Ecology of pollinators, pests and natural enemies in agricultural landscapes

- ensuring a sustainable supply of clover seeds

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Cover: Moss carder bee, *Bombus muscorum* L., on white clover, *Trifolium repens* L. Illustration by Johanna Yourstone.

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Abstract

The human population and its food consumption continues to grow and the environmental impact of meeting future food demands depends on how we expand the food supply. Already, agricultural intensification has led to biodiversity declines, which in turn can have direct and indirect effects on agricultural production. For future sustainable production there is a need to support ecosystems and their ability to provide ecosystem services. To do so, we need more knowledge on how functional insect groups such as pollinators, pests and natural enemies are affected by land use, and how they in turn affect agricultural systems and crop yields. Due to their ability to fix atmospheric nitrogen, clovers are grown worldwide for animal feed and for green manure. Clover is an important crop in organic farming where inorganic fertilizers are prohibited. However, producers of clover seeds are experiencing problems with varying yields. In this thesis I, therefore, investigated important factors affecting seed yield. I studied interactive effects of plant and pollinator traits on seed set in red clover in a common garden, and investigated spatiotemporal patterns of pollinators, pests and natural enemies in agricultural white clover seed fields. I found that pest abundance in white clover fields decreased with distance from the previous year's field. Furthermore, pest abundance increased, whereas parasitism rates provided by natural enemies decreased, with proportion arable land in the surrounding landscape. These results point to the potential for using spatial planning as a pest management tool in clover seed production. Abundance of pollinating non-*Apis* bees was higher in conventionally managed fields compared to organic fields, and abundance of honey bees and total number of bees in organic fields were negatively related to proportion semi-natural land. If these observations are due to more preferable habitats elsewhere needs further investigation. I further showed that white clover fields do not always have sufficient amounts of pollinators as previously believed. In white clover, short tongued bees are good pollinators, but in red clover, and especially tetraploid cultivars, flower visits by medium- or long-tongued bees resulted in a higher seed set. Unexpectedly, medium-tongued bees on a diploid red clover cultivar gave much higher seed set than other combinations of bee tongue length and clover ploidy. This shows the importance of a diverse pollinator fauna, as different pollinators are unequally able to pollinate different species of plants.

Keywords: Trifolium repens, Trifolium pratense, seed production, *Protapion fulvipes,* parasitoid, pollinator, land use, agricultural pest, dispersal, spatiotemporal distribution.

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Pollinatörer, skadedjur och naturliga fiender i jordbrukslandskapet

Abstract

Intensifiering av jordbruket har lett till minskning av biologisk mångfald, vilket i sin tur kan leda till konsekvenser i jordbruksproduktionen. För en framtida hållbar jordbruksproduktion finns det ett behov av att stödja ekosystemen och deras förmåga att tillhandahålla ekosystemtjänster. För att göra det behöver vi mer kunskap om hur funktionellt viktiga insektsgrupper påverkas av markanvändning och hur de i sin tur påverkar produktionen i jordbruket. Tack vare sin förmåga att binda atmosfäriskt kväve, odlas klöver över hela världen för produktion av djurfoder och som gröngödsling. Skörden för klöverfröproducenter varierar dock kraftigt från plats till plats och mellan år. I denna avhandling har jag studerat samspelet mellan egenskaper hos rödklöverplantor av olika sort och bin med olika tunglängd. Vidare har jag genom fältstudier undersökt ekologin hos bin, skadedjur och naturliga fiender i relation till markanvändning och omgivande landskap, samt effekter på fröproduktion i vitklöverfröodlingar. Jag fann att med kortare avstånd från föregående års närmsta vitklöverfält ökade antalet fröätande skadedjur i det nya klöverfältet. Antalet skadedjur ökade dessutom, medan effekten av naturliga fiender minskade, med andelen odlad jordbruksmark runt fälten. Dessa resultat pekar på möjligheten att använda rumslig planering som ett verktyg för skadedjurshantering i klöverfröproduktion. Antalet bin (borträknat honungsbin) var högre i konventionella fält än i ekologiska, och det totala antalet bin i ekologiska fält minskade med andelen obrukad gräsmark. Dock ökade artantalet av både bin och naturliga fiender med ökad andel obrukad gräsmark. I vitklöver är bin med kort tunga funktionella pollinatörer, men i rödklöver och i synnerhet tetraploid rödklöver observerades medel- och långtungade bin ge en högre frösättning. Kombinationen diploid rödklöver och bin med medel-lång tunga gav dock högst mängd frön. Mina resultat visade också att det inte alltid är tillräckligt med bin i vitklöverfröfält och att fler bin ökar frösättningen. Dock resulterade inte en högre andel bin i en högre slutgiltig vitklöverfröskörd eftersom skadedjurens fröätande hade större betydelse. Sammantaget visar jag i mina studier att genom att placera sitt vitklöverfält långt bort från föregående års fält kan man minska skadedjursangreppen, och att det är viktigt att vända trenden med minskning av bin med medellång och lång tunga för rödklöverfröskörden. Jag visar också att antalet skadedjur ökar, medan artrikedomen av nyttodjur minskar, med hög andel odlad mark i landskapet runt vitklöverfälten. För att motverka förlust av biologisk mångfald och minska skadedjursangrepp i jordbruksgrödor bör vi skapa mer variation i landskapet.

Keywords: Rödklöver, vitklöver, skadedjur, klöverfröproduktion, pollinering, markanvändning, naturliga fiender

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Dedication

To my family, my friends and to my teachers who have inspired me through the years.

In nature, nothing exists alone.
Rachel Carson

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 Hederström, V., Rundlöf, M., Birgersson, G., Larsson, M. C., Balkenius, A., Lankinen, Å. (2019). Do plant ploidy and pollinator tongue length interact to cause low seed yield in red clover? (manuscript)

II Lundin, O., Svensson, G. P., Larsson, M. C., Birgersson, G., Hederström, V., Lankinen, Å., Anderbrant, O., Rundlöf, M. (2017). The role of pollinators, pests and different yield components for organic and conventional white clover seed yields. Field Crops Research, 210, 1-8.

III Hederström, V., Nyabuga, F. N., Anderbrant, O., Svensson, G. P., Rundlöf, M., Lankinen, Å., Larsson, M. C. (2019). Dispersal distance and spatiotemporal distribution of the agricultural pest *Protapion fulvipes* in white clover fields. (manuscript)

IV Hederström, V., Johansson, S., Rundlöf, M., Svensson, G. P., Anderbrant, O., Lundin, O., Larsson, M. C., Lankinen, Å. (2019). Bee abundance, species richness and seed set in white clover fields in relation to local management and landscape context. (manuscript)

V Hederström, V., Berger, J., Svensson, G. P., Lundin, O., Rundlöf, M., Anderbrant, O., Lankinen, Å., Larsson, M. C. (2019). Interactions between white clover pests and their natural enemies: consequences for seed yield in different management and landscape contexts. (manuscript)

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The contribution of Veronica Hederström to the papers included in this thesis was as follows:

I Participated in the development of research question and design. Performed common garden experiments. Analysed the data and wrote the manuscript together with the co-authors.

II Planned and performed field work and participated in the writing of the paper which was led by OL.

III Developed research questions and design together with co-authors. Carried out and supervised field and lab work, and analysed the data. Wrote the manuscript with input from co-authors.

IV Developed research questions and design together with co-authors. Performed and supervised field and lab work. Compiled and analysed the data. Wrote the manuscript with input from the co-authors.

V Developed research questions and design in collaboration with co-authors. Performed and supervised field and lab work. Compiled and analysed the data. Wrote the manuscript with input from the co-authors

1 Introduction

Agricultural intensification has been a success in terms of increasing global food production, however, its severe impacts on the environment and biodiversity are evident and expected to have negative effects on sustained crop productivity (Matson et al., 1997). Meanwhile, the human population and its food consumption continues to grow (Godfray et al., 2010; United Nations, 2017) and the global food demand is expected to double from 2012 to 2050 (FAO, 2017). The environmental impact of meeting this demand depends on how we expand the food supply (Tilman et al., 2011). Further increasing the production of food could cause major changes to natural land and ecosystems (FAO, 2017). Already, the intensified land use of the 20th century, with increase of monocultures, fragmentation and loss of natural habitats as well as increased use of agrochemicals, has led to a reduction in biodiversity (Williams & Kremen, 2007; Kleijn & Raemakers, 2008; Rundlöf et al., 2008, Sánchez-Bayo & Wyckhuys, 2019). Loss of biodiversity is threatening the resilience of ecosystems (Steffen et al., 2015) and the ecosystem services that they provide (Chapin, 2000; Cardinale et al., 2012). Declines in abundance and presence of species, genes and functional traits can have direct and indirect effects on agricultural production, for example through the contribution of pollinators to yield in insect pollinated crops and the control of pests by their natural enemies. For future sustainable agricultural production, there is a need to support ecosystem resilience and its ability to provide ecosystem services. One piece of the puzzle in order to do so, is to understand the spatial and temporal patterns of functionally important biodiversity and its interactions with agricultural crops. Knowledge gaps still exist on how composition and dynamics of functional groups such as pollinators, pests and natural enemies interact with agricultural systems and in the end affect crop yields.

2 Background

2.1 Land use change and loss of biodiversity

Land use changes made during the last century have caused severe declines in biodiversity, and we are at the moment facing a mass extinction of species (Pimm et al., 2014; Ceballos et al., 2017). The intensification of agriculture in recent decades, with increased use of agrochemicals and monocultures, has led to fragmentation and loss of natural habitats (Williams & Kremen, 2007; Rundlöf et al., 2008) and is recognized as one of the key drivers for biodiversity loss (MAE, 2005, Sánchez-Bayo & Wyckhuys, 2019).

Loss of biodiversity is negative for several reasons, for example ethical: we do not have the right to extirpate species, cultural: biodiversity is part of our heritage, and it has pronounced benefits for our wellbeing, utilitarian: it is useful for us, and of importance for our survival. Loss of biodiversity can have cascading effects on ecosystem functioning and this may have direct consequences on agricultural production and on crop yield (FAO, 2019). We are dependent on ecosystems to stabilize the climate, generate and purify air and water, stabilize soils, prevent flooding, erosion and drought, and to provide us with goods for food, medicine, shelter and much more. Organisms as part of ecosystems create and maintain soils, decompose waste and recycle nutrients. Furthermore, a vast number of animals pollinate and fertilize plants, protect them from pests and spread their seeds. Degradation of ecosystems, following from loss of biodiversity, is therefore threatening the wellbeing of our own, and many other species. Political agreements on safeguarding biodiversity have been established (e.g. the Convention of Biological Diversity), however their actual implementation and success will be dependent on available knowledge and practical management solutions.

The most effective way to stop further biodiversity declines in areas with intensive agriculture, is probably to restore habitats while simultaneously enhancing the ecosystems functioning and reducing the input of agrochemicals. All while maintaining a sufficient production of food and other agricultural products.

2.2 Functional biodiversity in crop production: Pollinators, pests and natural enemies of pests

2.2.1 Pollinators

In both natural and human-managed ecosystems, animal-mediated pollination is a key ecosystem service (Kearns et al., 1998; Klein et al., 2007). Many plants are dependent on animal pollination for their sexual reproduction (Ollerton et al., 2011) and crops representing 35% of the human food supply benefit significantly from animal pollination (Klein et al., 2007). Insects, and among them bees, play a major role as pollinators (Klein et al., 2007; Abrol, 2012; Rader et al., 2016). They provide more than half of the global crop pollination (Rader et al., 2016). As the presence of pollinators with different traits (e.g. tongue length and phenology) and their interactions with flower traits can affect pollen transfer and thus seed set (Garibaldi et al., 2015), seed production in many wild plants as well as in several crops can be enhanced by a diverse pollinator community (Kearns et al., 1998; Fontaine et al., 2006; Biesmeijer et al., 2006; Hoehn et al., 2008). The decline of pollinating insects, therefore, poses a threat to both wild ecosystems and agricultural production (Potts et al., 2010).

Presence and community composition of pollinators are to a high degree driven by current land uses (Ricketts et al., 2008; Carre et al., 2009; Goulson et al., 2010; Potts et al., 2010; Kennedy et al., 2013). Because availability of pollen, nectar, nesting material and nesting sites are essential for pollinator survival and reproduction, the local habitat quality, farming practice and landscape context can have great impact on the bee community (Öckinger & Smith, 2007; Kennedy et al., 2013). Consequently, the land use changes and the agricultural intensification have caused pollinators to decline all over the world (Biesmeijer et al., 2006; Dupont et al., 2011; Bommarco et al., 2012; Bartomeus et al., 2013; Sánchez-Bayo & Wyckhuys, 2019, but see Carvalheiro et al., 2013). High coverage of large and homogenous agricultural land is linked to declines in pollinator populations (Senapathi et al., 2015), whereas presence of semi-natural habitats, which contributes with both nesting habitats and food resources, has been shown to promote bee species richness (Ricketts et al., 2008, Garibaldi et al., 2011, Rundlöf et al., 2008). Likewise, organic farming has been shown to enhance biodiversity and species richness of pollinators (Batáry et al., 2011; Tuck et al., 2014; Lichtenberg et al., 2017). However, the effect of organic farming may differ depending on landscape context (Rundlöf et al., 2008, Batáry et al., 2011).

