Integrating biological pest control techniques to enhance crop protection

Lucy Crowther

This thesis is submitted for the degree of Doctor of Philosophy

Lancaster University, Lancaster Environment Centre

March 2024

Abstract

Agricultural activities are directly responsible for a proportion of global biodiversity decline and the resulting decrease in local ecosystem services. Alongside changes in grower, retailer and consumer mindsets, legislation and insecticide resistance are limiting the availability of effective chemical controls. These changes put greater emphasis on identifying effective biological controls and understanding how they can work together in an integrated system. This thesis examines the interactive effects of two pest control methods in the field: floral field margins and entomopathogenic nematodes.

In chapter two, a meta-analysis investigates the variability in the success of floral field margins in support of biological control services, identifying the specific groupings of natural enemies which benefit from increased floral resource abundance and species richness. The findings were then used to inform the selection and establishment of floral field margins going forward. In chapter three, an organic brassica system was monitored for a cropping season to identify key pests of concern and understand conservation biological control actions. Following this, chapters four and five investigate the integrated pest control services of floral field margins and entomopathogenic nematodes to assess the efficacy of each method in the control of key brassica pests, while identifying any interactive effects.

Floral field margins had a positive influence on the local natural enemy communities. The entomopathogenic nematode *H. bacteriophora* was identified as a successful biological control agent for the control of the swede midge (*Contarinia nasturtii*). Neither method was an effective control of the cabbage root fly (*Delia radicum*). A significant interaction was found within the organic cropping system, where entomopathogenic nematodes showed consistent levels of efficacy in both vegetation treatments, despite a significantly lower control in the grass treatment. Due to the high economic cost of entomopathogenic nematodes, further work is warranted to increase the positive effect to match current synthetic chemical options.

Contents

List of Tables

List of Figures

Acknowledgments

This thesis was funded by the Waitrose CTP, thank you for providing the opportunity for this all to be possible and thank you, in particular, to Carly Stevens, Roz Wareing and Amanda Weston for your organisation and patience from start to finish.

Thank you to Andy Wilby and Ken Wilson for my supervisory support, you allowed me the opportunity to flex my independence and return for help when needed.

Thank you to the team at Barfoot Farms and Barfoots of Botley, specifically Emma Lilley, Jack Strange, Neil Cairns and Hazel Smith. Whom without I would have had no success in the field. I have learnt so much from you and look forward to learning much more.

Thank you to the iPeg team. I may have been in the wind for much of my time at Lancaster, but when I did appear, I was always welcomed with open arms and smiling faces.

Thank you to the Crowthers for constant support and interest, even though you may not understand why I am doing this.

And a final thanks to Jack, my rock.

Declaration

I declare that this thesis is entirely my own work and has not previously been submitted for the award of a degree elsewhere. Any sections of this thesis that have been published, or submitted for publication, have been clearly identified and collaboration with other researchers acknowledged.

Lucy Crowther

Chapter 1. Introduction

1. **Introduction**

1.1. The farming landscape

1.1.1. Development of agricultural

Agriculture is the science of farming; the preparation and management of the soil to grow crops or raise livestock (Harris and Fuller, 2014). First appearing in the Neolithic Revolution, over 10,000 years ago, the changing climatic conditions triggered a focus on the cultivation of wild plants into crops and the domestication of wild animals into livestock. Through selective breeding, humans were able to create predictable resources and inhabit a sedentary lifestyle (Mazoyer and Roudart, 2006; Swinton et al., 2007). Although the development of agriculture resulted in a significantly reduced diversity of food (and nutrients) being consumed by humans, it allowed for the evolution of a more complex and centralised society (Barker, 2006). Further agricultural revolutions pushed the science of farming forward, developing a more complex understanding of chemistry and soil science, culminating in the Green Revolution (1950s-1960s). Post-war developments introduced high-yielding varieties, mechanised farming and agrochemicals, allowing food production to overtake population growth (Evenson and Gollin, 2003; Washuck, Hanson and Prosser, 2022).

Agrochemicals, the umbrella term used to refer to chemical pesticides and synthetic fertilisers used in agricultural production, were introduced to farming to mitigate the effects of pests and disease and improve soil nutrient content to increase yield (Lamberth et al., 2013). In its most basic term, a pest is any unwanted organism. In farming, this could be an herbivorous pest, a disease or disease vector, a weed or a volunteer crop (Ehler, 2006). Pesticides, in some form, have been utilised within agriculture for a large portion of its existence, originally consisting of inorganic compounds and plant extracts, such as pyrethrins extracted from Chrysanthemums (van Driesche and Bellows, 1996). Although generally considered highly effective, scientific debate has begun to highlight that the yield increase linked to pesticide application is of relatively small consequence considering the long-term wider negative impacts (Washuck et al., 2022). Chemical pesticides have significant associated detrimental impacts on biodiversity, ecosystem services and biological processes (Stark and Banks, 2003; Geiger et al., 2010) and pollution of non-cultivated habitats through runoff or spray drift (i.e. eutrophication; Pavlidis and Tsihrintzis, 2018).

Chemical insecticides have been brought into the spotlight over recent years, with bans placed on specific compounds due to their associated detrimental effects on the wider environment and beneficial insects (Goulson, 2013). The most predominant groups of insecticides are neonicotinoids, organophosphates, organochlorines, carbamates and pyrethroids, with several additional minor groups. Insecticides have several modes of action, the four main modes being: contact, systemic, ingestion and fumigant, with a smaller number that act through growth regulation, gut disruption and repellent/anti-feeders (Thompson, 1996). As well as increasing regulation of their use, pesticide efficiency is decreasing over time due to a build-up of biological resistance (see Figure 1.1; Devine and Furlong, 2007). Resistance refers to the mechanisms an organism develops to overcome a toxin, thus making it ineffective for use in the control of that organism (Barzman et al., 2015; Bras et al., 2022). The reduction in the number of available pesticides leads to overuse, thus increasing the instances of resistance developing (Hillocks, 2012; Sparks and Nauen, 2015). For example, the peach-potato aphid (*Myzus persicae*), a globally economically important pest and plant virus vector, has developed resistance to most classes of insecticide, including pyrethroids, carbamates and organophosphates, due to widespread overreliance on chemical control (Bass et al., 2014). Current cultural, economic and political trends in both agriculture and ecology are pushing toward a reduction in agricultural chemical inputs for the benefit of public and environmental health (Hillocks, 2012; Schaub et al., 2020).

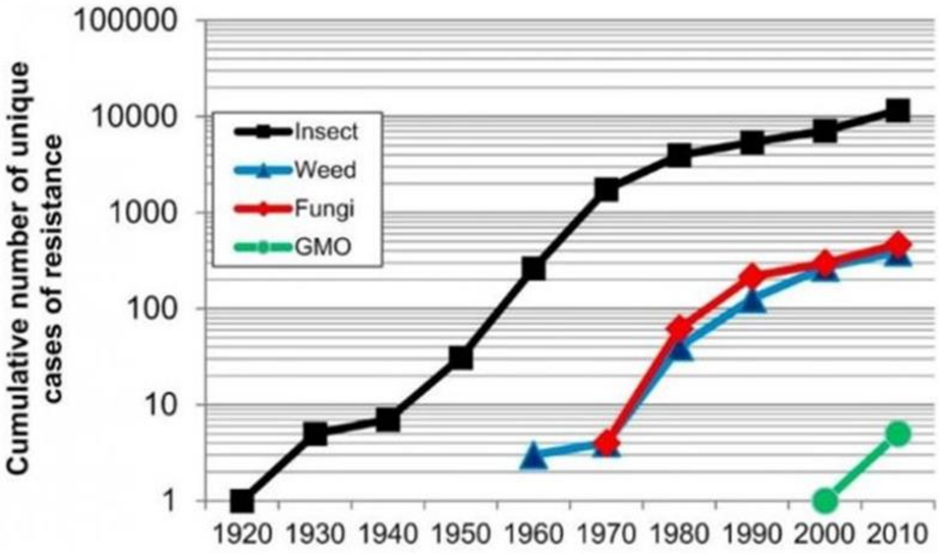

Figure 1.1. The cumulative increase in the number of cases of resistance for pesticide groups. Taken from Sparks & Nauen, 2015. In which data was provided by the Herbicide Resistance Action Committee and Fungicide Resistance Action Committee and Drs. David Mota-Sanchez and Mark Whalon (Michigan State University).

1.1.2. Ecosystem services

An ecosystem service is an environmental process that directly or indirectly benefits humans (Zhang et al., 2007; Noriega et al., 2018). The continuous trade-off for increased crop yield has resulted in a loss of biodiversity in agroecosystems and the ecosystem services they provide (Bianchi, Booji and Tscharntke, 2006; Power, 2010). There are four broad categories of ecosystem services:

a) Supporting services, which are the cycles that nature requires to function, such as pollination, seed dispersal and soil formation. These services support the delivery of other forms of service.
b) Provisioning services, which are services that provide the basic outputs of energy and physical resources, such as freshwater, food, and raw materials.
c) Cultural services, which are non-material services that humans specifically gain from interacting with environments, such as recreational and educational pursuits.
d) Regulatory services, which maintain and regulate the quality of the environment, such as carbon sequestration (Noriega et al., 2018; Schowalter, Noriega and Tscharntke, 2018).

In addition to these vital services, there are a number of disservices associated with biodiversity within ecosystems, such as pests, diseases and geophysical hazards (Schowalter et al., 2018). Invertebrates are the key drivers of several important ecosystem services, including pollination, decomposition, nutrient cycling and pest control (Prather et al., 2013). These beneficial invertebrates can be negatively affected by the application of chemical insecticides, due to the non-specific nature of most insecticides and toxin bioaccumulation (Calvo-Agudo et al., 2020). The niches occupied by beneficial invertebrates can result in these organisms being more greatly affected by chemical insecticides than the targeted pest. Chemical bioaccumulation and increased mobility across the landscape mean that beneficial insects are more likely to be exposed to multiple and/or acute applications (James and Xu, 2012).

1.2. Integrated Pest Management

In the 1950s, a new concept, although theoretically based on old practices was introduced; Integrated Pest Management (IPM) is the combination of multiple pest-control techniques to effectively limit pest populations, with an environmentally conscious emphasis (Barzman et al., 2015). IPM incorporates a number of strategies to limit pest populations, taking from chemical, biological, cultural and conservational toolboxes, holistically (Ehler, 2006; see Figure 1.2). Chemical pest control refers specifically to the use of synthetic chemicals to limit pest populations and/or crop damage, while biological refers to the use of living organisms to achieve this (Baker, Green & Loker, 2020). Cultural pest control encompasses a variety of methods to make the cropped habitat less suitable and reduce crop susceptibility, including actions such as growing resistant crop varieties, tillage, trapping and physical barriers (Glen, 2000). Finally, conservation pest control is the practise of supporting local natural enemies of crop pests to promote abundance and species richness, which is directly correlation with an increase in their biological control services (Ehler, 1998). Crucially, IPM does not aim for the total eradication of pest populations, which is often impossible and risks trophic collapse (Baker, Green and Loker, 2020). Instead, pest populations are maintained below the economic injury level (EIL; Finch and Collier, 2000; Tang and Cheke, 2008). The EIL, or economic threshold, is the smallest number of pests that can be supported before a loss in yield occurs (Peterson and Hunt, 2003).

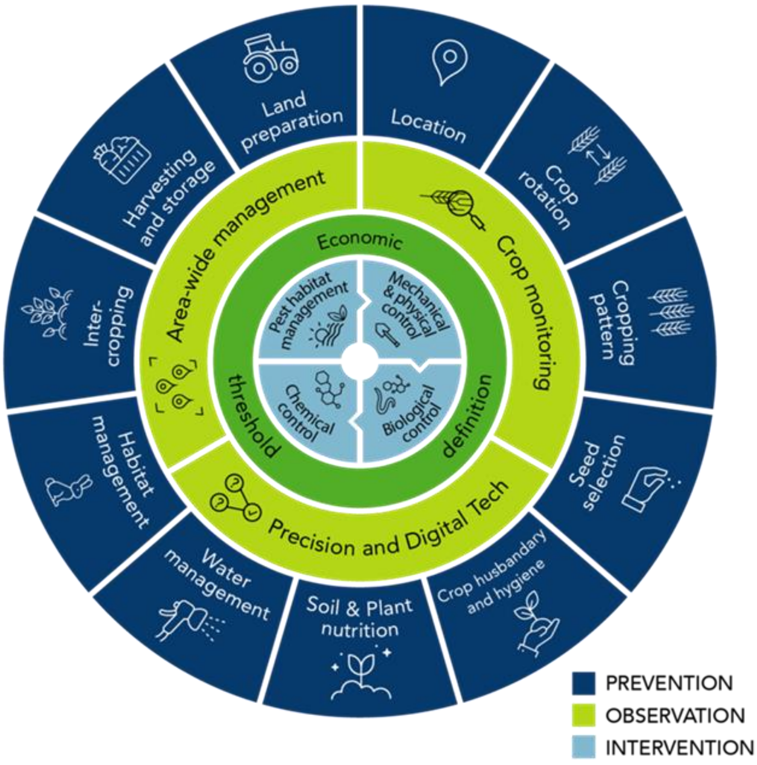

Figure 1.2. Strategies of Integrated Pest Management, taken from CropLife Europe (n.d.).

Barzman et al., (2015) describe eight principles of IPM, of which a combination is essential to create an effective management plan:

i) **Prevention** of pest outbreaks, through the design of a cropping system that has a reduced likelihood of pest infestation and significant economic loss.

ii) **Monitoring** of crops in the field, the use of available forecasting systems and upcoming weather predictions allow for an evaluation of current and forecasted pest infestations.

iii) **Decision-making** based on intervention thresholds, i.e. the point at which a control action must be taken to maintain a pest population below the EIL.

iv) **Non-chemical** control methods are given preference to chemical controls within an IPM system.

v) Considered **chemical pesticide selection**, but best utilised as a last resort.

vi) **Chemical pesticide application**; once a chemical pesticide has been selected, careful planning for application influences its effectiveness in control and its detrimental effects on the wider environment.

vii) **Limiting resistance** to pesticides with appropriate usage of chemical control options.

viii) **Evaluation** of the techniques used allows for adaptation and improvement

1.3. Biological pest control

Biological control can be described as the use of a beneficial organism (the natural enemy) to limit the population densities of another undesirable organism (the pest; Bale, van Lenteren and Bigler, 2007; Baker et al., 2020). The category of natural enemies include predators, pathogens, parasites and parasitoids of pest species (Wegensteiner, Wermelinger and Herrmann, 2015). Understanding the interactions between species is essential when using biological control. Factors such as life cycle, mode of travel and feeding characteristics, of both the pest and natural enemy, influence the effectiveness of each natural enemy as a biological control agent (Thies and Tscharntke, 2010). While many of these considerations are also made for chemical controls, biological controls require a much broader understanding of interactions between biotic and abiotic ecosystem components.

Biological control can be separated into three categories: i) classical biological control aims to establish a permanent population of a specialist natural enemy, outside its native range, to control a pest that is its prey/host species through establishing top-down trophic control

(Pearson and Callaway, 2005); ii) augmentative biological control repeatedly introduces additional individuals into an ecosystem where that natural enemy species is already present, supplementing the existing top-down trophic control (Collier and van Steenwyk, 2004; van Lenteren, 2011); iii) conservation biological control aims to improve the environment to benefit the existing community of natural enemies (Holland et al., 2016). Conservation biological control implements land management practices to provide habitats and resources, reduce environmental disturbance and alleviate the detrimental effect of agricultural intensification, to support local natural enemy populations (Begg et al., 2017). Historically, classical and augmentative biological controls have received more research focus than conservational. The temporary nature of specific crops and periodic landscape disruption prevents the development of the natural predator-prey dynamics that conservation biological control is based on (Symonson, Sunderland and Greenstone, 2002).

1.3.1. Augmentative and classification controls

Natural enemies can be grouped into two classes: i) generalist enemies, which are opportunistic feeders, with little discrimination between food sources, such as many Coleoptera, Chrysopidae, and Araneae, and ii) specialised enemies, such as hymenopteran parasitoids, which utilise a limited number of host species (Geiger, Wäckers and Bianchi, 2008). Generalist predators are widespread and abundant, but their complex trophic interactions can make them unpredictable biological control agents (Snyder and Evans, 2006). Intraguild competition or predation can inhibit the overall success of biological control services, but in other cases, the combined controls of generalist natural enemies can increase the overall suppression of the pest species (Snyder and Ives, 2003; Holland et al., 2016). Direct interaction between two natural enemies or two separate methods of biological control is little researched, though synergistic, antagonistic, additive or redundant interactions among natural enemies, or redundancies in their effect on the target pests, can have a huge influence on the success of a pest control system.

In addition to arthropod natural enemies, non-arthropod biocontrol agents can be purchased commercially to release into cropping systems in the form of entomopathogenic bacteria, fungi, and nematodes, such as *Bacillus thuringiensis* (Bt), *Beauveria bassiana,* and *Heterorhabditis bacteriophora,* respectively. While these entomopathogens have been identified as effective forms of pest control for some time, few have gained widespread acceptance, limited due to the financial cost of production (Federici, 2007). Toxins derived from the spores and crystals of the bacterium Bt have been utilised in commercially available products with the most success, these products are associated with lower environmental risk when compared to synthetic chemical alternatives (Raymond et al., 2010). Although these entomopathogens are more specific than chemical pesticides, there is still a risk that non-target organisms might experience mortality (Shah and Pell, 2003).

1.3.2. Entomopathogenic nematodes

Entomopathogenic nematodes (EPN: Steinernematidae and Heterorhadbitidae) are parasitic roundworms utilised for the biological control of herbivorous insect pests (Lewis et al., 2006). A symbiotic relationship with the bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. allows these genera of nematode to exploit invertebrate hosts to facilitate reproduction (Shapiro-Ilan and Dolinski, 2015). The third stage infective juvenile (IJ) or Dauer nematode enters the selected host through an orifice or the cuticle before releasing symbiont bacteria. Following this, the bacteria begin to proliferate and release toxins that adversely affect the invertebrate, reducing mobility and fertility and ultimately causing death. The EPN will then feed on the bacteria and reproduce. Once the subsequent offspring enter the third larval stage, they will leave the original host in search of another (Ehlers, 2001; see Figure 1.3). The success of each EPN species can vary considerably depending on factors such as foraging strategy, host range, kill time, mode of application and tolerance of environmental conditions (Lewis et al., 2006). EPNs are highly susceptible to biotic and abiotic conditions, including soil moisture and temperature, UV radiation and interaction with fungal and bacterial communities (Gauger, Lewis and Stuart, 1997). While EPNs have shown considerable potential as biological control agents, there is much more knowledge on biology, ecology, application and product development that is needed to improve efficacy such that they can rival currently available chemical measures.

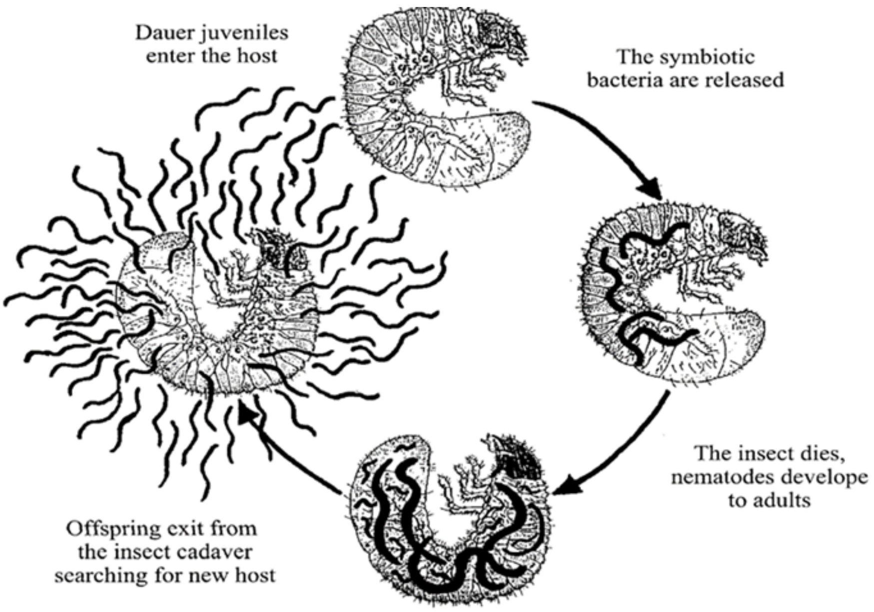

Figure 1.3. The basic stages of an entomopathogenic nematode life cycle (Ehlers, 2001). "Dauer juvenile" is synonymous with "infective juvenile" within the text.

There is a small number of EPN species currently available in the UK for biological pest control purposes; *Heterorhabditis bacteriophora, Steinernema feltiae,* and *S. carpocapsae*. *Phasmarhabditis californica* is an additional pathogenic nematode species available for mollusc pest control. The specific ecological niche and life history of each species have a direct effect on their potential for biological control (Shapiro-Ilan et al., 2006). Each EPN has a select range of host species, meaning it will only provide control of those species. To select a suitable EPN product, one must confirm that the pest is an acceptable host species, is in a susceptible life stage and will be present within the same space and time as the chosen EPN (Lewis et al., 2006). An EPN's kill time specifically depends on the speed of locating a susceptible host and the speed of host mortality. Two foraging strategies are utilised: ambush (sit-and-wait) or cruising (active hunting; Lewis et al., 2006; Grunseich et al., 2021). An EPN utilising the ambush technique will exhibit a behaviour termed "nictation", standing erect on its tail, and waving its head to make direct contact with or detect a host organism (Lortkipanidze et al., 2016). In contrast, cruising EPNs are highly mobile, traversing the environment to locate a host (Lewis et al., 2006). Cruising hunters have a greater chance of encountering stationary and inconspicuous hosts, while ambush hunters have a higher probability of encountering highly mobile hosts (Lewis et al., 2006). EPNs nictating in the upper soil are more likely to be exposed to potentially detrimental environmental conditions, such as dramatic changes in soil temperature and moisture, and physiological UV damage (Gaugler, Bednarek and Campbell, 1992).

EPNs can be mass-produced both *in vivo*, utilising insect cadavers, and *in vitro*, utilising artificial media (Ehlers, 2001). *In vitro* production will require the human-mediated addition of the necessary symbiotic bacteria to ensure biological control success (Cruz-Martinez et al., 2017). The majority of production is conducted using *in vitro* rearing, using a liquid media containing a carbon source, proteins yeasts and lipids. However, this method has a high risk of contamination (Ehlers, 2001). Storage capabilities of each species can vary significantly depending on each species' range of thermal tolerance, their optimum temperature and physiological ability to remain suspended over time (Grewal and Georgis, 1999). For EPN products, the formulation includes the process of turning a living nematode into a product that can be stored, transported and applied in the field without killing the nematode and maintaining high and consistent efficacy (Cruz-Martinez et al., 2017). This is predominantly achieved through stimulating reduced movement and respiration by maintaining a low-temperature environment (Grewal and Georgis, 1999).

As with chemical insecticides, product formulation can affect the efficacy of EPN-based products. The formulation of an EPN-based product is made up of the nematode (acting as the active ingredient), a carrier and potentially an adjuvant. The carrier component supports the active ingredient physically and maintains the optimal environment, which can be a solid (water-dispersible granules, synthetic sponges, activated charcoal, clay, vermiculite), liquid, gel (alginate) or insect cadavers, most often the larvae of *Galleria mellonella* (Shapiro-Ilan et al., 2006; Cruz-Martinez et al., 2017). An adjuvant is a non-lethal substance that can be added to a chemical or biological pesticide to increase its effectiveness, such as a humectant, surfactant, water conditioner, anti-microbial, or UV protectant (Holka and Kowalska, 2023). Adjuvants are used widely in tandem with chemical pesticides and have the same potential to significantly increase the efficacy of biologicals through alleviating the pressure associated with local detrimental biotic and abiotic influences. EPN products are generally applied through spray applicators or irrigation systems. Key consideration must be made to pressure and high or fluctuating temperatures, all of which can cause physiological stress and mortality to EPNs and resulting in reduced efficacy (Fife et al., 2005).

A number of existing peer-reviewed studies show EPN success in the control of a myriad of economically important crop pests, including *Diabrotica virgifera virgifera* (western corn rootworm; Toepfer et al., 2012), *Agriotes* larvae (wireworm; Ansari, Evans and Butt, 2009), Scarabaeidae larvae (Grewal et al., 2004), *Daktulosphaira vitifolia* (grape phylloxera; English-Loeb et al., 1999) and *Bradysia* fungus gnats (Harris, Oetting and Gardner, 1995). However, there is little taxonomic consistency between those pest species trialled and those which show susceptibility. Meta-analyses of this published research highlight apparent limitations of EPN-based control, attributing unsuccessful trials to factors such as inhospitable environments, physiological and morphological resistance and avoidant behaviours from the targeted pest species (Mrácek, 2002), or identifying specific failures in the control of foliage feeders and EPN-based control via foliar application (Arthurs, Heinz and Prasifka, 2004).

1.3.3. Conservation biological control

Modern agriculture has resulted in dramatic declines in agroecosystem biodiversity, particularly beneficial invertebrates. As well as the widespread use of agrochemicals, this loss can be attributed to the decline in semi-natural habitat (SNH) and fragmentation of the remaining SNHs (Landis, Wratten and Gurr, 2000; Parker and Nally, 2002). Habitat destruction to clear land for agricultural purposes has led to the significant oversimplification of landscapes through increasing field sizes and monoculture crop systems (Bianchi et al., 2006). Within such simplified landscapes, there is an absence of natural infrastructure to support a wide array of species, resulting in a lack of resource abundance and diversity, microclimate diversity and physical space. The promotion of greater landscape heterogeneity is associated with more habitat variation, which in turn supports a larger and more diverse supply of food and habitats (Geiger et al., 2008; Holland et al., 2016). Evidence suggests that actions that support agricultural landscape diversification, such as a reduction in field size, can increase local biodiversity without reducing productivity (Tscharntke et al., 2021).

Fragmentation of SNHs occurs when the remaining smaller areas of SNH are left isolated from one another (Haddad et al., 2015). The broader effect of habitat fragmentation is more complex than that of habitat destruction and so ecologists debate the extent that the negative impact of habitat fragmentation contributes to large-scale biodiversity loss. However, the isolation of communities will result in reduced dispersal and gene flow (Fletcher et al., 2018). In terms of biological pest control, due to cropped areas being temporally unstable, natural enemies must have the means to move around the landscape as crops are introduced and removed, and pest populations peak and fall. Natural enemy movement allows for the exploitation of alternative resources, avoidance of harmful agricultural activities and expansion of successful populations (Aviron et al., 2018).

There are a number of landscape management techniques that can be implemented to benefit biological services, such as crop rotation, minimum tillage, intercropping, companion planting and the creation of SNHs (Bianchi et al., 2006). SNHs such as hedgerows, field margins, woodland and ditches have been shown to support diverse natural enemy communities and provide the resources these communities need to thrive (Geiger et al., 2008; Holland et al. 2016). SNH that supports beneficial insects over winter allows for spillover and early colonisation of the cultivated areas at the beginning of the season (Geiger et al., 2008). A popular and widely implemented method of SNH creation is the establishment of floral field margins.

1.3.4. Floral field margins

Field margins are linear SNHs created along the boundary between a cultivated area and the field boundary. The establishment of field margins is one of the most widely incorporated practices aimed at mitigating any detrimental effects of agricultural activities (Zamorano et al., 2020). The practice of establishing field boundaries and areas of uncropped land at the edge of fields supports several major functions in terms of agronomy, environment and nature conservation. These services include increasing pollination and biological control services, stock-proofing, windbreaks for stock and crops, reducing agrochemical runoff and drift, limiting soil erosion, enabling nutrient and water cycling, as well as providing shelter, feeding and breeding sites to local wildlife and allowing their movement across the agricultural environment (Marshall and Moonen, 2002; Olson and Wäckers, 2006). To improve existing field margins, additional diversity can be created through the sowing of floral and fine grass species or the appropriate management to limit certain grass and weed dominance (Olson and Wäckers, 2006).

In terms of IPM, floral field margins provide three key resources to support natural enemies: 1) food resources, which may be pollen, nectar or alternative prey/hosts, 2) refuge, either overwinter or during times of agricultural activities, and 3) reproduction sites (Kinkler et al., 2010). The lack of these resources in agricultural landscapes is a significant limiting factor to biological control services. Most natural enemies are omnivores, either through life-stage omnivory, in which the natural enemy temporarily requires pollen and nectar resources of at least one stage of their life cycle, such as some hoverflies (Syrphidae) and green lacewings (Chrysopidae), or through true omnivory, when a mixed diet is permanently consumed (Orre et al., 2007). Arthropods require pollen for nutrients and proteins and nectar for an energy source (Wäckers, Romeis and van Rijn, 2007). Floral resources have been shown to support increased fitness in natural enemies; specifically increasing longevity (van Rijn and Wäckers, 2015) and fecundity (He et al., 2021). Through increasing floral resources within the landscape, the spatial distribution of natural enemies can be manipulated, as well as an overall increase in abundance and diversity within the crop (van Rijn and Wäckers, 2015). In practice, floral field margins provide positive, although variable, results in terms of pest control services (Shackelford et al., 2013; Albrecht et al., 2020; Crowther, Wilson and Wilby, 2023). Variations in field margin vegetative composition, management and surrounding landscapes can significantly influence the resources provided and success in supporting biological pest control within a crop (Mkenda et al., 2019).

