested (F. Wäckers, personal communication in 2014). This may imply that, in the field, aphid parasitoids may not use the floral subsidies that would be provided by habitat management. The observation of Aphidiinae feeding on flowers (Jervis, 1998; personal observation of *D. rapae* landing on buckwheat flowers in field conditions) suggest that parasitoid visit flowers and consume nectar, but it is not known whether these floral visits are the result of an occasional encounter or whether nectar sources are actively searched for. The examples of enhanced aphid parasitism rates near flowering vegetation (Araj et al., 2009; Ponti et al., 2007) support this idea, but

could also be due to confounding factors (e.g. parasitoid finding shelter and accumulating near floral plantings). Choice tests in olfactometers (Wäckers, 1994) or wind tunnel arenas (Siekmann et al., 2004) could test the preference of well-fed and starved *D. rapae* for either nectar sources or host patches. The aphid host used in such tests may influence its outcome, as only *M. persicae* honeydew can be fed upon by *D. rapae*, whereas *B. brassicae* honeydew cannot (Jamont et al., 2013). Girling et al. (2006) observed that *D. rapae* responded to odours emitted by plants after their infestation by *M. persicae*, rather than honeydew- or aphid-emitted odours. Choice tests could also include parasitoids with different feeding and oviposition histories. Indeed, parasitoids are able to associate odours with food or host rewards (Tertuliano et al., 2004), thus odours associated with host presence (Girling et al., 2006) may be associated with food reward if feeding occurs, and alter subsequent odour preferences. Parasitoids may show different responses to odour blends comprising different concentrations of herbivore-induced plant volatiles and floral scents. Such information on parasitoid perception and choice is crucial in predicting how attractive floral nectar can be to aphid parasitoids, and may further help deploying floral subsidies.

Conclusion

The present thesis has shown that feeding on buckwheat and OSR nectar enhanced the longevity of *D. rapae*, and feeding on buckwheat nectar also increased the time allocated to searching for aphids. This suggests that the longevity and search rate of *D. rapae*, and, consequently, the biocontrol of aphids could all three be enhanced if nectar was provided within crops. For the first time, the present thesis has also tested the potential competition between pollinators and parasitoids for nectar, and has shown that such competition, in OSR crops receiving floral subsidies, does not represent a major limitation of parasitism rate. This is encouraging for the deployment of floral subsidies in field conditions; however, the comprehension of this system, which should lead to practical guidelines for the enhancement of biocontrol, would be strengthened by the understanding of the structure and composition of food-webs associated with aphids, in particular with regard to the importance of hyperparasitoids.

References

- Alford, D. V., Nilsson, C., Ulber, B., 2003. Insect pests of oilseed rape crops, in: Alford, D. V (Ed.), *Biocontrol of Oilseed Rape Pests*. Blackwell Science, Oxford, pp. 9–42.
- Alhmedi, A., Haubruge, E., D'Hoedt, S., Francis, F., 2011. Quantitative food webs of herbivore and related beneficial community in non-crop and crop habitats. *Biol Control* 58, 103–112. doi:10.1016/j.biocontrol.2011.04.005
- Ali, M., Saeed, S., Sajjad, A., Whittington, A., 2011. In search of the best pollinators for canola (*Brassica napus* L.) production in Pakistan. *Appl Entomol Zool* 46, 353–361. doi:10.1007/s13355-011-0051-0
- Altieri, M.A., 1995. *Agroecology: the science of sustainable agriculture*, 2nd Editio. ed. Westview Press.
- Ambrosino, M.D., Luna, J.M., Jepson, P.C., Wratten, S.D., 2006. Relative frequencies of visits to selected insectary plants by predatory hoverflies (Diptera: Syrphidae), other beneficial insects, and herbivores. *Environ Entomol* 35, 394–400. doi:10.1603/0046-225X-35.2.394
- Anderson, J.C., Dubetz, C., Palace, V.P., 2015. Neonicotinoids in the Canadian aquatic environment: A literature review on current use products with a focus on fate, exposure, and biological effects. *Sci Total Environ* 505, 409–422. doi:10.1016/j.scitotenv.2014.09.090
- Andrew, R.L., Bernatchez, L., Bonin, A., Buerkle, C.A., Carstens, B.C., Emerson, B.C., Garant, D., Giraud, T., Kane, N.C., Rogers, S.M., Slate, J., Smith, H., Sork, V.L., Stone, G.N., Vines, T.H., Waits, L., Widmer, A., Rieseberg, L.H., 2013. A road map for molecular ecology. *Mol Ecol* 22, 2605–26. doi:10.1111/mec.12319
- Araj, S., Wratten, S.D., 2013. Floral resources effect on the longevity of the aphid parasitoid, *Aphidius ervi* Haliday (Hymenoptera : Braconidae) and its hyperparasitoid, *Alloxysta victrix* Westwood (Hymenoptera : Charipidae). *Jordan J Agric Sci* 9, 63–71.
- Araj, S., Wratten, S.D., 2015. Comparing existing weeds and commonly used insectary plants as floral resources for a parasitoid. *Biol Control* 81, 15–20. doi:10.1016/j.biocontrol.2014.11.003
- Araj, S.-E., Wratten, S., Lister, A., Buckley, H.L., 2008. Floral diversity, parasitoids and hyperparasitoids – A laboratory approach. *Basic Appl Ecol* 9, 588–597. doi:10.1016/j.baae.2007.08.001
- Araj, S.-E., Wratten, S., Lister, A., Buckley, H.L., 2009. Adding floral nectar resources to improve biological control: Potential pitfalls of the fourth trophic level. *Basic Appl Ecol* 10, 554–562. doi:10.1016/j.baae.2008.12.001
- Araj, S.-E., Wratten, S., Lister, A., Buckley, H.L., Ghabeish, I., 2011. Searching behavior of an aphid parasitoid and its hyperparasitoid with and without floral nectar. *Biol Control* 57, 79–84. doi:10.1016/j.biocontrol.2010.11.015
- Araj, S.-E., Wratten, S.D., Lister, A.J., Buckley, H.L., 2006. Floral nectar affects longevity of the aphid parasitoid *Aphidius ervi* and its hyperparasitoid *Dendrocerus aphidum*. *New Zeal Plant Prot* 59, 178–183.