Mass-flowering crops such as oilseed rape and clover, can be an important resource for pollinators (Westphal et al., 2009; Holzschuh et al., 2013; Rundlöf et al., 2014). However, they often constitute a resource highly concentrated in time and space, with strong effects on structure, habitat use and flower visitation of pollinator communities (Diekötter et al., 2010, 2014; Hanley et al., 2011; Holzschuh et al., 2013, 2016; Rundlöf et al., 2014; Riedinger et al., 2015; Montero-Castaño et al., 2016). Nonetheless, the abandonment of clover leys as soil fertilizers (replaced by artificial fertilizers) has been suggested to have played a significant role in the decline of bumble bees during the last century (Goulson et al., 2005, 2008, 2010, Carvell et al., 2006; Kleijn & Raemakers, 2008).

2.2.2 Pests

Crop yield losses to pests, pathogens and weeds, have despite extensive protection measures remained proportionally constant or even increased in some areas during the last century (Pimentel et al., 1991; Oerke et al., 1994; Oerke, 2006). Yield reduction caused by animal pests, primarily insects, was typically 5-15% in all major crops worldwide at the beginning of the century (Oerke & Dehne, 2004). Although the prognosis on yield losses to pests was somewhat better for Europe compared to some other areas (Oerke et al., 1994), challenges to meet the growing human population’s food demand, and the climate changes causing many species (including humans and pests) to move, are a global concern. Several factors drive changes in crop losses to pests. Among the most important ones are changes in land use at the landscape scale, agricultural management at the field scale (fertilization, pesticide use, crop breeding etc.) and climate (Porter et al., 1991; Chaplin-Kramer et al., 2011; Dávila-Flores et al., 2013; Lundin *et al.,* 2016).

For an herbivorous insect, a large agricultural field constitutes an excellent resource on which the insect will thrive and reproduce, and build up a large population. Unchallenged by natural control mechanisms, this insect population could cause damage to the crop to the extent that it will be considered a pest. Herbivore outbreaks are generally more likely to occur in agricultural monocultures compared to natural systems (Snyder & Ives, 2009). According to many researchers, this is to a great extent due to lack of biodiversity in agricultural systems which renders them instable (Pimintel, 1961; van Emden & Williams, 1974; Snyder & Ives, 2009). Measures to control pest populations is primarily accomplished by pesticide use, but also include the use of resistant plant breeds, biological control, intercropping, push and pull systems, mating disruptive pheromones, and relying on natural control provided by organisms inhabiting the surrounding semi-natural areas (Dent, 2000; Rebek et al., 2012).

The problem with pesticide application is that it does not only affect the target organism but also induces acute and chronic toxicity to non-target organisms (Geiger et al., 2010; Köhler & Triebskorn, 2013). Transitioning to more sustainable methods like integrated pest management (IPM) and ecological intensification (Bommarco et al., 2013), might be a way to counteract these problems. IPM is an environmentally sensitive approach to pest management that relies on a combination practices, with pesticide use only as a last backup option. By use of information on the life cycles of pests and their interaction with the environment, and a combination of available pest control methods, IPM is used to manage pest damage by the most economical means, and with the least possible hazard to people and the environment. IPM should since January 2014 be implemented in all member states of the European Union, and is regulated in the Directive 2009/128/EC.

In organic farming where agrochemicals are prohibited, and in general as a measure to decrease the use of insecticides, or the development of insect resistance to insecticides, crop rotation provide an essential tool. Growing the same crop in the same place several years in a row is known to cause depletion of nutrients, accumulation of crop diseases and pests (Bullock, 1992; Mohler & Johnson, 2009). For pest species with limited dispersal capacity, crop rotation can be an important measure to control population build-up by moving the resource in time and space (Bullock, 1992; Dent, 2000; Mohler & Johnson, 2009).

2.2.3 Natural enemies

Members of several arthropod groups, e.g. ground and rove beetles, lady birds, parasitoids, spiders and hover flies, can contribute to the suppression of pests in agricultural crops, and provide biological pest control i.e. act as natural enemies of crop pests (Barbosa, 1998; Losey & Vaughan, 2006). Biodiversity decline caused by agricultural intensification concerns also natural enemies (Bianchi et al., 2006, Sánchez-Bayo & Wyckhuys, 2019). The abundance and diversity of many natural enemies have been shown to be positively related to landscape complexity and to the amount of non-crop habitats in the landscape (Langellotto & Denno, 2004; Bianchi et al., 2006; Tscharntke et al., 2007; Attwood et al., 2008). Non-crop habitats such as field margins, semi-natural grasslands and pastures act as refuges with alternative food resources and nesting- and overwintering sites, from which the natural enemies can attack pests in the agricultural fields (Bianchi et al., 2006). Thus, increased landscape complexity generally enhances pest insect suppression (Thies & Tscharntke, 1999; Thies et al., 2003; Rusch et al., 2016; but see Zaller et al., 2008). While natural enemies respond positively to landscape complexity, pest abundance and the crop damage they cause show no such relationship (Chaplin-Kramer et al., 2011). Likewise, predatory insects, in contrast to herbivorous insects, more often respond positively to organic farming (Bengtsson et al., 2005), and biological control levels are reported to be higher under organic management (Crowder et al., 2010; Geiger et al., 2010; Porcel et al., 2018; Muneret et al., 2018). These reports show the potential for biological control as a pest management tool. Yet, the importance of natural habitats and farm management for pest control, and their actual benefits for crop yields, can vary depending on the type of crop, pest, natural enemy, and landscape composition (Tscharntke et al., 2016; Karp et al., 2018). A diverse natural enemy community has the potential to better control herbivorous arthropods (Letourneau et al., 2009), but there is no guarantee that a species-rich natural enemy community will provide effective pest control (Bianchi et al., 2006; Straub et al., 2008; Snyder & Ives, 2009). There are still knowledge gaps concerning how local management and landscape factors affect pests and natural enemies in different crops.

2.3 Clover

Both red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) have their origin in the eastern Mediterranean region, from where they have naturally spread into western Asia and to other parts of Europe (Baker & Williams, 1987). Their domestication is reported to have occurred in the south of Europe from the 16th century and onwards (Baker & Williams, 1987). Because clover can fix atmospheric nitrogen effectively and provide good forage for grazing livestock (Baker & Williams, 1987) it is grown worldwide for production of animal fodder and for provision of green manure. Their advantages made clovers very valuable, and leguminous crops such as clover have been highlighted as a key factor for the large increase in yields and the growing human population in Europe between 1770 and 1880 (Chorley, 1981). Clover gradually lost its importance over the 20th century as a consequence of the invention of the Haber-Bosch process, which enabled the production of artificial fertilizers (Taylor & Quesenberry, 1996). However, with implementation of more sustainable farming practices and increasing prices of chemical fertilizers and fossil fuels necessary for their production, clovers are again increasing in importance. Clover now constitutes an essential crop for the organic farming sector where inorganic fertilizers are prohibited (Stockdale et al., 2001; Thorup-Kristensen et al., 2003).

2.3.1 Red and white clover grown for seed production in Sweden

In Sweden, clovers are known to have occurred since the Middle Ages (Nordstedt, 1920) and were introduced as crops in the mid-1700s. Today, Sweden is one of the leading producers of organic ley seeds (Jordbruksverket, 2017) and in 2016, more than 40% of Sweden's arable land was sown with ley or fodder plants (often containing clovers in the mixture) (Jordbruksverket, 2016). However, growers producing the clover seeds are experiencing variable yields, as production is limited by both poor seed set and by seed predation. The variability in yield, from 0 to 1000 kg/ha (Jonsson, 2011), is so great among years and locations (Taylor & Quesenberry, 1996, Boelt et al., 2015), that seed companies are occasionally facing problems meeting demands (Öhlund, L., Lantmännen and Dahlqvist, T., Sveriges Frö- och Oljeväxtodlare, personal communication, 2018).

Red clover is an herbaceous, perennial but short-lived plant in the *Trifolium* genus within the legume family (*Fabaceae*) (Anderberg, 1999) (figure 1). It is a diploid species, but additionally bred as a tetraploid through sexual or asexual chromosome doubling (Annicchiarico et al., 2015), which is achieved through either use of colchicine, nitrous oxide or by gametic nonreduction (Taylor & Quesenberry, 1996). Red clover is the most commonly grown leguminous species in Sweden (Halling, 2012). Moreover, Sweden is one of the largest producers of red clover seeds in Europe, and in 2013 red clover fields for seed production covered 2000 ha of the country's area (Lundin, 2013). Red clover for seed production is a high risk crop, but advantageous to have in the crop rotation and yields a high price per kilo seeds (Lundin, 2013; Jonsson, 2011). Compared to other leguminous species grown as crops, e.g. alfalfa or white clover, red clover is more short-lived due to a higher susceptibility to pests and diseases (Taylor, 2008). To increase its persistence, red clover is part of breeding programs, and currently tetraploid

Figure 1. Red clover, *Trifolium pratense,* in bloom. Photo: Maria Blasi Romero.

cultivars appear to have better persistency, resistance and vegetative strength, as well as a higher forage yield and better content of sugars and proteins than diploid cultivars (Guy et al., 1989; Boller et al., 2010). The dissemination of tetraploid red clover cultivars is nevertheless slowed down by their lower seed production (Boller et al., 2010; Annicchiarico et al., 2015), which can be 20–50% lower than in diploid cultivars (Boller et al., 2010; Rundlöf et al., 2018).


Figure 2. White clover, *Trifolium repens*, in bloom. Photo: Veronica Hederström

White clover (figure 2) is the most important leguminous crop in grazed ley in Sweden (Jordbruksverket, 2018). As fodder, white clover is slightly richer in energy and contains comparatively more leaves than stalks at harvest compared to red clover (Jordbruksverket, 2018). In contrast, white clover has a shallow root system and is not as tolerant to drought and should therefore be grown on soils with good water holding ability (Jordbruksverket, 2010b). Just as red clover, white clover for seed production is a high risk crop, dependent on weather conditions during pollination and harvest (Jordbruksverket, 2010b).

Red and white clover for seed production are normally under-sown in a cereal crop in spring during the first year and harvested in the second year (Lundin, 2013, Jordbruksverket, 2010a, b; Svensk Raps, 2018). During the harvest year, white clover flowers in June-July and seeds are harvested in late July or early August, ca 28 days after the best pollination day (Jordbruksverket, 2010b). Red clover flowers later, in June-August, and the seeds are harvested 1.5-2 months after full bloom (Lundin, 2013, Jordbruksverket, 2010a). Although both clovers are perennial, usually only one harvest is taken from the seed production field. After the harvest year, the clover fields are rotated, and it is advised not to grow red clover or white clover, respectively, on the same field within 5 years, to reduce problems with pests and diseases (Jordbruksverket, 2010a, b).

2.3.2 Yield affecting factors

Several factors, among them both genetic (Herrmann et al., 2006) and environmental (Jordbruksverket, 2005, Boelt et al., 2015), are important for seed yield in clover. Although the causes of variation in yield are not fully understood, establishment, time of cutting, cultivar, pest control, pollination, harvesting method as well as weather conditions are all believed to be important

(Jordbruksverket, 2005; Lundin, 2013). Weather conditions during full bloom and during harvest is of essential importance in determining the clover seed yield. Clover is dependent on insect pollination to set seed (Darwin, 1859), and the pollinating bees in turn are dependent on the outside temperature and wind for their activity (Abrol, 2012a,b). Adding to this, overcast weather during flowering has a negative effect on flower fertility (Pasumarty & Thomas, 1990, 1998; Thomas, 1996). A major problem in clover seed production is seed eating pests (Langer & Rohde, 2005, Lundin et al., 2012, 2013). Both red and white clover are attacked by seed eating weevils which greatly reduce the seed yields; the reduction can reach levels of > 50% (Hansen & Boelt 2008, Lundin et al., 2012). Nonetheless, the most important yield-reducing factor according to Langer and Rohde (2005) is harvest success, which is often highly weather-dependent. Timing of harvest is important, and harvesting too late in wet conditions will lower the seed yield and quality as the seed easily germinates in the flower head during such conditions (Persson & Cristiansson, 2013). Weeds in the crop can lower the area on which clover is present and thereby lower the yield, but more important is that only a certain amount of some weed seeds are allowed in the final seed product (Jordbruksverket, 2010b; Larsson, 2016). The clover can be cut in April-May (or early June, depending on species cultivar and weather) of the harvest year to reduce weed growth, even the stand, increase the number of inflorescences and, to some extent, control pests (Hansen & Boelt, 2004; Jordbruksverket 2005; Jordbruksverket, 2010a, b). However, the timing of cutting, and whether mulch is left or removed can determine the success of this measure and also the regrowth and maturation of the crop (Hansen & Boelt, 2004; Jordbruksverket 2005).

