Attract, reward and disrupt: combining habitat manipulation and semiochemicals to enhance pest control in apple orchards

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Doctoral thesis
Swedish University of Agricultural Sciences
Alnarp 2019

Cover: Sown flower strip in apple orchard (left), *Anthocoris nemorum* feeding on an aphid, *Chrysoperla carnea* resting within a flower strip (centre) and apple bean intercropping (right).

   (photo: Joakim Pålsson and Marco Tasin)

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Abstract

Agricultural intensification entails negative effects on natural enemy populations and the pest regulation services they provide. Habitat manipulation holds potential to control pests with less negative effects on the environment than conventional methods. This can be achieved by the establishment of non-crop vegetation to support natural enemies and disfavour pests. Synthetic semiochemicals possess the capability of affecting arthropods behaviour and can be combined with habitat manipulation to increase pest control.

In this thesis I examine how biological control is affected in high (conventional) and low (organic) intensity apple production systems and how habitat manipulation with and without semiochemicals can be used to enhance biological pest control.

The intensity of management had a strong effect on the natural enemy community and their pest control potential. Organic apple orchards were able to support a higher number and diversity of natural enemies and presented a higher suppression of sentinel *Dysaphis plantaginea* colonies than integrated pest management orchards. The natural enemies which were most affected by management were predatory heteroptera with many species almost exclusively found in organic orchards. *D. plantaginea*, establishes a mutualism with ants as a protection strategy against natural enemies. By introducing bean plants infested with *Aphis fabae*, ants (*Lasius niger*) were diverted from *D. plantaginea* colonies, leaving them exposed to predation.

The possibility to attract natural enemies with synthetic herbivore induced plant volatiles (HIPVs) was tested in apple and barley. In both crops the HIPVs were able to attract green lacewings (*Chrysoperla carnea s.l.)* over four weeks. Significant increases in oviposition and larval abundance was recorded compared to the control as well as a higher reduction in two species of cereal aphids.

When HIPVs were deployed along with flower strips, the attraction of natural enemies including Miridae was enhanced. The combination of HIPVs, flower strips and mating disruption suppressed populations of lepidopteran pests below that of flower strips + mating disruption or mating disruption alone under a three-year study.

Keywords: Conservation biological control, codling moth, Leafrollers, Miridae, rosy apple aphid, Biological control, Mating disruption

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Dedication

To my family

Think of the solution, not the problem.
　　Terry Goodkind

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I   Porcel, M*, Andersson, GKS, Pålsson, J & Tasin, M. (2018). Organic management in apple orchards: Higher impacts on biological control than on pollination. *Journal of Applied Ecology*, vol. 55 (6), 2779-2789.

II   Pålsson, J., Porcel, M., Frimodt Hansen, M., Offenberg, J., Nardin, T., Larcher, R. and Tasin, M.* (2019). Aphid-infested beans divert ant attendance from the rosy apple aphid in apple-bean intercropping. (manuscript)

III Pålsson, J., Thöming, G., Silva, R., Porcel, M.,Dekker, T. and Tasin, M.* (2019). Recruiting on the spot: A biodegradable formulation for lacewings to trigger biological control of aphids *Insects.,* vol. 10, (6)

IV   Pålsson᾽ J., Porcel, M., Dekker, T. and Tasin, M.* (2019). Attract, reward & disrupt: responses of lepidopteran pests and natural enemies to combinations of habitat manipulation and semiochemicals in organic apple (manuscript)

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The contribution of Joakim Pålsson to the papers included in this thesis was as follows:

I   Collected field data on aphids. Identified the arthropods from suction samples together with the co-authors

II Collected the field data. Planned, analysed the data and wrote the manuscript together with the co-authors

III Collected the data from the apple orchards. Planed, collected and analysed odour collections and wrote the manuscript together with the co-authors

IV Planed, collected field data, identified the arthropods, analysed the data and wrote the manuscript together with the co-authors

Abbreviations

A	Attract
A+R	Attract and reward
AA	Acetic acid
CBC	Conservation biological control
HIPVs	Herbivore induced plant volatile
IP	Integrated production
IPM	Integrated pest management
MMD	Multipurpose mating disruption
MS	Methyl salicylate
PAA	Phenylacetaldehyde
R	Reward

1 Introduction

Land use has increased the in the last decades, together with use of fertilizers, irrigation and pesticides, and is predicted to increase even further in the future. Intensification of agriculture can lead to environmental pollution, pest resistance and highly homogeneous landscapes with small and fragmented non-cropped areas. This produces poor resilience against disturbances and low ecosystem services provision, which agriculture in many cases relies on (Beckmann et al., 2019; Loreau and de Mazancourt, 2013; Martin et al., 2019; Rockström et al., 2017; Tscharntke et al., 2005). While predators and pollinators decrease together with biodiversity in larger areas of monoculture, herbivores are expected to increase as host accessibility becomes more unrestricted (Gurr et al., 2012).

Natural enemies of pests can provide significant herbivore suppression in agroecosystems. A higher richness and evenness in natural enemy communities enhances biological control of pests (Crowder et al., 2010; Snyder et al., 2006; Snyder and Ives, 2003). The increase of biological control due to a diverse community is often caused by natural enemy compatibility, depending on their presence and activity over time and space. More ecologically complex landscapes and farms can support a higher diversity of natural enemies as they provide more recourses, such as pollen, nectar, alternative prey and shelter. This also contributes to a higher resilience to disturbances, e.g. pesticide use or tillage, at the local scale (Dainese et al., 2019; Gurr et al., 2012; Loreau and de Mazancourt, 2013; Tscharntke et al., 2005). Thus, in less complex landscapes, local management may have even higher impact on biodiversity with greater influence on depletion of local species (Dixon, 2003; Emmerson et al., 2016; Tscharntke et al., 2005). The ubiquity of pesticide use is one of the main limitations for implementing biological control in agriculture (Landis et al., 2000). A major challenge is to re-establish ecosystem services such as biological control to increase the sustainability of crop production (Tittonell, 2014).

1.1 Biological control

Biological control was defined by Eilenberg, Hajek and Lomer (2001) as: 'The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be'.

These authors further divided biological control in four strategies: classical biological control, inoculation biological control, inundation biological control and conservation biological control. Classical biological control is the introduction of an exotic organism with the purpose for long-term biological control, often of an exotic pest, through its establishment. Inoculation biological control includes the release of living organisms that will multiply and control the pest for a prolonged, but not permanent, period. Inundation biological control is when biological control is achieved exclusively by the released organisms, often through contact, and are commonly not active over a long period. The fourth strategy, conservation biological control (CBC), aims at protecting and enhancing naturally occurring biological control agents through modification of the environment or the adaptation of management practices. This includes not only the limited and selective use of pesticides, but also active processes such as the provision of refuges adjacent to crops or within crops, the facilitation of transfer of natural enemies between crops, and the direct provision of food and shelter to natural enemies within the crop.

1.2 Habitat manipulation

Altering the environment to benefit natural enemies is a form of CBC that is termed habitat manipulation or habitat management. Through habitat manipulation resources such as food for adult natural enemies, alternative prey or hosts, and shelter from hostile conditions can be provided to enhance the survival, fecundity, longevity and performance of natural enemies to increase their control capacity (Landis et al., 2000). These resources should be integrated spatially and temporally to favour the targeted natural enemies during the whole season, and ideally last for several years. The changes can be implemented on a landscape as well on a local (farm) scale.

The establishment of flowers strips is a habitat manipulation practice that introduces floral diversity into the farmland. Flower strips provide different resources that can be exploited by third trophic level arthropods to improve their performance as biocontrol agents. The presence of these resources can also contribute to their recruit and perdurance within and around the crop (Nilsson et al., 2016). This strategy has been shown to increase natural enemies presence in several crops, e.g. lepidopteran predators in grass (Frank and Shrewsbury, 2009)

and parasitoids and generalist predators in brassica (Pfiffner et al., 2009; Philips et al., 2014) and in apple (Cahenzli et al., 2019; Markó et al., 2013, 2012). It was also shown to provide alternative hosts to beneficial insects (Unruh et al., 2012). However, the reported increase of natural enemies is not necessarily consistent among sites and species (e.g. Pfiffner *et al.*, 2009) and has not always led to a higher pest control.

Flower strips also provide shelter which improve the microclimate. Establishment of shrubs can stabilise temperatures during winter/summer so overwintering or sensitive life stages may avoid extreme temperatures. Shelters may also allow natural enemies to avoid intraguild predation by providing a more complex structure to hide in from other predators. Even though establishment of non-crop vegetation can benefit natural enemies at farm scale, landscape complexity also affects species abundance and richness (Chaplin-Kramer and Kremen, 2012; Martin et al., 2019; Medeiros et al., 2018)

1.3 Semiochemicals

Semiochemicals are compounds mediating communication within and among species (Nordlund and Lewis, 1976). Semiochemicals can further be classified as pheromones and allelochemicals, depending on whether the interaction is intraspecific or interspecific. After semiochemical perception, a behavioral or physiological responses occurs (Nordlund and Lewis, 1976).

Allelochemicals can be further divided into subgroups, such as allomones, kairomones, synomones, and apneumones, depending on whether the emitter, the receiver, or both benefit from the interaction (Nordlund and Lewis, 1976). Kairomones are substances evoking a reaction which benefits the receiver but not the emitter. The opposite effect is triggered by allomones. Synomones are substances produced by an individual that benefit both the emitter and the receiver. Apneumones are substances produced by non-living material that are benefiting the receiver but not the organism living in or on the non-living material.

Semiochemicals are of interest in agriculture as they have the potential to affect the behaviour of arthropods. By permeating the atmosphere of a crop with a synthetic sex-pheromone, the encounter between sexes can be prevented or delayed. This approach is termed mating disruption. The use of mating disruption for direct control of lepidopteran pest species have been very successfully for over two decades in fruit and vine growing (Koul et al., 2008). By combining the sex-pheromone components of several pests in a single formulation, a wider pest control along with a reduction in pest application costs may be achieved (Porcel et al., 2015). Mating disruption is a highly species-

specific method to control pests, leading to reduced disturbance of natural enemies with the potential of increased biological control and resilience of the cropping system.

Besides sex-pheromones, herbivore-induced plant volatiles (HIPVs) are another group of semiochemicals with a potential use in pest management (Turlings and Erb, 2018). HIPVs can be classified as kairomones (Nordlund and Lewis, 1976) or synomones. HIPVs are produced and released into the atmosphere by plants upon herbivory, which induces a systemic defence in the whole plant (Heil and Ton, 2008; Ninkovic et al., 2016; Šimpraga et al., 2016). The release of HIPVs occurs exclusively upon herbivory and it represents an honest cue for pest's predators and parasitoids to follow (Braasch and Kaplan, 2012; Silva et al., 2017; Turlings and Erb, 2018).

Single or blends of synthetic HIPVs (e.g. methyl salicylate, acetic acid, 2-phenylethanol, cis-3-hexene-1-ol, linalool, phenyl acetaldehyde) are attractive to different natural enemies (e.g. Neuroptera, Syrphidae, predatory Heteroptera, Braconidae, Ichnemonoidea, Encyrtidae) (James and Grasswitz, 2005; Kessler, 2001; Rodriguez-Saona et al., 2011; Tóth et al., 2014; Ucchi et al., 2017). Some HIPVs have been shown not only to attract natural enemies, but also both sexes of lepidopteran pests such as tortricid moths (Knight et al., 2014). There are potential risks in the use of synthetic HIPVs as natural enemy attractants (Kaplan, 2012). It is possible that forth tropic level organisms are attracted, which may reduce the third trophic level populations (natural enemies of pests) which may release the pest from its predation pressure (Orre et al., 2010). There is also risk of aggregation of natural enemies around the HIPVs, whilst leaving other areas depleted, and thereby reducing pest control locally in these areas.

1.4 Attract and reward

When using HIPVs to attract natural enemies, if they do not associate the volatiles with a reward they may lose interest in that specific signal and, in the worst case scenario, the same attractant could become a repellent (Kaplan, 2012). This can be problematic because some single compounds (e.g. methyl salicylate) are attractive to several natural enemy species. A reduction of such an unwanted effect may be achieved through the combination of HIPVs with habitat manipulation, which provides alternative prey, nectar, pollen and shelter in case these resources are not otherwise present within the farm. The combination of HIPVs and flower resource has been tested in sweetcorn, broccoli and wine-grapes by Simpson et al. (2011a) and was coined "Attract and Reward" (A&R). Through such an approach, an increased recruitment and residency of natural enemies as well as an enhancement of biocontrol was

achieved. Although the preliminary results are promising, only a few studies have attempted an implementation of this approach in a cultivated field. Obstacles its use include, for example, the lack of knowledge on how to enhance specific natural enemies without increasing pest levels. At the same time, the disturbance due to pesticide use may mask the increase in biological control obtained via A&R. To overcome this limitation, the effect of such an approach should be investigated under a selective pest management strategy, using specific floral diversity and HIPVs.

2   Aim and objective

The general aim of this thesis was to examine how more sustainable pest control could be achieved through a combination of habitat manipulation and semiochemicals in apple orchards. Both of these methods have the capacity to increase and conserve biodiversity and alter insect abundance so that potentially higher pest control may be achieved. A higher resilience of the orchard agroecosystem towards pests is expected.

The first step was to examine how more sustainable management, i.e. organic management, which employs more specific and less disturbing pest control methods, affects beneficial insects and their potential for biological control. Natural enemy abundance and species evenness were examined using suction sampling, whilst biological control was assessed through sentinel aphid colonies (Paper I).

The next step was to employ habitat manipulation and the use of semiochemicals in organic orchards to determine their potential pest control. This was measured through three different experiments in apple orchards: (i) habitat manipulation to disrupt ant-aphid mutualism (paper II), (ii) attraction of lacewings to HIPVs and evaluation of biological control of aphids (paper III) and (iii) habitat manipulation using perennial flower strips, HIPVs and mating disruption to increase biological control of lepidopteran pests (paper IV).