- Ayal, Y., 1987. The foraging strategy of *Diaeretiella rapae*. *J Anim Ecol* 56, 1057–1068.
- Azzouz, H., Giordanengo, P., Wäckers, F.L., Kaiser, L., 2004. Effects of feeding frequency and sugar concentration on behavior and longevity of the adult aphid parasitoid: *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae). *Biol Control* 31, 445–452. doi:10.1016/j.biocontrol.2004.07.013
- Baker, H.G., 1977. Non-sugar chemical constituents of nectar. *Apidologie* 8, 349–356.
- Bale, J.S., van Lenteren, J.C., Bigler, F., 2008. Biological control and sustainable food production. *Philos Trans R Soc Lond B Biol Sci* 363, 761–76. doi:10.1098/rstb.2007.2182
- Balmer, O., Géneau, C.E., Belz, E., Weishaupt, B., Förderer, G., Moos, S., Ditner, N., Juric, I., Luka, H., 2014. Wildflower companion plants increase pest parasitism and yield in cabbage fields: experimental demonstration and call for caution. *Biol Control* 76, 19–27. doi:10.1016/j.biocontrol.2014.04.008
- Barbosa, P. (Ed.), 1998. *Conservation Biological Control*. Academic Press, San Diego, CA.
- Barrios, E., 2007. Soil biota, ecosystem services and land productivity. *Ecol Econ* 64, 269–285. doi:10.1016/j.ecolecon.2007.03.004
- Bartlett, B.R., 1962. The ingestion of dry sugars by adult entomophagous insects and the use of this feeding habit for measuring the moisture needs of parasites. *J Econ Entomol* 55, 749–753.
- Bass, C., Puinean, A.M., Zimmer, C.T., Denholm, I., Field, L.M., Foster, S.P., Gutbrod, O., Nauen, R., Slater, R., Williamson, M.S., 2014. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect Biochem Mol Biol* 51, 41–51. doi:10.1016/j.ibmb.2014.05.003
- Bassam, B.J., Gresshoff, P.M., 2007. Silver staining DNA in polyacrylamide gels. *Nat Protoc* 2, 2649–54. doi:10.1038/nprot.2007.330
- Begum, M., Gurr, G.M., Wratten, S.D., Nicol, H.I., 2004. Flower color affects tri-trophic-level biocontrol interactions. *Biol Control* 30, 584–590. doi:10.1016/j.biocontrol.2004.03.005
- Bender, R., Klinkenberg, P., Jiang, Z., Bauer, B., Karypis, G., Nguyen, N., Perera, M.A.D.N., Nikolau, B.J., Carter, C.J., 2012. Functional genomics of nectar production in the Brassicaceae. *Flora* 207, 491–496. doi:10.1016/j.flora.2012.06.005
- Bennett, E.M., Peterson, G.D., Gordon, L.J., 2009. Understanding relationships among multiple ecosystem services. *Ecol Lett* 12, 1394–404. doi:10.1111/j.1461-0248.2009.01387.x
- Bernal, J., González, D., 1993. Temperature requirements of four parasites of the Russian wheat aphid *Diuraphis noxia*. *Entomol Exp Appl* 69, 173–182.
- Bernal, J., González, D., 1997. Reproduction of *Diaeretiella rapae* on Russian wheat aphid hosts at different temperatures. *Entomol Exp Appl* 82, 159–166. doi:10.1023/A:1002929500786
- Bommarco, R., Kleijn, D., Potts, S.G., 2013. Ecological intensification: harnessing ecosystem services for food security. *Trends Ecol Evol* 28, 230–8. doi:10.1016/j.tree.2012.10.012
- Bommarco, R., Marini, L., Vaissière, B.E., 2012. Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia* 169, 1025–32. doi:10.1007/s00442-012-2271-6
- Bommarco, R., Miranda, F., Bylund, H., Björkman, C., 2011. Insecticides suppress natural enemies and

- increase pest damage in cabbage . J Econ Entomol 104, 782–791. doi:10.1603/EC10444
- Bowie, M.H., Gurr, G.M., Hossain, Z., Baggen, L.R., Frampton, C., 1999. Effects of distance from field edge on aphidophagous insects in a wheat crop and observations on trap design and placement. Int J Pest Manag 45, 69–73. doi:10.1080/096708799228076
- Bowie, M.H., Wratten, S.D., White, A.J., 1995. Agronomy and phenology of “companion plants” of potential for enhancement of insect biological control. New Zeal J Crop Hortic Sci 23, 423–427.
- Boyer, S., Brown, S.D.J., Collins, R. a, Cruickshank, R.H., Lefort, M.-C., Malumbres-Olarte, J., Wratten, S.D., 2012. Sliding window analyses for optimal selection of mini-barcodes, and application to 454-pyrosequencing for specimen identification from degraded DNA. PLoS One 7, e38215. doi:10.1371/journal.pone.0038215
- Boyer, S., Yeates, G.W., Wratten, S.D., Holyoake, A., Cruickshank, R.H., 2011. Molecular and morphological analyses of faeces to investigate the diet of earthworm predators: example of a carnivorous land snail endemic to New Zealand. Pedobiologia (Jena) 54, S153–S158. doi:10.1016/j.pedobi.2011.08.002
- Brodeur, J., Rosenheim, J.A., 2000. Intraguild interactions in aphid parasitoids. Entomol Exp Appl 97, 93–108. doi:10.1046/j.1570-7458.2000.00720.x
- Brown, S.D.J., Collins, R.A., Boyer, S., Lefort, M.-C., Malumbres-Olarte, J., Vink, C.J., Cruickshank, R.H., 2012. Spider: an R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. Mol Ecol Resour 12, 562–5. doi:10.1111/j.1755-0998.2011.03108.x
- Buntin, G.D., Raymerl, P.L., 1994. Pest status of aphids and other insects in winter canola in Georgia. J Econ Entomol 87, 1097–1104.
- Bunting, E.S., 1985. Oilseed rape in perspective, in: Scarisbrick, D.H., Daniels, R.W. (Eds.), Oilseed Rape. Collins, London, pp. 1–31.
- Carrié, R.J.G., George, D.R., Wäckers, F.L., 2012. Selection of floral resources to optimise conservation of agriculturally-functional insect groups. J Insect Conserv 16, 635–640. doi:10.1007/s10841-012-9508-x
- Carroll, A.B., Pallardy, S.G., Galen, C., 2001. Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). Am J Bot 88, 438–446.
- Carter, C., Thornburg, R.W., 2004. Is the nectar redox cycle a floral defense against microbial attack? Trends Plant Sci 9, 320–4. doi:10.1016/j.tplants.2004.05.008
- Casas, J., Pincebourde, S., Mandon, N., Vannier, F., Poujol, R., Giron, D., 2005. Lifetime nutrient dynamics reveal simultaneous capital and income breeding in a parasitoid. Ecology 86, 545–554.
- Cawoy, V., Kinet, J.L.J., 2009. Floral biology of common buckwheat (*Fagopyrum esculentum* Moench). Eur J Plant Sci Biotechnol 3, 1–9.
- Cawoy, V., Kinet, J.-M., Jacquemart, A.-L., 2008. Morphology of nectaries and biology of nectar production in the distylous species *Fagopyrum esculentum*. Ann Bot 102, 675–84. doi:10.1093/aob/mcn150

- Chagnon, M., Kreutzweiser, D., Mitchell, E.A.D., Morrissey, C.A., Noome, D.A., Van der Sluijs, J.P., 2014. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res Int.* doi:10.1007/s11356-014-3277-x
- Chiang, F., Hsu, K., Chen, W., Tseng, C., Tseng, Y., 1998. Determination of angiotensin-converting enzyme gene polymorphisms: stepdown PCR increases detection of heterozygotes. *Clin Chem* 44, 1353–1356.
- Close, R.C., Lamb, K.P., 1961. Trapping study of some winged aphid vectors of plant virus diseases in Canterbury, New Zealand. *New Zeal J Agric Res* 4, 606–618.
- Comba, L., Corbet, S.H., Hunt, L., Warren, B., 1999. Flowers, nectar and insect visits: evaluating British plant species for pollinator-friendly gardens. *Ann Bot* 83, 369–383.
- Conner, J.K., Davis, R., Rush, S., 1995. The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. *Oecologia* 104, 234–245.
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260. doi:10.1038/387253a0
- Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., Farber, S., Turner, R.K., 2014. Changes in the global value of ecosystem services. *Glob Environ Chang* 26, 152–158. doi:10.1016/j.gloenvcha.2014.04.002
- Cottier, W., 1953. *Aphids of New Zealand*. Departement of Scientific and Industrial Research, Wellington, New Zealand.
- Crowder, D.W., Harwood, J.D., 2014. Promoting biological control in a rapidly changing world. *Biol Control* 75, 1–7. doi:10.1016/j.biocontrol.2014.04.009
- Cullen, R., Warner, K.D., Jonsson, M., Wratten, S.D., 2008. Economics and adoption of conservation biological control. *Biol Control* 45, 272–280. doi:10.1016/j.biocontrol.2008.01.016
- Daily, G.C., 1997. *Nature's services: Societal dependance on natural ecosystems*. Island Press, Washigton, DC.
- Dale, V.H., Polasky, S., 2007. Measures of the effects of agricultural practices on ecosystem services. *Ecol Econ* 64, 286–296. doi:10.1016/j.ecolecon.2007.05.009
- Day, W.H., 1994. Estimating mortality caused by parasites and diseases of insects : comparisons of the dissection and rearing methods. *Environ Entomol* 23, 543–550.
- Deagle, B.E., Thomas, A.C., Shaffer, A.K., Trites, A.W., Jarman, S.N., 2013. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? *Mol Ecol Resour* 13, 620–33. doi:10.1111/1755-0998.12103
- Deagle, B.E., Tollit, D.J., 2007. Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition? *Conserv Genet* 8, 743–747. doi:10.1007/s10592-006-9197-7
- Decourtye, A., Mader, E., Desneux, N., 2010. Landscape enhancement of floral resources for honey bees in agro-ecosystems. *Apidologie* 41, 264–277.
- Derocles, S.A.P., Plantegenest, M., Simon, J.-C., Taberlet, P., Le Ralec, A., 2012. A universal method

for the detection and identification of Aphidiinae parasitoids within their aphid hosts. *Mol Ecol Resour* 12, 634–45. doi:10.1111/j.1755-0998.2012.03131.x