2.3.3 Red clover pollination

Clovers are pollinated by honey bees, bumble bees and solitary bees (Free, 1993), but bumble bees are thought to be more efficient pollinators of red clover compared to honey bees (Bohart, 1957; Peterson et al., 1960; Nørgaard Holm, 1966; Boller et al., 2010). It has been suggested that primarily long-tongued bumble bees are able to pollinate tetraploid red clover cultivars, because the more common and short-tongued bumble bees in the subgenus *Bombus sensu stricto* are supposedly less efficient due to the deep corollas (Boller et al., 2010), favouring robbing of nectar without pollen transfer (Free, 1965; Inouye, 1983, but see Maloof and Inouye, 2000). Small differences in corolla tube length have been found to cause differences in bee species visiting (Julén, 1954; Hänninen, 1962; Nørgaard Holm, 1966). Therefore, lower seed yield in tetraploid red clover cultivars is by many authors attributed to, among others, longer corolla tubes which may hamper many pollinators (Julén, 1954; Wexelsen & Vestad, 1954; Boller et al., 2010). This is worrying as studies from Sweden and Denmark have demonstrated fundamental shifts in composition and density of bumble bee communities since the first half of the 20th century (Bommarco et al., 2012; Dupont et al., 2011). They show a loss of long-tongued species and dominance of a few short-tongued generalist species, which may have direct consequences for crop yield stability in red clover (Bommarco et al. 2012).

However, plant traits have also been suggested to be the cause of lower seed set in tetraploid cultivars. Lower level of branching in tetraploids, leading to lower number of inflorescences per plant, has been put forward as a putative cause for lower seed yield (Taylor & Quesenberry, 1996), especially as seed yield per plant is shown to be strongly correlated with number of inflorescences per plant (Bond & Fyfe, 1968; Taylor & Quesenberry, 1996; Monks et al., 2014; Vleugels et al., 2014). The proportion of flowers that are ripe at harvest, which is often lower in tetraploid cultivars, is also correlated with seed yield (Taylor & Quesenberry, 1996). Although tetraploids have the same number of florets per inflorescence as diploids, they have been shown to have fewer seeds per inflorescence (Vleugels et al., 2015). Other reasons stressed in the matter of lower seed set in tetraploids are: inefficient pollination (Clifford & Scott, 1989; Bender, 1999), lower pollen viability (Kendall, 1967; Buyukkartal, 2003; Grebenisan & Savatti, 2011) and increased rates of anomalies and irregularities during meiosis (Mackiewicz, 1965; Buyukkartal, 2008), as well as a combination of all these factors (Buyukkartal, 2008).

2.3.4 The major white clover seed pest, *P. fulvipes* and its natural enemies

Weevil species known to feed on clover belong to the genera *Protapion*, *Hypera* and *Sitona*. In Denmark, *Hypera* spp. are reported as important pests in white clover seed production (Langer & Rohde, 2005, Hansen & Boelt, 2008). In Sweden, *Protapion* spp. weevils (Coleoptera: Apionidae) are considered to be the major pests in both red and white clover. Red clover seeds are mainly attacked by *P. trifolii* L., *P. apricans* Hbst. and *P. assimile* Kirby (Notini, 1935; Markkula & Myllymaki, 1964), while the white clover seed weevil, *Protapion fulvipes,* Geoffroy, 1785, is known as the major pest of white clover seeds (figure 3). Recent studies have investigated the ecology of clover seed weevils in red clover (Lundin et al., 2012, 2013, 2016) but less is known about white clover seed weevils.

Protapion fulvipes is an economically important pest, which substantially reduces seed yields of white clover (Gønget, 1997; Langer & Rohde, 2005;

Hansen & Boelt, 2008; Boelt et al., 2015). It is widely distributed, and in the Nordic countries it is the most common and abundant *Protapion* species. It is oligophagous on *Trifolium* spp. and other Fabaceae, e.g. *Ononis* and *Medicago* spp, but reproduces only on *T. repens* and *T. hybridum* (Markkula & Myllymaki, 1957, 1964; Gønget 1997). Overwintering adults of *P. fulvipes* emerge in early spring (mid-April), whereafter they locate and disperse to host plants on which they feed until sexually mature (Freeman, 1967). After mating, the female oviposits inside florets of developing clover inflorescences through mandible-cut holes (Freeman, 1967; Ohlsson, 1968; Gønget 1997). The timing of oviposition is important, i.e. Freeman (1967) found a preference for *P. fulvipes* females to oviposit in inflorescences with 25–75% open florets, when the ovules are in just the right stage. If eggs are deposited in a too young flower the larvae may damage the plant ovary, destroying its only food resource, and if the flower is too mature, the seeds will be too hard before the larvae have completed their development. During the oviposition period (ca 50 days starting with onset of flowering) females lay on average 4 eggs/day (Freeman, 1967). Eggs hatch after approximately 10 days and the larvae spend their lives (about 18 days) inside the inflorescences, feeding on ovules or developing seeds (Dieckmann, 1977). The larvae pupate in the old flower, mature for about 10 days (Dieckmann, 1977) and in early autumn, often coinciding with harvest of clover seeds, the young adults emerge from inflorescences, stay and feed on the leaves, and disperse to overwintering areas (Freeman, 1967). According to Gønget (1997), *P. fulvipes* is an active flyer, more so than relatives such as *P. apricans, P. assimile* and *P. trifolii* (Bovien & Jörgensen, 1936), that disperses twice a year; once in spring and once in autumn. During autumn dispersal *P. fulvipes* flies at low height and settles on the underside of low herbage and leaves of trees and herbs in woodlands (Gønget, 1997; Freeman, 1965; 1967). There it spends the late summer and autumn. For overwintering the species makes its way to the leaf litter in order to avoid lethal cold temperatures, and stays there until spring (Freeman, 1967, 65). At northern latitudes, *P. fulvipes* has only one generation per year (Jones, 1950; Freeman, 1967).

Protapion fulvipes is attacked by parasitoid wasps which lay their eggs on or inside the weevil eggs or larvae in florets of white clover inflorescences (Notini, 1935; Kruess, 1996; Sharkey, 1997; Pirouzeh et al., 2016). In south Sweden the primary parasitoid of *P. fulvipes* in white clover is *Mesopolobus incultus* Walker 1834 (Rosén, 1962). *Spintherus dubius* (Nees, 1834) (figure 3) is another parasitoid which is also commonly found in clover fields (Kruess & Tscharntke, 1994; Lundin, 2013). It is known as a primary parasitoid of *P. apricans* but is associated with both *T. pratense* and *T. repens* (Noyes, 2016), and is assumed to attack also *P. fulvipes*. There is, however, limited information on the abundance and the efficiency of these species in controlling pests in white clover, and whether it results in increased yield. Knowledge is also lacking on how relations between pests and parasitoids are affected by spatiotemporal relationships among white clover fields over consecutive years, i.e. by how the crop is moved in space among years.

Figure 3. The white clover seed weevil *P. fulvipes*, and one of its natural enemies, the parasitoid wasp, *Spintherus dubius*. Photo: Víťa Maňák.

3 Thesis aims

The aim of this thesis was to investigate factors affecting functionally important insects, i.e. pollinators, pests and natural enemies of pests, occurring in clover seed production, and their effect on seed yields. The overall goal of these investigations is to facilitate the sustainable supply of clover seeds, as an important asset for meeting future agricultural production demands while minimizing environmental impacts. The main focus of the project was to address two critical factors that cause low and variable seed yields in clover seed production: seed set and seed loss through insect pests.

In the first part of this thesis (paper I) the goal was to characterize factors that may cause poor seed set through insufficient pollination i.e. pollen viability, pollinator efficiency and their interaction. We focused on both plant and pollinator traits to understand how traits vary between red clover ploidy and pollinator species and how these traits relate to seed set. We particularly sought knowledge on why there is a poor seed set in tetraploid red clover.

In the second part of the thesis (paper II, III, IV, V) the aim was to examine causes for low and variable seed yield in white clover seed production. In paper III we investigated how spatiotemporal dynamics of the main clover seed pests (primarily clover weevils of the genus *Protapion*) affect seed yields through seed predation. We also explored the role of pollinators on seed set in white clover (paper II and IV) and how pollinator abundance and species richness in turn are affected by local management and landscape context (paper IV). In the final paper (V) we investigated the interactions between pests and natural enemies in white clover, under different management regimes and landscape contexts.

The following specific research questions are addressed in the papers that are included in the thesis:

(i) What are the causes of low seed set in tetraploid red clover? (paper I)

(ii) Do functional insects vary in density depending on local management (organic, conventional insecticide treated or conventional untreated) and landscape context (proportions of arable and semi-natural land), and how are these densities related to seed set? (paper II-V)

(iii) Where do clover seed pests overwinter, and when do they locate the new seed field? What is their movement range in the landscape and can this be exploited to prevent population build-up? Can the spatiotemporal distribution patterns of the pest help predict its effect on seed yield through seed predation? (paper III)

(iv) Can parasitism rates provided by natural enemies mitigate the effect of pests on seed set? (paper V)

4 Methods

Both common garden trials and field experiments were conducted to address the research questions. The common garden is situated at the Swedish University of Agricultural Sciences at Alnarp in southern Sweden, and the field experiments were conducted in 45 white clover seed fields spread in the landscape of Scania, the southernmost province of Sweden (figure 4).

4.1 Common garden trials on red clover

During three years, 2010, 2014 and 2015, plant and pollinator traits and their interactive effect on seed set were investigated on red clover cultivars of differing ploidy level, and pollinator species of differing tongue length, in a common garden (figure 5).

Plants from four diploid and five tetraploid red clover cultivars were compared regarding plant traits and seed production; inflorescences per plant, florets per inflorescence, floret size, colour, nectar volume, pollen germination and pollen tube growth, seed set and seed weight.

Pollinator presence and abundance were monitored by transect walks, and species specific behaviours of bumble bees and honey bees were evaluated by observation of flower handling time, and counting the number of florets visited per inflorescence. Single pollinator visits were observed in order to estimate the efficiency of pollinators with differing traits, i.e. we assessed the pollen deposited per stigma as well as seed set after visit by one individual bee.

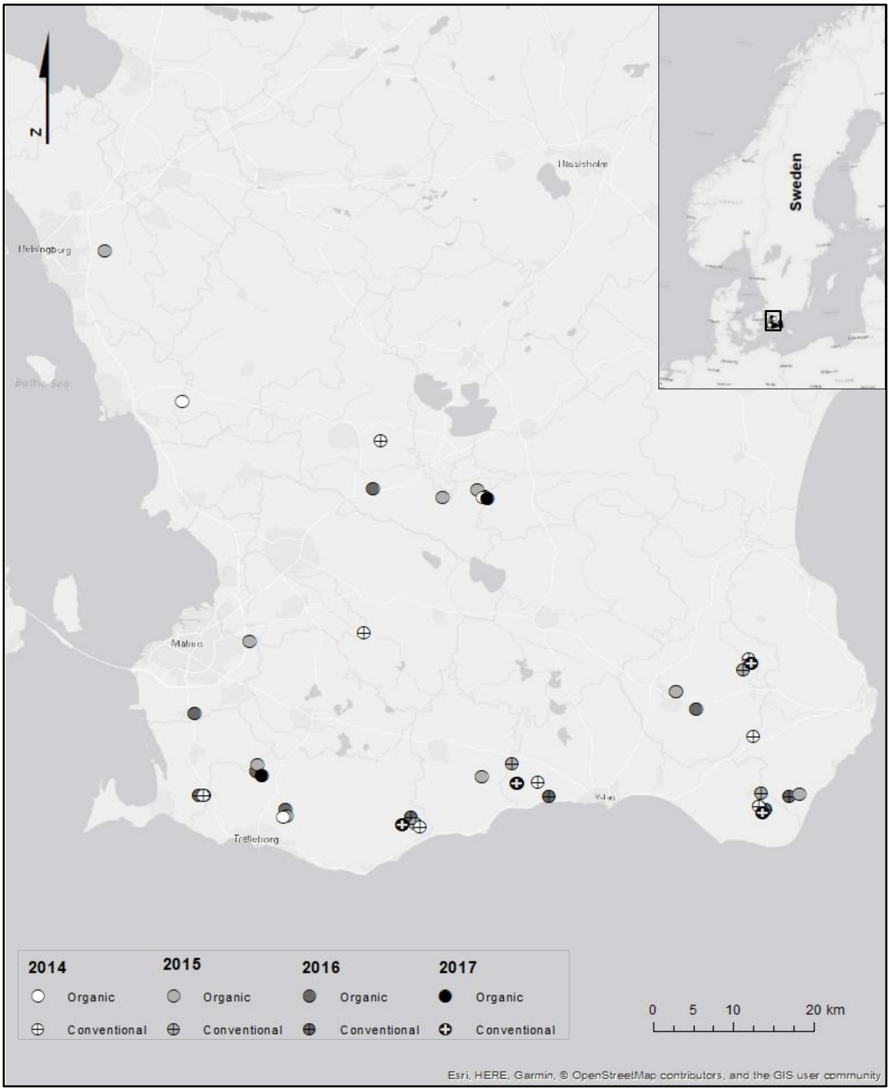

Figure 4. Map of Scania, southern Sweden, showing clover seed fields included in the study 2014-2017. Map of Sweden in the top right corner. Filling of circles depending on year: white = 2014, light grey = 2015, dark grey = 2016, black = 2017. Conventionally managed fields with a cross and organically managed fields without.