3 Management effect on biological control

(Paper I)

Integrated pest management (IPM) and organic farming are two different approaches commonly used in crop production. Less intense agriculture, i.e. organic farming, is expected to promote higher abundance and biodiversity of beneficial arthropods and, accordingly, higher biological control and pollination efficiency. Here we examined the management effect (IPM and organic) on natural enemy populations and on biological control of aphids in apple orchards.

3.1 Materials and methods

3.1.1 Orchards

The study was carried out in Skåne, Southern Sweden, the most important apple growing region in the country. It is characterized by a significant variation in landscape composition where 21.9% of the total area is covered by forest and 69.3% by cropland, primarily arable crops (32.7%). Apple orchards account for 0.2% (1,293 ha) of the total extension and are interspersed between field crops and forests.

The study was conducted in nine apple orchards managed by different growers. All orchards are situated at a minimum distance of 2 km from each other. Four orchards were managed under IP guidelines and five under organic management. A landscape index based on land-use data was generated to estimate the landscape effect.

3.1.2 Biological control

Biological control was measured over the whole cycle of the pest using sentinel aphid colonies. Rosy apple aphid (*Dyspahis plantaginea*) colonies were established on 2-year-old potted trees. The use of potted trees allows for homogeneous plant conditions ruling out differences in bottom-up processes affecting aphid colony development and biological control. Five trees were placed in each orchard to be colonized as early as possible by the arthropods present in them. In all orchards, potted trees were planted with their pot in lined 10 m apart along the border of the plot at approximately 5 m from the commercial tree lines to prevent insecticide applications from reaching them.

Aphid colonies were artificially established on the potted trees using custom manufactured clipcages (Figure 1). A total of four clipcages were placed on each

potted tree on separated branches (20 colonies per orchard). Additionally, on each tree, a natural enemy exclusion control (mesh bags) was established. All arthropods were knocked off the branch before placing the mesh bag on the tree. The zip was taped to prevent access to the branch. A clipcage was also placed inside each control bag (five per orchard).

Figure 1: Established *D. plantaginea* colonies on the trees with clipcage.

3.1.3 Natural enemies

Native natural enemies observed within the aphid colonies were identified to the lowest taxonomic level possible. Natural enemies were not removed from the colonies. Arthropods were collected each week from the canopy of the orchard with a field aspirator. Each sample consisted of 2 min aspiration on leaves up to 2 m, in approximately four trees, covering all the sides of the trees in a methodology adapted from Porcel et al. (2011) . Fifteen samples were taken per plot with a minimum distance of 10 m between groups of trees and always at 15 m from the edge of the plot. Samples were stored in a freezer (−18°C) on arrival to the lab and subsequently separated from debris and vegetal material under stereomicroscope. Main aphid natural enemies in this study, based on the species recorded in field observation of sentinel colonies, were identified at species level by using entomological keys.

3.2 Result and discussion

3.2.1 Biological control / sentinel aphids

We found a strong relationship between orchard management and biological control of aphids. Exposed aphid colonies declined over the duration of the experiment for both management systems while bagged controls grew exponentially with no tendency to disappear, except those that underwent predator invasions (Figure 2). However, colony suppression was higher in organic orchards than in conventional (Figure 2 and Figure 3a). Almost all colonies had disappeared within a month in organic orchards while in conventional orchards colonies lasted longer and reached bigger sizes (Figure 3a).

Natural enemies' presence around sentinel aphid colonies was higher in organic orchards, following the trend of the colony suppression, and responded quicker to the presence of sentinel aphids (Figure 3a-b). The most observed natural enemies in the colonies were: *Anthocoris nemorum*, the common European earwig, *Forficula auricularia*; and larvae of the predatory midge, *Aphidoletes aphidimyza*. Predatory mirids were exclusively observed in organic orchards. *A. nemorum* were mainly observed early after colony unclipping, sometimes roaming around the clip cage. *A. aphidimyza* and syrphid fly larvae occurred more in well-developed colonies later in the season. No parasitoids mummies were recorded during the experiment.

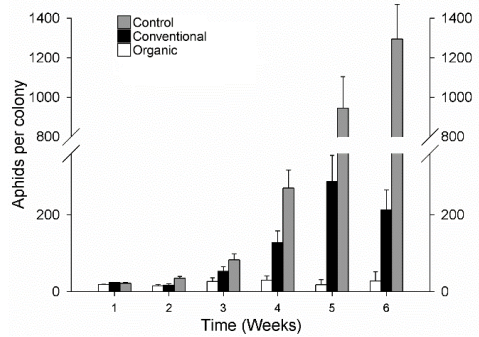*Figure 2:* Mean (± SE) number of *D. plantaginea* adults and nymphs (excluding alate adults) per colony and week in organic and conventional orchards and control colonies.
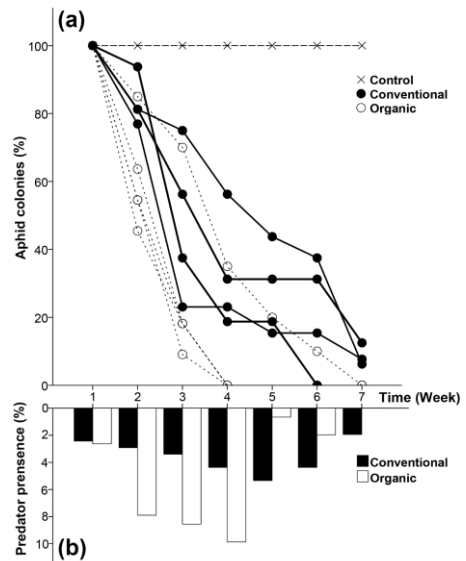

Figure 3: (a) Percentage of active *D. plantaginea* sentinel colonies out of total colonies established in the different conventional and organic apple orchards throughout the duration of the predation experiment. Control colonies are all grouped together.

3.2.2 Natural enemy community

Suction samples confirmed that organic management can support a higher abundance and richer predator community than IP. A total of 1 150 specimens of potential natural enemies of aphids were collected from different orders. The species collected were *A. nemorum*, *Orius minutus*, *Atractotomus mali*, *F. auricularia*, *Chrysoperla carnea s.l.*, *Hemerobius humulinus*, *Coccinella septempunctata*, *Adalia decempunctata*, *Adalia bipunctata*, *Propylea quatuordecimpunctata*, *Cantharis fusca*, *Cantharis lateralis*, *Cantharis livida*, *Cantharis nigricans*, *Cantharis obscura*, *Cantharis pallida* and *Cantharis rufa*. Adult individuals of syrphids, lacewings and predatory midges were not included as they do not predate aphids as adults. The total amount of these natural enemies was higher in organic orchards for all sampling dates. The same results were obtained comparing abundance on family level, with the exception of earwigs (Figure 4a-d). This difference could already be observed before the start of the experiment. Additionally, a higher natural enemy abundance and evenness index was measured at all sampling dates in organic orchards (Figure 5). Communities with higher natural enemy species evenness are assumed in general to show a higher resilience against pests (Tscharntke et al., 2005). For example, organic management in potato promoted predator species evenness leading to higher levels of biological control of potato beetle (Crowder et al.,
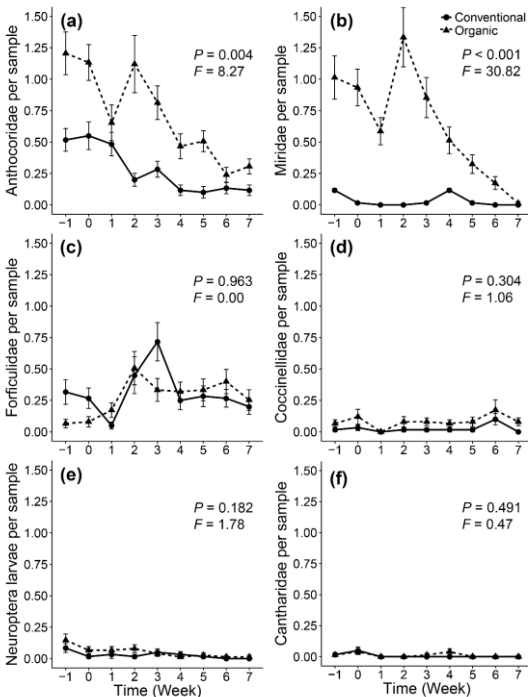
Figure 4: Mean (± SE) abundance per sample of (a) Anthocoridae (*A. nemorum* and *O. minutus*), (b) Miridae (*A. mali*) (d) Forficulidae (*F. auricularia*), (c) Neuroptera larvae (*C. carnea s.l.*, and *H. humulinus* larvae) (d) Coccinellidae (*C. septempunctata*, *A. decempunctata*, *A. bipunctata* and *P. quatuordecimpunctata*) and (e) Cantharidae (*Cantharis fusca*, *C. lateralis*, *C. livida*, *C. nigricans*, *C. obscura*, *C. pallida* and *C. rufa*) sampled in organic and conventional plots at different dates. F and *P*-values are shown for each group

Figure 5: (a) Mean (± SE) total abundance of predators per sample and (b) mean (± SE) species evenness per orchard (Pielou's evenness index) of the predatory species: *A. nemorum*, *O. minutus*, *A mali*, *F. auricularia*, *C. carnea s.l.*, *H. humulinus*, *C. septempunctata*, *A. decempunctata*, *A. bipunctata*, *P. quatuordecimpunctata*, *C. fusca*, *C. lateralis*, *C. livida*, *C. nigricans*, *C. obscura*, *C. pallida* and *C. rufa* sampled in organic and conventional plots in different dates.
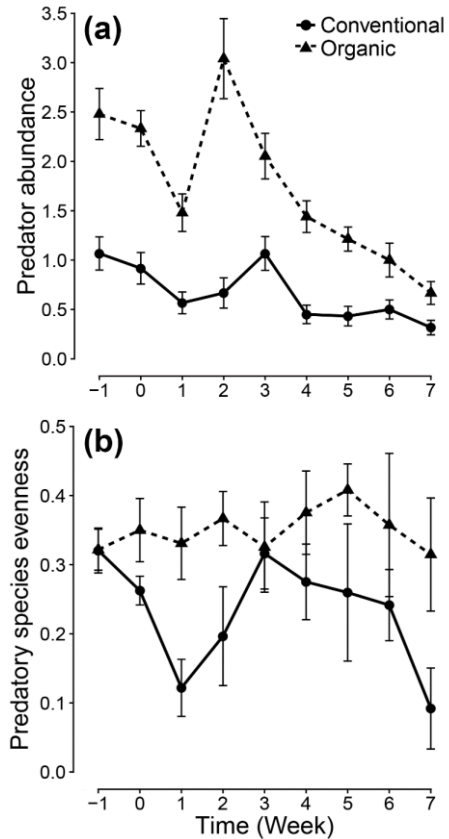
2010). Organic management also promoted higher biodiversity in cereal in several European countries, increasing carabid beetle species richness resulting in higher aphid consumption (Geiger et al., 2010).

The natural enemy group mostly affected by management in our study was predatory Heteroptera. Both families of Miridae and Anthocoridae (Figure 6) were the most frequently collected families in suction samples and they were numerically dominant in organic orchards (Figure 4a-b). Predatory Heteroptera are most likely the cause of the colony suppression found in this study. In particular, *A. nemorum*, which was frequently observed in sentinel colonies. The early activity of these species was essential to provide colony suppression while the aphid colonies were still at an early developmental stage.

Most of the natural enemies found in this study forage, reproduce and contribute to pest regulation during almost the entire growing season, presenting a significantly exposure to management impacts. The reduction of predatory Heteropterans in conventional management is likely due to systemic aphicides. These are often applied around flowering when *D. plantaginea* begin to feed. Because Miridae are omnivorous and often feed also from the plant (Johnsson, 1983), a strong population decrease is expected in sprayed orchards. In contrast, Anthocoridae are mainly zoophagous and overwinter as adult (Johnsson, 1983).

A faster and earlier recolonization of sprayed orchards is thus expected for Anthochoridae than from Miridae.

Figure 6: Predatory heteroptera on an apple leaf. Left figure: adult *Anthocoris nemorum*, Right figure *Heterotoma planicornis.*

4 Diversion of ant-aphid mutualism through habitat manipulation

(Paper II)

Dysaphis plantaginea benefits from a mutualistic relationship with the ant *Lasius niger*. Whereas aphids get protection against natural enemies, ants receive in exchange carbohydrates, i.e. honeydew (Way, 1963). Ant-attended aphids display an increased fitness compared to unattended conspecifics, leading to higher potential damage (Flatt and Weisser, 2000; Way, 1963). The mutualistic link between *L. niger* and *D. plantaginea* can be disrupted by offering alternative carbohydrate sources to the ants (Nagy et al., 2013). When ants-aphid mutualism is disrupted, more predators come into the colonies, resulting in higher aphid suppression. In this study we explored the possibility of using habitat manipulation, i.e. intercropping, to disrupt the ant-aphid mutualism on apple trees. To this purpose, bean plants carrying the bean aphid (*Aphis fabae*) were used to divert ants from apple trees. We hypothesized that the presence of an alternative honeydew source, released from *A. fabae* in a more diversified system, would compete for the attention of *L niger* resulting on less ant attendance of *D. plantaginea* and higher predation pressure.

4.1 Material and method

4.1.1 Experiment in the greenhouse

A single *Aphis fabae* female was transferred to the top of a potted bean, *Vicia faba* variety 'Gloria', to create an aphid colony per plant. The newly infested plant was covered with a perforated plastic bag as a protection measure.