- Desneux, N., Rabasse, J.-M., Ballanger, Y., Kaiser, L., 2006. Parasitism of canola aphids in France in autumn. *J Pest Sci* (2004) 79, 95–102. doi:10.1007/s10340-006-0121-1
- Desneux, N., Stary, P., Delebecque, C.J., Garipey, T.D., Barta, R.J., Hoelmer, K.A., Heimpel, G.E., 2009. Cryptic species of parasitoids attacking the soybean aphid (Hemiptera: Aphididae) in Asia: *Binodoxys communis* and *Binodoxys koreanus* (Hymenoptera: Braconidae: Aphidiinae). *Ann Entomol Soc Am* 102, 925–936.
- Dunn, R.R., 2010. Global mapping of ecosystem disservices: the unspoken reality that nature sometimes kills us. *Biotropica* 42, 555–557. doi:10.1098/rspb.2010.0340.F
- Ehler, L.E., 1998. Conservation biological control: past, present, and future, in: Barbosa, P. (Ed.), *Conservation Biological Control*. Academic Press, San Diego, CA., pp. 1–8.
- Ehrlich, P.R., Mooney, H.A., 1983. Extinction, substitution, ecosystem services. *Bioscience* 33, 248–254.
- Eisentraut, A., 2010. Sustainable production of second-generation biofuels. Potential and perspectives in major economies and developing countries. OECD/International Energy Agency, Paris.
- Ellis, S.A., Oakley, J.N., Parker, W.E., Raw, K., 1999. The development of an action threshold for cabbage aphid (*Brevicoryne brassicae*) in oilseed rape in the UK. *Ann Appl Biol* 134, 153–162.
- Faria, C.A., Wäckers, F.L., Turlings, T.C.J., 2008. The nutritional value of aphid honeydew for non-aphid parasitoids. *Basic Appl Ecol* 9, 286–297. doi:10.1016/j.baae.2007.02.001
- Feltham, H., Park, K., Goulson, D., 2014. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* 23, 317–323. doi:10.1007/s10646-014-1189-7
- Ferrer-Suay, M., Paretas-Martínez, J., Selfa, J., Pujade-Villar, J., 2012. Charipinae fauna from New Zealand with descriptions of two new species of *Alloxysta* Förster (Hymenoptera: Cynipoidea: Figitidae: Charipinae). *Aust J Entomol* 51, 229–238. doi:10.1111/j.1440-6055.2012.00859.x
- Fiedler, A.K., Landis, D.A., Wratten, S.D., 2008. Maximizing ecosystem services from conservation biological control: The role of habitat management. *Biol Control* 45, 254–271. doi:10.1016/j.biocontrol.2007.12.009
- Fischer, J., Manning, A.D., Steffen, W., Rose, D.B., Daniell, K., Felton, A., Garnett, S., Gilna, B., Heinsohn, R., Lindenmayer, D.B., Macdonald, B., Mills, F., Newell, B., Reid, J., Robin, L., Sherren, K., Wade, A., 2007. Mind the sustainability gap. *Trends Ecol Evol* 22, 621–4. doi:10.1016/j.tree.2007.08.016
- Foley, J.A., Defries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. *Science* (80-) 309, 570–4. doi:10.1126/science.1111772
- Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D.,

- O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M., 2011. Solutions for a cultivated planet. *Nature* 478, 337–42. doi:10.1038/nature10452
- Folke, C., Jansson, Å., Rockström, J., Olsson, P., Carpenter, S.R., Chapin, F.S., Crépin, A.-S., Daily, G., Danell, K., Ebbesson, J., Elmqvist, T., Galaz, V., Moberg, F., Nilsson, M., Österblom, H., Ostrom, E., Persson, Å., Peterson, G., Polasky, S., Steffen, W., Walker, B., Westley, F., 2011. Reconnecting to the Biosphere. *Ambio* 40, 719–738. doi:10.1007/s13280-011-0184-y
- Gagic, V., Hänke, S., Thies, C., Scherber, C., Tomanović, Z., Tschamntke, T., 2012. Agricultural intensification and cereal aphid-parasitoid-hyperparasitoid food webs: network complexity, temporal variability and parasitism rates. *Oecologia* 170, 1099–109. doi:10.1007/s00442-012-2366-0
- Gariepy, T.D., Messing, R.H., 2012. Development and use of molecular diagnostic tools to determine trophic links and interspecific interactions in aphid–parasitoid communities in Hawaii. *Biol Control* 60, 26–38. doi:10.1016/j.biocontrol.2011.06.011
- Gasparatos, A., Stromberg, P., Takeuchi, K., 2011. Biofuels, ecosystem services and human wellbeing: Putting biofuels in the ecosystem services narrative. *Agric Ecosyst Environ* 142, 111–128. doi:10.1016/j.agee.2011.04.020
- Gavolski, J., 2014. Guide to integrated control of insect pests of crops - Insect management in oilseed crops [WWW Document]. West Comm Crop Pests. URL [http://www.westernforum.org/Documents/WCCP/WCCP_documents/WCCP_Guidelines/WCCP_15/Oilseeds-WCCP-2015 \(2\).pdf](http://www.westernforum.org/Documents/WCCP/WCCP_documents/WCCP_Guidelines/WCCP_15/Oilseeds-WCCP-2015 (2).pdf) (accessed 3.5.15).
- Geiger, F., Bianchi, F.J.J.A., Wäckers, F.L., 2005. Winter ecology of the cabbage aphid *Brevicoryne brassicae* (L.) (Hom., Aphididae) and its parasitoid *Diaeretiella rapae* (McIntosh) (Hym., Braconidae: Aphidiidae). *J Appl Entomol* 129, 563–566. doi:10.1111/j.1439-0418.2005.01002.563
- George, D.R., King, L., Donkin, E., Jones, C.E., Croft, P., Tilley, L.A.N., 2013. Dichotomy of male and female responses to hoverfly-driven cues and floral competition in the parasitoid wasp *Aphidius ervi* Haliday. *Biol Control* 67, 539–547. doi:10.1016/j.biocontrol.2013.08.013
- Gibbons, D., Morrissey, C., Mineau, P., 2014. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environ Sci Pollut Res* 1–16. doi:10.1007/s11356-014-3180-5
- Girling, R.D., Hassall, M., Turner, J.G., Poppy, G.M., 2006. Behavioural responses of the aphid parasitoid *Diaeretiella rapae* to volatiles from *Arabidopsis thaliana* induced by *Myzus persicae*. *Entomol Exp Appl* 120, 1–9. doi:10.1111/j.1570-7458.2006.00423.x
- Gontijo, L.M., Beers, E.H., Snyder, W.E., 2013. Flowers promote aphid suppression in apple orchards. *Biol Control* 66, 8–15. doi:10.1016/j.biocontrol.2013.03.007
- Goulson, D., Chapman, J.W., Hughes, W.O.H., 2001. Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. *J Insect Behav* 14, 669–678.
- Goulson, D., Stout, J.C., Langley, J., Hughes, W.O.H., 2000. Identity and function of scent marks deposited by foraging bumblebees. *J Chem Ecol* 26, 2897–2911.