Figure 5. Some of the methods and system setups used for common garden trials. From top left corner: setup in 2011 (photo: Sofia Hydbom), measuring nectar volume (photo: Delphine Lariviere), counting pollen grains germinated in vitro and measuring pollen tubes (photo: Sofia Hydbom), counting pollen grains on stigma (photo: Delphine Lariviere), observing pollinator behaviour and single pollinator visits (photos: Maria Blasi Romero, Veronica Hederström), setup in 2015 (photo: Veronica Hederström).

4.2 Field studies in white clover

To collect data on pollinators, pest, natural enemies and yield components in white clover, field studies were performed in commercially grown white clover for seed production during 2011 and 2014-2017 (figure 6). Fields were both organically and conventionally managed and situated in Scania (figure 4). A timeline for a clover seed production field, in relation to our studies, is shown in figure 7.

We established two adjacent study plots in conventionally managed fields and one plot in organically managed fields in order to compare the effect of insecticide treatments in addition to the comparison between organic and conventional management; one of the plots in conventional fields was excluded from pesticide treatment whereas the other was treated as the rest of the field.

Pest insects emerging from overwintering sites were collected using soil emergence tents placed next to the previous year's clover field, next to the new seed field and at a mid-point in between these fields (figure 8). Tents were put up in early spring and taken down when no more insects were emerging from overwintering. In mid-May, before onset of flowering, pan traps for collection of pest insects were placed in the study plots inside clover fields to collect overwintered weevils immigrating in to the field and to follow the phenology of the pest population. Pollinators were monitored using transect walks inside the study plots on three occasions per field during the flowering period of white clover. To assess initial seed set in relation to the concurrent pollinator community, we marked inflorescences in full bloom (>80% open florets) during each pollinator survey and left them to mature in the field for two weeks, whereafter we collected the inflorescences, stored them in a freezer (to not lose seeds to seed eating pests) and later counted the number of seeds. To determine final seed set and to incubate flowers for emergence of newly hatched weevils and their natural enemies, we collected fully withered, mature inflorescences from each transect a few days before commercial harvest. To assess the flower resources for insects in the field, we determined the flowering density by counting the number of flowering and withered inflorescences in connection with every pan trap emptying, every pollinator survey and a final time during the collection of inflorescences. We used the final inflorescence density from the previous year's closest clover seed field, together with field size of the previous year's field, and number of emerged weevils from inflorescences from that field to calculate the pest pressure (pest load) from the previous year's clover seed field. We did the corresponding for parasitoids hatched at the previous year's field to calculate a "parasitoid load".

To assess the impact of land use and landscape structure on the functionally important insects and their effect on seed yield, proportion arable- and semi-natural land was calculated in buffer zones with 250-1000 m radius, surrounding the study plots. Calculations were made using ArcGIS software 10.6 (ESRI, 2017) and land use data from the Integrated Administration and Control System (IACS) from the Swedish Board of Agriculture.

Figure 6. Some methods used in field studies in white clover seed fields. From top left corner: trapping clover seed weevils with soil emergence tents when emerging from overwintering, emptying pan traps (photo: Sofia Hydbom), emptying pan traps (loosening tent pins keeping the trap in place), inflorescence marked with coloured thread during pollinator survey for assessment of initial seed set (photo: Delphine Lariviere), assessing flower density, surveying bees during transect walks (photo: Delphine Lariviere), incubation of inflorescences for emergence of new generation clover seed weevils. If nothing else stated, photo: Veronica Hederström.

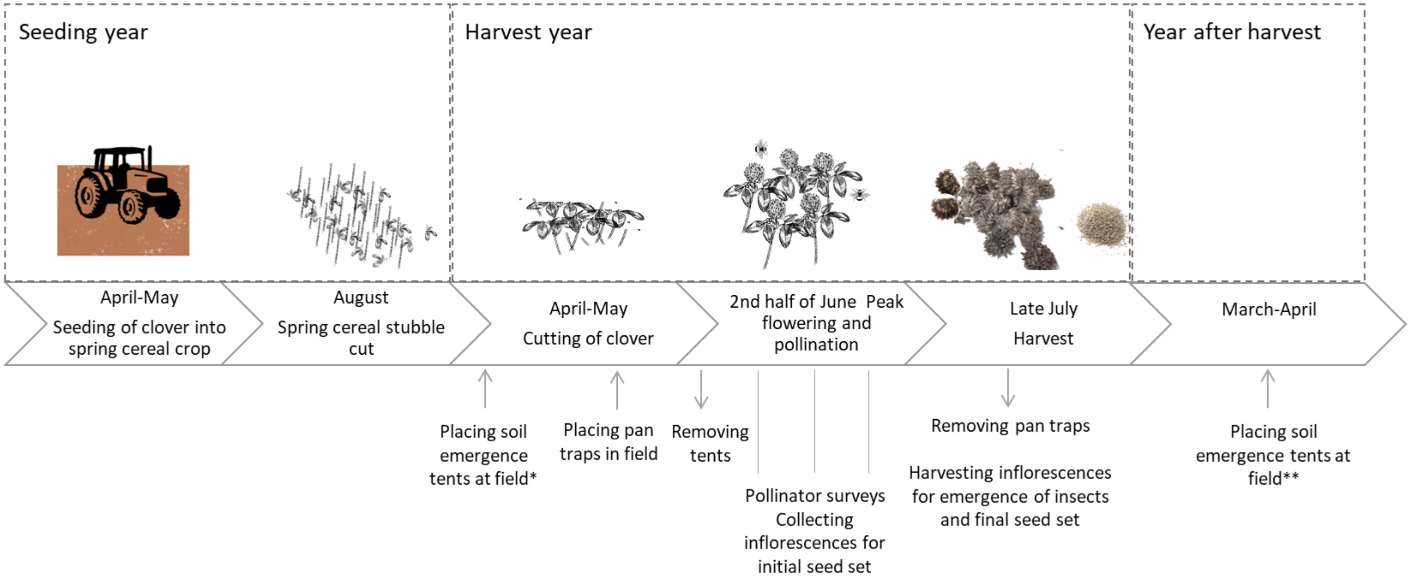

Figure 7. Timeline for a white clover seed field. In the year before harvest (seeding year) seeds of clover are sown into a spring (or autumn) cereal crop. The cereal is harvested during late summer and thereafter the stubble is cut short. The clover is cut in April-May (or early June) of the harvest year to even the stand, increase the number of inflorescences and to reduce weed growth. Peak flowering and peak pollination occur in the second half of June, and 25-30 days later, the seeds are harvested. In early spring of the harvest year we placed our soil emergence tents at the study field. Tents were removed from the field in early June. Pan traps were placed in the field in mid-May, and emptied once per week until removed shortly before harvest in mid-July. We harvested clover inflorescences for emergence of weevils and assessment of seed set around the same time. In the year after harvest we placed the soil emergence tents at the same place by the field again (now the field is called the source field).* = the field is called the study field in the year of its harvest. ** = the field is called the source field the year after its harvest.

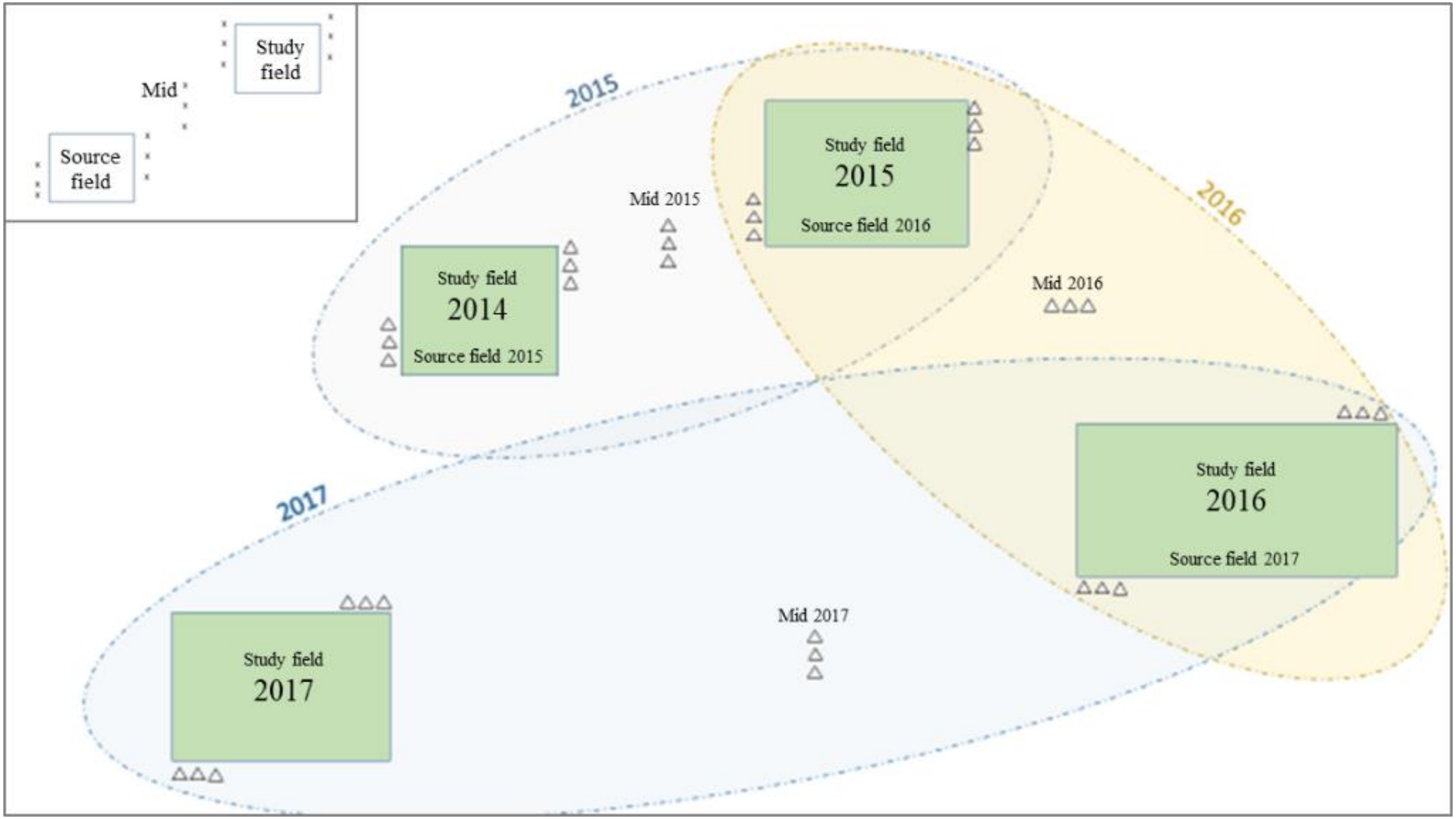

Figure 8. Visualization of placement of tents at one farm during three consecutive years. Dotted lines encloses fields and tents being part of the study one year. In top left corner: example of tent placement for one year at one farm. Each year, three tents were placed on two sides of the study field (the field which was to be harvested the current year), on two sides of the source field (the closest clover field harvested the previous year) and at a point in between these fields (mid).

5 Results and Discussion

Clovers (*Trifolium* spp.) are important forage and green manure crops, essential for nitrogen supply in organic farming systems (Stockdale et al., 2001). They are commercially important species, therefore a high and stable seed production is needed to meet market requirements (Boelt et al., 2015). Seed yields of both white and red clover are, however, known to vary greatly (Taylor & Quesenberry, 1996; Boelt et al., 2015). The reason for this variation, and possible solutions, were investigated in Paper I-V.

5.1 Interactive effects of plant and pollinator traits on seed set in red clover

In our study on plant traits and pollinator performance in diploid and tetraploid red clover (Paper I), we confirmed, in line with previous studies, that tetraploid cultivars produced fewer seeds than diploid cultivars (Julén, 1954; Taylor and Quesenberry, 1996; Boller et al., 2010). The production of seeds was half as large in tetraploids as in diploids. Even if taking seed weight into account, the yield was 21% lower in tetraploid cultivars. We found several factors possibly contributing to this lower seed set. First, tetraploids had fewer inflorescences per plant, longer corolla tubes and lower pollen viability. These results are in agreement with previous studies. Lower pollen germination rates in tetraploid cultivars was also found by Grebenisan & Savatti (2011). Relationships between seed yield and number of inflorescences per plant has been demonstrated in both diploids (Bond and Fyfe, 1968; Monks et al., 2014; Vleugels et al., 2014) and tetraploids (Herrmann 2006; Vleugels et al., 2015; 2016). Furthermore, number of mature inflorescences per plant (and number of seeds per inflorescence) have been suggested to be used as targets for breeding to increase seed yield in tetraploids (Vleugels et al. 2015, 2016). These previous studies did not, however, consider whether these traits were influencing seed yield as a consequence of insect pollination due to flower attractiveness and insect traits. Number of flowers per plant could influence the number of produced seeds per plant, but also influence the attraction of pollinators to the plant (i.e. have an additive effect). When we related pollinator abundance to clover cultivar and ploidy in paper I, the number of visiting bees was indeed (besides an interaction between tongue length and ploidy, see below) affected by the number of flowering inflorescences per plant.