Forty two-year-old potted apple trees (cv. Aroma) were maintained in 15 l pots under standard fertilization and disease and pest control conditions. Trees were kept in a net-house (to avoid ant nesting) or in different apple orchards within the Skåne region (Sweden) to allow *L. niger* to nest inside the pots at the base of the trees. Only the rooting system of trees exposed in the orchards which had an ant-nest was used in further experiments. The presence or absence of an active nest with a queen and eggs was verified by inspecting the soil of each pot. The root system with the ant nest was then removed in a block from the pot and transferred to an ant-secured starving-cardboard box. Ants were kept without food or water 14 hours before each run of the experiment.

The potted trees that were not exposed in orchards, and therefore had no ant nests, were treated with a natural pyrethrin extract to avoid presence of any undetected ant. A single *D. plantaginea* apterous virginoparae adult was collected from a field colony and inoculated on a shoot of a potted tree using a clip cage (same as in paper I). Bean and apple plants were inoculated with the respective aphid on the same day. At day 7 after inoculation, a *V. faba* plant with an established *A. fabae* colony was removed from its protective bag and planted into the same pot as the apple tree avoiding a direct contact between the canopy of the apple tree and the bean. The clip cage around *D. plantaginea* was removed after planting the bean. In order to prevent uncontrolled ant intrusion from outside, the pot with the two plants was placed into a masonry bucket with water. At 8:00, ant nests were removed from the starving box and placed inside a black plastic bag and thereafter back into its original pot. The pot was then placed into a masonry bucket with water. At 10:00 a.m., the two pots were connected with a wooden bridge allowing the ants to freely access the pot hosting the two plants. The bridge was situated equidistantly between the apple tree and the bean plant. The number of *D. plantaginea* aphids on apple, *A. fabae* on bean, and ants tending both colonies were counted six times in 2 h intervals from 10.00 a.m. to 8.00 p.m. Ten replicates of the experiment were run simultaneously each day for three consecutive days using ten different *L. niger* nests. Each apple and bean plants were used once and each ant nest was used three times in different days. A total of 30 replicates of the experiment were run.

4.1.2 Field experiment in the apple orchard

Four square plots (30 × 30 m) were established in an apple orchard in the Skåne region (Sweden). Plots included seven apple rows and were spaced a minimum of 25 m apart. Twenty-seven apple trees in the centre of each plot were artificially infested with a single apterous virginoparae of *D. plantaginea* following the protocol presented in Paper I. Five control colonies were

established using a mesh bag to exclude predators. The clip cages were removed after 7 days. In two plots, bean plants infested with *A. fabae* were planted underneath the *D. plantaginea* colonies (Figure 7). Each *D. plantaginea* colony was surrounded by three bean plants in the apple + bean treatment. The other two plots were regarded as control, with only apple trees. Bean plants were prepared following the same protocol as in the greenhouse experiment and were infested the same day as apple trees. After infestation they were kept in the net-house in Alnarp to acclimatize after growing in the greenhouse.

Figure 7: Planted bean infested with *A. fabae* in tree row to attract ants.

The number of aphids (*A. fabae* and *D. plantaginea*), ants and natural enemies associated to the aphid colonies was counted weekly during a four-week period. Not all *A. fabae* survived after the first week of exposure in the orchard. The uncolonised bean plants from the second to the fourth week of the experiment were used to evaluate if ants preferred infested over uninfested bean plant. Bean plants with no established colonies were excluded from the statistical analysis.

4.1.3 Collection of honeydew

Aphid honeydew (1-5 µl; N=6) was collected from active colonies of *A. fabae* and *D. plantaginea* by folding aluminium foil around the leaf hosting the colony during 6 hours. Collections were stored at -18° C inside microcapillaries until analysis. Sugar content was analysed through chromatographic separation using an ICS 5000 ion chromatography system (Dionex; Thermo Scientific, Waltham, USA) and amino acid content with a UHPLC Ultimate 3000 (Thermo Scientific, Waltham, Massachusetts, Stati Uniti) equipped with a fluorescence detector (Ex = 336 nm, Em = 445 nm). See Paper II for more details about honeydew analyses.

4.2 Results and discussion

4.2.1 Ant preference

In both greenhouse and field experiments, a higher number of ants were observed on *A. fabae* compared to *D. plantaginea*. In the greenhouse, the number of ants observed attending *A. fabae* was higher during the entire sampling period (10 hours). A significant increase of ant attendance occurred over time for both species, with a higher increase for *A. fabae* (Figure 8A). A similar trend was observed for the proportion of colonies attended (Figure 8B). At the final time of the experiment, 60.0% of the *A. fabae* colonies were ant-attended against only 6.6% of the *D. plantaginea* colonies. A similar ant behaviour was observed in the field experiment. Ants preferred *A. fabae* over *D. plantaginea* in the plot with infested bean plants intercropped with apple trees (Figure 9A-B). There were also significantly fewer ants attending *D. plantaginea* colonies in the apple + bean intercropping compared to the control plots without bean plants (Figure 9B). All of the observed ants were identified as *L. niger*.

Both greenhouse and field experiments showed a potential to disrupt ant-aphid mutualism. Previous studies have successfully disrupted this relationship using ant-feeders delivering a sugar solution at the trunk or within the apple canopy (Nagy et al., 2015, 2013). Placing large feeders with sucrose was as effective as ant exclusion with sticky barriers (Nagy et al., 2015). The authors argued that these can be caused, at least partially, by the reduced distance to the carbohydrate source near the trunk compared to the *D. plantaginea* colony on
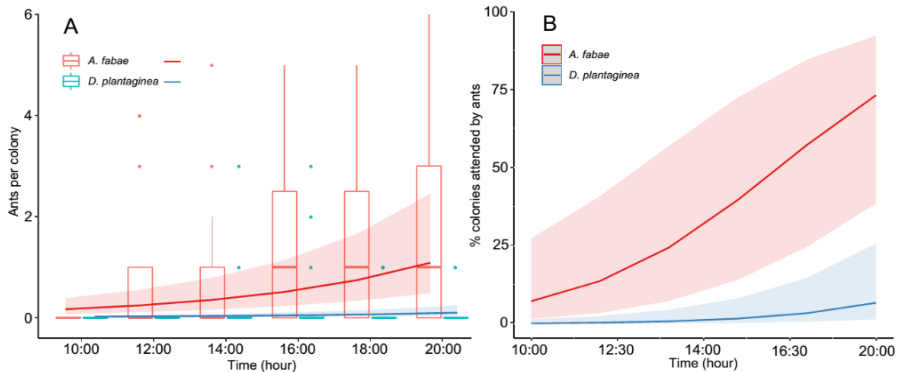
Figure 8: (A) Boxplot of the number of ants attending *D. plantaginea* and *A. fabae* colonies over time and predicted values (± 95% confidence intervals) of the GLMM.(B) Predicted values (± 95% confidence intervals) of the GLMM representing the % of *D. plantaginea* and *A. fabae* attended by *L. niger* over time. The increase in attendance and the differences between aphid species were significant for both models (GLMM: Wald test, $P < 0.01$).
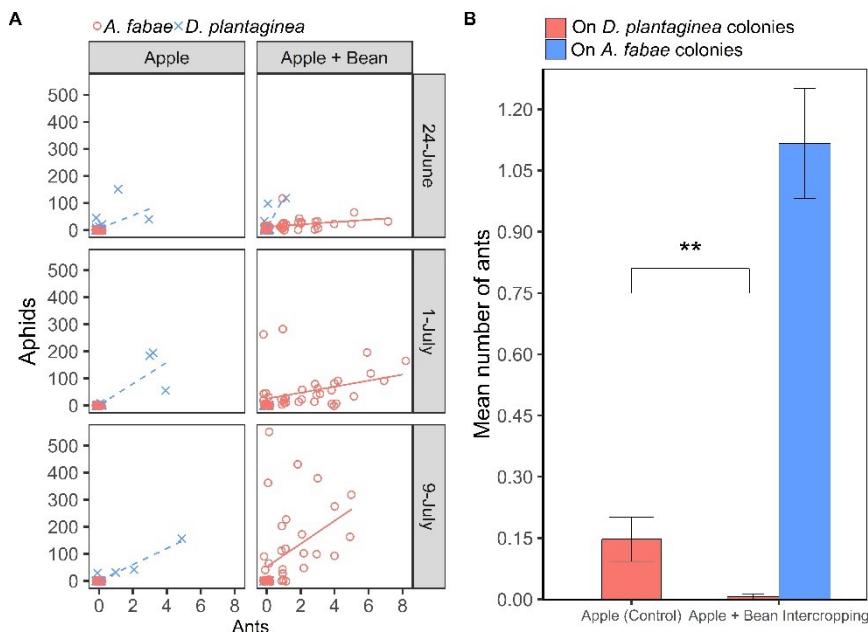
Figure 9: (A) Relation between the number of ants and the number of aphids in attended colonies of apple or apple + bean intercropping. (B) Mean number of ants (± SE) per plant attending aphid colonies in apple or apple + bean intercropping.

the canopy (Devigne and Detrain, 2006). This could also be the case in our study, with higher abundance of *L. niger* on *A. fabae* than on *D. plantaginea*. Although bean can produce extra flora nectar which may further increase the attraction of ants, in our field experiment ants were almost exclusively present on plants infested with *A. fabae*. The number of ants correlated with the size of the aphid colony (Figure 9A). This is in line with a previous study that found *L. niger* preferring *A. fabae* over extra floral nectar as it contained less sugar than the honeydew (Vantaux et al., 2011).

4.2.2 Chemical analysis of aphid honeydew

Sugar concentration and composition are important factors for *L. niger* preference. This ant is particularly sensitive to the presence of sucrose and melezitose (Detrain and Prieur, 2014), which contents depend on aphid species and host plant (Fischer and Shingleton, 2001; Vantaux et al., 2011). In addition to sugar, amino acids can increase ant attraction to lures in the laboratory (Madsen et al., 2017).

Our chemical analysis of aphid honeydew disclosed differences between *A. fabae* and *D. plantaginea* (Figure 10). The most dominant sugars in both species were glucose and fructose, with the latter more abundant in *A. fabae*. Melezitose,

Figure 10: Normalized sugar content (as proportion) in honeydew collected from *A. fabae* (Af) and *D. plantaginea* (Dp). *P*-values reported indicate statistically significant differences (Wilcoxon test, $P < 0.05$).
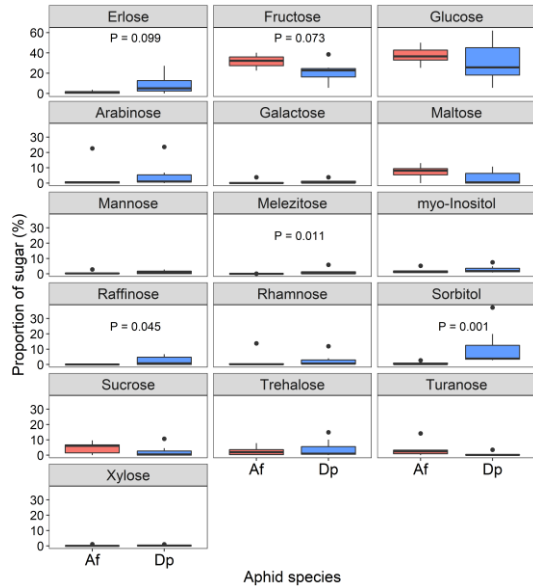
raffinose and sorbitol occurred in higher concentration in *D. plantaginea*. Although fructose and sucrose are often the dominant components of honeydew, it appears that melezitose may become very abundant depending on the aphid's genotype (Vantaux et al., 2011).

Our analysis highlighted 6 major, 5 minor and 6 "in traces" amino acids. Whereas asparagine was higher in *A. fabae,* the content of isoleucine was higher in *D. plantaginea. A. fabae* showed a higher variation in most of the amino acids than *D. plantaginea*. Although several studies have compared sugar composition in honeydew, less information is available on amino acids. While the addition of amino acids increased attraction of ants to sugar solutions (Madsen et al., 2017), the attendance of *L. niger* to *Metopeurum fuscoviride* was correlated with the amount of honeydew production but not with overall amino acid content (Fischer et al., 2002). This suggests that other factors, likely distance and amount of honeydew produced, are the main drivers of ant preference in our study.

4.2.3 Aphid control

Even though ant attendance of *D. plantaginea* was reduced when beans with *A. fabae* were intercropped under the apple trees, no difference in colony suppression was observed between treatments in the field (Figure 11A). *D. plantaginea* colony suppression increased similarly over the four-week period for both bean + apple and the apple treatments. However, the *D. plantaginea* colonies that survived until the end of the experiment were found exclusively in the apple treatment (Figure 9A). These colonies also grew bigger in size (Figure 11B). Furthermore, no colonies in the apple + bean treatment survived to the third week of the experiment. The natural enemies observed around sentinel colonies were mainly *A. nemorum, F. auricularia*, *C. carnea* s.l.
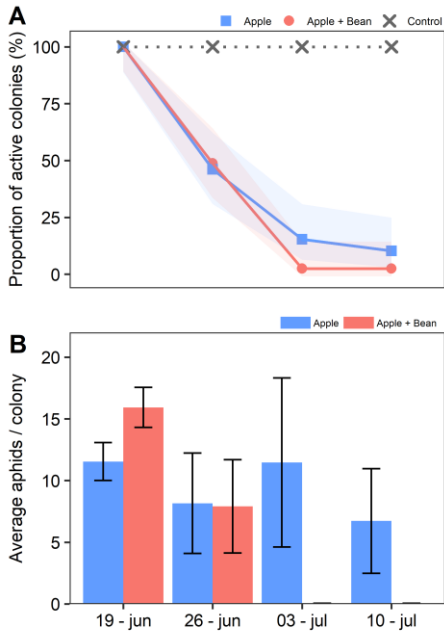
Figure 11: (A) Proportion of surviving *D. plantaginea* aphid colonies (± 95% Wald confidence interval) in apple and appple+bean treatments and (B) boxplot of the number of *D. plantagiena* aphids per colony in apple and appple+bean treatments in the field experiment.

larvae and ladybird larvae, which are often abundant in organic orchards (Paper I). Previous studies in which ant-aphid mutualism was disrupted with sugar feeders found clear reduction of *L. niger* attendance, a drop in *D. plantaginea* populations, and an increase in natural enemies in colonies on apple trees (Nagy et al., 2015, 2013). A similar effect was observed between *Aphis spiraecola* and *Lasius grandis* (Wäckers et al., 2017). In our study no difference in natural enemies was observed between treatments. This could be one of the reasons why a higher aphid suppression occurred in the two first weeks of the experiment, when many predators had their peak in organic orchards (Paper I). Some of these species are highly mobile and efficient at finding aphids, e.g. *A. nemorum*. This could have partially prevented the establishment of the ant-aphid mutualism on the small sentinel aphid colonies in the control treatment in our field setting. If naturally occurring colonies would have been used, this would have probably resulted in higher mutualism rates and a larger difference in predation between treatments due to mutualism disrupting. Furthermore, the colony suppression in both treatments was similar to that reported for organic orchards in Paper I, where trees were equipped with ant feeders releasing a sugar solution. This is an indication that ant attendance was low in our experimental conditions.