- Groeneveld, J.H., Klein, A.-M., 2014. Pollination of two oil-producing plant species: *Camelina* (*Camelina sativa* L. Crantz) and pennycress (*Thlaspi arvense* L.) double-cropping in Germany. *GCB Bioenergy* 6, 242–251. doi:10.1111/gcbb.12122
- Gurr, G.M., Wratten, S.D. (Eds.), 2000. *Biological control: measures of success*. Kluwer Academic Publishers, Dordrecht, the Netherlands.
- Gurr, G.M., Wratten, S.D., Snyder, W.E. (Eds.), 2012. *Biodiversity and insect pests: key issues for sustainable management*. John Wiley & Sons.
- Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W., Hebert, P.D.N., 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proc Natl Acad Sci U S A* 103, 968–71. doi:10.1073/pnas.0510466103
- Haviland, M., 1922. On the post-embryonic development of certain chalcids, hyperparasites of Aphides. *Q J Microsc Sci* 66, 321–338.
- Hayashi, K., 1991. PCR-SSCP: a simple and sensitive method for detection of mutations in the genomic DNA. *Genome Res* 1, 34–38. doi:10.1101/gr.1.1.34
- Heil, M., 2011. Nectar: generation, regulation and ecological functions. *Trends Plant Sci* 16, 191–200. doi:10.1016/j.tplants.2011.01.003
- Heimpel, G.E., Jervis, M.A., 2005. Does floral nectar improve biological control by parasitoids?, in: Wäckers, F.L., van Rijn, P.C.J., Bruin, J. (Eds.), *Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and Its Applications*. Cambridge University Press, Cambridge, pp. 267–304.
- Herrera, C.M., 1990. Daily patterns of pollinator activity, differential pollinating effectiveness, and floral resource availability in a summer-flowering Mediterranean shrub. *Oikos* 58, 277–288.
- Hogg, B.N., Nelson, E.H., Mills, N.J., Daane, K.M., 2011. Floral resources enhance aphid suppression by a hoverfly. *Entomol Exp Appl* 141, 138–144. doi:10.1111/j.1570-7458.2011.01174.x
- Höller, C., Borgemeister, C., Haardt, H., Powell, W., 1993. The relationship between primary parasitoids and hyperparasitoids of cereal aphids : an analysis of field data. *J Anim Ecol* 62, 12–21.
- Hopkinson, J.E., Zalucki, M.P., Murray, D. a H., 2013. Honeydew as a source of nutrition for *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae): effect of adult diet on lifespan and egg load. *Aust J Entomol* 52, 14–19. doi:10.1111/j.1440-6055.2012.00875.x
- Horner, H.T., Healy, R.A., Ren, G., Fritz, D., Klyne, A., Seames, C., Thornburg, R.W., 2007. Amyloplast to chromoplast conversion in developing ornamental tobacco floral nectaries provides sugar for nectar and antioxidants for protection. *Am J Bot* 94, 12–24.
- Horrocks, A., Davidson, M.M., Teulon, D.A.J., Horne, P.A., 2010. Demonstrating an integrated pest management strategy in autumn-sown wheat to arable farmers. *New Zeal Plant Prot* 63, 47–54.
- Hrcek, J., Miller, S.E., Quicke, D.L.J., Smith, M.A., 2011. Molecular detection of trophic links in a complex insect host-parasitoid food web. *Mol Ecol Resour* 11, 786–94. doi:10.1111/j.1755-0998.2011.03016.x

- Huang, J., Qiao, F., Zhang, L., Rozelle, S., 2000. Farm pesticide, rice production, and human health. A report for the Economy and Environment Program for Southeast Asia (EEPSEA).
- Hughes, R.D., 1963. Population dynamics of the cabbage aphid, *Brevicoryne brassicae*. *J Anim Ecol* 32, 393–424.
- Irvin, N.A., Pinckard, T.R., Perring, T.M., Hoddle, M.S., 2014. Evaluating the potential of buckwheat and cahaba vetch as nectar producing cover crops for enhancing biological control of *Homalodisca vitripennis* in California vineyards. *Biol Control* 76, 10–18. doi:10.1016/j.biocontrol.2014.04.006
- Jacometti, M., Jørgensen, N., Wratten, S.D., 2010. Enhancing biological control by an omnivorous lacewing: Floral resources reduce aphid numbers at low aphid densities. *Biol Control* 55, 159–165. doi:10.1016/j.biocontrol.2010.09.001
- Jamont, M., Crépellière, S., Jaloux, B., 2013. Effect of extrafloral nectar provisioning on the performance of the adult parasitoid *Diaeretiella rapae*. *Biol Control* 65, 271–277. doi:10.1016/j.biocontrol.2013.01.010
- Jamont, M., Dubois-Pot, C., Jaloux, B., 2014. Nectar provisioning close to host patches increases parasitoid recruitment, retention and host parasitism. *Basic Appl Ecol* 15, 151–160. doi:10.1016/j.baae.2014.01.001
- Jervis, M.A., 1998. Functional and evolutionary aspects of mouthpart structure in parasitoid wasps. *Biol J Linn Soc* 63, 461–493.
- Jervis, M.A., Ellers, J., Harvey, J.A., 2008. Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Annu Rev Entomol* 53, 361–85. doi:10.1146/annurev.ento.53.103106.093433
- Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A., Kidd, N.A.C., 2001. Life-history strategies in parasitoid wasps: a comparative analysis of “ovigeny.” *J Anim Ecol* 70, 442–458. doi:10.1046/j.1365-2656.2001.00507.x
- Jervis, M.A., Kidd, N.A.C., 1986. Host-feeding strategies in Hymenopteran parasitoids. *Biol Rev* 61, 395–434.
- Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T., Dawah, H.A., 1993. Flower-visiting by hymenopteran parasitoids. *J Nat Hist* 27, 67–105. doi:10.1080/00222939300770051
- Jones, R.A.C., Coutts, B.A., Hawkes, J., 2007. Yield-limiting potential of Beet western yellows virus in *Brassica napus*. *Aust J Agric Res* 58, 788. doi:10.1071/AR06391
- Jonsson, M., Wratten, S.D., Landis, D. a., Gurr, G.M., 2008. Recent advances in conservation biological control of arthropods by arthropods. *Biol Control* 45, 172–175. doi:10.1016/j.biocontrol.2008.01.006
- Juhler, R.K., Larsen, S.B., Meyer, O., Jensen, N.D., Spano, M., Giwercman, A., Bonde, J.P., 1999. Human semen quality in relation to dietary pesticide exposure and organic diet. *Arch Environ Contam Toxicol* 37, 415–423.
- Kant, R., Minor, M., Sandanayaka, M., Trewick, S., 2013. Effects of mating and oviposition delay on parasitism rate and sex allocation behaviour of *Diaeretiella rapae* (Hymenoptera: Aphidiidae).

Biol Control 65, 265–270. doi:10.1016/j.biocontrol.2013.01.012

- Kant, R., Minor, M.A., Trewick, S., 2012a. Fitness gain in a koinobiont parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) by parasitising hosts of different ages. *J Asia Pac Entomol* 15, 83–87. doi:10.1016/j.aspen.2011.08.011
- Kant, R., Minor, M.A., Trewick, S.A., 2012b. Reproductive strategies of *Diaeretiella rapae* (Hymenoptera: Aphidiinae) during fluctuating temperatures of spring season in New Zealand. *Biocontrol Sci Technol* 22, 1–9.
- Kavallieratos, N.G., Athanassiou, C.G., Stathas, G.J., Tomanović, Ž., 2002. Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) on citrus: Seasonal abundance, association with the species of host plant, and sampling indices. *Phytoparasitica* 30, 365–377. doi:10.1007/BF02979684
- Kavallieratos, N.G., Lykouressis, D.P., Sarlis, G.P., Stathas, G.J., Sanchis Segovia, A., Athanassiou, C.G., 2001. The Aphidiinae (Hymenoptera: Ichneumonoidea: Braconidae) of Greece. *Phytoparasitica* 29, 306–340.
- Kean, J., Wratten, S., Tylanakis, J., Barlow, N., 2003. The population consequences of natural enemy enhancement, and implications for conservation biological control. *Ecol Lett* 6, 604–612. doi:10.1046/j.1461-0248.2003.00468.x
- Kim, K.C., Byrne, L.B., 2006. Biodiversity loss and the taxonomic bottleneck: emerging biodiversity science. *Ecol Res* 21, 794–810. doi:10.1007/s11284-006-0035-7
- King, C., Spafford Jacob, H., Berlandier, F., 2006. The influence of water deficiency on the relationship between canola (*Brassica napus* L.), and two aphid species (Hemiptera : Aphididae), *Lipaphis erysimi* (Kaltenbach) and *Brevicoryne brassicae* (L.). *Aust J Agric Res* 57, 439–445.
- Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. *Proc Biol Sci* 274, 303–13. doi:10.1098/rspb.2006.3721
- Konrad, R., Wäckers, F.L., Romeis, J., Babendreier, D., 2009. Honeydew feeding in the solitary bee *Osmia bicornis* as affected by aphid species and nectar availability. *J Insect Physiol* 55, 1158–66. doi:10.1016/j.jinsphys.2009.08.012
- Koptur, S., 2005. Nectar as fuel for plant protectors, in: Wäckers, F.L., van Rijn, P.C.J., Bruin, J. (Eds.), *Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and Its Applications*. Cambridge University Press, pp. 75–108.
- Krischik, V.A., Landmark, A.L., Heimpel, G.E., 2007. Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera : Encyrtidae). *Environ Entomol* 36, 1238–1245.
- Kugimiya, S., Uefune, M., Shimoda, T., Takabayashi, J., 2010. Orientation of the parasitic wasp, *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae), to visual and olfactory cues of field mustard flowers, *Brassica rapa* L. (Brassicaceae), to exploit food sources. *Appl Entomol Zool* 45, 369–375. doi:10.1303/aez.2010.369
- Kyriakou, A., Close, R.C., Ashby, J.W., 1983. A strain of beet western yellows virus in Canterbury, New

