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**‘Attract and reward’: combining a floral resource
subsidy with a herbivore-induced plant volatile to
enhance conservation biological control**

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

Doctor of Philosophy

at

Lincoln University

by

G.U.S. Orre

Lincoln University

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“Don’t ask yourself what the world needs ask yourself what makes you come alive and then go and do that. Because what the world needs are people who have come alive.”

Dr Howard Thurma

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

**‘Attract and reward’: combining a floral resource subsidy with a
herbivore-induced plant volatile to enhance conservation
biological control**

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Abstract

Experiments were conducted to assess whether a concept termed ‘attract and reward’ (A&R) could enhance conservation biological control (CBC). In A&R, a synthetically-produced herbivore induced plant volatile (HIPV) (‘attract’) is combined with a floral resource (‘reward’). It is anticipated that the two will work synergistically attracting natural enemies into the crop (‘attract’) and maintaining them within the crop (‘reward’).

The study system consists of brassica, the most commonly occurring brassica herbivores, their natural enemies and higher order natural enemies. The HIPV deployed is methyl salicylate (MeSA) and the floral resource is buckwheat *Fagopyrum esculentum*.

The aim of the first two field experiments, in 2007 and 2008, was to evaluate the effects of MeSA and MeSA combined with buckwheat (A&R) on the abundance of arthropods from three trophic levels. In 2007, a field experiment was conducted using MeSA alone. The mean abundance of the leafmining fly *Scaptomyza flava*¹(TL2), the diamondback moth (TL2) (DBM) parasitoid *Diadegma semiclausum*²(TL3) and the hoverfly *Melangyna novaezealandiae* (TL3) was increased in MeSA-treatments by up to 300% and for the brown lacewing parasitoid *Anacharis zealandica* a maximum mean increase of 600% was recorded.

¹TL2 = trophic level 2

²TL3 = trophic level 3

Significantly more females of the *D. semiclausum* and *M. novaezealandiae* were attracted to MeSA than males.

When A&R was deployed in 2008, were arthropods from the third and fourth trophic levels affected. For none of the species was there a synergistic effect between ‘attract’ and ‘reward’ on their abundance. The brown lacewing *Micromus tasmaniae* (TL3), two parasitoids of DBM and one of cabbage white butterfly *Pieris rapae* (TL2) increased significantly in treatments with buckwheat. The hoverfly *Melanostoma fasciatum* (TL3) was significantly more abundant in treatments with MeSA, but significantly less abundant in treatments with buckwheat. The effect of MeSA on the fourth trophic level parasitoid *Anacharis zealandica*³(TL4) was inconsistent between years. Here it significantly decreased its abundance, while treatments with buckwheat increased it. Significantly fewer male than female *D. semiclausum* were attracted to MeSA only treatments.

These experiments show that MeSA and buckwheat can have unwanted effects on arthropod abundance which may disrupt CBC. To assess the effect of A&R on CBC a further field experiment evaluating herbivore densities, predation, parasitism and hyper-parasitism rates was conducted. The only effect was significantly higher aphid parasitism in treatments with MeSA.

Based on the results from the field experiments it remained unclear whether it was MeSA or a blend of volatiles produced by MeSA-induced host plants that were attractive to the arthropods. An olfactory experiment was conducted to evaluate if the aphid parasitoid *Aphidius colemani* can be attracted to two different concentrations of MeSA diluted in Synertrol oil. Significantly more parasitoids were attracted to 2.0% MeSA than to air while the parasitoid did not respond to the 0.5% concentration.

These results indicate that A&R has potential as a CBC technique, as long as any unwanted side effects can be managed. Although there were no synergistic effects between ‘attract’ and ‘reward’ on the abundance of individual natural enemies, combining MeSA and buckwheat could still be beneficial because the two techniques increase the abundance of different natural enemies.

Keywords

‘attract and reward’, herbivore-induced plant volatile, methyl salicylate, buckwheat, brassica, trophic level, habitat manipulation, conservation biological control.

³TL4 = trophic level 4

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Chapter 1: Introduction

1.1. General introduction

Ecosystem services (ES) are ‘the conditions and processes through which natural ecosystems, and the species that makes them up, sustain and fulfil human life’ Daily (1997). One such service is biological control (Daily 1997, Fiedler et al. 2008). Costanza et al. (1997) estimated the value of ES to be US\$33 trillion/year for the entire biosphere, with biological control alone worth US\$417 billion/year. Modern agriculture aims to maximise yield and profitability (Fiedler et al. 2008). Both the frequent disturbances linked with high intensity agricultural practices and the modification of the habitat to an environment low in physical and biological resources required by natural enemies of pests (Townsend 1972, Powell 1986, Letourneau 1998, Fiedler et al. 2008), result in a decline in the ES such as biological control (Fiedler et al. 2008).

The focus of this thesis is on biological control in the form of conservation biological control (CBC). The aim of CBC is to enhance natural enemy fitness – the contribution of a genotype to the next generation (Campbell et al. 1999), population size and effectiveness by manipulation of the environment to increase survival, fecundity, and longevity of the natural enemies and modify their behaviour (Landis et al. 2000). Manipulations may involve creating or maintaining physical refuges, providing alternative prey or hosts, or food sources such as nectar and pollen, moderating physical conditions for natural enemies through the use of ground covers, provision of shelter sites, or use of strip-harvesting methods (van den Bosch et al. 1967, Heidger and Nentwig 1989). CBC also includes minimizing pesticide induced mortality (Gurr et al. 2000b).

This thesis will evaluate a new concept in CBC termed ‘attract and reward’ (Jonsson et al. 2008, Khan et al. 2008). In ‘attract and reward’ the well established habitat manipulation technique of floral resource supplementation is combined with a more novel technique of herbivore-induced plant volatiles (HIPVs).

This introduction will explain the major concepts of biological control and CBC with an emphasis on floral resource supplementation and it will explain the main theory behind HIPVs. It will introduce the novel concept ‘attract and reward’ and provide an overview of the study system and the relationships between the studied species. Each research chapter is written in the style of a research paper and more detailed reviews of related literature will be given in the introduction and discussion to each of these.

1.2. Biological control

The concept of biological control (BC) has been defined multiple times. An early definition by De Bach (1964) defined it as “the action of parasites, predators or pathogens in maintaining another organism’s population density at a lower average than would occur in their absence”. Later, a commonly accepted definition was provided by Eilenberg et al. (2001) who defined it as “The use of living organisms to suppress the population of a specific pest organism, making it less abundant or less damaging than it would otherwise be”. However, these definitions mention a reduction in pest population densities but do not define a damage threshold or include the reduction of pesticide use. For biological control to be successful the pest needs to be reduced below some damage threshold (Gurr et al. 2000a). A concept that has been developed to determine if an organism needs to be controlled or not is called the economic injury level (EIL). This is defined as the lowest density of pest that will cause economic damage (Pedigo and Rice 2009). The economic threshold is normally set below the economic injury level. Once the pest population reaches this threshold control practices should begin (Hajek 2004). For the lucerne pest weevil *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) in New Zealand, the threshold may be around 60% of the pest’s natural equilibrium population. Consequently, reducing *S. discoideus* larval densities to about 40% through biological control by the introduced parasitoid *Microctonus aethiopoulos* Loan (Hymenoptera: Braconidae) is considered successful (Barlow and Goldson 1993). However, the control agent does not need to be the single solution to reducing pest densities to still be considered useful. A partially effective agent can still be a valuable component in integrated

pest management (IPM)-programs (Gurr et al. 2000a). In IPM-programs combine multi pest-control methods, such as cultural control, host plant resistance, biological control and the use of chemical control (Gurr et al. 2004). For example, pesticide use may lower the pest population, consequently reducing density-dependent, intra-specific competition within the pest population. In the absence of a biological-control agent, the pest population could recover quickly through compensatory higher fecundity or survival. As long as the biological control agent, even if only partially effective remains successful at low prey/host density it may prevent this pest population recovery (Gurr et al. 2000a).

In classical biological control new, usually exotic, natural enemies are introduced into an area in the hope that they will establish and provide ongoing control of the target pest (van Driesche and Bellows 1996). There are several examples of successes of controlling the target pest within classical biological control including arthropods, aquatic plants, terrestrial plants and vertebrates. For example, in several places the water hyacinth *Salvinia molesta* Mitchell (Saviniaceae), one of the worst aquatic weeds in the world, has commonly been permanently controlled through the introduction of the phytophagous weevil *Cyrtophagous salviniae* Calder and Sands (Coleoptera: Curculionidae) (van Driesche and Bellows 1996).

The success rate of classical biological control in the form of arthropod pest control by arthropods between 1880 and 1989 falls within 5-15% (Greathead and Greathead 1992). One way the natural enemy population suppression could possibly be increased is through the provision of resources other than the target pest, such as non-host foods used in CBC (Gurr and Wratten 1999). In CBC, habitat manipulation techniques are used to produce trophic cascades. These result in inverse patterns of abundance or biomass across more than one trophic level. In a three trophic level food chain, such as crop plants - herbivorous pests – natural enemies, enhancing the top predators (natural enemies) may result in lower abundance of mid level consumers (herbivores pest) and a higher abundance of basal producers (crop plants) (Carpenter and Kitchell 1993).

CBC utilises these ‘top-down’ effects to increase the natural enemy population (Gurr et al. 2000b). However, habitat manipulation can produce both ‘bottom-up’ and ‘top-down’ effects, consistent with the ‘resource concentration’ hypothesis and the ‘enemy’ hypothesis.

According to both these hypotheses, herbivores are predicted to be more abundant in simple systems i.e. monocultures, than in more complex ones (Root 1973). According to the ‘resource concentration’ hypothesis, the reduction in herbivore abundance in complex habitats is caused by mechanisms such as dilution of the contrast between a concentrated crop and the soil. This produces a dilution of the visual and chemical stimuli for the herbivore, resulting in

decreased colonisation rates and increased emigration rates and thereby a reduction in damage to the crop (Gurr et al. 2000b). As the herbivore population in the ‘resource concentration’ hypothesis is determined by a lower trophic level, the effects seen are ‘bottom-up’ effects. According to the ‘enemy’ hypothesis, predators and parasitoids are more numerous and/or effective in more diverse systems than in simple ones (Root 1973). As the herbivore population in the ‘enemy’ hypothesis is impacted by a higher trophic level, the effects seen are ‘top-down’ effects. These effects are utilised in CBC, which specifically involves maximisation of the impact of natural enemies by providing key ecological resources and by minimizing pesticide-induced mortality (Gurr et al. 2000b) (Figure 1-1).

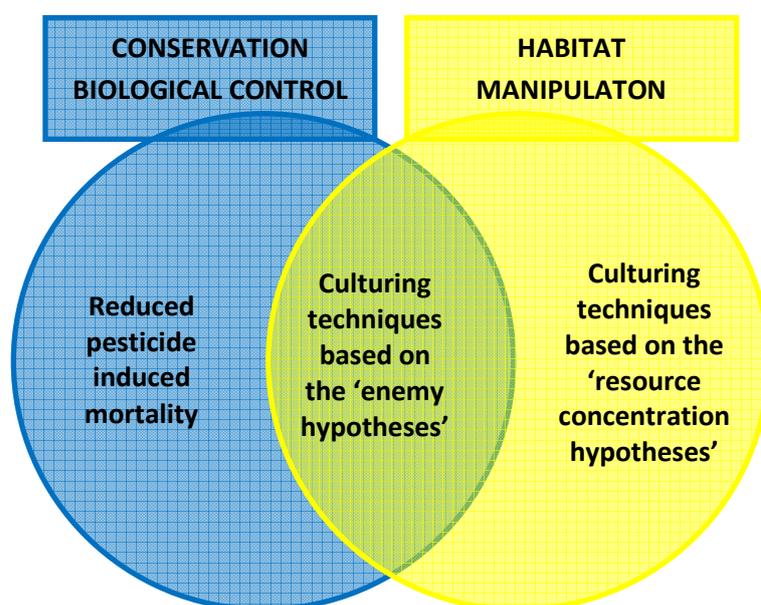


Figure 1-1. Conservation biological control shares common techniques with habitat manipulation but are not synonymous (From Gurr et al. (2000b)).

1.3. The application of floral resources within conservation biological control

One type of CBC is the provision of floral resources by non-crop plants grown within or around the crop and from which natural enemies can benefit (Tylianakis et al. 2004). The pest management purpose of these is to provide omnivorous natural enemies with alternative food sources, such as nectar and/or pollen. This may enable the natural enemies to remain in an area with temporarily low prey/host densities, preventing them from starving or emigrating (Polis and Strong 1996, Landis et al. 2000, Olson et al. 2005). Dietary floral supplementation can also increase longevity, fecundity (Landis et al. 2000, Berndt and Wratten 2005,

Lavandero et al. 2005, Scarratt 2005) and other components of ecological ‘fitness’ and lead to a change in sex ratio (Landis et al. 2000, Berndt and Wratten 2005, Scarratt 2005) and in dispersal ability of natural enemies (Wanner et al. 2006). For pro-ovigenic parasitoids, which emerge from the pupae with all or nearly all of their eggs mature (Flanders 1950), floral resources can lead to an increase in fecundity due to prolonged longevity (Gurr et al. 2005) while for synovigenic species, that continuously mature eggs throughout their reproductive life (Flanders 1950), floral resources can increase life-time fecundity due to prolonged longevity and egg-production rate (Gurr et al. 2005).

To optimise the provision of floral resources, these must be of the ‘right’ kind (Landis et al. 1999). The floral attractiveness (Wäckers 2005) as well as seasonal (Abe and Kamo 2003) and diurnal (Kovacs et al. 1995, Kovacs 1996) availability (Waller et al. 1981, Wäckers 2005), accessibility (Vattala et al. 2006) and suitability of the nectar and pollen for the natural enemy and also the effect on the pest need to be considered for each specific case when deploying floral resources (Wäckers 2005). A logical hierarchy for measures of success of using floral resource subsidies in CBC would be to answer the questions given by Gurr et al. (2007) (Table 1-1).

Table 1-1. The hierarchy for measure of success of using floral resource subsidies in CBC (From Gurr et al. 2007).

-
1. Aggregation of natural enemies at or near the flowers.
 2. An enhancement of the natural enemies' 'fitness' (longevity, fecundity and search efficiency).
 3. An increase in parasitism or predation rate of the pest.
 4. A decrease in the pest population density.
 5. The pest populations are brought below the relevant economic threshold (so avoiding the need to apply insecticides).
-

However, the level of dependence of natural enemies on non-prey foods varies between true omnivores that feed on prey and plant-provided resources in one life stage (Wäckers and van Rijn 2005) to life-history omnivores that feed on plant-provided food at least during part of its life-cycle (Polis and Strong 1996). For true omnivores, supplementation of floral resources may cause a decrease in predation rate because the predator becomes satiated through feeding

on plant resources (Jonsson et al. 2009a). Provision of nectar and pollen can decrease the predation rate by ladybeetles (Spellman et al. 2006, Brown and Mathews 2007), predatory mites (Wei and Walde 1997) and brown lacewings (Robinson et al. 2008). The developmental stage at which plant provided foods can be utilised and what type – nectar, pollen or both – varies within consumer groups. Adult green lacewings and hoverflies require both nectar and pollen (Stelzl 1991) while adult parasitoid flies and wasps use only nectar (Gilbert and Jarvis 1998). Ladybirds benefit from nectar and pollen as adults and juveniles (Pemberton and Vandenberg 1993), while parasitoids benefit from nectar only as adults (Wäckers and van Rijn 2005). Active pollen-feeding to supplement the diet is common among predacious arthropods such as hover flies, ladybirds, green lacewings and predatory mites (Phytoseiidae) (Wäckers 2005).

Some studies show how the application of floral resources to agro-ecosystems can increase pest control efficiency by natural enemies (Landis et al. 2000, Berndt and Wratten 2005, Lavandero et al. 2005, Scarratt 2005, Irvin et al. 2006). Jonsson et al. (2009a) found that between 1998 and 2007 the success rate of increasing predation or parasitism rate through habitat manipulation in the form of floral subsidies was clearly successful in seven out of eleven cases. For the same period, one of five studies showed a clear decrease in pest densities and one of two studies showed a reduction in crop damage when floral resources were deployed (Jonsson et al. 2009a).

1.4. The application of synthetically produced herbivore-induced plant volatiles within conservation biological control

Plants respond to herbivore attack through induced direct or indirect defences (Karban and Baldwin 1997). In direct defence the chemicals produced by the plant directly target the herbivore (Lou and Baldwin 2003) ('bottom-up' control), while in indirect defence the chemicals increase herbivore mortality through the recruitment of natural enemies ('top-down' control) (Kessler and Baldwin 2001). The production of herbivore-induced plant volatiles (HIPVs) is a form of induced plant defence that may influence both the abundance of natural enemies and of herbivores (Dicke et al. 1990a, Dicke et al. 1990b, Petterson et al. 1994). Production of HIPVs is induced by herbivore feeding damage (Dicke et al. 1990b, Geervliet et al. 1997, Geervliet et al. 1998) or by egg deposition on the plant (Hilker and Meiners 2002).

The effect of plant-produced HIPVs on the behaviour of natural enemies has been demonstrated in the laboratory for many systems. For example, many plant species release HIPV signals which attract natural enemies of their herbivores. These include Lima beans *Phaseolus lunatus* L. (Fabaceae) (Dicke et al. 1990a), maize *Zea mays* L. (Poaceae) (Turlings et al. 1990), cotton *Gossypium hirsutum* L. (Malvaceae) (McCall et al. 1993, McCall et al. 1994), tomato *Lycopersicon esculentum* L. (Solanaceae) (Thaler 1999), cabbage *Brassica oleracea* L. (Brassicaceae) (Agelopoulos and Keller 1994) and Brussels sprouts *B. oleracea gemmifera* L. (Brassicaceae) (Steinberg et al. 1992).

HIPVs can both attract natural enemies to herbivore-affected plants and trigger surrounding plants to ‘switch on’ their own production of direct or indirect defences (inter-plant communication) (Dicke and Bruin 2001). The compounds can also function as ‘primers’ signalling to surrounding un-damaged plants an impending herbivore attack without initiating a full defence response (Engelberth et al. 2004). Primed plants respond more efficiently once under herbivore attack (Engelberth et al. 2004, Heil and Kost 2006). HIPVs can both induce and prime the indirect defence mechanism of extrafloral nectar (EFN) production in Lima beans and they may also serve as a cue for the plant, enabling a more specific production of EFN by the plant (Heil and Kost 2006). Also plants’ floral nectar production can be affected by HIPVs. Black mustard *Brassica nigra* L. (Brassicaceae) treated with jasmonic acid reduces nectar secretion and its glucose and fructose concentrations (Bruinsma et al. 2008).

HIPVs can be synthetically produced and some of these attract natural enemies of herbivores (Thaler 1999, Kessler and Baldwin 2001, James 2003b, 2005, James and Grasswitz 2005). Several authors have pointed out the potential application of HIPVs for pest suppression within sustainable crop management regimes (Dicke et al. 1990a, Thaler 1999, Dicke and Hilker 2003, Kessler et al. 2004, Halitschke et al. 2008, Howe and Jander 2008, Khan et al. 2008). Caution is required, however, as it is possible that effects on second and fourth trophic levels may occur.

1.5. Effects of floral resources and herbivore-induced plant volatiles on arthropods from the second and fourth trophic level

Both HIPVs and floral resources may invoke unwanted side effects by attracting and/or enhancing the fitness of herbivores (Dicke and Minkenbergh 1991, Finidori-Logli et al. 1996, Baggen et al. 1999, Lavandero et al. 2006) and higher order parasitoids (Araj et al. 2008,

Jonsson et al. 2009b). Attraction of higher order parasitoids may cause un-wanted trophic cascades. These can occur in a four trophic level food web, such as crop plants - herbivorous pests – natural enemy - higher order natural enemy, when the floral resource enhances the top predator (higher order natural enemy) more than the lower level natural enemy. This results in lower abundance of the lower level natural enemy and a higher abundance of the mid level consumers (herbivorous pest) causing a reduction in the abundance of basal producers (crop plants) (Carpenter and Kitchell 1993).

Consequently, it is important to ensure the provided floral resources benefit natural enemies but not the pest or enemies of the natural enemies (Baggen et al. 1999, Adler and Bronstein 2004, Araj et al. 2006, Jonsson et al. 2009b). The effect of supplementation of floral resources on the population dynamics of the species involved is likely to differ according to the food web being investigated. To successfully supply floral resources to enhance biological control, knowledge of the system, including the biology of the herbivore, its enemies and their interactions with the resource subsidy is needed (Lavandero et al. 2006).

HIPVs represent a complex chemosensory information package and it is difficult to predict whether herbivores are attracted to them (Dicke and Minkenberg 1991, Finidori-Logli et al. 1996, Dicke and van Loon 2000) or repelled (Dicke and van Loon 2000). Usually, studies of the application of HIPVs in the field have focused on their capacity to attract natural enemies, even though HIPVs can affect the behaviour or physiology of organisms at multiple trophic-levels (Dicke and van Loon 2000) including herbivores (Halitschke et al. 2008). One explanation for the attraction of herbivores could be the large quantity of volatiles released from herbivore infested plants compared with from uninfested ones, making them more apparent to herbivores (Feeny 1976, Vet and Dicke 1992). HIPVs may also signify plants that have been weakened and are therefore more susceptible to herbivores (Dicke and van Loon 2000). However, more studies on how HIPVs affect second trophic level arthropods (Takabayashi and Dicke 1996, Dicke et al. 2003, Turlings and Ton 2006) under field conditions are needed (Takabayashi and Dicke 1996, Dicke et al. 2003).

Consequently, deploying synthetic HIPVs alone or combining their attraction property with floral resources in the ‘attract and reward’ concept needs research specific to each herbivore/natural enemy system as an effect on the second and/or fourth trophic level may either increase herbivore abundance or trigger an unwanted trophic cascade.

1.6. 'Attract and reward' - a concept to potentially enhance conservation biological control

CBC research has historically focused on manipulation of agro-ecosystems by supplying resources needed for the natural enemy population to flourish (Landis et al. 2000, Jonsson et al. 2008) and minimizing the pesticide induced mortality (Gurr et al. 2000b). However, one way to potentially increase the pest-reducing efficiency of the natural enemies further is by combining two aspects: 1) attraction of beneficial insects to the crop ('attract') and 2) maintaining the natural enemy population within the crop ('reward') and maximising its impact on the pest population. The 'attract' method, based on the use of synthetically produced HIPVs has shown good results in increasing natural enemy numbers near the crop (Thaler 1999, Kessler and Baldwin 2001, James 2003a, James and Grasswitz 2005). Natural enemies can benefit from floral resources ('reward'), for example, by increased 'ecological fitness' (longevity and fecundity) (Landis et al. 2000, Berndt and Wratten 2005, Lavandero et al. 2005, Scarratt 2005). Also, the sex ratio of the natural enemy population can go from a male bias towards a 1:1 female/male ratio (Landis et al. 2000, Berndt et al. 2002, Berndt and Wratten 2005, Scarratt 2005). It is hoped that the two 'eco-technologies' will work together synergistically, increasing the pest population reduction efficiency of the natural enemies.