1.4. UK Brassica production

The "cabbage tribe" or Brassicaceae (=Cruciferae) are a large group of important crops worldwide, both economically and nutritionally. The Brassica group provides more types of vegetables than any other, including *Brassica oleracea* (broccoli, Brussels sprouts, cabbage, cauliflower and kale), *B. napus* (oilseed rape; OSR) and *B. rapa* (turnip), with an extensive list of hybrids, subspecies and varieties (Højland et al., 2015). Within the *Brassica* genus, this extensive diversification and presence of undomesticated species in the wild have made it host to a plethora of invertebrate pest species, the presence of which can result in a loss of yield and a reduction in quality and marketability in cash crops (Lowenstein and Minor, 2018). Brassica pests come from a wide range of insect orders: Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, and Diptera, and feed through a wide range of pathways, thus limiting the effectiveness of any single control method (Williams, 2010; Razaq et al., 2011). Sprouting broccoli is a first-generation hybrid cultivar of Broccoli 'Calabrese' (*Brassica oleracea* var. *italica*) and 'Chinese kale' (*Brassica oleracea* var. *alboglabra*). Unlike generic whole-head broccoli, sprouting-type broccoli has a cluster of smaller speared florets forming a loose head and many additional elongated side shoots ending in florets. The plant is harvested three to seven times throughout the growing season, some varieties growing in flushes and others continually producing spears. Spears must be harvested once flowers have fully developed into beads but before they have started to open.

The UK vegetable brassica production covers approximately 30,000 hectares. The bulk of production occurs in Lincolnshire, although brassicas can be grown from Cornwall to Scotland. Thanks to this geographical spread, some brassica crop types can be harvested year-round. (Rakow, 2004). Throughout 2019 to 2024, farming has experienced numerous extreme weather events, changes in agrochemical legislation and dramatic fluctuations in demand around the COVID pandemic, all of which have been reflected in aspects of brassica crop production (planted area; Gov.uk, 2023). The overarching trend highlights that the area of land used for brassica production has been declining over time, interrupted by a upturn in 2020 and 2021 and returning to a pre-pandemic value by 2023. Over this time, yield (tonnes er hectare) across many brassica crops has been steadily declining; the rate of which being crop-dependent. Market price varies depending on the crop, with whole-head broccoli increasing over this period, while cauliflower decreased (Gov.uk, 2023), likely based on trends in end consumer preference.

1.5. Brassica pests

1.5.1. The swede midge

The swede midge, *Contarinia nasturtii,* Kieffer (Diptera: Cecidomyiidae) is a widespread, but relatively farm-specific, agricultural pest across Europe and Asia. The swede midge is invasive to Canada and North America, where it was first reported in 1996 and was confirmed four years later (Hallett and Heal, 2001; Olfert et al., 2006). It has since become a serious concern causing widespread damage and losses of up to 85% (Hallett and Heal, 2001). Due to the variation in morphology of the members of the Brassicaceae family, swede midge damage can present differently based on crop, potentially being misidentified as other pests, nutrient deficiencies and secondary rots. Swede midge larvae cause distortion in young shoots and petioles, brown scarring, development of side shoots and blindness (lack of formation of a head). This damage has variable effects on crop yield: from reduced marketability to total crop failure (Hallett and Heal, 2001; Chen and Shelton, 2007; Abram et al., 2012a).

The adult swede midge (1.5-2 mm) first appears in late May, with evidence that some overwintering larvae can remain in the soil to emerge the second year (Redshaw, 1966; Des Mateaux and Hallett, 2019). The adult midge has a maximum life span of three days, in which females produce approximately 100 fertilised eggs, laid directly onto new plant growth. In approximately three to nine days the transparent larvae (0.3-4 mm) hatch and enter the plant to feed within the meristem for seven to 21 days. The third instar larvae then move to the ground to pupate in the top 2 cm of the soil where they remain for one to three weeks, in earlier generations, or overwinter in the final generation (Readshaw, 1966; Chen et al., 2011). The multivoltine nature results in three to four generations annually, with developmental times of the full life cycle highly reliant on local environmental factors, such as local rainfall and temperature (Hallett, Goodfellow and Heal, 2007).

There are three key considerations for the use of IPM to control swede midge: larvae can reside in the soil for multiple years; the species exhibits overlapping generations; and larvae are partially protected by feeding within the plant tissue. Commercial chemical foliar sprays, chiefly neonicotinoids, are the current conventional control method in Canada and North America (Chen et al., 2011). However, the use of neonicotinoids is limited within the UK, with none currently permitted for use on brassica crops (accurate as of 2024; Gov.uk, 2024). Pyrethroid insecticides (Hallett et al., 2009; Evans and Hallett, 2016) and some organophosphates (Hallett et al., 2009) appear to be toxic to the midge in the lab (Chen and Shelton, 2007). The manner in which the midge resides within the plant protects it from direct pesticide contact, limiting the success of contact-acting insecticides. The AHDB (n.d. a) advises an action threshold for chemical control based on a cabbage cash crop system, at capturing more than four individual males on a pheromone trap per day.

A variety of nonchemical control techniques for swede midge continue to be trialled. Crop rotation requires a minimum of two to three-year intervals between cropping brassica, with suggestions of a minimum of one mile distance between cropping sites (Chen et al., 2011; Hodgdon et al., 2017). Chen, Li and Shelton (2009) simulated crop rotation systems using cauliflower-sweetcorn (*Brassica oleracea* var. *botrytis-Zea mays*) and cauliflower-kidney bean (*Brassica oleracea* var. *botrytis-Phaseolus vulgaris*) systems and successfully reduced the emergence of swede midge larvae in the subsequent year. Intercropping is often used to successfully increase diversity in large monoculture systems and disguise the crop through visual or olfactory disruption (Emery et al., 2021). Sweet alyssum (*Lobularia maritima;* a flowering plant in the Brassicaceae family), and love-in-a-mist (*Nigella damascene)* are successful intercropping partners to broccoli (*Brassica oleracea* var. *italica;* Brion (2015). Garlic, mint, thyme, and eucalyptus lemon essential oils (phylogenetically distant plants) can alter host-seeking behaviour in female midges, leading to reduced oviposition (Stratton et al., 2019). Studies trailing different planting times of OSR (*Brassica napus* var. *napus*) found that early-sown crops had higher larval damage and crops sown in wetter environments had a higher number of midge but showed low levels of damage (Soroka et al., 2018). Altering the timing of sowing, although successful, is difficult to fully integrate into conventional farming due to consumer demands. Floral field margins benefit local natural enemy communities; having the potential to not only increase the abundance and diversity of generalist natural enemies but also specialists, such as the swede midge's associated parasitoid wasp *Synopeas myles* (Walker) (Hymenoptera: Platygastridae; Abram et al., 2012a).

1.5.2. The cabbage root fly

The cabbage root fly (CRF) *Delia radicum*, Linnaeus (Diptera: Anthomyiidae) is an economically important pest of vegetable brassica crops across Europe and North America. The adult flies (5-10 mm) emerge from April and females lay groups of oblong-shaped eggs on the soil surface at the base of host plants. Approximately 100 individual eggs can be laid annually (Evans, 2017). After one week the eggs will hatch, and the larvae (known as root maggots) move into the host plant's root system where they feed for approximately three weeks before pupating (Santolamazza-Carbone et al., 2017). Larval feeding creates tunnels within the root, weakening the plant's ability to absorb and transport water and nutrients, resulting in wilting, discoloured foliage, stunted growth and reduced yields (Herbst et al., 2017). Young plants with less developed root systems are most susceptible to this damage and root vegetable brassica can have significantly reduced marketability at relatively low damage levels (Finch, 1993; Josso et al., 2013). Pupation occurs in the top eight to 12 cm of soil. The CRF will have between two and four generations overlapping annually. The number of generations and lengths of life stages during development are variable depending on climatic conditions, predominantly temperature (Wantulla et al., 2022).

Currently, there is no established treatment threshold based on the number of individual CRFs present. Instead, insecticide treatment is advised prior to transplantation (before the three to four-leaf growth stage), within four days post-module transplantation, or at seed emergency in crops drilled after the third week in April (Bayer, n.d.). Chemical control of the CRF has focused on organophosphates and carbamate insecticides, but these options are limited within the EU and UK (Wantulla et al., 2022). As a root feeder, CRF larvae are protected from contact control measures, limiting measures to predominantly seed treatments, granules or drench applications (Collier et al., 2020; Joseph and Iudice, 2020).

Alternative control methods include cultural controls such as crop covers, entomopathogenic microorganism products and the consultation of monitoring, forecasting and decision-making tools (Herbst et al., 2017; Collier et al., 2020). Companion cropping can limit the CRF’s ability to visually locate a host plant (Finch, Billiald and Collier, 2003) and trap cropping can be used to develop a “push-pull” strategy to manipulate oviposition behaviour (Lamy et al., 2017), although this requires an additional sacrificial brassica crop, which may not be economically possible. Semi-natural habitats have been highlighted as an important feeding resource for adult CRF (Hawkes, 1972), although these same areas support natural enemies. Natural enemies of the CRF include predatory beetles, which are responsible for a large portion of egg and larval mortality, as well as the larval and pupal parasitoids *Trybliographa rapae* (Hymenoptera, Figitidae), *Aleochara bilineata* and *A. bipustulata* (Coleoptera, Staphylinidae; Josso et al., 2013). The EPN *Steinernema feltiae* has consistently shown success in controlling CRF infestation in field and laboratory trials (Beck et al., 2014; Kapranas et al., 2020).

1.5.3. The cabbage stem flea beetle

The cabbage stem flea beetle (CSFB) *Psylliodes chrysocephala*, Linnaeus (Coleoptera: Chrysomelidae) is a major and widespread pest, native to Europe, North Asia and North Africa (Willis et al., 2020). The CSFB is a stem-mining pest, causing damage in both its larval and adult stages, most seriously affecting the European production of oilseed rape (Hoarau et al., 2022; Willis et al., 2020). There is one generation per year, with each female producing up to 1,000 eggs over its lifespan. Oviposition occurs in the Autumn, though, if temperatures remain between 4 and 12°C, it can continue through winter into spring (Højland et al., 2015). Larvae hatch in late September, enter a plant via the petioles and overwinter in the main stem and growing points. After pupating for eight to 12 weeks, adults emerge in late May and begin to feed on the stems and leaves (cotyledons and young true leaves) of Brassicaceae species (Hoarau et al., 2022). Adult CSFB feed on the cotyledons, chewing straight through in circles to cause the classic ‘shot holing’ pattern, while larval feeding causes tunnelling throughout the stems, causing weakening to the roots and stem (Seimandi-Corda et al., 2023; Godina et al., 2023). Primary infestation of the CSFB can result in increased susceptibility to fungal infections and frost damage (Seimandi-Corda et al., 2023). Evaluation of infestation rates, with a control threshold of five larvae per plant, is conducted through visual estimation of crop damage (Ortega-Ramos et al., 2021). Although accurate in assessing true abundance, the concealed mode of larval feeding means plant dissection is essential to obtain a larval count, a method inaccessible to many growers (Seimandi-Corda et al., 2023).

Previously, effective control of the CSFB was established using neonicotinoid seed dressings. However, current EU and UK bans prohibit the use of clothianidin, imidacloprid and thiamethoxam, due to detrimental effects on pollinators (Lundin et al., 2015; Rundlöf et al., 2015). After this loss, the only effective chemical insecticides remaining are pyrethroid-based. Overreliance on this singular remaining control has led to the development of pyrethroid resistance in CSFB populations (Højland et al., 2015). The first report of pyrethroid resistance was in 2018 with the reduced efficacy of lambda-cyhalothrin (Heimbach and Müller, 2013). Pyrethroid insecticides work through the disruption of nervous system functioning, preventing the closure of voltage-gated sodium channels, and causing eventual paralysis (Willis et al., 2020). As of 2020, UK populations can show total resistance to lambda-cyhalothrin (Willis et al., 2020), with no effective chemical alternative on the horizon.

Cultural controls can lessen the severity of infestation; crop rotation, specifically the geographical distance from previous years' crops reduces damage (Ortega-Ramos et al., 2021). For OSR, early or late drilling avoids adult migration and peaks in larval emergence respectively. Environmental conditions linked with high infestation rates include local air temperature exceeding 16°C increasing adult migrations, mild autumns and winters that allow for continuous egg-laying and drilling in unfavourable conditions (excessively dry or wet) which slows crop growth and prolongs vulnerable growth stages (AHDB, n.d. b). Companion cropping has been successful in reducing crop damage, with clover, volunteer cereals and buckwheat producing positive results (White et al. 2020; Ortega-Ramos et al., 2021). Experimental cultivation methods have demonstrated that reduced tillage systems can effectively mitigate larval infestations (Valantin-Morison et al., 2007), though whether this effect directly impacts larval mortality rates or indirectly through natural enemy support remains unclear (Ortega-Ramos et al., 2021). Entomopathogen-based biopesticide products have shown success; fungi species *Metarhizium robertsii* (formally *M. anisopliae*) and *Beauveria bassiana* can reduce CSFB populations in the field by 40-88% (Ortega-Ramos et al., 2021) and the nematode species *Steinernema feltiae* by 39% (Hokkanen et al., 2006). While *Heterorhabditis bacteriophora* has shown success in laboratory trials (Godina et al., 2023).

1.6. Thesis overview

This thesis set out to work towards the development of an Integrated Pest Management system for brassica pests, specifically investigating the interaction between multiple methods of pest control. In Chapter 2, a desk-based meta-analysis of existing, peer-reviewed data on the use of floral field margins in support of biological control services was carried out. Conducting this study prior to experimental trials enabled the findings of this study to be incorporated into the development of field margins within the experimental trials described in this thesis. In Chapter 3, a monitoring-based trial allowed for the identification of key pests of concern within the specific cropping system and understanding the cultivations and field operations needed to grow a successful sprouting brassica crop. In Chapter 4, the first phase of experimental field trials assessed field margins vegetation and three EPN species over varied planting dates to review their combined impact on pest control, crop damage, and yield loss, and identify any synergistic, additive, antagonistic or redundant interactions. In Chapter 5, the second stage of experimental field trials took the most successful EPN forward to trial in a large-scale conventional production system to identify effective application rates, compare it to synthetic chemical control, and again, identify any interactions between control methods. Finally, in Chapter 6, this thesis concludes with a summary of which methods were effective control methods for the key pests and how these methods may be used in tandem to allow for successful pest control. Additional consideration is given to an economic assessment for each control method and realistic options for EPN application to promote wider assimilation.

Chapter 2. The impact of field margins on biological pest control: testing the effects of margin type, development mode and feeding specialism via meta-analysis

This chapter has been published in the peer-reviewed journal BioControl; the following text is the accepted manuscript.

Crowther, L. I., Wilson, K. and Wilby, A. (2023). The impact of field margins on biological pest control: a meta-analysis. *BioControl.* 68, 387-396. doi: 10.1007/s10526-023-10205-6

Author contributions:

Crowther, L. I: Conceptualisation, methodology design, data curation, formal analysis, analysis interpretation, writing – original draft.

Wilson, K: Funding acquisition, conceptualisation, analysis interpretation, writing – review & editing, supervision.

Wilby, A: Funding acquisition, conceptualisation, analysis interpretation, writing – review & editing, supervision.

2.1 Abstract

Floral field margins are known to benefit invertebrate species diversity and abundance within agricultural landscapes, but variation in success limits widespread uptake. Understanding how variation within floral field margins and margins can affect certain entomological groupings is lacking but would allow for a more individualised design of margins to enhance biological control. This meta-analysis aims to answer the question; do floral field margins benefit biological pest control over grassy field margins? Finding that floral margins significantly benefit the natural enemy community and biological control services, relative to non-floral grass margins. We confirm that field margin type is linked to higher abundance and diversity of natural enemies, lower numbers of herbivorous invertebrate pests, and reduced crop damage. We consider whether specific characterisations of natural enemies and pest communities vary between these margin types; finding key differences in the abundances of aerial and epigeal enemies, the diversity of parasitoid and predatory enemies and pest abundances found in naturally regenerating and sown floral field margins. The findings here cement the implementation of floral field margins as a legitimate control method for crop pests in the face of pesticide losses and highlight design and management considerations for the success of floral margins.

2.2 Introduction

Over recent years, pest control in agriculture has become a growing concern. The decline in available forms of chemical pest control, through legislation and declining efficacy, has led to a rising reliance on biological control services provided by the local ecosystem (Chaplin-Kramer et al., 2011). However, the long-term overreliance on chemical control and widespread monoculture systems have caused considerable degradation to these services (Bommarco, Kleijn and Potts, 2013). Ecosystem services, the beneficial services obtained by humans from the environment, have significantly diminished as a whole within agricultural landscapes, due to habitat loss and fragmentation, and intensive agricultural production (Holland et al., 2017; Albrecht et al., 2020). Additional concerns over biodiversity declines on a larger scale have led to the promotion of Integrated Pest Management (IPM), which promotes sustainable, ecosystem-based pest control systems, that focus on long-term results, through the combination of multiple pest control techniques (Barzman et al., 2015). Such techniques include the promotion or restoration of diverse hedgerows, the creation of semi-natural habitats, crop rotation and the development of pest and disease-resistant varieties (Rusch et al., 2013;

Ramsden et al., 2015; Montgomery, Caruso and Reid, 2020). Such pest control techniques are classified as conservation biological control; a broad definition for techniques that reduce reliance on pesticides through promoting beneficiary invertebrates (Begg et al., 2017).

Establishment of floral field margins; linear areas of uncultivated herbaceous habitats, established between crops and field boundaries; is a widely implemented technique that has been identified as a successful approach to combat local biodiversity losses and preserve and restore associated ecosystem services (Marshall and Moonen, 2002; Bianchi and Wäckers, 2008; Bommarco et al., 2013; Albrecht et al., 2020). In the UK, maintaining uncultivated 'green cover' (i.e. grass margins) between boundary habitats or structures (e.g. hedgerows) and the cultivatable area, is incorporated within Cross Compliance; the rules farmers must stay within to receive rural payments (Ernoult et al., 2013). Such grass margins are commonly characterised by low floral diversity, potentially including some species considered agricultural weeds, such as docks (*Rumex* spp.), nettles (*Urtica* spp.) and thistles (*Cirsium* spp.), and a high abundance of competitive grasses (Marshall and Moonen, 2002). Grass field margins support essential resources required by naturally occurring enemies of crop pests, beyond those supplied by the crop (Ramsden et al., 2015; Bishoff et al., 2016). Such resources include shelter (over winter and during times of agricultural activity), oviposition or nesting sites to promote reproduction and food resources (Shackelford et al., 2013; Ramsden et al., 2015; Holland et al., 2016; Albrecht et al., 2020). Floral margins have been highlighted as a prospective option to increase the quantity and diversity of such resources (Karp et al., 2011).

Understanding the effectiveness of floral field margins and the mechanisms by which they contribute to the biocontrol of crop pests is a complex task but can lead to improvement in the establishment and management of future margins (Chaplin-Kramer et al., 2011), as well as adding to our understanding as to why sometimes conservation biological control measures fail (Karp et al., 2018). Consideration can be made to the nature of the establishment and continuous management of floral field margins: i) sown to promote diversity or ii) managed to promote diversity, often termed "naturally regenerated" or "weedy margins". A wide array of seed mixes are available commercially which allows quick establishment of floral field margins, and may be specifically designed to provide diverse floral resources over a long flowering period. Though these mixes can be effective in doing so, they can also introduce non-native species (Garland and Wells, 2020). An alternative method to increase diversity is to develop an appropriate management regime for an existing margin to foster the seed bank and increase species diversity, such as limiting grazing pressure and removal of annual cuttings (Fritch et al., 2011).

Another factor influencing the efficacy with which field margins support biological control is the assemblage of natural enemies delivering pest control. The natural enemies involved may constitute a relatively diverse suite of organisms with differing resource requirements. Within this group, functionally important subdivisions may also be formed depending on factors such as mode of action (pathogens, parasites and parasitoids, and predators), dietary specialism, and guild. The members of each of these groupings depend on different resources, over different timeframes, within the wider ecosystem. Thus, tailoring floral field margins to particularly effective groups, in terms of biocontrol, could be advantageous. Pathogens are microorganisms that cause disease, increasingly utilised by application for biocontrol, much like a pesticide (Lacey et al., 2015). While these organisms are likely to already be present in the environment, floral field margins have the potential to act as a refuge for pathogens, providing alternative hosts to promote continuous infection and a stabilised local microclimate (Baverstock, Clark and Pell, 2008). Many parasitoid adults require pollen and nectar resources for survival and reproduction, meaning the provisioning of a high quantity of quality open floral resources is imperative to promote their associated services (Ramsden et al., 2015). Dietary specialism refers to the broadness of a given species' diet/host range; generalist natural enemies will rely on a myriad of food resources, while specialists will have a narrower diet/host range, utilising a smaller group, or even a singular species (Hsu, Ou and Ho, 2021). Authors disagree with the labelling of some families as distinctly specialist or generalist, a general consensus can be found based on the majority of that group, though outliers may be included. Finally, the guild is a term here used to describe the area in which an arthropod carries out the majority of its activity; aerially (flying) or epigeal (on the soil surface; Martin et al., 2012).

Invertebrate pest species can also be categorised in several functionally relevant ways: for example, based on which life stage causes damage, the mode of feeding, or the morphological development pathway. Here, we use the classifications of endopterygota and exopterygota, reflecting morphological development characteristics. Endopterygota species are those that go through distinct larval, pupal and adult life-cycle stages, characterised by changes in morphology and behaviour. Invertebrate orders that fall into this grouping include Coleoptera, Diptera, Hymenoptera, and Lepidoptera. In contrast, exopterygota closely resemble adults throughout their lives, with small, gradual changes occurring between life stages (Wilby and Thomas, 2002). Hemiptera and Thysanoptera are key orders within exopterygota. The differences between these groupings may be important in terms of biological pest control; endopterygota may occupy entirely differing niches throughout their life cycles, thus there could be a different natural enemy assemblage associated with distinct stages (Strand and Obrycki, 1996; Bernays, 1998). It has been predicted that a greater diversity of natural enemies may be required to provide full control of endopterygota, in comparison to exopterygota, which utilise similar resources throughout their life cycle (Wilby and Thomas, 2002).

Recent meta-analyses confirm that specifically designed floral field margins can positively influence biological control as a whole (Dainese et al., 2019; Albrecht et al., 2020). In this meta-analysis, the addition of categorisation of natural enemies, crop pests and field margin type, will enable a more nuanced understanding of how floral margins influence the local invertebrate population. The main research question in this meta-analysis is: do floral field margins benefit biological pest control over grassy field margins? This will first be accessed using the abundance and diversity of the local arthropod natural enemy community, the abundance of invertebrate pests and crop damage. The following questions aim to further elucidate the role of field margins: (1) Do floral field margins benefit dietary specialists or generalist natural enemies?; (2) Do floral field margins affect natural enemies of differing guilds differently?; (3) Do floral field margins promote pest control services in the local landscape?; and (4) Do floral field margins best promote control of endopterygote or exopterygote herbivorous pests?

2.3 Materials and Methods

2.3.1 Literature search

Studies were selected based on a search of scientific articles in Web of Science Core Collection using the following search terms; (“biological control” OR “biocontrol” OR “pest control” OR “natural enem*” OR predator* OR parasite*) AND (“floral field margin*” OR “field margin*” OR “field border*” OR “field boundar*” OR “field edge*” OR “insectary strips” OR “field strip*” OR “wildflower strip*” OR “flower strip*” OR “grassy strip*”). The search was limited to the last 20 years (2000-2021). Additional searches were done of reference lists to ensure no appropriate studies were missed. Studies were included if they complied with the following criteria: i) made a comparison of species abundance and diversity of invertebrate natural enemies and/or pest species in field margins or adjacent crop, ii) used grass field margins as the control for comparison, iii) had a minimum of two replicates per treatment, and iv) reported test statistics, means and standard error or sample sizes needed to calculate test statistics, for comparative analysis (Pastor and Lazowski, 2017). These criteria were necessary to remove studies that did not fit the topic being reviewed (see Appendix 1, Figure A1.1). Where possible additional test statistics or necessary information was collected on crop damage and yield. Authors of studies that did not report all necessary information were contacted for the original data sets, if data sets were provided the study was included within the study. The search terms produced 556 results (as of 11/02/2021). A total of 171 test statistics were identified from 40 studies that met all criteria for analysis of the influence of field margin composition on pest and natural enemy communities. Studies could produce multiple test statistics if multiple response variables were measured. Three studies produced six test statistics of crop damage, and three studies produced four test statistics of yields (see Appendix 1, Table A1.1).

2.3.2 Statistical analysis

As is common practice (Shackelford et al., 2013; Dainese et al., 2019), for each study, Pearson's correlation coefficient r was calculated based on reported test statistics, or means, sample sizes and standard deviations, using the formulas found in Lipsey and Wilson (2000) and Borenstein et al (2009). This was used to provide a standard unit for comparison to be input into the model. This analysis was conducted using the *metafor* package (Viechtbauer, 2010) in R 4.0.3 (R Core Team, 2021). This package was chosen due to its ability to conduct meta-analyses and moderator (predictor variable) analyses, giving it the ability to fit meta-regression models using continuous or categorical predictor variables. Within *metafor*, the Pearson's correlation coefficient r values were transformed once more using Fisher's z-transformation ($z' = 1/2\ln[(1+r)/(1-r)]$; Hedges and Olkin, 1985) so that the data set was normally distributed. Fisher's z-transformation was selected in favour of Hedges' d, due to the potential bias associated with the latter (Hamman et al., 2018). This transformation function provided the Fisher's z value and a measure of the corresponding variance, based on sample size, to assign a weighting of precision to each study. Test statistics were split by response variable: natural enemy abundance, natural enemy diversity, pest abundance, crop damage and yield.

The meta-analysis was conducted using a random-effect meta-regression (rma function), with a restricted maximum-likelihood estimator to estimate heterogeneity (Viechtbauer, 2005). When testing the influence of predictor variables, a mixed-effect model was utilised (with study as the random effect), to account for multiple test statistics coming from the same studies, and the Wald χ^2 test was used, as a single model was being tested. For each response variable, the model was fit, and then each predictor variable was tested separately, see Appendix 1, Table A1.2 for predictor variable description. For the classifications of species features in the included studies see Appendix 1, Table A1.3.

A test for heterogeneity was conducted to establish variance across all studies; a diagnostic Baujat plot was used to visualise any particular studies influencing the overall heterogeneity, using diagnostics for checking the quality of regression fits. The included studies were reviewed for potential outliers and extreme outliers were removed from the analysis (Baujat et al., 2002; see Appendix 1, Figure A1.2). To assess publication bias, a funnel plot was generated to visually highlight any apparent bias, this visualised standard error against the correlation coefficients (Peters et al., 2008; see Appendix 1, Figure A1.3). Two tests were conducted to test for bias: regression test for funnel plot asymmetry and rank correlation test for funnel plot asymmetry, both giving non-significant results: P=0.609 and P=0.608, respectively, suggesting low publication bias.

2.4 Results

2.4.1 Natural enemy abundance

A total of 92 test statistics reported the difference in the abundance of natural enemy communities between grass and floral field margins. Overall, there was a significant difference in the abundance of natural enemies between grass margins and the floral margins (z=8.71, p<0.001). In the predictor variable analysis, natural enemy type, dietary specialism and floral margin type were found to have no significant influence on the difference found between floral and grass margins. However, the natural enemy guild classification (aerial versus epigeal species), was found to be a significant predictor of the difference between natural enemy abundances (χ^2 =12.921, df=1, p<0.001); see Figure 2.1 and Table 2.1). Although floral strips show higher abundances of both guilds, the difference is significantly greater for epigeal compared with aerial natural enemies.

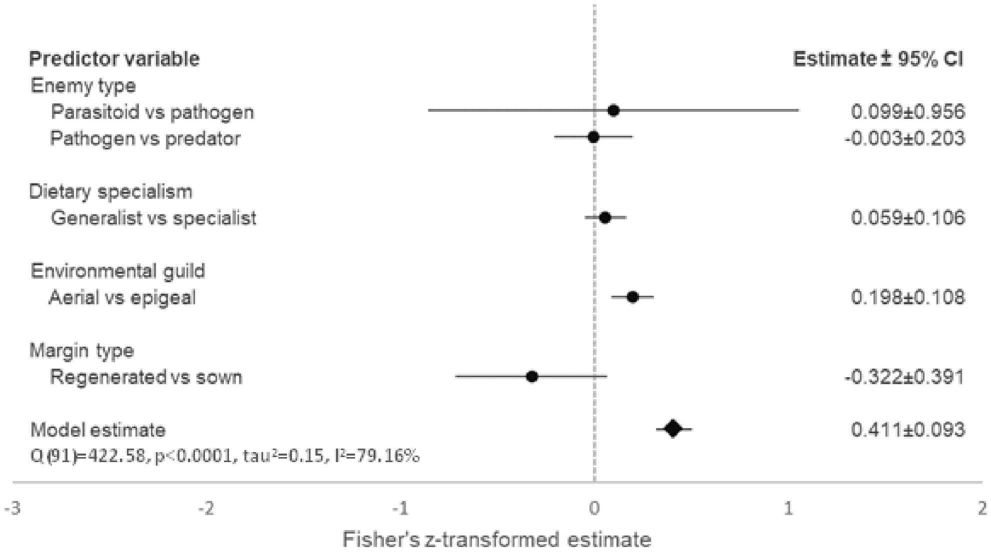

Figure 2.1. The effect estimates of the fixed-effect predictor variables on natural enemy abundance. Estimates were calculated using Fisher's z-transformation, with 95% confidence intervals (CI). An effect estimate is significantly different from zero if the associated CI range does not include zero. The model estimate (diamond symbol) is based on the random-effect model, comparing grass and floral field margins. Additional values are the model degrees of freedom, the test for heterogeneity, random-effect model significance value, tau^2 value (model generated estimate of total heterogeneity) and I^2 value (model generated total heterogeneity).

Table 2.1. Results of the test of moderators within the mixed-effect meta-analysis model, for each measure of effect. χ^2 indicates the results of the Wald-type χ^2 test. Significant p values are shown in bold.

Measure	**Predictor**	**Test of moderators**		
		df	**χ^2**	**p**
Natural enemy abundance	Type	2	0.045	0.978
	Diet	1	1.18	0.277
	Guild	1	12.921	**<0.0003**
	Margin	1	2.599	0.107
Natural enemy diversity	Type	1	5.952	**0.015**
	Diet	1	0.154	0.694
	Guild	1	0.507	0.476
	Margin	1	0.292	0.589
Pest abundance	Development	1	1.484	0.223
	Margin	1	20.748	**<0.0001**

2.4.2 Natural enemy diversity

Overall, the results of the random-effect model (n=24) showed that there is a significant difference in the species diversity between the grass control treatment compared to the floral field margin treatment (z=8.07, *P*<0.001). The predictor variables of natural enemy diet, guild, and type of floral margin showed to have no significant influence on the variability seen between the grass control margin and the floral margin treatments. The diversity of differing natural enemy type groupings did, however, account for some of the variability found (χ^2 =5.952, df=1, *P*=0.015; see Figure 2.2 and Table 2.1). Both parasitoid and predatory species diversity was higher in floral margins compared to grass margins. However, the greatest difference was found in species counts of parasitoid species.