The benefits of disrupting ants-aphid mutualism through habitat manipulation, rather than through ant-feeders, is that it would be easier to apply over larger areas in a natural system, avoiding the use of artificial materials such as plastic. In addition, beans can provide alternative food and nectar, which can enhance biological control of aphids on the trees. However, the possible suboptimal establishment rate of *A. fabae* on beans may limit the ant diversion effect and needs to be further investigated.

5   Semiochemicals to attract lacewings and increase biological control of aphids

(Paper III)

Upon herbivory, plants release HIPVs to induce systemic defence and recruit natural enemies of herbivores. As HIPVs are released exclusively upon herbivory they provide an honest cue for natural enemies to follow. Synthetic HIPVs have the potential of increasing biological control, together with other kairomones and synomones, through recruitment of natural enemies and with less negative effect on the environment as compared to conventional methods. In this study we measured natural enemies attraction (e.i. green lacewings) to two different formulations releasing HIPVs. In both apple and cereal system, we compare the standard polyethylene-based bags with a new formulation that is biodegradable and easier to distribute. We also evaluated whether the attracted lacewings could control native populations of aphids.

5.1 Material and method

5.1.1 Volatile Releasing Formulations

A reference device releasing the HIPVs was purchased from Csalomon (Plant Protection Institute, MTA ATK, Budapest, Hungary). It consisted of a cotton wick loaded with a 3-component blend of methyl salicylate (MS), phenylacetaldehyde (PAA), and acetic acid (AA) in a 1:1:1 ratio with a total load of 300 mg/device. The wick was placed into a sealed polyethylene bag, through which volatiles were slowly released (hereafter referred to as PE bag). We selected this formulation as a benchmark because field data on lacewing attraction and oviposition using this formulation were available at the time of

our study (Tóth et al., 2009). The new formulation was a novel product prepared in co-operation with ISCA Technologies (ISCA Technologies Inc., Riverside, CA, USA) and Bio-Innovate AB (Lund, Sweden). It consisted of a biologically inert biodegradable wax-water emulsion releasing paste loaded with the above-described blend at a concentration of 300 mg/mL. A single release point for this product constituted a 1 mL droplet applied with a plastic syringe (hereafter referred to as paste).

5.1.2 Measurement of Volatile Release

To compare over time the volatile release rate from the two formulations, PE bag and paste were hung within the canopy of apple trees in Alnarp (Lomma, Sweden) at a height of approximately 1.7 m from the ground in the beginning of May 2016. The releasing devices were retrieved from the trees at 1, 7, 14, 21, and 28 days after field exposure (5 devices per date). After collection, they were submitted to headspace collection via solid phase microextraction coupled to gas-chromatography mass-spectrometry.

5.1.3 Attraction Longevity

To determine the device's attraction longevity in the field, aged lures previously collected from the orchard were placed inside McPhail traps. Traps were subsequently hung in five apple orchards in Kivik (Sweden). Three orchards were organically certified and two were under integrated protection (IP). The orchards were situated a minimum of 1 km apart. In each orchard, 12 McPhail traps were placed in two different circles (diameter 14 m). Each circle was comprised of six traps loaded with either PE bag or paste aged at 1, 7, 14, 21, to 28 days and with a blank trap. The two circles were 30 m apart and at least 10 m from the orchard border. Traps were hung at a height of 1.6 m and 7 m apart. Trap position was randomized within each circle at the start of the experiment. Each trap was inspected twice a week over a two-week period. In order to avoid positional effects, traps were rotated two steps in a clockwise direction within each circle at each inspection. The collected specimens were stored in ethanol (70 vol. %) for species identification and sex determination.

5.1.4 Measurement of Biological Control

A field experiment was conducted in spring barley fields in Ås (Norway) in June and July 2016. Either a PE bag or a paste formulation was installed at the center of 25 m² plots (N = 12). Distances between plots were at least 5 m. Three different types of lure applications were tested: (1) PE bag dispenser (300 mg

total load) at vegetation height; (2) 1 mL paste-droplet with 300 mg total load on the plant (paste_1x); and (3) 3 mL paste-droplet with 900 mg total load on the plant (paste_3x). Four plots for each of the three formulation types were arranged randomly within the crop. The PE bag dispensers were hung on wooden sticks at approximately vegetation height. The wire enabled weekly adjustment to the height of the dispenser to mirror the vegetation height of the growing barley plants. The paste droplets (1 or 3 mL/plant) were applied with a 100 mL-syringe on leaves in the upper third of the barley plants. At a distance of 400 m, four control plots (25 m^2) without treatment were installed with at least 5 m between the plots, as mentioned above. The 400 m distance was used to reduce the influence from either of the treatments on the control plots, as range of effect is unknown for these formulations. In the middle of each plot, a marker point (wooden stick, 60 cm) was installed. In each plot, visual inspection of lacewings (eggs and larvae of *C. carnea s.l.*), aphids (nymphs and adults of *S. avenae* and *R. padi*), and other natural enemies (Coccinellidae larvae, Syrphidae larvae, and parasitized aphid mummies) was performed in five differentiated sectors. Sectors were established as dispenser or marker points (=Centre; C), and 30 cm distances in the directions north (N), south (S), west (W), and east (E) of the dispenser or marker points. The observations were performed on the three plants nearest to the five marked points (C, N, S, W, E). Counted lacewing eggs were marked with a small dot on the leaf to avoid repeated counts. At the phenological stage 13 (leaf development, 3 leaves unfolded, 1st of June) of barley plants, the sectors were checked for aphids, lacewings, and other natural enemies (first record). Then the different lure types and marker points were placed in the experimental barley fields. Over an experimental period of eight weeks, the sectors were checked weekly for lacewings, aphids, and other natural enemies, as described above. Dispensers and droplets were replaced once after four weeks. Our observations were carried out within a landscape with natural inter-field vegetation, where lacewings may have had access to floral resources, overwintering sites, and alternative prey.

5.2 Result and discussion

5.2.1 Volatile Release and Attraction Longevity

The release rates from of the loaded compounds, i.e. acetic acid, methyl salicylate and phenylacetaldehyde, were higher from the reference PE bag than
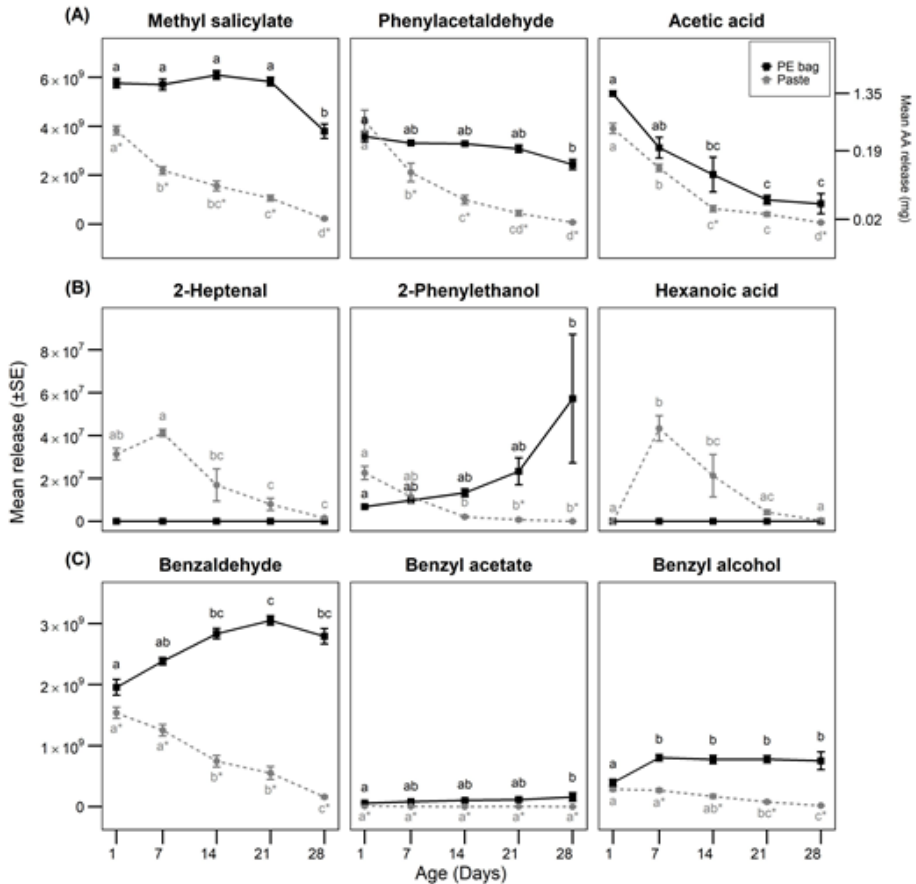
Figure 12: Mean release rate (±SE) of (A) methyl salicylate (MS), phenylacetaldehyde (PAA), and acetic acid (AA) from two different emitting devices at five different ages of field exposure (N = 5). The semi-quantitative release of AA is shown on the right y-axis. Letters above points indicate significant differences between ages of the same formulation and * significance between formulations at a given age (LRM and GLS, Tukey's test, $P <$ 0.05). (**B**) Mean release rate (±SE) of 2-heptenal, 2-phenylethanol, and hexanoic acid. (**C**) Mean release rate (±SE) of benzaldehyde, benzyl acetate, and benzyl alcohol. 2-heptenal and hexanoic acid were exclusively found in Paste

from the new paste over the four-week period, except for PAA at day 1 (Figure 12A). The PE bag release rate was stable for MS and PAA while AA decreased quickly over the four weeks. The paste had higher release rates of all three compounds in comparison to the PE bag. Release rates were significantly reduced over almost all weeks. Lacewing catches in apple orchards mirrored the release rates for each formulation. PE bag had a consistent attraction for lures of all ages while paste catches were reduced with age. (Figure 13). However, catches did only differ between the two formulations when they were 21 and 28 days old (Figure 13). Lacewing catches in our experiment were similar in
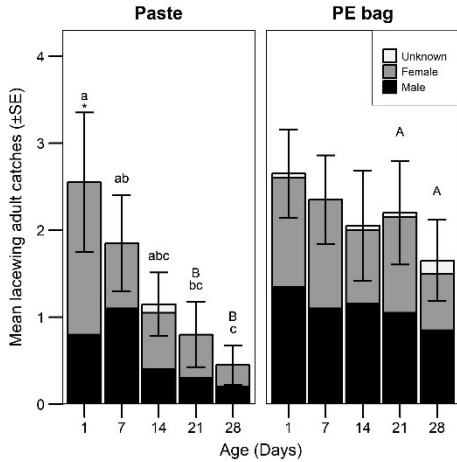
Figure 13: Mean catches (±SE) of lacewing adults in apple orchards by two different emitting devices at 1, 7, 14, 21, and 28 day of field exposure (N = 5). The lower part of the bar indicates the proportion of males, middle the proportion of females, and top the proportion of individuals that could not be sexed. Different uppercase letters above the bar indicates a significant difference of catches between the two formulations at a given age (GLMM, Tukey's test, $P < 0.05$). Different lowercase letters indicate significant differences between the same formulation (GLMM, Tukey's test, $P < 0.05$). An * above bars indicates significant higher ratio of females at the corresponding age (GLM, Tukey's test, $P < 0.05$)

number to those obtained in previous studies using the same blend (Koczor et al., 2010; Tóth et al., 2009).

Beside the three main components, both formulations emitted lower quantities of 2-phenylethanol, benzaldehyde, benzyl acetate, and benzyl alcohol, whereas 2-heptenal and hexanoic acid were released exclusively by the paste (Figure 12B-C). These compounds are present in lower concentrations than the main three compounds and are likely breakdown products or impurities derived from the synthesis. It is not clear how these impurities contributed to the differential attraction between the formulations.