1.7. The study system

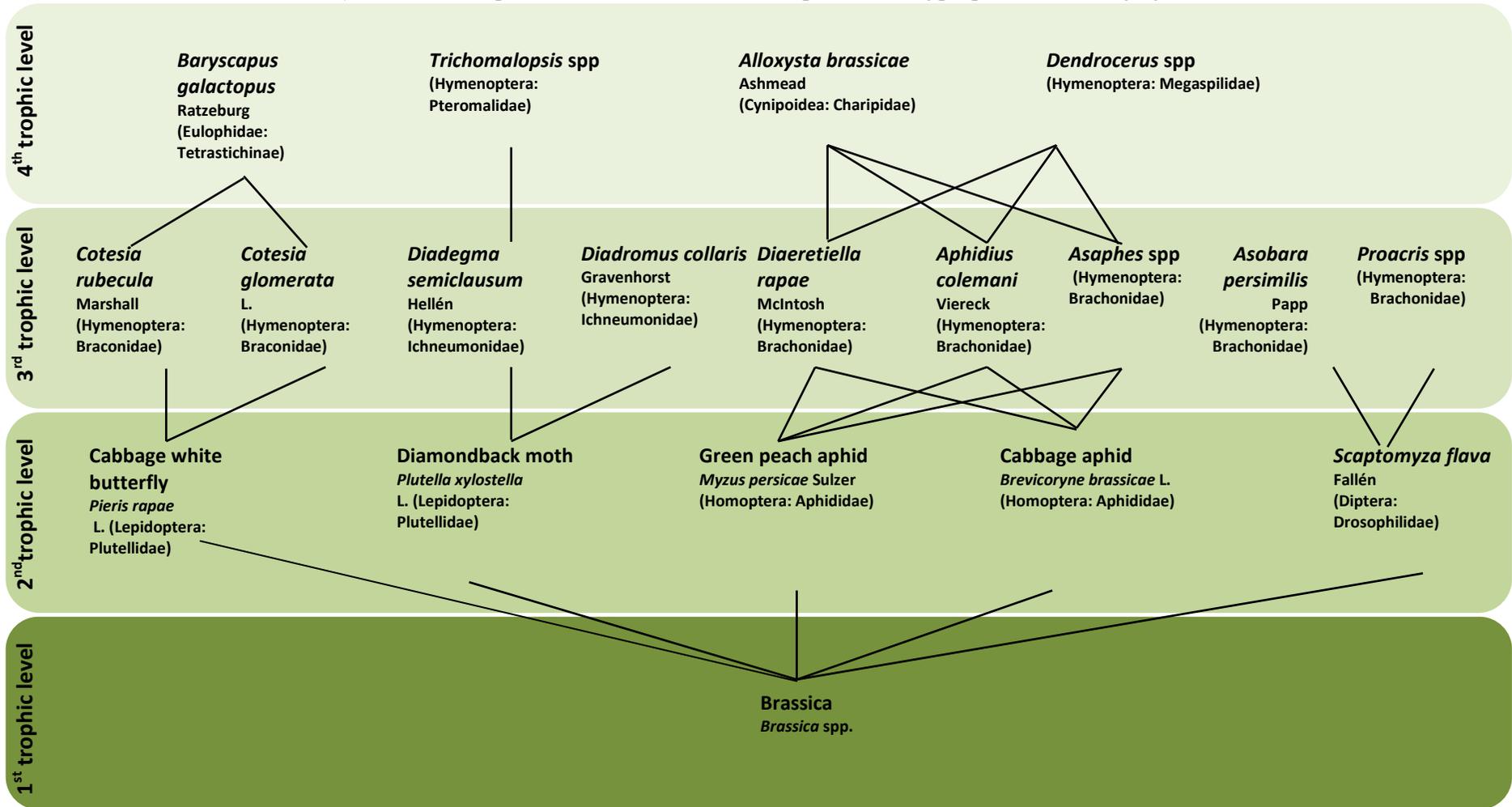
1.7.1. Brassicas and their most common pests and their natural enemies

The study crop throughout the work is forage brassica (*Brassica* spp (L.) Brassicaceae). This crop was chosen as forage brassicas comprise the largest area of cultivated crops in New Zealand with 250 000 ha grown per year (Wilson et al. 2004) and a relatively high number of abundant pests are easily sampled.

The focus of the studies was arthropods from three trophic levels namely, the main brassica pest species, their natural enemies and the natural enemies of the natural enemies based on known occurrence in New Zealand (Figure 1-2 a and b). Some of the most common herbivores reaching pest status in brassicas are the cabbage white butterfly *Pieris rapae* L. (Lepidoptera: Pieridae), the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), the cabbage aphid *Brevicoryne brassicae* L. (Cameron and Walker 2000, Kok 2004) and the green peach aphid *Myzus persicae* Sultzer (Homoptera: Aphididae) (Cameron and Walker 2000). The leafmining fly *Scaptomyza flava* Fallén (Diptera: Drosophilidae) is

readily found on some Brassicaceae in New Zealand (Martin et al. 2006) and a integrated pest management strategy has been developed in New Zealand for its control in European vegetable brassica crops (Cameron and Walker 2000). DBM has become one of the world's most destructive insects on cruciferous crops. The annual cost of managing this herbivore is estimated to be US\$ 1 billion (Talekar and Shelton 1993).

a) The four trophic level brassica-herbivore-parasitoid-hyperparasitoid study system



b) The four trophic level brassica-herbivore-predator-parasitoid study system

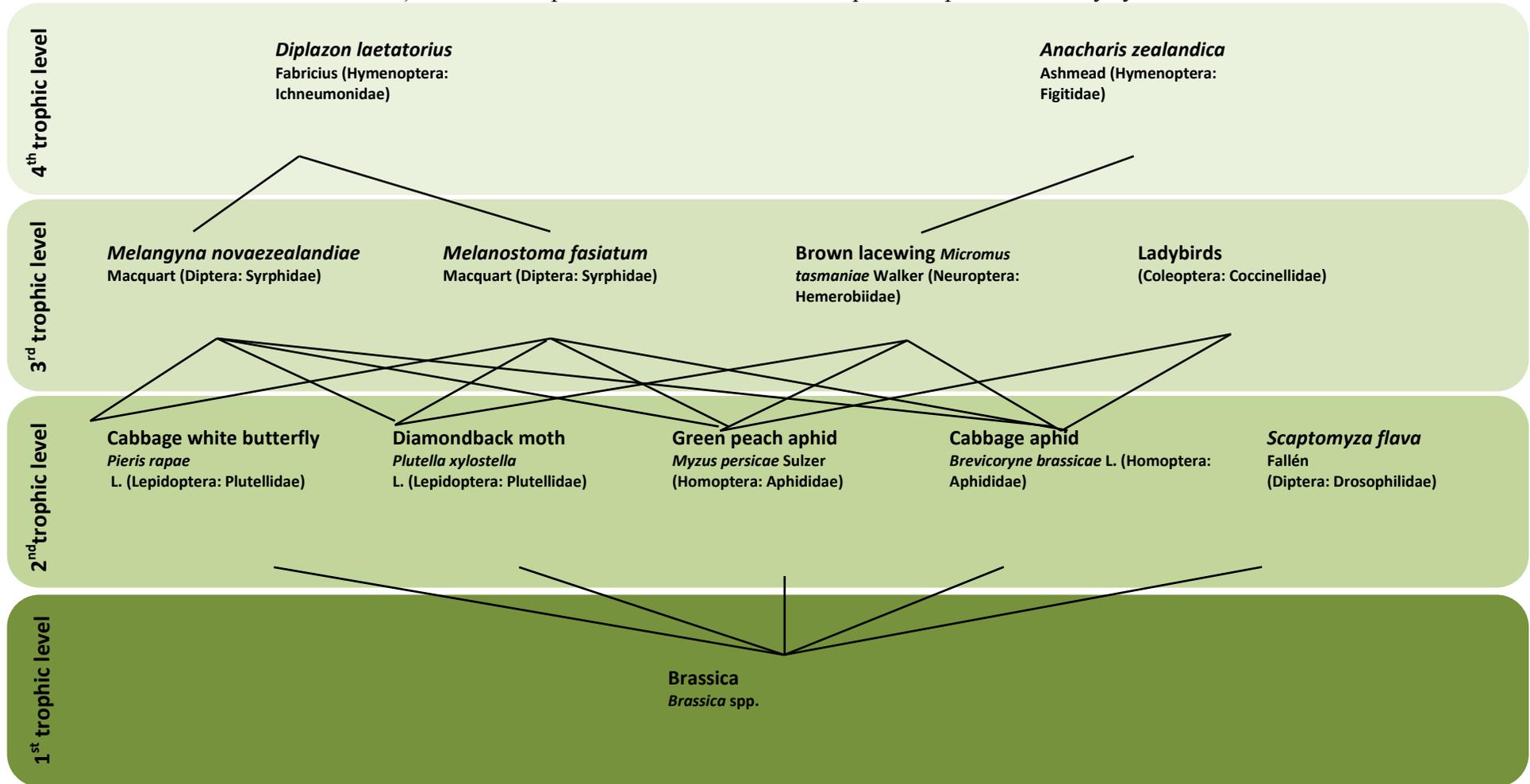


Figure 1-2. The relationship between the species involved in the four trophic level study system. As the system consists of both parasitic and predatory natural enemies (third trophic level), it has been split up between these (a and b respectively) to simplify the figure. Note, the lines between the predators and the herbivores are only indicative of the main prey consumed as these predators are not monophagous on a specific prey species. The first and second trophic level consists of the same species in both figures, with brassica being the crop.

1.7.2. The herbivore-induced plant volatile and the floral resource deployed in the study system

The HIPV used for the ‘attract’ part of ‘attract and reward’ is methyl salicylate (MeSA), a compound that is naturally produced by brassicas in response to herbivore damage (Wilson et al. 2004) and MeSA and salicylic acid (SA) are key compounds in the shikimic acid pathway involved in the induction of direct and indirect plant defences (Geervliet et al. 1997, van Poecke et al. 2001). Synthetically-produced MeSA increases the abundance of micro-Hymenoptera in grape vines and hop yards (Dicke et al. 2003). Two parasitoid species with hosts on brassicas, *Cotesia vestalis* Haliday and *C. glomerata* L. (Hymenoptera: Braconidae), perceive MeSA produced by herbivore-damaged Brussels sprouts *B. oleracea gemmifera*, L. (Brassicaceae) (James and Price 2004, James et al. 2005, James and Grasswitz 2005). This and the accessibility of synthetically produced MeSA in the form of slow release sachets on are the reasons why it is used throughout the work in this thesis.

The flowering plant used for the ‘reward’ part of this work is buckwheat *Fagopyrum esculentum* Moench (Polygonaceae). Lavandero *et al.* (2005) showed that the supplementation with flowering buckwheat in broccoli *Brassica oleracea* L. increased parasitism rate by *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) with up to twice as many DBM larva parasitized when buckwheat was supplied, compared to when none was present. In those experiments, the maximum parasitism rate for non-buckwheat treatments was 40% while the corresponding value was 75% for the buckwheat treatments (Lavandero et al. 2005). Consequently, buckwheat supplementation in the field can contribute to the pest management of DBM (Lavandero et al. 2005). This and other evidence (Stephens et al. 1998, Berndt et al. 2002, Tylianakis et al. 2004, Irvin et al. 2006) of the value of buckwheat in CBC are the reasons why it is selected in this work. It also has the agronomic and experimental advantage of its seed being inexpensive, rapidly germinating and producing abundant flowers within a few weeks of sowing (Bowie et al. 1995)

1.8. Aims

Until now ‘attraction’ and ‘reward’ have not been combined. Therefore in this thesis the combination of these two ‘eco-technologies’ is examined experimentally in the field and laboratory through the provision of a flowering floral resource, buckwheat and a synthetically produced HIPV, MeSA.

The overall aim is to explore how ‘attract and reward’ affects arthropod abundance across three trophic levels (pest, natural enemies of the pest and natural enemies of the natural enemies) and, as a consequence biological control in brassicas. The objectives of the experiments described in Chapters 2-5 are to find answers to the following questions:

Chapter 2: Does synthetically-produced MeSA attract arthropods from three trophic levels when applied in the field compared to when no MeSA is used? Does the sex ratio differ between the arthropods attracted to MeSA compared to when no MeSA is applied?

Chapter 3: Do any of the treatments: MeSA, buckwheat (BW) or the combined MeSA+ BW, attract more arthropods from three different trophic levels than the crop alone (C)? Does the application of ‘attract and reward’ have a synergistic effect on the abundance of arthropods from three trophic levels? Are there any interactions between MeSA and BW in attracting arthropods from three trophic levels?

Is there a difference in the sex ratio between the arthropods attracted to MeSA, BW, MeSA+BW and C? Does the deployment of ‘attract and reward’ (MeSA+BW) have a synergistic effect on the abundance of males/females from three trophic levels compared with when only BW or MeSA is deployed? Are there any interactions between MeSA and BW in attracting males/ females from three trophic levels?

Chapter 4: Are there differences in herbivore crop damage, natural enemy and herbivore densities, parasitism, hyper-parasitism and predation rates when the above four treatments are deployed? Are there any interactions between MeSA and BW on the crop damage, natural enemy and herbivore densities, parasitism, hyper-parasitism and predation rates?

Chapter 5: Is the third trophic level aphid parasitoid *Aphidius colemani* attracted to two different concentrations of synthetically-produced MeSA diluted in Synertrol oil?

This chapter is based on the following publication:

Orre, G.U.S., Wratten, S.D., Jonsson, M., Hale, R. 2010. Effects of a herbivore-induced plant volatiles on arthropods from three trophic levels in brassica. *Biological Control*, **53**:62-67.

Chapter 2: Effects of MeSA on arthropods from three trophic levels in turnip *Brassica rapa* L.

2.1. Abstract

Synthetically-produced herbivore-induced plant volatiles (HIPVs) attract arthropod natural enemies, thereby showing potential for enhancing biological control. However, HIPVs can also affect the behaviour of arthropod herbivores and possibly higher-order natural enemies, potentially complicating the use of HIPVs as a component of conservation biological control (CBC).

The aim of the work in this chapter is to understand the effects of HIPVs on the abundance of arthropods at three trophic levels. In particular, their effects on the abundance of herbivores and enemies of natural enemies are investigated experimentally. A field experiment was carried out in a commercial field of turnip *Brassica rapa* L. (Brassicaceae) (cv. Green Globe). A randomized block design was used with a treatment of synthetically-produced methyl salicylate (MeSA), in the form of slow-release sachets and a control consisting of the crop alone. Yellow sticky traps were used for sampling aerial brassica pests, their natural enemies and fourth trophic-level natural enemies within the crop.

The abundance of the diamondback moth parasitoid *Diadegma semiclausum* (TL3)¹, the hoverfly *Melangyna novaezealandiae* (TL3), the brassica leafmining pest *Scaptomyza flava* (TL2)² and the lacewing parasitoid *Anacharis zealandica* (TL4)³ increased significantly in the MeSA treatment compared to the controls. Significantly more *D. semiclausum* (TL3), *M. novaezealandiae* (TL3) females were attracted to MeSA than were males.

These results are highly relevant to potential future application of HIPVs in pest management, as the attraction of arthropods within ‘un-targeted’ trophic-levels may either increase herbivore abundance and/or disrupt unwanted trophic cascades.

¹TL3 = trophic level 3

²TL2 = trophic level 2

³TL4 = trophic level 4

2.2. Introduction

The effect of plant-produced HIPVs on the behaviour of natural enemies has been demonstrated in the laboratory for many systems (Steinberg et al. 1992, McCall et al. 1993, Agelopoulos and Keller 1994, Thaler 1999). However, it is difficult to predict whether HIPVs will attract or repel herbivores (Dicke and van Loon 2000). For example, the herbivorous beetle *Maladera matrida* Argaman (Coleoptera: Scarabaeidae) is attracted to volatiles emitted by herbivore-infested *Duranata repens* L. (Verbenaceae) under laboratory conditions (Vet and Dicke 1992) while the spider mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae) avoids intraspecific overcrowding by settling only on plant leaves with low levels of HIPV production (Dicke et al. 1990a). HIPVs can also act as oviposition repellents for lepidopteran herbivores (De Moraes et al. 2001, Kessler and Baldwin 2001, Sanchez-Hernandez et al. 2006).

Synthetically produced HIPVs attract natural enemies of herbivores in many cropping systems. Applying synthetically produced methyl salicylate (MeSA) and (Z)-3-hexanyl acetate (HA) to hops *Humulus* spp. L. (Cannabaceae) and grape *Vitis* spp. L. (Vitaceae) vines increases the abundance of several species of predators and parasitoids (James 2003b, 2005, James and Grasswitz 2005). Tomato plants induced with jasmonic acid can double the rate of field parasitism of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) by *Hyposoter exiguae* Viereck (Hymenoptera: Ichneumonidae), an endoparasitic wasp (Thaler 1999). Applying synthetic versions of HIPVs naturally produced by *Nicotiana attenuata* Torr. ex S. Watson (Solanaceae) to plants in the field resulted in an increased number of *Manduca sexta* L. (Lepidoptera: Sphingidae) eggs predated by a generalist predator and a reduction in oviposition rate of *Manduca* spp.. As a consequence, the HIPV-mediated plant responses reduced the number of herbivores by 90% (Kessler and Baldwin 2001).

HIPVs show potential to be deployed within pest management (Dicke et al. 1990a, Thaler 1999, Dicke and Hilker 2003, Kessler et al. 2004, Halitschke et al. 2008, Howe and Jander 2008, Khan et al. 2008). However, more attention needs to be given to the possible capacity of HIPVs to attract organisms at multiple trophic levels (Dicke and van Loon 2000) including herbivores (Halitschke et al. 2008) when deployed in the field. For example wild type *N. attenuata* plants producing HIPVs had higher damage by flea beetles in the field compared to transgenic ones unable to release HIPVs (Halitschke et al. 2008).

The aim of this work is to understand how synthetically-produced MeSA deployed within a brassica crop affects arthropods at three trophic levels. Of particular interest are the effects on the second and fourth trophic level, as effects on these may disrupt biological control.

2.3. Methods

The effect of synthetically-produced methyl salicylate (MeSA) on the abundance of arthropods in turnip *Brassica rapa* L. (Brassicaceae) (cv. Green Globe) was tested. The study was carried out from April 24 to June 12 2007 in a 400m x 470m turnip field near Burnham, Canterbury, New Zealand.

The experiment was set up as randomized block design with 12 blocks, each having one treatment with a slow-release sachet of MeSA (P-240-lure, ChemTica International, Zeta Industrial Park, La Valencia, Heredia, Costa Rica) and a control consisting of the crop alone. Blocks were separated by 67m (east-west) and 80m (north-south) and the treatment and control were separated by 67m within each block.

Yellow sticky traps (24cm x 20cm) (Trappit, Agrisense-BCS-Ltd., UK, sourced from Fruitfed Supplies Ltd, New Zealand) were used to sample the number of flying pests and natural enemies at each plot. Each trap was attached 10cm above the average height of the turnip plants between two stakes and moved upwards as the plants grew, to maintain the distance above the plants. In each replicate of the HIPV treatment, one MeSA sachet was attached 1m above ground to a string between the wooden stakes, to ensure consistent dispersal of the volatile. According to the manufacturers, the volatile would last 'a season' but to ensure that the chemical did not run out, the sachets were replaced twice, on May 10 and May 31. The traps were replaced on May 4, 10, 17, 24 and 31. During the experimental period, the number of cabbage grey aphids (TL2) *Brevicoryne brassicae*, green peach aphids (TL2) *Myzus persicae*, larvae of the diamondback moth (TL2) (DBM) *Plutella xylostella* and cabbage white butterfly (TL2) (CWB) *Pieris rapae* were counted on all the leaves of 20 randomly chosen crop plants in a radius of 1.5m from the trap on April 24, May 4, 10, 17, 24 and June 12.

The traps were assessed for the most common brassica herbivores and their associated natural enemies and fourth trophic-level parasitoids (see Chapter Section 1.7. for list of species recorded). The number of individuals on each trap was counted and their sex was determined when possible.

The identification and sex determination of *S. flava* (TL2) were carried out in two steps, due to the high number of individuals caught throughout the season (a total of 23,892). First, all individuals were identified based on the characteristic traits of Drosophilidae using standard keys and reference specimens and then counted. Then, a sub-sample was taken from the traps from six of the blocks for one sampling period, April 24 – May 5 as this period had the highest number of *S. flava* (Figure 2-1b). Thirty randomly-chosen Drosophilidae were removed from each trap, identified to species and the sex determined using an unpublished key for *Scaptomyza* in New Zealand supplied by Dr N. Martin (Plant & Food Research, New Zealand). To assure the Drosophilidae species on the remaining traps were *S. flava* and to detect any potential changes in the sex ratio for this species over time, a further subsample was taken for each of the sampling periods. For one randomly chosen trap on each sampling period, 25 individuals were removed randomly, identified and their sex determined. For the data from the first two sampling periods (April 24-May 4 and May 4-10), all individuals were identified and their sex determined, as the total number of individuals for each plot was fewer than 25.

The number of *D. semiclausum* (TL3) and *A. zealandica* (TL4) captured per trap throughout the sampling period fitted a negative binomial distribution. Consequently, these data were analyzed using a generalized linear mixed model (GLMM) with a logratio-link. A fixed model was used for the MeSA treatment, sex, different sampling dates and all the interactions between these. A random model was used for block within sampling dates and the interactions between these.

No *M. novaezealandiae* were caught on the traps from the control plots. Consequently to analyse differences between sex a GLMM was used on the number of individuals caught on the treatment traps. There was no difference in the female: male ratio caught on the traps throughout the sampling period ($\chi^2=2.538$, $df=5$, $p=0.771$). The data fitted a Poisson distribution and a GLMM with a logarithm-link and a fixed model for sex and different sampling dates and a random model for block was used.

The difference in the total number of *S. flava* (TL2) per sampling period was too variable to be analyzed with a GLMM; numbers captured ranged from 11 to 8714 per trap. Consequently, the data were \log_{10} -transformed and analyzed using a residual maximum likelihood model (REML). A fixed model was used for the MeSA treatment, the different sampling dates and the interactions between these. A random model was used for block within sampling dates and the interactions between these. The difference in the proportion of females between the treatments for *S. flava* was analyzed using the sub-sampled individuals from six blocks. The

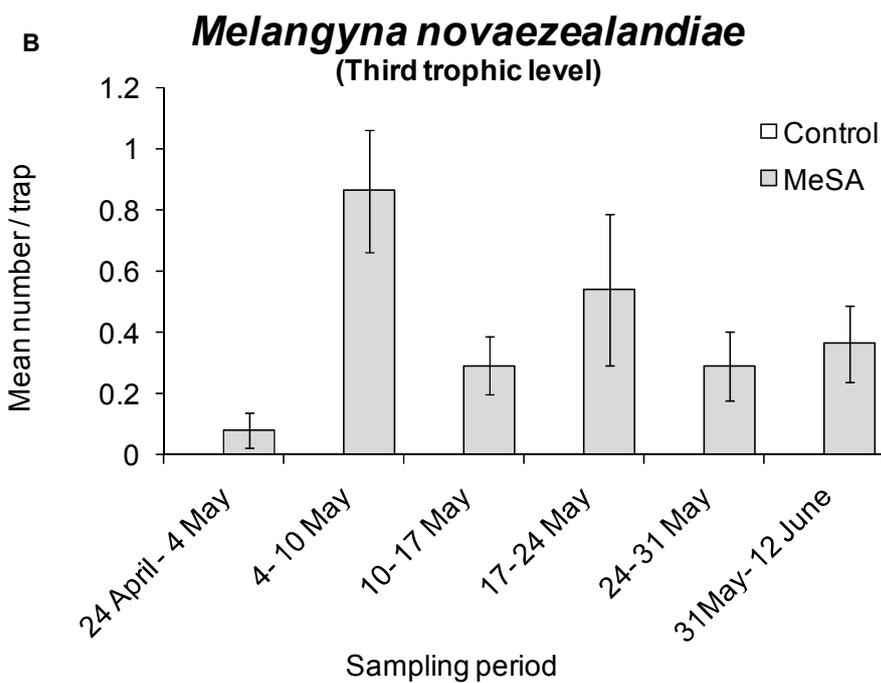
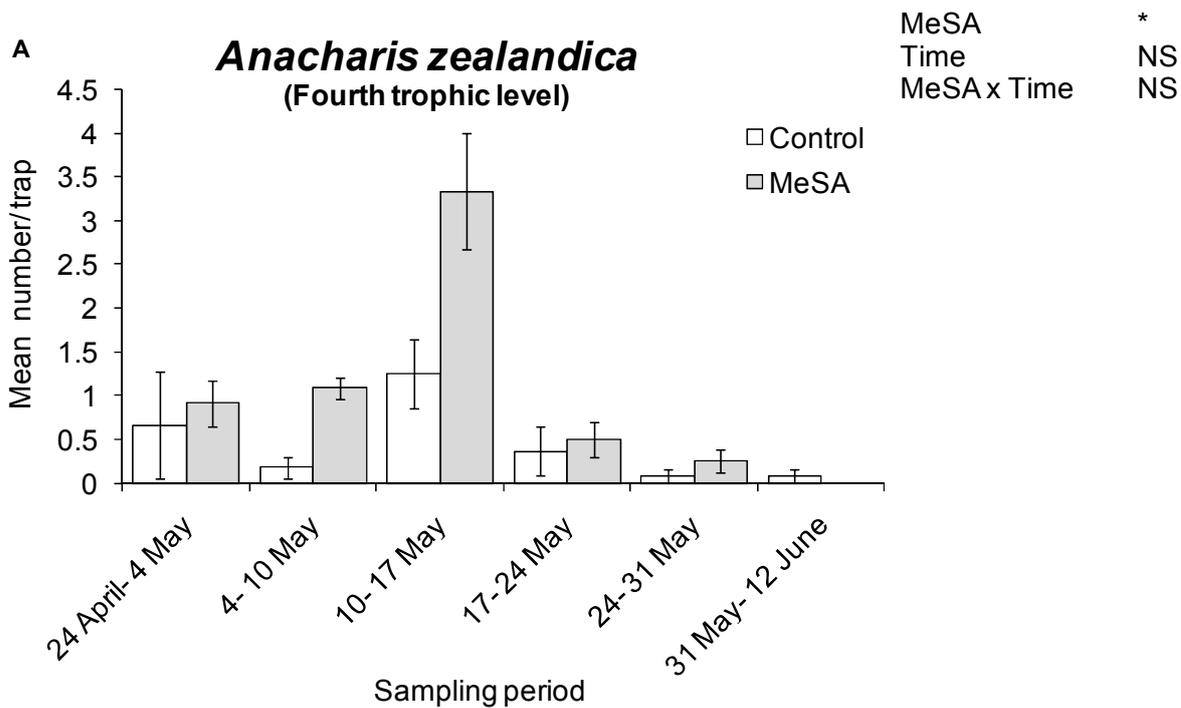
data were arcsine-transformed to fit the model's assumption of a normal distribution and homogeneous variances and were analyzed using a two-sample *t*-test.