Zealand. *New Zeal J Agric Res* 26, 271–277. doi:10.1080/00288233.1983.10427072

Lamb, K.P., 1959. Composition of the honeydew of the aphid *Brevicoryne brassicae* (L.) feeding on swedes (*Brassica napobrassica* DC.). *J Insect Physiol* 3, 1–13.

Lamb, R., 1989. Entomology of oilseed brassica crops. *Annu Rev Entomol* 34, 211–229. doi:10.1146/annurev.ento.34.1.211

Lammerink, J., 1968. A new biotype of cabbage aphid (*Brevicoryne brassicae* (L.)) on aphid resistant rape (*Brassica napus* L.). *New Zeal J Agric Res* 11, 341–344. doi:10.1080/00288233.1968.10431433

Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat Management to conserve natural enemies of arthropod pests. *Annu Rev Entomol* 45, 175–201.

Laubertie, E.A., Wratten, S.D., Hemptinne, J.-L., 2012. The contribution of potential beneficial insectary plant species to adult hoverfly (Diptera: Syrphidae) fitness. *Biol Control* 61, 1–6. doi:10.1016/j.biocontrol.2011.12.010

Lavandero, B., Wratten, S.D., Didham, R.K., Gurr, G., 2006. Increasing floral diversity for selective enhancement of biological control agents: a double-edged sword? *Basic Appl Ecol* 7, 236–243. doi:10.1016/j.baae.2005.09.004

Lavandero Icaza, B., 2004. Floral resources for the enhancement of the efficacy of *Diadegma semiclausum* (Helen) (Hymenoptera: Ichneumonidae), a parasitoid of *Plutella xylostella* (L.) (Lepidoptera: Hyponomeutidae) in broccoli (*Brassica oleracea* L. var. *italica*). PhD thesis, Lincoln University.

Lee, D., Zo, Y., Kim, S.-J., 1996. Nonradioactive method to study genetic profiles of natural bacterial communities by PCR – Single-Strand-Conformation Polymorphism. *Appl Environ Microbiol* 62, 3112–3120.

Lee, J.C., Andow, D.A., Heimpel, G.E., 2006. Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. *Ecol Entomol* 31, 470–480. doi:10.1111/j.1365-2311.2006.00800.x

Lee, J.C., Heimpel, G.E., 2002. Nectar availability and parasitoid sugar feeding, in: van Driesche, R.G. (Ed.), 1st International Symposium on Biological Control of Arthropods. Forest Health Technology Enterprise Team, Morgantown, West Virginia, pp. 220–225.

Lee, J.C., Heimpel, G.E., 2005. Impact of flowering buckwheat on Lepidopteran cabbage pests and their parasitoids at two spatial scales. *Biol Control* 34, 290–301. doi:10.1016/j.biocontrol.2005.06.002

Lee, J.C., Heimpel, G.E., 2007. Sugar feeding reduces short-term activity of a parasitoid wasp. *Physiol Entomol* 32, 99–103. doi:10.1111/j.1365-3032.2006.00555.x

Lee, J.C., Heimpel, G.E., 2008. Floral resources impact longevity and oviposition rate of a parasitoid in the field. *J Anim Ecol* 77, 565–572. doi:10.1111/j.1365-2656.2008.01355.x

Lee, J.C., Heimpel, G.E., Leibe, G.L., 2004. Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. *Entomol Exp Appl* 111, 189–199. doi:10.1111/j.0013-8703.2004.00165.x

- Lefort, M.-C., Boyer, S., Worner, S.P., Armstrong, K., 2012. Noninvasive molecular methods to identify live scarab larvae: an example of sympatric pest and nonpest species in New Zealand. *Mol Ecol Resour* 12, 389–95. doi:10.1111/j.1755-0998.2011.03103.x
- Legrand, M.A., Colinet, H., Vernon, P., Hance, T., 2004. Autumn, winter and spring dynamics of aphid *Sitobion avenae* and parasitoid *Aphidius rhopalosiphi* interactions. *Ann Appl Biol* 145, 139–144.
- Lessa, E.P., Applebaum, G., 1993. Screening techniques for detecting allelic variation in DNA sequences. *Mol Ecol* 2, 119–129.
- Letourneau, D.K., Altieri, M.A., 1999. Environmental management to enhance biological control in agroecosystems, in: Bellows, T.S. (Ed.), *The Handbook of Biological Control*. Academic Press, San Diego, CA., pp. 319–354.
- Letourneau, D.K., Armbrrecht, I., Rivera, B.S., Lerma, J.M., Carmona, E.J., Daza, M.C., Escobar, S., Galindo, V., Gutiérrez, C., López, S.D., Mejía, J.L., Rangel, A.M.A., Rangel, J.H., Rivera, L., Saavedra, C.A., Torres, A.M., Trujillo, A.R., 2011. Does plant diversity benefit agroecosystems? A synthetic review. *Ecol Appl* 21, 9–21.
- Lewis, W.J., van Lenteren, J.C., Phatak, S.C., Tumlinson, J.H., 1997. A total system approach to sustainable pest management. *Proc Natl Acad Sci U S A* 94, 12243–8.
- Lightle, D., Ambrosino, M., Lee, J.C., 2010. Sugar in moderation: sugar diets affect short-term parasitoid behaviour. *Physiol Entomol* 35, 179–185. doi:10.1111/j.1365-3032.2009.00718.x
- Littlejohn, C.P., Wratten, S.D., Curran, T.J., Hofmann, R.W., Dennis, S.J., 2014. Shelter provided by a bioenergy crop (*Miscanthus x giganteus*) increases pasture production on dairy farms, in: *Proceedings of the 5th Australasian Dairy Science Symposium 2014*. pp. 141–143.
- Lohaus, K., Vidal, S., Thies, C., 2013. Farming practices change food web structures in cereal aphid-parasitoid-hyperparasitoid communities. *Oecologia* 171, 249–59. doi:10.1007/s00442-012-2387-8
- Losey, J.E., Vaughan, M., 2006. The economic value of ecological services provided by insects. *Bioscience* 56, 311–323.
- Lucchetta, P., Desouhant, E., Wajnberg, E., Bernstein, C., 2007. Small but smart: the interaction between environmental cues and internal state modulates host-patch exploitation in a parasitic wasp. *Behav Ecol Sociobiol* 61, 1409–1418. doi:10.1007/s00265-007-0372-8
- Médiène, S., Valantin-Morison, M., Sarthou, J.-P., Tourdonnet, S., Gosme, M., Bertrand, M., Roger-Estrade, J., Aubertot, J.-N., Rusch, A., Motisi, N., Pelosi, C., Doré, T., 2011. Agroecosystem management and biotic interactions: a review. *Agron Sustain Dev* 31, 491–514. doi:10.1007/s13593-011-0009-1
- Morandin, L.A., Winston, M.L., 2006. Pollinators provide economic incentive to preserve natural land in agroecosystems. *Agric Ecosyst Environ* 116, 289–292. doi:10.1016/j.agee.2006.02.012
- Morrant, D.S., Schumann, R., Petit, S., 2009. Field methods for sampling and storing nectar from flowers with low nectar volumes. *Ann Bot* 103, 533–42. doi:10.1093/aob/mcn241
- Müller, C.B., Adriaanse, I.C.T., Belshaw, R., Godfray, H.C., 1999. The structure of an aphid-parasitoid community. *J Anim Ecol* 68, 346–370.

