Biological Control Strategies against the Cabbage Root Fly *Delia radicum*

Effect of Predators, Parasitoids and Pathogens

Linda-Marie Rännbäck
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Plant Protection Biology
Alnarp

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Cover: The image visualises the interactions in the studied system. From left to right: white cabbage plant, adult female cabbage root fly *Delia radicum* and natural enemies. Natural enemies from top to bottom: the generalist predator/pupal parasitoid *Aleochara bipustulata*, the specialist larval parasitoid *Trybliographa rapae* and generalist entomopathogenic fungi represented by *Metarhizium* sp. culture and sporulation on an insect cadaver. These fungi may infect both *D. radicum* and the natural enemies.

Photo: *Metarhizium* sp. culture taken by Dr. Bernhardt M. Steinwender, all other taken by Linda-Marie Rännbäck

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Abstract

Contemporary intensified agroecosystems are contributing to a reduction in natural enemy (NE) populations. In conservation biological control (CBC), NEs are favoured by providing supporting resources. CBC could be complemented with inoculation biological control (IBC) to enhance the effect of NEs. However, increased NE species richness may result in positive (e.g. niche complementarity [NC]) or negative (e.g. intraguild predation [IGP]) effects on pest suppression through species interactions. The research reported in this thesis investigated the potential of combining CBC with IBC as a strategy for cabbage root fly, *Delia radicum*, control. A three-year field experiment explored the combined effect of floral resources and perennial shelter habitats (i.e., conservation strips [CS]) on NEs of *D. radicum*. CS increased the abundance of hymenopteran parasitoids, but did not increase parasitism by either of the two dominant parasitoid species, *Trybliographa rapae* and *Aleochara bipustulata*. The entomopathogenic fungi *Metarhizium brunneum* and *Beauveria bassiana* were found to be pathogenic to both *D. radicum* and *T. rapae*, thus representing an IGP risk to *T. rapae*. However, the parasitoid laid more eggs in healthy than in *M. brunneum* infected *D. radicum* larvae in choice assays. Host plant habitats harbouring high densities of *M. brunneum* were avoided when given a choice, but host density was more important for attraction than fungal presence, indicating a trade-off between IGP risk and reproductive success. Despite the IGP risk, field cage studies showed that combining *T. rapae* and *M. brunneum,* at both low and high fungal densities, reduced *D. radicum* population levels more than when either NE acted alone. Selective ovipositioning in healthy larvae presumably led to this resource partitioning and resulting NC. However, fungal presence, particularly at high density, reduced the number of emerged F1 *T. rapae*. Consequently, combining CBC of *T. rapae* with IBC using a relatively low density of *M. brunneum* is recommended to ensure stable, long-term *D. radicum* control.

Keywords: Trybliographa rapae, *Aleochara bipustulata*, *Metarhizium brunneum*, *Beauveria bassiana*, conservation biological control, inoculation biological control, niche complementarity, intraguild predation, antipredator behaviour, parasitoid foraging

Author's address: Linda-Marie Rännbäck, SLU, Department of Plant Protection
Biology, P.O. Box 102, 230 53Alnarp, Sweden
E-mail: Linda-Marie.Rannback@ slu.se

Biologiska bekämpningsstrategier mot lilla kålflugan *Delia radicum*. Effekt av predatorer, parasitoider och patogener

Svensk sammanfattning

Dagens intensivt brukade jordbrukslandskap har medfört en minskning av nyttoinsekter, såsom pollinatörer och naturliga fiender. Avsaknad av stödjande resurser till dessa samt användningen av bredverkade insekticider har varit bidragande orsaker. De naturliga fienderna kan uppförökas och effektivare kontrollera skadeinsekter om de får tillgång till skydd och övervintringsplatser samt kompletterande föda som nektar och pollen. Att på detta sätt förbättra livsmiljön för befintliga naturliga fiender i odlingssystemet är syftet inom bevarande biologisk bekämpning (eng. conservation biological control). Denna strategi kan kompletteras med förstärkande biologisk bekämpning (eng. inundation and inoculation biological control), där naturliga fiender sätts ut vid lämplig tidpunkt. Genom att på detta sätt kombinera flera olika naturliga fiender kan den sammanlagda effekten öka additativt eller synergistiskt. Interaktioner mellan arterna kan dock även leda till att effekten minskar genom att de naturliga fienderna angriper varandra istället för skadegörarna, så kallad intraguild predation (IGP).

I min avhandling har jag studerat möjligheten att bekämpa lilla kålflugan (*Delia radicum*) genom att kombinera bevarande biologisk bekämpning med rovlevande skalbaggar och parasitoider, och förstärkande biologisk bekämpning med insektspatogena svampar.

Lilla kålflugans larver gör allvarlig skada på rötterna av kålväxter (Brassicaceae) dit ekonomiskt viktiga grödor hör, såsom t ex raps, blomkål, vitkål, kålrot och broccoli. Angreppen kan orsaka stort bortfall av småplantor samt kvalitetsskador på grödor där rötterna skördas. I EU minskar möjligheten att bekämpa lilla kålflugan kemiskt, bland annat på grund av minskad tillgång på godkända produkter samt ny lagstiftning. Biologisk bekämpning, tillsammans med förebyggande odlingsåtgärder, kan därför komma att bli ett alternativ eller ett komplement. Flera naturliga fiender angriper lilla kålflugans olika utvecklingsstadier. Rovlevande skalbaggar inom ordningarna kortvingar och jordlöpare konsumerar lilla kålflugans ägg och larver. Dessa skalbaggar kan gynnas av så kallade skalbaggsåsar med tuvbildande gräs som övervintringsplatser, för att de tidigt på säsongen ska kunna kolonisera fälten och äta skadeinsekter. Kortvingen *Aleochara bipustulata* är rovlevande som fullbildad, och som larv parasitoid på lilla kålflugans puppor. Parasitstekeln *Trybliographa rapae* parasiterar larver av lilla kålflugan och andra *Delia*-flugor. Burförsök i fält har visat att parasiteringsgraden av *T. rapae* kan ökas om denna parasitstekel får tillgång till nektar. Parasiteringsgraden av dessa båda parasitoidarter kan i fält kan uppgå till 50-80%. Insektspatogena svampar orsakar sjukdom och död hos insekter, och tidigare försök har visat att arter inom släktena *Metarhizium* och *Beauveria* har stor potential i en förstärkande biologisk bekämpningsstrategi, om de appliceras mot lilla kålflugans larver.

Under tre säsonger genomfördes ett fältförsök på Torslunda försöksstation, Öland, med syftet att undersöka effekten av bevarande biologisk bekämpning mot lilla kålflugan. Samtliga försöksrutor planterades med vitkål 2008 och 2010. Under 2009 planterades hälften med lök och hälften med vitkål. I mitten av försöksrutorna anlades perenna skalbaggsåsar, vilka kombinerades med årlig sådd av bovete och dill (s.k. blomstergräsremsa). Effekten av dessa blomstergräsremsor utvärderades jämfört med kontrollrutor med enbart annuellt kortklippt gräs. Blomstergräsremsorna ökade generellt förekomsten av parasitsteklar, men parasiteringsgraden av *T. rapae* och *A. bipustulata* ökade inte. Dock observerades en ökad förekomst av *A. bipustulata* i rutor med blomstergräsremsor under lilla kålflugans äggläggningsperiod under 2009, vilket kunde sättas i samband med ett minskat antal kålflugepuppor i denna behandling. Varken artsammansättning eller äggpredation av rovlevande skalbaggar påverkades av blomstergräsremsorna.

I laboratorium utfördes virulenstest vid olika koncentrationer med isolat av de insektspatogena svamparna *Metarhizium brunneum* och *Beauveria bassiana* på lilla kålflugan och *T. rapae*, genom att applicera dem på insekterna och sedan notera dödligheten över 14 dagar. Båda svamparna var patogena mot insektsarterna, men generellt var *M. brunneum* effektivare genom att döda både snabbare och vid lägre koncentration än *B. bassiana*. Detta indikerar att svamparna utgör en IGP-risk för *T. rapae*. Beteendestudier visade att *T. rapae* undvek att lägga ägg i larver som smittats med *M. brunneum* i en tvåvalssituation med friska larver. Värdväxtmiljöer med höga tätheter av *M. brunneum* undveks också. Dock undveks inte värdväxtmiljöer med svamp vid låg täthet av *M. brunneum*, vid tillsats av *B. bassiana* eller då värdtätheten på plantorna var hög. Detta visar på en avvägning mellan IGP-risk och möjlighet till reproduktion. Doftuppsamlingar från värdväxtmiljöer visade på skillnader i sammansättning av lättflyktiga kemiska ämnen beroende på närvaro, täthet och art av svamp, vilket kan förklara *T. rapae*s beteende. Sammanfattningsvis kunde *T. rapae* uppfatta och reagera på IGP-risk från *M. brunneum*, men inte *B. bassiana*.

I burförsök i fält undersöktes bekämpningseffekten mot lilla kålflugans larver med en kombination av *T. rapae* och *M. brunneum*. På vitkålsplantor tillsattes kålflugeägg som utvecklades till larver innan försöket startade. Försöket var faktoriellt upplagt med ± utsläppta *T. rapae* och/eller ± *M. brunneum* tillsatt till plantans rotsystem. Kombinationen av parasitstekel och svamp, både vid låg och hög svamptäthet, reducerade kålflugepopulationen mer än varje naturlig fiende var för sig. Effekten var additativ. Mekanismen bakom detta tros vara parasitstekelns förmåga att selektivt lägga ägg i friska larver, vilket lett till nischfördelning och komplementära effekter. Dock reducerades parasitstekelns F1-population av svamp, särskilt vid hög svamptäthet.

Kombinationen av bevarande biologisk bekämpning av *T. rapae* och inokulering med en relativt låg dos av *M. brunneum* har potential att leda till en långsiktigt stabil bekämpning av lilla kålflugan. Svampisolaten skulle kunna tillsättas i substratet vid plantuppdragningen. Detta behöver undersökas vidare i storskaliga fältförsök. För att optimera strategin krävs också mer forskning om *M. brunneum*s ekologi i rotsystemet, till exempel persistens och växt-svamp-interaktion.

Dedication

To my family, life and nature. I am doing my best

The more I learn, the more I realize how much I don’t know.

Albert Einstein

Hade det vart lätt, hade det vart gjort.

Ulf Nilsson

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 Nilsson, U., Rännbäck, L.-M., Anderson, P., Björkman, M., Futter, M. & Rämert, B. Effects of conservation strip and crop type on natural enemies of *Delia radicum*. (submitted)

II Rännbäck, L.-M., Cotes, B., Anderson, P., Rämert, B. & Meyling, N.V. (2015). Mortality risk from entomopathogenic fungi affects oviposition behavior in the parasitoid wasp *Trybliographa rapae*. *Journal of Invertebrate Pathology* 124, 78-86.

III Cotes, B., Rännbäck, L.-M., Björkman, M., Norli, H.R., Meyling, N.V., Rämert, B. & Anderson, P. (2015). Habitat selection of a parasitoid mediated by volatiles informing on host and intraguild predator densities. *Oecologia*. DOI 10.1007/s00442-015-3326-2

IV Rännbäck, L.-M., Cotes, B., Meyling, N.V., Anderson, P. & Rämert, B. Additive effects on prey regulation through niche partitioning between a below-ground parasitoid and an entomopathogenic fungus. (manuscript).

Paper II and III is published with Open Access.