2.4. Results

All sub-sampled Drosophilidae individuals were *S. flava* (TL2). Consequently, all the drosophilids were assumed to be *S. flava*. This was the only brassica herbivore found on the traps and the numbers of pests on the sampled crop plants were zero throughout the sampling period.

Of the third trophic level natural enemies expected on the traps, only *D. semiclausum* (TL3) and *M. novaezealandiae* (TL3) were caught in high enough numbers for the data to be analyzed. Only two brown lacewings and 31 *M. fasciatum* were caught on the traps. Consequently the numbers were too low for the data to be analyzed. *A. zealandica* was the only fourth trophic-level natural enemy found.

The third trophic-level parasitoid *D. semiclausum* and the hoverfly *M. novaezealandiae* (Figure 2-1b-c.), the fourth trophic-level parasitoid *A. zealandica* (Figure 2-1a) and the second trophic-level leafmining fly *S. flava* (Figure 2-1d) were significantly more abundant in the MeSA-treatment compared to the control (Table 2-1). Significantly more *D. semiclausum* (TL3) (Figure 2-2b) and *M. novaezealandiae* (TL3) (Figure 2-2c) females than males were attracted by MeSA. Both sexes were equally attracted for *A. zealandica* (TL4) ($p=0.923$, $df=1$) and *S. flava* ($p = 0.138$, $df = 10$, $t = 1.61$) (Table 2-1) (Figure 2-2a and d).



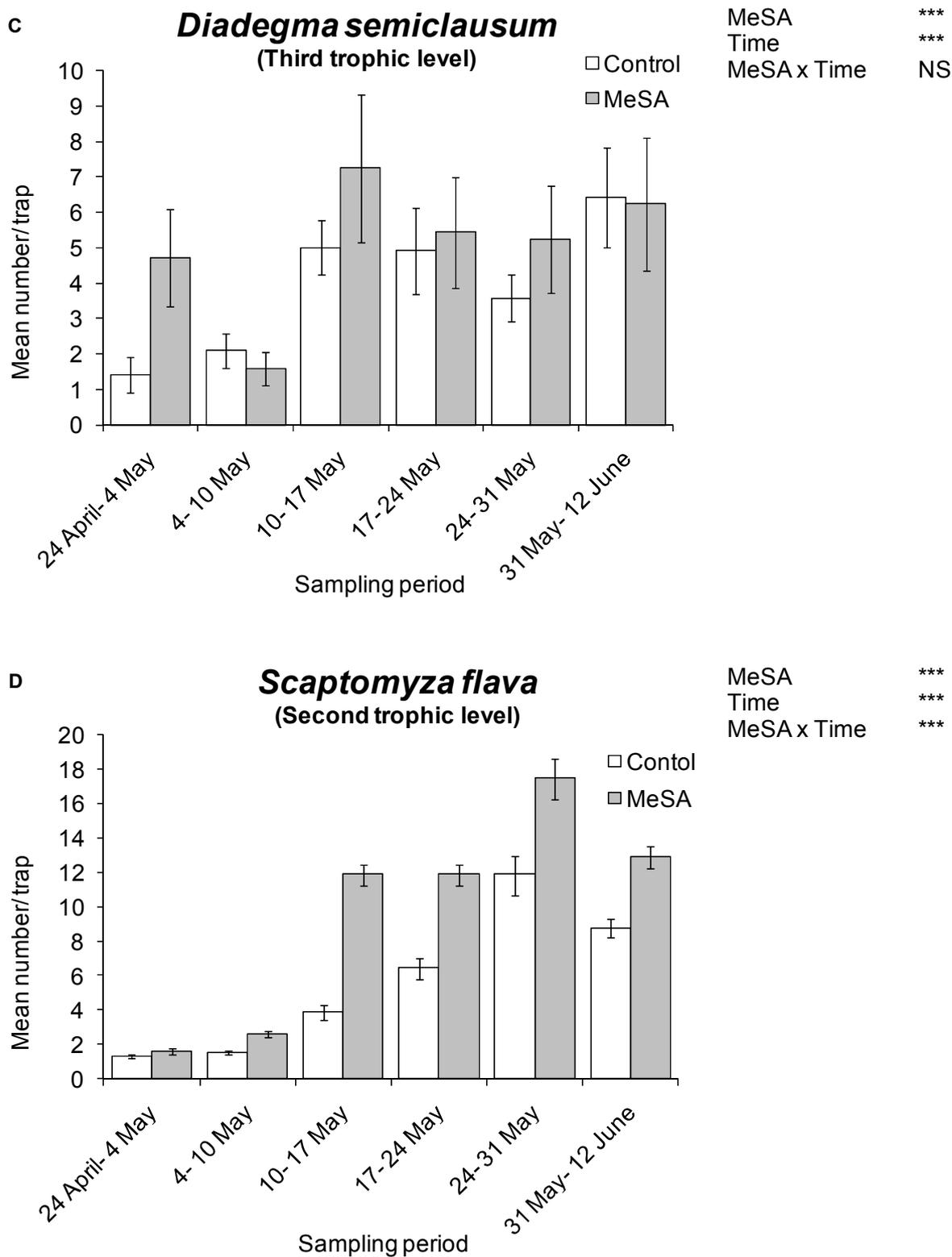


Figure 2-1. The Mean number (\pm SE) of individuals per trap for each species during each sampling period. The data for *S. flava* have been back-transformed from log₁₀-transformation.

Table 2-1. Generalized linear mixed model (GLMM) analysis for *D. semiclausum*, *M. novaezealandiae* and *A. zealandica* and a residual maximum likelihood model (REML) analysis for *S. flava* on the effect of fixed factors on their abundance. A two-sample *t*-test analysis for *S. flava* on differences in sex between treatments based on subsamples of 30 individuals taken for each treatment from six blocks. Treatment = the effect of either the MeSA-treatment + yellow sticky trap or the control consisting of the crop alone + yellow sticky trap.

Species	Factor	Wald statistic	df	p-value	Level of significance
<i>A. zealandica</i> (Fourth trophic level)	Treatment	5.35	1	0.021	*
	Sex	11.99	1	<0.001	***
	Time	10.13	5	0.071	NS
	Treatment x Sex	0.01	1	0.923	NS
	Treatment x Time	1.88	5	0.866	NS
	Sex x Time	0.68	5	0.984	NS
	Treatment x Sex x Time	0.00	5	1.00	NS
<i>D. semiclausum</i> (Third trophic level)	Treatment	55.73	1	<0.001	***
	Sex	3.54	1	0.060	NS
	Time	42.72	5	<0.001	***
	Treatment x Sex	4.89	1	0.027	*
	Treatment x Time	3.38	5	0.641	NS
	Sex x Time	41.85	5	<0.001	***
	Treatment x Sex x Time	4.25	5	0.514	NS
<i>M. novaezealandiae</i> (Third trophic level)	Sex	14.28	1	<0.001	***
	Time	16.61	5	0.005	**
<i>S. flava</i> (Second trophic level)	Treatment	204.49	1	<0.001	***
	Time	1033.83	5	<0.001	***
	Treatment x Time	28.23	5	<0.001	***
	Sex		10	0.138	NS

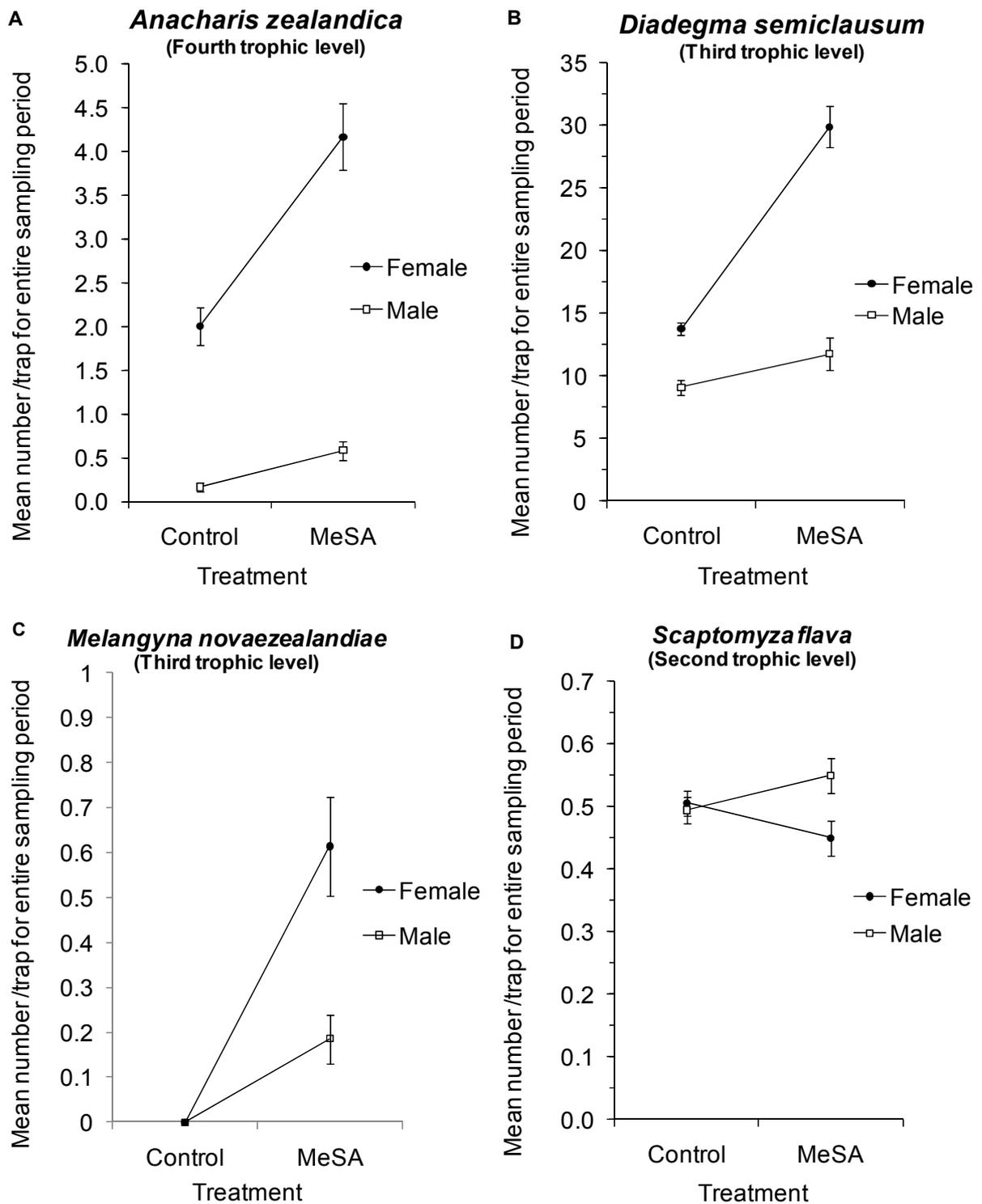


Figure 2-2. Interaction diagrams over the mean number (\pm SE) of males and females of three species caught per trap for the MeSA-treatment and the control traps over the entire sampling period. The data shown for *S. flava* are based on subsamples of 30 specimens taken for each treatment from six blocks. (The connecting lines between the data points do not represent a continuation between the data of each point but are used for ease of interpretation of the interaction diagram. (For further information on interaction diagrams see e.g. chapter 17 in Fowler (2008)).

2.5. Discussion

2.5.1. Attraction of arthropods from three trophic levels

Arthropods from three trophic levels were more abundant within the MeSA treatment than within the control in the field. This is the first time a higher-order parasitoid has been shown to be attracted to a synthetically-produced HIPV. The leafmining fly *S. flava* also responded positively to MeSA. Previous studies have shown herbivores to be attracted to HIPVs (Dicke and Minkenberg 1991, Finidori-Logli et al. 1996). Leafminers can induce the production of plant volatiles (Dicke and Minkenberg 1991, Finidori-Logli et al. 1996) and one explanation for the attraction of them could be the plants are more apparent to the herbivores (Feeny 1976, Vet and Dicke 1992) or weakened and are therefore more susceptible to them (Dicke and van Loon 2000). This is the first study showing that synthetically-produced MeSA increases the number of *D. semiclausum* (TL3) and *A. zealandica* (TL4). Hymenopterans have previously been shown to be affected by MeSA. The abundance of micro-Hymenoptera in grape vines and hop yards can be increased with synthetically-produced MeSA (James and Price 2004, James et al. 2005, James and Grasswitz 2005) and two other parasitoid species with hosts on brassica, *Cotesia vestalis* Haliday and *C. glomerata* L. (Hymenoptera: Braconidae), perceive MeSA produced by herbivore-damaged Brussels sprouts (Smid et al. 2002). Female *D. semiclausum* (TL3) were significantly more attracted to MeSA than males. Previous laboratory studies on *D. semiclausum* have shown female parasitoids to be more attracted to the chemical cues produced by host-infested cabbage plants than by un-infested ones (Ohara et al. 2003). However, the chemical structure of the cues attractive to *D. semiclausum* remains unknown (Ohara et al. 2003, Bukovinszky et al. 2005).

Specialist parasitoids can be expected to have evolved the capability to utilize host-specific volatiles for host search (Dicke and Hilker 2003, Zangerl 2003). The capability of natural enemies to learn to associate a specific HIPV or a blend of HIPVs with the presence of its prey/host has also been demonstrated for the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae) and its spider mite prey, *T. urticae* (Dicke et al. 1990c). Also, parasitoids ‘primed’ for host recognition increase their in-flight orientation towards host-related cues under laboratory conditions (Potting et al. 1999). A possible explanation for the increased numbers of *D. semiclausum* (TL3) females compared to males in response to MeSA may be that the former have evolved to associate MeSA with host presence.

M. novaezealandiae (TL3) was significantly more abundant in treatments with MeSA. Hoverflies have previously shown to be attracted to synthetically produced MeSA deployed in hop yards (James 2005).

MeSA has potential to function as a trigger for the plant's own production of HIPVs (Khan et al. 2008). It still remains unclear whether it is MeSA alone or a blend of volatiles produced by the host plant after the induction by MeSA that is attractive to the arthropods. It also remains unclear whether the significant results in number of arthropods captured were wholly a result of attraction from outside or within the field or whether there was a component of accumulation of arthropods through more staying than leaving the crop.

2.5.2. Prospects for biological control using MeSA

This work shows that the multi trophic attraction property of HIPVs can increase the abundance of both pests and the enemies of natural enemies, so HIPVs could potentially disrupt biological control. Consequently, the potential for HIPVs to be deployed in crop protection will strongly depend on the ability to manage compromising factors, such as the unintended attraction of additional herbivores (Turlings and Ton 2006) or higher-order parasitoids.

However, the data analysed in this chapter comprises trap catches only. The yellow sticky traps are attractive to herbivores (Sivapragasam and Saito 1986) and to natural enemies (Laubertie et al. 2006). Many insects are innately attracted to a few colours, in particular yellow, for which, for example, the CWB parasitoid, *Cotesia rubecula* Marshall (Hymenoptera: Braconidae) shows a preference when deprived of sugar foods (Wäckers 1994). To interpret the population and community consequences of the higher numbers of the arthropods caught from the second, third and fourth trophic levels requires on plant assessments of plant herbivore damage (trophic level two), predation/parasitism rates of herbivores by third trophic level natural enemies and parasitism rates of trophic level three by the fourth trophic level parasitoids. These questions will be addressed in Chapter 4.

Chapter 3: Attract and reward: the effect of combining buckwheat (*Fagopyrum esculentum*) and methyl salicylate on arthropods from three trophic levels in kale (*Brassica oleracea*) L.

3.1. Abstract

Floral resource subsidies can increase the ecological fitness of natural enemies of herbivores and are used as a habitat manipulation tool within conservation biological control (CBC). Herbivore-induced plant volatiles (HIPVs) attract arthropod natural enemies. One way to potentially enhance CBC is the combination of the two ‘eco-technologies’, synthetically produced HIPVs with the deployment of floral resource subsidies in a concept termed ‘attract and reward’. However, both HIPVs and floral resources can affect the behaviour of arthropod herbivores and higher-order natural enemies, potentially complicating the use of these within CBC. In this work, a synthetically produced HIPV, methyl salicylate (MeSA) is combined with a floral resource, buckwheat *Fagopyrum esculentum* Moench (Polygonaceae). The aim of the work in this chapter is to understand possible synergies in ‘attract and reward’ in influencing the abundance of arthropods at three trophic levels. A field experiment was carried out in a commercial field of kale *Brassica oleracea* L. (Brassicaceae) (cv. Sovereign). A factorial design was used with two treatments: with or without buckwheat and with or without MeSA. The control (without MeSA and without buckwheat) consisted of the crop alone. Yellow sticky traps were used for sampling aerial brassica pests, their natural enemies and fourth trophic-level natural enemies within the crop.

The abundance of the diamondback moth (DBM) *Plutella xylostella* (TL2) parasitoids *Diadegma semiclausum*¹(TL3), *Diadromus collaris* (TL3), the cabbage white butterfly²(TL2) (CWB) *Pieris rapae* parasitoid *Cotesia* spp. (TL3), the omnivorous brown lacewing *Micromus tasmaniae* (TL3) and its parasitoid *Anacharis zealandica*³(TL4) increased significantly in treatments with buckwheat compared to treatments with no buckwheat. The abundance of *A. zealandica* (TL4) was significantly lower in treatments with MeSA compared

¹TL3 = trophic level 3

²TL2 = trophic level 2

³TL4 = trophic level 4

to ones without. The hoverfly *Melanostoma fasciatum* (TL3) was significantly more abundant in treatments with MeSA than treatments without MeSA but significantly less abundant in treatments with buckwheat compared to ones with no buckwheat.

Significantly fewer male than female *D. semiclausum* (TL3) were attracted to the MeSA only treatments with a sex ratio of 1:1 for MeSA treatments while treatments with buckwheat had 60% males and 40% females.

There is a clear preference of natural enemies either to buckwheat or MeSA but none of the arthropod species showed a synergistic effect in their abundance when the A&R was deployed. Rather, in some cases one treatment could have a negative effect on the abundance of the natural enemy as in the case with *M. fasciatum* that appeared deterred by buckwheat. These results are highly relevant to potential future deployment of the concept ‘attract and reward’ as a pest management tool, as the attraction of arthropods in ‘un-targeted’ trophic-levels and the deterrence of some species of natural enemies may disrupt CBC.

3.2. Introduction

One form of CBC is the supplementation of dietary floral resource subsidies for natural enemies. These can increase natural enemy longevity, fecundity (Landis et al. 2000, Berndt and Wratten 2005, Lavandero et al. 2005, Scarratt 2005, Irvin et al. 2006) and other components of ecological ‘fitness’ (Chapter Section 1. 1.) and can lead to a change in sex ratio (Landis et al. 2000, Berndt and Wratten 2005, Scarratt 2005) and in dispersal ability (Wanner et al. 2006) and host/prey search area (Kean et al. 2003) of natural enemies. The results from field trials with floral resource subsidies have been mixed. For example, Jonsson et al. (2009a) found that of the 11 studies that were published between 1998- 2007, on the effect of floral subsidies on natural enemies of invasive pests in different agricultural systems, seven demonstrated an increase in predation/parasitism rates and one showed effects at pest population and crop level (Jonsson et al. 2009a).

In this chapter the potential to further increase the pest-reducing efficiency of natural enemies is explored. Floral resource subsidies are, for the first time combined with the natural enemy attraction properties of synthetically produced HIPVs (Thaler 1999, Kessler and Baldwin 2001, James 2003b, 2005, James and Grasswitz 2005) in a concept termed ‘attract and reward’ (A&R) (Jonsson et al. 2008, Khan et al. 2008) (Chapter 1.6.). Both HIPVs (Dicke et al. 1990a, Thaler 1999, Dicke and Hilker 2003, Kessler et al. 2004, Halitschke et al. 2008,

Howe and Jander 2008, Khan et al. 2008) and floral resources (Gurr et al. 2000b, Gurr et al. 2004, Heimpel and Jervis 2005, Wilkinson and Landis 2005, Jonsson et al. 2009a) show potential to be deployed within pest management. It is predicted that the natural enemy attraction properties of HIPVs would increase the movement of natural enemies into the crop from surrounding habitats ('attract') and that the floral subsidies deployed within the crop would be utilised by the attracted natural enemies which would remain longer within the crop ('reward'). The idea is that the two 'eco-technologies' will work together synergistically, increasing the natural enemies' efficiency to a level greater than when the two 'eco-technologies' are deployed separately. However, they can both invoke unwanted side effects by benefiting arthropods from second (Dicke and Minkenberg 1991, Finidori-Logli et al. 1996, Baggen et al. 1999, Lavandero et al. 2006) and fourth trophic levels (Araj et al. 2008, Jonsson et al. 2009b).

This chapter uses the same forage brassica model explored in Chapter 2 but here MeSA, the chosen HIPV to be deployed within the experiments of this thesis is combined with a floral resource supplementation in the form of buckwheat *Fagopyrum esculentum* (see Chapter Section 1.7.2.). Previous field studies on the application of synthetically produced MeSA have shown it to attract natural enemies of herbivores in hops and grapes (James 2003b, James and Grasswitz 2005). Floral resources have previously been deployed within brassica crops (Morris and Frank 2000, Lavandero et al. 2005, Lee and Heimpel 2005) and buckwheat has previously been successfully deployed within brassicas (Lavandero et al. 2005, Lee and Heimpel 2005). Supplementation with flowering buckwheat in broccoli *Brassica oleracea* L. increased the parasitism rate by *D. semiclausum* of the diamondback moth (DBM) with more than twice as many DBM larvae parasitized when buckwheat was supplied compared to when no buckwheat was present. The maximum parasitism rate was increased by 88% for buckwheat treatments compared to non-buckwheat treatments (Lavandero et al. 2005). In cabbage *B. oleracea* L. (Brassicaceae), flowering borders of buckwheat increased parasitism rates of *Voria ruralis* Fallén (Diptera: Tachinidae) on *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) larvae, *Cotesia rubecula* on cabbage white butterflies (CWB) and *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae) on DBM (Lee and Heimpel 2005).

The aim of the work in this chapter is to explore the concept of A&R and its effect on the abundance of arthropods from three trophic levels. Of interest are any synergistic effects between the two 'eco-technologies' and any negative interactions between the two. Also, effects on the second and fourth trophic level are of interest as these may disrupt biological control.

3.3. Methods

3.3.1. Field design and sampling methods

The effect of synthetically-produced methyl salicylate (MeSA), flowering buckwheat (BW) and the combination of the MeSA and BW on the abundance of arthropods in kale (*B. oleracea* cv Sovereign) was tested. The study was carried out from January 30 to March 3 2008 across three different kale fields (Figure 3-1) at Lincoln University Research Farm, Silverwood, Hororata, Canterbury, New Zealand. The experiment was set up as a randomized block design with four blocks in field 1, three in field 2 and two in field 3 (Figure 3-2).



Figure 3-1. Field locations on Silverwood farm. The experiment was carried out in three kale fields.