5.2.2 Measurement of Biological Control

Both the PE bag and the paste were able to attract lacewings which fed on and decreased aphid numbers in barley. A few other natural enemies were recorded, such as ladybirds (*C. septempunctata*), hoverfly larvae and parasitized aphid mummies. However, lacewings were the most abundant natural enemy and showed a strong attraction to the lure with a total of 983 eggs and 1 965 larvae recorded over 8 weeks. The other natural enemies occurred in much lower amounts (<100). The number of lacewing eggs and larvae changed over time and differed between treatments (Figure 14A). The most lacewing eggs and larvae were recorded with the reference formulation, followed by paste_1x and paste_3x. Very low numbers were found in control plots (Figure 14A). None of the other natural enemies showed a strong response to the tested formulations(Figure 14C). Eggs and larvae clustered close to the lure (sector C), while in the control plot they appeared evenly distributed in all sectors. These
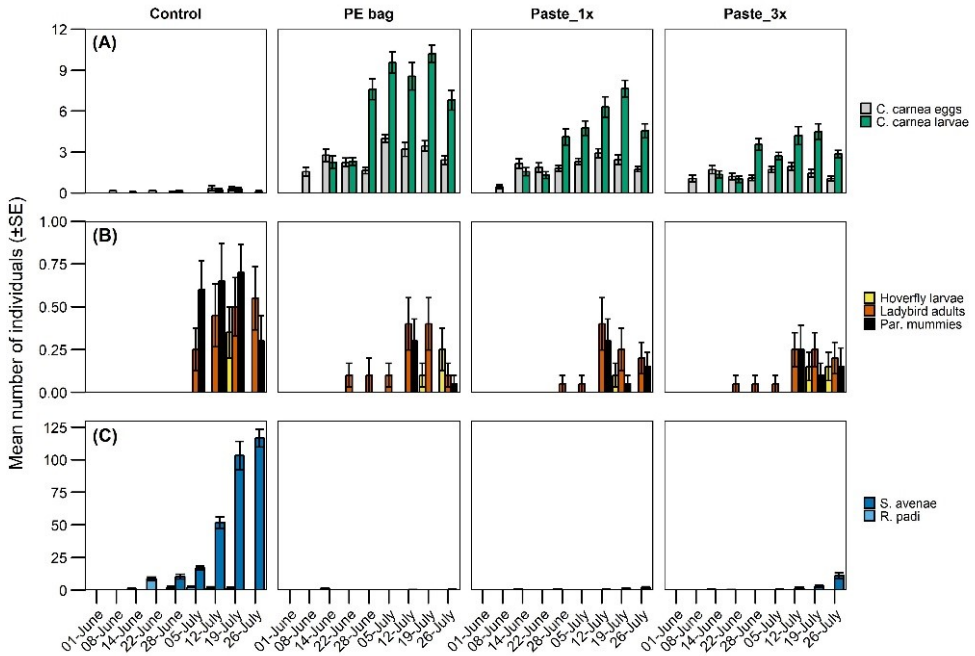
Figure 14: Mean number (±SE) of (A) lacewing, (B) other natural enemies, and (C) aphids observed on three barley plants during the experiment

results are in line with previous studies in which an increased lacewing oviposition was observed around the PE bag in apple, cherry, apricot and other three crops (Koczor et al., 2015; Tóth et al., 2009).

Two different aphid species, *S. avenae* and *R. padi*, were recorded in the field, with *S. avenae* as the dominant species (Figure 14C). Both aphid species were more abundant in the control and increased over time as compared to any of the treatment plots. *S. avenae* was almost totally absent in the PE bag and paste 1x treatments. *R. padi* abundance was independent of formulation or dollop density. The reduction of aphids in the treatments is most likely due to biological control provided by the increased presence of lacewings. The early arrival of lacewings allowed for an efficient aphid biocontrol, preventing the development of large colonies, which are more difficult to suppress by natural enemies. We cannot exclude the possibility that the HIPVs were repelling the aphids. For example, cis-jamone and MS were reported to repel aphid alates, including *R. padi* and *S. avenae* during their migration from winter host to barley (Bernasconi et al., 1998; Pettersson and Smart, 1994). In our experiment some aphids were recorded in all treatments (Figure 14C). However, repellency of pests and attraction of natural enemies in combination would provide an even higher biocontrol. Even though HIPVs show great potential, there are some risks associated to their use. The released synthetic HIPVs may mask those from

natural sources, causing repellency or starvation of natural enemies, or increasing intraguild predation due to the lack of prey (Kaplan, 2012). Attraction of the forth trophic level organisms may also occur (Orre et al., 2010). These risks can be reduced by adjusting the size of the paste droplet so that a shorter active time can be achieved. As an alternative, HIPVs can be combined with non-crop vegetation such as flowering plants, which can provide alternative food to natural enemies (Hatt et al., 2018; Simpson et al., 2011a)

6 Combining habitat manipulation with semiochemicals to increase control of lepidopteran pests

(Paper IV)

Arthropod abundance and biodiversity have dramatically decreased with agriculture intensification due to landscape homogenization and a growing use of agrochemical compounds. Arthropod species evenness and key species abundance are associated with several ecosystem services, including the natural regulation of pests (e.i. biological control). Habitat manipulation has been proposed as a strategy to restore biodiversity and abundance of natural enemies in agricultural landscape. Habitat manipulation must be necessarily coupled to a change in pesticide use towards pest management programs less harmful to beneficial arthropods. Semiochemicals are promising candidates to reduce insecticide disturbance in agroecosystems. A few studies have combined the use of HIPVs to attract natural enemies with flower strips to reward them. Such an approach, termed "attract and reward", showed positive results in increasing natural enemies. Here we established attract and reward in a perennial crop under a selective pest management strategy, i.e. replacing insecticides with semiochemicals.

6.1 Materials and methods

The study was conducted over a three-year period in five different organic orchards in Scania (Sweden). Population levels of pests and natural enemies were assessed in plots subjected to a progressive implementation of complementary intervention techniques for pest control from year 1 to year 3 (Figure 15). An initial and final evaluation of pest population was carried out on apple flower clusters in May 2015 and 2018, respectively.

Factors included in the field experiments were: multipurpose mating disruption (MMD), HIPVs as predator attractant (Attract), non-crop vegetation to provide food, shelter and alternative prey to natural enemies (Reward), and different combinations thereof. In year 1 (2015), we compared the effect of MMD with a control (untreated) plot. In the same year, the flower strips were sown. The effect of the flower strips and the HIPVs on the entomofauna of the orchard took place in the following seasons (2016-2018).

2015		2016		2017	
MMD		R + MMD	A&R	R + MMD	A&R + MMD
MMD		MMD	A	MMD	A + MMD

Figure 15: Schematic representation of the experimental set-up over the three years of the study. Treatments were added over the years while plots stayed the same in each orchard.

6.1.1 Sites

The size of the five orchards ranged from 2.2 to 7.1 ha. The two closest orchards were located 2.7 km apart, whilst the two furthest were separated by 113 km. Three of the orchards were in the same area near the town of Kivik (Sweden), where lacewing attraction was tested in Paper III. Four plots divided in two blocks were established in each orchard. Each plot consisted of 7 rows (3.5 m between rows; 25–30 m long) with a minimum distance of 10 m from the border. The plot-plot distance was 25 m. The two blocks were at least 50 meters apart. Although cultivars varied among orchards, the same cultivar was present in the four plots of a single orchard.

6.1.2 Treatments

Multipurpose mating disruption (MMD). A multipurpose pheromone formulation previously tested in Southern Sweden (Porcel et al. 2015) for population control of five leafroller species and the codling moth (Isomate CLS, manufactured by Shin-Etsu, Tokyo, Japan) was used. Dispensers were applied in apple orchards at the recommended dose of 800 per ha. Dispensers were hand applied in mid-April, prior to the onset of the first flight of the earliest target

pests (*C. pomonella*). Only one of the blocks had MMD 2015-2016 while in 2017 both blocks was covered with MMD.

Reward (R). In May 2015, flowers strips were sown in the drive alley between apple rows in two of the plots in each orchard to provide food, supplementary non-pest prey, shelter and overwintering sites for natural enemies (Figure 16). A mix of 33 different species was selected in co-operation with the European Core Organic Consortium "Eco-Orchard". The mix was custom prepared by the Danish seed company (Nykilde, Slagelse, Denmark) and was specifically designed to provide a perennial flower strip with a low maintenance. It consisted of functional agrobiodiversity (FAB) species together with grasses as stabilizers (see paper IV for species in the mix). The establishment of the strips as well as their effect on insect populations and biological control were measured during 2016-2018. Six and 12 weeks after sowing, the flower strips were cut at a height of 7 cm to avoid grass species domination. In the following year (2016), the flower strips were cut in the first week of May (pre-flowering of apple trees), in the last week of June, and at the end of August (pre-harvest) at the same height. The native grass in the non-flower plots was cut at a 3-4 week interval from mid-May to the end of August. In 2017, the flower strips were cut at the beginning of May, at the first week of July and at the first week of August at a height of 7 - 12 cm.

Attract (A). Csalomon lure (see Paper III) consisted of a 3-component blend of methyl salicylate (MS), phenylacetaldehyde (PAA), and acetic acid (AA) in a 1:1:1 ratio formulated on a cotton wick inside a polyethylene bag (Tóth et al., 2009). HIPVs dispensers were placed on the first, central and last three of the 5-centremost rows in the plots with the Attract treatment.

*Figure 16: T*illed soil ready for sowing the flower mix in 2015 (left), established flower strips in two different orchards in 2017 (centre and right).

6.1.3 Measured variables

Pheromone trap shutdown. The flight activity of six Tortricidae (*Adoxophyes orana*, *Archips podana, Archips rosana*, *C. pomonella*, *Pandemis heparana* and *S. ocellana*) was monitored through delta traps. Each plot contained one trap of each species. Rubber septa loaded with sex-pheromone were provided by Csalomon and were replaced every 40 days from May to September. Catches were recorded weekly from the onset of apple flowering to the end of the season, until no capture was recorded for two consecutive weeks. Because *H. nubiferana* damages apple inflorescence in Southern Scandinavian organic orchards, monitoring traps baited with the corresponding sex pheromone were also set in the orchards to measure the population density of this non-target species. The difference in captures between the control and the disrupted plots (trap shutdown) was taken as an estimate of the disruption effect in 2015 and 2016.

Sentinel egg cards. To estimate biological control of lepidopteran eggs, sentinel egg cards were used in the experimental orchards. Cards with 1 - 10 *A. orana* eggs were placed on a 1.5 × 3 cm cardboard card and placed in the middle of the 3 centre rows, 7 per row, of each plot in end of July with two replicates each year. Three cards per plot were also placed inside separate bagged branches to exclude natural enemies as a control. After 48 hours, cards were collected, and the removed and predated eggs were assessed under a stereo microscope.

The collected egg cards were placed in a rearing chamber (25 °C, 16:8 L:D period) to account for parasitoid emergence. The eggs were incubated for 2 months to estimate egg parasitism.

Measurement of arthropods in the canopy. Arthropods were collected from the tree canopy with a field aspirator to measure population levels of pests and natural enemies, as described in Paper I. Ten samples were collected in each plot from the 5 central rows and were stored at -18 °C for later identification of the individuals collected under stereomicroscope. The samples were taken in mid-June (fruit set development) and at the end of July (tortricids oviposition).

Evaluation of larval density in flower clusters. Flower clusters at the pink bud stage (BBCH 59) were sampled to evaluate emergence of overwintering pest populations. This methodology was carried out in 2015 as a baseline data before the experiment. The same sampling was done in 2018 to score the impact of two years of treatments on pest populations. A single replicate included 15 flower clusters from 3 adjacent trees. Ten replicates were taken from the 5 centre rows of each plot and all orchards were visited during the same day. Unidentified larvae were reared to adulthood to establish their species identity.

6.2 Result and discussion

6.2.1 Trap shutdown under Disruption, Attract and Reward

Mating disruption was able to significantly shutdown catches in sex-pheromone traps for all six targeted tortricid species (Figure 17). The most abundant species in 2015 and 2016 were *A. podana*, *H. nubiferana*, *P. heparana* and *S. ocellana*, whilst *A. orana*, and *C. pomonella* were caught in lower numbers (Figure 17). The addition of A, R or A+R did not affect the catches of tortricids compared to the MMD treatment alone. In 2017 the only species abundant enough for statistical analyses were *H. nubiferana*, *S. ocellana* and *C. pomonella* (Figure 17), which all occurred in similar amounts in all the treatments. While a suboptimal inhibition was observed for *C. pomonella* and *S. ocellana* as earlier reported in similar studies (Porcel et al., 2015; Porcel et al., 2017), a rather pronounced lack of inhibition was reported for *H. nubiferana*. This lack of trap shutdown recorded for *H. nubiferana* was expected as its pheromone was not included in the multispecies formulation.