The contribution of Linda-Marie Rännbäck to the papers included in this thesis was as follows:

I Assisted in developing and performing the field experiment with U. Nilsson. Wrote the paper together with the co-authors.

II Planned and performed bioassays with help from the co-authors. Analysed the data and wrote the paper together with the co-authors.

III Planned bioassays together with the co-authors. Wrote the paper together with the co-authors.

IV Planned and performed field-cage experiments with help from the co-authors. Analysed the data and wrote the paper together with the co-authors.

Abbreviations

CBC	Conservation biological control
CS	Conservation strip
HIP	Heavily-infested plant
HIPV	Herbivore-induced plant volatile
IBC	Inoculation biological control
IG	Intraguild
IGP	Intraguild predation
IPM	Integrated Pest Management
LC_{50}	Median lethal concentration, *i.e.* the concentration required to kill 50% of exposed insects in an experimental group
LC_{90}	the concentration required to kill 90% of exposed insects in an experimental group
L:D	Light:Dark
LIP	Lightly-infested plant
MST	Median survival time
UP	Uninfested plant
SDA	Sabouraud dextrose agar

1 Introduction

Loss of biodiversity is threatening the resilience of ecosystems (Steffen *et al.*, 2015) and contemporary agricultural intensification is leading to a reduction in natural enemy guild diversity and their ecosystem services (Hooper *et al.*, 2005; Tscharntke *et al.*, 2012; Crowder & Jabbour, 2014). Biological control is an alternative or a supplement to synthetic chemical pesticides, potentially increasing the long-term resilience to pest attacks of agroecosystems (Eilenberg *et al.*, 2001). The growing awareness of the adverse effects on the environment of extensive use of chemical pesticides has spurred the development of the Integrated Pest Management (IPM) concept (Kogan, 1998). IPM is an ecosystem-based, interdisciplinary strategy that focuses on long-term prevention of pest damage through a combination of measures such as cultural practices, biological control and chemical control. Pest control methods are selected and applied in such a way as to minimize risks to human health, beneficial and non-target organisms, and the environment (Kogan, 1998; Radcliffe *et al.*, 2009). The implementation of IPM in Europe has been hastened by recent European Union legislations on the sustainable use of pesticides, making IPM compulsory for its EU member states since 1st of January 2014 (EU, 2009).

1.1.1 Definitions and history

Biological control is defined as the use of living organisms to suppress pest populations in order to decrease the damage they cause (Eilenberg *et al.*, 2001). Four different strategies can be differentiated; classical biological control, inundation biological control, inoculation biological control and conservation biological control (Eilenberg *et al.*, 2001).

In classical biological control, suppression of an exotic pest is achieved by introducing one or more natural enemies from the native range of the pest, with the intention of permanent establishment and long-term pest control (Eilenberg *et al.*, 2001). In the early history of biological control, this was the main biological control strategy utilised. An example of successful biological control is the releases of the vedalia beetle ((*Rodolia* (*Vedalia*) *cardinalis* Mulsant (Coleoptera: Coccinellidae)) and a parasitoid (*Cryptochetum iceryae* (Williston) (Diptera: Cryptochetidae)) imported from Australia against the cottony cushion scale, *Icerya purchasi* Mask. (Homoptera: Margarodidae), which devastated citrus trees in California in the late 1880s (Caltagirone & Doutt, 1989). In recent times, another example is the plan to introduce the predator/parasitoid staphylinid beetle *Aleochara bipustulata* L. (Coleoptera: Staphylinidae) to control the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae), a pest on oilseed rape in the Canadian prairies (Andreassen *et al.*, 2009).

In inundation biological control, immediate pest control is expected, similar to the action of chemical pesticides, which is achieved exclusively by the released individuals themselves and not by subsequent generations (Eilenberg *et al.*, 2001). The use of microbial biological control organisms often falls into this category. Research efforts expanded after the discovery of the 'green muscardine' fungus *Metarhizium anisopliae* (Metsch.) Sorokin (Ascomycota: Hypocreales) on grain beetles (*Anisoplia austriaca* Herbst) (Coleoptera: Scarabaeidae) by Metchnikoff in 1879. Modern examples of biological control programmes includes the bacteria *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) to control Lepidoptera larvae and the use of the fungus *Metarhizium anisopliae* for control of a complex of spittlebugs (Cercopidae) in South American sugarcane and pastures (Lord, 2005; de Faria & Wraight, 2007).

1.2 Inoculation biological control

In inoculation biological control (IBC), the biological control organism is expected to multiply and control the pest for an extended period (Eilenberg *et al.*, 2001). This strategy is used *e.g.* in greenhouse production systems, where early season and periodic releases are made with a mixture of arthropod biological control agents for season-long control of pest complexes. The use of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) in more than 20 countries to control whitefly, thrips and mites in many greenhouse cultures is an example (van Lenteren, 2012). The use of entomopathogenic fungi may also fall into this category if the intention is for these fungi to persist, multiply and provide long-term control (Eilenberg *et al.*, 2001)

1.3 Conservation biological control

Conservation biological control (CBC) involves modifying existing management or the environment in the agroecosystem to protect and enhance specific indigenous natural enemies in order to reduce pest attacks (Landis *et al.*, 2000; Eilenberg *et al.*, 2001; Jonsson *et al.*, 2008). Habitat manipulation within the field or nearby areas, by providing shelter habitats protecting natural enemies from adverse conditions and/or habitats with alternative food resources, is included in the CBC strategy (Landis *et al.*, 2000; Jonsson *et al.*, 2008). Gurr *et al.* (2012) summarised the needs of natural enemies with the acronym ‘SNAP’ – Shelter, Nectar, Alternative prey and Pollen, to facilitate dialogue and adoption by farmers.

Shelter habitats

Agricultural intensification with *e.g.* an increase in field size has led to fragmentation and destruction of semi-natural habitats (Tscharntke *et al.*, 2007). Managing field margins or providing within-field habitats may be one solution for sustaining natural enemy populations. Raised perennial grass ridges, so-called ‘beetle banks’, were first developed in cereals to provide overwintering habitats for generalist predators against cereals aphids. The tussock-forming grasses *Dactylis glomerata* L. (Poaceae) and *Holcus lanatus* L. (Poaceae) decrease temperature fluctuations during winter, and are reported to harbour high overwintering densities of spiders (Aranea), ground beetles (Carabidae) and rove beetles (Staphylinidae) (Thomas *et al.*, 1991; 1992; MacLeod *et al.*, 2004). Creating beetle banks within the crop enables predators to colonise the crop early and provide early season pest control, and may thus prevent pest outbreaks (Collins *et al.*, 2002; Frank & Shrewsbury, 2004). Sown perennial flower strips may also be important overwintering sites, since high abundance and natural enemy species diversity have been found in this habitat when sampled during the winter (Pfiffner & Luka, 2000).

Flower strips

Providing natural enemies with floral food (nectar and pollen) can greatly increase their abundance and performance. Nectar is an important carbohydrate source for adult hymenopteran parasitoids to increase their longevity, fecundity, parasitism rates and search efficacy (Winkler *et al.*, 2006; Vattala et al., 2006; Géneau *et al.*, 2012; Wäckers & van Rijn, 2012). Winkler *et al.* (2006) offered *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae) flowering *Fagopyrum esculentum* (Moench) (Polygonaceae) as a nectar source in field-cages, which increased the parasitism rate on its host (*Plutella xylostella* L., (Lepidoptera: Plutellidae)) compared with starved individuals. Sowing flower strips in crop fields has been shown to increase parasitism in several agroecosystems, such as wheat, cabbage, apple and vine (Tylianakis *et al.*, 2004; Lee & Heimpel, 2005; Berndt *et al.*, 2006; Irvin *et al.*, 2006; Pfiffner *et al.*, 2009; Balmer *et al.*, 2014).

However, care must be taken to choose plants for flower strips that selectively favour the target natural enemy more than the pest insect, based on *e.g.* flower attractiveness, accessibility and nutritional suitability (Wäckers, 2004; Winkler *et al.*, 2009; Nilsson *et al.*, 2011; Nilsson *et al.*, 2012; Wäckers & van Rijn, 2012). As hymenopteran parasitoids generally have short mouthparts, their feeding is limited to flowers with shallow corollas and easily accessible nectaries (Jervis *et al.*, 1993; Patt *et al.*, 1997; Vattala *et al.*, 2006). Plants proven suitable include *e.g. Fagopyrum esculentum*, *Lobularia maritima* Desv. (Brassicaceae) and numerous Apiaceae, such as *Anethum graveolens* L. (Jervis *et al.*, 1993; Fiedler *et al.*, 2008; Wäckers & van Rijn, 2012).

1.4 Ecological interactions between natural enemies

A high diversity of natural enemies has in general been shown to lead to improved prey suppression (Letourneau *et al.*, 2009; Griffin *et al.*, 2013). More efficient biological control of pest insects may thus be achieved by combining multiple natural enemy species (Cardinale *et al.*, 2003; Stiling & Cornelissen, 2005; Snyder *et al.*, 2006). However, increased natural enemy species richness can cause positive, neutral or negative effects, which have been attributed mainly to the ecological mechanisms niche complementarity, functional redundancy and intraguild predation (Casula *et al.*, 2006; Straub *et al.*, 2008).

Natural enemy interactions should be considered when implementing biological control strategies. For example, CBC could be complemented with timely inoculative or inundative releases of functionally complementary natural enemies. Moreover, combining natural enemy species that respond differently to abiotic conditions may provide a functional insurance under environmental fluctuations (Loreau & de Mazancourt, 2013).

1.4.1 Intraguild predation and its avoidance

When two species which share a common prey resource interact with each other in terms of competition and predation *per se*, this is known as intraguild predation (IGP) (Polis *et al.*, 1989). The interaction can be unidirectional, where one species is the IG predator, preying on the other, or bidirectional, where the roles as IG predator and IG prey can shift. An example of a unidirectional IGP system is when a pathogen can infect both a herbivore and its parasitoid (Rosenheim *et al.*, 1995; Straub *et al.*, 2008).

Natural enemies of herbivores can utilise volatile secondary metabolites emitted by plants attacked by herbivores, *i.e.* herbivore-induced plant volatiles (HIPVs), when searching for prey (Dicke & Baldwin, 2010). HIPVs and other host-derived cues have been shown to guide female hymenopteran parasitoids to locate and evaluate host habitats before oviposition (Vet & Dicke, 1992; Mills & Wajnberg, 2008; Wäschke *et al.*, 2013), *e.g.* based on host density (Hemachandra *et al.*, 2007b; Girling *et al.*, 2011). It is also recognised that the host habitat selection behaviour of natural enemies of herbivores may be influenced by rhizosphere-inhabiting microbes via induced changes in the composition of released HIPVs (Schausberger *et al.*, 2012; Pineda *et al.*, 2013; Soler *et al.*, 2013).

Parasitoid host foraging assumes trade-offs between profitable oviposition sites (*i.e.* expected fitness gain) and the risk of predation (Weisser *et al.*, 1994; Roitberg *et al.*, 2010). In addition to host-derived cues, parasitoids may also respond to predator-derived infochemicals and avoid predation risks by diverting foraging to ‘safer’ habitats, by shifting their temporal or spatial foraging (Raymond *et al.*, 2000; Dicke & Grostal 2001; Nakashima *et al.*, 2004; Meisner *et al.*, 2011), or exerting selective oviposition behaviour (Brobyn *et al.*, 1988; Fransen & van Lenteren, 1993; Takizawa *et al.*, 2000). Thus, by exhibiting variable anti-predator behaviour in this manner, parasitoids can partly or fully avoid the potential disruptive impact of IGP (Snyder & Ives, 2008).