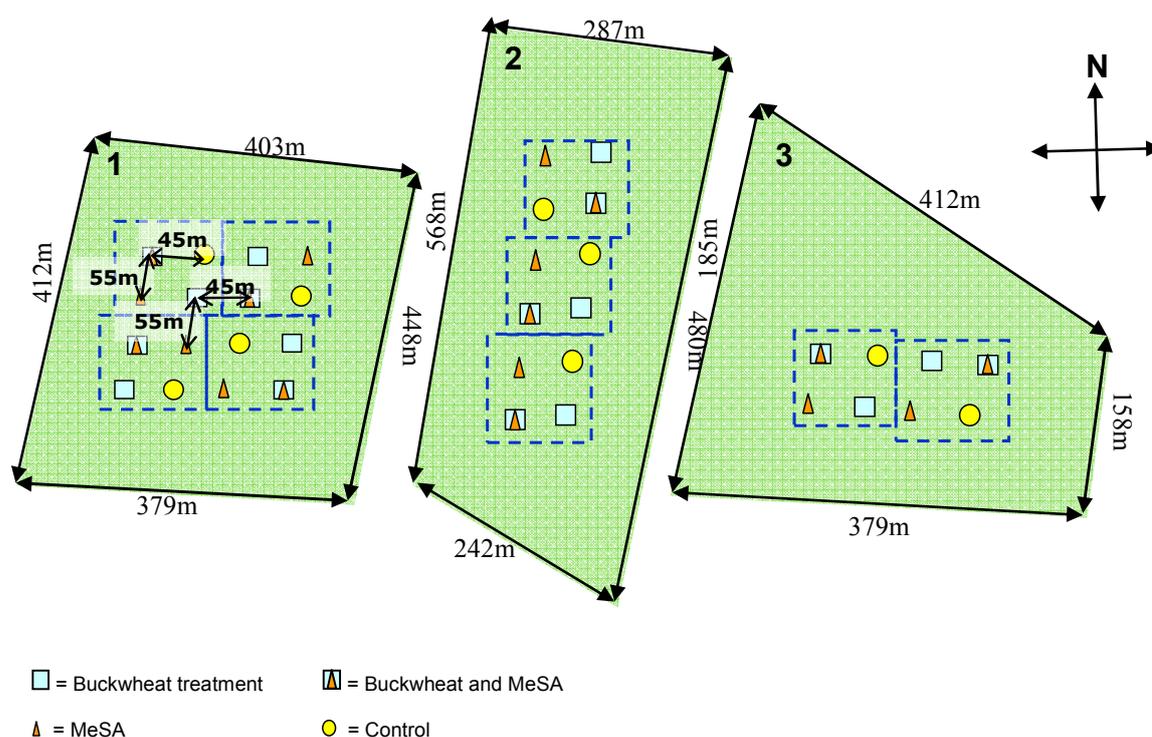


Figure 3-2. The location of the blocks and the plots in the three fields. Each block was separated by 45m (east-west) and 55m (north-south) and the centre of each plot within a block was separated by the same distances. Dotted blue line - - - encloses the four plots each block consisted of. Note: figure not to scale.

Each block consisted of three treatments and a control. The treatments were one treatment with a slow-release sachet of MeSA (MeSA) (P-240-lure, ChemTica International, Zeta Industrial Park, La Valencia, Heredia, Costa Rica), one with a 3m x 3m flowering buckwheat plot (BW), one with a slow release sachet of MeSA combined with a 3m x 3m plot of flowering buckwheat (MeSA+BW) and a control (C) consisting of the crop alone. A 50 m margin was left around the edge of each field to avoid possible edge effects. Blocks were separated by 45m (east-west) and 55m (north-south) and the treatments and control plots within each block were separated by the same distances respectively (Figure 3-2).

Yellow sticky traps (24cm x 20cm) (Trappit, Agrisense-BCS-Ltd., UK, sourced from Fruitfed Supplies Ltd, New Zealand) were used to sample the number of aerial brassica pests and their natural enemies at each plot. Each trap was attached 10cm above the mean height of the kale plants between two stakes and moved upwards as the plants grew, to maintain the distance above the plants. In each replicate of the HIPV treatment, one MeSA sachet was attached 1m above ground to a string between the wooden stakes, to ensure the volatile would disperse throughout the field (Figure 3-3). According to the manufacturers, the volatile would last ‘a season’ but to ensure that the chemical did not run out, the sachets were replaced twice, once on February 13 and once February 27. The traps were replaced on February 6, 13, 20 and 27.

In treatment plots with buckwheat, buckwheat seeds were sown in 3 m x 3 m squares in the centre of each plot. The first lot of buckwheat seeds were sown on December 19, 2007. Space was left between the lines of buckwheat and new seeds were sown on January 9 and 30 2008, to ensure continuous presence of flowering plants during the experimental period.

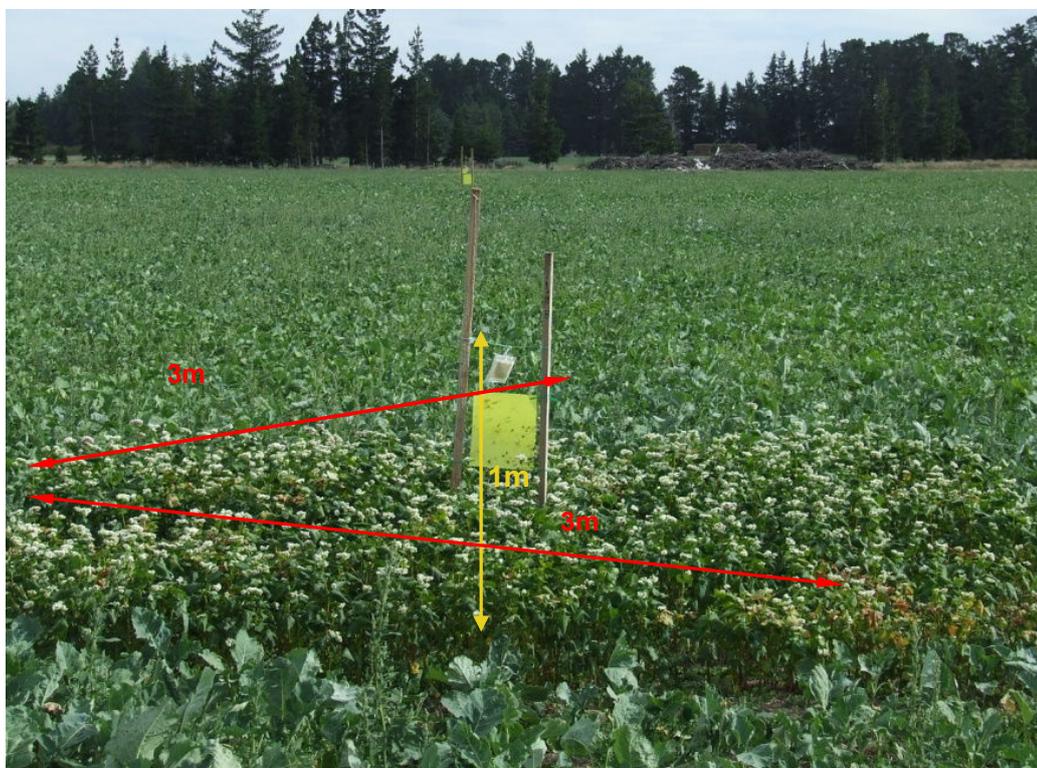


Figure 3-3. The layout of a plot with the combined treatment of MeSA and a 3m x 3m plot of buckwheat.

During the experimental period the number of cabbage grey aphids, green peach aphids, larvae of the DBM and CWB and *Scaptomyza flava* leaf mines were counted on all the leaves of 20 randomly chosen kale plants in a radius of 1.5m from the trap on March 11.

The traps were assessed for the most common brassica herbivores and their associated natural enemies and fourth trophic-level parasitoids (see 1.7. for list of species recorded). The number of individuals on each trap was counted and the sex was determined for the species that could be sex determined without being removed from the traps these were *D. semiclausum* (TL3), *D. collaris* (TL3), *M. fasciatum* (TL3), *M. tasmaniae* (TL3) and *A. zealandica* (TL4).

3.3.2. Statistical analysis

The number of *D. semiclausum* (TL3), *D. collaris* (TL3), *Cotesia* spp. (TL3), *M. fasciatum* (TL3), the brown lacewing *M. tasmaniae* (TL3) and *A. zealandica* (TL4) captured per trap throughout the sampling period fitted a negative binomial distribution. Consequently, these data were analyzed using a generalized linear mixed model (GLMM) with a logarithm-link. A

fixed model was used for the MeSA-treatment, BW-treatment, sex, different sampling dates and all the interactions between these and a random model for block within sampling dates and the interactions between these. Sex was excluded from the fixed model for *Cotesia* spp. (TL3) and the brown lacewing (TL3) for which sex could not be determined on the traps.

However, only 7 *D. collaris* (TL3) were caught on the traps during the last two sampling periods (Figure 3-4b). To be able to include sex as a variable in the analysis of the species these two sampling periods were not included. Only a total of 36 *A. zealandica* (TL4) were caught on the traps during the total sampling period. This was too few to be able to include sex as a variable in the analysis.

The level of significance was Bonferroni-adjusted to $p < 0.0083 = *$, $p \leq 0.0008 = **$ and $p \leq 0.0002 = ***$ due to the high number of species (six) analysed per trap.

The number of pests and leaf mines on the plants from the on plant pest count was too low to be analysed.

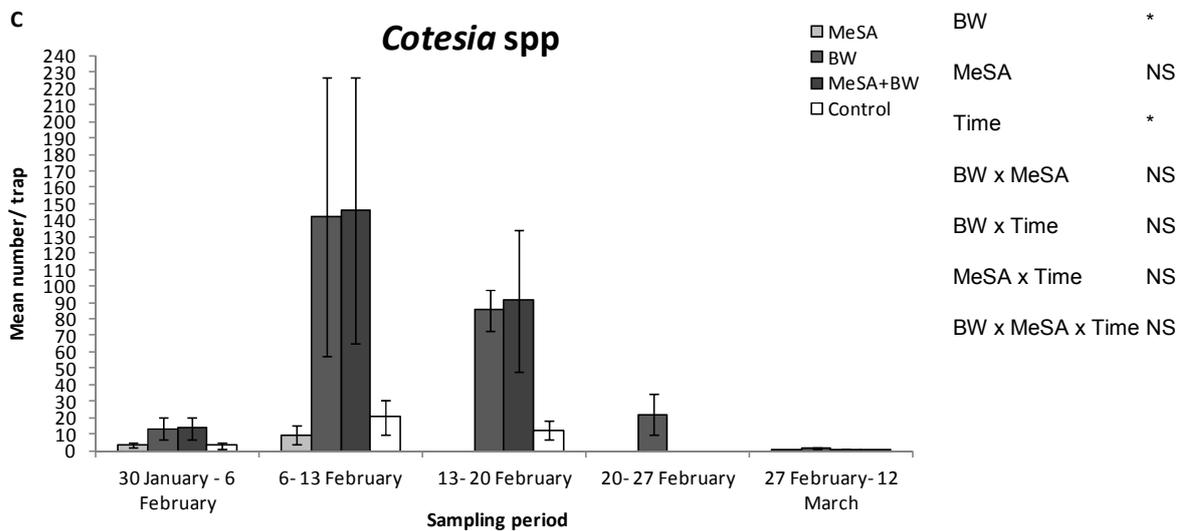
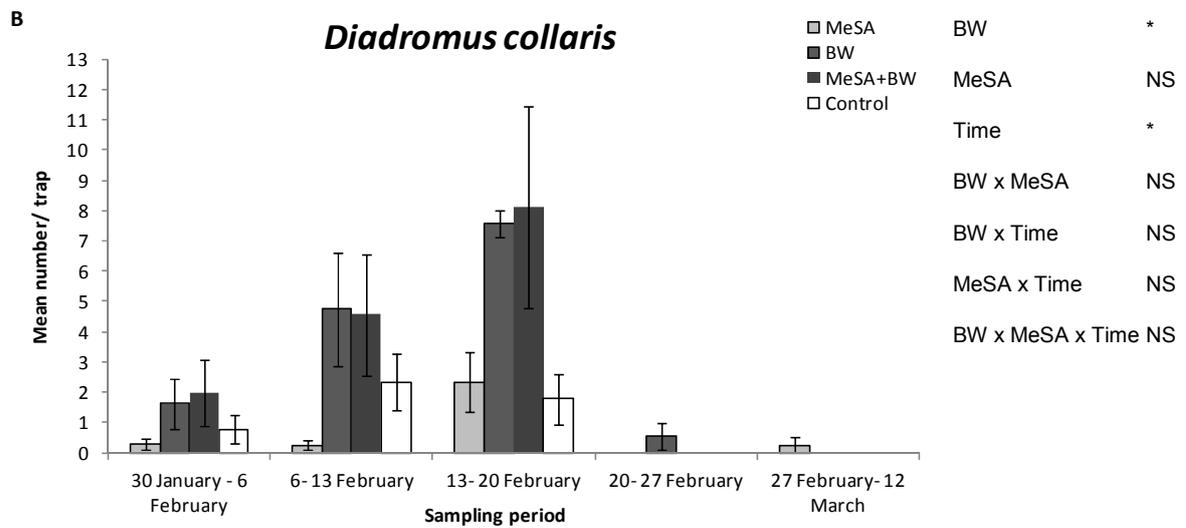
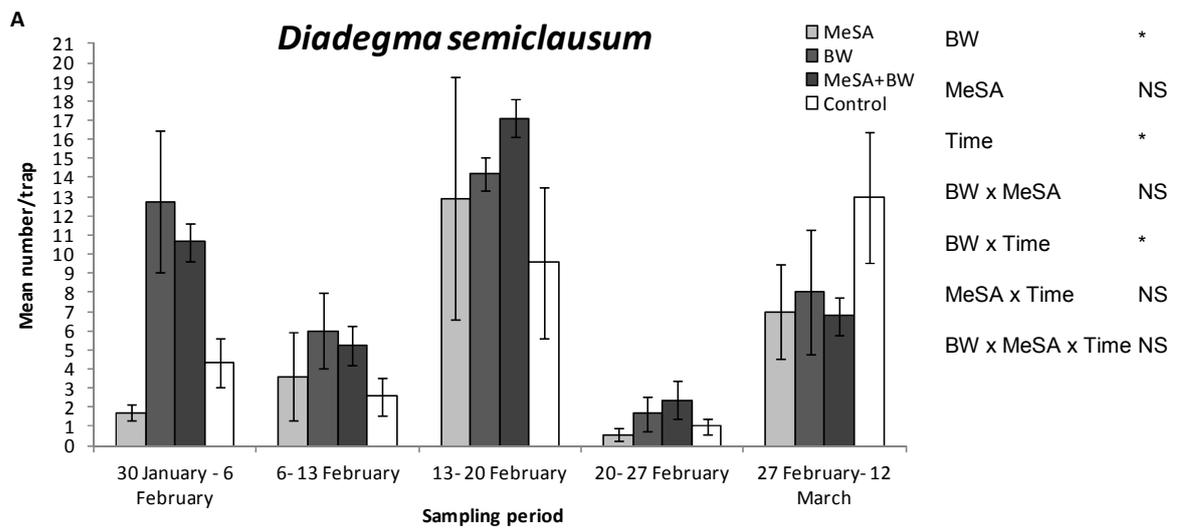
3.4. Results

Of the third trophic level natural enemies expected on the traps (see Chapter Section 1.7. for list of species recorded), *D. semiclausum*, *D. collaris*, *Cotesia* spp., *M. fasciatum* and the brown lacewing were caught in high enough numbers for the data to be analysed. *A. zealandica* was the only fourth trophic-level natural enemy found.

Treatments with buckwheat had a significantly higher abundance of the two diamondback moth (TL2) (DBM) parasitoids *D. semiclausum* (TL3) (Figure 3-4a), *D. collaris* (TL3) (Figure 3-4b), the cabbage white butterfly (TL2) (CWB) parasitoid *Cotesia* spp. (TL3) (Figure 3-4c), the omnivorous brown lacewing *M. tasmaniae* (TL3) (Figure 3-4e) and its parasitoid *A. zealandica* (TL4) (Figure 3-4f) (Table 3-1). The abundance of *A. zealandica* (TL4) was significantly lower in treatments with MeSA compared to ones without (Table 3-1). The hoverfly *M. fasciatum* (TL3) was significantly more abundant in treatments with MeSA than treatments without but significantly less abundant in treatments with buckwheat compared to ones with no buckwheat (Figure 3-4d) (Table 3-1).

Significantly fewer male than female *D. semiclausum* (TL3) were attracted to the MeSA only treatments (Table 3-1) with a sex ratio of 1:1 for MeSA treatments while treatments with buckwheat had 60% males and 40% females (Figure 3-5).

The numbers of brassica herbivores on the sampled crop plants from March 11 were too few to be analysed.



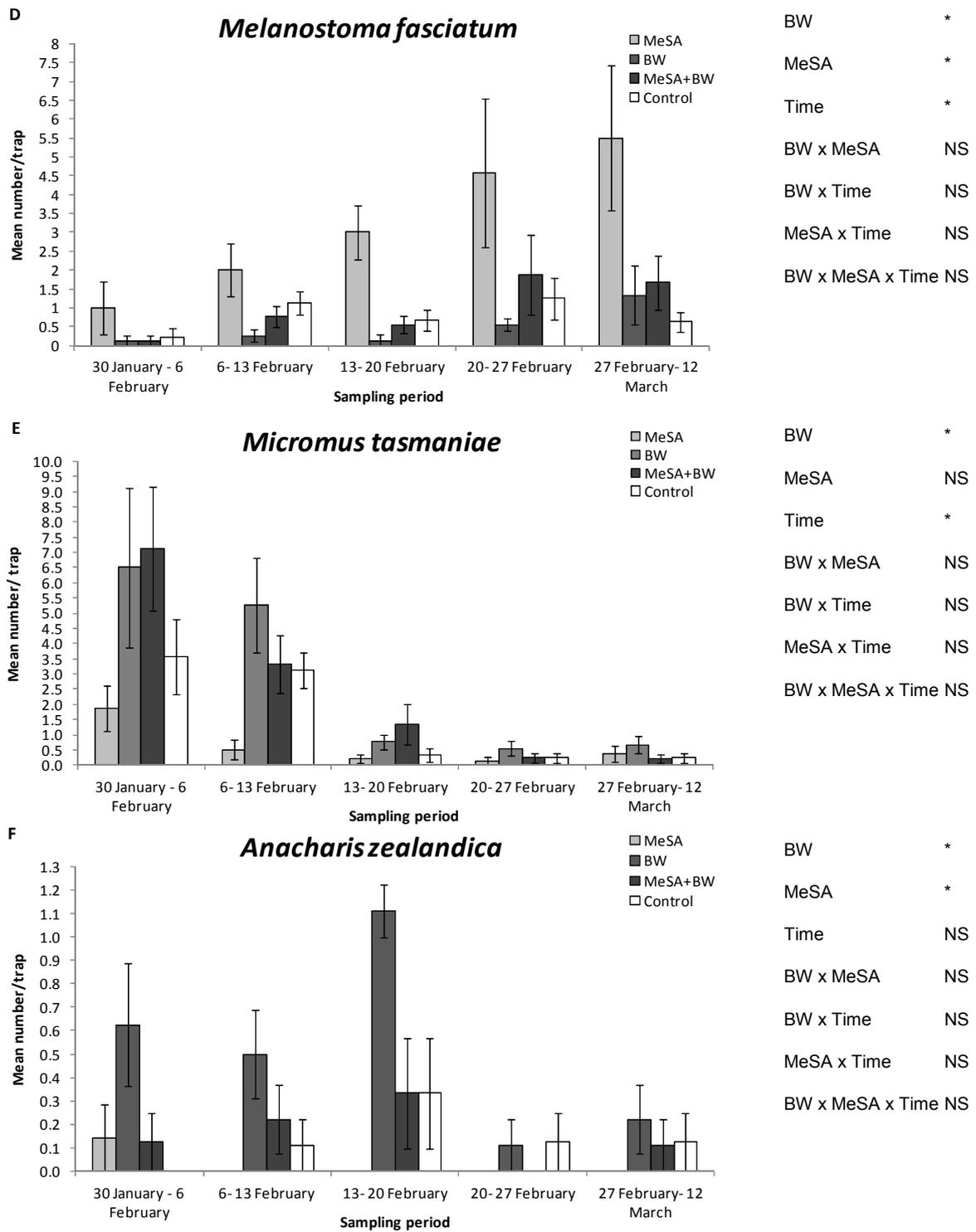


Figure 3-4. The mean number (\pm SE) of individuals per trap for each species during each sampling period. The significance levels have been Bonferroni-adjusted $p < 0.0083 = *$, $p \leq 0.0008 = **$ and $p \leq 0.0002 = ***$

Table 3-1. Generalized linear mixed model (GLMM) analysis for *Diadegma semiclausum*, *Diadromus collaris*, *Cotesia* spp. *Melanosoma fasciatum*, *Micromus tasmaniae* and *Anacharis zealandica*. MeSA = the effect of treatments with MeSA + yellow sticky trap. BW= the effect of treatments with buckwheat + yellow sticky trap. The significance levels have been Bonferroni-adjusted $p < 0.0083 = *$, $p \leq 0.0008 = **$ and $p \leq 0.0002 = *$**

Species	Factor	Wald statistic	df	p-value	Level of significance
<i>Diadegma semiclausum</i> (Third trophic level)	BW	9.52	1	0.002	*
	MeSA	5.06	1	0.025	NS
	Sex	26.87	1	<0.001	*
	Time	32.45	4	<0.001	*
	BW x MeSA	2.83	1	0.092	NS
	BW x Sex	1.15	1	0.283	NS
	MeSA x Sex	8.51	1	0.004	*
	BW x Time	29.13	4	<0.001	*
	MeSA x Time	2.07	4	0.723	NS
	Sex x Time	33.99	4	<0.001	*
	BW x MeSA x Sex	2.39	1	0.122	NS
	BW x MeSA x Time	9.95	4	0.041	NS
	BW x Sex x Time	0.52	4	0.972	NS
MeSA x Sex x Time	1.41	4	0.843	NS	
BW x MeSA x Sex x Time	4.33	4	0.363	NS	
<i>Diadromus collaris</i> (Third trophic level)	BW	81.3	1	<0.001	*
	MeSA	0.19	1	0.664	NS
	Sex	28.37	1	<0.001	*
	Time	21.66	2	0.005	NS
	BW x MeSA	3.75	1	0.054	NS
	BW x Sex	1.26	1	0.264	NS
	MeSA x Sex	4.07	1	0.045	NS
	BW x Time	3.75	2	0.156	NS
	MeSA x Time	1.12	2	0.572	NS
	Sex x Time	17.93	2	<0.001	*
	BW x MeSA x Sex	2.46	1	0.118	NS
	BW x MeSA x Time	9.78	2	0.009	NS
	BW x Sex x Time	0.24	2	0.885	NS
MeSA x Sex x Time	1.27	2	0.531	NS	
BW x MeSA x Sex x Time	0	2	1.000	NS	
<i>Cotesia</i> spp (Third trophic level)	BW	116.36	1	<0.001	*
	MeSA	0.01	1	0.926	NS
	Time	46.72	4	<0.001	*
	BW x MeSA	2.00	1	0.160	NS
	BW x Time	6.17	4	0.195	NS
	MeSA x Time	12.91	4	0.015	NS
	BW x MeSA x Time	12.45	4	0.018	NS
	<i>Melanosoma fasciatum</i> (Third trophic level)	BW	47.65	1	<0.001
MeSA		30.49	1	<0.001	*
Sex		0.39	1	0.535	NS
Time		17.8	4	<0.001	*
BW x MeSA		6.13	1	0.013	NS
BW x Sex		1.56	1	0.212	NS
MeSA x Sex		0.15	1	0.696	NS
BW x Time		2.29	4	0.682	NS
MeSA x Time		2.63	4	0.622	NS
Sex x Time		12.6	4	0.013	NS
BW x MeSA x Sex		5.66	1	0.017	NS
BW x MeSA x Time		4.59	4	0.332	NS
BW x Sex x Time		1.27	4	0.866	NS
MeSA x Sex x Time	9.34	4	0.053	NS	
BW x MeSA x Sex x Time	0.23	4	0.994	NS	
<i>Micromus tasmaniae</i> (Third trophic level)	BW	23.25	1	<0.001	*
	MeSA	4.37	1	0.039	NS
	Time	93.14	4	<0.001	*
	BW x MeSA	5.39	1	0.022	NS
	BW x Time	1.91	4	0.752	NS
	MeSA x Time	4.86	4	0.308	NS
	BW x MeSA x Time	4.08	4	0.400	NS
<i>Anacharis zealandica</i> (Fourth trophic level)	BW	19.96	1	<0.001	*
	MeSA	16.4	1	<0.001	*
	Time	3.3	4	0.521	NS
	BW x MeSA	2	1	0.160	NS
	BW x Time	9.36	4	0.059	NS
	MeSA x Time	2.28	4	0.685	NS
BW x MeSA x Time	0.07	4	0.999	NS	

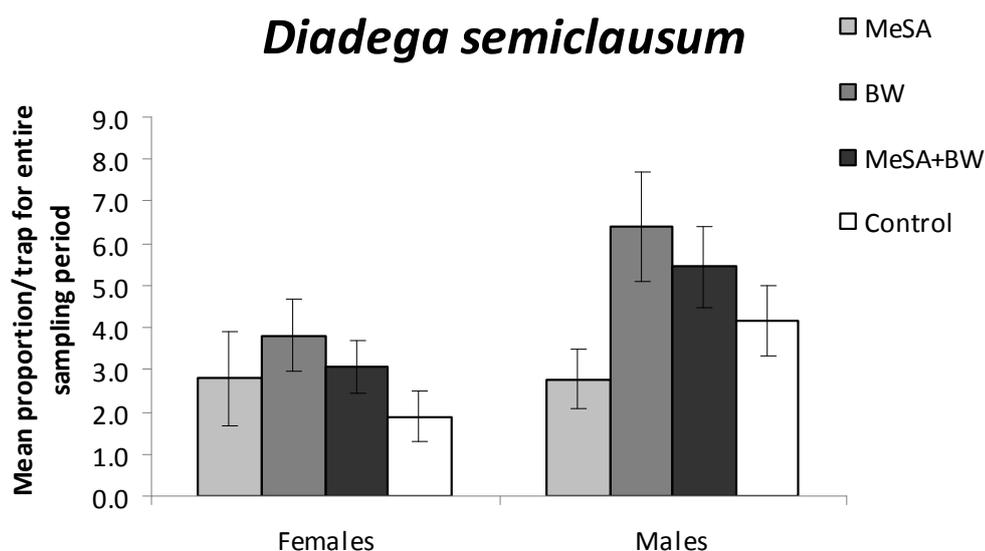


Figure 3-5. The mean number (\pm SE) of females and males of *Diadega semiclausum* caught per trap for the MeSA, buckwheat, MeSA + buckwheat-treatments and the control over the entire sampling period.