- Nematollahi, M.R., Fathipour, Y., Talebi, A.A., Karimzadeh, J., Zalucki, M.P., 2014. Parasitoid- and hyperparasitoid-mediated seasonal dynamics of the cabbage aphid (Hemiptera : Aphididae). *Environ Entomol* 43, 1542–1551.
- Nougadère, A., Sirot, V., Kadar, A., Fastier, A., Truchot, E., Vergnet, C., Hommet, F., Baylé, J., Gros, P., Leblanc, J.-C., 2012. Total diet study on pesticide residues in France: levels in food as consumed and chronic dietary risk to consumers. *Environ Int* 45, 135–50. doi:10.1016/j.envint.2012.02.001
- Oliver, K.M., Moran, N.A., Hunter, M.S., 2005. Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proc Natl Acad Sci U S A* 102, 12795–800. doi:10.1073/pnas.05061311102
- Olson, D.M., Fadamiro, H., Lundgren, J.G., Heimpel, G.E., 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiol Entomol* 25, 17–26.
- Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K., Sekiya, T., 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A* 86, 2766–2770.
- Östman, Ö., Ekbohm, B., Bengtsson, J., 2003. Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecol Econ* 45, 149–158. doi:10.1016/S0921-8009(03)00007-7
- Patel, S.R., Awasthi, A.K., Tomar, R.K.S., 2004. Assessment of yield losses in mustard (*Brassica juncea* L.) due to mustard aphid (*Lipaphis erysimi* Kalt.) under different thermal environments in eastern central india. *Appl Ecol Environ Res* 2, 1–15.
- Patt, J.M., Hamilton, G.C., Lashomb, J.H., 1997. Foraging success of parasitoid wasps on flowers: interplay of insect morphology, floral architecture and searching behavior. *Entomol Exp Appl* 83, 21–30. doi:10.1046/j.1570-7458.1997.00153.x
- Pickett, J.A., 2008. Sustainable biofuels: prospects and challenges. The Royal Society, London.
- Pierre, J.-S., 2011. Neuroeconomics in parasitoids: computing accurately with a minute brain. *Oikos* 120, 77–83. doi:10.1111/j.1600-0706.2010.18603.x
- Pike, K.S., Stary, P., Miller, T., Allison, D., Graf, G., Boydston, L., Miller, R., Gillespie, R., 1999. Host range and habitats of the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) in Washington state. *Environ Entomol* 28, 61–71.
- Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. *Mol Ecol* 21, 1931–50. doi:10.1111/j.1365-294X.2011.05403.x
- Ponti, L., Altieri, M. a., Gutierrez, A.P., 2007. Effects of crop diversification levels and fertilization regimes on abundance of *Brevicoryne brassicae* (L.) and its parasitization by *Diaeretiella rapae* (McIntosh) in broccoli. *Agric For Entomol* 9, 209–214. doi:10.1111/j.1461-9563.2007.00330.x
- Porter, J., Costanza, R., Sandhu, H.S., Sigsgaard, L., Wratten, S.D., 2009. The value of producing food, energy, and ecosystem services within an agro-ecosystem. *Ambio* 38, 186–93.
- Power, A.G., 2010. Ecosystem services and agriculture: tradeoffs and synergies. *Philos Trans R Soc Lond B Biol Sci* 365, 2959–71. doi:10.1098/rstb.2010.0143

- Rakow, G., 2004. Species origin and economic importance of Brassica, in: Pua, E.-C., Douglas, C.J. (Eds.), Brassica. Springer Berlin Heidelberg, pp. 3–11. doi:10.1007/978-3-662-06164-0_1
- Ramsden, M.W., Menéndez, R., Leather, S.R., Wäckers, F., 2014. Optimizing field margins for biocontrol services : The relative role of aphid abundance, annual floral resources, and overwinter habitat in enhancing aphid natural enemies. *Agric Ecosyst Environ* 199, 94–104. doi:10.1016/j.agee.2014.08.024
- Ratnasingham, S., Hebert, P.D.N., 2007. BOLD: The Barcode of Life Data System. *Mol Ecol Notes* 7, 355–364. doi:10.1111/j.1471-8286.2006.01678.x
- Rauch, S.A., Braun, J.M., Barr, D.B., Calafat, A.M., Khoury, J., Montesano, M.A., 2012. Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. *Environ Health Perspect* 120, 1055–1060.
- Ridgway, H.J., Amponsah, N.T., Brown, D.S., Baskarathevan, J., Jones, E.E., Jaspers, M. V., 2011. Detection of botryosphaeriaceous species in environmental samples using a multi-species primer pair. *Plant Pathol* 60, 1118–1127. doi:10.1111/j.1365-3059.2011.02474.x
- Robinson, K.A., Jonsson, M., Wratten, S.D., Wade, M.R., Buckley, H.L., 2008. Implications of floral resources for predation by an omnivorous lacewing. *Basic Appl Ecol* 9, 172–181. doi:10.1016/j.baae.2007.01.002
- Root, R.B., 1973. Organization of a Plant-Arthropod Association in Simple and Diverse Habitats : The Fauna of Collards (*Brassica Oleracea*). *Ecol Monogr* 43, 95–124.
- Rougerie, R., Smith, M.A., Fernandez-Triana, J., Lopez-Vaamonde, C., Ratnasingham, S., Hebert, P.D.N., 2011. Molecular analysis of parasitoid linkages (MAPL): gut contents of adult parasitoid wasps reveal larval host. *Mol Ecol* 20, 179–86. doi:10.1111/j.1365-294X.2010.04918.x
- Rusch, A., Suchail, S., Valantin-Morison, M., Sarthou, J.-P., Roger-Estrade, J., 2012. Nutritional state of the pollen beetle parasitoid *Tersilochus heterocerus* foraging in the field. *Biol Control* 58, 17–26. doi:10.1007/s10526-012-9463-1
- Russell, M.C., 2015. A meta-analysis of physiological and behavioral responses of parasitoid wasps to flowers of individual plant species. *Biol Control* 82, 96–103. doi:10.1016/j.biocontrol.2014.11.014
- Saeed, N.A., Razaq, M., 2014. Effect of sowing dates within a season on incidence and abundance of insect pests of canola crops. *Pak J Zool* 46.
- Saleh, A.A.A., Desuky, W.M.H., Mohamed, N.E., 2009. Studies on some parasitoids of the cowpea aphid *Aphis craccivora* Koch (Homoptera: Aphididae) in Egypt. *Egypt J Biol Pest Control* 19, 11–16.
- Sampaio, M. V., Bueno, V.H.P., Soglia, M.C.M., De Conti, B.F., Rodrigues, S.M.M., 2006. Larval competition between *Aphidius colemani* and *Lysiphlebus testaceipes* after multiparasitism of the host *Aphis gossypii*. *Bull Insectology* 59, 147–151.
- Satar, S., Kersting, U., Ulusoy, R., 2005. Temperature dependent life history traits of *Brevicoryne brassicae* (L.) (Hom., Aphididae) on white cabbage. *Turk J Agric For* 29, 341–346.
- Saville, D.J., 1990. Multiple comparisons procedures: the practical solution. *Am Stat* 44, 174–180.