1.4.2 Niche complementarity

Niche complementarity may arise when natural enemies reduce interspecific competition for prey through either of the two mechanisms resource partitioning or facilitation (Straub *et al.*, 2008). In resource partitioning, the shared prey population is divided between the enemy species based on *e.g.* differential temporal and spatial occurrence (Straub & Snyder, 2008; Northfield *et al.*, 2010; Gable *et al.*, 2012), exploitation of different prey densities (Snyder & Ives, 2003), prey qualities or developmental stages (Wilby *et al.*, 2005), which leads to additive effects on prey suppression (Casula *et al.*, 2006). In facilitation, the attack rate on prey by one enemy species is facilitated by the presence of another enemy species, leading to more than additive effects, *i.e.* synergy (Losey & Denno, 1998; Ramirez & Snyder, 2009).

Selective foraging based on IGP risk may also result in partitioning of a prey resource. Niche complementarity of natural enemies, despite potential for IGP, has been demonstrated in above-ground systems (*e.g.* Losey & Denno, 1998; Snyder & Ives, 2003; Cardinale *et al.*, 2003; Gable *et al.*, 2012). Similar effects have only recently been revealed in a below-ground context (*e.g.* Ramirez & Snyder, 2009; Jabbour *et al.*, 2011).

2 Objectives

The overall objective of this thesis was to explore the possibility to combine different biological control strategies against the immature stages of the important Brassicaceae pest, the cabbage root fly *Delia radicum* L. (Diptera: Anthomyiidae), in order to develop a pest-resilient agroecosystem approach on a farm-scale level. The work was conducted in two main stages. First, conservation biological control (CBC) to enhance the effect of naturally occurring parasitoids and predators in a crop rotation system was investigated. Second, the prospect of complementing CBC measures with inoculation biological control (IBC) with entomopathogenic fungi was studied.

The natural enemies studied were *Trybliographa rapae* Westwood (Hymenoptera: Figitidae), the most important larval parasitoid of *D. radicum*, the egg-larval predator/pupal parasitoid *Aleochara bipustulata* L. (Coleoptera: Staphylinidae) and the generalist entomopathogenic fungal species *Metarhizium brunneum* Petch (included in the cryptic species complex *Metarhizium anisopliae* (Metsch.) Sorokin *sensu lato*) and *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), targeting the *D. radicum* larvae.

For the CBC approach, the effect of conservation strips (CS), comprising a combination of perennial shelter habitats and floral resources, on the abundance and performance of the natural enemy guild was studied specifically. A crop rotation designed to disrupt the life cycle of the pest, but at the same time support the natural enemy guild, was also employed. These habitat manipulation strategies were evaluated in the field on *D. radicum* and its natural enemies by studying the impact of:

- ➢ Conservation strips on parasitism by *Trybliographa rapae* and *Aleochara bipustulata*.

- Conservation strips on the abundance and diversity of epigeal predators (such as Carabidae and Staphylinidae (including *Aleochara bipustulata*)) and hymenopteran parasitoids.
- Conservation strips on predation on the immature stages of *Delia radicum*.
- Crop rotation on the parasitism and abundance of natural enemies of *D. radicum*.

For the IBC objective, the effect of introducing entomopathogenic fungi to the system on *D. radicum* and *T. rapae* mortality and longevity, as well as on *T. rapae* behavioural responses, was investigated. The interactions in the system were studied in an ecological theoretical framework of intraguild predation, resource partitioning and niche complementarity. This was evaluated in the laboratory and in field-cages by examining:

- The susceptibility of *Delia radicum* and *Trybliographa rapae* to the two species of entomopathogenic fungi.
- *Trybliographa rapae* oviposition behaviour during host foraging when the entomopathogenic fungi are present either as infected hosts or as infective propagules in the host environment.
- *Trybliographa rapae* behaviour during host habitat selection when different species and densities of fungi are present, and how that response is modulated by host larval density.
- Whether host habitat selection by *Trybliographa rapae* is linked to changes in the composition of volatile emissions from the host habitats.
- The effect of combining *Trybliographa rapae* and *Metarhizium brunneum* on *Delia radicum* populations.
- The impact of *Metarhizium brunneum* on *Trybliographa rapae* F1 populations.
- The influence of environmental conditions on the efficacy of *Trybliographa rapae* and *Metarhizium brunneum*.

3 Organisms studied

3.1 Herbivore *Delia radicum*

The cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae), is a noxious pest on cruciferous crops (*e.g.* Chinese cabbage, Brussels sprouts, white cabbage, cauliflower, turnip, radish, oilseed rape) in temperate climates throughout the Holarctic region. The 6-7 mm greyish coloured female fly (Fig 1) oviposits in the soil close to the stem base and the larvae (Fig 1) feed by burrowing into the roots, causing wilting, death and/or quality damage. The larvae feed for around four weeks, during which time they develop through three larval instars, and subsequently pupate in the soil a few centimetres from the plant. Overwintering occurs as a pupa within a puparium (Fig 1). In Northern Europe, two to three generations occur per year. The adults of the overwintered first generation emerge in April-May, and the second generation appears in late June to July (Block *et al.*, 1987; Coaker & Finch, 1971; Finch, 1989).

Preventive management practices to decrease *D. radicum* populations and larval damage include cultural and physical methods; *e.g.* crop rotation, deep ploughing to bury puparia, avoiding sowing/planting during the main egg-laying periods, considering crop or cultivar resistance, crop covering with cloth or netting (Coaker & Finch, 1971; Finch & Skinner, 1980; Finch & Collier, 2000), mulching (Hellqvist, 1996) and companion planting (intercropping/ undersowing/trap cropping) (Langer, 1996; Rousse et al., 2003; Finch & Collier, 2012). Trapping the adults for monitoring purposes or direct control, with traps attractive in colour, shape, size and/or odour, has also been tested (Finch, 1989; Meadow, 2003). Recently, the use of synthetic HIPVs to attract natural enemies was seen to lead to a reduction in *D. radicum* infestation levels (Ferry et al., 2009; Kergunteuil *et al.*, 2012).

Chemical control includes insecticide application after prediction of peak insect activity by a forecasting system, *e.g.* by modelling the relationship between temperature and rates of insect development and/or assessing trap catches and/or crop inspection. Insecticide film coating of seeds and/or incorporating granular insecticides into the soil (of transplants) may also be employed (Finch & Collier, 2000). However, recent legislation (EU, 2009), resistance development in *D. radicum* populations, toxicity to natural enemies and decreased availability of environmentally sound insecticides require the development of additional approaches in an IPM strategy (Finch & Collier, 2000). Biological control strategies may complement, replace or reduce use of insecticides against *D. radicum*. CBC as a means to favour and enhance the naturally occurring natural enemy populations may constitute an important corner-stone in future pest management strategies in the Brassicaceae agroecosystem, which may be complemented with IBC and other environmentally benign approaches (Eilenberg & Meadow, 2003; Ferry *et al.*, 2009; Kergunteuil *et al.*, 2012; Josso *et al.*, 2013; Meyling *et al.*, 2013).

Figure 1. Delia radicum. From left to right: adult female, larvae on white cabbage and a fly emerging from its puparium. Photo: Linda-Marie Rännbäck

3.2 Natural enemies

3.2.1 Predators

Many generalist feeding epigeal predators potentially consume immature stages of *D. radicum* (Coaker & Finch, 1971), among which ground beetles (Coleoptera: Carabidae) and rove beetles (Coleoptera: Staphylinidae) are particularly well-studied (Coaker & Finch, 1971; Jonasson, 1994; Finch, 1996; Finch & Elliott, 1999; Finch *et al.*, 1999; Hartfield & Finch, 2003; Prasad & Snyder, 2004; Eyre *et al.*, 2009; Hummel *et al.*, 2012). Predation rate in the field can range between 30-95%, depending on the context and availability of alternative prey (Coaker & Williams, 1963; Coaker & Finch, 1971; Finch, 1989). Important egg predators include several small to medium-sized ground beetles; *Bembidion* spp., *Agonum* spp., *Amara* spp. and *Harpalus* spp. (Coaker

& Williams, 1963; Coaker & Finch, 1971; Finch, 1996; Finch & Elliott, 1999). Adult rove beetles of *e.g. Aleochara* spp. also serve as predators on immature stages (Fuldner, 1960; Coaker & Williams, 1963), particularly the larvae (Hartfield & Finch, 2003). Inoculative releases of adult *Aleochara bilineata* (Gyllenhal) (Coleoptera: Staphylinidae) in attempts to control *D. radicum* larvae have been tested (Maisonneuve *et al.*, 1996; Hartfield & Finch, 2003). Releasing 2-8 beetles per plant reduced the number of recovered fly pupae by more than 60% (Hartfield & Finch, 2003). Epigeal predators may be favoured within production fields by providing shelter habitats, such as ‘beetle banks’, which protect against disturbance from agricultural practices as well as serving as overwintering habitats, enabling early season crop colonisation (Collins *et al.*, 2002; Frank & Shrewsbury, 2004; MacLeod *et al.*, 2004).

3.2.2 Parasitoids

Several parasitoids of *D. radicum* have been recorded (Coaker & Finch, 1971; Jones, 1986). The most important parasitoids of *D. radicum* include the larval parasitoid *Trybliographa rapae* Westwood (Hymenoptera: Figitidae) (Fig. 2) and the pupal parasitoids *Aleochara bipustulata* L. (Fig. 2) and *Aleochara bilineata* (Gyllenhal) (Coleoptera: Staphylinidae) (Wishart & Monteith, 1954; Fuldner, 1960).

Trybliographa rapae

Trybliographa rapae is a proovigenic koinobiont parasitoid that attacks all three larval instars of *D. radicum*, although the early third instar is preferred (Wishart & Monteith, 1954; Jones, 1986; Neveu *et al.*, 2000). It also parasitizes larvae of other *Delia* spp. (Wishart & Monteith, 1954). The parasitoid is shiny black, 2-4.5 mm long. The sexes are most easily differentiated by the number of antennae segments; females have 13, while males have 15 (Wishart & Monteith, 1954; Forshage, 2009). In northern Europe two generations occur each year (Jones, 1986; Block *et al.*, 1987).

The parasitisation rate of *D. radicum* in production fields varies from a few per cent up to >50% (Wishart & Monteith, 1954; Bromand, 1980; Hemachandra *et al.*, 2007a; Meyling *et al.*, 2013). Host habitat choice by *T. rapae* is based on volatile cues released from plants infested with *D. radicum* larvae (Brown & Anderson, 1999; Neveu *et al.*, 2002; Nilsson *et al.*, 2012), informing about *e.g.* host density (Jones & Hassell, 1988; Hemachandra *et al.*, 2007b) and attack by other herbivores (Pierre *et al.*, 2011). Once in the host habitat, subsequent host location is mediated by larval movement and larva-related cues, perceived by sensory organs on the antennae and ovipositor upon probing (Butterfield & Anderson, 1994; Brown & Anderson, 1998).

Acceptance of suitable hosts and oviposition follows the evaluation of quality traits such as instar (Neveu *et al.*, 2000) and feeding status (Brown & Anderson, 1999), presumably through gustatory responsiveness of the ovipositor to factors present in host haemolymph (Brown & Anderson, 1998).