3.5. Discussion

Arthropods from two trophic levels were affected by the deployment of elements from ‘attract and reward’. For none of the species was there a synergistic effect between ‘attract’ (MeSA) and ‘reward’ (BW) on their abundance. The natural enemies were attracted either to buckwheat or MeSA. In one case, the natural enemy, *M. fasciatum* appears repelled by buckwheat.

Natural enemies that were more abundant in buckwheat treatments were *D. semiclausum*, *D. collaris* and *Cotesia* spp. and the brown lacewing *M. tasmaniae*. Parasitoids have previously been shown to benefit from buckwheat (Stephens et al. 1998, Berndt et al. 2002, Tylianakis et al. 2004, Lavandero et al. 2005, Lee and Heimpel 2005, Irvin et al. 2006). Both *Cotesia rubecula* (Lee and Heimpel 2005) and *D. semiclausum* (Lavandero et al. 2005) have in field studies shown an increased parasitism rate with access to buckwheat. *D. semiclausum* that has fed on buckwheat can have an increased maximum parasitism rate from 40% with no buckwheat to 75% (Lavandero et al. 2005).

Significantly fewer male than female *D. semiclausum* (TL3) were attracted to the MeSA only treatments with a sex ratio of 1:1 for MeSA treatments compared with 60% males and 40% females with buckwheat. Parasitoids can learn to associate a specific HIPV or a blend of

HIPVs with the presence of its host (Takabayashi and Dicke 1996). A specialist parasitoid, such as *D. semiclausum* may have evolved the capability to utilize host-specific volatiles for host search (Dicke and Hilker 2003, Zangerl 2003) and *D. semiclausum* females may have evolved to associate MeSA with host presence. *D. semiclausum* feeds on buckwheat (Lavandero et al. 2005). A possible explanation for the increased numbers of *D. semiclausum* males in treatments with buckwheat may be that the males ‘prefer’ feeding to mating. However, information on the feeding status (fed/unfed) and mating status (mated/unmated) of the males and females attracted are needed.

The effect of floral resources on the second trophic level true omnivorous brown lacewing depends on prey density, such as aphids (Jonsson et al. 2009b). Buckwheat has previously, under laboratory conditions shown to increase the longevity (Robinson et al. 2008) and in the field the number of eggs oviposited by the brown lacewing (Jonsson et al. 2009b) when aphid densities were low. Consequently, buckwheat can help lacewings survive periods with low density of prey and it can help them maintain a high rate of oviposition as long as some prey are available (Jonsson et al. 2009b). The density of aphids was low in this field study based on the data from the pest count on March 11. Therefore, the brown lacewing’s preference to buckwheat could be due to lack of aphids so the lacewing needed the buckwheat for survival.

The hoverfly *M. fasciatum* (TL3) was more abundant in treatments with MeSA than those without, while it was less abundant in treatments with buckwheat. The attraction of hoverflies to synthetically produced MeSA is in accordance with James’ (2005) findings, where synthetically produced MeSA deployed in a hop yard attracted species of hoverflies. In Chapter 2 *Melangyna novaezealandiae* (TL3) was attracted to MeSA. Hoverflies utilise buckwheat as a floral resource (Laubertie 2007). The lower number of hoverflies caught on the traps in the buckwheat treatments could be due to the hoverflies were more attracted to the flowers than to the sticky trap and therefore was caught in lower numbers on the traps.

A. zealandica, the fourth trophic level parasitoid of the omnivorous brown lacewing was more abundant in treatments with buckwheat than ones without. This supports the yellow water-trap data of Stephen et al. (1998). The parasitoid feeds on floral nectar of buckwheat and under laboratory conditions, both male and female *A. zealandica* parasitoids with access to buckwheat live 7-8 times longer than ones with access to water only (Jonsson et al. 2009b). In this study the brown lacewing, the host of *A. zealandica* was more abundant in treatments with buckwheat than the ones with none. Consequently, the reason for the parasitoids preference to buckwheat could be due to the presence of both host and food resource within these treatments. Contrary to the results from Chapter 2, *A. zealandica* was significantly lower

in treatments with MeSA compared to ones without. A potential explanation could be linked to its host, the brown lacewing. In the study from Chapter 2 the only two lacewings were caught on the traps throughout the sampling period. Hence, *A. zealandica* would have experienced a lack of hosts and was reacting on the only signal present within the crop, the treatment of synthetically produced MeSA. Other hymenopterans have previously been shown to be affected by MeSA and their abundance can be increased by the deployment of the synthetic version of volatile in the field (James and Price 2004, James et al. 2005, James and Grasswitz 2005). However, in this case there was a presence of host within the buckwheat and *A. zealandica* can benefit from the buckwheat (Jonsson et al. 2009b). Consequently, the parasitoid would have preferred buckwheat treatments to treatments with MeSA alone which lacked both hosts and a food resource.

MeSA has potential to function as a trigger for the plant's own production of HIPVs (Khan et al. 2008) it still remains unclear whether it is MeSA alone or a blend of volatiles produced by the host plant after the induction by MeSA that is attractive to the arthropods.

3.6. Prospect for biological control using 'attract an reward'

This work shows that both HIPVs and buckwheat can increase the abundance of most natural enemies. However, in none of the cases did the combined deployment of buckwheat and MeSA work synergistically on the abundance of any individual natural enemy species but rather, the natural enemies 'preferred' buckwheat or MeSA alone. In one case a deterrent effect of buckwheat on the natural enemy (TL3) could be seen, as in the case of *M. fasciatum*. Consequently, deploying buckwheat and MeSA together might compromise biological control due to deterrence or other negative interactions between the treatments decreasing the abundance of some natural enemies.

Buckwheat increased the abundance of enemies of natural enemies (TL4). Consequently, the potential for buckwheat and HIPVs to be deployment in crop protection will strongly depend on the ability to manage compromising factors such as the unintended attraction of additional herbivores (Turlings and Ton 2006) or higher order parasitoids (Baggen et al. 1999, Lavandero et al. 2006, Jonsson et al. 2009b).

As in Chapter 2 the data analysed in this chapter comprises trap catches only. The yellow sticky traps are attractive to herbivores (Sivapragasam and Saito 1986) and natural enemies (Laubertie et al. 2006). Many insects are innately attracted to a few colours, in particular

yellow, for which, for example, the parasitoid, *Cotesia rubecula* Marshall (Hymenoptera: Braconidae) shows a preference when deprived of sugar foods (Wäckers 1994). To interpret the population and community consequences of the negative interactions between the two treatments, the deterrence of some natural enemies and the increase in on-plant populations and in abundance of the second and fourth trophic level species requires assessment of increased rates of plant damage by herbivores and predation and parasitism rates by both third and fourth trophic-level natural enemies. These questions will be addressed in Chapter 4.

Chapter 4: ‘Attract and reward’: the effect of combining buckwheat (*Fagopyrum esculentum*) and methyl salicylate on herbivore densities, predation, parasitism and hyper-parasitism rates

4.1. Abstract

An increased natural enemy abundance is not enough to show that habitat manipulation techniques, such as the combination of floral resource supplementation with the deployment of synthetically produced herbivore-induced plant volatiles (HIPVs), are successful tools for improving conservation biological control (CBC). To be able to conclude that the habitat manipulation techniques are successful in bringing the herbivore population below a damage threshold, their effects on crop damage and on pest and higher-order natural enemy population densities (and ideally their impacts) need to be assessed.

Floral resource supplementation increases herbivore parasitism rates and attracts predators of herbivores. HIPVs can increase both parasitism and predation rates of herbivores in the field. In this work, a synthetically produced HIPV, methyl salicylate (MeSA) is combined with a floral resource, buckwheat (BW) *Fagopyrum esculentum* to test the ‘attract and reward’ (A&R) concept.

The aim of the work presented in this chapter is to understand the population and community consequences of A&R by assessing its effect on herbivore crop damage, natural enemy and herbivore densities and parasitism, hyper-parasitism and predation rates. A field experiment was carried out in a commercial field with a mixture of kale *Brassica oleracea* and swedes *Brassica napus*. A two factorial design was used with two treatments: with or without BW and with or without MeSA. The control (without MeSA and without BW) consisted of the crop alone. Herbivore damage by the leafmining fly *Scaptomyza flava*¹(TL2), densities of the cabbage aphid *Brevicoryne brassicae* (TL2), the green peach aphid *Myzus persicae* (TL2) and larvae of the cabbage white butterfly (TL2) (CWB) *Pieris rapae* and of the diamondback moth (TL2) (DBM) *Plutella xylostella* were assessed by on-plant counting on brassica plants.

¹ TL2 = trophic level 2

² TL3 = trophic level 3

The density of larvae of the natural enemies: ladybirds²(TL3) (Coleoptera, Coccinellidae), hoverflies (TL3) (Diptera: Syrphidae) and lacewings (TL3) (Neuroptera: Hemerobiidae) and aphid parasitism rates were also assessed. Rates of aphid hyper-parasitism were assessed by collecting parasitized aphids (aphid mummies) and rearing them to parasitoid emergence. For 24h predation rates, eggs of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) were used as sentinel prey.

Aphid densities, hyper-parasitism and predation rates were not significantly different between treatments while there was a significant increase in aphid parasitism rates for treatments with MeSA compared to treatments without. However overall, aphid parasitism rates were low, with a mean parasitism of 0.08 (SE±0.01) per plant, independent of treatment. This may explain the lack of treatment effect on aphid densities.

4.2. Introduction

For biological control to be successful, the pest population needs to be reduced to below an established damage threshold (Gurr et al. 2000a). Gurr et al. (2007) pointed out the importance of assessing the effect of habitat manipulation techniques on pest populations and crop damage rates to evaluate the success of conservation biological control (CBC) (Chapter 1 Table 1-1). However, few studies have included the effect CBC on the first, the second (Jonsson et al. 2009a) and the fourth trophic level (Gurr et al. 2000b). Previous work on the deployment of synthetically-produced herbivore-induced plant volatiles (HIPVs) within CBC has mainly focused on the increased abundance of natural enemies within the crop (James 2003b, a, James and Price 2004, James 2005, James et al. 2005, James and Grasswitz 2005), only a few studies have shown effects on predation or parasitism rates (Thaler 1999, Kessler and Baldwin 2001). However, to be able to draw any conclusions regarding the potential success of deploying any type of habitat manipulation technique within CBC, both direct (increased predation and/or parasitism rates) and indirect effects (increased higher-order parasitism rates) of the manipulation on pest populations and the crop needs to be assessed.

This chapter uses the same forage brassica model explored in Chapters 2 and 3 and combines MeSA with buckwheat (BW) *Fagopyrum esculentum* the HIPV and floral resource deployed within the experiments of this thesis in the concept termed ‘attract and reward’(A&R) (Jonsson et al. 2008, Khan et al. 2008) (Chapter Section 1.6.).

The application of floral resources as a tool in CBC increases pest parasitism rates in different agricultural systems including brassicas (Jonsson et al. 2009a, Lavandero et al. 2005, Lee and Heimpel 2005).

There are no studies on the effect of buckwheat supplementation in brassicas on aphid parasitism rates. However, under laboratory conditions alyssum L. *Lobularia maritima* (Brassicaceae), buckwheat, coriander *Coriandrum sativum* L. (Apiaceae) and phacelia *Phacelia tanacetifolia* Bentham (Bolraginaceae) significantly increase the parasitism rate of pea aphids *Acyrtosiphon pisum* Harris (Homoptera: Megaspilidae) by *Aphidius ervi*, with buckwheat having the significantly greatest effect on parasitism rates. The aphid hyper-parasitoid *Dendrocerus aphidium* Rondani (Hymenoptera: Megaspiliae) also showed an increase in parasitism rate with access to buckwheat, coriander and phacelia (Araj et al. 2008).

Synthetically-produced HIPVs can attract natural enemies of herbivores (Thaler 1999, Kessler and Baldwin 2001, James 2003b, 2005, James and Grasswitz 2005) and this could potentially be utilised for pest suppression within sustainable crop management regimes (Dicke et al. 1990a, Thaler 1999, Dicke and Hilker 2003, Kessler et al. 2004, Halitschke et al. 2008, Howe and Jander 2008, Khan et al. 2008). In the field, tomato plants induced with jasmonic acid can double the rate of field parasitism of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) by *Hyposoter exiguae* Viereck (Hymenoptera: Ichneumonidae), an endoparasitic wasp (Thaler 1999). Applying the synthetic versions of HIPVs naturally produced by *Nicotiana attenuata* Torr. Ex S. Watson (Solanaceae) to plants in the field resulted in an increased number of *Manduca sexta* L. (Lepidoptera: Sphingidae) eggs predated by a generalist predator *Geocoris pallens* Stål (Heteroptera: Lygaeidae) and a reduction in oviposition rate of *Manduca* spp.. As a consequence, the HIPV-mediated plant responses reduced the number of herbivores by 90% (Kessler and Baldwin 2001).

Results from Chapters 2 and 3 have shown the capacity of an HIPV, MeSA and floral resources, buckwheat not only to increase the abundance of natural enemies but also of herbivores and fourth trophic level natural enemies. Effects of A&R on pest densities, parasitism rates at multiple trophic levels and on crop damage need to be determined before A&R can be considered to be a viable pest management strategy.

The aim of the work in this chapter is to understand the effects of A&R on herbivore crop damage, herbivore densities, parasitism, hyper-parasitism and predation rates. This is important as unwanted effects of A&R on second and fourth trophic level arthropods or any

deterrent or negative interaction between MeSA and buckwheat may disrupt the intended improvement in biological control efficiency.

4.3. Methods

4.3.1. Experimental design of field experiment

This experiment tested the effect of synthetically-produced MeSA, flowering BW and the combination of MeSA and BW on the density of natural enemies and herbivores, egg predation rates and on aphid parasitism and hyper-parasitism rates in a commercial field with a mixture of kale *B. oleracea* and swedes *B. napus*. The study was carried out from December 5 2008 to March 31 2009 in a 170m x 220m brassica field near Selwyn Huts, Canterbury, New Zealand.

The experiment was set up as a randomized block design with eight blocks each of which consisted of three treatments and a control. The treatments were one treatment with a slow-release sachet of MeSA (MeSA) (P-240-lure, ChemTica International, Zeta Industrial Park, La Valencia, Heredia, Costa Rica), one with a 3m x 3m flowering buckwheat plot (BW), one with a slow release sachet of MeSA combined with a 3m x 3m plot of flowering buckwheat (MeSA+BW) and a control (C) consisting of the crop alone. Blocks were separated by 34m (east-west) and 24m (north-south) and the treatment and control plots within each block were separated by 34m (east-west) and 24m (north-south) within each block (Figure 4-1).

The first buckwheat seeds were sown on December 5, 2008. A 15 cm space was left between the rows of buckwheat. New seeds were sown in these on December 12 2008, January 6 and February 24 2009 5 cm from the row sown at the previous date, to ensure continuous presence of flowering plants during the experimental period.

4.3.2. Sampling methods

Three different sampling methods were used to assess herbivore crop damage, natural enemy and brassica pest densities, aphid parasitism and hyper-parasitism and egg predation rates.

The number of leaf mines from *S. flava* (TL2), cabbage aphid (TL2) *Brevicoryne brassicae* and the green peach aphid (TL2) *Myzus persicae*, aphid mummies (parasitized aphids) and larvae of DBM (TL2), CWB (TL2), ladybirds (TL3), hoverflies (TL3) and lacewings (TL3) were counted *in situ* on all the leaves of 12 plants per plot in four directions 1.5m from the

centre of each plot (Figure 4-1) on three occasions, January 9, February 25 and March 23 2009.

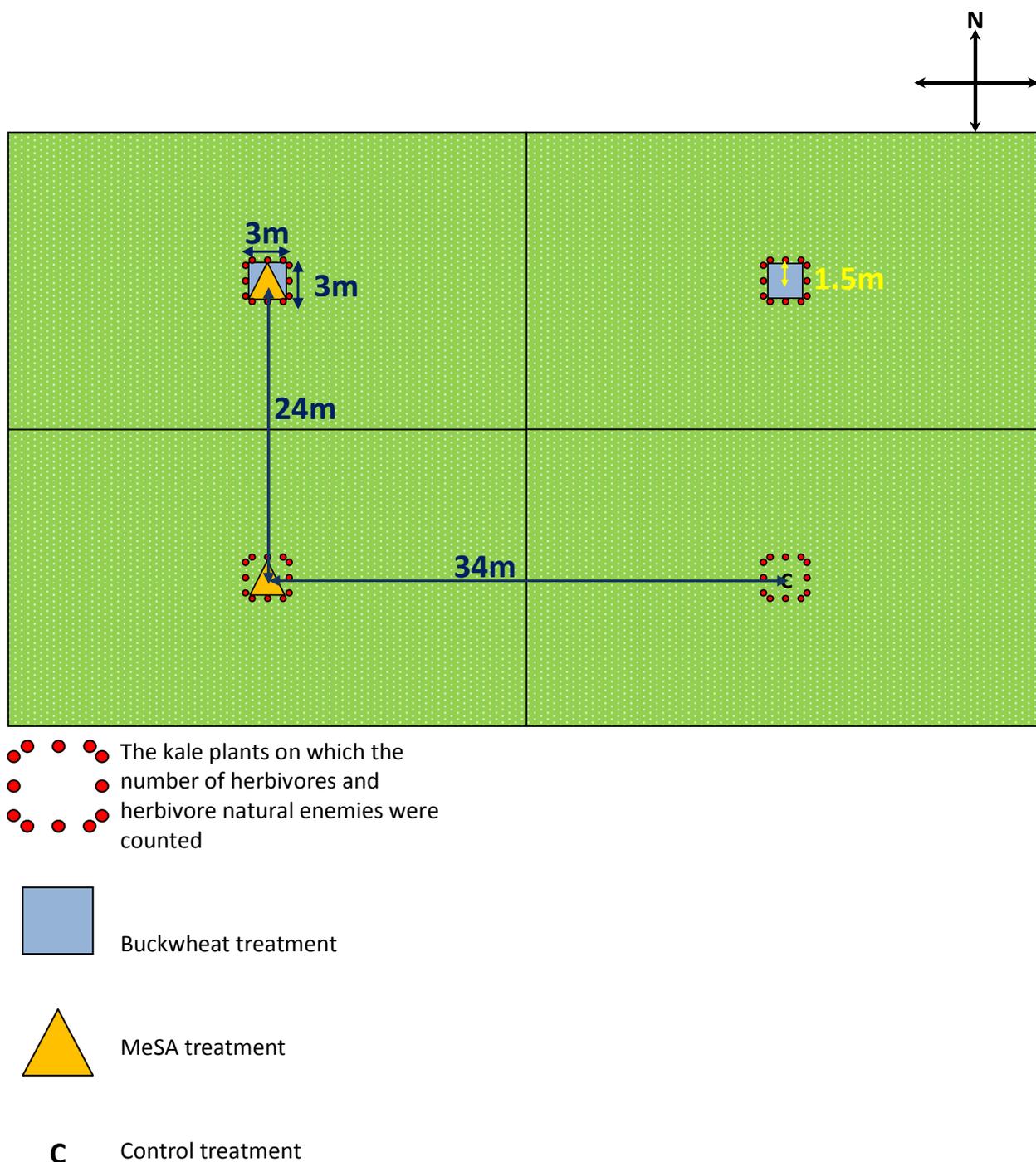


Figure 4-1. The location of the 12 plants per plot on which pest and natural enemy counts were done. The plants were located 1.5m from the centre of each plot. The plants on each side within a plot were separated by 1m.

Aphid mummies were collected to estimate aphid hyper-parasitism rates on February 26 2009. A total of 100 mummies per plot were collected within a 0.5m wide strip between 1.5m and

2m from the centre of the plot on randomly chosen plants (Figure 4-2). Only one colony per plant was collected. A colony was defined as aphids clustering together on a leaf. In the field, the leaves with the mummies were removed and brought back to the laboratory. A maximum of five randomly chosen mummies per colony was then removed and stored in 0.6ml vials to parasitoid emergence. The emerged parasitoids were identified, counted and the proportion of hyper-parasitized aphids was determined.

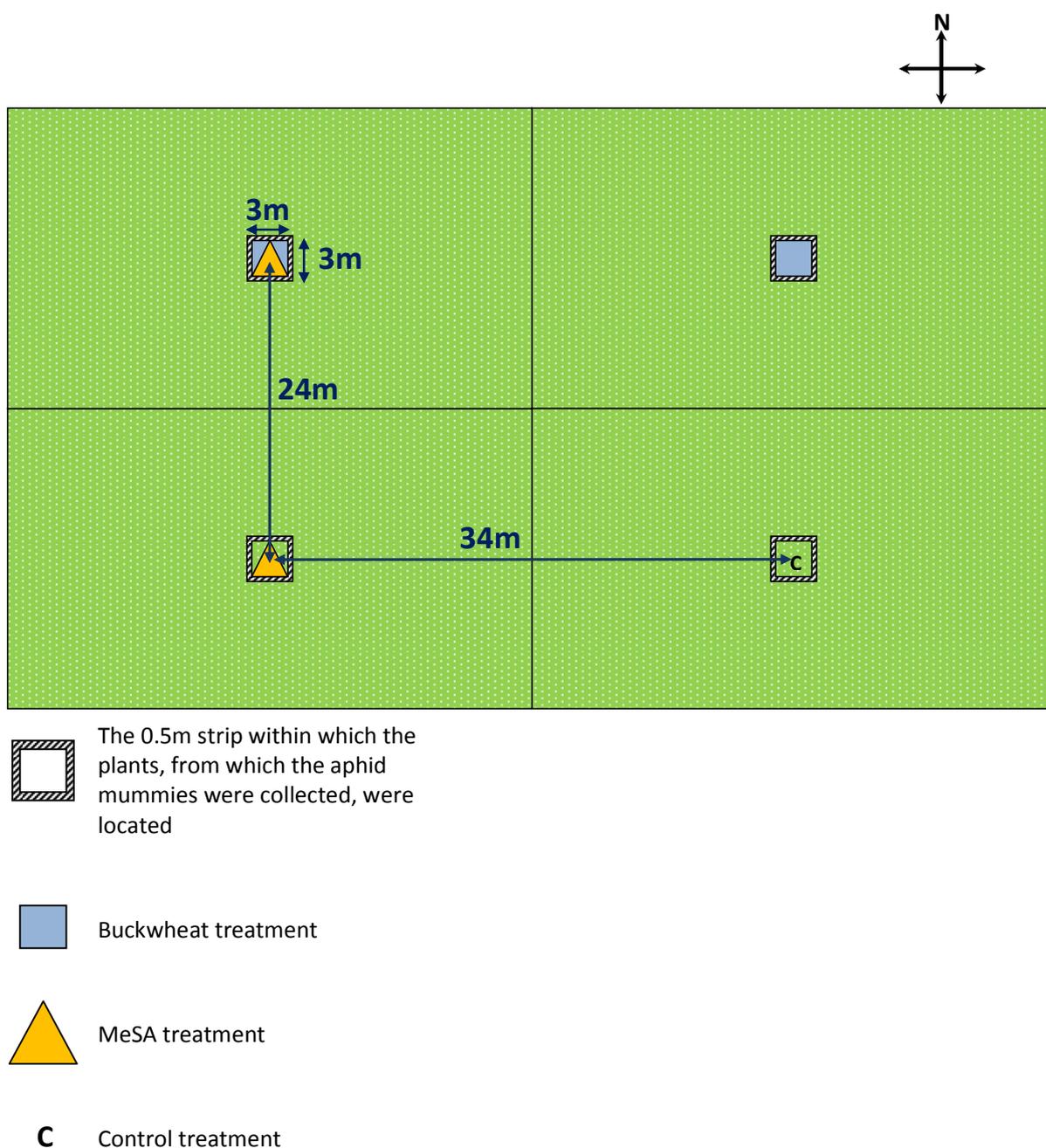


Figure 4-2. The plants on which aphid mummies were collected were located between 1.5m and 2m from the centre of each plot.