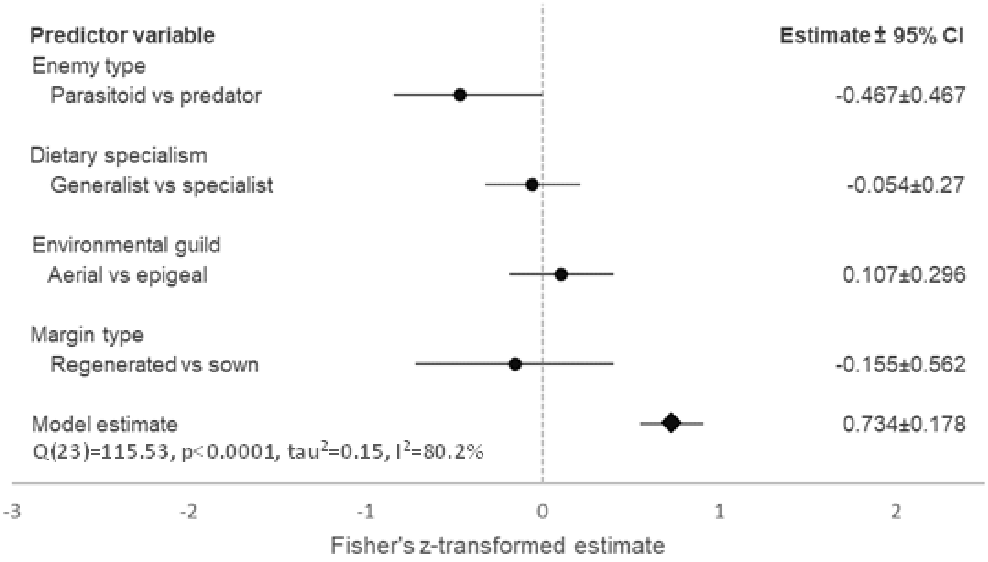

Figure 2.2. The effect estimates of the fixed-effect predictor variables on natural enemy diversity. Estimates were calculated using Fisher's z-transformation, with 95% confidence intervals (CI). The model estimate (diamond symbol) is based on the random-effect model, comparing grass and floral field margins. Additional values are the model degrees of freedom, the test for heterogeneity, random-effect model significance value, tau^2 value (model generated estimate of total heterogeneity) and I^2 value (model generated total heterogeneity).

2.4.3 Pest abundance

Forty test statistics were generated based on the difference between pest abundance in floral and grass margins. Overall, there was a significant difference in pest abundance between areas associated with grass margins and floral margins (z=4.53, *P*<0.001). The abundance of individuals in the pest community was assessed using the predictor variables; morphological development (χ^2=1.484, df=1, *P*=0.223) and the margin type (sown or regenerated; χ^2 =20.748, df=1, *P*<0.001; see Figure 2.3 and Table 2.1). The type of floral field margin (sown to regenerated) accounted for the largest variation found in pest abundances, with regenerated margins contributing slightly more to this variation found between the grass control than sown margins. This result is one of the key results in terms of farmer and grower interest; a reduced number of pests within crops associated with the establishment of both types of floral field margins, and specifically regenerated margins, continuously managed to promote diversity.

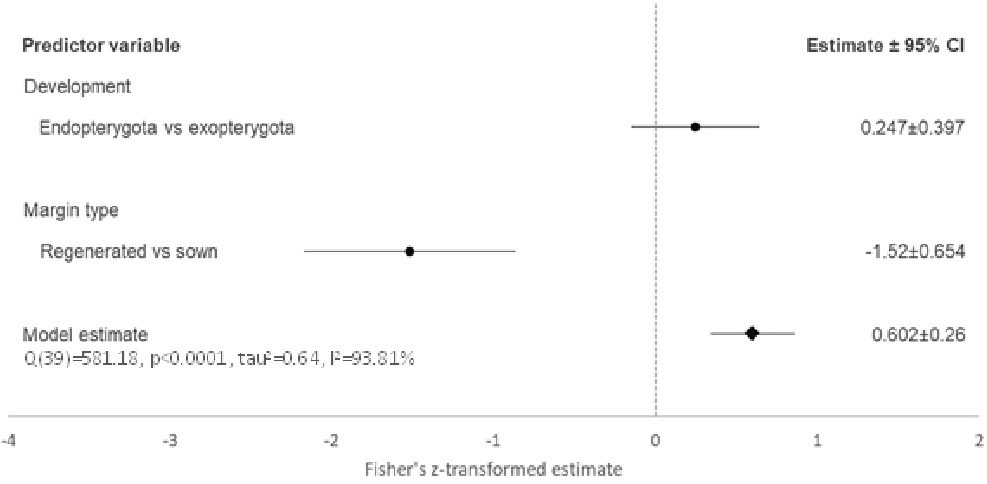

Figure 2.3. The effect estimates of the fixed-effect predictor variables on pest abundance. Estimates were calculated using Fisher's z-transformation, with 95% confidence intervals (CI). The model estimate (diamond symbol) is based on the random-effect model, comparing grass and floral field margins. Additional values are the model degrees of freedom, the test for heterogeneity, random-effect model significance value, tau^2 value (model generated estimate of total heterogeneity) and I^2 value (model generated total heterogeneity).

2.4.4 Crop damage

Analysis of crop damage was limited due to sample size (n=6). Thus, no predictor variable analysis was conducted. Overall, there was a significant difference in crop damage associated with grass (control) field margins and floral field margins, as a whole (z=3.63, *P*<0.001), being lowest when the field margins were floral. As with pest abundance, this is another key metric for farmers and growers, confirmation that floral margins benefit the control of damage in the crop in comparison to grass field margins is imperative for the continuous establishment and management of floral margins.

2.5 Discussion

This analysis of published studies demonstrates a conclusive positive response from biological control services based on the establishment of floral field margins, relative to standard grass margins. We conclude that floral field margins are associated with a significant increase in abundance and species diversity of natural enemies of crop pests, as well as a significant decrease in pest abundance and crop damage. This confirmation that the establishment of specific non-crop habitats increases biological control services is hugely important, not only for farmers and growers in terms of yield quantity and quality but also as a broader incentive to manage habitats in a wildlife-friendly way to benefit ecosystem services (Chaplin-Kramer et al., 2011; Albrecht et al., 2020; Hatt et al., 2020).

2.5.1 Natural enemy communities

Both abundance and the species diversity of the natural enemy community were found to be significantly greater in areas associated with floral field margins compared to grass field margins. This result supports the thought that floral field margins are able to better support beneficial arthropod communities through greater resource provisioning and the creation of stable micro-ecosystems within an unstable landscape (Gardner et al., 2021). It is important to note that a more diverse community can benefit a community's adaptive capacity in the face of local environmental change (i.e. agricultural activities; Hellmann et al., 2016). This is due to a greater number of fulfilled niches, with species that can each tolerate a differing variety of environmental conditions (Tilman, Lehman and Bristow, 1998). Previous meta-analyses highlight the wider local landscape heterogeneity as a key consideration when evaluating the success of floral field margins. Complex agroecosystems offer a higher abundance and diversity of habitats and therefore resources and can act as a source for rapid migration of beneficial invertebrates to new habitats (Shackelford et al., 2013; Holland et al., 2017).

The predictor analysis results concluded that grouping the natural enemy community by enemy type (predator or parasitoid) and guild (aerial or epigeal) can aid us in understanding how natural enemy communities respond to the establishment of floral field margins. In terms of natural enemy abundance, epigeal natural enemies benefitted more than aerial, though both were significantly more abundant in association with floral margins compared to grass. This was unexpected, given aerial enemies largely encompass parasitoid wasps which directly benefit from increased floral diversity (Lavandero et al., 2006; Géneau et al., 2012). However, both ground beetles and spiders, two of the largest groups of epigeal natural enemies, are known to significantly benefit from increased plant diversity, with the benefits being based more on microclimate, vegetation structure and ease of mobility, over nectar and pollen resources (Meek et al., 2002; Ditner et al., 2013).

For natural enemy diversity, the enemy type classification was a significant predictor of the difference between floral and grass margins, with the difference being found in parasitoid (rather than predator) species diversity. Our understanding of the reasoning behind parasitoid diversity benefiting more so from an increased floral diversity is relatively straightforward; a large proportion of this grouping requires pollen and nectar resources in their adult stage to power reproduction and maintain fitness (Lavandero I et al., 2006; Wäckers and van Rijn, 2012). Thus, greater abundance and diversity of floral resources equates to greater resource variability and availability (Ramsden et al., 2015). The predictor variables dietary specialism and floral margin types did not significantly describe any of the differences found between floral and grass margins. It appears that both specialist and generalist natural enemies are significantly supported by floral margins of any kind (McCabe et al., 2017).

2.5.2 Pest abundance & crop damage

Counts of pest abundance were found to be significantly different between grass field margins and floral field margins, with floral margins playing host to fewer individual pests. Likewise, crop damage was shown to be significantly reduced in association with floral field margins, though the limited number of studies that assessed crop damage prevented further analysis of predictor variables. Both pest abundance and cop damage are key for farmers and growers when considering the effectiveness of a control measure; here we can successfully say that floral field margins can benefit biological control services, through the reduction in pest counts and crop damage, more so than standard grass field margins (Letourneau et al., 2011).

Predictor variable analysis found that the type of floral field margin had a significant influence on arthropod pest responses. Of the two types of floral margin, the naturally regenerated margins were shown to support fewer individual pests than sown margins. The reason for this finding could be two-pronged: 1) naturally regenerated margins support more natural enemies and so manage the number of pests, and/or 2) sown margins support more pest species than regenerated, though still fewer than grass margins (Letourneau et al. 2011). There is evidence that herbivorous pests can benefit from the increase in floral species diversity, in much the same way as natural enemies would, as there is some overlap in resource requirements (Winkler et al., 2010; Wäckers and van Rijn, 2012; Karp et al., 2018). Though the predictor variable of morphological development proved to be non-significant, that overall significant increase in both natural enemy abundance and diversity might shroud this result.

2.5.3 Knowledge gaps

The data set collated here was distinctly biased towards predator natural enemies, over parasitoids and pathogens. This imbalance could be due to differences in the ease of surveying and identifying predators, and the total abundance of predators and parasitoids over known beneficial pathogens. The analysis of existing studies identified several areas where more research is needed; studies that included information regarding crop damage and crop yield were generally lacking. The failure to measure these outcomes of increasing local floral diversity highlights the redirection needed in future research, as these are the variables that quantify the effectiveness of floral field margins and increased implementation by persuading farmers and growers (Chaplin-Kramer et al., 2011; Albrecht et al., 2020). The addition of such data into the academic and public sphere will only continue to increase our understanding of floral field margins, and their ability to promote local biological control services. Though one size may never fit all when it comes to cultural biological controls, increasing and collating our knowledge in such ways as this meta-analysis will allow us to understand our failures and develop a reliable methodology to establish floral field margins for biological control.

Chapter 3. An examination of natural enemy and pest communities in an agricultural field: a case study in organic Brassica

3.1. Abstract

Integrated pest management (IPM) is a strategy for pest control that combines multiple techniques to achieve effective and long-term control with minimal negative effects on the wider ecosystem. To utilise this approach effectively, an in-depth understanding of the local environment, biodiversity and associated processes and interactions is required. The conservation biological control method of field margins provides consumable resources, refuge and reproductive sites to beneficial invertebrates, which promotes biological control services. This case study examines an organic brassica production system to review how invertebrate communities, specifically crop pests and their natural enemies, interact with the crop and field margin habitats over a cropping season, identifying floral resources and considering survey methods. The same pest species were identified in both crop and field margin habitats; however, abundances were significantly higher in the cropped area. Natural enemy richness was highest in the field margin, although abundance was highest in the crop, indicating an overspill of natural enemies into the crop to provide biological control services. Pest species abundances fluctuated significantly over the cropping period, while natural enemies remained stable throughout. Pan trapping was the most effective trapping method, collecting the highest abundance and richness of arthropods. However, it is not an appropriate surveying technique for collecting ground-dwelling organisms.

3.2. Introduction

With the decline of semi-natural habitats around the UK, and globally, beneficial invertebrates and their associated ecosystem services have followed suit (Smith et al., 2007; Zamorano et al., 2020). In terms of agricultural production, key invertebrate-driven ecosystem services include biological control of crop pests, crop pollination, and nutrient cycling (Mkenda et al., 2019). As production demand is expected to continue to increase, pressure will continue to be put on agricultural systems to provide higher yields. Crop breeding and the development of agrochemical products can work towards this target, but pesticide resistance is a growing concern (Smith, 2006; Ingrao et al., 2017). The development of new chemical pesticides is a costly and lengthy process, with trends in public perceptions turning against synthetic chemicals, making alternative non-chemical controls more important (Woodcock et al., 2016).

Integrated Pest Management (IPM) is a strategy for pest control that focuses on combining multiple methods to promote long-term, environmentally beneficial control (Barzman et al., 2015). One method widely used in IPM programmes is field margins. These are established alongside cropping habitats to support beneficial invertebrates through the provisioning of vital resources, such as sustenance (pollen, nectar and alternative prey/hosts), refuge habitats, and oviposition sites (Holland et al., 2017; Albrecht et al., 2020). Floral field margins have been found to increase beneficial invertebrate richness and abundance (Smith et al., 2007; Albrecht et al., 2020). However, variations in margin characteristics including botanical composition, total area, management techniques and proximity to other semi-natural habitats, means that margins have varying levels of effectiveness (Woodcock et al., 2016; Ingrao et al., 2017; Zamorano et al., 2020).

When assessing invertebrate communities, no single surveying method can be relied upon to collate a representative sample of all groupings (Ikemoto et al., 2021). Many surveying techniques have been developed and can be categorised into active and passive methods (Hutchinson et al., 2021). Passive techniques, such as pitfall and pan trapping, can be used to obtain data over numerous sites simultaneously, with relatively little human input (Vrdoljak and Samways, 2012). Active methods, such as sweep netting and vacuum sampling require more human input, so data collection is slower. For each trapping method, it must be considered that the collated sample represents a certain proportion of the community, only those susceptible to that given trap, under the conditions in which it has been deployed (Southwood and Henderson, 2009). Existing comparisons of trapping techniques largely focus on flower-visiting insects, specifically bees (Laubertie, Wratten and Sedcole, 2006; Vrdoljak and Samways, 2012) and often on semi-natural habitats only, excluding agriculturally cultivated areas (Hutchinson et al., 2021).

This case study aims to compare arthropod abundance and richness in a field under organic brassica production and its associated naturally regenerating field margin to gain an understanding of how pest and natural enemy communities interact with each other within the two habitats. Three key points were reviewed during this study: 1) How do crop pests and natural enemy communities differ between a permanent field margin habitat and a cultivated habitat? 2) How do arthropods and floral resources change over the cropping season? 3) How do the survey methods differ in their representation of arthropod communities?

3.3. Materials and Methods

3.3.1. Study site

An organically managed field in West Sussex (50.850432, -0.736831), part of a large commercial sprouting broccoli production system, was monitored over the cropping season in

June, July, and September 2021 (an earlier survey was prevented by COVID-19 restrictions). At approximately eight weeks old, plants are treated with an organic insecticide containing 44.03% Spinosad (from *Saccharopolyspora spinosa*) at 12 ml per 1000 modules and transplanted into the cultivated area. Post transplantation, a ferric phosphate-based molluscicide was applied at 5 kg per hectare.

Along the field boundary was a well-established, six-metre-wide, naturally regenerated field margin. The botanical makeup of this field margin included common grasses: common soft brome (*Bromus hordeaceus* subsp. *Hordeaceus*), cocksfoot (*Dactylis glomerata*) and perennial ryegrass (*Lolium perenne*), and common floral species: ox-eye daisy (*Leucanthemum vulgare*), common poppy (*Papaver rhoeas*), white clover (*Trifolium repens*) and common vetch (*Vicia sativa*; for full species list see Appendix 2, Table A2.1).

Ten pairs of survey points were located along two parallel 325-metre transects: one situated 30 metres into the crop and one three metres into the field margin. The survey points began 50 metres along each transect and spaced 25 metres apart. Three survey methods were used at each point; sweep netting, pan trapping and pitfall trapping. The arthropods collected were placed in 70% ethanol for later identification. All arthropods were identified to at least order classification, natural enemies specifically were identified to family and pests to family or species, according to Chinery (1993). Species were classified as pests and natural enemies according to the Encyclopaedia of Pests and Natural Enemies in Field Crops (AHDB, n.d. c).

3.3.2. Invertebrate sampling

Sweep net surveys were conducted between 10 am and 5 pm, under specific weather conditions: air temperature above 13°C, no rainfall, at least 60% clear sky, and low wind speeds (Carvell et al., 2016). At each survey point, 25 figure-of-eight sweeps were carried out within a 10-metre radius of each survey point. This was an arbitrary number of sweeps that remained the same at each sampling point. A triangle of three colours of pan traps (white, yellow, and blue) was set at each survey point. Pan traps measured 19 cm in diameter and were half-filled with 10% saline water and a few drops of unscented detergent (ECOVER Zero) and set at the height of vegetation/crop at the time of surveying. For pitfall trapping, a plastic cup (10 cm diameter), was buried to 1 cm below the soil surface and covered with 4 cm metal mesh. Pitfall traps were half filled with a 10% saline water and unscented detergent mixture. Traps were set for 48 hours per visit.

3.3.3. Data analysis

Analysis was conducted using a Generalised Linear Mixed Model, Maximum likelihood and normal error distribution, using the *lme4* package (Bates et al., 2015) in R version 4.0.3. (R Core Team, 2022). A number of measures of the invertebrate communities were compared between field and margin: total invertebrate abundance and richness to the classification order, natural enemy (predators and parasites/parasitoids) abundance and richness to the classification family and pest abundance. Three explanatory variables were identified; *habitat* (field or margin), *survey method* and *date* surveyed, with survey point and date also set as random terms. All two-way interactions were included in the original model, non-significant interactions were dropped.

3.4. Results

3.4.1. Crop pests

The same major pest species were sampled from both habitats; fleas beetles, including the cabbage stem- and the striped- flea beetles (*Psylliodes chrysocephala* and *Phyllotreta striolata*), aphids (Aphidoidea), the swede midge (*Contarinia nasturtii),* the cabbage root fly *(Delia radicum)*, and the large and small cabbage white butterflies (*Pieris brassicae* and *P. rapae*; see Figure 3.1). Additional minor pests were identified in negligible abundance: the turnip sawfly (*Athalia rosae*) and the cranefly (larvae known as leatherjacket; *Tipula paludosa* and *T. oleracea.*). The three pest species found in the highest abundance were the swede midge, the cabbage root fly (CRF) and the cabbage stem flea beetle (CSFB).

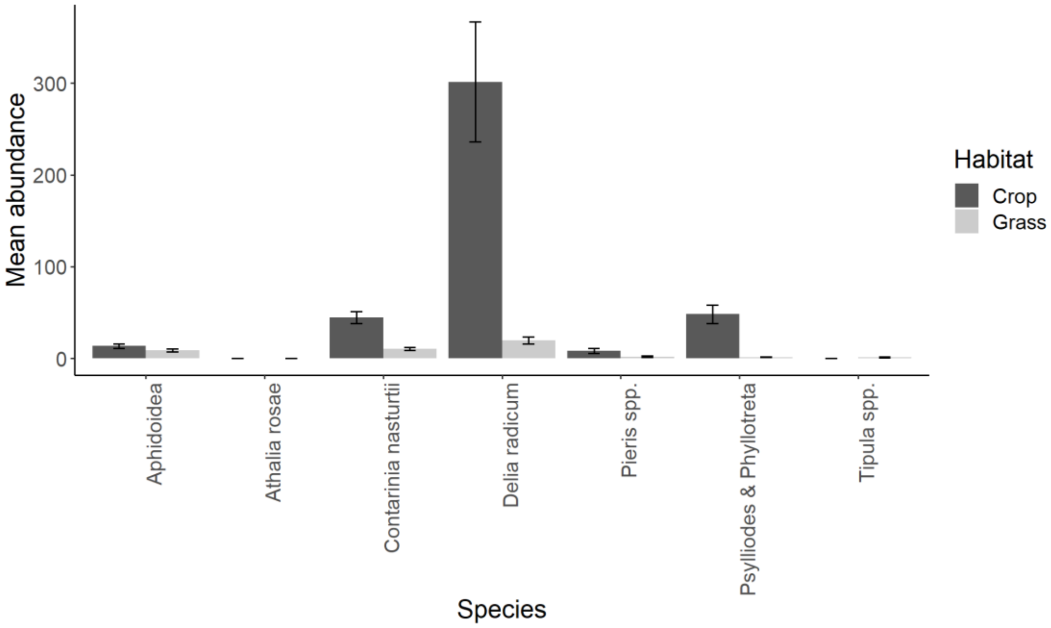

Figure 3.1. Mean abundance of common brassica pests across the entire survey period; *Delia radicum* (the cabbage root fly), Aphidoidea (peach-potato aphid and potato aphid, no cabbage aphids were found), *Contarinia nasturtii* (the swede midge), *Pieris brassicae* and *P. rapae* (large and small cabbage white butterflies combined), *Psylliodes chrysocephala* and *Phyllotreta striolata* (the cabbage stem and striped flea beetles combined), *Athalia rosae* (the turnip sawfly) and *Tipula paludosa* and *T. oleracea* (the cranefly). Error bars indicate standard error.

Statistical comparison of both pest abundance ($F_{1,300}$=11.01, *P*=0.002) and species richness ($F_{1,300}$=28.74, *P*<0.001), found significantly higher counts in the cultivated habitat (see Figures 3.2a and b). When analysed individually, abundances of the flea beetles, aphids, the swede midge, the CRF and the cabbage white butterflies (large and small combined) were found to all be significantly higher in the crop habitat (see Table 3.1). Only the turnip sawfly (*Athalia rosae*) and the cranefly (*Tipula* spp.) were found in greater abundance within the field margin.

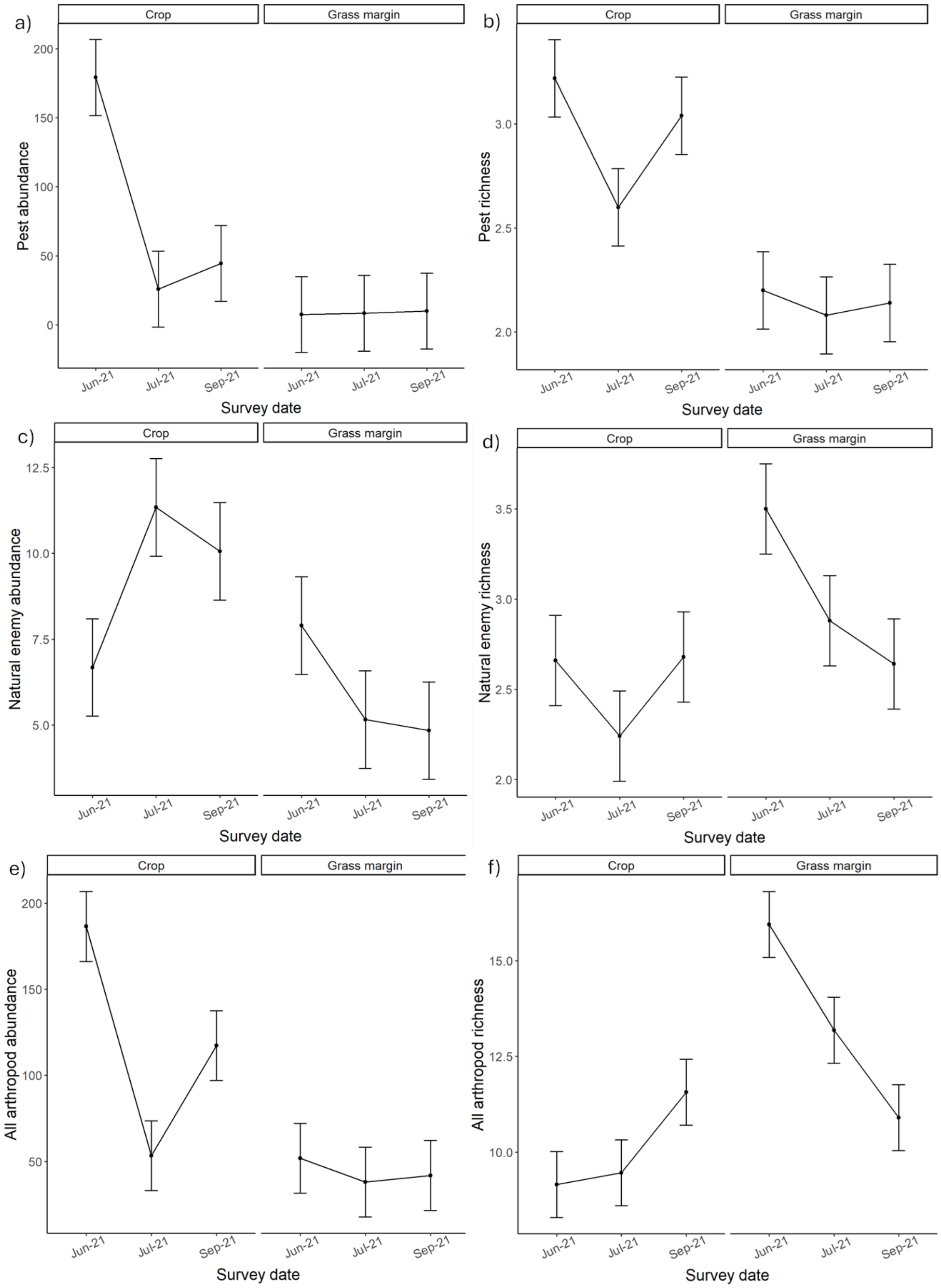

Figure 3.2. GLMM model generated estimates: a) pest abundance, b) species pest richness, c) natural enemy abundance, d) natural enemy group richness, e) all arthropod abundance and f) all arthropod order richness, for the interaction between habitat (cropped area and grass field margin and survey date. Error bars indicate standard error.

Table 3.1. Results of Generalised Linear Mixed Model, fitted with Gaussian error and maximum likelihood estimation. *Date* set as a random factor. Significant variables highlighted in bold.

Measure	**Variable**	**df**	**F**	**P**
Pest abundance	Habitat	1,300	11.01	**0.002**
	Survey method	4,300	3.07	**0.02**
	Date	2,300	4.53	**0.03**
	Habitat x date	2,300	4.72	**0.02**
Pest species richness	Habitat	1,300	28.74	**<0.001**
	Survey method	4,300	19.06	**<0.001**
	Date	2,300	2.06	0.15
	Habitat x date	2,300	0.99	0.39
Natural enemy abundance	Habitat	1,300	8.52	**0.006**
	Survey method	4,300	7.58	**<0.001**
	Date	2,300	0.26	0.77
	Habitat x date	2,300	3.99	**0.03**
Natural enemy family richness	Habitat	1,300	5.75	**0.02**
	Survey method	4,300	10.01	**<0.001**
	Date	2,300	2.53	0.2
	Habitat x date	2,300	1.77	0.19
All arthropod abundance	Habitat	1,300	11.371	**0.002**
	Survey method	4,300	4.913	**0.003**
	Date	2,300	3.929	**0.03**
	Habitat x date	2,300	2.697	0.08
All arthropod order richness	Habitat	1,300	18.566	**<0.001**
	Survey method	4,300	15.256	**<0.001**
	Date	2,300	3.557	**0.04**
	Habitat x date	2,300	7.653	**0.002**

3.4.2. Natural enemies

A total of 931 individuals from 21 families were found in the field margin habitats, compared to 1,501 individuals from 18 families in the crop (see Figure 3.3). Analysis of variation between the two *habitats* found a significantly higher abundance in the crop habitat ($F_{1,300}$=8.52, *P*=0.006, Figure 3.2c), while the margins supported significantly higher family richness ($F_{1,300}$=5.75, *P*=0.02, Figure 3.2d). The most common natural enemies across both habitats were parasitoid wasps (Chalcidoidae, Cynipoidae and Ichneumonidae; 705 combined), dung flies (Scathophagidae; 387), rove beetles (Staphylinidae; 294) and wolf spiders (Lycosidae; 243).