Carbohydrates from *e.g.* nectar increase the longevity of *T. rapae* (Jones, 1986; Nilsson *et al.*, 2011), which may lead to increased life-time fecundity. Nilsson *et al.* (2011) showed in a laboratory study that *Fagopyrum esculentum* Moench. (Polygonaceae) was attractive to female *T. rapae,* and that the flowers of *F. esculentum*, *Anethum graveolens* L. (Apiaceae) and *Lobularia maritima* Desv. (Brassicaceae) were successfully exploited. Furthermore, longevity increased with flowering *F. esculentum* and *A. graveolens*, and in field-cages, a higher parasitism rate on *D. radicum* larvae was found with *F. esculentum* flowers than without (Nilsson *et al.*, 2011). As *T. rapae* is reported to be a poor flier (Jones, 1986), providing in-field floral resources may be vital for increasing its longevity, fecundity and efficacy.

Aleochara spp.

As larvae, *A. bipustulata* and *A. bilineata* are parasitoids on *D. radicum* puparia (Fuldner, 1960). The beetles are 5-7 mm long and black, and *A. bipustulata* often bears two reddish spots on the elytra (Fuldner, 1960; Andreassen *et al.*, 2007). In Sweden, three generations have been recorded for *A. bipustulata* and two for *A. bilineata* (Jonasson, 1994). The parasitisation rate of *D. radicum* puparia in production fields varies from a few per cent up to >80% (Bromand, 1980; Hemachandra *et al.*, 2007a; Meyling *et al.*, 2013).

The adult females are attracted to host patches by volatile cues released from plants damaged by *D. radicum* larvae, whereupon they oviposit in the soil close to the plant (Fournet *et al.*, 2001; Ferry *et al.*, 2007). A major constituent of these volatile cues is dimethyl disulfide, which is attractive to *A. bipustulata* and *A. bilineata* (Ferry *et al.*, 2007). The first instar beetle larva searches for a host puparium, into which it gnaws a hole and enters. The larva feeds on the host and subsequently pupates inside the host puparium (Fuldner, 1960).

The species differ in host range and preference. *Aleochara bilineata* has a narrow host range, comprising primarily *D. radicum, D. floralis, D. antiqua* and *D. platura*. Besides the phytophagous *Delia* spp., *Psila rosae* and *Pegomya* spp., *A. bipustulata* may also parasitise certain dung and carrion feeding Diptera (Andreassen *et al.*, 2007; 2009). However, *A. bipustulata* generally prefers smaller puparia than *A. bilineata* (Ahlström-Olsson, 1994; Jonasson, 1994).

Aleochara bipustulata overwinters as a free-living adult in grassy habitats and in Sweden becomes active at the end of May, while *A. bilineata* hibernates as a first instar larvae inside the host puparium until the arrival of spring, when it starts to feed on the host and subsequently emerges in the end of June (Fuldner, 1960; Jonasson, 1994). Consequently, the occurrence of eggs and larvae of the first generation *D. radicum* coincides with the predatory activity of *A. bipustulata* rather than *A. bilineata* (Jonasson, 1994).

Figure 2. Trybliographa rapae (left) and *Aleochara bipustulata* (right). Photo: Linda-Marie Rännbäck

3.2.3 Entomopathogenic fungi

A pathogen group that offers potential for control of *D. radicum* is the entomopathogenic fungi (Eilenberg & Meadow, 2003). Entomopathogenic fungi infect their host insects by producing spores that adhere to, germinate and may form an appressorium which by the action of mechanical pressure and cuticle degrading enzymes penetrate the host cuticle. Inside, the fungi overcome host immune responses and proliferate in the hemolymph as single- or multi-celled structures often without cell walls, sometimes producing toxins. This growth and/or toxin production eventually kill the host. The success and speed of the infection process is influenced by several factors, *e.g.* temperature and relative humidity, as well as nutritional, chemical and physical conditions. After host death saprophytic outgrowth occurs through intersegmental areas of the host cuticle and either more infective spores are produced or sometimes resting structures for longer term persistence are formed (Vega *et al.*, 2012).

Eilenberg and Meadow (2003) suggested that inundation biological control with a highly virulent isolate of *Metarhizium anisopliae* (Metsch.) Sorokin *sensu lato* or *Beauveria bassiana* (Balsamo) Vuillemin *sensu lato* (Ascomycota: Hypocreales) (Fig. 3) would be an efficient strategy against the immature stages of *D. radicum*. Several isolates of these two genera have been screened through laboratory, greenhouse and field trials for their efficacy in controlling *D. radicum*, targeting larvae and pupae (Vänninen *et al.*, 1999a; 1999b; Bruck *et al.*, 2005; Chandler & Davidson, 2005; Razinger *et al.*, 2014;

Myrand *et al.*, 2015), and adults (Meadow *et al.*, 2000). Incorporating high fungal densities into the soil around cabbage plants reduced *D. radicum* emergence by >90% (Chandler & Davidson, 2005) and root damage by <70% (Vänninen *et al.*, 1999a), killed 85% of larvae (Bruck *et al.*, 2005) and yielded an 80% reduction in pupae numbers (Vänninen *et al.*, 1999b).

Metarhizium anisopliae s.l. and *B. bassiana s.l.* occur ubiquitously in the soil environment world-wide, in both natural and managed ecosystems (Meyling & Eilenberg, 2007). Both species complexes are considered generalists, as they have broad host ranges (Meyling & Eilenberg, 2007), and as such may pose a risk to arthropod natural enemies (Roy & Pell, 2000; Furlong & Pell, 2005). Since entomopathogenic fungi can affect the behaviour of insect natural enemies in different ways (Roy *et al.*, 2006; Baverstock *et al.*, 2010), it is likely that some insects have the ability to evaluate the IGP risk posed by fungal infected hosts and contaminated environments (Brobyn *et al.*, 1988; Fransen & van Lenteren, 1993; Meyling & Pell, 2006; Ormond *et al.*, 2011).

Recent expansions on the knowledge frontier of the ecology of these fungi (Vega *et al.*, 2009; Bruck, 2010; Meyling *et al.*, 2011; Behie *et al.*, 2015) offer the potential to select strains suitable for the habitat of the target pest. It appears that *M. anisopliae s.l.* mostly occurs below ground, while *B. bassiana s.l.* is more common above ground (Meyling *et al.*, 2011). Both species complexes have endophytic characteristics and are rhizosphere competent (Vega *et al.*, 2009; Wyrebek *et al.*, 2011; Sasan & Bidochka, 2012; Razinger *et al.*, 2014). However, *Metarhizium* spp. preferentially colonise the roots, while *B. bassiana* are found endophytically throughout the plant (Behie *et al.*, 2015). These ecological characteristics of *Metarhizium* spp. could be utilised in an IBC strategy in order for the fungus to pre-establish in the root system before herbivore attack, and persist for a prolonged time. This can be achieved either by incorporating the fungus into the compost module when raising transplants for subsequent planting in the field (Chandler & Davidson, 2005; Razinger *et al.*, 2014; Klingen *et al.*, 2015) or seed coating (Keyser *et al.*, 2014). Evidence is accumulating that some isolates may even proliferate in the rhizosphere and increase in abundance over time (*e.g.* Bruck & Donahue, 2007; Klingen *et al.*, 2015). An additional benefit of *Metarhizium* spp. is their potential plant growth promoting effect (Liao *et al.*, 2014).

Figure 3. Fungal sporulation on insect cadavers. *Metarhizium anisopliae s.l.* (left) with olive green laterally adhering conidial chains forming prismatic columns, a ‘crust’, on top of the mycelium and *Beauveria bassiana s.l.* (right) with conidia clustered into balls in its white mycelium (Humber, 2012). Photo: Linda-Marie Rännbäck

4 Conservation biological control of *Delia radicum*

(Paper I)

Delia radicum is a severe pest of cruciferous crops, but the adverse environmental effects of contemporary pest management practices require the development of more sustainable approaches that may be used in an IPM context. Using CBC as a means to favour and enhance naturally occurring natural enemy populations may constitute an important corner-stone in future pest management strategies in the Brassicaceae agroecosystem. Thus the work described in Paper I examined how natural enemies against *D. radicum* respond to CBC measures in a crop rotation system.

4.1 Materials and methods

Field site and experimental design

A field experiment was performed at Torslunda research station, Öland, south-east Sweden in 2008, 2009 and 2010. Sixteen experimental plots, spaced 40 m apart, were established and divided into four randomised blocks. Each plot measured 21 m x 10 m, with a 1.5 m wide strip in the middle. In control treatments, the central strip was sown entirely with cocksfoot grass (*Dactylis glomerata*, wild type), which was cut regularly during summer and ploughed down in autumn. Conservation strip (CS) treatments were sown annually with dill (*A. graveolens* cv. Tetra) and buckwheat (*F. esculentum*, wild type), and a 25 cm wide strip on each side of the flowers was sown with perennial grass (*D. glomerata*), which was then left undisturbed for the remainder of the experiment (Fig 4). During 2008 and 2010, all plots were planted with white cabbage (*Brassica oleracea* var. *capitata* f. *alba* cv. Castello L.), while in 2009 half the plots were planted with onions (*Allium cepa* cv. Hytech Eco L.).

Sampling and assessment

To assess the effect of CS and crop rotation on the abundance and performance of natural enemies, the following were monitored:

- *Abundance and diversity of hymenopteran parasitoids*, using two yellow-coloured pan traps fitted with a plexiglass window per plot, positioned 0 and 7 m from the CS, and emptied after 48 h during peak blooming. In 2008 one plot per treatment and block were assessed and in 2010 all plots were assessed. Specimens were determined to superfamily level.
- *Abundance and diversity of epigeal predators,* using four pitfall traps per plot positioned 4 and 7 m away from the CS. The traps were emptied once a week in June-August and the Carabidae and Staphylinidae were determined to species level.
- *Egg predation rates* in June-August in 2009 and 2010 in one control and one CS plot per block with cabbage, by placing two batches of 20 *D. radicum* eggs in the field 4 and 7 m away from the CS close to a cabbage stem for 24 h, and subsequently counting removed and bite-marked eggs.
- *Parasitism* at the end of each season, by collecting *Delia* spp. puparia from 20 crop root systems per plot and incubating them in the laboratory (20°C, L:D 16:8 h) for emergence and identification of parasitoids, and calculation of parasitism rate.

Figure 4. Conservation strip comprising a combined flower strip (*Fagopyrum esculentum* and *Anethum graveolens*) and beetle bank (*Dactylis glomerata*) at Torslunda, Sweden. In the background, a plot with a control strip (*D. glomerata*) can be seen. Photo: Linda-Marie Rännbäck

4.2 Results and discussion

Abundance and diversity of hymenopteran parasitoids

The overall abundance of hymenopteran parasitoids (*i.e.* Parasitica spp.) was higher in plots with CS than in controls for both 2008 and 2010. At superfamily level Cynipoidea spp., to which *T. rapae* belongs, were also more abundant in plots with CS in those years. This was also observed for Ichneumonidea spp. in 2008 (Fig. 5). In 2010, Parasitica spp. and Cynipoidea spp. were caught more often in the trap position next to the CS/control strip, but Ichneumonidea spp. were found more often in traps positioned 7 m away from the strips. Similarly, other studies have shown that the abundance of hymenopteran parasitoids increases in the vicinity of flower plants (Pfiffner *et al.*, 2003; Frank & Shrewsbury, 2004). Parasitoids are expected to feed in the field to increase their longevity and performance (*e.g.* Wäckers & van Rijn, 2012). In addition, the CS may have enhanced BC of other cabbage pests noted in the field, as the Ichneumonidea include important parasitoids, *e.g. Cotesia* spp. parasitising *Pieris* sp. and *Diadegma* spp. parasitising *P. xylostella*.