H. armigera Hübner (Lepidoptera: Noctuidae) eggs (obtained from Anne Barrington, HortResearch, Auckland, New Zealand) were used as sentinel baits to assess 24h predation rates. Baits were deployed in the field on two occasions, February 27 and March 25 2009. Two eggs were glued (3M, Repositionable 75, Spray Adhesive) on a diagonal to a 5 cm x 5 cm cards of sandpaper (P150 Tufbak) (Figure 4-3) to simulate eggs from herbivorous lepidopterans, such as the CWB. This density was used as CWBs lay their eggs singly (personal observation). Two cards of sandpaper were attached to the back of a kale leaf 1.5m from the centre of the plot in four directions (Figure 4-4).

The baits were left in the field for 24h before collection. The numbers of eggs remaining on the sandpapers were counted and missing eggs were assumed predated. Mean 24h predation rate was calculated based on mean proportion predated eggs per plot for both sampling dates.

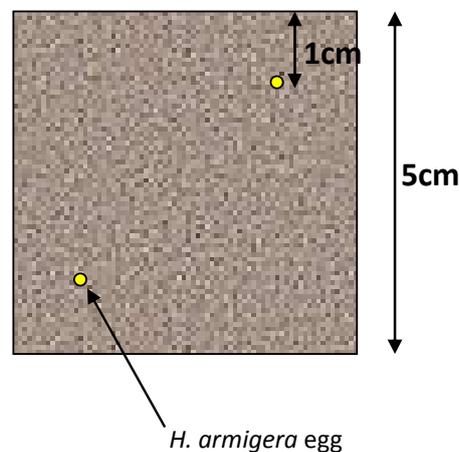


Figure 4-3. Two *H. armigera* eggs were glued onto cards of sandpaper 1cm from the edge of the card.

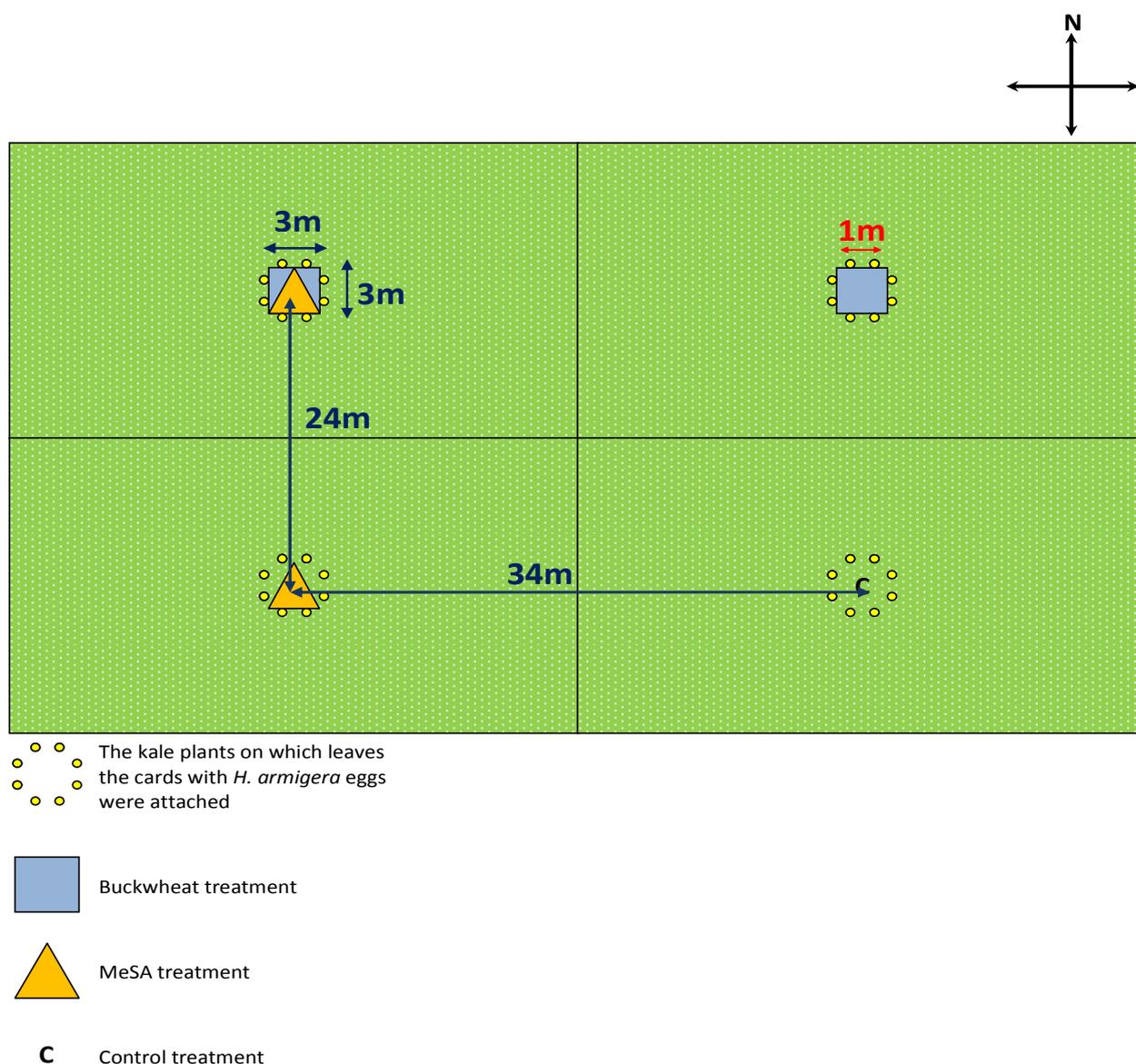


Figure 4-4. The location of the plants within the plots on which the sandpaper cards with *H. armigera* eggs were attached. The plants on each side within a plot were separated by 1m.

4.3.3. Analysis

The effect of the three different treatments on aphid density and proportion parasitized aphids was analysed for the data from pest count number two, February 25. The effect of the three treatments on aphid hyper-parasitism levels was analysed for data from the aphid mummy collection on February 26. Block 8, the MeSA+BW in block 1 and the BW treatment in block 4 were excluded from all analysis as treatments with buckwheat did not have flowering buckwheat.

For the total number of aphids per plant the sum of aphids and aphid mummies was used. To analyse aphid density an unbalanced two-way ANOVA was conducted on mean number of

aphids per plant for each plot. To satisfy the assumption for equal variance and normal distribution for the ANOVA model, all analysis was conducted on square root transformed data.

Proportion parasitized aphids was calculated based on the total number of aphid mummies out of the total number of aphids (aphids + aphid mummies) per plot. A GLM with a binomial distribution and a logit-link function was conducted to analyse mean proportion of aphids parasitized per plot. There was evidence of over-dispersion of the data so the program estimated the dispersal parameter.

Hyper-parasitism rates were calculated based on proportion hyper-parasitized mummies out of the 100 mummies collected per plot. An unbalanced two-way ANOVA was conducted on mean proportion of aphids parasitized per plot to analyse hyper-parasitism rate. To satisfy the assumption for equal variance and normal distribution for the ANOVA-model all analysis was conducted on arcsine (\sqrt{x}) transformed data as this fitted the data best to a normal distribution.

The effect of the tree treatments on predation rates was tested based on proportion predated eggs per plot for both dates. The data was arcsine (\sqrt{x}) transformed to fit a normal distribution and a repeated measures REML was conducted on proportion of eggs predated per plot.

The number of aphids was too low from the previous pest count on, January 9 to be analysed. On the third pest count, on March 23 the mean parasitism level was extremely high with 79% (SE \pm 3%) of the aphids being parasitized and with an average of 74% (SE \pm 2%) of the plants having 100% parasitism. Consequently, the data could not be analysed. The number of leaf mines, larvae of DBM and CWB and of hoverflies, ladybirds and lacewings counted on the plants were too low during the entire sampling period to be analysed.

4.4. Results

There was no significant difference in the aphid densities for the different treatments (Figure 4-5) (Table 4-1).

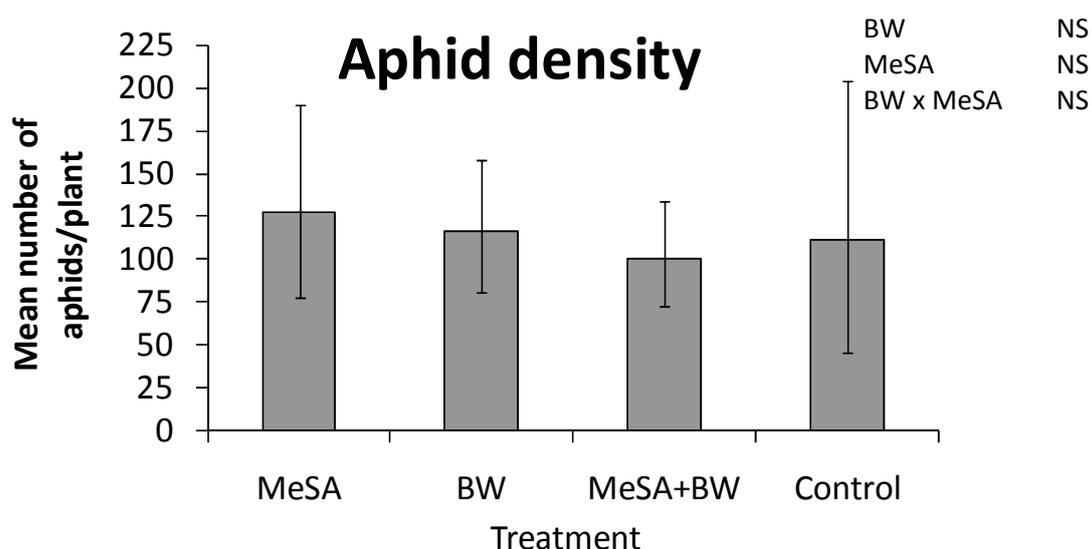


Figure 4-5. The mean number (\pm SE) of aphids per plant for the MeSA, buckwheat, MeSA + buckwheat-treatments and the control. The data have been back-transformed from square root-transformation. MeSA= MeSA only treatment, BW=buckwheat only treatment, MeSA+BW=combined treatments with buckwheat and MeSA and C=control treatment.

Significantly more aphids were parasitized in treatments with MeSA compared to treatments with no MeSA (Figure 4-6) (Table 4-1).

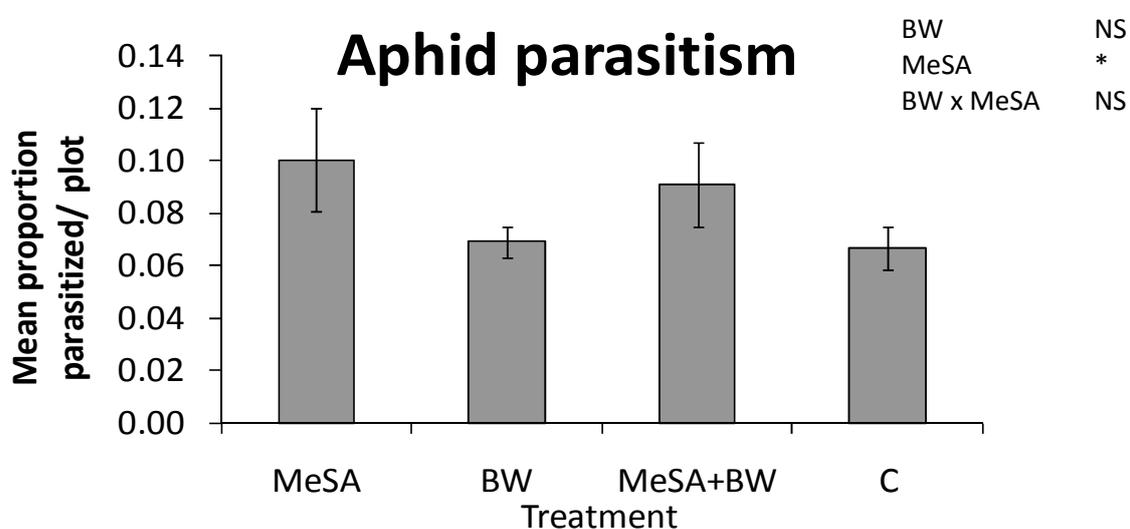


Figure 4-6. The mean number (\pm SE) of parasitized aphids per plant for the MeSA, buckwheat, MeSA + buckwheat-treatments and the control.

The level of hyper-parasitism was independent of treatments (Table 4-1) with a back-transformed mean proportion of 91% (SE +1.4%, -1.7%) hyper-parasitized aphids (Figure 4-7).

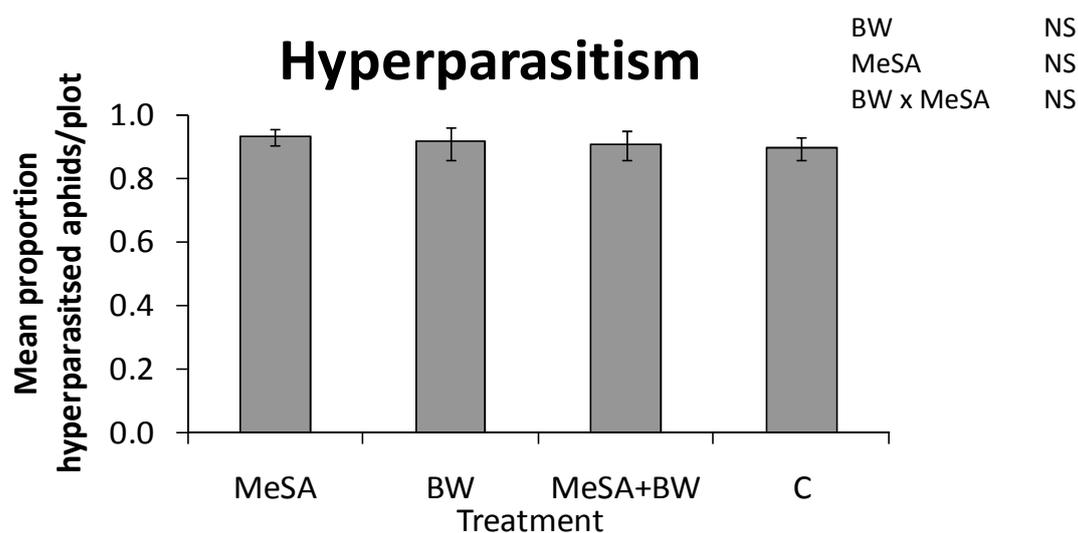


Figure 4-7. The mean (\pm SE) proportion of hyper-parasitized aphids per plant for the MeSA, buckwheat, MeSA + buckwheat-treatments and the control. The data have been back-transformed from arcsine-transformation. MeSA= MeSA only treatment, BW=buckwheat only treatment, MeSA+BW=combined treatments with buckwheat and MeSA and C=control treatment.

Back-transformed mean daily predation rate based on both dates was 77% (SE +12%, -15%), independent of treatment (Table 4-1) (Figure 4-8).

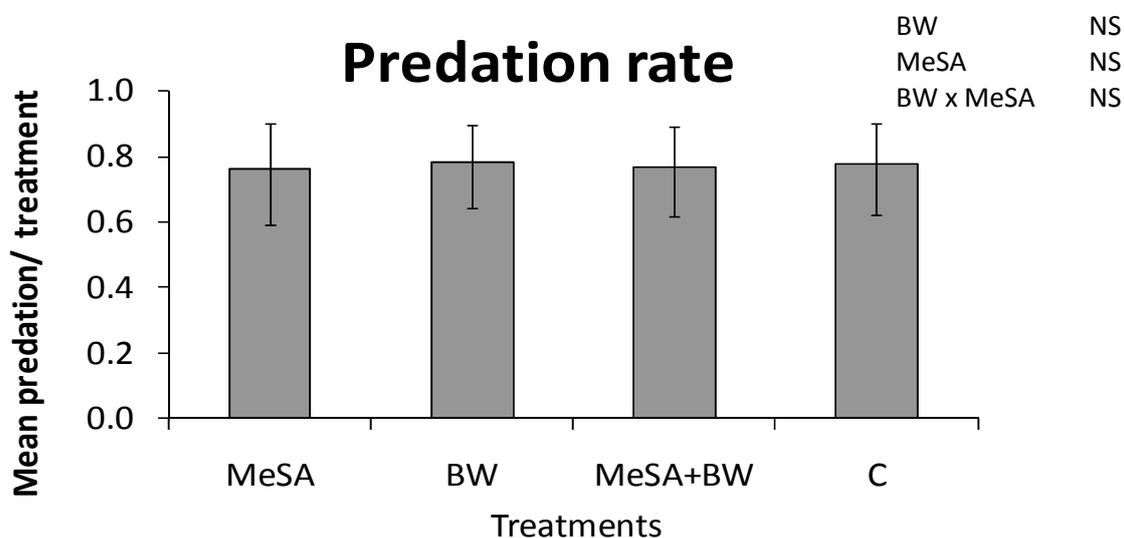


Figure 4-8. The mean (\pm SE) predation rate per plot for the MeSA, buckwheat, MeSA + buckwheat-treatments and the control for the two sampling dates. The data have been back-transformed from arcsine-transformation.

The number of brassica herbivores, leaf mines from *S. flava* and larvae of ladybirds, hoverflies and lacewings on the sampled crop plants were too low for the data to be analysed.

Table 4-1. An un balanced two-way ANOVA analysis for aphid densities and aphid hyper-parasitism, GLM analysis for aphid parasitism and a repeated measures REML for egg predation rates. MeSA = the effect of treatments with MeSA , BW= the effect of treatments with buckwheat.

	Factor	Wald statistic	df	F-value	Level of significance
Aphid density	BW		1	0.385	NS
	MeSA		1	0.882	NS
	BW x MeSA		1	0.690	NS
Aphid parasitism rater	BW		1	0.930	NS
	MeSA		1	0.026	*
	BW x MeSA		1	0.359	NS
Aphid hyperparasitism rate	BW		1	0.718	NS
	MeSA		1	0.339	NS
	BW x MeSA		1	0.271	NS
Predation rate	BW	0.00	1	0.960	NS
	MeSA	0.13	1	0.716	NS
	Time	0.26	1	0.638	NS
	BW x MeSA	0.00	1	0.972	NS
	BW x Time	0.11	1	0.740	NS
	MeSA x Time	1.26	1	0.269	NS
	BW x MeSA x Time	3.17	1	0.083	NS

4.5. Discussion

Aphid parasitism rates were significantly higher in treatments with MeSA than in those with none. Behavioural work on aphid parasitoids has demonstrated the role of plant volatiles in their foraging (Powell and Zhang 1983, Powell and Wright 1992, Wickremasinghe and van Emden 1992, Grasswitz and Paine 1993, Guerrieri et al. 1997, Powell et al. 1998) and the aphid parasitoid *Aphidius ervi* uses HIPVs in the search for its host *Acyrtosiphon pisum* Harris (Homoptera: Aphididae) (Du et al. 1996, Guerrieri et al. 1997) under laboratory conditions. However, this is the first time the effect of a synthetically produced MeSA has been shown to increase parasitism rates in the field. But, the increased parasitism rate in treatments with MeSA seems not to have affected aphid densities, as these were not significantly different between treatments.

Aphid parasitoid parasitism rates were not significantly affected by the treatments. However, the overall hyper-parasitism rates were close to 100%, with a back-transformed mean proportion of 91% (SE +1.4%, -1.7%) hyper-parasitized aphids per plot. Consequently, the results could be due to a lack of hosts and hence any effects of the treatments could have been over-ridden by host limitation. This is also supported by the observed high levels, 74% (SE \pm 2%) of aphid colonies with 100% parasitism on March 23.

Both buckwheat (Pontin et al. 2006) and synthetically produced HIPVs (Kessler and Baldwin 2001) have previously been shown to accumulate arthropod predators of herbivores. However, in this study, there was no significant effect of treatments on predation rates of the egg of *H. armigera*. The deployment of *H. armigera* eggs within the field was to simulate eggs from herbivorous lepidopterans such as the CWB. CWB eggs in cabbage fields often suffer from quite high mortality from arthropod predation (Schmaedick and Shelton 1999). Also in this case could a high predation rate of the *H. armigera* eggs be seen with a back-transformed mean daily predation rate of 77% (SE +12%, -15%), independent of treatment. The lack of significant treatment effects could be due to the arthropod predators not utilising the buckwheat (the presence of any egg predators on the buckwheat flowers was never observed during this study) or responding to MeSA. The depositing of lepidopteran eggs on plants can induce the production of HIPVs (Hilker and Meiners 2002). However, it is not known if MeSA is one of the volatiles induced by egg deposition, neither is it known if synthetically-produced MeSA is one of the volatiles involved in previously-observed increases in predation as a response to HIPVs (Kessler and Baldwin 2001). Consequently, predatory arthropods may not have learnt to associate the presence of MeSA with the presence of prey (Dicke et al. 1990c) and hence have not responded to the presence of the volatile in these experiments.

4.5.1. Prospects for biological control using ‘attract and reward’

This work shows that MeSA could potentially increase aphid parasitism rates. However, to be able to conclude the effect this may have on aphid densities the experiment would have to be repeated throughout a season as there is a time lag between aphid parasitism and a reduction in number of aphids. Overall aphid parasitism rates were low with a mean parasitism of 8% (SE±1%) per plant, independent of treatment and with only a 3% increase in mean parasitism rate between the lowest (buckwheat and control, mean parasitism of 7% per plot) and the highest (MeSA, mean parasitism of 10% per plot) (Figure 4-6). Consequently, the increase in parasitism would probably not have an effect on aphid densities and the effect of MeSA on parasitism rates in this case is low.

As in a previous chapter (Chapter 3), MeSA has been shown to have a positive effect on some aspects of CBC, in this case aphid parasitism rate, while the presence of buckwheat had no effect on aphid parasitism. This is contrary to expectations, as the aim of the A&R concept is to combine the two ‘eco-technologies’ to work synergistically, increasing pest population reduction rates by natural enemies.

It still remains to be shown that the observed increased abundance (usually on traps) of some arthropod natural enemies of pests in response to A&R (Chapter 3) can be translated into increased pest reduction rate by the natural enemies, followed by a lower rate of herbivore damage of the crop. This information is needed to be able to conclude whether the concept can be considered successful or not as a tool to improve CBC (Gurr et al. 2007).

Chapter 5: Attraction of the aphid parasitoid *Aphidius colemani* to synthetically produced methyl salicylate in Synertrrol oil

5.1. Abstract

Aphids can cause both direct damage to brassica crops and be vectors of viruses thereby reducing crop yields. *Aphidius colemani* parasitizes *Myzus persicae*, an aphid commonly occurring within brassica crops. Aphid feeding can induce the production of methyl salicylate (MeSA) which is an herbivore-induced plant volatile (HIPV) that can function as an attractant to natural enemies of the aphids.

Synthetically-produced methyl salicylate has potential to trigger the plant's own production of HIPVs. It still remains unclear whether it is MeSA alone or a blend of volatiles produced by the host plant after the induction by MeSA that is attractive to the arthropods in the field experiments in Chapter 2 and 3.