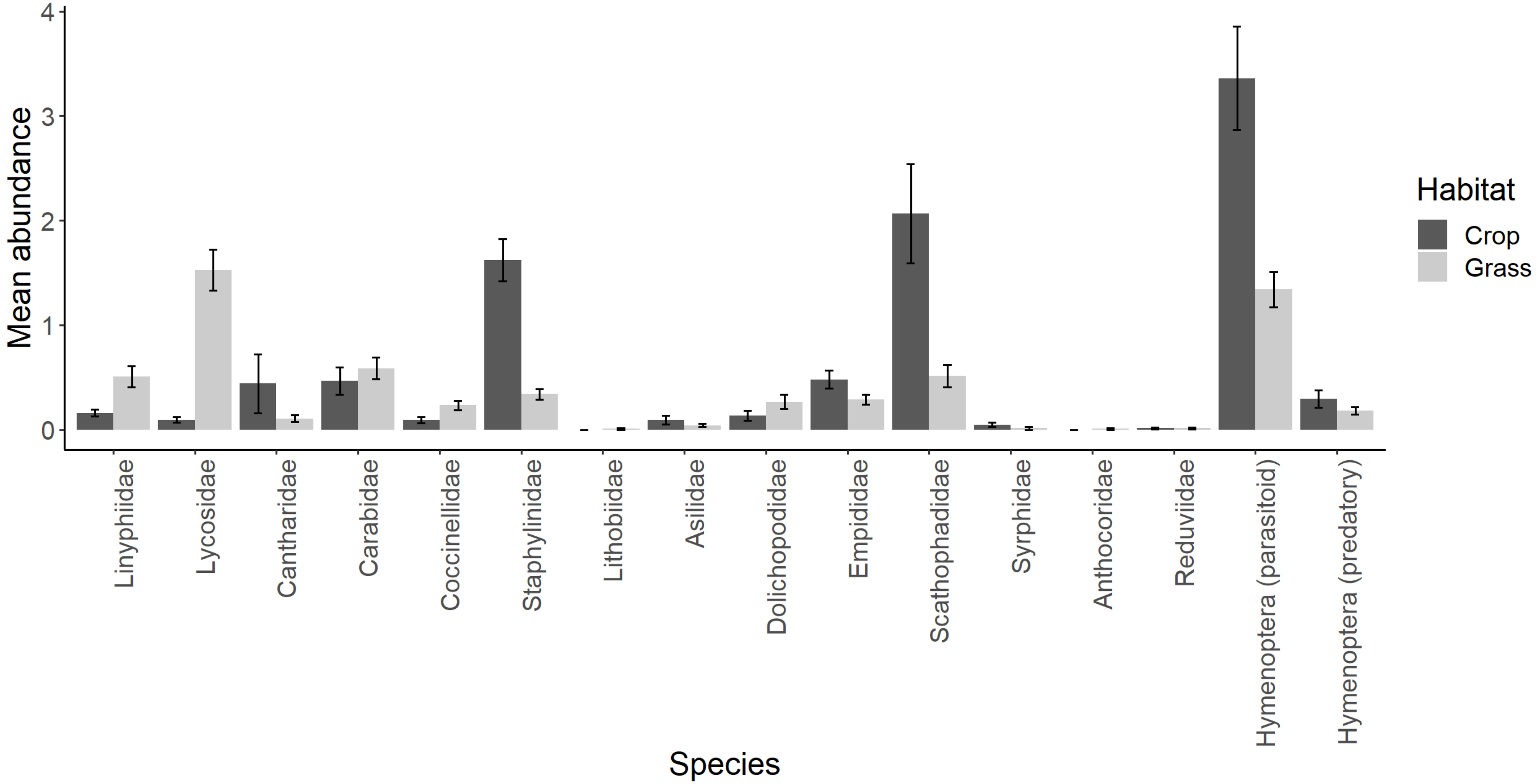

Figure 3.3. Mean abundance of natural enemies families from the crop and the grass field margin habitat. Predatory Hymenoptera includes Pompiloidae and Vespula, parasitoid Hymenoptera includes Chalcidoidae, Cynipoidae and Ichneumonidae. Error bars indicate standard error.

3.4.3. All arthropods

A total of 25,266 organisms were caught across the entire survey period. A significantly higher abundance of individual invertebrates was found in the cultivated area compared to the field margin ($F_{1,300}$=11.37, P=0.002, Figure 3.2e), totalling 18,674 individuals to 6,581. However, the majority of which are classified as pests. While a significantly higher family richness was found in the field margin ($F_{1,300}$=18.57, P <0.0001; see Figure 3.2f).

3.4.4. Phenology

Comparing measures over the timeline of the study, the analysis found that for pest communities, *date* surveyed significantly influenced abundance ($F_{2,300}$=4.53, P=0.03), but did not species richness ($F_{2,300}$=2.06, P=0.15). For natural enemies, *date* surveyed did not have a significant influence on abundance or richness ($F_{2,300}$=0.26, P=0.77; $F_{2,300}$=2.53, P=0.2). Any significant time-based fluctuations in pest and natural enemy abundance occurred in the cultivated habitat only (see Figure 3.4). There was a significant interaction between *habitat* and *date* on abundance only, for pests ($F_{2,300}$=4.72, P=0.02) and natural enemies ($F_{2,300}$=3.99, P=0.03), indicating that the degree of difference in abundance between habitat types varied throughout the survey season.

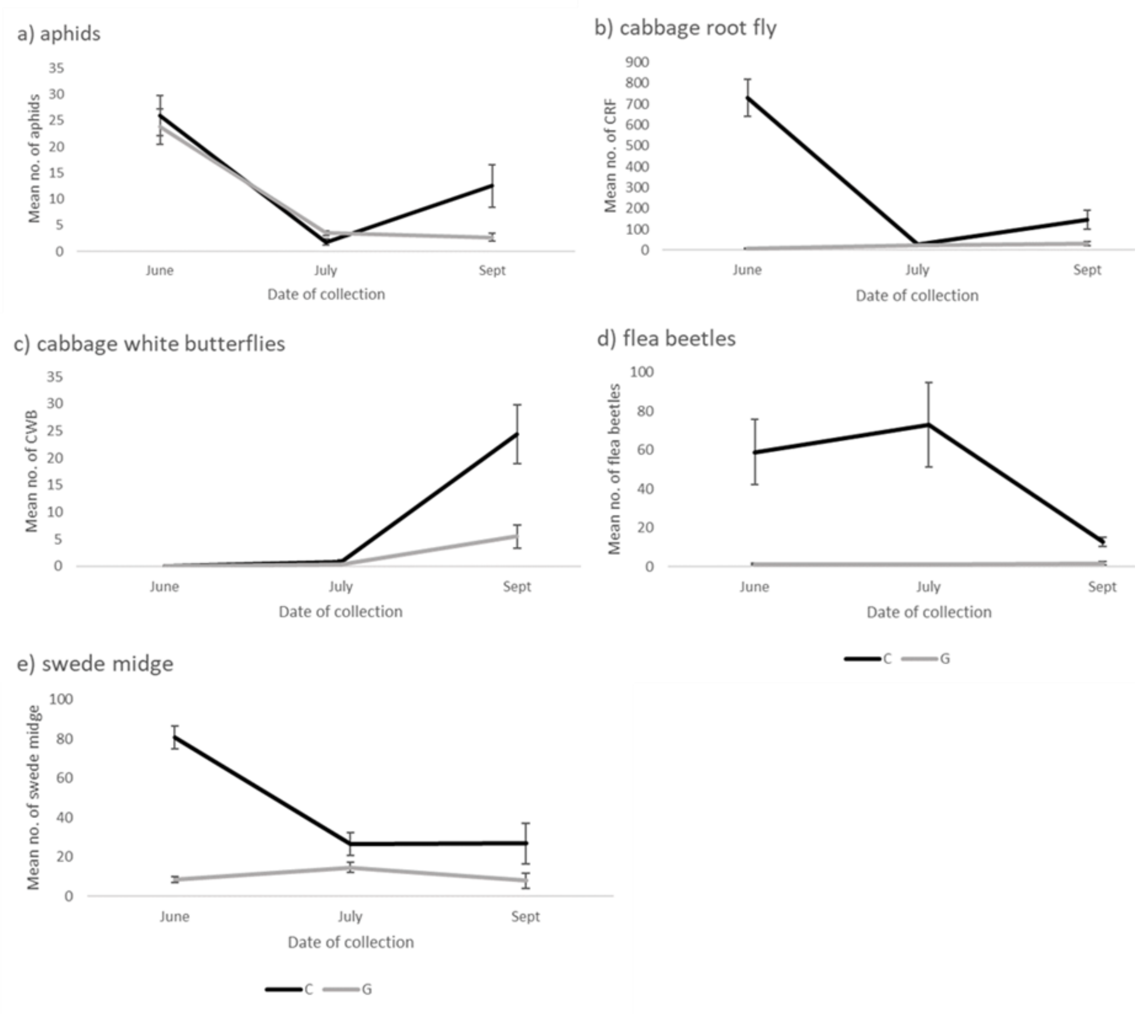

Figure 3.4. Mean abundances of key pests collected from the grass field margin habitat (G) and crop (C) over the entire study period. a) aphids (Aphidoidea), b) cabbage root fly (*Delia radicum*), c) cabbage white butterflies (*Pieris brassicae* and *P. rapae*), d) flea beetles (*Psylliodes chrysocephala* and *Phyllotreta striolata*) e) swede midge (*Contarinia nasturtii).* Error bars indicate standard error.

Vegetation surveying of the field margin identified several naturally occurring floral species. The majority of recorded species flower over June, July and August, with at least one species in flower from April to October (see Figure 3.5). One species that is a member of the Brassicaceae family, the hedge mustard (*Sisymbrium officinale*) appears in the field margin, this species is of note as it has the potential to act as a host to Brassica pests.

Figure 3.5. Floral resources bloom chart of species found in the grass field margin. *Indicates members of the Brassica family.

3.4.5. Survey method

The surveying method utilised had a significant influence on the abundance and richness in the samples collected (see Table 3.1). Mean counts show the highest abundance and richness was collected by pan trapping (averaged over all three colours), followed by sweep netting and pitfall trapping, although the difference between the latter was negligible. Breaking down samples by arthropod Orders: pan trapping attracted the highest levels of abundance and richness in Coleoptera, Diptera and Lepidoptera, Hymenoptera and abundance of Hemiptera. Pitfall trapping attracted the highest abundance and richness of Arachnids and abundance of Hymenoptera, though this was skewed by Formicidae captures. Considering the colour of pan traps, yellow pan traps consistently collected the greatest richness and second highest counts of abundance. On average, white traps collected the highest overall abundance, however, this was skewed due to a high capture rate of CRF.

3.5. Discussion

3.5.1. Do crop pest populations differ between a permanent field margin habitat and a cultivated habitat?

Monitoring identified the same community makeup of pest species within both habitats, likely indicating some degree of movement between both areas. Three pests were sampled in higher abundances than all others: the swede midge, the CRF and the CSFB, combined with anecdotal evidence this identifies them as pests of significance in this cropping system. The higher abundances of pest species found in the cultivated area were expected, given these insects utilise Brassica plants as food sources at some stage of their life cycle, for most species this is the larval stage (Denys and Tscharntke, 2002; Olson and Wäckers, 2006; Ingrao et al., 2017). When considering the high richness of pest species residing within the grass margin habitat, this could be due to one or all of the following processes: a) pest overspill from the cultivated habitat into the field margin or, b) pests shelter in the margin habitat and move into the cultivated habitat when an appropriate host crop is established, and/or c) pests reside in the field margin all year round. For field margins to support a large and diverse natural enemy community a prey population must remain available within the margin itself (Bischoff et al., 2016; Pollier et al., 2018; Albrecht et al., 2020). Thus, a controlled pest population residing within the field margin is not a wholly negative result.

3.5.2. Do natural enemy communities differ between a permanent field margin habitat and a cultivated habitat?

For natural enemy abundances, data interpretation is slightly more complex; more rove and solider beetles (Staphylinidae and Cantharidae), predatory flies (Empididae, Scathophagidae and Syrphidae) and parasitoid wasps were collected from within the cultivated habitat compared to the field margin, which demonstrates that invertebrate-driven biological control services are likely being provided to the crop. The field margin habitat was favoured by predatory spiders (Linyphiidae and Lycosidae) and carabid beetles (Carabidae). These groups are effective natural enemies, existing literature finds that spiders favour grass habitats over cultivated areas (Plath et al., 2021), and carabid communities in crop habitats can vary little in response to surrounding semi-natural habitats (Werling and Gratton, 2008). Predatory bugs were found in comparably low abundances in both habitats. However, the benefits of semi-natural habitats for predatory bugs are under-investigated in comparison to other biological control agents (Atakan, 2010).

This high abundance of many natural enemies within the cultivated habitat is most likely a result of overspill from the field margin, where natural enemies shelter over winter and during agricultural activities (Dennis and Fry, 1992; Ramsden et al., 2015). Natural enemy species richness and diversity has been linked directly with conservation biological control services; a greater number of species promotes more effective biocontrol (Holland et al., 2016). Found here in high numbers, rove beetles (Staphylinidae) are omnivorous feeders. This dietary versatility allows them to reside within cultivated habitats before pest infestations, subsisting on vegetation, fungi and seeds (Balog, Mehrparvar and Weisser, 2013). Parasitoid wasps (Chalcidoidae, Cynipoidae and Ichneumonidae) were also abundant, aerial mobility allows wasps to be early colonisers (Mansion-Vaquié et al., 2017). Thus, aerial natural enemies are one of the most effective biological control agents (Holland et al., 2008).

3.5.3. How do invertebrate communities and floral resources change over the cropping season?

The assessment of pest and natural enemy phenology shows fluctuations in abundance over the survey season. While pest infestation rates vary significantly in the crop, the same species remain in low and stable numbers in the field margin (Figure 3.3). The heterogeneity of a habitat is linked with the stability of arthropod communities (Holland et al., 2016). Increasing habitat complexity, at the landscape- and field-level, enables a stable community to regulate itself through the development of predator-, or parasite-prey interactions and so limiting peaks in individual populations (Zhao et al., 2015; Morgan et al., 2017). Cycles of pest outbreaks in agricultural systems demand constant monitoring and application of pesticides to limit peaks, which in turn have detrimental effects on beneficial invertebrates (Bommarco et al., 2011; Ndakidemi, Mtei and Ndakidemi, 2016). Focusing on an increase in non-crop habitats, or crop type (polyculture) can promote in-field insect community stability and so reduce the reliance on pesticides (Rusch et al., 2013).

An agriculturally cultivated field is a temporary habitat, changing throughout the year based on cropping activities and from year to year based on crop rotation, thus continuous re-colonisation is needed (Frouz and Kindlmann, 2015). Source-sink dynamics describe one habitat of "high quality" (the source) supporting a growing population that can supply immigrants to a habitat of "low quality" (the sink; Rosenheim, 2001; Cohen and Crowder, 2017). In this case, quality may not refer directly to resources, but to longevity. Field margins are a source habitat, supplying natural enemies that migrate into the cultivated habitat. Organic management can allow beneficial invertebrates to move into cultivated habitats with higher rates of survival than in conventionally managed landscapes (Muneret et al., 2018; Török et al., 2021). The negative side to this, in agricultural terms, is that pest communities can do the same, moving to shelter in field margins post-harvest, and reappearing when appropriate crops are established (Fusser et al., 2016; Ingrao et al., 2017).

Recording the vegetative composition of the field margin allows us to build an image of which resources are being provided and when, depending on the species. To be beneficial, the introduced resources need to temporally overlap with the natural enemy's needs (Ingrao et al., 2017). Field margins that include a mix of native or naturalised herbaceous species and tussock grasses have been shown to also provide synchronised resources, alternative prey resources and shelter habitats (Torretta and Poggio, 2013; Ramsden et al., 2015). As previously mentioned, a wild brassica species, hedge mustard (*S. officinale)* is present in the field margin habitat. The presence of wild, related species has negative implications for pest control; related weeds can interfere with crop rotation, supporting pests between plantings (Haramoto and Gallandt, 2007). It can be determined, based on flowering times, that this field margin will provide floral resources from approximately May to October. High colour diversity in floral resources has been linked with high invertebrate diversity (Hoyle et al., 2018). The most common colour to floral species is yellow, which is regarded as the most widely visited of colours, highly detectable by foraging insects (Vrdoljak and Samways, 2012).

The selection of survey method is hugely influential on the abundance and richness of arthropods collected. Considering where within the habitat strata a species resides, feeding behaviours (hunting versus ambush, foraging), and how a species moves throughout its environment, all contribute to which technique is most likely to intercept that species (Shrestha et al., 2019). Pitfall trapping was best able to collect Arachnida (spiders, mites and harvestmen), and Carabid and Staphylinid Coleoptera (ground and rove beetles). Such groupings classically reside at ground level and are active hunters, moving about the environment across the ground and so intercepted by the pitfall method. Here, pan traps proved to be the most successful trap type; differing colours showed variable performance, with yellow and white traps proving most successful at gathering the largest and most diverse sample. Colour is known to attract different arthropod taxa, though the context is important; background contrast, angles, and texture (Ikemoto et al., 2021). In this study, yellow and white were the most contrasting colours to the brown and green landscape, which could explain their success.

3.5.4. Future research

A key outcome of this case study investigation was to highlight gaps in farming practice that need to be addressed to create a tailored Integrated Pest Management for this cropping system. Based on the results here it appears naturally-regenerating grass field margins can support natural enemies, but do the services associated with this grouping translate into the cultivated area and reduce the detrimental effects to the crop? Integrated Pest Management systems are most successful when multiple techniques are utilised in collaboration, ideally working in synergy to have an even greater positive effect (Gurr and Kvedaras, 2010; Barzman et al., 2015). While it is well known that field margins can benefit biological control services as a whole, they lack species-specific and timely control of key agricultural pests (Winkler et al., 2010). Additional non-chemical pest management methods include companion planting/ intercropping, application of entomopathogenic fungi and nematodes, physical barriers and soil preparation (Gurr and Kvedaras, 2010) have the potential to improve pest control in the field greatly.

3.6. Conclusions

This study demonstrates that naturally-regenerating grass field margins can foster a large and diverse natural enemy community and provide refuge within the agricultural landscape. Natural enemies were found in high family richness within the cultivated habitat, potentially through overspill from the margin, and so, theoretically supply biological control services to the organic cropping system. This study confirms that pest populations are significantly larger within the cultivated habitat in comparison to the field margin and demonstrates how these populations fluctuate over the cropping season. While populations within the crop significantly fluctuate, populations in the field margin remain more stable over time. The surveying method of pan trapping, specifically yellow pan traps, was shown to be most successful in capturing the highest abundance and richness of arthropods in the agricultural landscape.

Chapter 4. Combining biological control approaches for managing insect crop pests in the field can generate interactive effects

This chapter has been published in the peer-reviewed journal Agriculture & Forest Entomology; the following text is the accepted manuscript.

Crowther, L. I., Wilby, A. and Wilson, K. (2024). Combining biological control approaches for managing insect crop pests in the field can generate interactive effects *Agricultural & Forest Entomology.* 26(4), 470-484. doi: 10.1111/afe.12639

Author contributions:

Crowther, L. I: conceptualisation, methodology design, data collection, formal analysis, analysis interpretation, writing – original draft.

Wiby, A: funding acquisition, conceptualisation, writing – review & editing, supervision.

Wilson, K: funding acquisition, conceptualisation, writing – review & editing, supervision.

4.1. Abstract

With the loss of effective chemical controls of crop pests, a move toward biological controls is a way to future-proof our agricultural system. Floral field margins have shown successes in reducing crop pests, though the effect may not be precise enough to control infestations in commercial crops. Entomopathogenic nematodes may provide a more species- and time-specific control, and combined these methods may have synergy. Three in-field vegetation margins, divided into four plots each (two floral and two grass), were established in March 2022. Three strips of sprouting broccoli were planted parallel in April, May and June. Each strip had a split-plot design, with eight replicates of four nematode treatments placed alongside each floral/grass plot: *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* and a nematode-free control. Pest pressure was assessed using adult counts. Assessments of crop yield and crop damage associated specifically with the swede smidge (*Contarinia nasturtii*) were also made. We found that utilising a combination of control measures was successful in controlling a wider range of pests, though the success of each control method was highly dependent on the time of crop plantings. Field margins had a significant influence on crop damage associated with the swede midge. The entomopathogenic nematode *H. bacteriophora* was an effective control method for the cabbage stem flea beetle and the swede midge, as well as reducing crop damage and yield loss. Field margins and EPNs combined have an interactive effect on crop yield, highlighting the need for control methods to be tested in combination in future integrated pest management research to accurately understand their effects within an integrated system.

4.2. Introduction

Integrated pest management (IPM) is a system that uses a combination of multiple pest control techniques (cultural, biological, physical, and chemical) to limit agricultural pest populations, while considering ecosystem functioning (Deguine et al. 2021). A key component of IPM is species phenology, monitoring and use of forecasts and thresholds (Barzman et al. 2015; Collier et al. 2020). An over-reliance on synthetic chemical pest control has reduced the wider implementation of IPM methods, though rapid declines in pesticide availability, due to legislation, and in efficacy, due to the development of resistance, have changed this more recently (Deguine et al. 2021).

Many IPM practices have been tested individually, rather than in combination as would be appropriate for an in-field IPM system. However, this lack of research reviewing the integration of actions means many synergistic, antagonistic, additive or redundant effects are currently being overlooked (Stenberg, 2017). A combination of multiple pest control techniques could allow for stronger management of a species or broader control of a range of species (Barzman et al. 2015). The timing of crop planting can be manipulated to avoid periods of high pest abundance coinciding with susceptible growth stages, thus limiting damage to the crop (Wilson & Barnett, 1983). This method requires knowledge of key pest phenology and general ecology, crop rotations, land availability and commercial demands (Karungi et al. 2000).

A widely adopted IPM technique is the use of floral field margins to support local biological control agents (Albrecht et al. 2020). Floral field margins are diverse herbaceous areas of wildflowers and/or tussock grasses between the cultivated area and field boundary or within the cultivated area alongside the crop (Marshall, 2004). The reported effectiveness of supporting biological control services does vary, but consensus identifies significant benefits to natural enemy communities (increased abundance and species richness) and reductions in pest abundance in cultivated habitats (Holland et al. 2016; Albrecht et al. 2020; Crowther et al. 2023). The variation in the effectiveness of this method is likely due to variations in floral composition, management, and/or the surrounding semi-natural habitat (Albrecht et al. 2020).

Biological protective agents (bacteria, viruses, fungi, nematodes and botanicals, for example) are a mainstay of IPM programmes. Entomopathogenic nematodes (EPNs) are microscopic roundworms, of which several species can be key agents in biological pest control. Symbiotic relationships with bacteria (*Photorhabdus* spp. and *Xenorhabdus* spp.) allow EPNs to utilise soil-dwelling invertebrate hosts for reproduction (Shapiro-Ilan, Leite & Han, 2023). Applied onto, or injected into the soil, an EPN third-stage infective juvenile (IJ) will infect host life stages which reside within the soil. Infection by an EPN causes behavioural changes, interrupted morphological development, sterility, and increased mortality in the host (Koppenhöfer et al. 2020). EPNs (Heterorhabditidae and Steinernematidae) have been commercialised for some time, though they are generally utilised only in small-scale production (Abdel-Razek & Abd-Elgawad, 2021). Each EPN varies in their predatory behaviour, with *Heterorhabditis* using active hunting, and *Sterinernema* species, such as *S. carpocaspae*, using ambush (Ehlers, 1996). As a whole, EPNs have been demonstrated to infect approximately 250 species, although individual EPN species have a more limited host range (Sharmila, Subramanian & Poornima, 2018).

In an organic Brassica crop, many crop pests of economic significance could be susceptible to EPNs. Here, we consider three species of key concern:

a) The swede midge (*Contarinia nasturtii*)

In Europe, the swede midge is a relatively farm-specific brassica pest, whose larval feeding causes a significant reduction in crop quality and marketability (Chen et al. 2011; Collier et al. 2020). In conventional systems, pyrethroid insecticides are effective against swede midge, but organic systems rely on cultural controls and certified products such as Spinosad, containing a derivative from the bacterium *Saccharopolyspora spinosa*, which is toxic to invertebrates (Ester et al. 2003). The timing of insecticide application is especially important for the control of swede midge due to many short and overlapping generations (Chen et al. 2011; Abram et al. 2012b). The EPN *H. bacteriophora* (Corlay, Boivin & Bélair, 2007), *S. carpocapsae* and *S. feltiae* (Evans et al. 2015) have been linked to increased mortality of the swede midge.

b) The cabbage stem flea beetle (*Psylliodes chrysocephala*)

The cabbage stem flea beetle (CSFB) can cause losses through seed predation, 'shot-holing' young leaves, larval feeding to petioles and stems and cosmetic damage caused by adult feeding (Ortega-Ramos et al. 2021). In the UK and Europe, the effective forms of chemical control of the CSFB have recently been lost through legislative regulation of neonicotinoids and resistance to pyrethroids (Scott & Bilsborrow, 2019; Willis et al. 2020; Hoarau et al. 2022). The CSFB has been classified as a minor pest to vegetable brassicas, though anecdotally, control of the CSFB in vegetable brassicas had benefitted from the widescale application of chemical controls to oilseed rape (*Brassica napus* subsp. *napus*; AHDB, n.d. c). With the pyrethroid resistance limiting the control of the CSFB and new synthetic insecticides likely to have wider detrimental environmental effects, biological control methods must play a larger role. Several EPN species have been identified for their potential as effective management of the CSFB; *S. carpocapsae* (Hou et al. 2001), *S. feltiae* and *H. bacteriophora* (Trdan et al. 2008). H. bacteriophora has caused up to 75% mortality, while *S. feltiae* and *S. carpocaspae* have caused 80% and 85% mortality in under laboratory conditions (Price, Campbell & Pope, 2023).

c) The cabbage root fly (*Delia radicum*)

Larvae of the cabbage root fly (CRF) feed on and damage the roots of brassica crops, appearing in April in the UK (Collier et al. 1991). Young plants or specific crops with smaller root systems are most susceptible to larval damage and, as a result, plants wilt and die (Collier et al. 2020). Pests that reside in the soil or plant tissue are difficult to control as they are protected from contact-acting pesticides in foliar sprays, thus reducing the number of pathways available for control (Collier et al. 2020). Chemical control of the CRF is limited; previously used organophosphate and carbamate insecticides are no longer available for use (Ester et al. 2003; AHDB, n.d. e). The EPN *S. feltiae* has been identified for its potential to effectively control CRF in the UK (Beck et al. 2014; Kapranas et al. 2020)

This study aims to quantify the efficiency of floral field margins and EPNs as part of an IPM system in the control of three Brassica pests: the swede midge, the CSFB and the CRF. Trials were conducted over three plantings, not only to allow the experiment to run over an entire cropping season but to also test each control method in differing climatic conditions and consider the time of crop planting as a method of pest control in its own right. Four research questions were addressed while considering how each method works within different planting dates: 1) Can entomopathogenic nematodes reduce pest abundance? 2) Do entomopathogenic nematodes and floral field margins have any effect on crop damage and crop yield? 3) Do floral field margins affect pest and natural enemy abundance and species richness? 4) Do entomopathogenic nematodes and floral field margins interact, resulting in synergistic, antagonistic, additive or redundant effects?

4.3. Materials and Methods

4.3.1. Study site and trial design

The study was conducted in an organic field in West Sussex (N 50.850462, E -0.736513). The field had been planted with sprouting broccoli the previous year (2021), so was selected to increase the likelihood of pest presence for the trial. Sprouting broccoli is a first-generation hybrid cultivar of Broccoli 'Calabrese' (*Brassica oleracea* var. *italica*) and 'Chinese kale' (*Brassica oleracea* var. *alboglabra*). Three, five-metre-wide vegetation margins were sown in March 2022. Each of the three margins was split into four plots (see Figure 4.1a), sown alternately with a floral mix, "BGM4 Wildflowers and fine grasses" at a rate of 40kg/ha, or a grass mix of "Grazing Paddock" at a rate of 30kg/ha, as recommended by the producers/retailers (for full species lists see Appendix 3, Table A3.1). Prior to vegetation establishment, the field was disc cultivated twice: the first pass to remove the previous year's crop residue and the second pass after weed emergence. The field was then ploughed at a depth of 25 cm and power harrow cultivated in tandem with drilling. Post-drilling, the field was rolled to ensure seed-soil contact. The grass mix was used as a control treatment within this study as it is commonplace for a grass margin to be present in commercial farm systems.

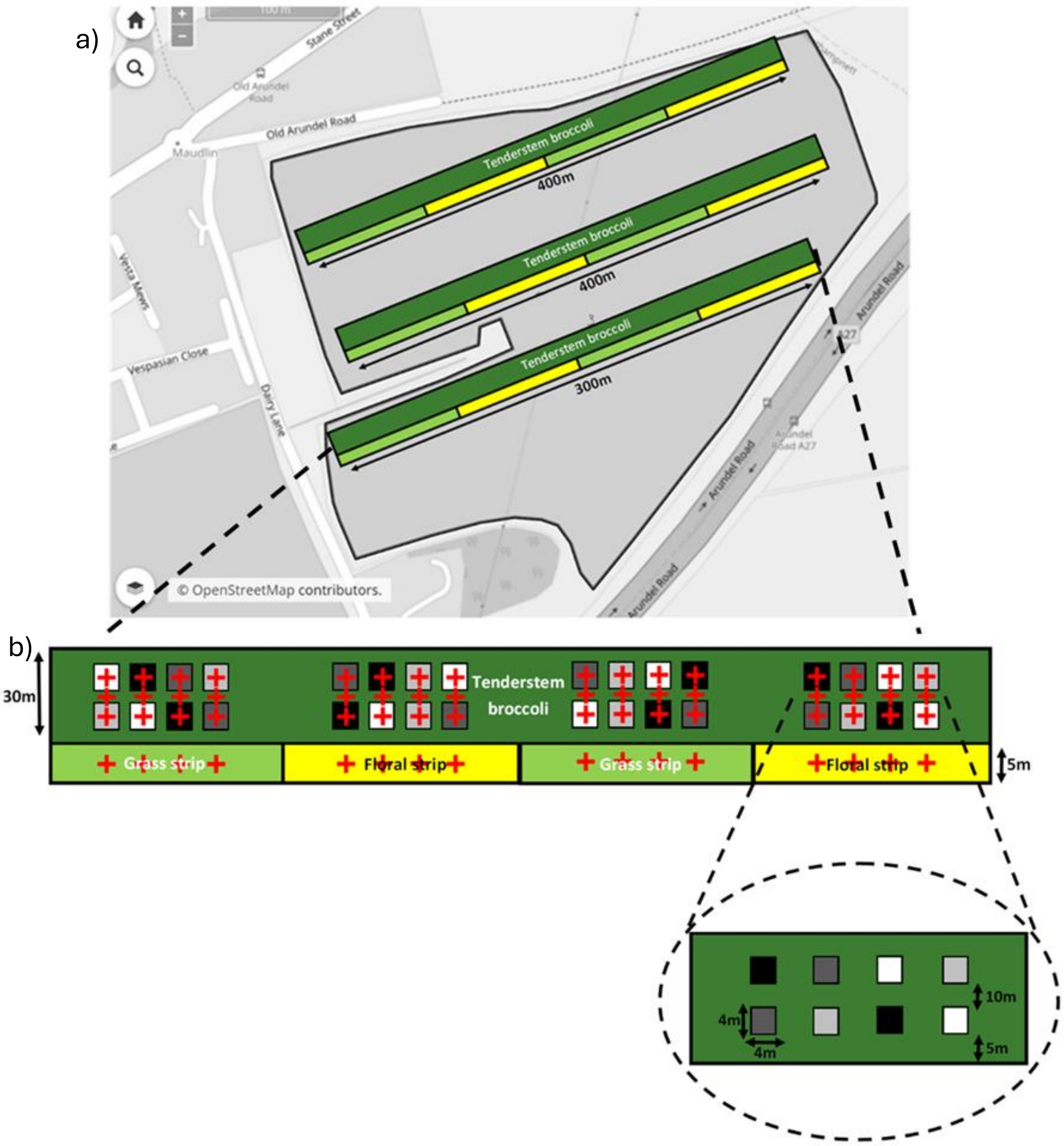

Figure 4.1. Field trial design, showing a) whole-field trial and b) in-crop trial. Greyscale square plots represent the random design of nematode treatments; *Steinernema feltiae*, *S. carpocapsae*, *Heterorhabditis bacteriophora*, and the untreated control. Red crosses indicate invertebrate sampling locations.