- Schwieger, F., Tebbe, C., 1998. A new approach to utilize PCR-single strand-conformation-polymorphism for 16S rRNA gene-based microbial community analysis. *Appl Environ Microbiol*.
- Searle, K.R., Hobbs, N.T., Gordon, I.J., 2007. It's the "Foodscape", not the Landscape: Using Foraging Behavior to Make Functional Assessments of Landscape Condition. *Isr J Ecol Evol* 53, 297–316. doi:10.1560/IJEE.53.3.297
- Settle, W.H., Ariawan, H., Astuti, E.T., Cahyana, W., Hakim, A.L., Hindayana, D., Lestari, A.S., Pajarningsih, 1996. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. *Ecology* 77, 1975–1988.
- Severinghaus, L.L., Kurtak, B.H., Eickwort, G.C., 1981. The reproductive behavior of *Anthidium manicatum* (Hymenoptera : Megachilidae) and the significance of size for territorial males. *Behav Ecol Sociobiol* 9, 51–58.
- Shelton, A.M., Nault, B.A., 2004. Dead-end trap cropping: a technique to improve management of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Crop Prot* 23, 497–503. doi:10.1016/j.cropro.2003.10.005
- Shennan, C., 2008. Biotic interactions, ecological knowledge and agriculture. *Philos Trans R Soc Lond B Biol Sci* 363, 717–39. doi:10.1098/rstb.2007.2180
- Shik, J.Z., Kay, A.D., Silverman, J., 2014. Aphid honeydew provides a nutritionally balanced resource for incipient Argentine ant mutualists. *Anim Behav* 95, 33–39. doi:10.1016/j.anbehav.2014.06.008
- Shonnard, D.R., Williams, L., Kalnes, T.N., 2010. Camelina-derived jet fuel and diesel: sustainable advanced biofuels. *Environ Prog Sustain Energy* 29, 382–392. doi:10.1002/ep
- Siekmann, G., Keller, M.A., Tenhumberg, B., 2004. The sweet tooth of adult parasitoid *Cotesia rubecula*: ignoring hosts for nectar? *J Insect Behav* 17, 459–476. doi:10.1023/B:JOIR.0000042535.76279.c7
- Šimenc, J., Potočnik, U., 2011. Rapid differentiation of bacterial species by high resolution melting curve analysis. *Appl Biochem Microbiol* 47, 256–263. doi:10.1134/S0003683811030136
- Steenblik, R., 2007. Biofuels – at what cost? Government support for ethanol and biodiesel in selected OECD countries [WWW Document]. *Int Institue Sustain Dev*. URL <http://www.iisd.org/PUBLICATIONS/pub.aspx?pno=895> (accessed 6.5.12).
- Stevens, M., McGrann, G., Clark, B., 2008. Turnip yellows virus (syn Beet western yellows virus): an emerging threat to European oilseed rape production ?, *HGCA Research Review*.
- Struik, P., Kuyper, T., Brussaard, L., Leeuwis, C., 2014. Deconstructing and unpacking scientific controversies in intensification and sustainability: why the tensions in concepts and values? *Curr Opin Environ Sustain* 8, 80–88. doi:10.1016/j.cosust.2014.10.002
- Sullivan, D.J., Völkl, W., 1999. Hyperparasitism: multitrophic ecology and behavior. *Annu Rev Entomol* 44, 291–315. doi:10.1146/annurev.ento.44.1.291
- Sunnucks, P., Wilson, A.C., Beheregaray, L.B., Zenger, K., French, J., Taylor, A.C., 2000. SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. *Mol Ecol* 9, 1699–710.

- Takasu, K., Lewis, W.J., 1995. Importance of adult food sources to host searching of the larval parasitoid *Microplitis croceipes*. *Biol Control* 5, 25–30.
- Tazerouni, Z., Talebi, A.A., Rakhshani, E., 2012. Comparison of development and demographic parameters of *Diuraphis noxia* (Hem., Aphididae) and its parasitoid, *Diaeretiella rapae* (Hym., Braconidae: Aphidiinae). *Arch Phytopathol Plant Prot* 45, 886–897.
doi:10.1080/03235408.2011.599150
- Tena, A., Pekas, A., Wäckers, F.L., Urbaneja, A., 2013. Energy reserves of parasitoids depend on honeydew from non-hosts. *Ecol Entomol* 38, 278–289. doi:10.1111/een.12018
- Tertuliano, M., Olson, D.M., Rains, G.C., Lewis, W.J., 2004. Influence of handling and conditioning protocol on learning and memory of *Microplitis croceipes*. *Entomol Exp Appl* 110, 165–172.
doi:10.1111/j.0013-8703.2004.00132.x
- Teulon, D.A.J., Stufkens, M.A.W., 1998. Current status of New Zealand indigenous aphids. *Conserv Advis Sci Notes* 216, 23.
- Thomson, D., 2004. Competitive interactions between the invasive european honey bee and native bumble bees. *Ecology* 85, 458–470.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature* 418, 671–677.
- Tompkins, J.-M.L., 2010. Ecosystem services provided by native New Zealand plants in vineyards. PhD thesis, Lincoln University.
- Tompkins, J.-M.L., Wratten, S.D., Wäckers, F.L., 2010. Nectar to improve parasitoid fitness in biological control: Does the sucrose:hexose ratio matter? *Basic Appl Ecol* 11, 264–271.
doi:10.1016/j.baae.2009.12.010
- Traugott, M., Bell, J.R., Broad, G.R., Powell, W., van Veen, F.J.F., Vollhardt, I.M.G., Symondson, W.O.C., 2008. Endoparasitism in cereal aphids: molecular analysis of a whole parasitoid community. *Mol Ecol* 17, 3928–38. doi:10.1111/j.1365-294X.2008.03878.x
- Tylianakis, J.M., Didham, R.K., Wratten, S.D., 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology* 85, 658–666.
- Tylianakis, J.M., Tschardtke, T., Lewis, O.T., 2007. Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature* 445, 202–5. doi:10.1038/nature05429
- Valentini, A., Pompanon, F., Taberlet, P., 2009. DNA barcoding for ecologists. *Trends Ecol Evol* 24, 110–7. doi:10.1016/j.tree.2008.09.011
- van Alphen, J.J.M., Bernstein, C., Driessen, G., 2003. Information acquisition and time allocation in insect parasitoids. *Trends Ecol Evol* 18, 81–87. doi:10.1016/S0169-5347(02)00035-6
- van den Bosch, R., Messenger, P.S., 1973. *Biological Control*. Intertext Educational Publishers.
- van Emden, H.F., Eastop, V.F., Hughes, R.D., Way, M.J., 1969. The ecology of *Myzus persicae*. *Annu Rev Entomol* 14, 197–270.
- van Emden, H.F., Harrington, R. (Eds.), 2007. *Aphids as crop pests*. CABI, London.

- Van Handel, E., 1985. Rapid determination of glycogen and sugars in mosquitoes. *J Am Mosq Control Assoc* 1, 299–301.
- van Rijn, P.C.J., Kooijman, J., Wäckers, F.L., 2013. The contribution of floral resources and honeydew to the performance of predatory hoverflies (Diptera: Syrphidae). *Biol Control* 67, 32–38. doi:10.1016/j.biocontrol.2013.06.014
- Varenes, Y.-D., Boyer, S., Wratten, S.D., 2014. Un-nesting DNA Russian dolls - the potential for constructing food webs using residual DNA in empty aphid mummies. *Mol Ecol* 23, 3925–3933. doi:10.1111/mec.12633
- Varenes, Y.-D., Gonzalez Chang, M., Boyer, S., Wratten, S.D., 2015. Nectar feeding increases exploratory behaviour in the aphid parasitoid *Diaeretiella rapae* (McIntosh). *J Appl Entomol In Press*. doi:10.1111/jen.12281
- Vattala, H.D., 2005. Enhancement of the efficacy of the parasitoid, *Microctonus hyperodae* Loan by provision of floral resources to improve biological control of its host, the Argentine stem weevil (*Listronotus bonariensis*) (Kuschel). PhD thesis, Lincoln University.
- Vattala, H.D., Wratten, S.D., Phillips, C.B., Wäckers, F.L., 2006. The influence of flower morphology and nectar quality on the longevity of a parasitoid biological control agent. *Biol Control* 39, 179–185. doi:10.1016/j.biocontrol.2006.06.003
- Vattala, H.D., Wratten, S.D., Phillips, C.B., Wäckers, F.L., Worner, S.P., 2005. Measuring parasitoid carbohydrate levels to improve biological control. *New Zeal Plant Prot* 58, 135–139.
- Vollhardt, I.M.G., Bianchi, F.J.J.A., Wäckers, F.L., Thies, C., Tschardt, T., 2010. Nectar vs. honeydew feeding by aphid parasitoids: does it pay to have a discriminating palate? *Entomol Exp Appl* 137, 1–10. doi:10.1111/j.1570-7458.2010.01025.x
- Wäckers, F.L., 1994. The effect of food deprivation on the innate visual and olfactory preferences in the parasitoid *Cotesia rubecula*. *J Insect Physiol* 40, 641–649.
- Wäckers, F.L., 2000. Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars. *Oikos* 90, 197–201.
- Wäckers, F.L., 2001. A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *J Insect Physiol* 47, 1077–1084.
- Wäckers, F.L., 2005. Suitability of (extra-)floral nectar, pollen, and honeydew as insect food sources, in: Wäckers, F.L., van Rijn, P.C.J. (Eds.), *Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and Its Applications*. Cambridge University Press, Cambridge, pp. 17–75.
- Wäckers, F.L., van Rijn, P.C.J., 2012. Pick and mix: selecting flowering plants to meet the requirements of target biological control insects, in: Gurr, G.M., Wratten, S.D., Snyder, W.E. (Eds.), *Biodiversity and Insect Pests: Key Issues for Sustainable Management*. John Wiley & Sons.
- Wäckers, F.L., van Rijn, P.C.J., Heimpel, G.E., 2008. Honeydew as a food source for natural enemies: making the best of a bad meal? *Biol Control* 45, 176–184. doi:10.1016/j.biocontrol.2008.01.007
- Walker, G.P., Davis, S.I., Macdonald, F.H., Herman, T.J.B., 2012. Update on diamondback moth