The synthetically-produced MeSA sachets deployed within the previous chapters of this thesis is mixed with organic oil in a commercial product. However, the concentration of the volatile in the sachets is not known. The aim of this chapter is to evaluate if *Aphidius colemani* can be attracted to synthetically-produced MeSA mixed with Synertrrol oil. Using a Y-tube olfactometer, the response of *A. colemani* to 0.5% (w/v) and 2.0% (w/v) MeSA diluted in Synertrrol oil compared to air was investigated. Significantly more parasitoids were attracted to 2.0% MeSA than to air while the parasitoid did not respond to the lower concentration.

This is the first time that synthetically-produced MeSA has been shown to be attractive to a parasitoid in an olfactometer experiment.

5.2. Introduction

One commonly occurring herbivore in brassica crops is the green peach aphid *Myzus persicae*. Colonies of the aphids can reduce the vigour and the yield potential of the crop and transmit viruses (Ferguson and Blake 1985), such as the turnip yellows virus (TuYV) (Stevens et al. 2008). TuYV is probably the most important viral disease of oilseed rape in the United

Kingdom and is probably the main reasons why commercial oilseed rape crops do not reach their genetic yield potential. The main virus vector of TuYV is *M. persicae* (Stevens et al. 2008). Field experiments on oilseed rape *Brassica napus* in Australia have shown that crops that suffer 96% and 100% infection of TuYV had a 46% and 37% yield reduction respectively (Jones et al. 2007). Managing TuYV spread and hence the virus from causing similar losses in the United Kingdom during mild winters could raise the average yield at individual crop level from 3.3 t/ha to between 4.4 t/ha and 6.0 t/ha. Assuming between 10-15% of the losses could be prevented by controlling TuYV the value of the yield improvement would be around £60-90 million/year for UK oilseed rape growers. Similarly at 50% occurrence of the virus, the value of the yield improvement could be £30-40 million/year (Stevens et al. 2008). TuYV is also present in New Zealand in turnip, sugar beet and fodder beet and *M. persicae* is an efficient vector of the virus, with a minimum acquisition period of 5min feeding, a minimum inoculation feeding period of 3min and a latent period of 9-27h in Canterbury, New Zealand (Kyriakou et al. 1983). Other important viruses spread by the green peach aphid in New Zealand are different types of mosaic virus (Lamb 1960). The cauliflower mosaic virus can for example cause up to 30% yield loss in cauliflowers *Brassica oleracea* in some parts of New Zealand (Fry 1952).

Aphidius colemani is a parasitoid of *M. persicae*. It is commercially produced for biological control of *M. persicae* in glasshouses (Grasswitz and Paine 1993) and is commercially available in New Zealand (from e.g., Zonda Resources Ltd. Auckland, New Zealand and Bioforce Ltd, Drury, New Zealand). The parasitoid is a polyphagous parasitoid of aphids which probably originates from northern India or Pakistan and has spread throughout most of the world (Starý 1975).

Aphid feeding by the pea aphid *Acythosiphon pisum* can influence the release of HIPVs in broad beans *Vicia faba* (Du et al. 1998). Aphids produce little feeding damage and are perceived as pathogens by the plant (Walling 2000). Salicylic acid (SA) is a key component in the shikimic pathway that is thought to be responsible for defence against aphids and other phloem feeders (Walling 2000, Kaloshian and Walling 2005). Green peach aphid feeding can trigger SA-dependent gene responses in *Arabidopsis* (Brassicaceae) (Moran and Thompson 2001) and may therefore induce volatiles that attract the aphid parasitoid *Diaretiella rapae* (Girling et al. 2006). Soybean plants *Glycine max* L. (Fabaceae) releases significantly more methyl salicylate (MeSA) when infested by soybean aphids *Aphis glycines* Matsumura (Hemiptera: Aphididae) and synthetically produced MeSA attracts in the field significantly more *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) that predate on this aphid

(Zhu and Park 2005). Applying synthetically produced SA to *M. persicae* damaged Brussels sprouts *B. oleracea* var. *Gremmifera* L. (Brassicaceae) increased the proportion of aphids parasitized by *A. colemani*, with almost twice as many aphids parasitized when 10mM SA was applied to the foliage of the Brussels sprouts, compared to no SA (Karatolos and Hatcher 2009).

The synthetically-produced MeSA sachets deployed within the previous chapters of this thesis is mixed with organic oil in a commercial product. It still remains unclear if the observed increase in natural enemy abundance in response to the synthetically-produced MeSA is due to MeSA alone attracting the arthropods or due to the plants being induced by the volatile to produce their own HIPVs (Khan et al. 2008) (Chapters 2 and 3).

The aim of this chapter is to evaluate if two concentrations, 0.5% (w/v) and 2.0% (w/v), of synthetically-produced MeSA mixed with the organic oil, Synertrrol can be attractive to *A. colemani* compared to clean air. These concentrations have been shown to increase the abundance of hymenopterans in broccoli *B. oleracea* and sweet corn *Zea mays* L. (Poaceae) (Simpson et al. 2009).

5.3. Methods

The experiment was carried out using methods similar to the ones used by Girling et al. (2006) on *Diaretiella rapae* and Pareja et al (2009) on *Aphidius ervi*. Green peach aphids *M. persicae* reared on Chinese cabbage *Brassica rapa* and parasitized by *A. colemani* were obtained from Zonda Resources Ltd. (Auckland, New Zealand). The parasitized aphids (aphid mummies) were removed with soft forceps from the Chinese cabbage plants and allowed to emerge in a chamber. This was a dark 250ml container in which the mummies were placed. A hole was made in the lid and a ventilated Perspex cylindrical cage was placed over the hole of the container. The cage was lit from above and the emerged wasps were collected from it (Girling et al. 2006) (Figure 5-1). The chamber was kept at 21°C and in a 16:8h light: dark regime. Emerged parasitoids were fed 10% (w/v) glucose solution with 3µl of yellow food colouring (Hansells, yellow colouring, colour 102, Old Fashion Foods Ltd, Auckland, New Zealand) on cotton wool and allowed to mate (Pareja et al. 2009). The yellow food colour has shown to increase the attraction of parasitoids to the sugar solution (Personal communication, Graham Walker, Plant and Food Research, Auckland, New Zealand).

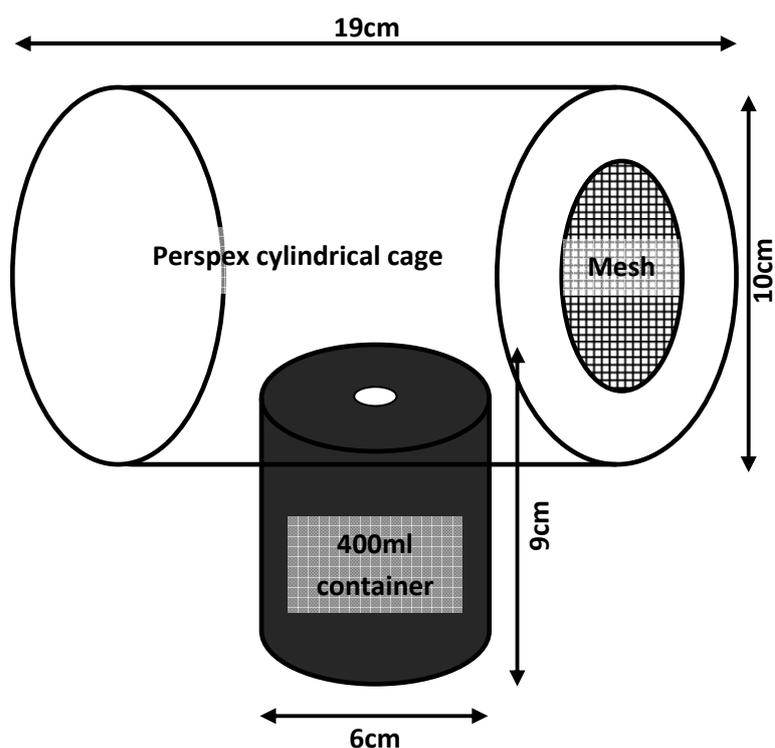


Figure 5-1. The chamber used for the collection of the emerged *A. colemani* from the aphid mummies.

The experiment was conducted over three days with two concentrations of MeSA diluted in Synertrol oil (Organic Crop Protectants, NSW Australia, www.ocp.com.au). These were 0.5% (w/v) and 2% (w/v). On day 1 and 2 was the parasitoid's response to the MeSA concentration of 0.5% (w/v) tested and on day 3 was the response to the 2.0% (w/v) concentration tested. One day old fed (Girling et al. 2006, Pareja et al. 2009) and mated females were used (Pareja et al. 2009). The females were separated from males and 'primed' as this can increase aphid parasitoids' response to an odour source (Girling et al. 2006). 'Priming' was done by giving the females experience of 100-150 *M. persicae* combined with synthetically-produced MeSA. The females were transferred into a cylindrical (19cm x 10cm diameter) ventilated Perspex cage with aphids and a filter paper (Whatman, 4.25cm diameter) with 1 μ l of the tested concentration of MeSA 1 h prior to the experiment (Girling et al. 2006).

Dual-choice tests were carried out using an olfactometer set-up (Analytical Research Systems Inc, Florida, USA) with a glass Y-tube olfactometer with a 3.5cm internal diameter, a 22cm stem and 30cm arms at a 70° angle (Figure 5-3). Air was pumped through a hydrocarbon filter (1) and divided by a T-junction into two separate humidifying bubblers (2), containing deionised water, through Teflon tubing (3) into two separate flow meters (4), which regulated the flow rate to 400ml/min (Girling et al. 2006). The air then passed through Teflon tubing (5)

into two volatile collection chambers (6) into which the volatile sources to be tested were placed. From here, the air flowed into the arms of the Y-tube (7) (Figure 5-2).

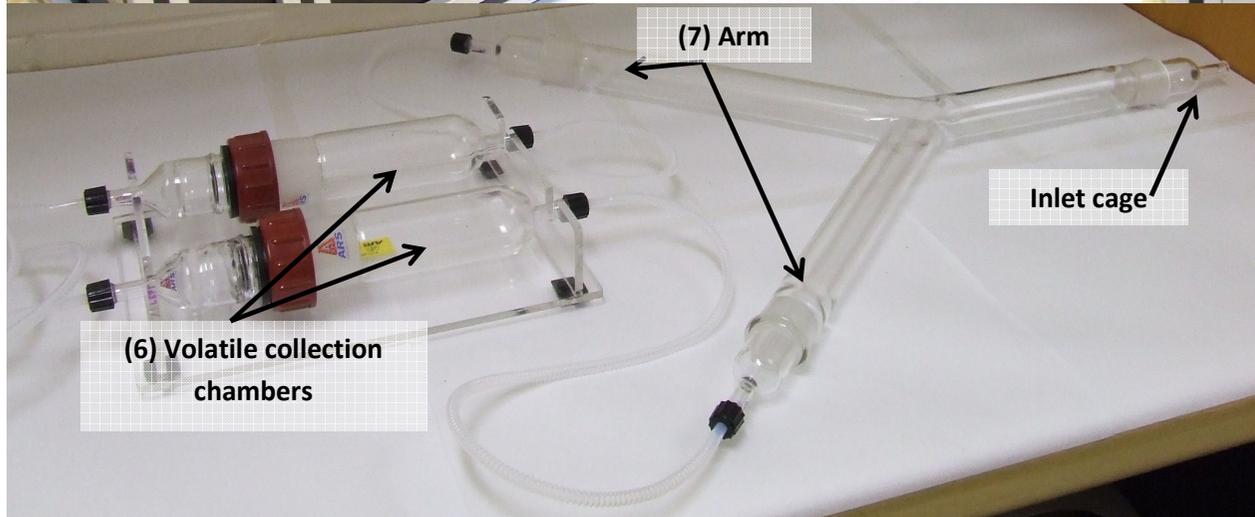
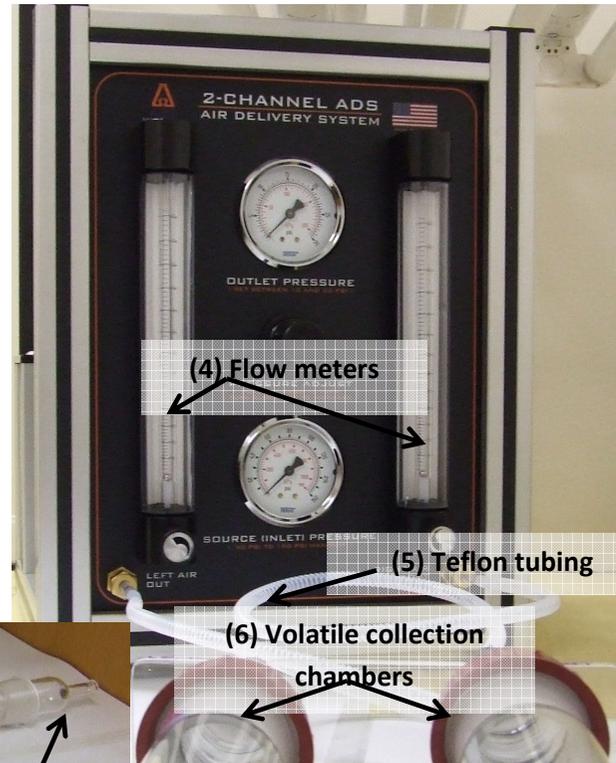
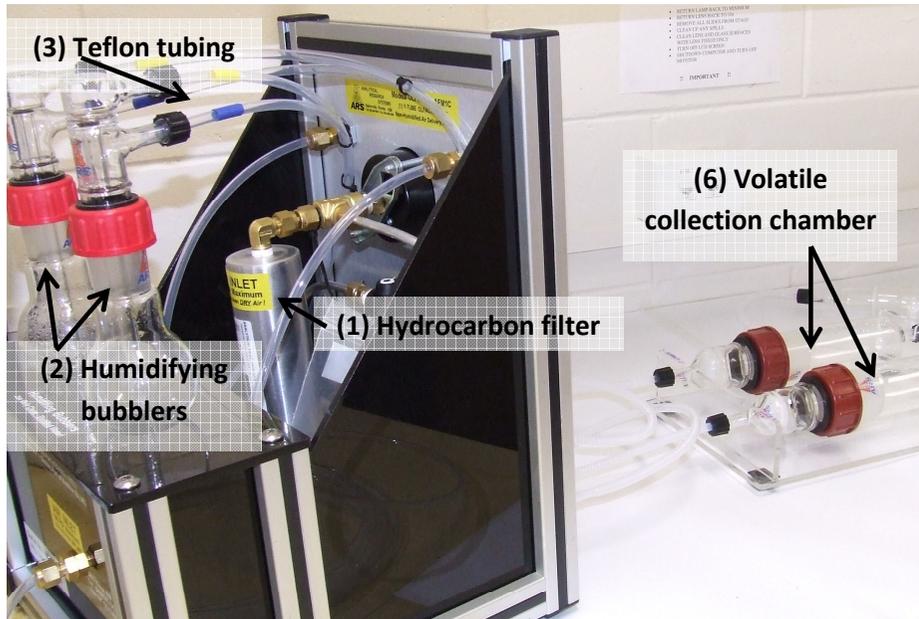


Figure 5-2. The olfactometer set-up.
60

The Y-tube was placed on a white bench and lit from above with artificial light (6 Lumilux cool daylight L30W/865 20000h) (Pareja et al. 2009). All experiments were conducted at 23°C (Girling et al. 2006). Both the wasps, in a cage and the volatile, in an air-tight vial was placed in the experimental room under the lamp at least 1h before the experiments were conducted to allow the wasps to acclimatize to the light level and temperature and the volatile to adapt to the temperature (Girling et al. 2006).

The olfactometer was divided into three sections in accordance with the methods used by Girling et al (2006). The first section was the 'stem', defined as the first 21cm of the main arm of the Y-tube. The next section was the 'choice zone', the area in which the parasitoid made its 'choice' between the two arms of the Y-tube. It was defined as the first 3cm of each arm of the olfactometer from the end of the stem. The final section was the 'arms' of the olfactometer, the remaining 27cm of the Y-tube (Figure 5-3) (Girling et al. 2006). A single 'primed' female parasitoid was introduced to the Y-tube in a removable inlet cage at the bottom of the stem (Figure 5-2). Each female was given 5min to make a choice (Girling et al. 2006, Pareja et al. 2009) between 1µl of MeSA diluted in Synertrol oil (MeSA+ Synertrol oil) of respective concentration applied to filter paper (Whatman, 4.25cm diameter) (Koschier et al. 2000) and clean air. A female was considered to have made a 'choice' when it crossed the 'choice line' and first entered one arm (Girling et al. 2006, Pareja et al. 2009).

After each individual was tested, the Y-tube was turned over for the next test, to eliminate any directional biases by the parasitoid (Girling et al. 2006). The Y-tube was first cleaned with detergent, then distilled water and finally with 100% acetone (Pareja et al. 2009), and dried in an oven at 50°C (Kvedaras et al. 2009) between every third run (Pareja et al. 2009) to remove any volatiles adhered to the glass. For the 0.5% (w/v) MeSA concentration was a total of 52 wasps tested and for the 2% concentration a total of 51 wasps.

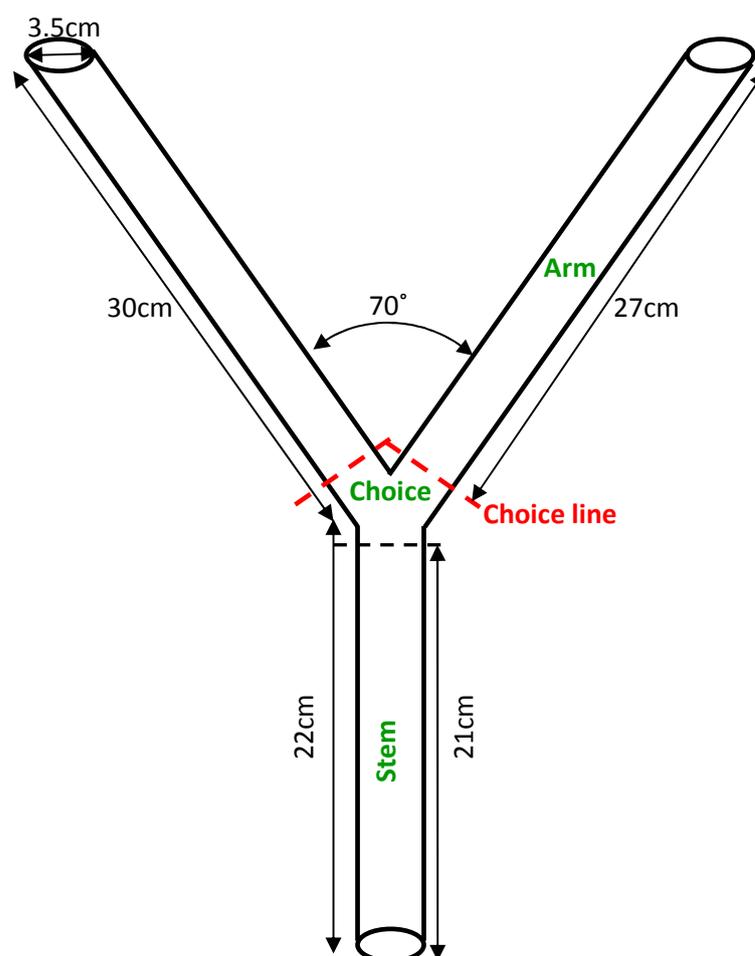


Figure 5-3. The dimensions of the Y-tube and the three zones it was divided into.

The number of *A. colemani* making a choice was analysed based on the null hypothesis that the probability of the response to tested compound or the clean air is equal to 50%.

Consequently, differences in response were analysed using a one-sample binomial test with a proportion success of 0.5 and a 95% confidence interval.

5.4. Results

The percentage of *A. colemani* that did not respond in the experiment using 0.5% (w/v) MeSA diluted in Synertrol oil (MeSA +Synertrol oil) compared to clean air was high, 71.2% (Figure 5-4). The parasitoid was not differentially attracted to either source with 15.4% choosing MeSA +Synertrol oil compared to 13.5% choosing clean air (Figure 5-4).

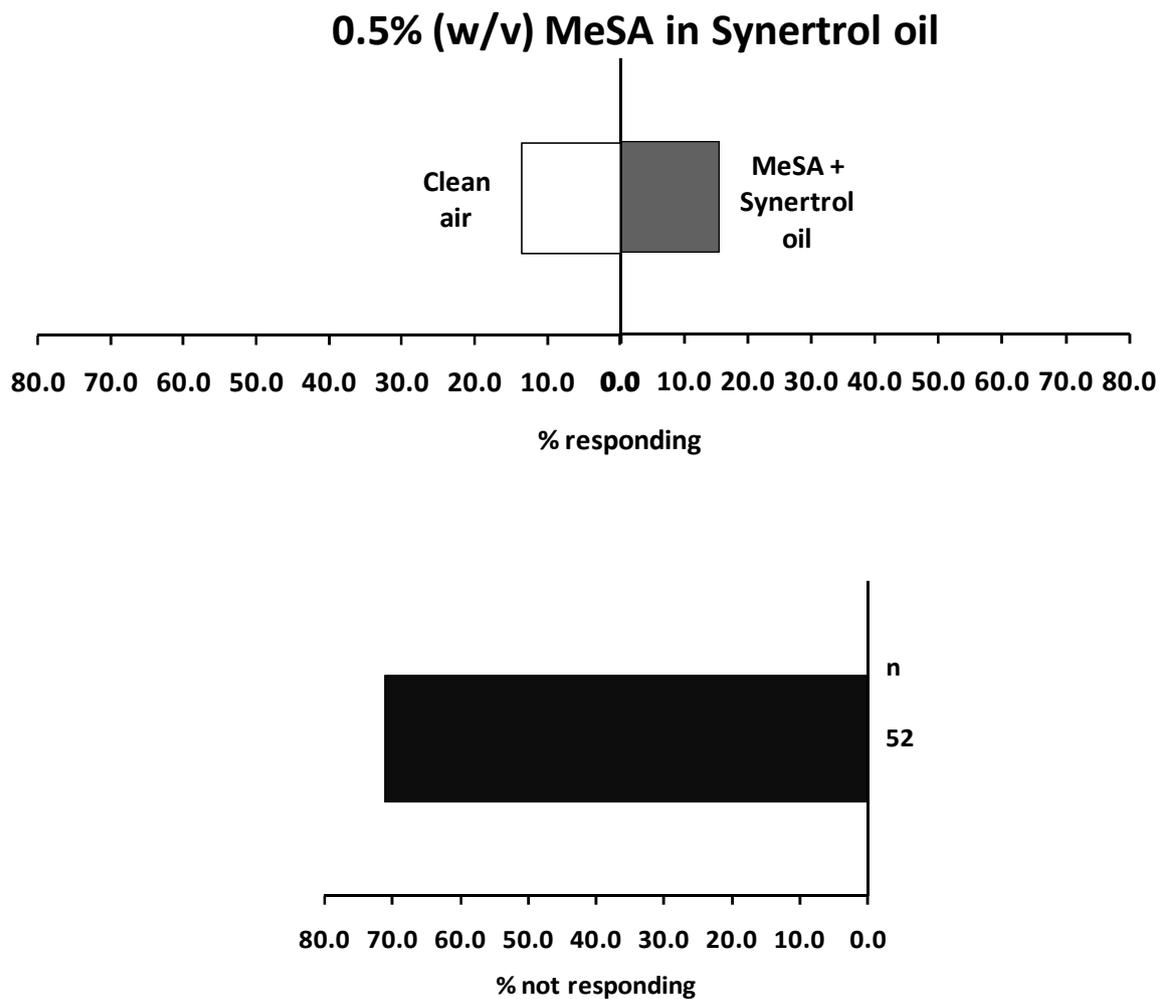


Figure 5-4. Response of *A. colemani* in the Y-tube to 0.5% (w/v) MeSA mixed with Synertrol oil (MeSA+ Synertrol oil) compared to clean air. n=the number of individuals.

Significantly more *A. colemani* were attracted to 2.0% (w/v) MeSA diluted in Synertrol oil compared to clean air ($p=0.035$) with 32.7% choosing 2.0% MeSA+ Synertrol oil compared to 11.5% choosing clean air. 53.8% of the wasps did not respond (Figure 5-5).