A 30-metre-wide strip of sprouting broccoli was planted parallel with each vegetation margin at differing timing, at a rate of 35,000-50,000 plants per hectare. The early planting (300 m) was planted in the week commencing 4th April 2022, mid-planting (400 m) was planted in the week commencing 16th May 2022 and late-planting (400 m) was planted in the week commencing 4th July 2022. Sprouting broccoli plants were grown in peat-based modules in an offsite nursery. At approximately eight weeks old, plants were treated with an organic insecticide (Tracer, active ingredient Spinosad) at 12 ml/1000 plants, to control caterpillars, and transplanted into the trial field. Slug pellets (active ingredient ferric phosphate) were applied post-planting at a rate of 5 kg/ha. The early and mid-plantings were covered with fleece post-planting until the middle of May, as protection against frost. Within each of the sprouting broccoli plantings, 32 4x4 m sub-plots, containing approximately 100 plants each, were created in four blocks of eight sub-plots; one block adjacent to each floral or grass field margin treatment (see Figure 4.1b). Each block of eight sub-plots was designed in two rows of four, to which three nematode treatments and an untreated control were randomly allocated within each row.

4.3.2. Nematode application

Three species of nematode were applied: *Steinernema carpocaspae*, *S. feltiae* and *Heterorhabditis bacteriophora*, purchased from Koppert Biological Systems under the product names Capsanem, Entonem and Larvanem, respectively. Nematodes were stored at 4°C until 30 minutes before application. Each block of eight sub-plots had two replicates of each nematode treatment. For each species of nematode, a total of 100 million IJs were applied per planting (12.5 million IJ per sub-block) over two applications, as recommended by Shapiro-Ilan et al. (2006). The first nematode application was directly post-planting, and the second application was two weeks later. For each application, 50 million IJ were hydrated in eight litres of water, five minutes before application and continuously agitated, as per manufacturers' instructions. Nematodes were applied to the base of plants using a hand-pumped pressure sprayer, with the mesh nozzle removed. Eight litres of water were applied to the control plot for consistency, equating to 500,000 IJ and 80ml water per plant.

4.3.3. Invertebrate sampling

Sampling began in May 2022 and continued once a month to October 2022. Each planting was surveyed twice at five to eight and 10 to 13 weeks after planting; thus, early planting was surveyed in May and July, mid planting in July and August, and late planting in September and October.

a) Nematode treatment monitoring

For every survey, one yellow pan trap was used to sample pest insects in each subplot. Yellow pan traps (19 cm diameter) were half-filled with 10% saline water and unscented detergent (brand ECOVER Zero) and positioned at vegetation level (traps originally started on the ground and as the plants grew taller were attached to wooden stakes). Pan traps were positioned in a triangle formation at the centre of the plot for 48 hours. Only counts of the three key pests were collated from these traps.

b) Vegetation treatment monitoring

The abundance and species richness of all arthropods, natural enemies and pests were assessed in sixteen paired survey points, demarcated along each planting, four per alternating vegetation block. In each pair, one survey point was positioned centrally (2.5 m from the edge) in the vegetation margin and one survey point was positioned centrally (15 m from the crop edge) in the sprouting broccoli strip. For early planting, survey points were approximately 18 m apart and for mid and late plantings, survey points were approximately 25 m apart.

At each survey point, three sampling techniques were used; sweep netting, pitfall trapping and pan trapping. Sweep-net sampling was conducted between 10 am and 5 pm, and windy and rainy days were avoided. Twenty figure-of-eight sweeps were conducted within 10 m to either side of each survey point. For pitfall sampling, one 10 cm diameter plastic cup was buried just below the soil surface. The traps were partially filled with 10% saline water and unscented detergent and covered in 4 cm metal mesh. For pan-trap sampling, three 19-cm diameter pan traps (one blue, one white and one yellow) were deployed at each survey point. These were positioned in a triangle formation, approximately 10 cm apart, at vegetation height. Pan traps were half-filled with 10% saline water and unscented detergent. Both pitfall and pan traps were set for 48 hours.

4.3.4. Crop damage and yield surveying

Crop damage related to swede midge larvae and crop yield were surveyed three times over the survey season (once per planting), in June, August and October 2022. In each sub-plot, ten sprouting broccoli plants were sampled at random. Crop damage associated with the swede midge was counted as presence/absence per plant and was classified by the typical symptoms exhibited in sprouting broccoli: distorted shoots, petioles and leaf tissue, swelling and death of the florets, and brown, corky scarring on the petioles and florets (Hallett, 2007; Chen et al. 2011). Yield data were collected through a count of the number of florets per these ten plants prior to commercial harvest. Here, the main head was counted as a single floret, with additional florets sprouting from new stems and leaf junctions. This may not directly correspond with commercial yield as multiple harvests are conducted so not all florets would be fully grown.

4.3.5. Vegetation surveying

In early July, a vegetation survey was carried out to compile the species composition of each vegetation treatment. Five 1 x 1 m quadrats were positioned at random in each of the four plots in each margin (10 for each treatment). Using the DOMIN scale all species and percentage cover were recorded, including the percentage of bare ground (Appendix 3, Table A3.2).

4.3.6. Statistical analysis

All analyses were conducted using the lme4 package in R, version 4.0.3 (R Core Team, 2022). Separate Generalised Linear Mixed Models (GLMM) were created for the results gathered from nematode treatment plots, which assessed counts of the three key pests, crop damage and crop yield, and the vegetation margin plots, which assessed abundance and species richness for all arthropods, natural enemies and pests. For each, a full model was created with all two and three-way interactions, which was simplified through the removal of the non-significant interactions. Interactions including vegetation treatment and nematode were retained, as these were of specific interest. Maximum Likelihood estimate was used throughout, and averaging count data allowed for Gaussian error distribution to be used. Planting and date were set as random factors to account for the nesting due to different planting dates and the repeated sampling. Abundances of the three key pests, swede midge associated crop damage and crop yield were averaged for each nematode treatment, per vegetation treatment type before analysis to prevent pseudoreplication. Swede midge abundance and total pest abundance and species richness were log-transformed.

The effect of different vegetation treatments and the timing of planting on all invertebrates, all natural enemies and all pests were analysed using the variable vegetation treatment (grass and floral), with planting (April, May or July), habitat (vegetation margin or cultivated area) and date set as random factors. Invertebrate counts across all survey types were combined to assess the total natural enemy abundance and species richness, and mean values were calculated for each of the four vegetation blocks per margin. *A priori* contrasts were used to compare each level of nematode treatment individually to the untreated control treatment.

4.4. Results

Within the nematode-treated subplots 5,514 swede midge adults, 3,913 CSFB adults and 2,184 CRF adults were collected. A total of 82,105 invertebrates were collected from the vegetation margin monitoring; 41,269 were associated with the floral mix, and 40,836 were collected in association with the grass mix. A number of additional brassica pests were sampled from the field trial; the striped and crucifer flea beetles (*Phyllotreta striolata* and *P. cruciferae*), the pollen beetle (*Brassicogethes aeneus*), the turnip sawfly (*Athalia rosae*), aphids (total Aphidoidea), both large and small white butterflies (*Pieris brassicae* and *Pieris rapae*), as well as one cabbage moth (*Mamestra brassicae*) collected in the grass vegetation treatment, and two diamondback moth (*Plutella xylostella*) from the floral. A myriad of natural enemies was collected from across the field; these insects were classified based on the AHDB (n.d. c), and include ground, rove, solider and ladybird beetles (Carabidae, Staphylinidae, Cantharidae and Coccinellidae), money and wolf spiders (Linyphiidae and Lycosidae), green lacewing (Chrysopidae), several predatory flies and true bugs, and predatory and parasitoid wasps.

4.4.1. Effect of nematode on pest abundance

EPNs were an effective control of both the swede midge ($P<0.001$) and the CSFB ($P<0.001$). Post hoc analysis identified *H. bacteriophora* as the only EPN species that significantly reduced the abundance of both species compared to the untreated control treatment (see Figures 4.2a and b). *Nematode* had no significant control of the CRF (see Figure 4.2c). The interaction between *nematode* and *planting* was significant for the CSFB alone ($P<0.001$; see Table 4.1), highlighting the variable influence between *planting dates*, with mid and late plantings associated with the greatest effects (see Figures 4.3a and b).

Entomopathogenic *nematodes* were an effective control of both the swede midge ($P<0.001$) and the CSFB ($P<0.001$). Post hoc analysis identified *H. bacteriophora* as the only EPN species that significantly reduced the abundance of both species compared to the untreated control treatment (see Figure 4.2a, and b). *Nematode* had no significant control of the CRF (see Figure 4.2c). The interaction between *nematode* and *planting* was significant for the CSFB alone ($P<0.001$; see Table 4.1), highlighting the variable influence between *planting* dates, with mid and late *plantings* associated with the greatest effects (see Figure 3a and b).

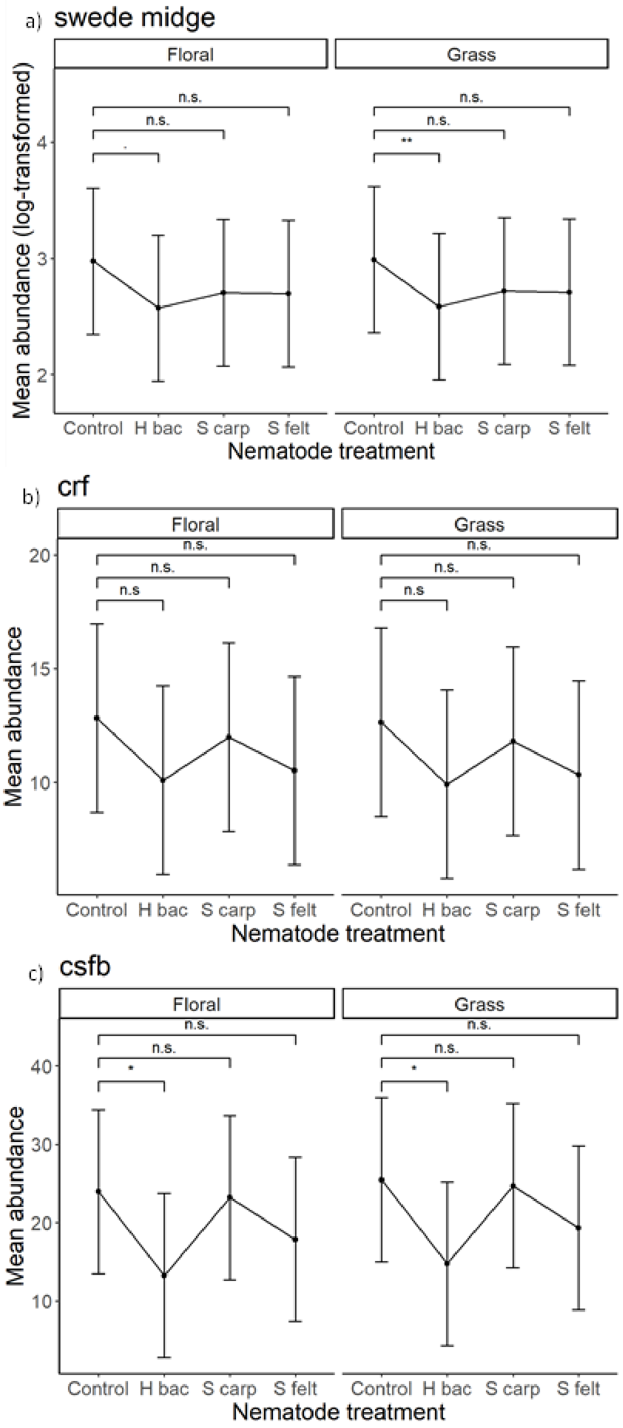

Figure 4.2. GLMM generated estimates depicting the nematode*vegetation type interaction for the swede midge (*Contarinia nasturtii*), the cabbage root fly (CRF; *Delia radicum*) and the cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*). The nematode treatments: *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *S. feltiae* and the untreated control. Annotations: n.s. = non-significant (>0.1), . = non-significant (>0.05), * = significant (<0.05), ** = significant (<0.01), *** = significant (<0.001). Error bars indicate 95% confidence intervals.

Table 4.1. Results of Generalised Linear Mixed Models on EPN biological control data, with normal error distribution. Describing the effect of the variables: nematode species, vegetation type, planting, date of survey, and key interactions. Significant variables are shown in bold.

Measure	Variable	df	F val	P
Swede midge abundance (log-transformed)	Nematode spp.	3,48	8.56	**<0.001**
	Vegetation type	1,48	0.06	0.81
	Planting	2,48	308.57	**<0.001**
	Date	5,48	218.8	**<0.001**
	Nematode x Veg type	3,48	0.8	0.5
	Nematode x Planting	6,48	1.64	016
	Veg type x Planting	2,48	2.4	0.1
Cabbage stem flea beetle abundance	Nematode spp.	3,48	6.67	**<0.001**
	Vegetation type	1,48	0.59	0.45
	Planting	2,48	38.64	**<0.001**
	Date	5,48	40.81	**<0.001**
	Nematode x Veg type	3,48	1.28	0.3
	Nematode x Planting	6,48	5.57	**<0.001**
	Veg type x Planting	2,48	0.38	0.68
Cabbage root fly abundance	Nematode spp.	3,48	1.4	0.26
	Vegetation type	1,48	0.03	0.86
	Planting	2,48	30.07	**<0.001**
	Date	5,48	16.56	**<0.001**
	Nematode x Veg type	3,48	0.54	0.66
	Nematode x Planting	6,48	1.57	0.18
	Veg type x Planting	2,48	3.24	**0.04**
Crop damage (swede midge)	Nematode spp.	3,24	14.19	**<0.001**
	Vegetation type	1,24	20.1	**<0.001**
	Planting	2,24	2102.37	**<0.001**
	Nematode x Veg type	3,24	1.21	0.33
	Nematode x Planting	6,24	4.98	**0.01**
	Veg type x Planting	2,24	5.02	**0.001**
Crop yield	Nematode spp.	3,24	60.15	**<0.001**
	Vegetation type	1,24	2.91	0.1
	Planting	2,24	2130.27	**<0.001**
	Nematode x Veg type	3,24	10.62	**<0.001**
	Nematode x Planting	6,24	31.35	**<0.001**
	Veg type x Planting	2,24	3.21	0.057

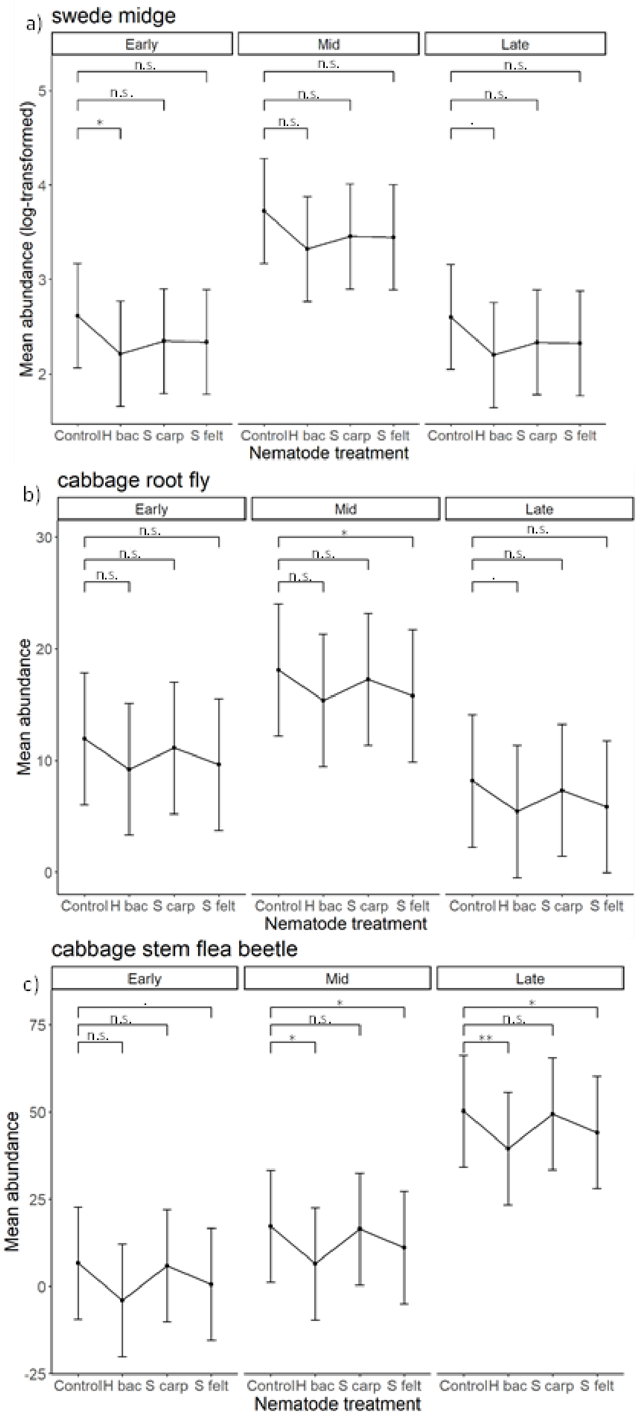

Figure 4.3. GLMM generated estimates depicting the nematode*planting interaction for the swede midge (*Contarinia nasturtii*), the cabbage root fly (CRF; *Delia radicum*) and the cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*). The nematode treatments: *Heterorhabditis bacteriophora*, *Steinernema carpocapsae, S. feltiae* and the untreated control. Annotations: n.s. = non-significant (>0.1), . = non-significant (>0.05), * = significant (<0.05), ** = significant (<0.01), *** = significant (<0.001). Error bars indicate 95% confidence intervals.

4.4.2. *Crop damage and yield*

Crop damage caused by swede midge was found to be significantly affected by all three experimental factors: *nematodes* ($P<0.001$), *vegetation treatment* ($P<0.001$) and most strongly by *planting* ($P<0.001$; Table 4.1). Both *nematode* and *vegetation treatment* had a significant interaction with *planting*, highlighting the variation in efficacy based on the time of *planting*, though they did not have a significant interaction with each other (see Table 4.1). Damage within *nematode* treatments was consistently highest in the untreated control, post hoc analysis identified only the EPN *Heterorhabditis bacteriophora* treatment to have significantly lower counts of damage in the grass vegetation treatment, compared to the control (see Figure 4.4a). For *vegetation treatment*, the percentage of damaged plants was significantly lower in crop associated with the floral vegetation treatment.

Crop yield, measured by the mean number of florets, was significantly affected by *nematode* treatment ($P<0.001$) and *planting* ($P<0.001$; Figure 4.4b). A significant interaction between these two factors, again, demonstrates that the effectiveness of a *nematode* treatment can vary based on the time of *planting*. Yield was lowest in the untreated control and highest in the *H. bacteriophora* treatment. *Vegetation treatment* alone had no significant effect on crop yield overall, though there was a significant interaction between *nematode* and *vegetation treatment* that influenced the average yield only ($P<0.001$; Figure 4.4b). Although mean counts suggest that, overall, the floral treatment had the highest yields, post hoc analysis found that *nematode* treatments were significantly different to the control in the grass treatment only.

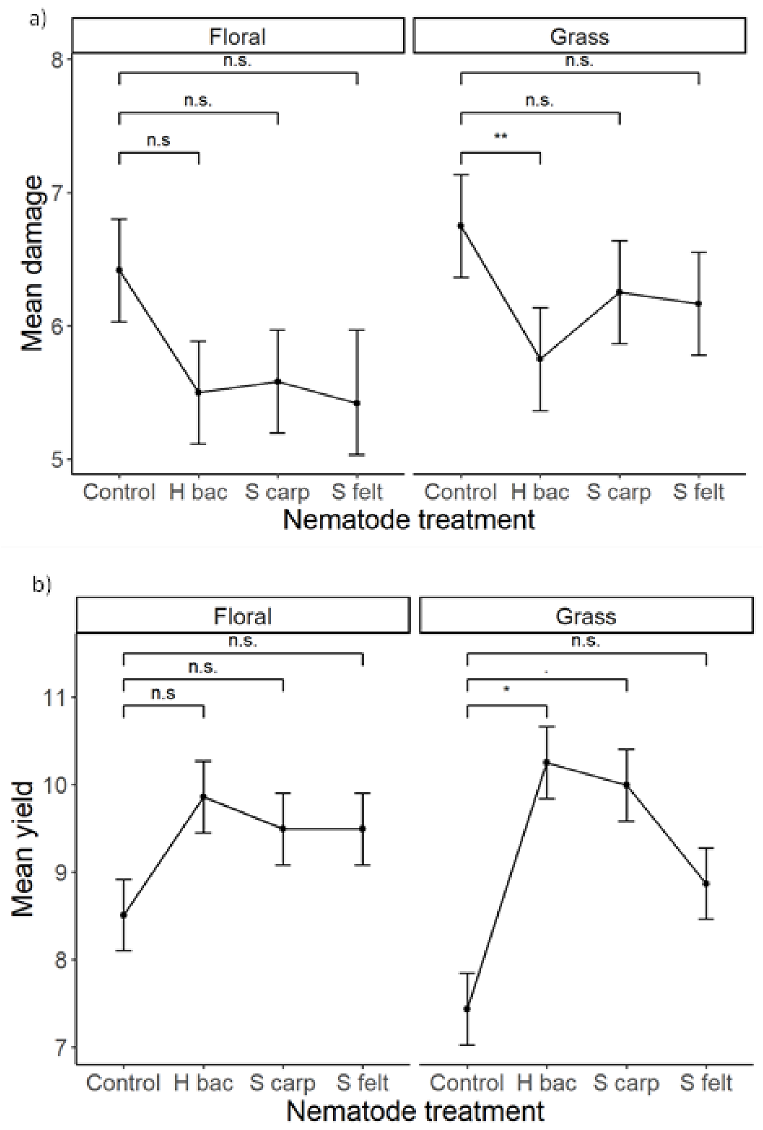

Figure 4.4. GLMM generated estimates of the nematode*vegetation type for a) mean damage caused by the swede midge and b) mean yield (average number of florets prior to first commercial harvest). The nematode treatments: *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *S. feltiae* and the untreated control. Annotations: n.s. = non-significant (>0.1), . = non-significant (>0.05), * = significant (<0.05), ** = significant (<0.01), *** = significant (<0.001). Error bars indicate 95% confidence interval.

4.4.3. Effect of vegetation type on invertebrate communities

Adequate establishment of the floral treatment was a barrier to the success of this method for biological control; both *vegetation treatments* contained a significant proportion of agricultural weeds (Appendix 3, Table A3.2), making the distinction between treatments less defined than expected. Both *vegetation treatments* were dominated by chickweed (*Stellaria media*), with the grass treatment having slightly more perennial ryegrass (*Lolium perenne*), and the floral treatment containing black medick (*Medicago lupulina*), red clover (*Trifolium pratense*) and sainfoin (*Onobrychis viciifolia*) from the seed mix.

Counts of total pest abundance and species richness were not significantly different between *vegetation treatments* (Figures 4.5a and b). However, counts for all pests were lower in areas associated with the floral treatment, excluding aphids and some Lepidoptera: the large and small white butterflies (*Pieris brassicae* and *P. rapae*) and total lepidoptera larvae (see Figure 4.6). For natural enemies, differences between *vegetation treatments* were also non-significant (Figures 4.5c and d). The abundance and species richness of all arthropods varied little between *vegetation treatments*, though were significantly influenced by *planting* (P<0.001 and P<0.001; see Table 4.2). *Habitat* (vegetation margin or cultivated habitat) had a significant influence on arthropod species richness only (P<0.001), more species were found in the vegetation margin compared to the cropped area. *Planting* was a significant influence on the abundance and species richness of arthropods and natural enemies (see Table 4.2).

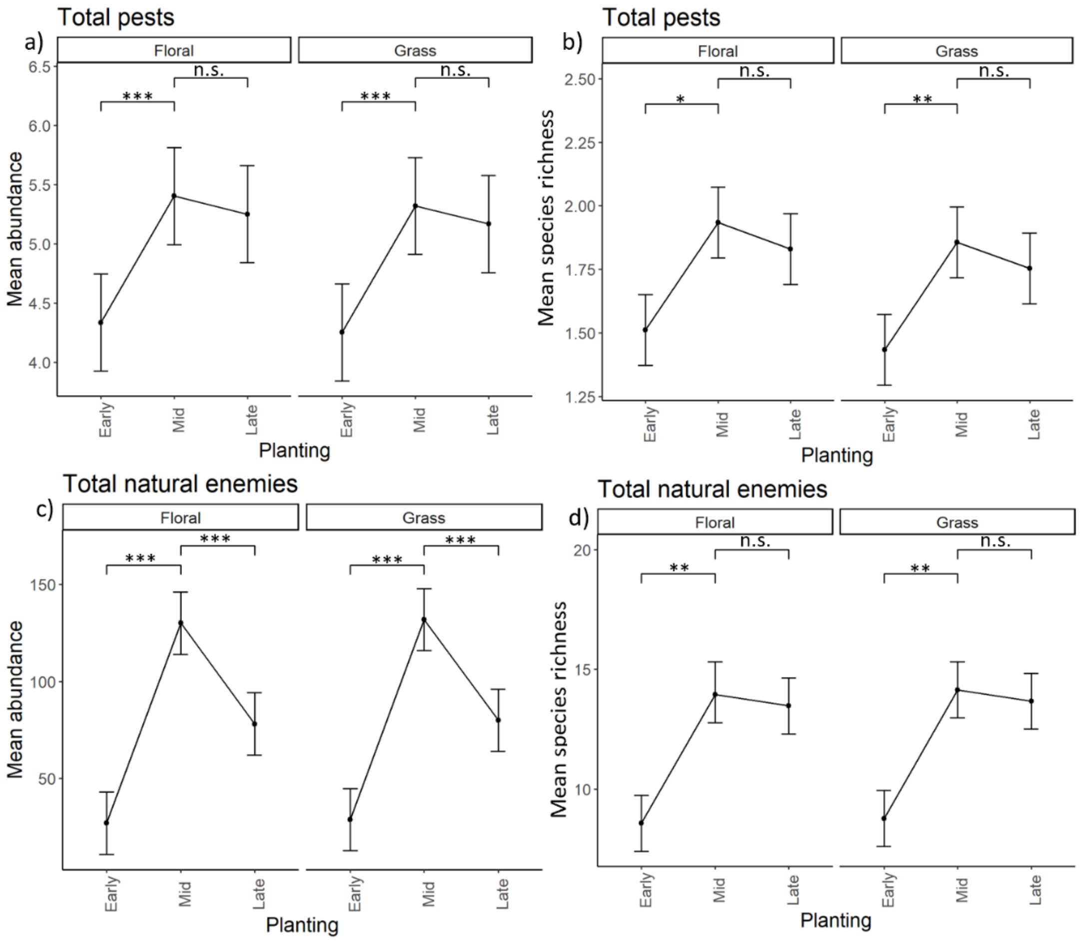

Figure 4.5. GLMM generated estimates for the mean abundance and species richness of all pests and natural enemies from the vegetation strips and cultivated area. Counts were grouped by vegetation strip treatments: floral and grass. Annotations: n.s. = non-significant (>0.1), . = non-significant (>0.05), * = significant (<0.05), ** = significant (<0.01), *** = significant (<0.001). Error bars indicate 95% confidence intervals. Graphs depicting all arthropod abundance and richness, and the abundance of the key pests can be found in Appendix 3, Figure 3.1.