It has long been discussed that the longer corollas in tetraploid cultivars lead to difficulty for pollinators to reach the nectar (Julén, 1954; Wexelsen & Vestad, 1954; Boller et al., 2010), and to more frequent flower visitation by short-tongued pollinators in diploid clover (Nørgaard Holm, 1966; Dennis & Haas, 1967a, b). Our results were in line with this, and we observed that both short- and medium-tongued pollinator species showed a preference for diploid cultivars when given a choice, whereas long-tongued pollinators showed no preference (figure 9a). While we found a clear interaction between plant ploidy and pollinator tongue length on pollinator abundance, pollinator behaviour showed more uniform patterns across plant ploidy. Three out of seven species spent longer time per floret on tetraploids compared to diploids, but in general short-tongued species spent more time per floret, regardless of clover ploidy, compared to medium-tongued species (figure 9b). Although we did not see a

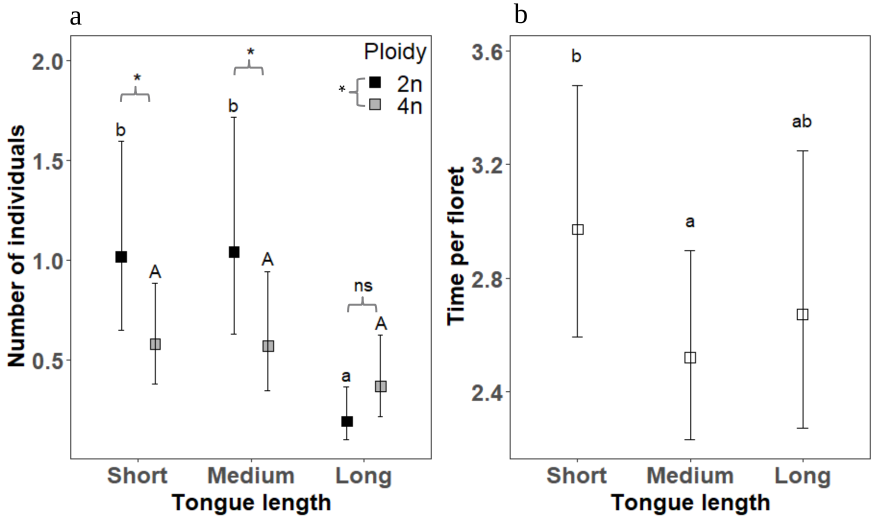

Figure 9. Pollinator abundance and time spent per floret. Comparison of model estimated means and confidence limits (95%) on (a) number of observed pollinator visitors from different tongue length classes in relation to clover ploidy (2n, diploid; 4n, tetraploid), and (b) time spent per floret (s) for bees of different tongue lengths. Pairwise comparisons of estimated means are indicated by stars, ns or letters. Means sharing a letter are not significantly different. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., $P > 0.05$.

difference among bees of different tongue lengths in number of florets visited per inflorescence, the longer time spent per floret could lead to a reduction in the total number of flowers the bee has time to visit. Dennis & Haas (1967b) state that the longer the tongue of the bee is, the faster the bee works and the greater is its value as a pollinator of red clover.

Our study revealed that two efficiency traits were independent of plant ploidy. Long-tongued species deposited more pollen on the stigmas of both diploid and tetraploid cultivars after a single visit. A larger pollen load could be an indication of higher pollinator efficiency, but it should be noted that we do not know the relationship between pollen load size and seed set for red clover. The probability to set at least one seed per inflorescence was generally higher after a visit by a pollinator with medium- or long tongue, compared to one with a short tongue independent of clover ploidy. The last investigated efficiency trait – number of seeds produced per inflorescence after a single visit – was instead determined by a combination of plant ploidy and pollinator tongue length. In the tested diploid cultivar (Ares), seed set was highest when pollinated by medium-tongued pollinators (figure 10a). In contrast, in the two tested tetraploids the trend was that most seeds were produced when pollinated by longer-tongued pollinators. This effect was only significant in one cultivar and here both

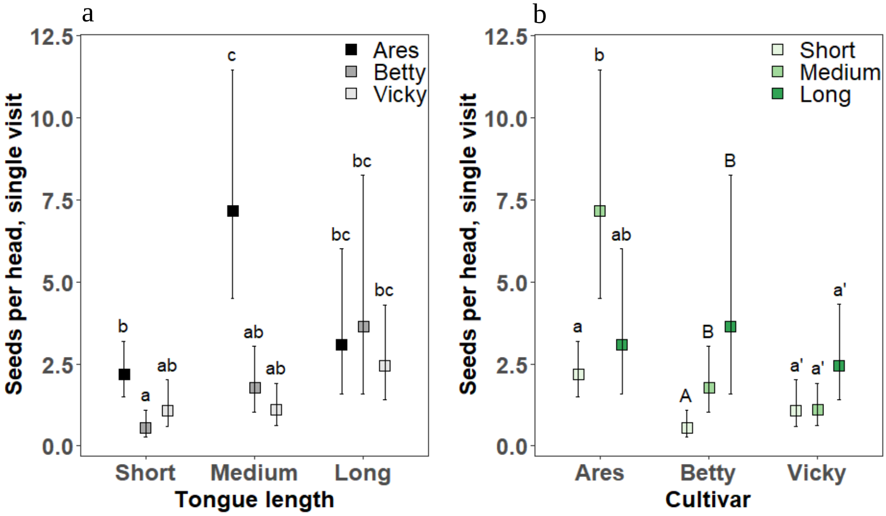

Figure 10. Seed set after single pollinator visits. Model estimated means and confidence limits (95%) for single visits. In (a) number of seeds produced per flower head after one visit by pollinators of different tongue lengths in relation to clover cultivar (black = 2n, Ares; grey = 4n, Betty and white = 4n, Vicky), letters showing pairwise comparisons among all, and, (b) same as in a but with cultivar on the x-axis and tongue length represented by colours (light green = short, darker green = medium, dark green = long), pairwise comparisons within cultivar. Pairwise comparisons of estimated means are indicated by letters. Means sharing a letter are not significantly different.

medium- and long-tongued bees produced more seeds per flower head than short-tongued after a single visit (figure 10b).

The higher efficiency for medium- and long-tongued bees in general, and for seed set after visits by medium-tongued in diploids, could be related to weight or size of the bee (as the pollen spurts out of the floret when exposed to pressure/pull), and should be getting attention in future studies. Especially the much higher seed set after a visit by a medium- compared to a short-tongued species on a diploid plant (figure 10a) needs further investigation.

In a realistic seed field setting, bees will not have the choice between tetraploid and diploid red clover cultivars, as these would not be mixed in a seed field. To some extent wild plants adjacent to the clover field might be more attractive for bees having trouble reaching nectar in tetraploid red clover florets. However, I believe instead that the slower working speed of short-tongued bees, and the lower seed setting after a visits by a short-tongued bee in combination with lower pollen viability and fewer inflorescences per plant in tetraploid red clover, is the cause of ploidy yield difference in red clover. The most common bees observed in red clover fields in the agricultural landscape are short-tongued species (> 80%; Dupont et al., 2011, Bommarco et al., 2012). If these bees are what a red clover field has at its disposal, this will result in a tetraploid field having less flowers pollinated, i.e. a given number of bees, during a limited amount of time, can only do so much work, and if the bees present in the landscape are less efficient in the field with tetraploid plants, compared to a field with diploid plants, this will result in less seeds being produced in the tetraploid field. In addition, there are less inflorescences per plant in the tetraploid fields, i.e. fewer flowers can be pollinated and give rise to seeds, and the pollen grains of tetraploid plants to a larger extent fail to germinate. Adding more honey bees and commercial *Bombus terrestris* to fields with tetraploid cultivars could perhaps result in more seeds as a higher abundance of bees could compensate for their lower efficiency, but a more sustainable and reliable approach would be to promote the more efficient medium- and long-tongued pollinators. It has been suggested that the reduction in diversity and change in community composition of pollinating insects in the agricultural landscape over the past decades may contribute to decreased and more variable seed yield in red clover (Bommarco et al., 2012). This relates particularly to the decrease in pollinators with longer tongues in favour of a few short-tongued species in the Swedish and Danish agricultural landscape (Wermuth & Dupont, 2010; Bommarco et al., 2012). The high pollination efficiency of medium- and long-tongued pollinators in red clover pinpoints the significance of conserving these species, in order to counteract the changes in bumble bee community evenness to get a higher and more stable yield of red clover seeds.

5.2 Causes of low and variable seed yield in white clover

We aimed to identify factors causing the variable seed set in white clover, and also to determine why organic yields were considerably lower than conventional yields. We found (paper II and III) in line with Nyabuga et al. (2015), that *P. fulvipes* was the most abundant clover herbivore in white clover and that there were very few individuals of other *Protapion* species present. In contrast to studies conducted in white clover in Denmark (Langer & Rohde, 2005), we found the seed eating *Hypera* spp. to be less of a problem, as they were only found in very low numbers (Paper II, III). Our studies in paper II showed that seed set in white clover seed fields was negatively related to abundance of both *P. fulvipes* and *Hypera* spp. weevils, with *P. fulvipes* being the most damaging. We also concluded that the abundance of *P. fulvipes* was higher in organic fields compared to conventional fields, i.e. that pesticides efficiently supressed the pest in conventional fields. Thus, we confirmed in both Paper II, III and V, in line with previous studies (Langer & Rohde, 2005) that *P. fulvipes* causes great damage to clover seeds.

5.2.1 Ecology and pest management of the white clover seed weevil

In paper III and V we aimed at finding alternative pest control measures for organic production, and also to minimize insecticide use in conventional production in order to maintain an efficient pest control while minimizing environmental impacts. However, in order to manage a pest it is important to know its ecology and population dynamics (Dent, 2000). It is known that the white clover seed weevil *P. fulvipes* is fairly specific in its host range (Markkula & Myllymaki, 1957, 1964; Gønget, 1997), nonetheless there is little information available as to where the species overwinters, when it locates the new clover seed field, and how mobile this pest species is. Key factors that determine if for example, crop rotation, i.e. movement of a crop in time and space, can be an efficient pest management strategy, is the host specificity and mobility of a pest, as well as its persistence through dormant life stages (Mohler & Johnson, 2009). In paper III, we mapped the spatial and temporal population dynamics of *P. fulvipes*, using soil emergence tents, pan traps and incubation of inflorescences, with the objective to inform pest management and depict the potential to reduce crop yield loss through spatial planning.

We found that *P. fulvipes* overwintered to a higher extent close to where it developed, i.e. it did not disperse to the new clover seed field until early spring next year (paper III). Furthermore, there was no difference in abundance of overwintering weevils in the landscape in between fields compared to adjacent to the new seed field, which could have potentially acted as an attractor for dispersing weevils looking for overwintering sites. As the season progressed there was an increased immigration of overwintered *P. fulvipes* into the new seed field. There was high potential for local amplification of the pest population in the new seed field at several steps. (ii) A higher pest load, i.e. the total number of new generation weevils emerging from inflorescences, from the source field, in one year, resulted in an increase of overwintered weevils in nearby study fields in the following year. (ii) Higher pan trap catches, i.e. higher numbers of immigrating overwintered weevils, in turn resulted in higher pest abundance in the mature inflorescences and severely reduced numbers of seeds per pod during harvest. This effect was greatly mitigated by insecticide treatments in conventional fields, but also by increased distances between fields in subsequent years. An increased distance from the source field resulted in a decreased number of overwintering weevils, as well as a reduced number of weevils caught

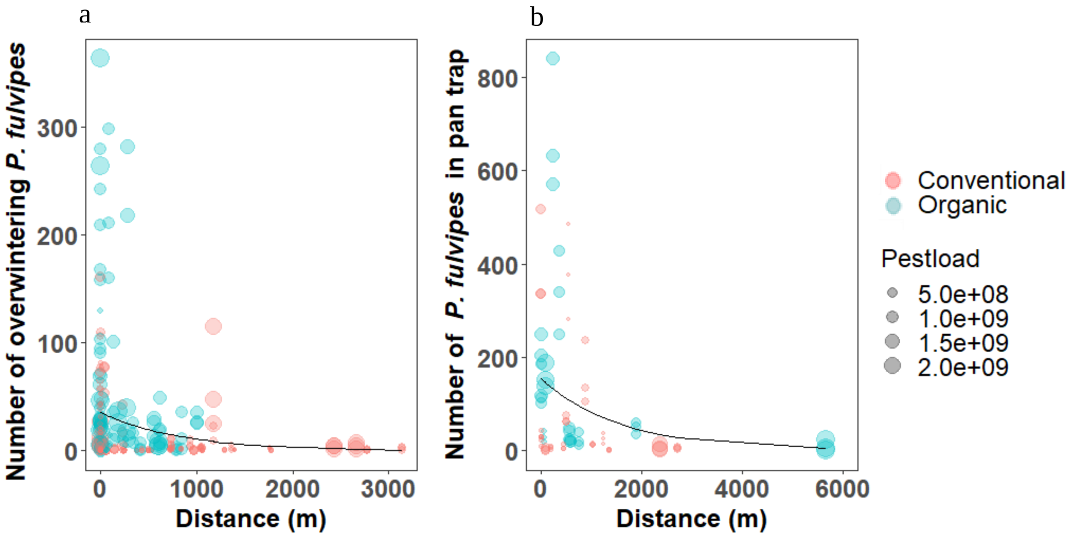

Figure 11. Pests in relation to distance and pest load from the previous year's clover field. (a) Number of *P. fulvipes* emerging from overwintering in soil emergence tents, and (b) number of overwintered *P. fulvipes* getting caught in pan traps in relation to distance and pest load from the previous year's closest clover field. Lines in figures comes from simple general mixed models (with negative binomial distribution and log link) with only one predictor (distance) for illustration purpose only. Size of circles represents pest load. Pink = conventional, blue = organic. Values on x-and y-axis differ between figures. Distance in a, was measured from the previous year's field to each tent, which were placed on both sides of the study field and at a midpoint between the previous year's field and the study field. In b, distance was measured as the closest point between the previous year's field and the study field.

in pan traps in the new seed field (figure 11a, b) or emerging from inflorescences inside the new seed field later in the season. Similarly, in other studies, seed fields of red clover tended to have higher abundances of *P. trifolii* if located closer than 800 m from red clover fields in the previous year (Lundin et al., 2016), and higher numbers of *P. fulvipes* was found in white clover fields in the presence of a source field within 1 km (Langer & Rohde, 2005).