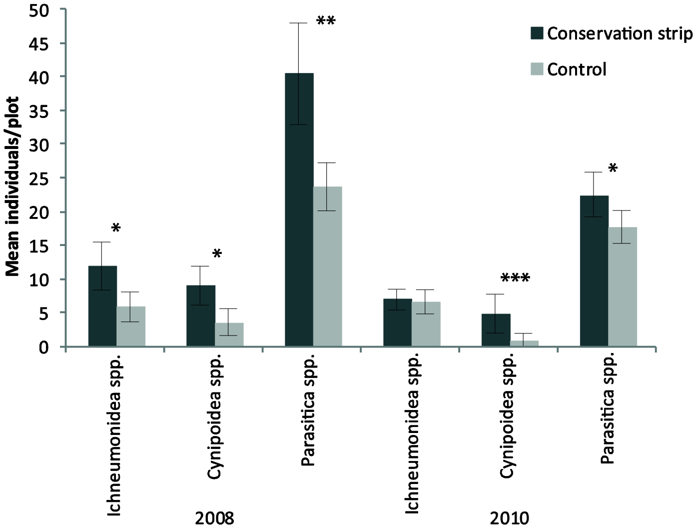

Figure 5. Mean number (± SEM) of different hymenopteran parasitoids caught in pan traps in 2008 and 2010 in plots with conservation strips and without (Control). Parasitica spp. includes all hymenopteran parasitoids caught. The figures shown are based on back-transformed values. (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

Abundance and diversity of epigeal predators

A total of 10 735 carabids from 55 species and 28 092 staphylinids from 163 species were recorded during the three years of sampling. The individual carabid species caught most frequently over the three years was *Broschus cephalotes* L., while *A. bipustulata* dominated among the staphylinids. No specimens of *A. bilineata* were found during the study period.

The species assemblage distribution of epigeal predators, which was analysed for the 21 most abundant species, was best explained by seasonal period, followed by year and, to a low extent, crop type, while the CS treatment had no effect. Great fluctuations within and between years in species assemblages of epigeal predators are commonly reported (*e.g.* Luff, 1987). In contrast to our findings, several studies have previously shown the importance of shelter habitats for epigeal predators (Thomas *et al.*, 1991; 1992; Collins *et al.*, 2002; MacLeod *et al.*, 2004; Frank & Shrewsbury, 2004). The effect of CS may have been masked by cross-treatment movement. Furthermore, the structurally complex landscape, with a high proportion of non-crop habitats, in which our field experiment was situated may also have masked the effectiveness of the CS (Tscharntke *et al.*, 2012). In particular, generalist species with great dispersal capacity, such as carabids, respond at large spatial scales and may be less affected by on-farm habitat manipulation in complex landscapes (Chaplin-Kramer *et al.*, 2011).

For the three-week period following the start of *D. radicum* egg laying, there were higher numbers of Staphylinidae spp. beetles in plots with conservation strips in 2009 (Figure 6). There was no treatment effect in either of the other years tested, or for Carabidae spp. In 2009, the three most commonly caught (>70% of catches) staphylinid beetles were *A. bipustulata*, *Aloconota gregaria* and *D. angustula*. However, an effect of CS was seen only for *A. bipustulata*. As *A. bipustulata* is a predator of both *Delia* spp. eggs and larvae, the species may have contributed to the reduced number of *D. radicum*-infested plants in plots with CS found in 2009 (Nilsson *et al.*, 2012).

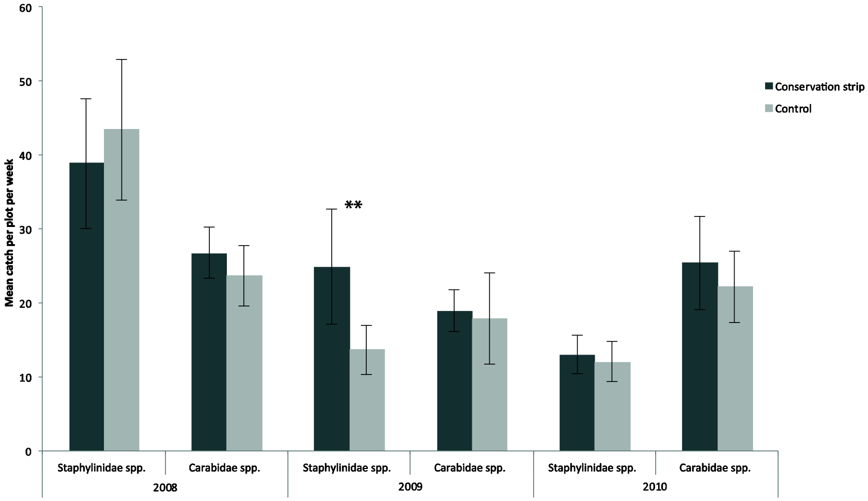

Figure 6. Numbers of carabid and staphylinid beetles (mean ± SEM) caught in pit fall traps in the experimental field during the three weeks after the start of *Delia radicum* egg laying 2008, 2009 and 2010. The figures shown are based on back-transformed values (*$P < 0.05$, **$P < 0.01$).

Egg predation

There was no difference in egg predation between plots with CS or the control in either 2009 or 2010. The mean predation rate on *D. radicum* eggs was 48% in 2009 and 32% in 2010. Egg predation differed between weeks for both years, with the highest predation rates noted in early July (week 27 in 2009 and week 28 in 2010). This large variation in egg predation over the season is in agreement with earlier studies (Björkman *et al.*, 2010; Meyling *et al.*, 2013). There was a positive correlation between the activity density of the small-sized carabid *Bembidion femoratum* Sturm in 2009 and *B. quadrimaculatum* L. in 2010 and egg predation rates. Likewise, *Bembidion* spp. have previously been reported to be *Delia* egg predators (*e.g.* Coaker & Williams, 1963; Finch & Elliott, 1999). The generally weak association between ground active beetles and egg predation was discussed by Finch & Collier (2007), who suggested that their importance is overestimated because of methodology issues and, being generalists, distraction of alternative prey.

Parasitism

Conservation strips did not increase parasitism by either of the two dominant parasitoid species, *T. rapae* or *A. bipustulata*, in any study year (Fig. 7). Laboratory and semi-field studies have revealed the importance of nectar for *T. rapae* fitness (Nilsson *et al.*, 2011) and *T. rapae* is known to visit flowers in the field (Jervis *et al.*, 1993), but the effect of flowers in Paper I may have been masked by cross-treatment movement (Lee & Heimpel, 2005). In fact, higher parasitism was found for both parasitoid species in control plots in 2009 (Fig. 7). This could be explained by the higher level of *D. radicum* infestation in control plots in that year (Nilsson *et al.*, 2012), potentially leading to greater emissions of HIPVs and thus higher patch detectability by both parasitoid species (*e.g.* Neveu *et al.*, 2002; Hemachandra *et al.*, 2007b; Ferry *et al.*, 2007). The crop rotation practice, with an onion crop in 2009, did not have any effect on parasitism by either parasitoid species in 2010.

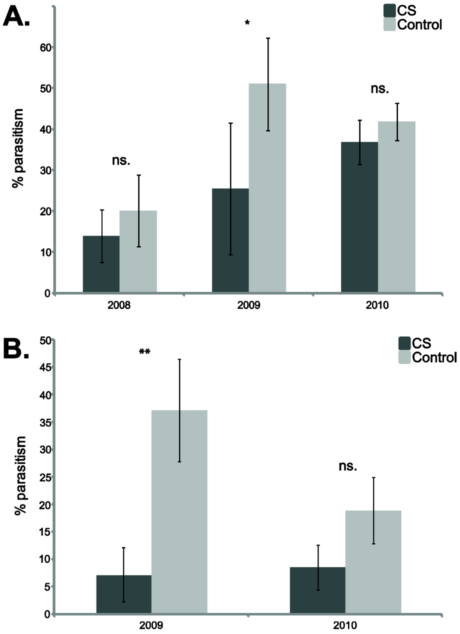

Figure 7. Mean parasitism rates (±SEM) in (A) host larvae of *Delia radicum* parasitised by *Trybliographa rapae* and (B) host pupae parasitised by *Aleochara bipustulata*, in plots with conservation strips (CS) and without (Control). Based on the proportion of *D. radicum* infested cabbage plants that were parasitised by at least one parasitoid. The figures shown are based on back-transformed values (*$P < 0.05$, **$P < 0.01$, *ns.* = not significant).

5 *Trybliographa rapae* behavioural response to intraguild predators

(Papers II & III)

Trybliographa rapae is one of the most important parasitoids of *D. radicum*. Conservation biological control has the potential to increase its population size and performance (Paper I; Nilsson *et al.*, 2011), but to achieve the greatest control efficacy against *D. radicum*, CBC may be complemented with IBC. However, since combining several natural enemies could lead to decreased control through IGP (Straub *et al.*, 2008), it is vital to study interactions between natural enemies before designing biological control strategies. Consequently, in Paper II and III laboratory studies were conducted to investigate the behavioural responses of *T. rapae* upon encountering potential IG predators, the entomopathogenic fungi *M. brunneum* isolate KVL 04-57 and *B. bassiana* isolate KVL 03-90, on its host and in its host habitat.

5.1 Materials and methods

Insects, fungi and plants (Papers II & III)

Trybliographa rapae and *D. radicum* were reared in the laboratory at 19 ± 1°C under L:D 16:8 h, as described by Nilsson *et al.* (2011). *Delia radicum* early third instar larvae for bioassays were reared in darkness, on thin turnip slices to facilitate removal. The *T. rapae* individuals used were 1-2 days old for dose-response bioassays (50:50 males:females) and 2-4 days old for other bioassays (only females).

The fungi were cultured for 20 days at 20 ± 1°C in 90 mm diameter petri dishes on 4% Sabouraud dextrose agar (SDA) and the conidia were then harvested by flooding the cultures with sterile 0.05% Triton-X 100 and scraping them off. The resulting suspension was centrifuged twice and the supernatant replaced by new 0.05% Triton-X 100. The concentration of the suspension was established by enumeration in a haemocytometer, then diluted to give the concentrations used in the bioassays. Conidial viability was assessed for suspensions used in all bioassays by counting the proportion of ungerminated conidia after 24 h growth on SDA dishes under the microscope. Germination was above 92% for all bioassays.

White cabbage plants were raised in potting soil mixed with a slow-release fertiliser in 1.5 L and 3.0 L pots under greenhouse conditions (22 ± 1°C, 75% RH, L:D 16:8 h). When the plants reached two months of age, one of two *D. radicum* infestation levels was established; lightly-infested plants (LIP) with 15 *D. radicum* eggs (hatching into 1-10 larvae) or heavily-infested plants (HIP) with 30 eggs (hatching into 15-30 larvae). Nine days later, HIP plants under fungal treatment were inoculated with fungi by pouring fungal suspension around the stem base. The plants in 3.0 L or 1.5 L pots received 40 mL or 20 mL of fungal suspension, respectively, and in addition 20 or 10 mL, tap water to flush the suspension down into the root system. The same volumes of tap water were used for uninfested plants (UP). Two different densities were established of *B. bassiana* (*B. bassiana*-High: 1×10^9 conidia mL^{-1} and *B. bassiana*-Low: 1×10^8 conidia mL^{-1}) and *M. brunneum* (*M. brunneum*-High: 1×10^8 conidia mL^{-1} and *M. brunneum*-Low: 5×10^7 conidia mL^{-1}) and the bioassays were conducted 48 h later.