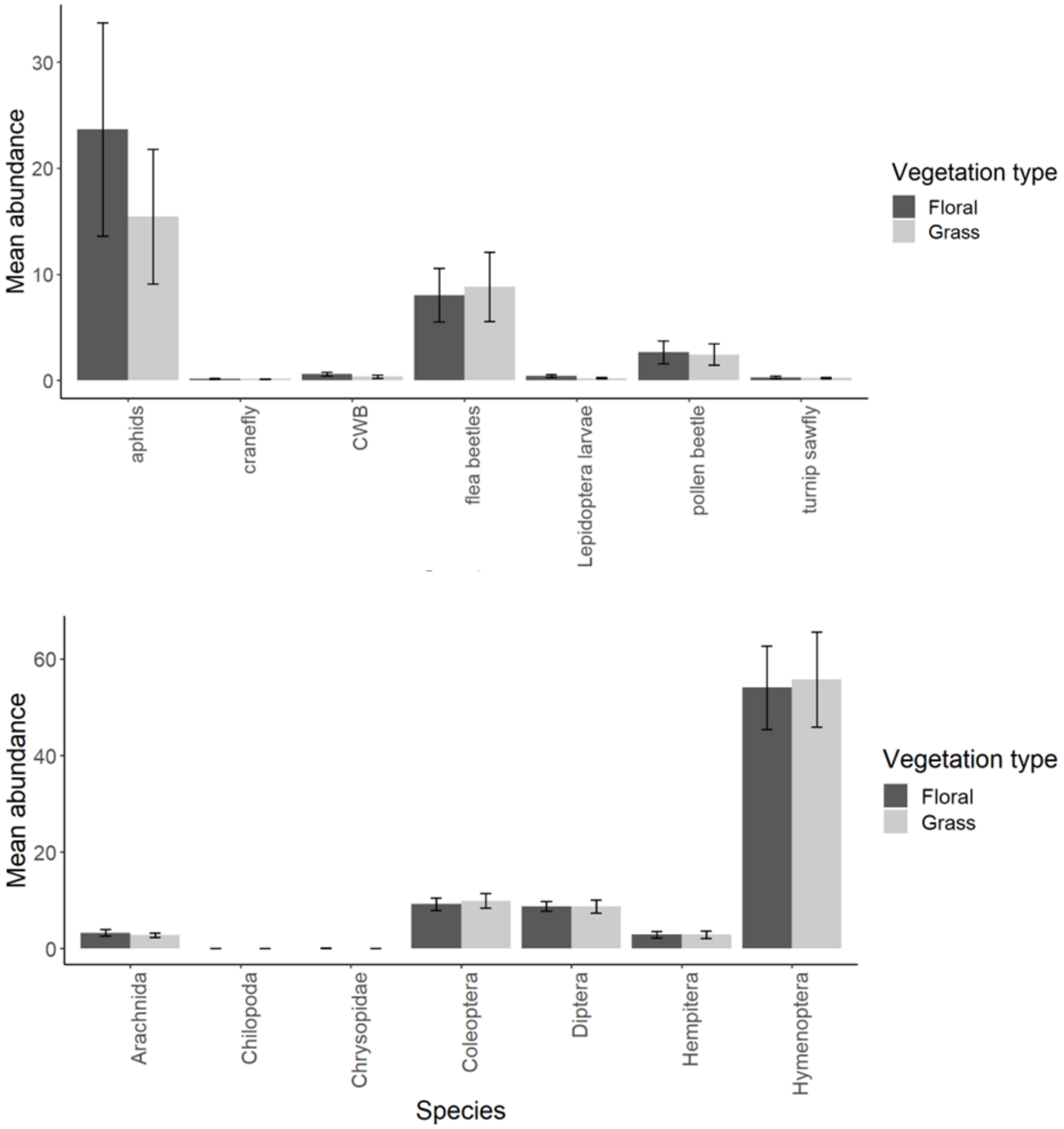

Figure 4.6. Mean abundance per vegetation type each survey visit, of a) minor brassica pest species, flea beetles (*Phyllotreta* spp. and *Psylliodes* spp.), pollen beetle (*Brassicogethes aeneus*), turnip sawfly (*Athalia rosae*), aphids (total Aphidoidea), cranefly (*Tipulidae* spp.), and cabbage white butterflies (CWB; *Pieris brassicae* and *P. rapae*), and b) natural enemy groupings, Coleoptera includes Coccinellidae larvae, in whole-field trials. Error bars indicate standard error.

Table 4.2. Results of Generalised Linear Mixed Models on vegetation strip control data, with normal error distribution. Describing the effect of the variables: vegetation type, planting, habitat (location of survey; vegetation strip or cultivated area) date of survey, and key interactions. Significant variables are shown in bold. Individual analysis of the three key pests can be found in Appendix 3, Table A3.4.

Measure	Variable	df	F val	P
Natural enemy abundance	Vegetation type	1,48	0.02	0.89
	Planting	2,48	34.2	**<0.001**
	Habitat	1,48	19.5	**<0.001**
	Date	3,48	30.76	**<0.001**
	Veg type x	2,48	0.14	0.87
	planting	2,48	7.54	**0.001**
	Habitat x planting			
Natural enemy family richness	Vegetation type	1,48	0.29	0.59
	Planting	2,48	8.67	**<0.001**
	Habitat	1,48	37.7	**<0.001**
	Date	3,48	23.73	**<0.001**
	Veg type x	2,48	0.25	0.78
	planting	2,48	0.69	0.51
	Habitat x planting			
Pest abundance	Vegetation type	1,48	0.7	0.41
	Planting	2,48	39.26	**<0.001**
	Habitat	1,48	5.6	**0.02**
	Date	3,48	18.19	**<0.001**
	Veg type x	2,48	0.51	0.6
	planting	2,48	47.32	**<0.001**
	Habitat x planting			
Pest species richness	Vegetation type	1,48	1.69	0.19
	Planting	2,48	1.43	0.25
	Habitat	1,48	1.16	0.29
	Date	3,48	1.94	0.14
	Veg type x	2,48	1.31	0.29
	planting	2,48	0.79	0.46
	Habitat x planting			
All Arthropod abundance	Vegetation type	1,48	0.004	0.95
	Planting	2,48	28.07	**<0.001**
	Habitat	1,48	0.05	0.82

	Date	3,48	8.27	**<0.001**
	Veg type x planting	2,48	0.28	0.76
	Habitat x planting	2,48	12.29	**<0.001**
All Arthropod order richness	Vegetation type	1,48	0.002	0.97
	Planting	2,48	16.74	**<0.001**
	Habitat	1,48	116.09	**<0.001**
	Date	3,48	56.81	**<0.001**
	Veg type x planting	2,48	0.42	0.66
	Habitat x planting	2,48	0.39	0.68

4.5. Discussion

EPN application had a significant effect on the adult populations of the swede midge and the CSFB, effectively reducing counts in comparison to the untreated control. As well as this, findings indicate that crop damage associated with the swede midge can be limited by both EPNs and floral vegetation treatments, though no synergism was found here. For crop yield, EPN was effective in mitigating losses, though vegetation treatment was not a significant control method alone, it did have an interactive effect with EPN control of loss of crop yield.

The time of crop planting was a significantly influential factor on pest pressure and the efficacy of EPN and floral field margins. Based on phenological cycles, each pest species will experience a population peak when conditions are optimum for reproduction, which would explain some of the variation seen throughout this study. Manipulating the timing of planting can allow for the avoidance of highly susceptible growth stages coinciding with periods of high pest pressure, limiting the need for pest control interventions (Lundin et al. 2020).

4.5.1. Can entomopathogenic nematode reduce pest abundance?

The results found here show that EPNs can be utilised as a successful method to reduce the number of adult swede midge and CSFB. For the swede midge, *H. bacteriophora* was the most effective species, achieving 37 to 55% control when successful. This EPN control proved to be unsuccessful at reducing adult swede midge abundance in the middle planting (May), which was when overall counts were highest. This may highlight that EPN-driven pest control is a preventative option, rather than a curative (Georgis et al. 2006). However, mitigation of loss of crop yield was most successful at this time, indicating that adult distribution may not accurately depict crop damage distribution. For the CSFB, although abundances did increase throughout the season, most likely as a result of migration (Hoarau et al. 2022), the EPN *H. bacteriophora* consistently limited CSFB adult abundance at 34-44% less than the untreated control. H. bacteriophora was most successful in the late planting. At this time of year, later larvae will be undergoing pupation and some adults moving into diapause, thus EPN application is not in line with when most larvae are in what was assumed to be the most susceptible life stage. Although this mechanism is not clear, this result may identify that adult CSFB as susceptible to EPN attack. Previous research on EPN-driven control of CSFB in the field is limited; Hokkanen et al. (2006) found treating one to two weeks prior to pupation resulted in 60-73% reductions in CSFB treated with *S. feltiae.* Noosidum et al. (2021) found that EPN treatments significantly reduced radish crop damage in associated with the striped flea beetle (*Phyllotreta sinuata*) in experiments running from April to May and June to July.

Counts of the CRF were trending lower in EPN treatments, though this was not significant overall. EPNs should not be discounted as a potential method to control CRF as greater concentrations of EPN products and the addition of adjuvant products to increase survival in the field could increase the effectiveness of EPN biological control (Shapiro-Ilan & Dolinski, 2015).

4.5.2. Do entomopathogenic nematodes and floral field margins have any effect on crop damage and crop yield?

The number of plants exhibiting swede midge-associated crop damage was found to be significantly reduced by the application of nematode treatments, most successfully by *H. bacteriophora*, which achieved up to 20% fewer plants exhibiting damage. Crop damage was highest in the two latter plantings, in which *H. bacteriophora* was less effective, achieving 16% and 12% reduction, respectively. This drop-off in EPN efficacy may be a result of a lack of longevity in the EPN population, suggesting additional applications may be necessary to retain peak control services (Sáenz-Aponte et al. 2020). Floral field margins significantly reduced crop damage throughout the study, although the degree to which varied between plantings. They were most successful in the late planting, achieving a 10% reduction in damaged plants, suggesting that natural enemies need time to colonise and increase in numbers and species richness in new areas of semi-natural habitat, supporting the conclusion that the efficacy of floral field margins will increase with time (Pellissier & Jabbour, 2018).

In the case of yield, *H. bacteriophora* can prevent loss of yield between 7 and 51%, depending on the time of planting. *H. bacteriophora* achieved the highest rate of protection during the mid planting, when, as previously mentioned, the swede midge adult abundance was at its highest. Variabilities in the effectiveness of the nematode treatments may be due to climatic variations; temperature, rainfall and humidity (Shapiro-Ilan & Dolinksi, 2015).

4.5.3. Do floral field margins affect pest and natural enemy abundance and species richness?

It must be reiterated that the initial establishment of the vegetation treatments was a hurdle in this study, as both vegetation treatments were dominated by agricultural weeds, though this is an issue experienced on many farms (Wietzke et al. 2020). The floral field margins provided variable, though trending positive results, as is found in the existing literature (Chaplin-Kramer, 2011; Holland et al. 2016; Albrecht et al. 2020; Crowther et al. 2023). For the swede midge, CRF, CSFB and total pest abundance numbers were trending lower in the floral vegetation treatment, identifying potential, though no significant differences were found.

Natural enemy abundance and species richness did not significantly differ between the two vegetation treatments, though abundance varied somewhat. The mean counts of predatory Coleoptera were higher in the grass treatment, given that this habitat type can provide a greater area of shelter habitat known to be used by this grouping, this is understandable (MacLeod et al. 2004). Natural enemies that can move across the landscape easily, for example, parasitoid Hymenoptera and Diptera, showed little difference across treatments. With the domination by agricultural weeds, open nectar floral resources were lacking, meaning the resources identified to benefit these groupings were not available (Zhu et al. 2020). Mean counts of the parasitic wasp family Platygastridae, which included *Synopeas myles,* a parasitoid of the swede midge, were not different between the vegetation margin treatments.

4.5.4. Do entomopathogenic nematodes and floral field margins have a synergistic, antagonistic, additive or redundant effect?

While EPN-driven biological control and vegetation margins did not interact synergistically to increase the control of any one pest, the combination of methods here allowed for significant control across a range of pests, each control method being significantly effective upon a different pest species (Zehnder et al. 2007). For crop yield alone there was a significant interactive effect between nematode treatments and the vegetation treatment, though vegetation treatment alone had no significant influence. Post hoc analysis identified that the significant interaction arose because the difference in yield between *H. bacteriophora* and control was statistically significant in the grass vegetation treatment but non-significant in the floral treatment (Fig 3). Although yield in the *H. bacteriophora* treated plots was similar in the floral and grass treatments, the control yield in the floral treatments was elevated compared to the grass treatment. This may reflect a sub-additive interaction between nematode and vegetation treatments, with *H. bacteriophora* resulting in similar yield irrespective of vegetation treatment. There is, theoretically, the potential that improvements to soil associated with field margin habitats, such as decreased compaction and increased organic matter content, may benefit EPNs (Jaffuel et al. 2017). While this wasn't specifically demonstrated in this study, a long-term study assessing EPNs in permanent field margins would provide a useful test of this prediction.

Here, we find that the application of the EPN species *H. bacteriophora* can significantly reduce the abundance of adult swede midge and CSFB in sprouting broccoli crops. Crop damage caused by the infestation of the swede midge and loss of crop yield were both reduced to the greatest extent by the application of *H. bacteriophora*. In the case of crop damage, floral field margins significantly reduced the percentage of plants exhibiting damage. While for crop yield, grass field margins were able to increase the efficacy of *H. bacteriophora*. Although mean counts of damaged plants remained lower in the floral treatment. The effectiveness of biological pest control varied for each method across crop planting dates, highlighting how the time of planting can be manipulated to increase control or allow for detailed forecasts.

Chapter 5. The Efficacy of Alternative Pest Control Methods in Brassicas: A Comparison of Entomopathogenic Nematodes, Cyantraniliprole Insecticide and Floral Field Margins

5.1. Abstract

The ability to rely on synthetic chemical insecticides in the agricultural sector is weakening, as legislation, resistant pest populations and increasing economic costs limit effective use. Alternative methods of biological control or support of existing biological control services must be evaluated in an attempt to fully understand and perfect their usage. Pest control methods are largely tested in solitary, meaning we miss out on identifying any synergistic, antagonistic, additive or redundant interactions they may have. Here, we assess floral field margins in support of biological control agents and the entomopathogenic nematode *Heterorhabditis bacteriophora* for the control of the swede midge (*Contarinia nasturtii*) and the cabbage root fly (*Delia radicum*). Floral field margins did successfully support a more diverse natural enemy community. No alternative control effectively reduced the abundance of the cabbage root fly. The cyantraniliprole-based insecticides successfully limited the abundance of the swede midge and the associated crop damage. *Heterorhabditis bacteriophora* also significantly decreases swede midge abundance, while matching or exceeding the effect of the chemical insecticide when mitigating yield loss and reducing total pest abundance. No interactive effects were identified between the two methods, although the time of crop planting had a significant influence on the success of both floral field margin and entomopathogenic nematode driven control.

5.2. Introduction

Integrated pest management is a pest control strategy utilised in agricultural systems that aims to minimise any detrimental influence on health, environment and farm economics by combining chemical, cultural and biological controls (Barzman et al., 2015). Cultural pest control focuses on reducing the susceptibility of a crop to pest pressures (Schellhorn, Harmon and Andow, 2000), while biological pest control is the control of a pest population using natural enemies of the given pest species (Barratt et al., 2017). Natural enemies can be encouraged through environmental management (conservation biological control), have their populations increased through the addition of individual organisms (augmentative biological control), or be introduced to an entirely new environment (classical biological control; Stenberg et al., 2021). A key component of IPM is the combination of multiple techniques to provide an effective and broad level of pest control, as well as spreading the risk of failure across more than one method (Barzman et al., 2015; Stenberg, 2017). The recent losses from the chemical toolbox, through more strict legislation and increased instances of pesticide resistance in pest populations, have put greater focus on the two remaining options: cultural and biological control (Højland et al., 2015; Galimberti et al., 2020).

In terms of conservation biological control, floral field margins are reported to support the local naturally occurring natural enemy community, through the provision of food resources, shelter from agricultural activities and reproduction sites (Ramsden et al., 2015; Albrecht et al., 2020). Increasing local semi-natural habitats, and specifically floral habitats, has consistently been shown to increase local biodiversity (Winkler et al., 2010; Karssemeijer et al., 2023). Such increases in abundance and richness of biodiversity are linked with a benefit to biological control and additional ecosystem services, such as pollination and nutrient cycling (Mkenda et al., 2019; Albrecht et al., 2020). For many natural enemies, their predatory behaviour is linked to just one life stage, and outside of this, they may require food resources beyond what the crop can support (e.g. Syrphidae larvae are predatory, while adults feed on floral resources; Cowgill, Sotherton and Wratten, 1993). In tandem with invertebrate-supported ecosystem services, floral field margins can support ecosystem disservices; the same resources that support and promote beneficial insects can also support herbivorous pests (Winkler et al., 2010). Increasing understanding of floral field margin composition and management can allow us to create the best balance of these services and disservices, promoting natural enemies to manage pest populations, while not losing those pest populations entirely, as they are needed to support the natural enemy community long term (Barzman et al., 2015).

Entomopathogenic nematodes (EPNs) are microscopic, non-segmented roundworms that are obligate insect parasites (Kaya and Gaugler, 1993). EPNs are well suited to biological control as they persist within the soil, are safer for the human applicator and environment in comparison to many chemical alternatives and have the potential to be more effective than chemical alternatives in certain circumstances (Inman III, Singh and Holmes, 2012). EPNs come from two families: Steinernematidae and Heterorhabditidae, which have formed mutually beneficial relationships with the insect-pathogenic bacteria, *Xenorhabdus* and *Photorhabdus*, respectively (Thanwisai et al., 2022). Several species of both EPN families have been highlighted as an effective form of biological control against some economically important pests, the key to their effectiveness being the speed at which they can locate and kill the host (Ehlers, 2001). The EPN *Heterorhabditis bacteriophora* (Poinar, Rhabditida: Heterorhabditidae) is of particular interest due to its reasonably wide host range, rapid kill time in comparison to other species and reported effectiveness in laboratory, glasshouse and field-based experiments (Susurluk, 2008; Kamali et al., 2013; Chapter 4). Depending on the species, EPNs can be considered either augmentative or classical biological control; as a whole EPNs are ubiquitous, although species and strains can vary across the globe, with *H. bacteriophora* specifically being geographically widespread (Bhat, Chaubey and Askary, 2020). To complete its life cycle, the third-stage infective juvenile (IJ) EPN enters an insect host through the cuticle or any natural openings; juveniles of Heterorhabditidae have a dorsal tooth to facilitate entrance into the host (Kaya and Gaugler, 1993). The symbiotic bacteria are then released from the gut to rapidly reproduce and release lethal metabolites, this causes septicaemia and mortality of the host (Kaya and Gaugler, 1993). Foraging behaviours vary between species of EPN, each either implementing ambush or cruising strategies, or a combination of the two. EPNs that favour the cruising technique will actively transverse the local environment in search of an insect host, or a host's chemical cues (Zoroa et al., 2011). In contrast, ambush-hunting nematodes will implore a sit-and-wait tactic, remaining stationary but alert and exhibiting nictation behaviour (Lortkipanidze et al., 2016; Grunseich et al., 2021). Of the more commonly utilised EPN, the species *H. bacteriophora* is a cruising hunter, and *Steinernema carpocapsae* is an ambush predator (Lortkipanidze et al., 2016; Grunseich et al., 2021).

This study builds on the findings of Chapter 4, in which the nematode *H. bacteriophora* was found to significantly decrease the percentage of plants damaged by the swede midge and limit the associated loss of crop yield. Here we explore this IPM approach further by quantifying the impact of EPN dose on its efficacy, as well as comparing the efficacy of this biological control system with that of a chemical pesticide. Two key pests will be considered individually, the swede midge (*Contarinia nasturtii*) and the cabbage root fly (*Delia radicum*) throughout. Four questions will be addressed: 1) Can floral field margins reduce the abundance of brassica pests, crop damage and loss of yield? 2) Can the EPN *H. bacteriophora* effectively control key brassica pests in conventional sprouting broccoli crops? 3) What is the most effective application rate of EPN for the control of key brassica insect pests? 4) Do EPN and floral field margins have any synergistic, antagonistic, additive or redundant effects in the control of key brassica pests?

5.3. Materials and Methods

5.3.1. Field trial design

Trials were conducted over the 2023 cropping season in three sprouting broccoli fields in West Sussex, United Kingdom, which were part of a large commercial production system. The soil type was sandy clay loam. As part of a rotational system, all fields followed a winter cereal crop. In each trial field was established three 6-metre-wide vegetation strips, sown in October 2022. Each vegetation strip was split into two treatments; a floral treatment sown with an AB8 mix of traditional grasses and wildflowers, at a rate of 20kg/ha and a grass control treatment sown with a ryegrass dominant grazing mixture, at a rate of 14kg/ha (see Appendix 4, Table A4.1). The AB8 mix was selected as it is compliant with Countryside Stewardship and Sustainable Farm Initiative, and so is a widely used mix. A 15-metre-wide strip of non-chemically treated sprouting broccoli plants was planted parallel to each vegetation strip (see Figure 5.1). As is normal practice, plants were transplanted robotically at a variable rate of 35,000 to 50,000 plants per hectare, dependent on the planting date. The first trial field was planted on 19/04/2023, the second on 17/05/2023 and the third on 30/07/2023. This variation in timing allows for sequential harvest to meet commercial demands. An application of molluscicide (active ingredient ferric phosphate), was applied post-planting at a rate of 5 kg/ha.

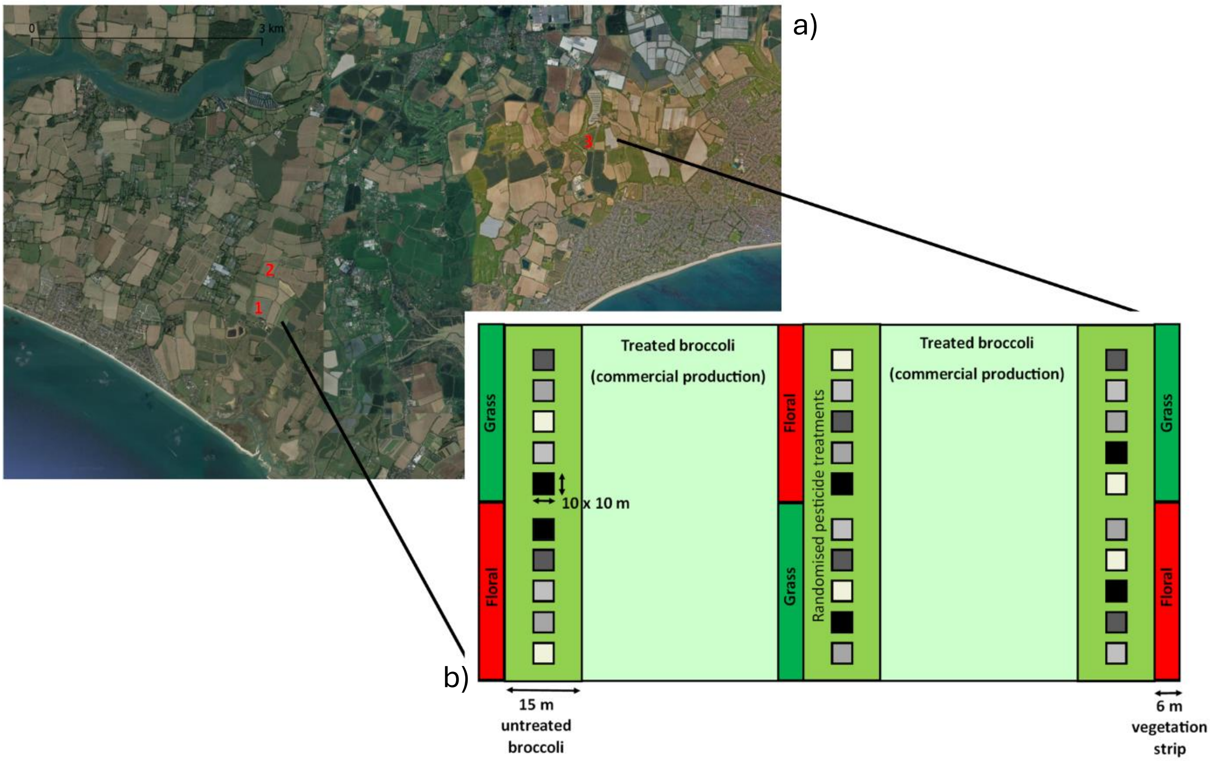

Figure 5.1. a) a map showing trial field locations, numbers indicating the order of planting date and b) the field trial experimental design depicting each field's three 6-metre-wide vegetation strips, split between grass (green) and floral (red) treatments. Three 15-metre-wide sprouting broccoli strips, each split into 10 randomised 10 m x 10 m entomopathogenic nematode (EPN) treatment plots (grey gradient visualises the EPN application rate concentration gradient). The five levels of EPN treatments were a completely untreated control, a no EPN cyantraniliprole-based insecticide treated control and three application rates of the EPN *Heterorhabditis bacteriophora* (50 million IJ/10m^2, 25 million IJ/10m^2 and 12.5 million IJ/10m^2. The surrounding sprouting broccoli in commercial production was treated with the cyantraniliprole-based insecticide.

5.3.2. Entomopathogenic nematode & insecticide application

Each sprouting broccoli strip had 10 treated plots of broccoli, five per vegetation treatment, measuring 10 x 10 m. A consistent minimum buffer of 10 m of untreated broccoli was maintained between plots and the field edges (see Figure 5.1). Five levels of EPN treatment were applied to the sprouting broccoli strip for each vegetation treatment type: a completely untreated control; a "business as usual" control of a cyantraniliprole-based insecticide applied at 18.66% active ingredient concentration, 12 ml per 1000 plants; and *Heterorhabditis bacteriophora* applied at double the recommended rate (50 million IJ/10m^2), full recommended rate (25 million IJ/10m^2) and half rate (12.5 million IJ/10m^2).. These rates were generated based on the recommended application rate by the manufacturer for the product *Larvanem* (Kopperts UK) at 86% *H. bacteriophora*. EPNs were applied within five days post-planting. The EPNs were refrigerated at 4 °C and removed to acclimatise at ambient temperature for 30 minutes prior to application. The product was mixed with water at 15-20 °C, at a rate of 3 litres/10m^2 and the solution was agitated consistently for five minutes. The EPN application was made using a hand-pumped sprayer, three bar maximum spray pressure, with no filter. EPN application took place in the evening to limit UV light exposure, which has been shown to increase nematode mortality (van Dijk et al. 2009). Prior to planting the cyantraniliprole-based insecticide treatment was applied as a drench. Cyantraniliprole is a receptor disruptor utilised in broad-spectrum insecticides for the control of chewing and sucking pests (Selby et al., 2013). All other treatments were applied post-planting. The cyantraniliprole-based insecticide control and untreated control treatment plots received an equal application of three litres of water.

5.3.3. Data collection

Pest surveys were conducted at two, six, 10- and 14-weeks following crop planting. One survey point was positioned centrally within the 10 x 10 m treated plot, where one yellow plant trap was positioned at foliage height. The pan trap was partially filled with a 10% saltwater mix, with a couple of drops of unscented detergent (Ecover, Zero), and deployed for 48 hours at a time. Species were classified as pests based on the AHDB Pest Encyclopaedia (AHDB n.d. c).

Crop damage and yield surveys were conducted once for each field, prior to the first commercial harvest. This was approximately eight weeks following crop transplanting. A random selection of ten plants was selected from each 10 x 10 m plot. Each plant was assessed for swede midge-associated damage (distortion in young shoots and petioles, brown scarring) and scored in presence/absence. A total count of the number of spears to assess yield, this assessment may not directly correspond with commercial yield as multiple harvests are conducted over a longer period.

Natural enemies were surveyed over three periods during the full cropping season: May, July and September. Each planting was surveyed twice. Assessments were carried out at each of the aforementioned survey points using pitfall trapping, pan trapping and sweep netting. For pitfall trapping, a 10-cm diameter plastic cup was buried 1 cm below the soil surface, partially filled with a 10% saltwater unscented detergent mix and covered in 4-cm metal mesh. For pan trapping, a yellow 19-cm diameter pan was positioned at vegetation height and partially filled with a 10% saltwater unscented detergent mix. Both pitfall and pan traps were deployed for 48 hours at a time. For sweep netting, 15 figure-eight sweeps were conducted within the 10 x 10 m plot. Natural enemies were classified based on the AHDB (n.d. c) and identified to family classification. Earwigs (Dermaptera) were included as a natural enemy; although earwigs have the potential to cause damage in some crops, they are also important predators of specific pests (Alins et al. 2023).

The species composition of field vegetation treatments was ascertained through vegetation surveys of each strip. For each vegetation type, five 1 m^2 quadrats were deployed at random, totalling 10 quadrats per vegetation strip, or 15 quadrats per vegetation treatment in each field. Percentage cover of each species and bare ground was recorded with an associated DOMIN scale cover score in each quadrat.

5.3.4. Data analysis

Pest counts were analysed using a generalised linear mixed model (GLMM). The included fixed factors were *vegetation type*, *EPN* application (which included the three EPN application rates, cyantraniliprole and untreated control), *crop age* and *field*, with *field* and *strip* as random factors. Counts of crop damage and yield were analysed using a separate GLMM. This model included only the fixed variables *vegetation type*, *EPN application* and *field,* while the random factors remained the same. For analysis of the natural enemy community counts from all three survey methods were combined for each survey point to obtain a full assessment of the community. Analysis was conducted using a third GLMM. For this model, the variables *vegetation type*, *EPN application* and *field* were included. The same random factors remained as in previous models.

Each full model included all two-way interactions. Any non-significant interactions were removed as the models were simplified through single-term deletions using Satterthwaite's method (Kuznetsova, Brockhoff and Christensen, 2017). Non-significant interactions were not reported within the main text (see Appendix 4, Tables A4.2, A4.3 and A4.4), excluding *vegetation type*EPN application*, which is of specific interest. *A priori* contrasts were used to compare levels with the *EPN application* treatment for all pest-related measures. Comparisons were set for each level individually and all three *H. bacteriophora* application rates as a group to compare against the untreated control and cyantraniliprole-based insecticide. Maximum Likelihood estimate and normal (Gaussian) error distribution were used for all models. All analyses were conducted using the lme4 package in R, version 4.0.3 (R Core Team, 2022).

5.4. Results

A total of 7870 individual insects classified as crop pests were collected over the entire cropping season, these were predominantly made up of the two key pests, 3698 adult swede midge and 2450 adult cabbage root fly. The remaining pest assemblage consisted of the cabbage stem beetle and other flea beetle species (genera Psylliodes and Phyllotreta), pollen beetle (Meligethes), turnip sawfly (*Athalia rosae*), cranefly (*Tipula paludosa* and *Tipula oleracea*), frit fly (*Oscinella frit*), diamondback moth (*Plutella xylostella*), and the small and large white butterflies (*Pieris rapae* and *P. brassicae*). The natural enemy community collected over the entire survey period included 31 families from the following groupings: predatory and omnivorous Coleoptera, predatory and parasitic Diptera, predatory and parasitoid Hymenoptera, predatory Hemiptera, predatory Arachnida, Chysopidae and Dermaptera.

5.4.1. The effect of vegetation on pest counts, yields and crop damage

Vegetation type had no significant influence on the majority of responses measured within this study. The pest-based responses all showed no significant difference between values associated with the floral and grass treatments: the swede midge (*P*=0.393; see Table 5.1), the CRF (*P*=0.571), total pest abundance and species richness (*P*=0.798 and *P*=0.586). For all of these variables, mean counts varied little between *vegetation type* treatments. Crop damage and yield were not significantly influenced by the differing *vegetation type* treatments.