Our results indicate that effects of population amplification of *P. fulvipes* in the agricultural landscape are limited to a local context and points to spatial management as a promising solution to mitigate local population build-up of this pest. This would be especially valuable within organic farming, where insecticide treatments are not an option, but also to reduce insecticide use in conventional farming. Based on our observation that both distance and size of pest load from the previous year's clover field had an effect all the way from immigrating overwintered weevils to the resulting number of weevils and seeds in harvested clover inflorescences at the end of the season (paper III), we suggest that keeping clover seed production fields with a large enough distance between fields and years could help to spatially escape pest weevils. For example, it has been suggested in potato production that moving crop fields at least 500 m between years can greatly reduce the abundance of Colorado potato beetles attacking the crop (Follet et al., 1996). In paper III, the pest abundance seemed to be greatly reduced at a distance of 1500 m, and even lower at a distance of 2000 m, from the previous year's clover seed field. If farms are too small to allow placement of fields with great enough distance between years, grower cooperation would be necessary. To cope with problems with pollen beetle damage in oil seed rape, researchers have suggested crop rotation management at the landscape scale, an intervention which would occur at scales greater than farm units (Skellern & Cook, 2018 a, b). The implementation of spatial planning would be mitigated if occurring as a cooperation between farmers and the seed companies assigning farmers to grow clover for seed production, as well as cooperation among growers themselves.

We showed in both paper II and V that the abundance of pests was much lower in insecticide treated conventional fields compared to unsprayed control plots inside conventional fields, and also compared to organic fields. However, agrochemicals can have negative effects on biodiversity and the target species may evolve resistance to such treatment. It is therefore essential to find alternative or complementary measures. Natural enemies are especially important in organic farming (as insecticide use in conventional farming keeps pests at bay). The efficiency of natural enemies to control pests differs depending on pest species and crop system. Subsequently, in paper V, we investigated whether natural enemies can provide effective control of *P. fulvipes* in white clover seed production. We found that seed set in white clover inflorescences was negatively related to both abundance of pests and parasitoids, and also to parasitoid species richness. Since the pests eat the clover seeds and the abundance of parasitoids is determined by the abundance of hosts, this result is not surprising. Likewise, a higher species richness of natural enemies can increase both the potential for facilitative or synergistic effects among natural enemies (Losey & Denno, 1998; Cardinale et al., 2003; Schmidt et al., 2003; Snyder & Ives, 2003, 2009). Perhaps the observation of seed set being negatively related to parasitoid species richness, is a consequence of synergistic effects among parasitoids, or merely a result of a larger pest population resulting in more parasitoid species. This, however warrants further investigations. We found no effect of parasitism rate on seed set, i.e. the pest control provided by parasitoids was not great enough to translate into higher seed set with higher levels of parasitism rate. The mean parasitism rate observed during the study in paper V was only 12%. Perhaps reflecting that the landscape setting around our experimental fields was relatively simple, with high proportions of arable land (in most cases >50%) and low proportions of semi-natural land (often <20%), suggesting that there could be a limited potential for recruiting enough specialized parasitoids from the surrounding landscape. In systems were one actively adds natural enemies to control pests, it is judged that parasitism rates below 32% have no significant effect on seed yield, i.e. the natural enemies are too few or too “inefficient” to control the pest (Hawkins & Cornell, 1994).

Bianchi et al. (2006) concluded that diversified landscapes hold most potential for conservation of biodiversity and thereby sustaining pest control function (i.e. control by natural enemies). Natural enemies of several *Protapion* species, including *P. fulvipes*, have been shown (in red clover) to be more sensitive to lack of connectivity between habitats than their hosts (Kruess and Tscharntke, 1994). In paper V we found that, in contrast to pest abundance, the rate of parasitism exerted on *P. fulvipes* by parasitoid wasps was unrelated to distance to the previous year’s closest clover field. Parasitism rates were, furthermore, independent of management practice (paper V). Parasitoid species richness and diversity was on the other hand, higher in organic than in conventional insecticide treated plots. We showed that parasitism rate decreased, whereas number of clover seed pests increased, with proportion arable land surrounding the clover field (figure 12). A similar relation to proportion arable land have been observed for abundance of *P. trifolii* in red clover fields (Lundin et al., 2016). The same study also found a lack of relation between parasitism rates and distance to the previous year’s red clover field. That distance does not impact parasitism rates, suggest that placing clover fields further away from the

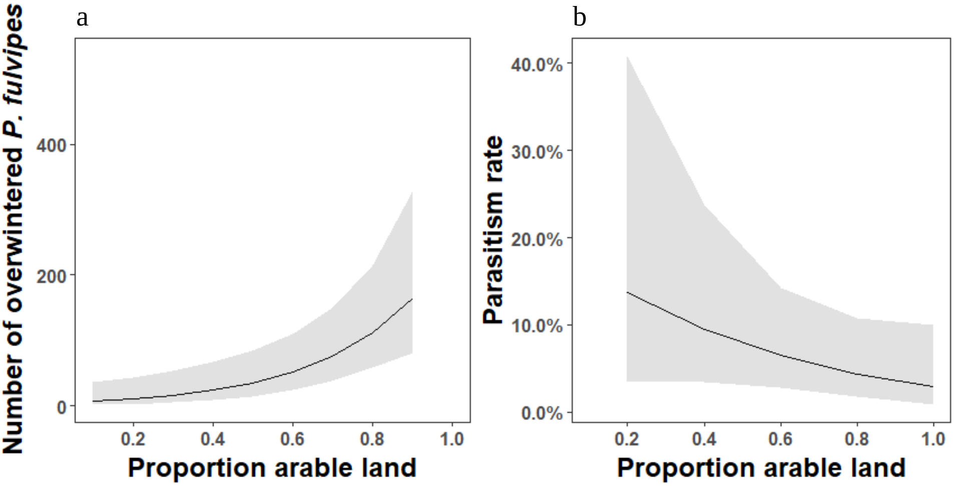

Figure 12. Pests and parasitism rate in relation to proportion arable land. Model estimated means and confidence limits (95%) for (a) abundance of overwintered *P. fulvipes* per plot (total number of pan trap captured *P. fulvipes* divided by the number of traps per plot), and (b) parasitism rate in relation to proportion arable land within 500 m.

previous season's field, would reduce pest abundance but not biological control exerted by parasitoid wasps.

In addition to larger distances between fields, keeping smaller seed production fields or skipping years to reduce the population build-up of *P. fulvipes*, is also a possible management solution. Using smaller fields could have both positive and negative effects depending on several factors. With smaller field sizes the agricultural landscape would become more diversified, and with smaller fields one could also reduce the distance from the field interior to habitats that could harbour pest natural enemies (Bianchi et al., 2006). However, if the same area for clover seed production is required, dividing the total area into smaller fields could mean smaller distance between fields, which would facilitate dispersal of pests to new fields. The practice with smaller fields and remained sown area would therefore only work positively for seed yield if the natural enemies of *P. fulvipes* are efficient at controlling the pest. Further investigations are therefore needed to conclude if more complex landscapes result in higher parasitsim rates and if this translate into less damaged seeds.

5.2.2 Pollinators and seed set in white clover fields in relation to local management and landscape context

Land use such as local field management and composition at the landscape scale can have strong effects on pollinator communities (Ricketts et al., 2008; Carre et al., 2009; Goulson et al., 2010; Potts et al., 2010; Kennedy et al., 2013). In turn, pollinating bees affect seed set both in crops and in wild plants (Kearns et al., 1998). When we compared bee abundance between organic, conventional treated and conventional untreated plots in white clover seed fields we found a higher abundance of non-*Apis* bees in insecticide treated conventional plots compared to organic plots in paper II. When we increased the study to include additional years and fields (paper IV), the pattern was the same, however, one difference was that also untreated transects in conventional fields had higher abundance of non-*Apis* bees compared to organic fields (figure 13a). In addition we found that the pattern was the same for bee species richness (figure 13a). These results are in contrast to studies showing that organic management increases bee abundance (Kennedy et al., 2013), and other studies reporting an increase in biodiversity from organic farming (Batáry et al., 2011; Tuck et al., 2014; Lichtenberg et al., 2017). Nevertheless, positive effects on abundance and diversity is not always found in organic farming systems (Clough et al., 2005; Ekroos et al., 2008; Purtauf et al., 2005; Brittain, 2010). Organic farming had much stronger effects on abundance and diversity of several species groups in cereal crops than in orchards and vegetable crops, which might be a reflection

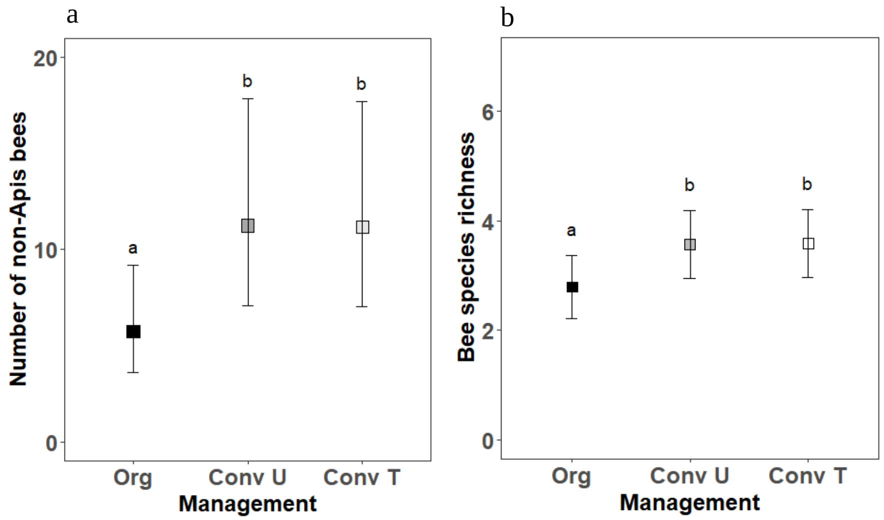

Figure 13. Abundance of non-*Apis* bees and species richness in relation to local management. Model estimates and confidence limits (95%) for (a) number of non-*Apis* bees and (B) bee species richness per transect and survey round in relation to local management (Org = organic, Conv U = conventional untreated, Conv T = conventional insecticide treated). Means sharing a letter not significantly different (p > 0.05).

of the intensive management of conventional cereal crops (Tuck et al., 2014). Tuck et al. (2014) showed that the effect of organic farming was even lower, but, in contrast to our results, still positive in pastures and permanent or semi-permanent leys, which in general are less intensively managed. In paper II we suggested that the detected lower bee abundance in organic white clover fields might be related to the attraction and stimulation of bees to low doses of neonicotinoids (Cutler & Rix, 2015; Kessler et al., 2015), which are one of the pesticide types used in conventional white clover fields. However, the observation in paper IV of bee abundance and species richness in organic transects differing from that in both treated and untreated conventional transects, indicates that this result is independent of the insecticide use. The high abundance of pests also in untreated conventional transects (paper III) and the result that honey bee- and total bee abundance was equally high in conventional treated and untreated transects contradicts another theory proposed in paper II, viz. of bees being deterred by pest damaged flowers (Strauss & Irwin, 2004) in organic plots and that less pest damaged flowers in conventional fields attracted more bees from the surroundings. Although bee abundance and species richness were positively related to inflorescence density, inflorescence density or number of florets per inflorescence did not explain the differences between management types. This observed lower abundance of non-*Apis* bees and bee species richness in organic fields deserves further attention. Factors changing the appearance and attractiveness of inflorescences would be of interest to investigate, e.g. nectar rewards and flower maturation potentially influenced by soil nutrients, soil water content and frequency of soil cultivation and cutting, height and timing of cutting as well as regrowth after cutting. Other possible explanations for lower abundance of non-*Apis* bees in the organic white clover fields, could be the presence of preferred alternative foraging habitats around the field. Semi-natural areas and flower rich field borders are known to harbour floral and nesting resources for pollinators (Persson & Smith, 2013; Petersen et al., 2006; Williams & Kremen, 2007; Öckinger & Smith, 2007), and field borders around organic fields often have a higher plant species richness compared to conventional fields where weeds are efficiently controlled with herbicides (Petersen et al., 2006). However, the flower density in field borders or surrounding habitats was not controlled for in paper II and IV and neither did we assess bee abundance or species richness outside the agricultural fields. In future studies it would be of interest to also quantify the quality of resources in the area surrounding the white clover fields.