Dose-mortality and time-mortality bioassays (Paper II)

To assess the virulence, *i.e.* the IGP risk, of the two fungal isolates on *D. radicum* early third instar larvae and *T. rapae* adults, dose-mortality and time-mortality bioassays were set up. *Delia radicum* larvae were inoculated in 5 mL fungal suspension at concentrations of 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia mL^{-1}, and a control was established with sterile 0.05% Triton-X 100, by submerging batches of 10 larvae for 20 s. *Trybliographa rapae* adults were inoculated individually by applying 1 µL suspension on the thorax of the same treatments as *D. radicum* except 1×10^4 conidia mL^{-1}. For both insect species, each individual was incubated singly in 30 mL cups and monitored every 24 h up to 14 days for death and subsequent fungal outgrowth.

Host patch quality choice and no-choice bioassays (Paper II)

Experiments were designed to test whether *T. rapae* can assess IGP risk in a host patch, as represented by free fungal conidia in a substrate. Host patches consisted of 10 third instar *D. radicum* larvae feeding (for 18 h) on a 35 × 35 × 6 mm piece of turnip, which was placed in 55 mm diameter petri dishes surrounded by vermiculite. The larvae were inoculated in 0.05% Triton-X 100 and incubated for 24 h, as described above, before introducing them onto the turnip piece. The vermiculite was inoculated with either 1.5 mL 0.05% Triton-X 100 (control) or 1.5 mL 1 × 10^8 conidia mL^{-1} fungal suspension. Two host patches were introduced to experimental boxes. In the choice situation, one control and one fungal host patch were introduced. In the no-choice situation, both host patches inside the box consisted of the same treatment. Female *T. rapae* were allowed to forage for 24 h (20 ± 1°C, L:D 16:8 h, 4200 lux inside the boxes), after which the larvae were removed and dissected for parasitoid eggs. The *T. rapae* were then transferred individually to 30 mL cups and monitored daily during 14 days for mortality and fungal outgrowth.

Host quality choice bioassays (Paper II)

Tests were also performed on whether *T. rapae* females can discriminate between healthy and fungal infected hosts, representing an IGP risk to the developing offspring. The same experimental set-up and procedures as in the host patch quality experiments above were used. The vermiculite was inoculated with 1.5 mL deionised water. The larvae were inoculated with either 0.05% Triton-X 100 (Control) or a suspension of 1 × 10^8 conidia mL^{-1} of *M. brunneum* or 1 × 10^9 conidia mL^{-1} of *B. bassiana*. These concentrations were seen to result in infection in all exposed *D. radicum* larvae (above the LC_{90}).

Host habitat choice bioassay in olfactometer (Paper III)

To evaluate longer range host habitat assessment under IGP risk, a dual-choice olfactometer was used to assess the host habitat selection of *T. rapae* females under different host densities and fungal densities on white cabbage plants (at 24°C, 60% RH, 1300 lux). The combinations tested are shown in Fig. 10. The host habitats used as odour source in the olfactometer were enclosed in cooking bags and attached to the set-up. An air flow pushed the odours through the system into a Y-shaped glass tube into which the parasitoids were introduced and their choice within 5 min assessed (Nilsson *et al.*, 2011).

Volatile collection and chemical analysis (Paper III)

In a headspace collection set-up, volatiles from the host habitats (including roots and soil) were trapped for 4 h on adsorbents. The following treatments were sampled (only 3 L pots): empty bags, soil, UP, HIP and HIP with *B. bassiana*-High, *M. brunneum*-High and *M. brunneum*-Low (n = 5 for HIP, N = 8 for the other treatments). The adsorbents were eluted with hexane and an internal standard mixture was added. The samples were analysed by gas chromatography – mass spectrometry (GC-MS) for compound identification, as described by Thöming *et al.* (2014), and the relative amounts of identified compounds were calculated. Compounds found in empty bags, soil and UP were excluded from further analysis.

5.2 Results and discussion

Dose-mortality and time-mortality bioassays (Paper II)

Both fungal species were pathogenic to *D. radicum* and *T. rapae*, thus representing an IGP risk. Increasing fungal concentration resulted in an increase in mortality. For *D. radicum* larvae exposed to fungi, the median lethal concentration (LC_{50}) values were 2.44×10^6 for *M. brunneum* and 1.08×10^7 conidia mL^{-1} for *B. bassiana*. Inoculation of adult *T. rapae* with *M. brunneum* or *B. bassiana* resulted in LC_{50} values of 1.57×10^7 and 1.83×10^7 conidia mL^{-1}, respectively. Increasing fungal concentration also resulted in decreased median survival time (MST). At 1×10^9 conidia mL^{-1} the MST was 4 days for *M. brunneum* for both *D. radicum* larvae and *T. rapae*, while *B. bassiana* resulted in MST of 5 days for *D. radicum* and 6 days for *T. rapae*. Taken together, these results indicate that *M. brunneum* is more virulent than *B. bassiana*, thus representing a greater IGP risk to foraging *T. rapae*.

Host patch quality choice and no-choice bioassays (Paper II)

In the choice between fungal inoculated and non-inoculated host patches, *T. rapae* females did not discriminate between either *M. brunneum* and control, or *B. bassiana* and control. This indicates that *T. rapae* may not be able to avoid free conidia in a substrate. Studies on other insects have shown an avoidance response to free conidia of virulent fungi in laboratory arenas simulating their natural habitats (Meyling & Pell, 2006; Mburu *et al.*, 2009; Ormond *et al.*, 2011). In the no-choice situation, more eggs were laid in hosts in *M. brunneum* inoculated patches than in control patches or in host patches inoculated with *B. bassiana* (Fig 8). Females that later succumbed to mycosis caused by either fungus laid more eggs than non-mycosed females. This response to a realised time limitation due to lethal infection shows that *T. rapae* perceives fungus as a risk, which may be more pronounced for *M. brunneum*. When life expectancy decreases, insects may accept lower quality hosts than normal, and may also oviposit more before death, rather than retaining eggs (Roitberg *et al.*, 1993; Fletcher *et al.*, 1994; Javois & Tammaru, 2004).

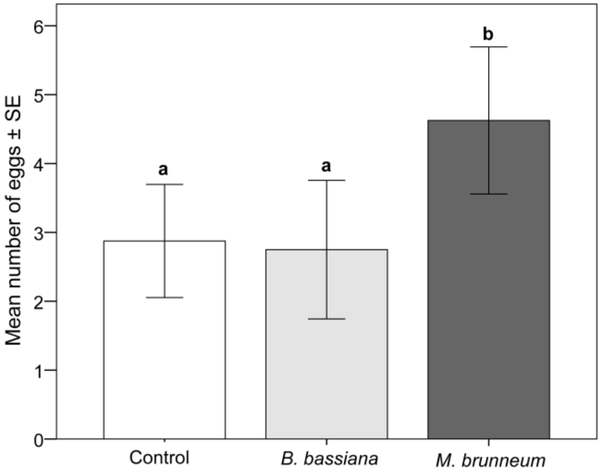

Figure 8. Mean numbers of eggs (± SE) laid per female of *Trybliographa rapae* in 24 h in no-choice bioassays on host patch quality (n = 16). Bars followed by different letters are significantly different (Poisson GLMM, $P < 0.05$).

Host quality choice bioassays (Paper II)

Females laid a higher proportion of eggs in healthy host larvae than in *M. brunneum* infected larvae, but no difference was found between healthy host larvae and those infected by *B. bassiana* (Fig. 9). Hosts infected by *M. brunneum* may thus have been perceived as being of inferior quality and a risk to developing offspring. Similarly, fungal infected hosts are rejected for oviposition by other parasitoids (Brobyn et al., 1988; Fransen & van Lenteren, 1993).

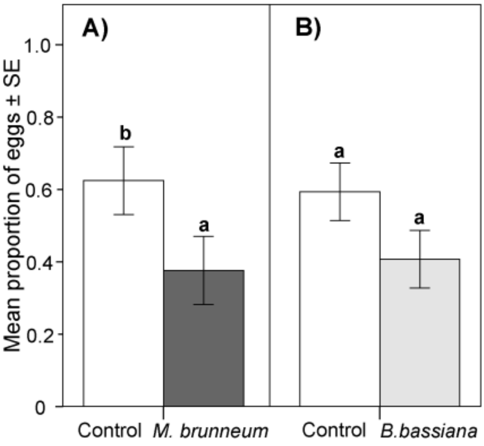

Figure 9. Mean proportions of eggs (± SE) laid per female of *Trybliographa rapae* in 24 h in the dual-choice bioassays on host quality. A) Control versus *Metarhizium brunneum* infected larvae (n = 24). B) Control versus. *Beauveria bassiana* infected larvae (n = 24). Bars followed by different letters are significantly different (binomial GLMM, $P < 0.05$).

Host habitat choice (Paper III)

The *T. rapae* choice of host habitat depended on the host density on the plant, with HIP being more attractive than LIP or UP (Fig. 10a). When given the choice between UP and HIP with any fungus, *T. rapae* preferred the host-infested plants, despite fungal presence (Fig. 10b). On choosing between HIP and HIP with fungus added, females showed an avoidance response only for *M. brunneum*-High. Neither *B. bassiana* at any density nor *M. brunneum*-Low affected the choice by *T. rapae* between HIP and HIP with fungus (Fig. 10c).

In host habitats with fungal presence, modelling revealed that differences in the density of *D. radicum* larvae between plant pairs significantly influenced parasitoid choice, irrespective of fungal species or fungal density (Fig. 10b, 10c). Furthermore, despite the larval density effect, the attraction to HIP with fungus was significantly different depending on fungal species and fungal density. In the presence of *M. brunneum*-High, the probability of attraction towards a fungal infested habitat was significantly lower than when *T. rapae* encountered *B. bassiana*-High. This effect was less significant in the presence of *M. brunneum*-Low and non-significant in the presence of *B. bassiana*-Low.

The fungi represent an IGP risk and the lower attraction to high densities of the most virulent fungus, *M. brunneum* (Paper II), indicates ability by *T. rapae* to evaluate host habitat quality and risk to itself and offspring. However, a trade-off occurs, since *T. rapae* encountering high-density host patches may ignore fungal presence in the habitat. The importance of host density in host habitat selection has previously been demonstrated (Hassell, 2000; Hemachandra *et al.*, 2007b; Girling *et al.*, 2011). Similarly, parasitoid avoidance of habitats with IG predators has been observed in other systems (Raymond *et al.*, 2000; Namashima *et al.*, 2004; Meisner *et al.*, 2011).

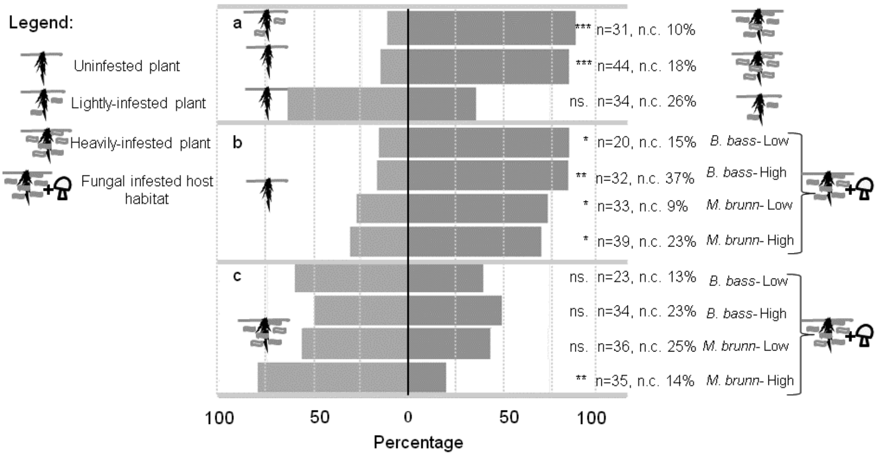

Figure 10. Behavioural response of *Trybliographa rapae* females offered a) different infestation densities of *Delia radicum* larvae, b) uninfested plants (UP) versus heavily-infested plants (HIP) with low or high density of *Beauveria bassiana* or *Metarhizium brunneum*, and c) HIP versus HIP with low or high density of *B. bassiana* or *M. brunneum*. Test on two-tailed binomial distribution, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, *ns.* = not significant, *n* = numbers of female responders, *n.c.* = percentages of female responders not making a choice (excluded from the statistical analysis).