Table 5.1. Results of Generalised Linear Mixed Model, with *a priori* contrast analysis, for the measures adult swede midge abundance, adult cabbage root fly abundance and total pest abundance and species richness. Describing the effect of the variables: *vegetation type, EPN application*, *field*, *crop age*, and the *vegetation type*EPN application* treatment interaction. Values for additional two-way interaction can be seen in Appendix 4, Table A4.2. Half = 12.5 million IJ/10m^2, full = 25 million IJ/10m^2, double = 50 million IJ/10m^2 *Heterorhabditis*

bacteriophora, chemical = cyantraniliprole. Significant explanatory variables are shown in bold.

Measure	Explanatory variable	df	t	F	P
Swede midge	Vegetation type	1		0.731	0.393
	EPN application	4		4.747	**<0.001**
	Control v (half, full, double)	350.662	2.036		**0.042**
	Control v half	350.662	-1.133		0.258
	Control v full	350.662	0.736		0.462
	Control v double	350.662	1.807		0.072
	Control v chemical	350.662	2.206		**0.028**
	Chemical v (half, full, double)	350.662	0.195		0.846
	Field	2		7.883	**0.011**
	Crop age	3		7.876	**<0.001**
	Vegetation type*EPN application	4		0.677	0.609
Cabbage root fly	Vegetation type	1		0.321	0.571
	EPN application	4		0.67	0.614
	Control v (half, full, double)	360.00	0.75		0.453
	Control v half	360.00	0.544		0.587
	Control v full	360.00	-0.686		0.493
	Control v double	360.00	0.697		0.486
	Control v chemical	360.00	0.708		0.48
	Chemical v (half, full, double)	360.00	0.127		0.899
	Field	2		35.758	**<0.001**
	Crop age	3		14.574	**<0.001**
	Vegetation type* EPN application	4		1.428	0.224
Pest abundance	Vegetation type	1		0.066	0.798
	EPN application	4		3.134	**0.013**
	Control v (half, full, double)	360.00	1.667		0.438
	Control v half	360.00	-0.777		0.96
	Control v full	360.00	0.066		0.947
	Control v double	360.00	1.874		0.062
	Control v chemical	360.00	1.783		0.075
	Chemical v (half, full, double)	360.00	1.171		0.864
	Field	2		10.772	**<0.001**
	Crop age	3		4.8	**0.003**
	Vegetation type*EPN application	4		0.364	0.834
Pest species richness	Vegetation type	1		0.297	0.586
	EPN application	4		1.349	0.251
	Control v (half, full, double)	360.00	-0.716		0.474
	Control v half	360.00	-1.153		0.25
	Control v full	360.00	-0.947		0.344
	Control v double	360.00	0.7		0.485

Control v chemical	360.00	0.935		0.054
Chemical v (half, full, double)	360.00	-1.497		0.135
Field	2		12.198	**<0.001**
Crop age	3		26.157	**<0.001**
Vegetation type*EPN application	4		0.714	0.583

5.4.2. The effect of EPN on pest counts, yields and crop damage

The *EPN application* variable had a significant influence on the abundance of adult swede midge (*P* <0.001) and total pest abundance (*P*=0.013; see Table 5.1). Using *a priori* contrasts the variation between the levels could be identified. For the swede midge, the chemical cyantraniliprole treatment was most effective in lowering swede midge abundance compared to the control (t=2.206, *P*=0.0281) and double *H. bacteriophora* application rate (50 million IJ/10m^2) showed a marginally significant decline compared to the control (t=1.807, *P*=0.072). The model-generated estimates showed that treatment with the EPN *H. bacteriophora,* at any application rate, reduced swede midge numbers. As the applied concentration rate of the EPN was increased, the mean count of adult midge captured decreased (see Figure 5.2a). When grouped, the EPN treatments significantly lowered the abundance of the swede midge in comparison to the control (t=2.036, *P*=0.042). For total pest abundance, contrast analysis identified a close to significant reduction in pests associated with the 50 million IJ/10m^2 *H. bacteriophora* (t=1.874, *P*=0.062) and the chemical cyantraniliprole treatments (t=1.783, *P*=0.075). This showed that when applied at 50 million IJ/10m^2 *H. bacteriophora* can reduce overall pest abundance more effectively than a cyantraniliprole alternative (see Figure 5.2c). The counts of the CRF (*P*=0.614) and total pest richness (*P*=0.251) were not significantly influenced by *EPN application*, but the cyantraniliprole treatment consistently showed the lowest values throughout (Figures 5.2b and c).

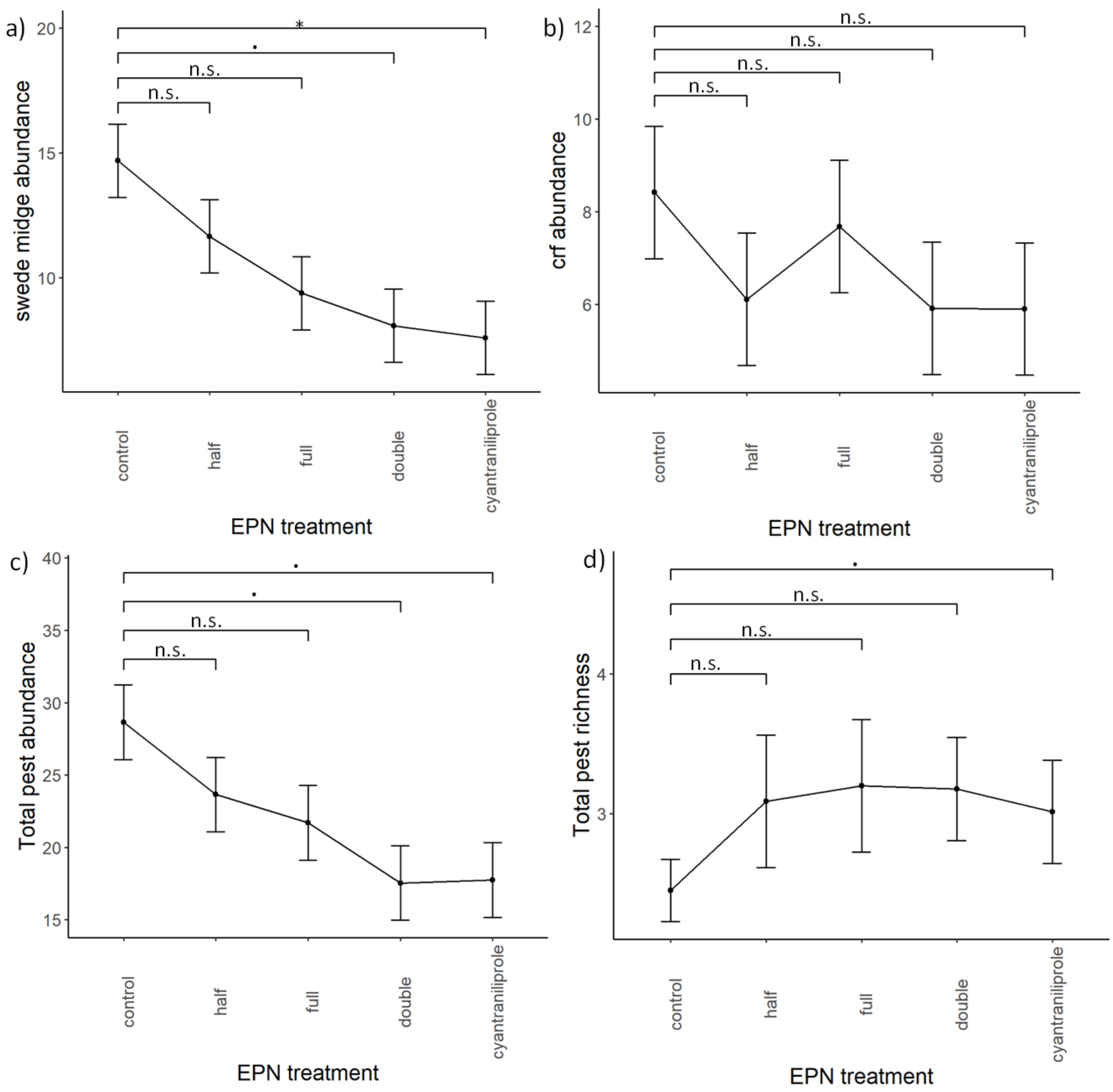

Figure 5.2. GLMM model generated estimates for: a) adult swede midge abundance, b) adult cabbage root fly (crf) abundance, c) total pest abundance and d) total pest species richness. Half = 12.5 million IJ/10m^2, full = 25 million IJ/10m^2, double = 50 million IJ/10m^2 *Heterorhabditis bacteriophora*. Annotations: n.s. = non-significant (>0.1), . = non-significant (>0.05), * = significant (<0.05), ** = significant (<0.01), *** = significant (<0.001). Error bars indicated standard error.

For crop damage, *EPN* treatment proved significant (F=5.013, *P*=0.001; see Table 5.2), with the contrast analysis showing that the chemical cyantraniliprole treatment was associated with significantly lower percentage damage than the untreated control treatment (t=2.895, *P*=0.004). The trend of percentage crop damage seen in the model-generated estimates mirrors that of swede midge adult abundance: the highest counts associated with the untreated control, the lowest with the cyantraniliprole treatment (see Figure 5.3a). For crop yield, the EPN application did not prove to have any significant influence (Figure 5.3b).

Table 5.2. Results of Generalised Linear Mixed Model, with *a priori* contrast analysis, for the measures swede midge-associated crop damage and crop yield of sprouting broccoli. Describing the effect of the variables: *vegetation type*, *EPN application*, *field* and the *vegetation type*EPN application* treatment interaction. Values for additional two-way interaction can be seen in Appendix 4, Table A4.3. Half = 12.5 million IJ/10m^2, full = 25 million IJ/10m^2, double = 50 million IJ/10m^2 *Heterorhabditis bacteriophora*, chemical = cyantraniliprole. Significant explanatory variables are shown in bold.

Measure	**Explanatory variable**	**df**	**t**	**F**	**P**
Crop damage	Vegetation type	1		0.603	0.44
	EPN application	4		5.013	**0.001**
	Control v (half, full, double)	80.868	1.526		0.131
	Control v half	80.868	-1.394		0.167
	Control v full	80.868	0.751		0.455
	Control v double	80.868	2.985		0.202
	Control v chemical	80.868	2.895		**0.005**
	Chemical v (half, full, double)	80.868	-5.09		0.612
	Field	2		10.537	**0.004**
	Vegetation type*EPN application	4		0.123	0.974
Crop yield	Vegetation type	1		1.575	0.194
	EPN application	4		0.436	0.694
	Control v (half, full, double)	80.999	-0.683		0.496
	Control v half	80.999	0.242		0.801
	Control v full	80.999	-0.127		0.899
	Control v double	80.999	0.627		0.532
	Control v chemical	80.999	0.627		0.532
	Chemical v (half, full, double)	80.999	-0.125		0.9
	Field	2		26.673	**<0.001**
	Vegetation type*EPN application	4		3.0209	0.159

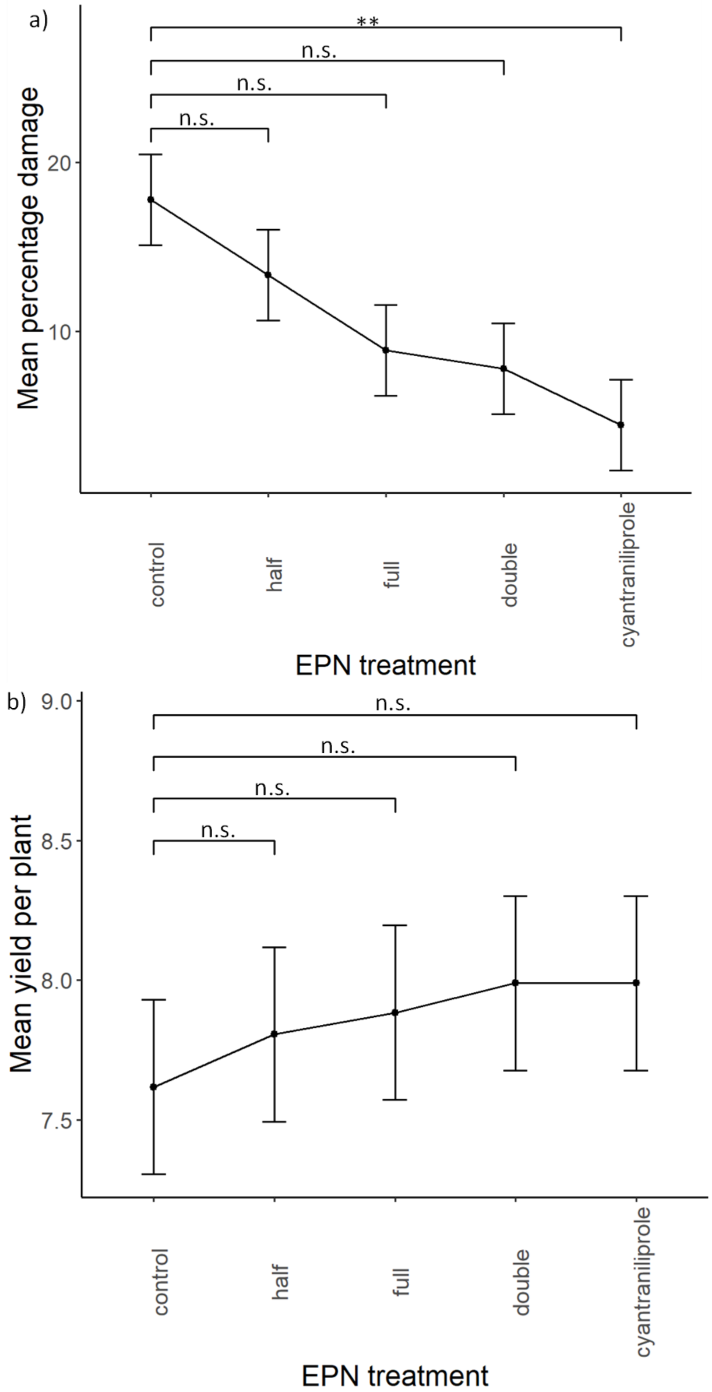

Figure 5.3. GLMM model generated estimates for: a) mean percentage swede midge-associated crop damage in sprouting broccoli, b) mean yield (mean number of spear florets per plant at first harvest), n=90. Half = 12.5 million IJ/10m^2, full = 25 million IJ/10m^2, double = 50 million IJ/10m^2 *Heterorhabditis bacteriophora*. Annotations: n.s. = non-significant (>0.1), . = non-significant (>0.05), * = significant (<0.05), ** = significant (<0.01), *** = significant (<0.001). Error bars indicated standard error.

5.4.3. The effect of crop age and field

Field (geographic location) and *crop age* (weeks since transplanting), both showed to have a significant influence on many of the responses measured within this study. Significant variations between trial fields were identified for all measures, excluding natural enemy richness (see Tables 5.1, 5.2 and 5.3) indicating that it is only the richness of natural enemies that did not vary across the locations included within this study. The key measures are the adult abundance of the swede midge (P=0.011) and the cabbage root fly (P<0.001), crop damage and yield (P=0.004 and P<0.001). However, the trial fields associated with the highest rates of swede midge-associated damage showed to be the highest yielding.

Within this experimental design *crop age* was only an appropriate explanatory variable for pest-based responses, of which all were significantly variable based on the age of the crop: the swede midge (P<0.001), the CRF (P<0.001) and total pest abundance and species richness (P=0.003 and P<0.001). The pest species exhibited predictable phenological patterns to varying degrees. For the swede midge, counts peaked at six to 10 weeks post-transplanting and returned to a lower abundance at week 14. The phenological pattern of the CRF was less clear, with greater discrepancy seen between fields. Mean counts of the CRF remained relatively low throughout the study, with a single outlying peak in mean counts in two weeks post-planting of the latest cropped field.

5.4.4. Natural enemies

The abundance of total natural enemies was not significantly affected by either pest control measure: *vegetation type* (P=0.853) and *EPN application* (P=0.912), thus no variation could be attributed to either pest control method (see Table 5.3). Variation within the abundance of the community was significantly influenced by the *field* alone (P=0.001), the highest mean count sampled in the trial fields cropped earlier in the season. Total natural enemy richness was significantly influenced by *vegetation type* (P=0.002), with mean counts showing the floral vegetation treatment supports a greater richness of the natural enemies of crop pests. *EPN application* treatment and *field* showed no difference in influence on the natural enemies' richness (P=0.22 and P=0.3). A comparison of the model-generated estimates suggests that the cyantraniliprole treatment may have a non-significant negative effect on the local natural enemy community as this treatment boasts both the lowest counts of abundance and richness of natural enemies (see Figure 5.4).

Table 5.3. Results of Generalised Linear Mixed Model, with *a priori* contrast analysis, for the natural enemy abundance and species richness. Describing the effect of the variables: *vegetation type*, *EPN application*, *field* and the *vegetation type*EPN application* treatment interaction. Values for additional two-way interaction can be seen in Appendix 4, Table A4.4. Half = 12.5 million IJ/10m^2, full = 25 million IJ/10m^2, double = 50 million IJ/10m^2 *Heterorhabditis bacteriophora*, chemical = cyantraniliprole. Significant explanatory variables are shown in bold.

Measure	**Explanatory variable**	**df**	**F**	**P**
Natural enemy abundance	Vegetation type	1	0.0346	0.8527
	EPN application	4	0.2465	0.9115
	Field	2	15.3814	**0.001**
	Vegetation type*EPN application	4	0.0819	0.9879
Natural enemy richness	**Vegetation type**	1	9.2859	**0.002**
	EPN application	4	1.4472	0.2203
	Field	2	1.2112	0.3001
	Vegetation type*EPN application	4	0.3805	0.8224

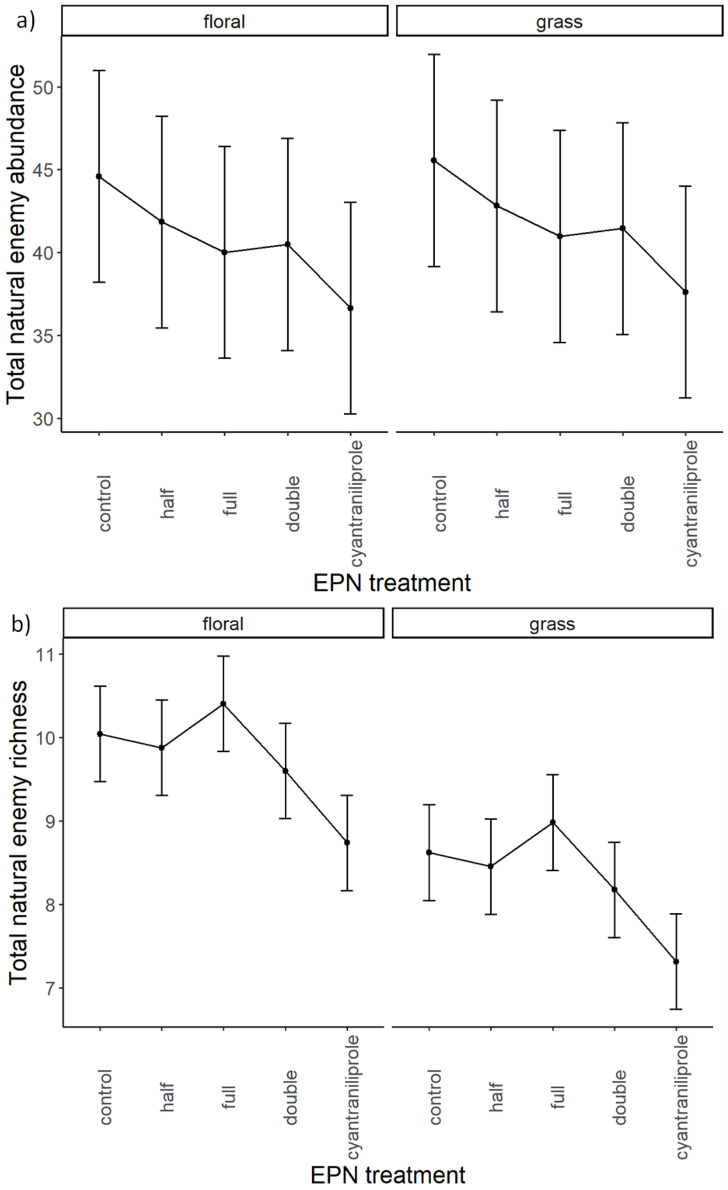

Figure 5.4. GLMM model generated estimates for: a) natural enemy abundance, b) natural enemy family richness. Half = 12.5 million IJ/10m^2, full = 25 million IJ/10m^2, double = 50 million IJ/10m^2 *Heterorhabditis bacteriophora*. Error bars indicated standard error.

5.5. Discussion

This study sought to answer four questions: 1) Can floral field margins reduce the abundance of brassica pests, crop damage and loss of yield? 2) Can the EPN *H. bacteriophora* effectively control key brassica pests in conventional sprouting broccoli crops? 3) What is the most effective application rate of EPN for the control of key brassica insect pests? 4) Do EPN and floral field margins have any synergistic, antagonistic, additive or redundant effects in the control of key brassica pests? Based on the results of this study it cannot be concluded that floral field margins significantly reduce the abundance and richness of brassica pests (swede midge, CRF or total pests), the percentage crop damage or loss of yield, although poor floral establishment should be noted. The EPN *H. bacteriophora* can significantly and effectively limit the overall abundance of brassica pests, specifically the swede midge. The most effective application rate is the highest dose, 50 million IJ/10m^2, which in the case of crop yield and adult swede midge abundance came close to matching the effectiveness of the chemical alternative and in the case of total pest damage exceeded it. In contrast to Chapter 4, no significant interactive effects were identified between the *vegetation* treatment and *EPN* treatments.

5.5.1. Vegetation strips

The successful establishment of the floral field margin treatment was a limiting factor in this assessment of biological control services. This lack of success in establishment in some trial fields and differences in vegetation composition between fields can be attributed to a myriad of factors, such as soil type, tillage, existing seed bank and agrochemical history, specifically residual herbicides in the soil (Schnee et al., 2023). The significant influence of the differing trial fields throughout this study may account for some degree of the difference in vegetation community composition. However, trial fields were in differing geographical locations and planted at different times throughout the growing season so this effect cannot be entirely attributed to a single factor. Unsurprisingly, given the poor establishment of the floral seed mix, this study found that floral field margins had a neutral impact on local pest abundance. While this contrasts with the overarching consensus of existing research, several meta-analyses highlight a lack of consistency in responses from herbivorous pests to semi-natural habitats (Shackelford et al., 2013; Holland et al., 2016). The assessments of yield and damage within the crop showed no difference in effect between the two *vegetation type* treatments.

The floral vegetation treatment was significantly associated with a significantly higher number of natural enemy species, but not with the number of individuals. Although variable, these results do demonstrate the positive impact increasing local floral diversity can have on the local natural enemy community (Chaplin-Kramer et al., 2011; Albrecht et al., 2020; Crowther et al., 2023). A more species rich natural enemy community should, in theory, provide control services over a greater range of pest species. However, if lacking in abundance, this service is limited (Crowther et al., 2023). Greater floral diversity is linked with the ability to provide a wider variety of resources for an extended time, each species having a unique phenological pattern (Ramsden et al., 2015). Ecological theory suggests that greater species diversity, as seen here, is linked to increased community stability over time and increased productivity (Tilman, 1999).

5.5.2. Entomopathogenic nematode

This study found that the entomopathogenic nematode *H. bacteriophora* is a successful control technique for the swede midge (see Figure 5.2). *Heterorhabditis bacteriophora* resides within the soil and so targets the swede midge during pupation. As a result, the EPN will not reduce the first larval infestation and associated damage (Kung, Gaugler and Kaya, 1990). Although *H. bacteriophora* did not match the efficacy of the cyantraniliprole-based insecticide, the 50 million IJ/10m^2 application rate came close, achieving a 45% reduction of adult swede midge abundance compared to the control, while the cyantraniliprole-base insecticide achieved a 52%. The increase in the applied concentration of *H. bacteriophora* was shown to have an increasing effect on the adult pest population, from a 45% reduction at 50 million IJ/m^2 to 36% at 25 million IJ/10m^2 and 21% at 12.5 million IJ/m^2, compared to the untreated control. With the addition of adjuvants, as advised by Beck et al., (2013) and Portman, Krishnankutty and Reddy (2016), the EPN 50 million IJ/10m^2 application rate has legitimate potential to provide control at the level of chemical alternatives with further research. As the EPN mode of action is via host larval parasitism, the reduction seen in adult swede midge within the vicinity can be interpreted as a reduced number of larvae successfully maturing to adulthood (McGaw and Koppenhöfer, 2008).

For total pest abundance, *H. bacteriophora* applied at 50 million IJ/10m^2 proved to be more effective than the cyantraniliprole control, both being close to statistically different from the untreated control treatment. 50 million IJ/10m^2 achieved a 39% reduction in mean pest abundance in comparison to the untreated control, while the cyantraniliprole treatment achieved a 38% reduction. This highlights the ability of EPN-based biological control to match existing chemical alternatives (Giblin-Davis, Peña and Duncan, 1996). There was no statistically significant evidence for the control of the CRF in the field by either the EPN *H. bacteriophora* or cyantraniliprole, trends in mean counts do show that both cyantraniliprole and *H. bacteriophora* reduced the mean number of adult flies. The fact that CRF did not show any significant relationship with either pest control method is supportive of the findings presented in Chapter 4, where none of the three EPN species tested, *H. bacteriophora, Steinernema feltiae* and *S. carpocapsae*, or the floral vegetation treatment showed any significant effect on the CRF abundance.

The percentage of crop damage was significantly reduced by the application of the cyantraniliprole treatment. A clear trend was present suggesting a positive reduction in crop damage associated with *H. bacteriophora* application, this effect increasing as the application rate was doubled. The cyantraniliprole treatment achieved a 75% reduction in crop damage compared to the untreated control, while *H. bacteriophora* achieved a 56% reduction at 50 million IJ/10m^2, 50% at 25 million IJ/10m^2 and 25% reduction at 12.5 million IJ/10m^2. This measure specifically will be reflected in the crop quality and marketability. Crop yield was not significantly affected by *EPN application*. Mean counts of yield do indicate a positive trend associated with EPN treatment, though this was non-conclusive in this study. A number of meta-analyses highlight the need for pest control assessment studied to include crop damage and yield as a metric for success to truly gain a more valuable understanding of the effect that control measures have on crop production (Chaplin-Kramer et al., 2011; Crowther et al., 2023).

The effect of the EPN and chemical treatments on the natural enemy community was assessed to identify any if any negative impacts were present. The influence of EPNs as a whole on natural enemies has been largely overlooked when reviewing their effectiveness in the field (Garriga et al., 2019). Any lack of compatibility identified would reduce the uptake of EPNs as an alternative pest control measure to chemical insecticides, as this is a key criticism of the latter. This study found that neither the EPN *H. bacteriophora* nor the cyantraniliprole-based insecticide had significant detrimental effects on the local natural enemy community. This supports the previous analysis of EPN-natural enemy compatibility, finding *H. bacteriophora* to have no significant negative effect on natural enemies (Garriga et al., 2019).

5.6. Conclusions

This study suggests that floral vegetation can significantly increase the richness of the natural enemy community. However, no positive influence was seen linked to floral field margin, in comparison to grass margin, for any pest measures, crop damage or yield. It must be noted that the establishment of flora vegetation was relatively unsuccessful in this study and so this result may not be representative of the full scope of potential associated biological pest control services. Both the EPN *H. bacteriophora* and cyantraniliprole-based insecticide proved to be significantly effective control measures for the swede midge. In a comparison of *H. bacteriophora* application rates, the highest rate at 50 million IJ/10m^2 was most effective for swede midge control, with efficacy decreasing relative to reducing the application rate. For total pest abundance, *H. bacteriophora* 50 million IJ/10m^2 followed by cyantraniliprole showed the greatest reduction in comparison to the untreated control, although both treatments were non-significant individually. The chemical treatment of cyantraniliprole was the only treatment to significantly reduce crop damage. Although positive trends for *H. bacteriophora* for both crop damage and yield warrant further study.

Chapter 6. Final findings and an economic assessment of floral field margins and entomopathogenic nematodes for biological control

6. **Discussion**

The overarching aim of this thesis was to increase the understanding of key pest control methods as part of an Integrated Pest Management system for insect brassica pests. To do this, four questions have remained throughout: 1) Do floral field margins support natural enemies and their associated biological control services in limiting pest populations, crop damage and loss of yield? 2) Can entomopathogenic nematodes limit pest populations, crop damage and loss of yield? 3) How do these pest control methods perform over different planting dates? And finally, 4) do these pest control methods have any synergistic, antagonistic, additive or redundant interactions?

In general, the communication of results of scientific-based studies back into farming communities is relatively unsuccessful, due to barriers in language, a lack of networking, or a lack of integration due to the complex motivations of farmers (Rust et al. 2021). Throughout this thesis, it has been imperative that trial design, although rigorous, must be practical for on-farm implementation, and any findings must be written in an accessible manner. As a result, it should provide realistic and appropriate advice on management implications that can work within farmers' more nuanced understanding of agroecosystems to achieve effective and applicable results. Understanding the cost-effectiveness of IPM systems is a complex task: the cost associated with different IPM actions can vary considerably, success can also vary based on the number and quality of actions in practice and existing data are scarce (Lefebvre, Langrell & Gomez-y-Paloma, 2014; Bottrell & Schoenly, 2018). Although a full evaluation of economic returns from an IPM system is beyond the scope of this thesis, here capital costs have been collated in order to understand any disparities between the costs of integrating either method into an IPM system and identify the progression needed to support such integration.

6.1. Key findings

6.1.1. Meta-analysis

Based on existing research, floral field margins can provide resources that support biological control services, although significant variability within the efficacy of floral margins as a whole is a constant caveat (Winkler et al. 2010; Balzan & Moonen, 2013; Rodenwald et al. 2023). An initial desk-based meta-analysis was conducted to understand where this variability stems from using unique groupings based on vegetation composition and entomological characteristics. The results of this meta-analysis found that both the abundance and species richness of natural enemy communities were significantly higher in floral field margins than grass field margins, while pest abundance was significantly lower in association with floral field margins. To understand this difference further, contrasts were introduced. For natural enemies, comparisons of type (parasitoid/parasite, pathogen or predator), dietary spectrum (generalist or specialist) and environmental guild (aerial or epigeal) were made. For pests, developmental mode (holometabolous shown by the endotergyota vs hemimetabolous shown by exopterygota) was used to further understand the difference. For both natural enemies and pests, vegetation composition, termed field margin type (naturally regenerated or specifically sown) was an additional contrast.