Alternative foraging resources elsewhere might also be an explanation for the interactive negative relationship we found of semi-natural land on honey bee-

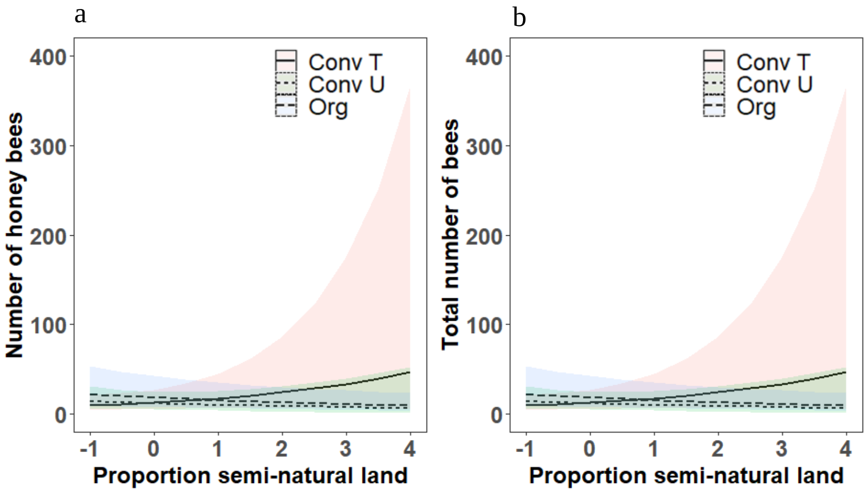

Figure 14. Bee abundance in relation to local management and proportion semi-natural land. Showing model estimated mean and confidence limits (95%) for (a) number of honey bees, and (b) total number of bees, per transect and survey round, in relation to local management and proportion semi-natural land (standardized to zero mean and unit variance) within 1 km from the study transect. Org = organic, dashed line and blue background, Conv U = conventional untreated, dotted line and green background, Conv T = conventional insecticide treated, solid line and pink background. Only the line symbolizing organic transects in a and b deviates from zero.

and total bee abundance in organic transects (figure 14). Generally, semi-natural rich landscapes are associated with higher bumble bee densities and higher species richness (Rundlöf et al., 2008; Ricketts et al., 2008; Garibaldi et al., 2011), whereas landscapes dominated by intensively used arable land harbour less biodiversity (Stoate, 2001; 2009). Although our result on bee abundances are not concurrent with this, we found that bee species richness increased with proportion semi-natural land and decreased with proportion arable land. The lack of a correlation between proportion arable land and bee abundance may be due to that the most common species observed, honey bees, *B. terrestris* and *B. lapidarius* are not affected by the amount of arable land at the investigated scale. Just like many other species pollinating agricultural crops, *B. terrestris* and B. *lapidarius*, are generalists that often do not have special habitat requirements but may live in most types of agricultural landscapes (Kleijn et al., 2015). Honey bees are placed in the landscape by bee keepers, and therefore to a certain extent not dependent on the proportion arable land. As mentioned, the farms included in our studies were surrounded by a high proportion of arable land, in general higher than in many other studies (e.g. in Rundlöf et al., 2008). This restricted the gradient over which the effect of the proportion arable land could be studied, and also gave different prerequisites; i.e. a higher proportion of arable land might not make a difference at these already high levels of arable land.

Even though we found effects of local management and landscape context on species richness, bee diversity (represented by Shannon‘s diversity index) was unaffected by both these factors. The lack of an effect on diversity could, again, be due to our fields being situated in intensively used land with a high proportion of arable land, i.e. simple landscapes (Batáry et al., 2011), with a smaller species pool and low species evenness. A finer tuning of the proportion of landscape types, depending on local flower and nesting resources throughout the season, may well provide better knowledge on the abundance and community composition of bees in mass-flowering crops.

Final seed set was highest in conventional treated transects, intermediate in conventional untreated and lowest in organic transects (figure 15). When exploring the earlier stages of seed set leading up to final yield in paper IV by separating the effect of pollinators from that of seed-eating pests, we found a positive effect of honey bee- and total bee abundance on initial seed set (figure 15 a, b) but, in both paper II and IV, we found no effect of bee abundance on final seed set. This difference in results between initial and final seed set is most likely caused by seed eating pests. Yet, a higher initial seed set does not necessarily result in higher final seed set when excluding pests, due to e.g., seed abortion (see Bos et al., 2007). When investigating further, we observed that final seed set was lower than initial seed set in organic fields, however there was no difference between initial seed set and final seed set calculated from undamaged pods only (figure 15 d). Indicating that the lower final seed set (in all pods) observed in our study, were caused by weevils, and not due to seed abortion. We observed a higher final seed set in undamaged pods in conventional fields compared to organic fields, possibly caused by indirect effects of weevil damage, but we cannot fully exclude that some seeds were aborted due to lack of resources in organic fields.

We know from before that pollinators in white clover seed fields are of utmost importance (Darwin, 1859), but our results in paper IV are not consistent with the previous believe that yields in white clover seed fields are rarely limited by insufficient pollination (Palmer-Jones et al., 1962; Free, 1993; Thomas, 1996; Goodwin et al., 2011). Our continued investigations in paper IV, building on results from paper II, revealed that an increased pollinator abundance may contribute to higher seed set in white clover. Although the final seed yield at the moment is more dependent on seed-eating pests, our results stress the importance of both achieving efficient pest management and at the same time increase pollinator abundance.

We found no relation between seed set and higher bee species richness nor diversity, potentially reflecting that the most common species (the short-tongued honey bees, *B. terrestris* and *B. lapidarius*) are efficient pollinators in white clover, and that addition of a few individuals of the rarer long-tongued species does not improve seed set. This is in contrast to red clover, where pollinators with medium- and long tongues are important for seed set (paper I). Still, a diversity of pollinators could have some influence on seed yield in white clover in the case of unfavourable weather, as honey bees are more sensitive to cold weather than bumble bees (Lundberg, 1980; Corbet, 1993). A more diverse pollinator community would also provide more resilience in case of environmental change or outbreak of pollinator diseases.

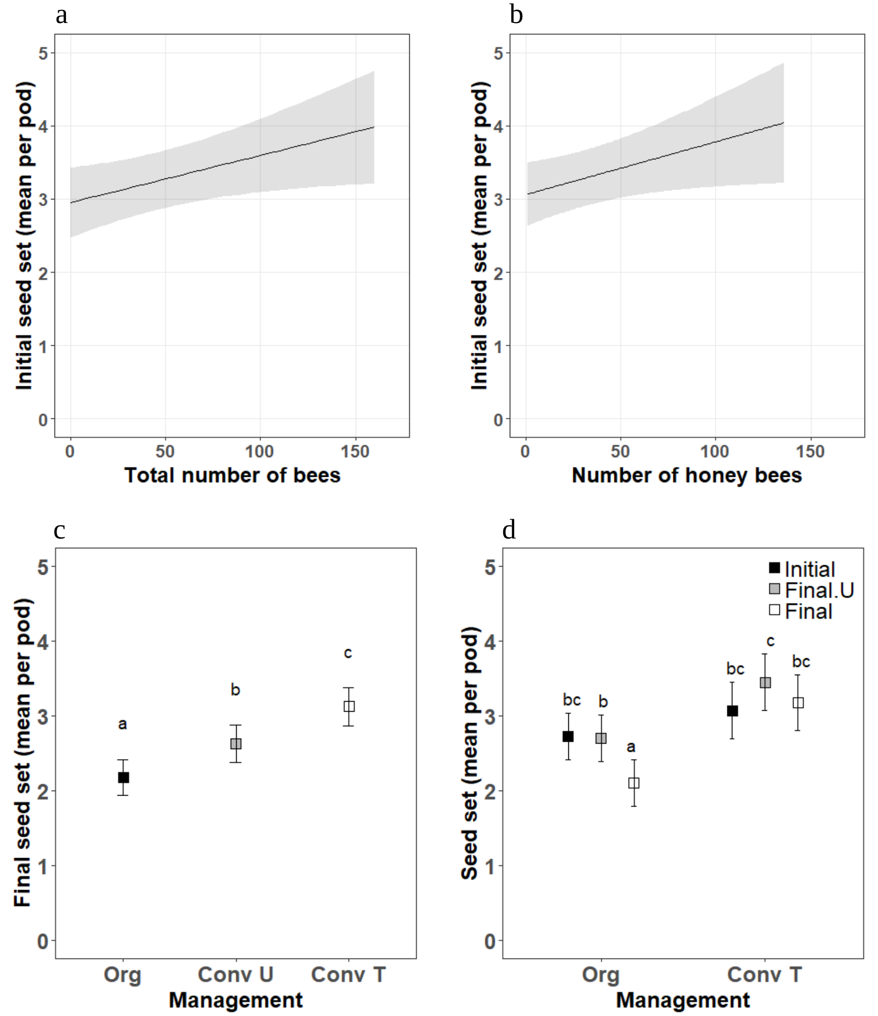

Figure 15. Initial and final seed set. Model predicted means and confidence limits (95%) for (a) initial seed set in relation to abundance of all bees and (b) in relation to honey bee abundance, (c) final seed set in all pods in relation to management (Org = organic, Conv U = conventional untreated, Conv T = conventional insecticide treated), (d) mean number of seeds depending on management and seed measure (initial = initial seed set, Final.U = final seed set calculated from only undamaged pods, Final = final seed set calculated from both damaged and undamaged pods). Pairwise comparisons of estimated means are indicated by letters in c and d, with means sharing a letter not significantly different ($p > 0.05$).

6 Conclusions and future perspectives

The surface of our planet is an immense network of integrated ecosystems, ranging from the microscopic to the global scale. The inhabitants of the planet are dependent on the proper functioning of these ecosystems. Ecosystems stabilize the climate, generate oxygen, purify air and water, stabilize soils, and prevent flooding, erosion and drought. Organisms that are part of the ecosystems create and maintain soils, decompose waste and recycle nutrients. A vast number of animals pollinate and fertilize plants, protect them from pests and spread their seeds. Ecosystems provide goods which we humans use and trade for food, shelter, medicinal, aesthetic, cultural and many more purposes. Proper functioning of our planet's ecosystems is critical for human survival. However, the sixth mass extinction is ongoing and this time it is us, humans, causing it. Degradation of ecosystems, which follows from loss of biodiversity, is threatening the well-being of our own species, and many, many other species. To turn this trend it is not enough to protect areas here and there. Species and the threats they are facing know no such things as protected area boundaries, they move freely across. Furthermore, to help protect crops and production of food and other products in agricultural systems it is not enough with a protected area far off, instead we must try and implement a sustainable thinking in all our use of natural resources. Insecticides, for now, keep pests at bay, but constantly we run in to problems with development of insect resistance to insecticides. Although development of resistance could potentially be delayed or never occur given proper management, the way we use pesticides today is not sustainable. Instead of relying, *de facto*, almost exclusively on pesticides, it would be more sustainable to work with nature and facilitate functions already provided. In this thesis I show that placing white clover fields further away from where the previous year's clover was grown can help prevent pest population build up. Natural enemies in the form of parasitoid wasps attack pests of clover seeds, but we did not find that an increased parasitism rate increased seed set. The potential for biological control exerted by parasitoids on *P. fulvipes* in the investigated area is at the moment not very high. I believe that this is due to the intensively used landscapes in which we performed our field studies. The proportion of arable land was almost always above 50% and the parasitism rate was in 75% of the cases below 15%. As we found that pest abundance increased, whereas parasitism rates decreased, with proportion arable land, it would be interesting to perform similar and extended studies in less cultivated areas. We also observed that larger pest loads (a combination of field size, inflorescence density and number of emerged weevils from the previous year's field) provided a larger pest population for the next year's seed field. Having smaller fields could both reduce the build-up of pest populations and render the ratio parasitoid-pest abundance more even, as perhaps more parasitoids could be available in the surrounding and able to find the pests, and hopefully this could result in higher parasitism rates. Smaller fields, in general, would give a more diversified landscape, and more border areas with a diverse flora and fauna promoting both pollinators and natural enemies.