- (*Plutella xylostella*) insecticide resistance and the vegetable brassica insecticide management strategy. *New Zeal Plant Prot* 65, 114–119.
- Walker, J., T.S., Park, N.M., Clothier, B.E., Manktelow, D.W.L., van den Dijssel, C.W., Hodson, A.J., Barley, M.J., Hodson-Kersey, L.R., 2009. Progress in pesticide risk reduction in New Zealand horticulture. *New Zeal Plant Prot* 62, 321–327.
- Walther-Hellwig, K., Fokul, G., Frankl, R., Buchler, R., Ekschmitt, K., Wolters, V., 2006. Increased density of honeybee colonies affects foraging bumblebees. *Apidologie* 37, 517–532.
- Wang, N., Qian, W., Suppanz, I., Wei, L., Mao, B., Long, Y., Meng, J., Müller, A.E., Jung, C., 2011. Flowering time variation in oilseed rape (*Brassica napus* L.) is associated with allelic variation in the FRIGIDA homologue BnaA.FRI.a. *J Exp Bot* 62, 5641–58. doi:10.1093/jxb/err249
- Wang, W., Liu, Y., Chen, J., Ji, X., Zhou, H., Wang, G., 2009. Impact of intercropping aphid-resistant wheat cultivars with oilseed rape on wheat aphid (*Sitobion avenae*) and its natural enemies. *Acta Ecol Sin* 29, 186–191. doi:10.1016/j.chnaes.2009.07.009
- Weissensteiner, T., Lanchbury, J.S., 1996. Strategy for controlling preferential amplification and avoiding false negatives in PCR typing. *Biotechniques* 21, 1102–1108.
- Welch, K.D., Harwood, J.D., 2014. Temporal dynamics of natural enemy–pest interactions in a changing environment. *Biol Control* 75, 18–27. doi:10.1016/j.biocontrol.2014.01.004
- Wezel, A., Bellon, S., Doré, T., Francis, C., Vallod, D., David, C., 2009. Agroecology as a science, a movement and a practice. A review. *Agron Sustain Dev* 29, 503–515.
- White, T.C.R., 2013. Experimental and observational evidence reveals that predators in natural environments do not regulate their prey: they are passengers, not drivers. *Acta Oecologica* 53, 73–87. doi:10.1016/j.actao.2013.09.007
- Wiedenmann, R.N., Smith, J.W., 1997. Attributes of natural enemies in ephemeral crop habitats. *Biol Control* 10, 16–22.
- Williams, I.H., 1978. The pollination requirements of swede rape (*Brassica napus* L.) and of turnip rape (*Brassica campestris* L.). *J Agric Sci* 91, 343–48. doi:10.1017/S0021859600046438
- Williams, I.H. (Ed.), 2010. *Biocontrol-based integrated management of oilseed rape pests*, 2010th ed. Springer, Dordrecht, the Netherlands.
- Winkler, K., 2005. *Assessing the risks and benefits of flowering field edges - Strategic use of nectar sources to boost biological control*. Wageningen University.
- Winkler, K., Wäckers, F., Bukovinszkyne-Kiss, G., van Lenteren, J., 2006. Sugar resources are vital for *Diadegma semiclausum* fecundity under field conditions. *Basic Appl Ecol* 7, 133–140. doi:10.1016/j.baae.2005.06.001
- Winkler, K., Wäckers, F., Pinto, D.M., 2009a. Nectar-providing plants enhance the energetic state of herbivores as well as their parasitoids under field conditions. *Ecol Entomol* 34, 221–227. doi:10.1111/j.1365-2311.2008.01059.x
- Winkler, K., Wäckers, F.L., Kaufman, L. V., Larraz, V., van Lenteren, J.C., 2009b. Nectar exploitation by herbivores and their parasitoids is a function of flower species and relative humidity. *Biol*

Control 50, 299–306. doi:10.1016/j.biocontrol.2009.04.009

- Woodcock, B.A., Edwards, M., Redhead, J., Meek, W.R., Nuttall, P., Falk, S., Nowakowski, M., Pywell, R.F., 2013. Crop flower visitation by honeybees, bumblebees and solitary bees: Behavioural differences and diversity responses to landscape. *Agric Ecosyst Environ* 171, 1–8. doi:10.1016/j.agee.2013.03.005
- Wratten, S.D., Gillespie, M., Decourtye, A., Mader, E., Desneux, N., 2012. Pollinator habitat enhancement: benefits to other ecosystem services. *Agric Ecosyst Environ* 159, 112–122. doi:10.1016/j.agee.2012.06.020
- Wyckhuys, K.A.G., Strange-George, J.E., Kulhanek, C.A., Wäckers, F.L., Heimpel, G.E., 2008. Sugar feeding by the aphid parasitoid *Binodoxys communis*: how does honeydew compare with other sugar sources? *J Insect Physiol* 54, 481–491. doi:10.1016/j.jinsphys.2007.11.007
- Yokoi, T., Fujisaki, K., 2007. Repellent scent-marking behaviour of the sweat bee *Halictus (Seladonia) aerarius* during flower foraging. *Apidologie* 38, 474–481.
- Zhang, W., Ricketts, T.H., Kremen, C., Carney, K., Swinton, S.M., 2007. Ecosystem services and dis-services to agriculture. *Ecol Econ* 64, 253–260. doi:10.1016/j.ecolecon.2007.02.024
- Zhang, W.Q., Hassan, S. a., 2003. Use of the parasitoid *Diaeretiella rapae* (McIntoch) to control the cabbage aphid *Brevicoryne brassicae* (L.). *J Appl Entomol* 127, 522–526. doi:10.1046/j.1439-0418.2003.00792.x
- Zhu, P., Gurr, G.M., Lu, Z., Heong, K., Chen, G., Zheng, X., Xu, H., Yang, Y., 2013. Laboratory screening supports the selection of sesame (*Sesamum indicum*) to enhance *Anagrus* spp. parasitoids (Hymenoptera: Mymaridae) of rice planthoppers. *Biol Control* 64, 83–89. doi:10.1016/j.biocontrol.2012.09.014
- Zhu, P., Lu, Z., Heong, K., Chen, G., Zheng, X., Xu, H., Yang, Y., Nicol, H.I., Gurr, G.M., 2014. Selection of nectar plants for use in ecological engineering to promote biological control of rice pests by the predatory bug, *Cyrtorhinus lividipennis* (Heteroptera: Miridae). *PLoS One* 9, e108669. doi:10.1371/journal.pone.0108669