Host habitat volatile collection (Paper III)

Compound identification and subsequent analysis of the host habitat volatile profiles revealed differences between treatments. Fungal species and fungal density affected the odour profile exhibited by HIP. The volatiles from the plant samples clustered in distinguishable groups (Fig. 11). The HIP and *M. brunneum*-Low groups were differentiated from *M. brunneum*-High and *B. bassiana*-High. For example, COMP-14 (1,3-di-tert-butylbenzene) was highly related to plants with *M. brunneum*-High, while compounds such as COMP-2 (dimethyl disulfide) were more representative of HIP (Fig. 11). Furthermore, there were differences in the relative amounts of individual compounds emitted from the different treatments, with 1,3-di-tert-butylbenzene differentiating high fungal densities of both fungal species from HIP and plants with *M. brunneum*-Low. This compound has previously been detected in emissions from fungal cultures, *e.g. B. bassiana* (Crespo *et al.*, 2008). Its presence in the host habitats tested in Paper III may indicate actively growing mycelium, but no firm conclusion could be drawn on whether it originates from an interaction with cabbage roots, growth on larval cuticles or a combination. Dimethyl disulfide was found in all treatments except *M. brunneum*-High. This compound is emitted by plants heavily infested with *D. radicum* larvae and has been found to be attractive to the main predators of *D. radicum* in the field (Ferry *et al.*, 2007). The absence of dimethyl disulfide in *M. brunneum*-High, presumably due to a reduction in feeding by *D. radicum* larvae, may have contributed to the reduced attraction of *T. rapae* to this treatment. These different treatment compound profiles was linked to the behavioural response observed for *T. rapae* in the host habitat choice bioassays, where host habitats representing high IGP risk were avoided. This indicates that *T. rapae* females may use volatile cues informing on host density and host habitat quality, *e.g.* presence of an IG predator, when making foraging decisions to increase reproductive success.

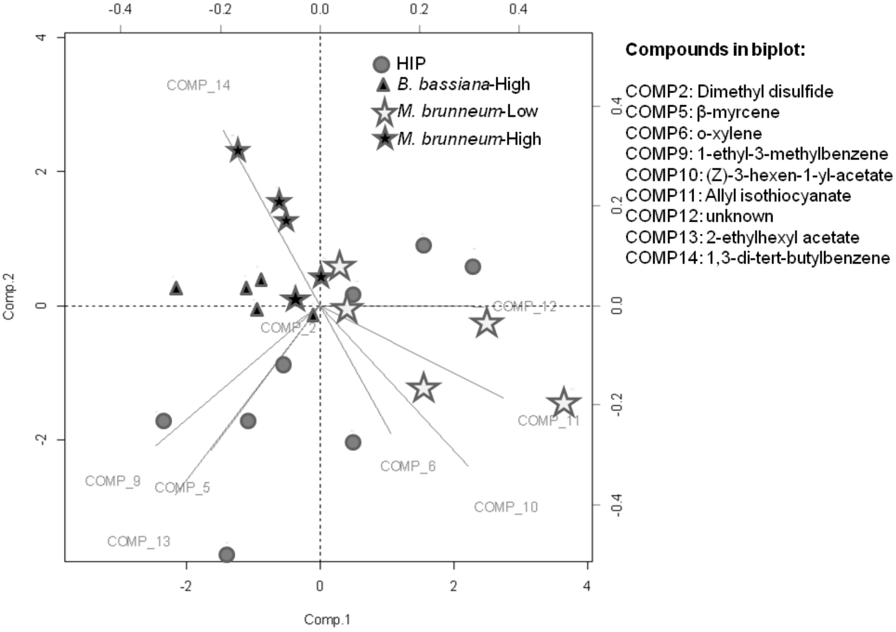

Figure 11. Principal Component Analysis biplot for nine volatile compounds identified by GC-MS (lines) and the 23 plant samples. Treatments represented are: heavily-infested plants (HIP) (circles); plants heavily-infested with high density of *Beauveria bassiana* (*B. bassiana*-High) (triangles); plants heavily-infested with low density of *Metarhizium brunneum* (*M. brunneum*-Low) (grey stars); and plants heavily-infested with high density of *M. brunneum* (*M. brunneum*-High) (black stars). The first three principal components (PCs) accounted for 62.40 % of the total variation of the dataset. The biplot represents the principal components PC1 and PC2. The distance between the points shows the similarity in the observations (plants) based on the volatile compounds (lines). The projection of a point on a line represents the contribution of a certain compound to the volatile profile of that specific plant.

6 Complementary biological control of *Delia radicum*

(Paper IV)

To achieve the greatest biological control efficacy against *D. radicum,* CBC may be further strengthened by IBC with functional complementary natural enemies. As *T. rapae* exerted selective oviposition behaviour on healthy *D. radicum* larvae when presented together with *M. brunneum* infected larvae in the laboratory (Paper II), Paper IV investigated whether this would also be the case in a semi-field situation. In field-cages the pest suppressive effects of combining *T. rapae* and inoculation with *M. brunneum* were investigated, to determine whether positive effects (niche complementarity) through prey partitioning could be achieved despite the potential for IGP.

6.1 Materials and methods

Insects, fungi and plants

Trybliographa rapae and *D. radicum* were reared in the laboratory as described in Section 5.1. *Metarhizium brunneum* was cultured, harvested and the concentration of the suspension established as described in Section 5.1. Conidial viability was above 98% for all replications.

White cabbage plants were raised in 3 L pots in potting soil amended with leca granules and a slow-release fertiliser under greenhouse conditions (20 ± 1°C, 65% RH and in 16:8 L:D h) for seven weeks. The plants were then infested by placing 25 *D. radicum* eggs at the stem base and covering with moist soil and fibre cloth. Nine days later, plants were inoculated by pouring around the stem base either a 40 mL conidial suspension of 5×10^7 (Low density) or 1×10^8 (High density) conidia mL^{-1} of *M. brunneum* in 0.05% Triton-X 100, with 40 mL 0.05% Triton-X 100 or 40 mL of tap water as controls. To flush the conidia further down into the root system, an additional 20 mL tap water was used for all treatments. After 24 h, the plants were transferred to the field-cage experiment.

Field site and experimental design

The experiments were conducted in June-August 2011 and 2012 at the Swedish University of Agricultural Sciences in Alnarp, Sweden (55°39’42’’N, 13°05’13’’E). Field size was 40 × 70 m and within the field two rows (*i.e.* two blocks) of four cages were established 10 m apart and at 10 m from the field edge. The cages were constructed with dimensions 1.2 × 1.5 m and 1.2 m high, and covered by 4.2 × 4 m insect nets (0.8 x 0.8 mm mesh size). In each cage, four empty 3 L pots were buried flush with the soil surface 40 cm apart and 25 cm from the net, with a fifth soil-filled 3 L pot in the cage centre. Four cabbage plants with *D. radicum* larvae were placed in the empty pots. The treatments in the experiments were laid out in an additive 2 × 2 factorial design, where the Low and High fungal densities comprised independent data sets, performed in alternating weeks:

- *D. radicum* larvae (Control)
- *D. radicum* larvae + *M. brunneum*
- *D. radicum* larvae + *T. rapae*
- *D. radicum* larvae + *M. brunneum* + *T. rapae*

One or both blocks were utilised each week. In weeks in 2011, when there were empty cages, additional treatments with plants inoculated with 0.05% Triton-X 100 were added to exclude the possibility that the detergent had any effect on the number of developed *D. radicum* or *T. rapae,* and these treatments were compared with their respective water control.

In treatments with parasitoids, four mated 2-4 days old *T. rapae* females were introduced, together with cups with sucrose solution and water.

Assessment

The experiments were terminated after 72 hours, and *D. radicum* larvae picked from the root system and incubated individually in darkness at 20°C in 30 mL cups with moist sand and a turnip piece. The larvae and resulting pupae were monitored for mortality and mycosis every 48 h for 12 days. After pupation, the pupae were checked once a week to note hatching of *D. radicum* or *T. rapae* (F1 generation), or mycosis from *M. brunneum*. The *T. rapae* recovered from the experimental cages were kept in 30 mL cups with sucrose and water, and monitored daily for mortality and mycosis. The microclimate in the cages was monitored during the experiment for air temperature and air relative humidity using dataloggers. The soil temperature was monitored by placing the datalogger 2 cm below the soil surface in the soil-filled pot.

6.2 Results and discussion

Impact of the natural enemies on D. radicum

The detergent 0.05% Triton-X 100 had no effect on either the number of developed *D. radicum* or the number of developed *T. rapae*. At the low fungal density, the combination of *M. brunneum* and *T. rapae* resulted in fewer developed *D. radicum* compared with either natural enemy alone (Fig. 12A). At the high fungal density, *M. brunneum* and *T. rapae* were equally efficient in reducing the number of developed *D. radicum*. However, the combination of high fungal densities of *M. brunneum* and *T. rapae* resulted in fewer developed *D. radicum* (Fig 12B). The reduction in mean proportion of the *D. radicum* population on combining *T. rapae* and *M. brunneum* compared with the control was 78% at the high fungal density and 63% at the low fungal density. No interactions were observed between *T. rapae* and *M. brunneum* at either low or high fungal density, indicating additive effects. Additive effects demonstrate resource partitioning between natural enemies (Cardinale *et al.*, 2003; Casula *et al.*, 2006). In our system, the decisions made by the foraging *T. rapae* female to avoid IGP risk by selecting healthy larvae (Paper II) led to resource partitioning and the observed niche complementary effects. Under open field conditions, the complementary effects could be enhanced if the parasitoid were to avoid host habitats with high fungal densities (Paper III).