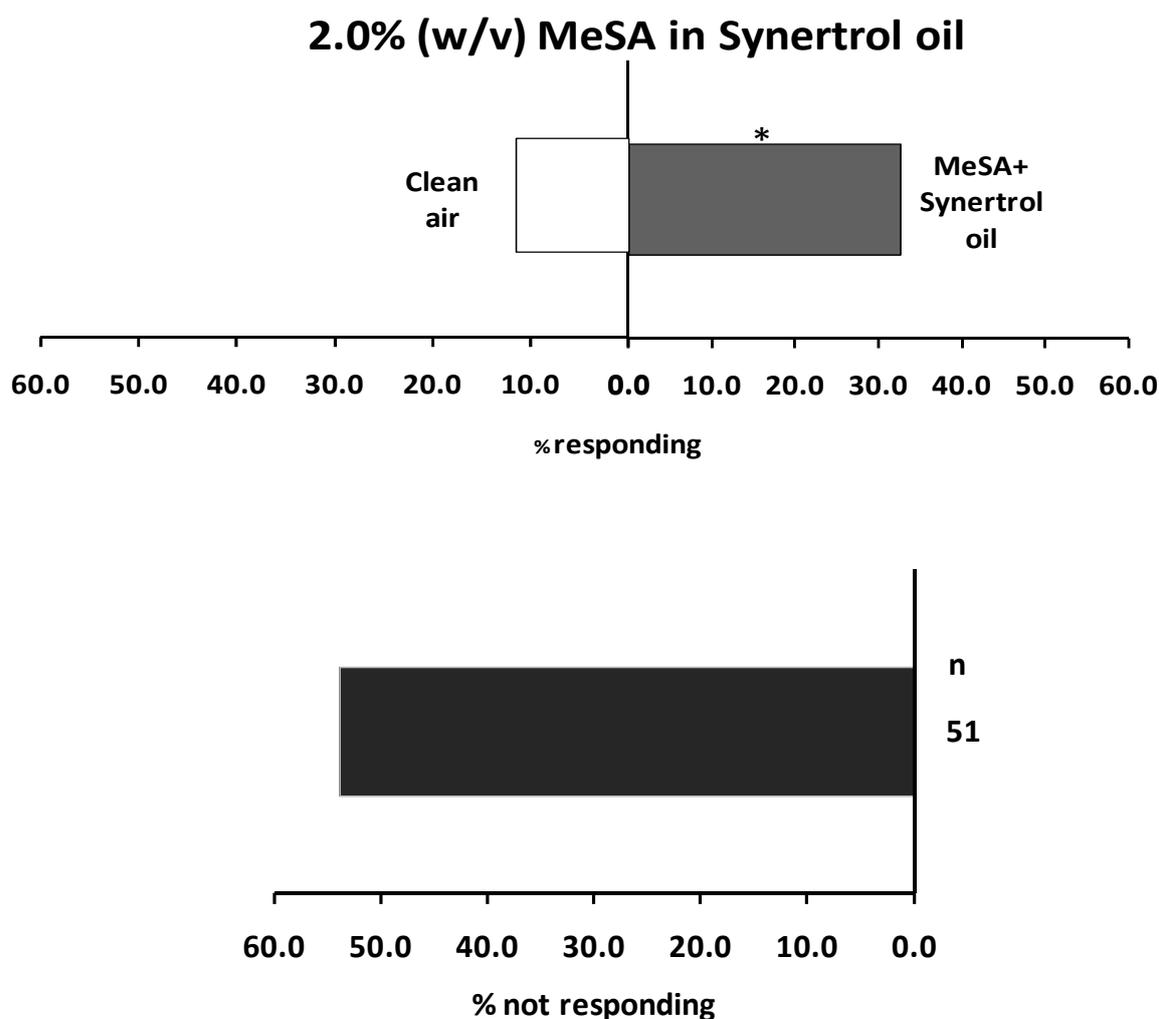


Figure 5-5. Response of *A. colemani* in the Y-tube to 2% (w/v) MeSA mixed with Synertrrol oil (MeSA+ Synertrrol oil) compared to air. n= the number of individuals.

5.5. Discussion

Significantly more *A. colemani* were attracted to 2.0% (w/v) MeSA+ Synertrrol oil than to clean air. 53.8% of the parasitoids did not respond. This is similar to the proportion that did not respond in experiments with a significant result in Girling et al.(2006). 71.2% of the parasitoids did not make a choice when exposed to 0.5% (w/v) MeSA + Synertrrol oil and of the ones making a choice 8 (15.4%) individuals chose MeSA and 7(13.5%) air. These proportions are similar to those observed by Girling (2006), who concluded that *D. rapae* was not attracted to either of the odour sources. Thus, this study suggests that *A. colemani* is attracted by synthetically-produced MeSA alone, at least if deployed at certain concentrations. However, this does not exclude the possibility that this parasitoid also responds to other HIPVs induced by MeSA in the field.

This is the first time synthetically-produced MeSA has been shown to be attractive to a parasitoid in an Y-tube olfactometer experiment Karatolos and Hatcher (2009) were the first to study the effect of synthetically-produced SA on the aphid *M. persicae* and the aphid parasitoid, *A. colemani* in a glasshouse laboratory study. Previous olfactory studies using synthetically produced HIPVs have included assessing the ability of female parasitoids to learn to associate a volatile with host presence (D'Alessandro et al. 2006) and the attraction of different volatile compounds to herbivorous thrips (Koschier et al. 2000).

Previous studies have shown that very low concentrations of an HIPV are needed to attract arthropods (Koschier et al. 2000, Karatolos and Hatcher 2009). Karatolos and Hatcher (2009) found that a concentration of 10mM of SA was enough to increase the number of *M. persicae* parasitized by *A. colemani*, but, at this concentration there was a reduction in parasitoid emergence. Also, the aphid mortality was increased at this concentration (Karatolos and Hatcher 2009). The release rate of MeSA in Synertrol oil at 23°C is un-known and consequently the concentration of the volatile that the wasp experienced in the Y-tube is also un-known. A concentration of 0.5% (w/v) of MeSA in Synertrol oil might attract *A. colemani* at a different, higher temperature.

Hymenopterans can be attracted to the Synertrol oil alone when deployed in sweet corn although the same attraction has not been seen for the oil deployed in broccoli (Simpson et al. 2009). However, if the oil was attractive to *A. colemani* in the present study the wasp should have reacted positively on the lower concentration of MeSA, 0.5% (w/v) as this concentration contained 99.5% oil compared to the 2.0% (w/v) that contained 98% oil. Consequently, it can be assumed that the attraction of the parasitoid was to MeSA and not the Synertrol oil.

5.5.1. Suggestion for future olfactometer Y-tube experiments

There is a need for more extensive experiments before any firm conclusions can be drawn regarding the attractiveness of synthetically-produced MeSA to *A. colemani*.

Experiments comparing the attraction of *A. colemani* to Synertrol oil alone on filter paper and Synertrol oil mixed with synthetically produced MeSA on filter paper are needed to eliminate the possibility that the observed attraction is not due to the wasp being attracted to the oil or the filter paper. Tests comparing the two concentrations against each other would further confirm that *A. colemani* is not attracted to MeSA with a concentration of 0.5%.

We still do not know if certain HIPVs induced by MeSA are attractive to *A. colemani*. This could be tested by comparing the parasitoids response to odours from MeSA-exposed and

non-exposed plants in a Y-tube olfactometer and then collect the volatiles from the same plants used in the bioassays and analyse their volatile profiles with gas chromatography (GC) using methods similar to those of Pareja et al.(2009).

Chapter 6: Discussion

6.1. Introduction

The effect on the ecological ‘fitness’ of herbivore natural enemies by the deployment of floral resources within conservation biological control (CBC) has been increasingly studied.

However, supplementation with floral resources has not always improved the efficiency of natural enemies enough to see an effect cascade down to the abundance of the target herbivore pest and the extent of crop damage (Jonsson et al. 2009a). Effects on these trophic levels are needed to be able to evaluate the success of CBC (Gurr et al. 2007). Research on how to optimise the provision of floral resources has included studies of the nectar and pollen availability (Waller et al. 1981, Wäckers 2005), accessibility (Vattala et al. 2006) and suitability for natural enemies. However, one concern is that the supplementation of floral resources only redistributes the natural enemies within the crop, increasing their abundance only close to the deployed resources rather than throughout the crop. One way to potentially attract natural enemies into the crop from surrounding habitats, such as field margins, could be the use of herbivore-induced plant volatiles (HIPVs), as these have been shown to attract natural enemies (Dicke et al. 1990a, Turlings et al. 1990, Steinberg et al. 1992, McCall et al. 1993, Agelopoulos and Keller 1994, McCall et al. 1994, Thaler 1999). Synthetically-produced HIPVs can increase natural enemy abundance in the field (James 2003b, a, James and Price 2004, James 2005, James et al. 2005, James and Grasswitz 2005). Combining the attraction properties of synthetically-produced HIPVs (‘attract’) with floral resource supplementation (‘reward’) has been termed ‘attract and reward’ (A&R) (Chapter 1) (Jonsson et al. 2008, Khan et al. 2008). The aim of the work in this thesis is to evaluate, in a brassica cropping system, the effects of A&R on the abundance of arthropods from three trophic levels: herbivores (trophic level II), natural enemies (trophic level III) and natural enemies of the natural enemies (trophic level IV) and as a consequence biological control in brassicas. It is hoped that the two ‘eco-technologies’ will work together synergistically, to enhance the pest population reduction efficiency of the natural enemies.

In this discussion, the results of the experiments described in this thesis are discussed in the context of CBC. First, the changes in the targeted natural enemy populations, due to the deployment of MeSA and buckwheat are compared against a hierarchy used to evaluate success within CBC. Then, potential effects on CBC by the increased abundance of

arthropods from untargeted trophic levels are addressed. Finally, potential future research questions which have arisen from this thesis are considered.

6.2. Levels of success reached using MeSA and buckwheat to enhance CBC

As discussed in Chapter 1, there exists a hierarchy of outcomes of habitat manipulation which researchers hope to achieve when conducting CBC work (Gurr et al. 2007). To evaluate the success rate of the A&R concept studied in this thesis the results from each of the experiments can be compared against these five steps (Table 6-1). The first step is to increase the abundance of natural enemies near the flowering plants. This is the first study where synthetically-produced methyl salicylate (MeSA) has been deployed within brassicas. Consequently, one of the aims of this thesis is to test if the habitat manipulation tool ‘attract’ in the form of MeSA will increase the abundance of natural enemies near the source of the volatile.

In Chapter 2, the abundance of the diamondback moth ¹(TL2) (DBM) *Plutella xylostella* parasitoid *Diadegma semiclausum* ²(TL3) and the predatory hoverfly *Melangyna novaezealandiae* (TL3) was increased in the plots with MeSA; thus the first step of the hierarchy was achieved (Table 6-1) (Figure 6-1). Also, more females than males were attracted by the volatile. This could have further positive effects on the efficiency of *D. semiclausum* as a DBM biological control agent.

A&R were combined in the form of MeSA and buckwheat (BW) *Fagopyrum esculentum* in Chapter 3. Here also the first step of the hierarchy was achieved (Table 6-1). Significantly more brown lacewing *Micromus tasmaniae* (TL3), parasitoids of the DBM (TL2), *D. semiclausum* (TL3) and *Diadromus collaris* (TL3) and of the cabbage white butterfly (TL2) (CWB) *Plutella xylostella*, *Cotesia* spp. (TL3) were caught in treatments with buckwheat compared to treatments with none. *Melanostoma fasciatum* (TL3) was significantly more abundant in treatments with MeSA but significantly less abundant in treatments with buckwheat (Figure 6-2). However, no synergistic effect was seen on the abundance of natural enemies when the two technologies were combined (Figure 6-2). Significantly fewer male than female *D. semiclausum* (TL3) were attracted to the MeSA only treatments with a sex ratio of 1:1 for MeSA treatments compared with 60% males and 40% females with

¹TL2 = trophic level 2

²TL3 = trophic level 3

buckwheat. The apparent tendency of MeSA to attract to achieve a lower proportion of males could be important as a tool within CBC. However, as discussed earlier (Chapter 2 and 3) catching flying insects on sticky traps or other trapping devices reveals nothing on the dynamics or efficiency of biological control, including pest population suppression or improved yield or quality of the crop. To address this the capability of A&R to accomplish the third and fourth step in the hierarchy (Table 6-1) was assessed in Chapter 4 in which the effect of A&R on crop damage, natural enemy and herbivore (aphid) density, predation rate of egg sentinel baits and on aphid parasitism and hyperparasitism rates were evaluated. Treatments with MeSA lead to a significant increase in aphid parasitism rates and the third step in the hierarchy was therefore achieved (Table 6-1) (Figure 6-3). However, once again there was no synergism between the two treatments.

Table 6-1. The extent to which work in this thesis achieved the five steps in the hierarchy used to measure success within CBC (From Gurr *et al.* 2007). NB this does only apply to some of the species studied.

	Chapter 2	Chapter 3	Chapter 4
1. Aggregation of natural enemies at or near the flowers.	✓	✓	
2. An enhancement of the natural enemies' 'fitness' (longevity, fecundity and search efficiency).			
3. An increase in parasitism or predation rate of the pest.			✓
4. A decrease in the pest population density.			
5. The pest populations are brought below the relevant economic threshold (so avoiding the need to apply insecticides).			

		MeSA	
4 th trophic level	<i>A. zealandica</i>	+	
3 rd trophic level	<i>D. semiclausum</i>	+	
	<i>M. novaezealandiae</i>	+	
2 nd trophic level	<i>S. flava</i>	+	
1 st trophic level	Turnip <i>Brassica rapa</i>	NA	

Figure 6-1. The effect of MeSA on multiple trophic levels from Chapter 2. + = an increase in abundance of individuals from the species in treatments with MeSA, 0 = no effect of MeSA on the abundance of individuals from the species. Effect on the 1st trophic level, the crop, was measured as difference in herbivore damage between the treatment and the control.

		MeSA	BW	MeSA+ BW
4 th trophic level	<i>A. zealandica</i>	-	+	0
3 rd trophic level	<i>D. semiclausum</i>	0	+	0
	<i>Cotesia</i> spp.	0	+	0
	<i>D. collaris</i>	0	+	0
	<i>M. tasmaniae</i>	0	+	0
	<i>M. fasciatum</i>	+	-	0
2 nd trophic level	<i>S. flava</i>	0	0	0
1 st trophic level	Kale <i>Brassica oleracea</i>	NA	NA	NA

Figure 6-2. The effect of ‘attract and reward’ on multiple trophic levels from Chapter 3. + = an increase in abundance of individuals from different species in the treatment. - = a negative effect of the treatments on the abundance of individual from the species. 0 = no effect of the treatment on the abundance of individuals from the species. Effect on the 1st trophic level, the crop, was measured as difference in herbivore damage between the treatments and the control.

		MeSA	BW	MeSA +BW
4 th trophic level	Aphid hyperparasitism	0	0	0
3 rd trophic level	Aphid parasitism rate	+	0	0
	Predation rate	0	0	0
2 nd trophic level	Aphid density	0	0	0
1 st trophic level	Kale <i>Brassica oleracea</i> and swedes <i>Brassica napus</i>	NA	NA	NA

Figure 6-3. The effect of ‘attract and reward’ on aphid density, parasitism and hyperparasitism rate and on predation rate of egg sentinel baits from Chapter 4. + = an increase in rate/density in the treatment. - = a decrease in rate/density in the treatment. 0 = no effect of the treatment on rate/density. Effect on the 1st trophic level, the crop, was measured as difference in herbivore damage between the treatments and the control.

6.3. Effects of MeSA and buckwheat on arthropods from untargeted trophic levels

According to the above evaluation of the steps achieved in the hierarchy of success (Table 6-1), A&R could have a high success rate within CBC even though there is no synergistic effect between the two treatments. Some natural enemies ‘prefer’ buckwheat while some ‘prefer’ MeSA. As long as there are no negative interactions between the two treatments on the natural enemies, the result could still be an increase in biological control efficiency. However, the effects of the habitat manipulation on the abundance of arthropods from the fourth and second trophic levels have been a complicating factor in the above interpretation. These effects need to be evaluated as unwanted side effects involving these trophic levels may disrupt biological control. Consequently, as mentioned earlier, one of the aims in this thesis is to study the effects of the treatments on multiple trophic levels.

The effect of MeSA on the abundance of arthropods from the second and the fourth trophic levels has often been overlooked, even though HIPVs can affect the behaviour or physiology of organisms at multiple trophic-levels (Dicke and van Loon 2000). Populations of the brassica leafmining pest *Scaptomyza flava* (TL2) and the lacewing parasitoid *Anacharis zealandica*³(TL4) increased significantly in the MeSA treatment compared to the controls in Chapter 2 (Figure 6-1). However, the increase in abundance of *S. flava* (TL2) on traps, did not translate into crop damage. Consequently, the effect on the crop of the increase in herbivore numbers may not be as negative for biological control. HIPVs may signify plants that have been weakened and are therefore more susceptible to herbivores (Dicke and van Loon 2000). HIPVs can also function as ‘primers’ of surrounding plants (Chapter 1.4.) (Engelberth et al. 2004), enhancing the plants’ response to later herbivore attacks (Engelberth et al. 2004, Heil and Kost 2006). One explanation for the lack of crop damage in response to the increased abundance of *S. flava* could be due to ‘false advertisement’ by the synthetic MeSA. The herbivore may have ‘learnt’ to associate (Dicke et al. 1990c) the presence of MeSA or the HIPVs induced by MeSA (Chapter 1.4) with weakened plants. However, in the current work MeSA is of the synthetic version potentially sending out a ‘false’ signal that the crop has been weakened. Consequently, the crop is still fully capable of defending itself against herbivores, withstanding the herbivore; also the potential ‘priming’ capability of the plants by the synthetic MeSA could result in the brassicas responding even more efficiently to the leafminer’s attack, although there was no evidence in this work that the herbivore did actually attempt to oviposit in, or even land on the brassicas.

The effect of the increase in *A. zealandica* (TL4) abundance on the brown lacewing (TL3) population cannot be evaluated as only two lacewings were caught on the traps throughout the sampling period (Chapter 2).

An attraction of arthropods from an untargeted trophic levels also occurred when A&R was deployed (Chapter 3) (Figure 6-2). The abundance of *A. zealandica* (TL4) increased in treatments with buckwheat. However, it was repelled by treatments with MeSA in Chapter 3 (Figure 6-2). Potentially, this repellence property of MeSA to some arthropods could be utilised as a ‘push’ component in the ‘push-pull’ strategy within an integrated pest management programme (IPM) (Cook *et al.* 2007). It could also be deployed in A&R, repelling arthropods occupying unwanted trophic levels (Cook *et al.* 2007), as long as it is not at the expense of decreasing the abundance of target natural enemies.

³TL4 = Trophic level 4

6.4. Future research

Treatments with MeSA and with buckwheat resulted in an increased abundance of natural enemies. However, to be able to interpret what the consequences are of the increase on CBC, information on the feeding status (fed/unfed) and mating status (mated/unmated) of the males and females attracted are needed. An increased proportion of newly-emerged unmated females can be expected to prefer nectar/ pollen feeding to mating and host/prey search and hence only numbers on traps are not a good indicator of increased CBC. Also, unmated hymenopteran females produce males (haploid) (Noda 2000), which may cause a skewed sex ratio in the next generation and hence reduce long-term improvement of CBC. On the other hand, mated and fed females can be expected to search for host/prey or nectar/pollen feeding and hence an increased proportion of these would be a good indicator of increased CBC. Mated hymenopteran females can produce both male and female (diploid) offspring and hence have a greater potential for achieving successful CBC. Newly-emerged males can be expected to favour nectar/pollen rather than mating and prey search and hence large number of these are consequently not a good indicator of increased CBC. Older males can be expected to favour mating and prey search and would consequently be good indicators of increased CBC, as long as males are not the dominant sex attracted.

Similar studies on *A. zealandica* (TL4) are needed to evaluate the effect of the increased abundance of the parasitoid on the lacewing population. Buckwheat can, under laboratory conditions, increase the lacewing's longevity (Robinson *et al.* 2008) and in the field, increase the number of eggs oviposited by it (Jonsson *et al.* 2009b) when aphid densities are low. Similarly, buckwheat can increase the longevity of *A. zealandica* (TL4) under laboratory conditions (Jonsson *et al.* 2009b). However, the extent to which buckwheat affects the 'fitness' of the parasitoid compared to the lacewing in the field is not known (i.e. more or equal to that of the lacewing).

As discussed earlier (Chapter 1), MeSA has the potential to trigger the plant's own production of HIPVs (Khan *et al.* 2008) but it still remains unclear whether it is MeSA alone and/or a blend of volatiles produced by the host plant after induction by MeSA that is attractive to the arthropods. This question is addressed in Chapter 5, where the attraction of mated and fed *A. colemani* (TL3) females to synthetic MeSA is tested. However, further work testing the attraction of parasitoids with different feeding and mating status are needed as these have consequences for the increase of CBC. Also, including other natural enemies that show and increased abundance in response to the synthetic MeSA treatments in the field, such as *D. semiclausum* (TL3) and the two hoverfly species (TL3), would be relevant.

The potential induction of the plants' own production of HIPV still remains to be addressed. Experiments on the volatile production of plants exposed to synthetically-produced MeSA compared to unexposed plants are needed.

The success of CBC is expected to be enhanced through the A&R concept by increasing the attraction of natural enemies into the crop from surrounding habitats, avoiding redistribution of already existing natural enemies within the crop. However, the observed increased abundance of natural enemies (on traps in the current work) in response to MeSA could still be due to redistribution of them within the crop. Marking and tracking, for example with rubidium, (Pickett et al. 2004, Scarratt and Wratten 2004) of the natural enemies in neighbouring habitats are needed to show that the natural enemies do indeed enter the crop in higher numbers when 'attract' is used.

Floral resource supplementation can be optimised by supplying the natural enemies with flowering plants that have optimal nectar and pollen availability (Waller et al. 1981, Wäckers 2005) and suitability for the natural enemies. Buckwheat *Fagopyrum esculentum* is considered to have nectar and pollen well suited for many natural enemies (Bowie et al. 1995, Stephens et al. 1998, Berndt et al. 2002, Tylianakis et al. 2004, Irvin et al. 2006, Lavandero et al. 2006). Jasmonic acid (JA)/ methyl jasmonate (MeJA) is involved in the octadecanoid pathway that plays an important role in the induction of defences such as some HIPVs (Bruinsma *et al.* 2008). Synthetically produced MeJA can increase the abundance of natural enemies in the field (James and Grasswitz 2005). However, JA treated black mustard plants *Brassica nigra* L. (Brassicaceae) have reduced nectar secretion and a lower concentration of glucose and fructose of the nectar than control plants. HIPVs can affect the surrounding plants by altering their chemical composition (Dicke and Bruin 2001). Deploying synthetically-produced MeSA in the field could potentially alter the nectar quality and quantity of the buckwheat deployed in A&R, consequently reducing its quality as a food resource. Experiments on the nectar and pollen quality of buckwheat exposed to synthetically-produced MeSA would therefore be relevant.

6.5. Conclusions

Overall, the results from this study indicate that A&R could potentially be deployed as a habitat manipulation technique within CBC, even though there were no synergistic effects between MeSA and buckwheat on the abundance of natural enemies as initially hypothesised.

Combining A&R increases the abundance of natural enemies, some due to effects of buckwheat others because of MeSA. However, the results from this study have demonstrated that the abundance of arthropods from untargeted trophic levels is also increased by A&R. The consequences of these increases on CBC still remain to be fully evaluated. There still remain steps in the hierarchy of success in CBC (Gurr et al. 2007) that the A&R concept needs to achieve (Table 6-1) and the section (6.3.) above points out some of the additional questions remaining to be answered before the A&R concept as a tool to enhance CBC can be considered ready to be deployed by end users. In other words, a service providing unit (SPU) (Luck et al. 2003) cannot be developed based on the data in this thesis. An SPU is the unit required to provide an ecosystem service (ES) (Chapter 1.1.), such as biological control, at some defined temporal or spatial scale with the consequences of the deployment defined (Luck et al. 2003). Also, the current work addressed brassicas and their pest and natural enemy complexes. For other crop systems, the potential caveats identified here would need to be explored through new field experimentation. The latter should not use only trapping methods but should address, as in this work, the non-targeted trophic levels and the dynamics or the predation/parasitism processes involved and, most importantly levels of crop damage. Future approaches to sustainable pest management need to be based on well-researched ecological science to develop technologies which are readily adopted by agriculturalists and which have few external costs (Cullen et al. 2008). The work in this thesis is a contribution to that approach; it is increasingly urgent and this is supported by recent reviews on the future of world agriculture (Jordan et al. 2007, Landis et al. 2008, Vitousek et al. 2009).

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