Bee abundance and species richness in mass flowering white clover fields were influenced by both local management and landscape context. While clover is a food resource for bees, our study does not support that organic management favors bee abundance and diversity in white clover seed fields; on the contrary it shows lower abundance and species richness in organic fields, possibly caused by presence of preferred alternative foraging habitats around the field, or perhaps less rewarding inflorescences within the field due to environmental factors and management. This however needs further investigation. Although honey bee- and total bee abundance in organic fields were negatively related to semi-natural land, we found that proportion semi-natural land was positively correlated with bee species richness, independent of management.

In our studies, pollinator abundances and richness had no effect on the final white clover seed set, i.e. when pests had also affected the seed set. However the initial seed set, before pests had their go at the seeds, was higher with increased abundance of bees. This indicates that white clover fields do not always have sufficient amounts of pollinators as previously believed. In white clover, abundance of short-tongued bees seems to be enough to render a good seed set, but in red clover, and especially the tetraploid red clover cultivars, flower visits by medium- or long-tongued bees resulted in higher seed set. Surprisingly, medium-tongued bees on the investigated diploid clover cultivar gave much higher seed set than other combinations of bee tongue length and clover ploidy. Short-tongued bees worked slower and their visits resulted in lower seed production compared to medium- and long-tongued bees. This shows the importance of a diverse pollinator fauna, as different species are unequally able to pollinate different species of plants. This was concluded already by Darwin

(1862), who saw that flower shapes and pollinator tongues fitted together in a special manner, and that they were coevolving. The threat of a declining bee fauna should be taken into consideration both for the sake of the crop seed production and for a stable biodiversity in general. By increasing the amount of semi-natural land in the area surrounding agricultural fields we could support a diverse wild bee and natural enemy community and ensure resilient future crop production. As suggested by Bengtsson et al. (2005), the most important factor determining biodiversity at the farm level is probably the attitude of individual farmers and their concrete measures taken to facilitate biodiversity, rather than the farming system used. Therefore, efforts to enhance biodiversity in agricultural landscapes will, in addition to subsidy systems that reward environmentally sound management practices, need the participation of interested and well informed farmers.

Despite the fact that researchers have studied the issue of variable yields in clover seed production for so long, it is still difficult to know how to tackle the problem and ensure a high and even yield. In line with previous studies we found that *P. fulvipes* causes great damage to clover seeds, and this thesis affirms that preventing weevils from finding the new seed field in the first place is of great importance. Although seed-eating pests rather than pollinator visitation is more determining for seed yield in white clover seed production, the detected positive effect of bee abundance on initial seed set, i.e. in flowers collected prior to most seed damage, suggests that seed yield can be increased if we can mitigate the effect of pests and increase bee abundance in white clover seed fields. We found most seeds per pod in conventional treated plots, and as the greatest loss to seed eating weevils and the lower abundance of non-*Apis* bees occurred in organic fields, the implementation of increased distance between fields and reduced field sizes could provide a measure to increase yields especially for organic growers. However, insects are mobile organisms and they are affected by circumstances on scales far greater than farm units, and as all growers in the European Union should be implementing IPM, my findings can be of use to all clover seed growers.

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Populärvetenskaplig sammanfattning

Den mänskliga befolkningen och dess livsmedelskonsumtion fortsätter att öka i en snabb takt och den globala efterfrågan på mat väntas fördubblas från 2005 till 2050. Miljöpåverkan av att möta denna efterfrågan beror på hur vi utökar livsmedelsförsörjningen.

Vårt behov av en ökad matproduktion har länge drivit utvecklingen av jordbruket. Vi har gått från ett småskaligt, traditionellt jordbruk med ett lapptäcke av åkrar, betesmarker, och ängar till ett mer storskaligt, intensivt produktionslandskap. Moderniseringen av jordbruket har resulterat i en större matproduktion men har också orsakat miljöproblem i form av övergödning, utsläpp av växthusgaser, föroreningar, jorderosion och förlust av biologisk mångfald. Det är framförallt fragmentering och förlust av naturliga livsmiljöer samt ökad användning av kemikalier som har lett till denna minskning av biologisk mångfald. Förlust av biologisk mångfald hotar ekosystemens motståndskraft och funktion. Vår planets invånare är beroende av att dessa ekosystem fungerar ordentligt. Ekosystemen stabiliserar klimatet, genererar syre, renar luft och vatten, stabiliserar marken, förhindrar översvämning, erosion och torka. Organismerna som är del av ekosystemen skapar och underhåller jordar, sönderdelar avfall och återvinner näringsämnen. Ett stort antal djur pollinerar och befruktar växter, skyddar dem från skadedjur och sprider deras frön. Ekosystem tillhandahåller produkter som vi människor använder och handlar med för mat, skydd, medicin, estetiska, kulturella och många andra syften. Förlusten av biologisk mångfald hotar således välbefinnandet hos vår egen art och många, många andra arter. För att vända denna trend och möta framtiden på ett varaktigt sätt är det därför viktigt att utveckla ett mer hållbart jordbruk samtidigt som vi bibehåller matförsörjningen för den globalt växande befolkningen. Politiska överenskommelser om att skydda den biologiska mångfalden har signerats, men det faktiska genomförandet och dess framgång är beroende av tillgänglig kunskap och praktiska lösningar. Det finns fortfarande kunskapsbrister angående hur dynamik och sammansättning av funktionella grupper som pollinatörer, skadedjur och naturliga fiender påverkar jordbrukssystem och vice versa.

En gröda som redan nu är viktig ur ett hållbart-jordbruks-perspektiv är klöver. Klöver är en viktig gröda inom både ekologiskt och konventionellt jordbruk, där den används som vallväxt för foder och gröngödsling. I ekologiska jordbruk, där konstgödning är förbjuden, utgör klöver en viktig resurs eftersom den i samarbete med bakterier inuti rötterna kan binda kväve från luften och göra det tillgängligt i marken till nytta för både sig själv och andra växter. Tillgången på klöverfrö är därför viktig inom jordbruket.

Produktion av ekologiskt vit- och rödklöverfrö har sedan 90-talet ökat markant i Sverige, som idag är en världsledande producent av ekologiskt klöverfrö. Dock varierar skördarna kraftigt från år till år och från plats till plats. Denna stora variation innebär negativa ekonomiska konsekvenser för både odlare och fröföretag, bl.a. genom osäkerhet i produktion, lagerkostnad och brist på utsäde. Väder, andra abiotiska faktorer samt skördemetod har stor påverkan på fröskörden, men två av de huvudsakliga faktorerna som orsakar skördeförluster är troligen dålig frösättning på grund av pollineringsproblem, samt skadegörare. Det är dessa två områden som jag har undersökt i denna avhandling.

Eftersom klöver är beroende av insektspollinering för att sätta frö är tillgången på framförallt humlor och honungsbin viktiga under blomningen. Men en stor begränsande faktor är förlust av frön till skadeinsekter. Frö-ätande klöverspetsvivlar av släktet *Protapion*, kan orsaka stora skördeförluster. I konventionell odling kan vivlarna idag bekämpas framgångsrikt med neonikotinoider, en grupp växtskyddsmedel som fungerar som nervgift för insekter. Dessa medel är dock omdebatterade eftersom de har negativa effekter även på pollinatörer och andra nyttoinsekter. Framtida möjligheter att kontrollera vivlar med neonikotinoider hotas därför dels av förbud mot dess användning samt av resistensutveckling hos skadegörarna (något som ofta sker och som skett mot de tidigare använda bekämpningsmedlen). Idag finns det dessutom inget bekämpningsalternativ som kan användas mot klöverspetsvivlar inom den ekologiska klöverfröodlingen. Förekomsten av vivlarnas naturliga fiender, parasitoider, i klöverodlingar tyder dock på att det finns en potential för biologisk kontroll.

Inom rödklöverfröodling har man ytterligare problem med att kromosomfördubblade (så kallade tetraploida) rödklöversorter ger sämre frösättning än så kallade diploida sorter. De tetraploida sorterna är mycket eftertraktade i vallodling pga. sin härdighet och höga produktion av grönmassa.

För att komma till bukt med problemen som klöverfröodlare tampas med och bidra till både en hållbar tillgång på klöverfrö och ett hållbart jordbruk i allmänhet har jag i denna avhandling sökt hitta kunskap om ekologi och biologi hos både nytto- och skadedjur. Jag har bland annat studerat deras spridningsmönster och det omgivande landskapets inverkan på deras populationer, samt relaterat detta till frösättning i klövergrödan.

I studie I undersökte jag dels hur klöversorter med olika kromosomantal (diploida v.s. tetraploida) skiljer sig från varandra i blomegenskaper, samt hur bin med olika längd på tungan samspelar med de olika klöversorterna. Tetraploida sorter hade färre blomhuvud per planta, längre blompip och sämre pollenkvalité (dvs. pollenkornen var sämre på att gro) jämfört med de diploida sorterna. Man har länge vetat att de längre blompiporna hos tetraploida rödklöversorter kan göra att bin med kortare tunga har svårt att nå ner till nektarn och antingen väljer andra blomsorter, biter ett hål och tjuvar nektar underifrån utan att pollinera på ett ”korrekt” sätt, eller får kämpa längre. Jag noterade att bin med kort och medellång tunga föredrog diploida sorter framför tetraploida sorter om de hade ett val, och att bin med kort tunga i allmänhet var långsammare under sina besök på blomhuvuden än bin med medellång och lång tunga. Efter ett besök av ett bi med lång tunga deponerades mer pollen på blommornas pistill. När jag jämförde hur många frön det blev per blomhuvud efter att bara en enda individ besökt blomhuvudet, som ett mått på pollinatörens effektivitet, observerade jag att bin med medellång och lång tunga gav upphov till fler frön per blomhuvud jämfört med bin med kort tunga.

Under realistiska fältförhållanden kommer bin inte att ha valet mellan tetraploid och diploid rödklöver på samma sätt som i våra försök gjorda på plantor i ett trädgårdslaboratorium. Detta eftersom man inte blandar diploida och tetraploida sorter i ett frö-fält. I viss utsträckning kan vilda växter intill klöverfältet vara mer attraktiva för bin som har problem att nå nektarn i tetraploida rödklöverblommor. Jag tror emellertid att den långsammare arbetshastigheten hos bin med kort tunga och den lägre frösättningen efter ett besök av ett bi med kort tunga, i kombination med lägre pollenkvalité och färre blomhuvuden per planta hos tetraploida rödklöversorter, är orsaken till avkastningsskillnad mellan diploida och tetraploida rödklöversorter. De bin som är vanligast i rödklöverfält i dagens jordbrukslandskap är just bin med kort tunga. Man har i andra studier visat att på 1940-talet var det en mer jämn fördelning i humlesamhällena, med fler individer av också medel- och långtungade arter. Idag tillhör mer än 80 % av humlorna som observeras i rödklöverfält de korttungade arterna. Om dessa korttungade bin är vad ett rödklöverfält har till sitt förfogande kommer detta att resultera i att fält med tetraploida plantor får färre blommor pollinerade. D.v.s. en viss mängd bin, under en viss tid kan endast utföra ett visst arbete, och om bina som finns i fälten är mindre effektiva på ett fält med tetraploida plantor jämfört med ett fält med diploida plantor, kommer det resultera i att färre frön produceras i det tetraploida fältet. Till det ska man då lägga att det finns färre blomhuvud per planta i de tetraploida fälten, dvs. färre blommor kan pollineras och ge upphov till frön, samt att pollenkornen från tetraploida plantor i större utsträckning misslyckas med att gro. Man skulle kunna tänka sig att utplacering av fler honungsbin och fler kommersiella humlebon kan hjälpa till viss del, men eftersom dessa pollinatörer är korttungande så är det mer hållbart och tillförlitligt att främja de mer effektiva medel- och långtungade bina.

I studierna II-V utförde jag fältförsök i 45 vitklöverfröodlingar sprida i Skåne. Jag följde hur frö-ätande vivlar förflyttade sig i landskapet över flera säsonger, och också hur bin, vivlar och naturliga fiender påverkades av odlingsform och landskap, samt hur de i sin tur påverkade frösättningen.

Jag fann precis som man sett förut, att antalet vivlar var högre i ekologiska fält och att det resulterade i färre frön. Till min förvåning hittade jag färre bin (exkl. honungsbin) och färre bi-arter i ekologiska odlingar, vilka annars är kända för att bidra till både högre antal och högre artrikedom av både bin och andra insekter. Artrikedomen av vivlarnas naturliga fiender var dock högre i ekologiska odlingar jämfört med insektsbehandlade konventionella odlingar. Anledningen till det lägre antalet bin i ekologiska odlingar behöver undersökas vidare, men skulle kunna bero på mer lockande blommor i landskapet utanför de ekologiska fälten, eller kanske på att blommorna i fältet hade mindre nektar eller såg mindre attraktiva ut på grund av någon brist. Mängden blommor i fälten skiljde sig inte åt mellan ekologiska och konventionella fält, så det bör inte ha varit orsak till skillnaden i antal bin.

Antalet bi-arter i fälten ökade med andelen obrukad mark inom 1 km runt fälten och minskade med andelen brukad mark runtom. Antalet av honungsbin och det totala antalet bin i ekologiska fält minskade dock med andelen obrukad mark i omgivningen. Återigen, skulle detta behöva undersökas vidare för att utröna varför, men skulle kunna bero på lockelser på annat håll.

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