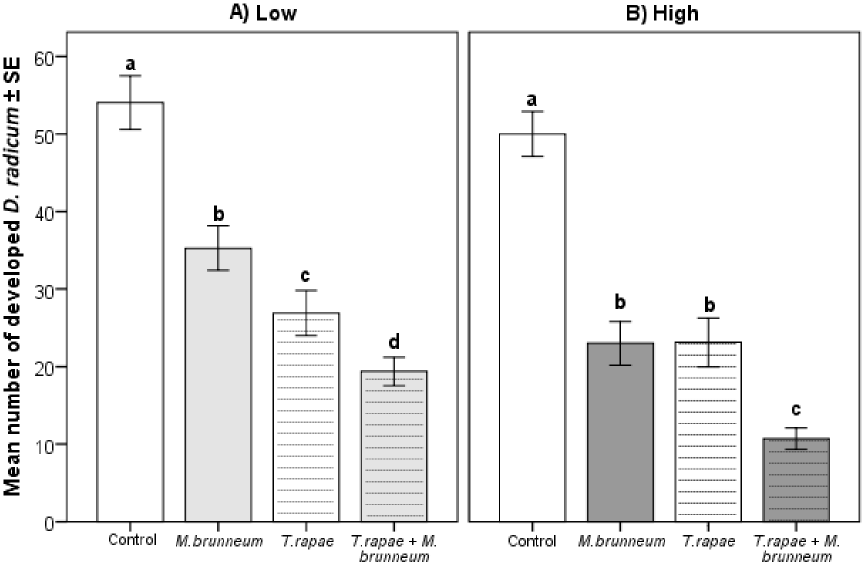

Figure 12. Mean number (±SE) of developed *Delia radicum* with or without *Metarhizium brunneum* and/or *Trybliographa rapae* at A) low fungal density (5 × 10^7 conidia mL^{-1}; n = 18) and B) high fungal density (1 × 10^8 conidia mL^{-1}; n = 17). Bars with different letters within the subfigure are significantly different (Poisson GLMM, $P < 0.05$)

Effect of environmental conditions

Soil temperature and air relative humidity were included as continuous variables in additional modelling, together with presence/absence of *M. brunneum* (at both fungal densities) and *T. rapae* to reveal what combination of factors and interactions best explained the number of developed *D. radicum*. At low fungal density, the presence of *M. brunneum* led to a reduction in number of developed *D. radicum*. Furthermore, at low fungal density the number of developed *D. radicum* decreased (*i.e.* parasitism increased) with increasing soil temperature in the cages where *T. rapae* was present, and increased (*i.e.* parasitism decreased) as air humidity increased. In previous studies, parasitoid foraging has been observed to increase with sunshine and decrease during rainfall (Hilker & McNeil, 2008). At the high fungal density, presence of *M. brunneum* and of *T. rapae* caused a reduction in the number of developed *D. radicum*. Neither environmental factor was influential at high fungal densities, probably since a dominant effect of *M. brunneum* mycosis masked any influence of environmental variables on *T. rapae*. These results suggest that combining natural enemy species that respond differently to environmental conditions may provide functional insurance under environmental fluctuations (Loreau & de Mazancourt, 2013).

Impact of fungus on parasitoid

In the fungal treatments, for both densities, 98% of the parasitoids recovered after experiment termination succumbed to mycosis by *M. brunneum* within 5 days. The mean number of developed F1 *T. rapae* was reduced in cages where *M. brunneum* was present, for both low (Fig. 13A) and high fungal density (Fig. 13B). The reduction in mean proportion of the *T. rapae* population was 29% at the low fungal density and 52% at the high fungal density. Thus it may be favourable to combine CBC of *T. rapae* with inoculation of a relatively low density of *M. brunneum*, leading to more stable long-term *D. radicum* control, since the IGP risk to F1 adults of *T. rapae* is relatively low.

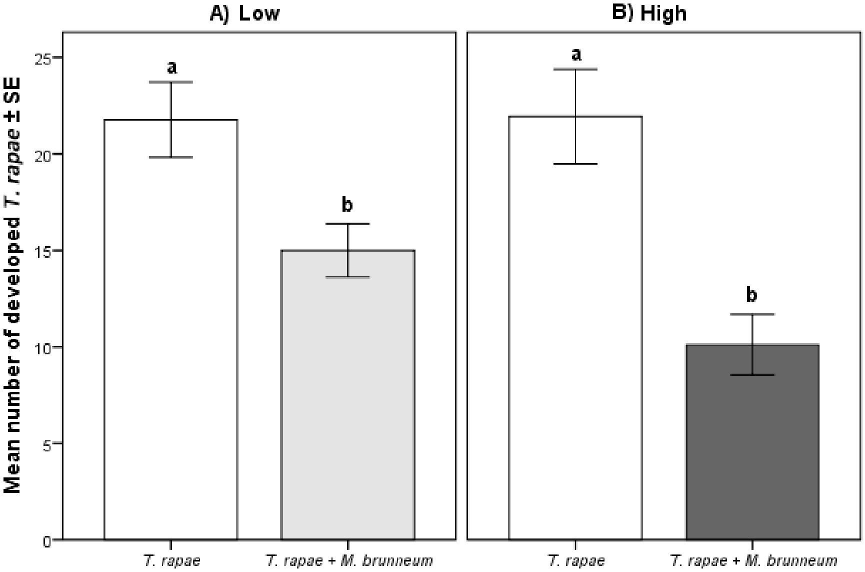

Figure 13. Mean number (±SE) of developed *Trybliographa rapae* with or without *Metarhizium brunneum* at A) low fungal density (5 × 10^7 conidia mL^{-1}; n = 18) and B) high fungal density (1 × 10^8 conidia mL^{-1}; n = 17). Bars with different letters within the subfigure are significantly different (Poisson GLMM, $P < 0.05$).

7 Conclusions and future perspectives

The results presented in this thesis demonstrated the potential for enhancing the population size and performance of natural enemies against *Delia radicum* in the field through conservation biological control (CBC) with floral resources and perennial shelter habitats. Introduction of conservation strips (CS) increased catches of hymenopteran parasitoids, but this did not translate into increased parasitism by *T. rapae*. Moreover, CS did not increase parasitism by *A. bipustulata,* although during the egg laying peak of *D. radicum*, higher numbers were observed in CS in one study year. In addition, CS did not increase egg predation and did not explain the species assemblage of coleopteran predators. Cross-treatment effects caused by too short distances between plots cannot be ruled out. Moreover, CBC requires a landscape perspective, as the highly complex landscape surrounding the study field may have masked the effectiveness of CS, since natural enemies in the surroundings could have continuously migrated into the field. It also necessitates evaluation on longer time scales and in different landscape contexts. Nevertheless the findings in this thesis make an important contribution to management of *D. radicum* and to the CBC knowledge base in the Brassicaceae agroecosystem.

CBC measures could be complemented with inoculation biological control (IBC) with entomopathogenic fungi in order to enhance the biological control effect against *D. radicum*. The *M. brunneum* isolate KVL 04-57 tested here is a promising biological control candidate against *D. radicum* larvae, as laboratory studies revealed higher virulence compared with the *B. bassiana* isolate KVL 03-90. However, both fungal isolates also showed pathogenicity against adult *T. rapae,* indicating that there is an intraguild predation (IGP) risk associated with foraging in fungal contaminated host habitats. Upon encountering fungal infested habitats and hosts, however, *T. rapae* showed an ability to perceive and react to the IGP risk posed by *M. brunneum*, while *B. bassiana* was not avoided to the same extent.

At close range, when infective propagules were present in the host environment in a no-choice situation, a higher oviposition rate was observed in *M. brunneum* inoculated host patches compared with either the control or *B. bassiana*. For both fungi, higher oviposition rates were seen in individuals that were later mycosed. These results indicate that *T. rapae* perceived decreased life expectancy and allocated resources to oviposition rather than retention of eggs before succumbing to mycosis. In addition, when presented with a choice between healthy and fungal infected hosts, *T. rapae* females laid more eggs in healthy larvae than in *M. brunneum* infected larvae. This effect was less pronounced for *B. bassiana*.

During host habitat location, *T. rapae* avoided cabbage plant host habitats inoculated with high densities of *M. brunneum*, but not lower densities or any density of *B. bassiana*. A dispersal strategy to visit patches unexploited by fungi may favour parasitoids by allowing them to forage in an enemy-free space and thus most likely achieve greater reproductive success, and potentially increased biological control. However, it was found that parasitoids encountering high-density host patches may ignore fungal presence in the habitat. This makes it difficult to predict the trade-off between IGP risk and reproductive success in open field situations where *T. rapae* will encounter other larval and fungal densities than those tested in this thesis. The behaviour of *T. rapae* could be explained by the differing volatile compound profiles, depending on fungal presence, species and density. In particular, the compounds dimethyl disulphide, representing a host density cue, and 1,3-di-tert-butylbenzene, representing a cue of fungal IGP risk, may play key roles.

Overall, *M. brunneum* seems to be perceived as a greater IGP risk than *B. bassiana*, and *T. rapae* females react to this IGP threat by different means to reduce the risk of IGP to themselves and their offspring.

In the field-cage study, there were additive effects of combining inoculation with low and high fungal densities of *M. brunneum* with releases of *T. rapae* in reducing the *D. radicum* population. This may be driven by the behavioural response of the parasitoid to avoid IGP at foraging, by avoiding fungal infected host larvae and selecting healthy ones, which leads to partitioning of the host resource between fungus and parasitoid, and thus niche complementarity. At low fungal densities, the *T. rapae* parasitisation rate increased with increased temperature, while it decreased with increased air relative humidity. Such an environmental response was not seen for *M. brunneum*. Thus, by combining biological control agents with different responses to fluctuating environments, greater control resilience can be achieved.

However, even though high fungal densities seemingly led to a greater reduction in *D. radicum* numbers compared with low fungal densities, they also led to a greater reduction in *T. rapae* F1 numbers. Furthermore, high fungal densities may reduce *D. radicum* infestation levels below *T. rapae* detection. Thus using high fungal densities may compromise the long-term survival and stability of the *T. rapae* population at farm level especially since the poor dispersal ability of the parasitoid may limit between-field migration over larger distances.

Based on the overall findings reported in this thesis, it can be suggested that combining CBC of *T. rapae* with application, *e.g.* into the substrate of transplants, of the minimum dose of *M. brunneum* required for adequate BC of the first generation of *D. radicum* may lead to more stable long-term control, since spatial refuges are created for *T. rapae* (healthy larvae), while simultaneously the infection risk to F1 adults of *T. rapae* is reduced. Co-existence of *D. radicum*, *T. rapae* and *M. brunneum* can thus be achieved, leading to an agroecosystem resilient to pest outbreaks that is also economically stable and profitable for farmers. However, this complementary biological control strategy needs to be tested at larger spatiotemporal scales during several generations of *D. radicum* and natural enemies, in a crop rotation system in commercial cabbage fields in different landscape contexts.

Future research on developing pest management strategies against *D. radicum* could expand on the findings reported in this thesis and investigate further the implications in the system of: *Metarhizium* rhizosphere competence, the chemical ecology of plant-fungus-insect interactions, additional ecological interactions and spatiotemporal complementarity between natural enemies in this system when including more species.

The persistence of *M. brunneum* in the rhizosphere over the field season should be correlated with population development of *D. radicum* and *T. rapae*. In applying *M. brunneum* in a preventive approach against the first *D. radicum* generation, the fungal inoculum may have decreased to less harmful levels when a larger second generation of *T. rapae* occurs later in the season to control the second generation of *D. radicum*. In contrast, some fungal isolates may proliferate and increase in abundance in the rhizosphere over time, and a ‘low’ density applied may increase to ‘high’ levels perceived as risky by *T. rapae*. One approach for increasing the spatial complementarity between *M. brunneum* and *T. rapae*, and thus providing an enemy-free space, would be to plant some rows in the field free of fungi. In addition, the effect of *M. brunneum* on potentially important key egg and larval coleopteran predators, such as *Aleochara* spp. and *Bembidion* spp., needs to be studied further.

Additional studies should also address the chemical ecology and role of the fungus-plant association on the behaviour of herbivores and natural enemies.

For example, it remains to be elucidated whether the endophytic root growth of *Metarhizium* spp. induces the plant to alter its volatile profile, or whether it is the fungal mycelia growing on roots or insect cuticles that influences insect behaviour. It is also unknown how *D. radicum* reacts to fungal presence in the root system when selecting oviposition sites.

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