Figure 17: Average accumulated catches (±SE) of Tortricidae during 2015 - 2017. Bars capped with the same letter did not significantly differ within a given year (GLMM, Tukey's test, *p* < 0.05). Ao = *Adoxophyes orana*, Ap = *Archips podana*, Ar = *Archips rosana*, Cp = *Cydia pomonella*, Hn = *Hedya nubiferana*, Ph = *Pandemis heparana*, So = *Spilonota ocellana*. Ao was caught in insufficient numbers for analysis in 2015-2017. Ap, Ar and Ph were caught in insufficient numbers for analysis in 2017.
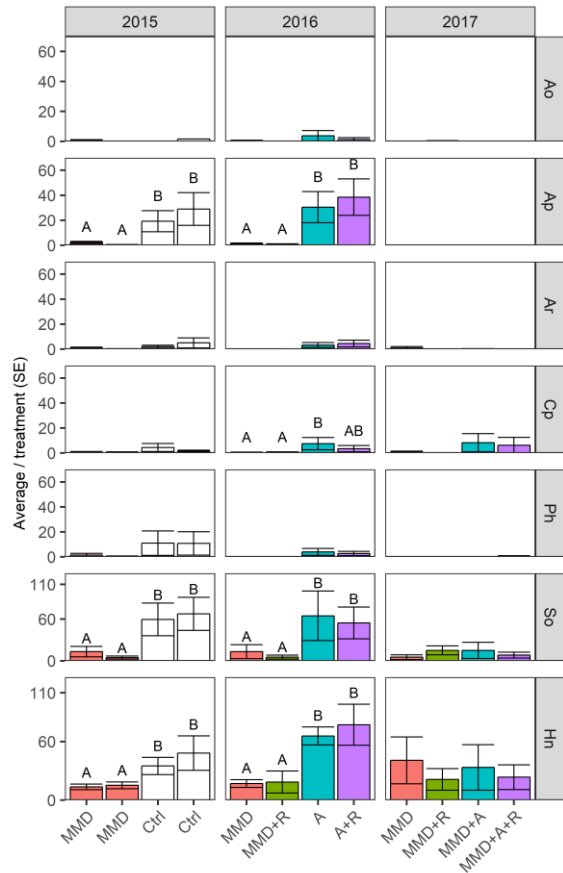
6.2.2 Egg predation under Attract and Reward

Sentinel cards with *A. orana* eggs showed predation in all treatment with an increase of total predation from 2016 to 2017 (Figure 18). There were greater differences between treatments in 2016 when egg predation was positively affected in the R treatment while negatively affected by A. In 2017, no differences between treatments were observed although A+R had highest predation levels. The result from 2016 could indicate a possible repellence of predators, that was also observed in Paper III for lacewings. Unlike in the experiment presented in Paper III, the repellent effect in the current experiment was likely caused by the higher dispenser density and not the increase in single point release. This has also been observed in vineyards after placing dispensers loaded with MS at a high density (James and Price, 2004). However, this effect was not observed in 2017 when A and A+R showed the highest increase over the years (Figure 18). Another possibility is that the HIPVs masked the eggs from the natural enemies. This could also maybe explain why no parasitoids (which often rely on olfactory ques to find their hosts) emerged from the eggs, even though *A. orana* larvae emerged indicating healthy eggs. It could also be that the egg parasitoid population was too low to be measured. However, the lack of difference in egg predation in 2017 between the four treatments may be misleading. A possible mismatch with the period of main activity of key natural enemies could have occurred as sentinel eggs were exposed only once in the season. In addition, predation on larvae is likely to be uncorrelated with predation on eggs. A similar finding was recently reported by (Cahenzli et al., 2019), who found sentinel egg cards unsuitable to reflect density of predators in general. Therefore, our result on sentinel eggs should be taken with caution.
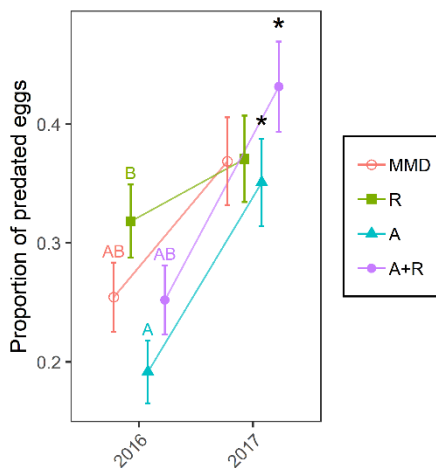

Figure 18: Average % of egg removal (±SE) indicating predation. All 4 variants were treated with mating disruption in 2017. Different letters indicate significant differences in the same year. Asterisk indicates significant higher removal for treatments between years (GLMM: Tukey's test, $P < 0.05$).

6.2.3 Larval density in flower clusters

Leafroller infestation in apple flower clusters increased from 2015 to 2018 with the highest increase observed in the R as compared to the other treatments (Figure 19). In 2015 there were no differences between treatments while in 2018 significantly fewer larvae were present compared to R that recorded the most (Figure 19). Two tortricid species could be identified as larvae of *H. nubiferana* and *S. ocellana*. The most abundant tortricid larvae found in 2018 was *H. nubiferana* (50.3 %) and when they were excluded from the analyses there was no difference in leafroller infestation between treatments (Figure 20). *H. nubiferana* population increase over time may be a result of competitive release as they are not affected by the mating disruption treatment established. In addition, *H. nubifera* may be benefitting from the R treatment as infestation levels were higher in this treatment while they were disfavoured in A (Figure 19). *S. ocellana* larval density followed the pattern of *H. nubiferana* with higher abundance in R and less in A, although their adult population decreased slightly over the years, which was also ascertained in trap shutdown (Figure 17 and Figure *19*).

One interesting aspect concerning tortricid larval population (excluding *H. nubiferana*) is that spring populations did not changed over the three years under the different treatments, with mating disruption applied to the whole orchards in 2017. A decrease in the larval population was to be expected as observed for trap catches. It is possible that females were able to

Figure 19: Average number of total leafrollers (LR), leafrollers without *H. nubiferana* (LR-Hn), *H. nubiferana* (Hn), *S. ocellana* (So) and geometrid larvae per flower cluster (±SE) at BBCH 59 in 2015 and 2018. Different letters indicate statistically significant differences within the year and an asterisk indicates statistically significant higher abundance in that specific years (GLMM: Tukey's test, $P < 0.05$).
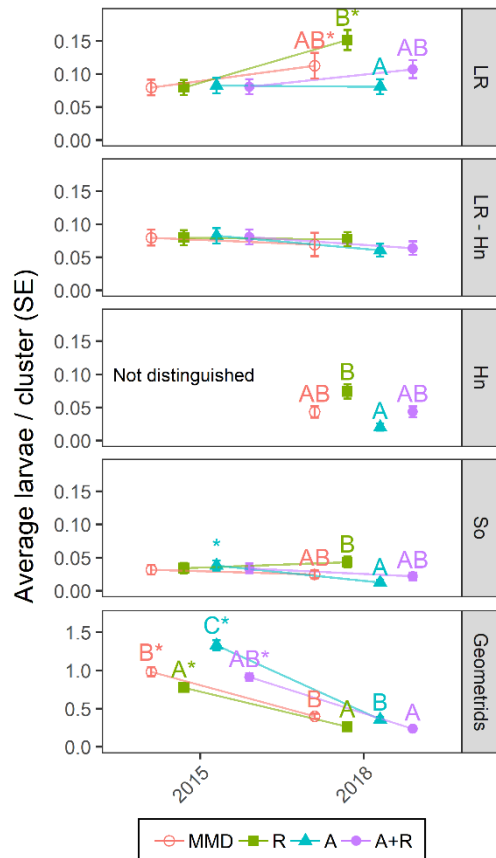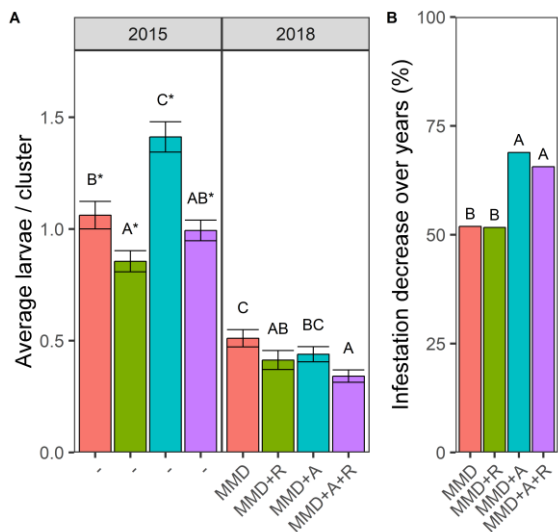
Figure 20: (A) Average number of feeding larvae per flower cluster (±SE) in the first (2015) and in the last year of the study (2018). (B) Relative decrease in infestation from 2015 to 2018. Bars capped with the same letter do not significantly differ from each other within the same year (GLMM: Tuk-ey's test, P < 0.05). Bars capped with an asterisk significantly differed between years (GLMM: Tukey's test, P < 0.05).

mate outside of the orchards, where there was no MMD, and then migrate into the orchards.

Apart from tortricids, geometrid larvae were also found in the flower cluster and in greater amounts. Their population decreased significantly from 2015 to 2018 in all plots (Figure 19). In 2018 there were significantly fewer geometrids in R and A+R treatments. The most abundant geometrid in 2018 was *Operophtera brumata* (91.0%) which pupates in the soil around June, to emerge in October/November to mate, and oviposit on the apple canopy (Borer et al., 2009; Klemola et al., 2012). Flower strips may potentially foster ground dwelling natural enemies, such as carabid beetles, able to prey on the pupae (Klemola et al., 2012), although this was not verified in our study.

Total lepidopteran larvae population followed the trend of geometrids in 2015 (Figure 20A). In 2018, A+R had the lowest infestation followed by R, A and MMD, with significant differences only between A+R and MMD (Figure 20A). Comparing the results from the statistical models for each treatment between the years shows an important reduction in A and A+R (Figure 20B). These results suggest that MMD is controlling the main target tortricid pests by itself but has no effect on non-target lepidopteran pests, which is not surprising. Adding A+R to MMD provides some resilience against the pest populations not targeted by MMD (*H. nubiferana* and geometrids). However, drawing conclusions from the comparisons between the two years should be taken with care as there was a high variation in pest populations between plots in 2015, mainly in geometrids. Species such as *O. brumata*, which is reported to be the main geometrid in orchards (personal conversation with growers), have a

population cycle of 7-11 years (Hittenbeck et al., 2019). If, hypothetically, they were sampled at their peak in 2015, and followed a natural decline towards 2018, it might be difficult to distinguish this cycle from the effect of the treatment. However, it is not likely to affect the treatment effects observed in 2018 for lepidopteran larvae.

6.2.4 Arthropod density in the canopy

The total amount of predators (sum of Miridae, Anthocoridae, Cantharidae, Coccinellidae, Staphylinidae, Neuroptera, Dermaptera, predatory acari and Formicidae) was higher in the first sampling event in both years, while overall parastiods (sum of Bethylidae, Braconidae, Ceraphronidae, Cynipidae, Diapriidae, Ichneumonidae, Megaspilidae, Platygastridae, Proctoruptidae, Scelionidae and Chalcidoidea) occurred in higher numbers later in the season (Figure 21). The total amount of natural enemies was most benefited by the A+R, which did not differ from A (Figure 21). Flower strips (R) alone did not increase natural enemy or predator abundance in the canopy in any of the sampling event. Instead R was close to MMD. A+R increased the abundance of predators in all sampling events (Figure 21). However, there was no difference between A and A+R in 2017. Predatory heteropterans was the most abundant predatory group with *A. mali* (Miridae) and *H. planicornis* (Miridae) as the most abundant species (Figure 22). Both species showed an increase in A+R, however, this increase was not significant.
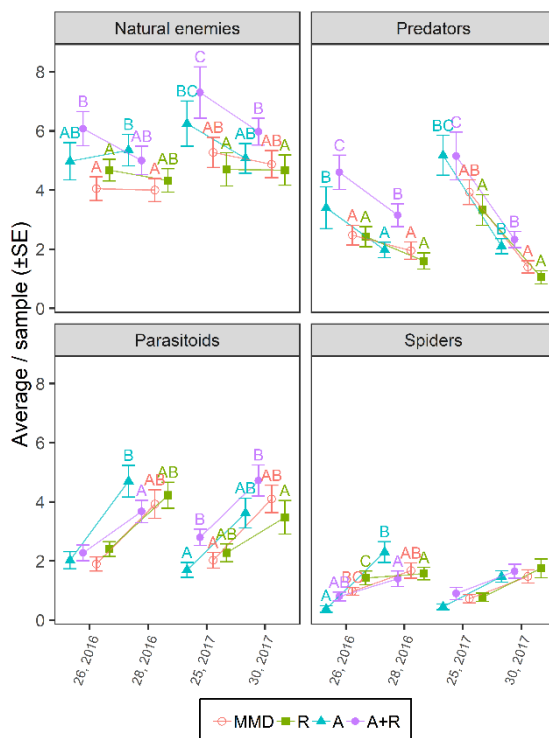

Figure 21: Average number of natural enemies, predators, parasitoids and spiders (± SE) collected through suction sampling. All 4 variants were treated with mating disruption in 2017. Different letters indicate statistically significant difference within a given week (GLMM, Tukeys's test, $P < 0.05$).

The variation between orchards was in general considerably higher than between treatments within orchards. Statistical differences between treatment were not detected at species level, possibly due to the limited number of individuals collected for individual species. However, looking on total Miridae there is a strong treatment effect with a higher abundance in A+R in 2016, and in A+R and A in 2017 (Figure 22). Nonetheless, Miridae populations did not increase in R but showed a significant increase when flowers were combined with HIPVs. Furthermore, R recorded lower values than other treatments. A possible explanation is that Miridae could be affected negatively by one or several of the plants in the mixture. Plants repelling arthropods are known, mainly exploited for pest control, as the well-known example of the "push-pull system" (Cook et al., 2007). Aromatic plants have also been used to repel scarab beetles in pear (Tang et al., 2013). To the best of my knowledge no repellent plants were included in the sown plant mix. Earwigs, lacewings and predatory acari were
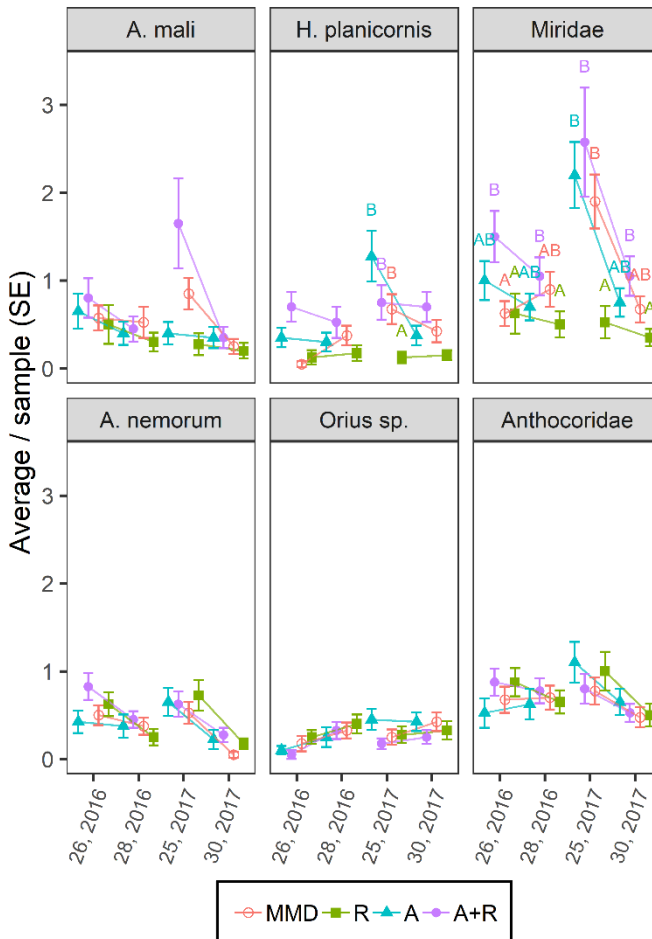

Figure 22: Average number of predatory heteropterans (± SE) collected with suction sampling. All 4 variants were treated with mating disruption in 2017. Different letters indicate significant difference between treatments within the same sampling occasion (GLMM, Tukeys's test, $p < 0.05$).

also collected but did not show trend as clear as predatory Heteroptera. When all generalist predators were pooled together a more pronounced effect was observed for A+R in 2016, and for both A and A+R in 2017 (Figure 23). This indicates that R could have increased generalist predator populations slowly over time while A provided a quicker response following the application of the treatment.

No clear candidate within the natural enemy community could be identified as main responsible for egg removal from the sentinel egg cards, which was significantly higher in R in 2016 and with no differences in 2017. Mainly *A. mali* and earwigs were observed on the egg cards. However, night active species might have not been detected. Miridae populations in 2017 could instead be more connected with *H. nubiferana,* and to some degree to *S. ocellana* larval infestation in flower clusters in 2018. *H. nubiferana* oviposition period roughly coincided with the first suction sampling, in which Miridae were the most abundant predator group (Figure 22). The lowest larval abundance of both *H. nubiferana* and *S. ocellana* were recorded in A, which was possibly due to a higher total natural enemy abundance (Figure 19). Additionally, the highest abundance of natural enemies was in A+R in which the total lepidoptera larval infestation was the lowest, although not different from A alone.

Concerning parasitoids, it was mainly Chalcidoidea which showed to benefit from A or A+R depending on the year (Figure 24A). Ichneumonidae and Braconidae varied between treatments and year, and only when pooled showed an increase in A (Figure 24A). Egg parasitoids, like Scelionidae, Encyrtidae and Eulophidae, also showed a trend of higher abundances in treatments that included R in both sampling
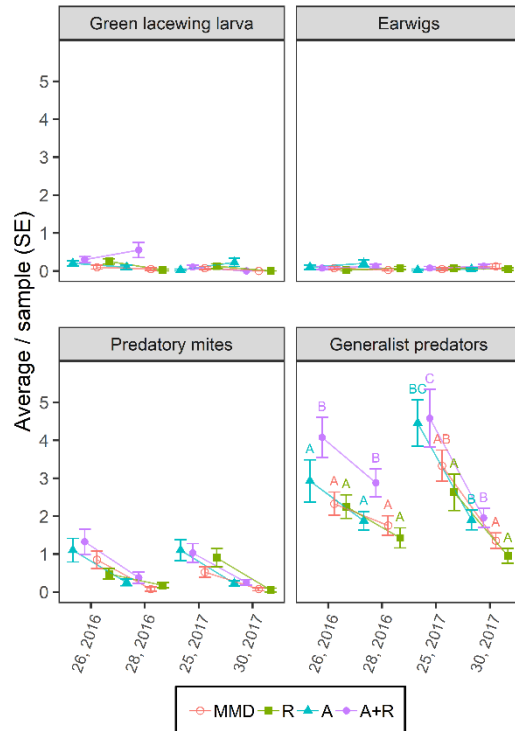

Figure 23: Average number of *C. carnea s.l.* larvae, earwigs, predatory acari and generalist predators (± SE) collected with suction sampling. All 4 variants were treated with mating disruption in 2017. Different letters indicate significant differences within the same sampling occasion (GLMM, Tukeys's test, $p < 0.05$).
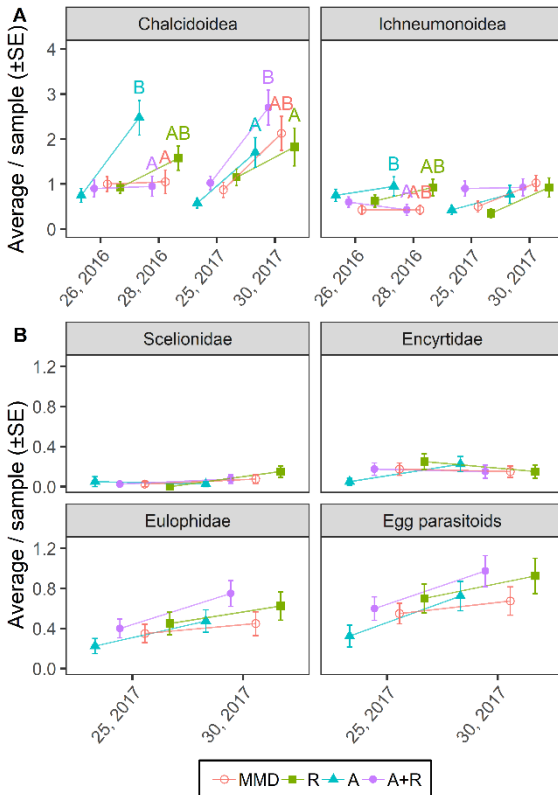
Figure 24: (A) Average number of parasitoids belonging to Ichneumonidae and Chalcidoidea (± SE). (B) Average number of egg parasitoids as single families and as total, collected in suction sampling. All 4 variants were treated with mating disruption in 2017. Different letters indicate significant differences at each sampling occasion (GLMM, Tukeys's test, $p < 0.05$).

events (Figure 24B). However, this was not supported statistically. Floral resources have been shown to enhance parasitoids performance resulting in higher parasitism rates in vineyards, although this effect was site-dependent (Berndt, Wratten, & Scarratt, 2006). Furthermore, Ichneumonid and Braconid wasps were found to be relevant parasitoids of leafrollers in German orchards, where *A. orana, H. nubiferana* and *P. heparana* were parasitized by a high number of species in comparison to *S. ocellana* (Kienzle, Zebitz, Brass, & Athanassov, 1997). Ichneumonid and Braconid wasps were the only families that emerged from larvae collected from flower clusters when larvae were reared for identification in this study. Larval parasitism rates were not measured in the field but could be expected to increase in R and A+R as shown in other studies, e.g. on stink bugs in a cotton-peanut system (Tillman and Carpenter, 2014) and leafrollers in apple (Unruh et al., 2012).

Flower strips have been observed to enhance parasitoid over predator densities (Gurr et al., 2012). In this study it is was corroborated for total parasitoids, but not for parasitoid families that are important for pest control in apple. A stronger treatment effect was detected the first year for both predators and parasitoids, during which both HIPVs and flower strips were active for the first time, as compared to the second year. Potentially, there could be a higher recolonization the second year, with a higher overwintering survival, either in the treatment plots or in the surrounding landscape. Parasitoids and flying

predators are known to be able to cover high distances, at least 100 m (Gontijo et al., 2013). Long-term studies with stable non-crop vegetation are needed because the build-up of beneficial arthropod populations is assumed to be a long-term process. In Canada, for example, five years from flower strips establishment were necessary to achieve a substantial damage reduction in apple orchards (from 95.2% to 9.2%) (Markó et al., 2012).

Our findings on the effect of habitat manipulation, HIPVs and their combination, are partly in line with previous studies on A+R conducted in other crops, even though these studies did not include mating disruption in their design. When A+R was tested in broccoli, sweetcorn and grapevine, flower strips increased HIPVs attraction for both predators and parasitoids and were able to retain the natural enemies attracted longer compared with the no-reward treatments (Simpson et al., 2011a, 2011b). Even though a general increase in attraction and retention of natural enemies was achieved, there were still important differences between site and crop. Another study in brassica found that natural enemies rather prefer either attract or reward treatments with indications that buckwheat inhibits MS attraction of natural enemies (Orre Gordon et al., 2013). A study in bean found that coriander and MS attracted different natural enemy communities, and when combined in a single treatment the two different communities were merged (Salamanca et al., 2018). Further study into A+R would consist on longer experiments under selective pest management to evaluate the response of secondary pests and other herbivores. The release of the natural enemies which have shown the strongest response in each cropping system should also be examined. Predator and parasitoid augmentation strategies are more suitable for greenhouse cultivation as compared to open fields due to a more than probable spill-out effect in the open. A+R may potentially retain and support them over a longer period so sufficient pest control is achieved.

7 Conclusion and future perspectives

In this PhD thesis local management significantly affected the abundance and biodiversity of natural enemies and the associated provision of an ecosystem service essential to agriculture, biological control. When comparing IPM and organic orchards, species evenness and diversity of natural enemies, together with biological control, were lower in the more intense management. The most affected natural enemies were generalist predatory Heteroptera. It needs to be remarked that this difference was observed under disruption of ant mutualism through ant-feeders containing sugar solution. Besides using ant-feeders, we attempted the diversion of ants through habitat manipulation. Bean plants with *A. fabae* were intercropped with apple trees to divert ant attendance from *D. plantaginea*. Although we could not show an increased aphid suppression by natural enemies following mutualism disruption, a significant lower ant attendance to *D. plantaginea* was recorded. As the migration of *A. fabae* from its winter host to bean plants occurs when *D. plantaginea* colonies are at an early stage of development, further research is required to minimize the gap between *A. fabae* establishment and ant diversion. Because plant species and varieties affect honeydew content, additional varieties of bean as well as host plants of *A. fabae* should also be evaluated within the apple-bean intercropping, with a view to increase ant diversion. Alternatively, beans could be replaced by other legume plant to be added to the flower mix tested in Paper IV, provided that such species will be capable of sustaining aphids and diverting ants from the apple trees as beans do.

In order to provide a higher biological control of aphids and caterpillars, a blend of synthetic HIPVs was used. Whereas this blend triggered lacewing oviposition and biological control of aphids, possible negative effects on predator populations cannot be exclude. For example, attraction of natural enemies to HIPVs in the absence of the target herbivore prey may on the long-term lead to their starvation with unpredictable consequences on the ecosystem. In addition, while a higher density of predators is likely to occur in the field

where attractants are deployed, a dilution effect may occur in adjacent areas from where the same predators come from. Potentially, there are fewer risks with the application of HIPVs in shorter periods, only just before pest peaks. Accordingly, significant landscape- and population-level questions need to be investigated before this method can be effectively and ethically utilized at large scales.

Drawbacks associated with HIPVs deployment can be potentially mitigated by the inclusion of non-crop flowering vegetation, which provides additional resources such as alternative prey, nectar, and pollen to predators and parasitoids. In addition to that, sex-pheromone mating disruption for the control of tortricid species can be implemented in the same orchards to provide population control of a wider range of species. In our experimental conditions, mating disruption alone was able to control tortricid populations with no negative effects on natural enemies. Whereas HIPVs and floral resources increased the abundance of natural enemies independently, a broader and more stable enhancement of predators and parasitoids was obtained by the co-occurrence of both A&R. The combination of the selective pest control of mating disruption with the A&R approach suppressed overall larval populations of geometrids and tortricids below that of mating disruption. Although HIPVs and flower strips were only evaluated for two years, yet a quick response of generalist predators was observed, especially in the A&R combination. Such a response was somehow expected, because of their higher ability to disperse over large areas and exploit a broader range of resources in comparison with specialists. It remains nonetheless unknown how the balance between pests and natural enemies will evolve on the long term in the presence of A&R components. In general, the natural enemy community can take a long time to build up, especially in the case of rare species within a homogeneous landscape. The addition of HIPVs to increase their biocontrol efficiency within the crop may not guarantee a faster colonisation. Accordingly, habitat manipulation via A&R should not only consider local management practices, but also the composition of the surrounding landscape.

Although we showed the potential of combining semiochemicals and habitat manipulation in enhancing pest control, additional long-term studies are needed to fully realize synergies of practical relevance between the components of the Attract, Reward and Disrupt approach. In particular, long-term (> 3 years) interdisciplinary research programs are encourage aiming at understanding how surrounding landscape, orchard management as well as priorities of local stakeholders can be tailored into sustainable pest control methods.

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Populärvetenskaplig sammanfattning

Människans aktiviteter de senaste decennierna har haft en stor miljöpåverkan med många negativ effekter på landskapet och den biologiska mångfalden, vilket inkluderar många nyttodjur så som naturliga fiender till skadedjur, pollinatörer och nedbrytare, som är viktiga i ett fungerande ekosystem. Lantbruket är den mänskliga aktivitet som har tagit mest mark i bruk för att kunna möta behovet hos den växande befolkning. Även användandet av konstgödsel och bekämpningsmedel mot ogräs och skadeinsekter har ökat för att ytterligare öka lantbrukets produktivitet. Detta har lett till ett fragmenterat landskap, där människans monokulturer dominerar och endast en bråkdel naturliga habitat kvarstår som vilda djur och växter kan leva i. Lantbruket är beroende av många ekosystem tjänster så som pollenering, nedbrytning av organisk matrial, rent vatten och motståndskraft mot skadegörare. Dessa tjänster har ofta kunnat relateras till hög biodiversitet. Hög biodiversitet innebär att det finns en stor mångfald av alla arter, vilket i sin tur leder till att arterna kompletterar varandra. Ofta styrs de till stor del av att det finns ett komplext landskap med en hög artmångfald av växter vilket kan förse de övre trofiska nivåerna; växtätare, predatorer och parasitoider, med nektar, pollen, byten och skydd under hela året vilket i sin tur ökar artmångfalden på de övre nivåerna. I ett sådant miljö kan nyttodjur lättare återkolonisera områdena efter störning så som plöjning eller växtskydd.

Predatorer och parasitoider kontrollerar växternas populationer i naturliga ekosystem. Skadedjurens naturliga fiender kan gynnas genom att etablera gräs- och blomsterremsor, häckar och dungar i jordbrukslandskapet, detta leder ofta till effektivare biologisk bekämpning. Naturliga fiender kan även gynnas genom att man ändrar bekämpningsmetoder, antingen genom byte av preparat, tidpunkt eller hur man applicerar det. Båda dessa åtgärder är viktiga strategier inom bevarande biologisk bekämpning (eng: Conservation Biological Control, CBC). En annan möjlighet att kontrollera skadeinsekter är att använda syntetiska doftämnen (eng. semiochemicals) som påverkar insekters beteende. Feromoner

har använts länge i äpple-, päron- och vinodlingar för att hindra eller fördröja parningen av flera olika arter av vecklar-fjärilar med bra resultat. Det finns även andra doftämnen som kan attrahera naturliga fiender eller repellera skadegörare. En grupp av doftämnen som hyser stor potential för ökat växtskydd är de som utsöndras av växter när de blir attackerade av växtätare, så kallade HIPVs (eng. Herbivore Induced Plant Volatiles).

Målet med denna avhandling var att undersöka hur naturliga fiender och deras förmåga att kontrollera skadeinsekter i äppelodling kan främjas genom insådd av växter samt användandet av olika lättflyktiga doftämnen. Detta för att uppnå en mer hållbar produktion

Det finns flera olika skadeinsekter inom äppelodling som kulminerar under olika delar av säsongen. Röd äpplebladlus (*Dysaphis plantaginea*) är den som kulminerar tidigast på våren. Den börjar förekomma redan på senvåren strax innan blomning då äpple är dess vintervärd. Under blomning eller strax där efter börjar lusen föröka sig och om inte den kontrolleras kan den orsaka stora skador på både äpplen och trädet om invasionen är stor. Under sommaren är det främst olika arter av vecklarefjärilar som behöver kontrolleras då deras larver kan äta på äpplena. De vecklare som är mest problematiska är äpplevecklaren (*Cydia pomonella*) och Lövträdsknoppvecklare (*Spillonota ocellana*), fast det är stor variation på artsammansättnigen av vecklar populationen mellan områden.

I första delen av min avhandling undersökte jag hur dagens brukande av äppelodlingar, konventionella(IP)- och ekologiskodling, påverkar naturliga fiender och deras förmåga för att kontrollera röd äpplebladlus. Kolonier av röda äpplebladlöss etablerades på äppelträd i kruka i nio olika äppelodlingar som antingen var ekologiska eller IP. Var vecka räknades bladlössen och naturliga fiender samlades in från odlingarnas träd. Resultatet påvisade att en högre artrikedom av naturliga fiender förekom i ekologiska odlingar jämfört med IP odlingar. Rovlevande ängsstinkflyn var den grupp predatorer som påverkades mest negativt av IP, de förekom uteslutande i ekologiska odlingar. Även andra naturliga fiender så som tvestjärtar, nyckelpigor, guldögonsländor, näbbskinnbaggar påträffades i båda odlingssystemen, men deras populationer var lägre i IP odlingar under hela sommaren. Tillsammans med den högre artrikedomen så var även den biologiska bekämpningen av de etablerade bladluskolonierna högre i ekologiska odlingar. Alla kolonier var döda efter tre veckor i ekologiska odlingar medan i IP odlingar så överlevde några kolonier i 7 veckor. Kolonierna i IP var större. Den predatorn som oftast observerades i bladluskolonierna var allmänt näbbstinkfly (*Anthocoris nemorum*) och dess population i odlingarna visade starkt samband med minskningen av de etablerade bladluskolonierna.

Den röda äpplebladlusen lever i mutualism med svartmyror (*Lasius niger*) som sköter och skyddar lössen i utbyte kolhydrater som finns i lössens honungsdagg. Den biologiska bekämpningen som uppmättes i de ekologiska och IP odlingarna skedde under förhållandet då denna mutualism var bruten. Det gjordes genom att förse myrorna med en 20 % sockerlösning vid trädstammen bas. Vi hypoteserade att det var möjligt att bryta mutalismen även med hjälp av samodling med andra växter. I ett labb- och fält försök etablerades röd äpplebladlus på äppelträd som samodlades med åkerböna där bönbladlöss (*Aphis fabae*) hade etablerats. Labbförsöket visade att svartmyror föredrog bönlusen över röd äpplebladlus redan efter några timmar. Detta bekräftades också i ett fyra veckor långt fältförsök i en äppelodling. Färre myror förekom i de etablerade äppelbladluskolonierna som samodlades med bönorna jämfört med kontroll kolonier som inte samodlades med bönor. Dock skedde det ingen ökat biologisk bekämpning av äpplebladlusen om de samodlades med bönor. Äppelbladluskolonierna var dock större och överlevde längre, upp till fyra veckor, när åkerbönor inte var planterade under äppelträden. Det är möjligt att de etablerade bladlössen blev angripna av predatorer innan svartmyrorna hittat dem och kunde börja sköta och försvara dem.

HIPVs och feromoner har föreslagits som växtskyddsmetoder som potentiellt kan ersätta de mer traditionella insekticiderna. HIPVs är lovande då de kan inducera snabba och starka reaktioner hos insekter. Olika HIPVs har visat sig kunna attrahera flera olika naturliga fiender. I ett försök att öka biologisk bekämpning av bladlöss i spannmål placerade vi ut en HIPVs blandning av ättiksyra, metylsalicylat och fenylacetataldehyd i kornfält. Två olika dispenserar provades, en ny innovation bestående av pasta som är lätt att applicera och en standard dispenser bestående av bomullsveke i en polyeten påse. Även dispensernas livslängd undersöktes. Utsöndringen de tre HIPVs mättes från 1-28 dagar gamla dispensärerna, dessa hängdes sedan även ute i äppelodlingar för att se hur länge de attraherade naturliga fiender. I både äppelodlingar och kornfält attraherades nästintill enbart guldögonsländor (*Chrysoperla carnea s.l.*). I kornfältet så förekom det mycket högre antal guldögonsländs ägg och larver i behandlingarna med HIPVs jämfört med kontrollen rutor utan någon HIPVs. Det var ingen skillnad i antal ägg eller larver mellan de två olika typerna av dispenserar. Ytterligare så förekom det nästan inga bladlöss i HIPVs behandlingarna medan i kontrollen så skedde en exponentiell ökning under de åtta veckor som försöket pågick. Med största sannolikhet så var det guldögonsländs larver som bidrog till det låga antalet bladlöss. Dock så kan det inte helt uteslutas att HIPVs repellerade bladlössen då metylsalicylat har visat sig kunna repellera bladlöss under deras migration in i korn. I äppelodlingarna visade fällfångsterna att båda HIPVs dispensrarna kunde attrahera

guldögonsländor i minst fyra veckor. Standard dispensern hade mer stabil frigivning av alla ämnen, utom ättiksyran, i jämförelse med den nya pastan. Fångsterna av guldögonsländor var lika upp till 2 veckor, därefter så fångades fler med standard dispensern.

Det finns vissa risker med att använda syntetisk HIPVs. Normalt utsöndras de endast när växter är attackerade av herbivorer vilket förser dess naturliga fiender med en ärlig signal att följa. Om HIPVs används när det finns liten tillgång på byten så kan attraherande effekten minska eller i värsta fall orsaka svält för de attraherade naturliga fienderna med potentiella negativa effekter på deras population. De potentiella riskerna kan minskas om HIPVs används tillsammans med CBC strategier, tex. etablering av blomsterremsor, i grödan eller i jordburkslanskapet som förser naturliga fiender med mer resurser. Denna här kombinationen har benämnts attrahera och belöna (eng. attract and reward, A&R) och har visat sig kunna attrahera och bibehålla fler naturliga fiender över tid än när HIPVs eller blomsterremsor förekommer var för sig i äpple-, vin- och kålodling. En högre biologisk bekämpning har också mätts upp i A&R.

I ett treårigt experiment som startade 2015 undersöka vi hur A&R påverkar biologisk bekämpning av fjärilslarver under feromon-förvirring (eng. Mating disruption) av vecklare i äppelodling. Första året utvärderades feromon-förvirringens förmåga att kontrollera de sex vanligaste arterna av vecklarefjärilar. De sex arterna övervakades med fällor betade med respektive arts sex-feromon. Minskning eller avsaknad av fångst i behandlingen med feromon-förvirring jämfört med kontrollen visade att hanen inte kunde använda sig av honans feromon för att hitta henne och para sig. De kommande åren utvärderades blomsterremsor och HIPVs, var för sig och även i kombination med feromon-förvirring, förmåga att gynna naturliga fiender samt deras förmåga för biologisk bekämpning. Den biologiska bekämpningen uppskattades genom att placera kort med vecklar ägg medan de naturliga fiender samlades in med hjälp av sugprov. Blomster kluster från äppelträden samlades även in 2015 och 2018 för att mäta fjärilslarvs populationen på våren före och efter alla behandlingar. Resultaten visar att feromon-förvirring kan inhibera alla sex arter som inkluderades i förvirringen, framförallt sista året då hela odlingen var behandlat med feromon-förvirring. Kombinationen av feromon-förvirring och blomsterremsor, HIPVs eller A&R påverkade inte fångsterna av vecklarfjärilarna. Blomsterremsorna hade positiv påverkan på den biologisk bekämpning som uppskattades med ägg korten medan HIPVs hade en negativ effekt 2016. Skillnaden mellan behandlingarna försvann följande år, dock skedde en stor ökning av predation i behandlingen med A&R mellan åren.

Sugproven visar att naturliga fiender förekom mer i A&R, och i vissa perioder även A, än andra behandlingar. Predatorer, främst generalister, ökade

mest och de förekom i större antal i början av sommaren medan parasitoider förekom mer på sen-sommaren. De predatorer som främst gynnades av HIPVs eller A&R var rovlevande ängsskinnbaggar (Miridae), dock påverkades de inte alls när blomsterremsorna förekom själv. Tredje året förekom det fler naturliga fiender, dock så minskade skillnaden mellan behandlingarna, vilket kan indikera att populationen gynnas över alla behandlingarna. Det är dock svårt att bedöma om ökningen beror på att alternativa födan och skyddet som blomsterremsorna ger eller om det sker på grund av en ökad migration från omgivningen in i behandlingarna när hela odlingen är under feromon-förvirring. Det kan även vara en kombination av de två.

I äppelblomster klustren minskade den totala mängden fjärilslarver mellan 2015 och 2018 i alla behandlingar. Störst minskning skedde när HIPVs var själv eller kombinerat med blomsterremsor i A&R, dock så var de dessa försöksrutor som hade flest larver 2015 vilket gör att man ska vara försiktig med slutsatserna. Det är ändå tydligt de fjärilsarterna som inte var inkluderade i feromon-förvirringen ökade. Det var främst mätarlarver, där frostfjäril var den dominerande arten, och Äppleknoppvecklarens (*Hedya nubiferana*) larver som fanns i blomsterklustren. Äppleknoppvecklaren förekom mest i blomsterremsorna medan densamme missgynnas av HIPVs, vilket var de behandlingar som rovlevande ängsstinkflyn förekom minst respektive mest i. Mätarlarver däremot förekom minst i behandlingarna med blomsterremsor och A&R. Frostfjärilen förpuppar under sommaren för att para sig på hösten. Det är inte omöjligt att blomsterremsorna försvårade förpuppningen eller gynnande naturliga fiender som kunde attackera larverna när de gräver ner sig eller attackerar själva puppan. Detta har dock inte blivit undersökt utan behöver undersökas i mer detalj för att säkerställa vad som sker.

Bevarande biologisk bekämpning, så som A&R, under selektivt växtskydd (feromon-förvirring) har stora potential att långsiktigt kontrollera skadegörare genom att gynna dess naturliga fiender. Flera olika växter kan användas för att gynna olika insekter. Dock är det fortfarande oklart hur populationerna påverkas över längre tid med A&R. Det kan ta flera år för vissa naturliga fiender att etablera sig, särskilt för de mer ovanliga arterna i mer homogena landskap. I sådana fall är det inte säkert att attraktion med HIPVs kan påskynda etableringen av naturliga fienders i grödan. Etablering av växter och habitat behöver även ske på större skala än på fält nivå för att bygga upp nyttodjurens population. Detta för att få en högre motståndskraft mot störningar på de lägre nivåerna (fält/gård), som vid tillexempel användning av växtskyddsmedel. Mer forskning behöver göras för att ta reda på detta och bör göras över flera discipliner tillsammans med lokala intressenter.

Acknowledgements

I wish to thank everyone who have helped we with big and small things. Some have helped me with inspiration for my work while others rescued me from disasters, more or less.

First of all, I would like to thank *Marco*, *Mario* and *Teun*. I have been lucky to have all of you as supervisors. We have had good discussions about everything, and I feel you always supported me in my work and you have helped me when I needed it, from stats and writing to field work.

Thanks to all the field assistants who helped me to collect all the data and for the nice discussions while traveling to Österlen. Especially to *Giulia* with the bean experiment, without your hard work it would not have been possible.

A big thanks to all the grower who let us have our experiments in their orchards and taught me a bit about how to grow apples.

Thanks to the people from the Garden Lab for borrowing things, watering plants and the nice talks about gardening.

Sebastian, thanks for showing me how to work more efficiently with R and how to make plots in a nice way.

To my office mates, *Daniela* and *Mette*, for the small talk that helped reducing some stress.

Elin, Kalle, Raj and *Ryan* for nice discussions about barley and sharing your latest creations, it provided well-needed breaks in the long working days.

Mikael and *Björn* for conversations about insects and how important goulash soup is. And *Mikael*, we should really start beekeeping.

Thanks to all the other colleagues from the V-house who enriched my lunches and gave me ideas on how to improve my work.

Lastly, I would like to thank my family. Sorry for working so much, but this summer I will not work, and I will not have any insects in the freezer. Big thanks to my kids, *Alma* and *Elsa,* who forced me to leave the computer and do more important things, like painting, baking with clay and selling our creations in the store, having water fights or just relaxing reading or watching TV.