For natural enemy communities, the significant difference found between grass and floral field margins in existing research was described by type, guild and field margin type. Both parasitoid and predatory species richness was significantly higher in floral field margins, although this was not the case for pathogens. This could be due to a greater ease of mobility in the predatory and parasitoid groups (Wäckers & van Rijn, 2012). Within the environmental guild, aerial enemies were found to occur at higher abundances in floral field margins compared to grass. This difference is likely due to aerial species relying more strongly on floral resources in the adult life stage (Ramsden et al. 2015). For pests and natural enemies, field margin type explained a significant proportion of variance. A larger difference was found in the number of individuals present in crops associated with naturally regenerated field margins compared to grass margins than when comparing sown to grass. These findings suggest that naturally-regenerated field margins are more temporally synchronised with natural enemies' life stage requirements to provide specific resources when needed (Alcalá Herrera et al. 2021), thus, enabling greater associated biological control service to limit pest abundance. None of the significant differences found in pest communities between grass and floral field margins were described by developmental mode, i.e. whether a pest goes through distinct larval, pupal and adult life stages or not. It was theorised that a greater richness of natural enemies would be needed to control endopterygota pests, due to differing morphology with each life stage (Wilby & Thomas, 2002). While a significantly lower rate of crop damage could be found associated with floral field margins when compared to grass, this result was from a relatively small data set (n=6), highlighting one of the main advancements needed; assessments of pest control services need to include a measure of crop damage and yield.

6.1.2. Identifying pests of significance

To develop and test an IPM strategy a basic understanding of the cropping system, in this case, both organic and conventional sprouting broccoli production on a commercial scale was needed. In Chapter 3, monitoring over a cropping season (with a slightly delayed start due to COVID-19 guidelines), provided the opportunity to learn about the current practices of pest control, as well as identifying the key pests of significance. The subsequent trials described in Chapters 4 and 5 aimed to implement appropriate non-chemical control measures to assess their efficacy in an IPM system. The results of the monitoring-based study described in Chapter 3 identified three key pests of focus within the organic production: the swede midge (*Contarinia nasturtii*), the cabbage root fly (CRF; *Delia radicum*) and the cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*). Focus on the CSFB was dropped from later trials as it was not a pest of concern in conventional cropping. The abundance of adults in all three of these key pests was highest in the cropped area compared to the six-metre-wide naturally-regenerated grass field margin. The richness of natural enemies was higher in the field margin, although abundance was highest in the crop, suggesting that overspill from the margins may have been occurring (Gladbach et al. 2011). Analysis of phenology highlighted that pest abundance fluctuated significantly over the cropping season, whereas natural enemy abundance remained more stable throughout. It was clear that the field margin supported biological control by natural enemies through the lower pest abundance in the margin itself, though this did pose the question as to whether this field margin effect translated to lower pest pressure (pest abundance, crop damage and yield loss) within the cropped area.

6.1.3. The swede midge

Through farmer commentary and identification in the trial described in Chapter 3, the swede midge was classified as the most serious of the three key pests discussed here. In Chapter 3, adult swede midge abundance was 77% higher in the cropped area compared to the field margin. The ecosystem disservices of field margins must be considered when implementing field margins as a pest control strategy as brassica pests may benefit from the same resources as their natural enemies (Winkler et al. 2010). The efficacy of floral field margins for use in supporting control of the swede midge is not definitive. In chapters 4 and 5, the establishment of floral field margins/strips was a significant limiting factor in the success of the technique. In these studies, the cause of this lack of establishment was likely due to; 1) floral mix seed quality, 2) dominance of the existing seed bank and 3) the artificially high nutrient content of the soil (Kirmer et al 2009). Despite this, floral margins did show some efficacy. In Chapter 4, floral margins were responsible for up to a 10% reduction in crop damage, compared to grass field margins. In Chapter 5, however, the floral field margins were less successful, having no significant influence on pest abundance, yield or crop damage.

When introduced as a biological pest control method in Chapter 4, three entomopathogenic nematodes (*Steinernema feltiae, S. carpocapsae* and *Heterorhabditis bacteriophora*) were tested for the control of the swede midge. The latter EPN species was the most consistently effective across all measures, successfully reducing crop damage and limiting loss of yield associated with the swede midge. These results demonstrated that *H. bacteriophora* could achieve up to 55% reduction in adult swede midge abundance, up to 20% reduction in crop damage associated with the swede midge and maintain up to 51% higher yields than the untreated control in organic production. When taken forward into the trial described in Chapter 5, *H. bacteriophora* was tested in a conventional cropping system against the current chemical control (a cyantraniliprole insecticide at 18.66% active ingredient concentration). Although not quite as effective as the chemical alternative in control of the swede midge, *H. bacteriophora* significantly reduced the abundance of adult midge when compared to the untreated control, achieving up to 45% reduction in adult midge abundance and 56% reduction in crop damage. Of the three application rates tested, 50 million infective juveniles (IJ)/10m^2 proved to consistently be the most effective, even achieving greater control of total pest abundance than the chemical alternative.

6.1.4. The cabbage root fly

Floral field margins did not prove to be an effective control measure of the CRF; in Chapter 4, adult abundance of the CRF was significantly higher within the vegetation margins in comparison to the cropped area, posing the question as to whether the vegetation draws the CRF out of the crop, or whether the vegetation is a refuge where the CRF can survive and then move into the crop. The results of the trial described in Chapter 5 also found no positive influence of floral field margins on CRF control, suggesting that annual floral field margins do not support effective natural enemies of the CRF and are not an appropriate pest control technique. Likewise, EPNs were not a successful control measure against this pest species, despite existing research identifying *S. feltiae* as a likely biological control option in the field (Beck et al. 2013). Application rates may have been a limiting factor in EPN success, although due to the high associated financial costs of EPN products, application above the recommended ranges is not a practical and cost-effective control option. Additionally, soil type is known to significantly impact EPN success in biological control, proving more effective in heavier soils due to higher moisture levels and so success may be found in different trial locations, with heavier soil types (Shapiro et al., 2000).

6.1.5. The cabbage stem flea beetle

Pest control techniques for the CSFB were only tested in Chapter 4. This was due to the CSFB only appearing as a significant pest within the organic sprouting broccoli system, dictated either by geographic location or lack of organically certified control. Each EPN species exhibited varying levels of success in CSFB; *H. bacteriophora* alone caused a significant difference in CSFB abundance, causing up to 44% reduction in beetles. *S. feltiae* and *S. carpocapsae* did not cause a significant reduction in counts of CSFB. It is imperative to consider the phenological pattern of the CSFB, which differs from the other pest species tested here. The CSFB is univoltine, having a single generation per year and oviposition activity is carried out through autumn and into early spring if conditions remain mild (Hoarau et al. 2022). Thus, the majority of larvae can begin appearing from September. Application of the EPN did not occur until spring onwards (April, May and July applications), meaning a large portion of larvae will have been missed with this later application. However, the CSFB may well still be residing within the soil at this time, just in a different life stage, either pupation or adult diapause. Thus, the findings here identify that these life stages may well be susceptible to EPN attack. Floral field margins were not an effective control of the cabbage stem flea beetle. However, once again it should be noted that the floral vegetation treatments across both chapters 4 and 5 were unsuccessfully established so this result may not be conclusive.

6.1.6. Interactions

A consistent downfall with IPM research, and wider pest control research as a whole, is that control methods are tested in isolation (Lefebvre et al. 2014; Stenberg, 2017). A key component of IPM is to integrate multiple methods of control to provide the most effective and reliable control of pests (Barzman et al. 2015). Thus, solitary testing does not provide a holistic understanding of interactions within the cropping system. When assessing the interactions of pest control methods, it was evident from Chapter 4 that when used in tandem floral field margins had an interactive effect with EPN on crop yield, though this interactive effect was complex to interpret. The untreated EPN control in the grass vegetation treatment experienced a higher yield loss than its counterpart in the floral vegetation treatment. Mitigation of this loss of yield by *H. bacteriophora* at 50 million IJ/10m^2 retained an equal yield in both floral and grass vegetation treatment. This resulted in a sub-additive interaction between the EPN and floral treatment as the difference in yield between the control and treated was greater in the grass. In Chapter 5, which was conducted in a conventional cropping system as opposed to an organic one, no interactive interactions were identified.

The most consistent interaction found throughout the trials described within this thesis is the interaction between each pest control method and the time of planting. Pest phenology and climatic conditions have a significant influence on the abundance of pests present within the crop over the cropping season (Mayanglambam, Singh & Rajashekar, 2021). It is known that manipulation of planting date to avoid highly susceptible growth stages coinciding with peaks in pest activity is an effective form of pest control (Abbas et al. 2019). While it does seem simple, avoidance of such times cannot always be implemented due to the demands of commercial fresh vegetable production. Understanding which control methods perform best on certain planting dates can allow for a more effective IPM system, lowering economic costs and lessening negative environmental connotations. Practically, this would involve utilising control methods that may be more expensive, such as EPNs, in planting dates that they are most effective and relying on chemical control in plantings with the highest pest pressure or when alternative measures are not effective.

6.2. Economic assessments

The economic cost of growing a spouting broccoli crop includes seed, plant raising, nutritional inputs (fertilisers, nitrate inhibitors, trace elements, soil conditioners, e.g. lime), crop protection inputs (herbicides, insecticides, molluscicides, fungicides, adjuvants, water conditioners), irrigation, fleece, labour (forecast planning, agronomic advice, field operations, fleece laying and removal, hand-harvesting), associated machinery hire and/or maintenance and fuel. These inputs amount to a reported cost of approximately £80.00 per hectare for conventional production (based on costs reported by trial host farm). For a brassica crop, the additional cost of land rent is often applicable, due to specific diseases such as clubroot needing a broad rotation to control. The average annual land rental payment under a Farm Business Tenancy is £228.00 per hectare (Gov.uk, 2024b). The average yield for this variety group of sprouting broccoli is seven tons of fresh product per hectare, though highly dependent on where the crop sits with the planting season. Any damage and/or yield loss caused by insects, birds, disease, harvest/field operations and customer-imposed specifications are intercepted in the field, before reaching the factory, so little data on rejections are recorded. However, based on the data collected here, yield loss attributed to the swede midge alone can reduce yields by up to 35%, if left untreated, resulting in a potential income loss of up to £7766.50 per hectare.

6.2.1. Floral field margins

The cost of establishing an annual floral field margin is made up of seed purchase, ground cultivation, seed drilling and operator labour. Over the trials conducted in Chapters 4 and 5, the floral seed mixes were selected based on suitability for the AB8 Countryside Stewardship action. This mix is now also applicable to the Sustainable Farming Incentive 2023 IPM2 action. This was assumed to be the most likely mix established on farms due to wide involvement in the schemes, accessibility and generally lower financial costs. As well as this, the floral species within this mix provide both open and closed nectar sources, both of which are needed to support a wider range of natural enemies (Russell, 2015). Capital outlay for a seed mix that meets the requirements of these actions ranges from £127.00 to £399.40 per hectare (at a sowing rate of 20kg/ha), based on supplier.

Based on the mechanical operations utilised to establish the floral field margins assessed in Chapters 4 and 5, the standard procedure would be to use a combination power harrow to create a seed bed and drill the seed in one pass, followed by a rolling operation, to establish good seed contact. For organic cultivations, an additional power harrow operation is utilised prior to drilling to reduce weed pressure. The average cost of these operations, carried out by the farm itself (i.e. not contracted) would be £38.78/ha for conventional cropping and £87.80/ha for organic cropping. The associated average labour cost would be £8.59/ha for conventional cropping and £15.47/ha for organic cropping (Redman, 2023). Using the lowest cost of seed, the total cost of establishing an annual floral field margin is £174.37/ha to £230.27/ha, dependent on the cropping system.

As reiterated throughout this thesis, floral vegetation establishment was a significant issue within these experimental trials. This is not an uncommon occurrence when attempting to establish wildflowers in soils that have experienced consistent artificial manipulation of nutrient levels (Kirmer et al. 2009). To improve establishment several actions could be considered: pre-drilling herbicides, pre-drilling tillage and seed selection (Fritch et al. 2011). Commercial seed mixes can contain foreign ecotypes, selected based on ease of propagation and high fecundity, but have high failure rates and can cause wider ecosystem disruption through hybridisation with local ecotypes (Kirmer et al 2009; Schmidt et al. 2021). Selection of a seed mix composed of native and local species, that have undergone quality assurance procedures, has a higher associated cost. As a whole, the monetary payoff of conservation biological control is hard to equate due to the indirect benefits acquired (Naranjo, Ellsworth & Frisvold, 2015).

6.2.2. Entomopathogenic nematodes

As a result of the findings of chapters 4 and 5, the entomopathogenic nematode *Heterorhabditis bacteriophora* at a rate of 50 million IJ/10m^2 has been identified as an effective control measure for the swede midge (*Contarinia nasturtii*). There are remaining limiting factors to the widespread utilisation of EPN for biological pest control including economic cost and application technologies.

The capital cost of the successful EPN, *H. bacteriophora*, ranges from £105.48 to £497.80 for 500 million IJ, depending on the supplier. Based on the product with the lowest associated economic cost, and the application rate of 50 million IJ/10m^2, as identified in Chapter 5, treatment with *H. bacteriophora* would cost £1055/ha. Treatment with the cyantraniliprole-based chemical insecticide at a maximum application rate of 0.6 litres/ha is £195.73/ha. The organically certified control Spinosad-based insecticide, though considered non-effective based on the damage seen in the trial described in Chapter 4, also at a maximum application rate of 0.6L/ha, costs £205.62/ha. At approximately five times the cost, EPN is not currently an economically realistic alternative to chemical controls. However, as the cost of chemical insecticides is trending upward, their effectiveness and availability trending down, and with increased demand reducing the cost of EPN, this price gap is likely to close (Abd-Elgawad, 2019).

Since EPNs are significantly more expensive than chemical alternatives, the application must be efficient to keep costs as low as possible. The cost of application of EPN should not differ from that of chemical control as the same equipment can be utilised with minor adjustments. When considering application, the objective is to deliver the agents evenly and directly to the pest. EPNs can be applied using a range of conventional methods, for both aerial and ground-focused applications, which are currently in use for chemical alternatives (Nilsson & Gripwall, 1999). The use of sub-optimal application methods has potentially contributed to some recorded failures or sub-optimal performance of EPNs in the field (Mason, Matthews & Wright, 1998). The equipment selection to apply EPNs can vary based on crop spacing, plant morphology (specifically foliage cover) product formulation, pest location and life stage. Additional consideration needs to be made based on the specification of the available equipment; holding capacity, pressure and spray distribution patterns (Shapiro-Ilan et al. 2006).

In most situations, EPN as a biological control option is selected based on the pest having at least one life stage within, or permanently residing, within the soil. EPN application for use against foliar pests has shown a notably lower success rate, predominantly due to exposure to unfavourable abiotic factors; high temperature, low humidity and UV radiation (Mason et al. 1998). Pre- and post-application irrigation is an effective way to retain EPN efficacy, high humidity and soil moisture content increases ease of movement and reduces exposure to detrimental factors (Georgis, 1990). Belowground application reduces the risk of UV light damage and desiccation (Ulu & Erdoğan, 2023)

Pressurised spraying is the most common mode of application for chemical pesticides. Retaining and or modifying existing equipment can keep EPN-based control costs more in line with the cost of current control measures. Key considerations need to be made to pressure (kPa) and temperature, EPN infective juveniles have been shown to retain viability up to 5 x 10^3 Kpa (Nilsson & Gripwall, 1999) and 6.9 x 10^3 kPa (Dutky, 1974), through nozzles with a greater than 50 µm diameter (Nilsson & Gripwall, 1999; Fife et al. 2003; Shapiro et al. 2006). Although lower pressure is associated with higher survival rates, thus a pressure of less than 2 x 10^3 kPa is advised (Fife et al. 2003). In pressurised systems, heat is produced by the pump. Any high temperatures, or large fluctuations in temperature, can result in reduced EPN viability (Shapiro-Ilan et al. 2006). Temperatures of 32°C and above will have detrimental effects on the EPN, reducing product efficacy (Georgis, 1990). A drench application is commonly used for chemical insecticide applications and entomopathogenic fungi inoculation (Tefera & Vidal, 2009). Drench application can allow for the use of less product, retaining efficacy at less than 10% of the application rate needed for a whole field application (Collier et al. 2016). Finally, precision fertiliser application systems can be utilised to apply EPNs directly to the plant roots during transplantation. This method reduces cost as the product is being applied in a more concentrated area, meaning volume can be reduced (Pawas et al. 2023).

If applied by spraying the application will cost between £9.36 and £11.91/ha, based on the size of the sprayer boom (24 m and 36 m), and if applied through liquid fertiliser systems the application cost will be an average of £11.24/ha. The labour costs associated with these operations would cost £0.6 to £1.11/ha if applied using the sprayer equipment and £1.26/ha if using the liquid fertiliser equipment (Redman, 2023). On a global scale, augmentative biological control has an estimated end-user market value of greater than $200 million (van Lenteren, 2011). Largely reported as resulting in lower returns compared to current chemical options, augmentative control remains either less efficacious, more expensive, or both

(Redman, 2023). Where augmentative biological control is most economically viable is within glasshouse production, especially organic systems. Here, cost-benefit ratios are reportedly comparative with that of synthetic chemical insecticides (1:2-5; Naranjo et al, 2015). Long-term persistence of EPNs in the soil for more than one cropping season would contribute to reducing associated costs. *H. bacteriophora* can survive up to 23 months in the field (Susurluk & Ehlers, 2008). However, persistence may not equal effective pest control. It is likely that adjuvants, reduced soil disruption (i.e. minimum tillage) and over-winter cover crops would positively contribute to EPN persistence, limiting physical stress and maintaining a micro-ecosystem that protects the EPN from desiccation and UV radiation (Smits, 1996).

6.3. Further work

To continue the development of this IPM strategy several further works could be carried out. Firstly, due to the clear successes of floral field margins within the literature, the continuation of attempts to establish such areas of high floral diversity within farming landscapes would be advised. While a generic methodology and advice on establishment do exist, the vast number of biotic and abiotic influences vary so greatly between geographical regions, soil types and even farm-to-farm that a personalised methodology and species composition could allow significantly better establishment. To further this, research points to the efficacy of floral field margins in supporting pest control to increase over time. Moving from establishing annual to permanent field margins could allow for dominance over the existing seed bank as the desired floral species composition increases (Cordeau et al. 2017) and colonisation of a larger and more diverse natural enemy community (Noordijk et al. 2010). Not only could the efficacy of floral field margins increase for the arthropod natural enemies through greater above-ground resource provisioning, but this could also continue the highlighted synergistic effect on the below-ground EPN-based biological control, influencing EPN efficacy and persistence.

Further development of EPN biological control would be in the form of trialling adjuvants, meaning additives such as humectants, surfactants and UV radiation blockers. Existing research suggests that such products can reduce sedimentation (Schroer, Ziermann, & Ehlers, 2005), facilitate spreading (Beck et a. 2013) and reduce desiccation (Lacey, Shapiro-Ilan & Glenn, 2010). Although, such products have not yet been shown to be effective in field-based production. In addition to this, as theorised above, the identification of the optimal application technique is imperative to ensure the success of EPN-driven biological control. Considering the delivery of the EPN to the intended site, prevention of mortality caused by equipment, reducing waste, retaining the efficacy and integration of EPN products with existing agronomic products and regimes.

Finally, when conducting research such as this, several external variables can influence the results to a degree that we are not able to control, only develop experimental designs around to reduce their impact. Influences such as spatial scale, (i.e. field, farm and wider landscape attributes) crop type and, as touched on previously, farm management systems (i.e. organic versus conventional) vary so greatly that these will always be a limiting factor when reviewing results. Continuation of such research through more growing seasons, in different crops and in more geographical locations will ultimately produce more rigorous and reliable results. With one of the aims of this study being to investigate the interactions of IPM techniques, it should be highlighted that each IPM method or each IPM system involving multiple methods, will interact different based on the influences listed above and many more, making the results presented here and the understanding of wider IPM systems yet more nuanced.

6.4.Conclusion

Within this thesis, it has been identified that EPNs are an effective option for the control of both the swede midge and the CSFB. While the mechanism by which the CSFB is less clear, for the swede midge the EPN *H. bacteriophora* consistently significantly reduced adult abundance and associated crop damage and demonstrated the ability to limit loss of crop yield. It has also been confirmed that EPNs are not an effective control option for the CRF in the field. Economic assessments identified that effective EPN-mediated control is upwards of five times the cost of current chemical measures, meaning it is not appropriate as an immediate alternative. Success of floral field margins was variable and lower than expected. The efficacy of both floral field margins and EPN was highly variable depending on crop planting date, which is a key consideration when developing an IPM system, suggesting the use of chemical control in timings with low biological control efficacy and high pest pressure. The most effective application rate of *H. bacteriophora,* of the three trialled within this study, was identified as 50 million IJ/10m^2, further research in adjuvant products to increase the efficacy or reduce the application rate while maintaining efficacy would be advised to reduce the economic cost gap between EPN and chemical control measures.

References

Abbas, G., Younis, H., Naz, S., Fatima, Z., ur Rehman, A., Hussain, S., Ahmed, M. and Ahmad, S. (2019). Effect of planting dates on agronomic crop production. In Hasanuzzaman, M. (eds). *Agronomic Crops Volume 1: Production Technologies*. Singapore, Springer Singapore. Pp 131-147.

Abd-Elgawad, M. M. M. (2019). Towards optimization of entomopathogenic nematodes for more service in the biological control of insect pests. *Egyptian Journal of Biological Pest Control.* 29(77), 358-369.

Abdel-Razek, A. S. and Abd-Elgawad, M. M. M. (2021). Spinosad combined with entomopathogenic nematode for biocontrol of the Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]) on citrus. *Egyptian Journal of Biological Pest Control.* 21, 112-117.

Abram, P. K., Haye, T., Mason, P. G., Cappuccino, N., Boivin, G. and Kuhlmann, U. (2012a). Biology of *Synopeas myles*, a parasitoid of the swede midge, *Contarinia nasturtii,* in Europe. *BioControl.* 57, 789-800.

Abram, P. K., Haye, T., Mason, P. G., Cappuccino, N., Boivin, G. and Kuhlmann, U. (2012b). Identity, distribution, and seasonal phenology of parasitoids of the swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae) in Europe. *Biological Control.* 62(3), 197-205.

AHDB. (n.d. a). Swede midge control methods. Available at: https://horticulture.ahdb.org.uk/knowledge-library/swede-midge-control-methods [Accessed 11/10/2023].

AHDB. (n.d. b). Why cabbage stem flea beetle (CSFB) is an issue in oilseed rape. Available at: https://ahdb.org.uk/knowledge-library/why-cabbage-stem-flea-beetle-csfb-is-an-issue-in-oilseed-rape [Accessed 11/10/2023].

AHDB. (n.d. c). Encyclopaedia of pests and natural enemies. Available at: https://ahdb.org.uk/pests. [Accessed 22/01/2021 to 25/10/2023].

AHDB. (n.d. d). Cabbage stem flea beetle (CSFB) and its management in oilseed rape. Available at: https://ahdb.org.uk/knowledge-library/cabbage-stem-flea-beetle-csfb-and-its-management-in-oilseed-rape [Accessed 13/09/2023].

AHDB. (n.d. e). Cabbage root fly biology and management in brassica crops. Available at: https://ahdb.org.uk/knowledge-library/cabbage-root-fly-biology-and-management-in-brassica-crops [Accessed 12/09/2023].

Albrecht, M., Kleijn, D., Williams, N. M., Tschumi, M., Blaauw, B. R., Bommarco, R., Campbell, A. J., Dainese, M., Drummond, F. A., Entling, M. H., Ganser, D., de Groot, A., Goulson, D., Grab, H., Hamilton, H., Herzog, F., Isaacs, R., Jacot, K., Jeanneret, P., Jonsson, M., Knop, E., Kremen, C., Landis, D. A., Loeb, G. M., Marini, L., McKerchar, M., Morandin, L., Pfister, S. C., Potts, S. G., Rundlöf, M., Sardiñas, H., Sciligo, A., Thies, C., Tscharntke, T., Venturini, E., Veromann, E., Vollhardt, I. M. G., Wäckers, F., Ward, K., Westbury, D. B., Wilby, A., Woltz, M., Wratten, S. and Sutter, L. (2020). The effectiveness of flower strips and hedgerows on pest control, pollination services and crop yield: a quantitative synthesis. *Ecology Letters.* 23(10), 1488-1498.

Alcalá Herrera, R., Cotes, B., Agustí, N., Tasin, M. and Porcel, M. (2021). Using flower strips to promote green lacewings to control cabbage insect pests. *Journal of Pest Science.* 95, 669-683.

Alins, G., Lordan, J., Rodríguez-Gasol, N., Arnó, J and Peñalver Cruz, A. (2023). Earwig releases provide accumulative biological control of the woolly apple aphid over the years. *Insects*. 14(11), 890

Ansari, M. A., Evans, M. and Butt, T. M. (2009). Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control. *Crop Protection*. 28(3), 269-272.

Arthurs, S., Heinz, K.M. and Prasifka, J.R. (2004). An analysis of using entomopathogenic nematodes against above-ground pests. *Bulletin of Entomological Research*. 94, 297–306.

Atakan, E. (2010). Influence of weedy field margins on abundance patterns of the predatory bugs *Orius* spp. and their prey, the western flower thrips (*Frankliniella occidentalis*), on faba bean. *Phytoparasitica*. 38, 313-325.

Aviron, S., Lalecheré, E., Duflot, R., Parisey, N. and Poggi, S. (2018). Connectivity of cropped vs. semi-natural habitats mediates biodiversity: A case study of carabid beetles communities. *Agriculture, Ecosystems & Environment.* 268, 34-43.

Badenes- Pérez, F. R. (2019). Trap crops and insectary plants in the order Brassicales. *Annals of the Entomological Society of America*. 112(4), 318-329.

Baker, B. P., Green, T. A. and Loker, A. J. (2020). Biological control and integrated pest management in organic and conventional systems. *Biological Control.* 140, 104095.

Bale, J. S., van Lenteren, J. C. and Bigler, F. (2007). Biological control and sustainable food production. *Philosophical Transactions of the Royal Society B: Biological Science*. 363(1492), 761-776.

Balog, A., Mehrparvar, M. and Weisser, W. W. (2013). Polyphagous predatory rove beetles (Coleoptera: Staphylinidae) induce winged morphs in the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae). *European Journal of Entomology.* 110(1), 153-157.

Balzan, M. V. and Moonen, A-C. (2013). Field margin vegetation enhances biological control and crop damage suppression from multiple pests in organic tomato fields. *Entomologia Experimentalis et Applicata.* 150(1), 45-65.

Barker, G. (2006). *The Agricultural Revolution in Prehistory: Why Did Foragers Become Farmers?* Oxford University Press.

Barratt, B. I. P., Moran V. C., Bigler, F. and van Lenteren, J. C. (2017). The status of biological control and recommendations for improving uptake for the future. *BioControl.* 63, 155-167.

Barzman, M., Bàrberi, P., Birch, A. N. E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J. E., Kiss, J., Kudsk, P., Lamichhane, J. R., Messéan, A., Moonen, A-C., Ratnadass, A., Ricci, P., Sarah, J-L. and Sattin, M. (2015). Eight principles of integrated pest management. *Agronomy for Sustainable Development*. 35, 1199-1215.

Bass, C., Puinean, A. M., Zimmer, C. T., Denholm, I., Field, L. M., Poster, S. P., Gutbrod, O., Nauen, R., Slater, R. and Williamson, M. S. (2014). The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae. Insect Biochemistry and Molecular Biology*. 5, 41-51.

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*. 67(1), 1–48.

Baujat, B., Mahé, C., Pignon, J-P. and Hill, C. (2002). A graphical method for exploring heterogeneity in meta-analyses: application to a meta-analysis of 65 trials. *Statistics in Medicine*. 21(18), 2641-2652.

Baverstock, J., Clark, S. J. and Pell, J. K. (2008). Effect of seasonal abiotic conditions and field margin habitat on the activity of *Pandora neoaphidis* inoculum on soil. *Journal of Invertebrate Pathology*. 97(3), 282-290.

Bayer. (n.d.). Cabbage root fly. Available at: https://cropscience.bayer.co.uk/agronomy-id/pest-and-slugs/cabbage-root-fly [Accessed: 01/03/2024].

Beck, B., Brusselman, E., Nuyttens, D., Moens, M., Pollet, S., Temmerman, F. and Spanoghe, P. (2013). Improving foliar applications of entomopathogenic nematodes by selecting adjuvants and spray nozzles. *Biocontrol Science and Technology*. 23(5), 507-520.

Beck, B., Spanoghe, P., Meons, M., Pollet, S., Temmerman, F. and Nuyttens, D. (2014). Improving control of the cabbage root fly with entomopathogenic nematodes. *Aspects of Applied Biology*. 122, 125-134.

Begg, G. S., Cook, S. M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., Lövei, G. L., Mansion-Vaquie, A., Pell, J. K., Petit, S., Quesada, N., Ricci, B., Wratten, S. D. and Birch, A. N. E. (2017). *A functional overview of conservation biological control.* Crop Protection. 97, 145-158.

Bernays, E. A. (1998). Evolution of feeding behavior in insect herbivores. *BioScience*. 48(1), 35-44.

Bhat, A. H., Chaubey, A. K. and Askary, T. H. (2020). Global distribution of entomopathogenic nematodes, Steinernema and Heterorhabditis. *Egyptian Journal of Biological Pest Control*. 30(31), 227-236.

Bianchi, F. J. J. A. and Wäckers, F. L. (2008). Effect of flower attractiveness and nectar availability in field margins on biological control by parasitoids. *Biological Control*. 46(3), 400-408.

Bianchi, F. J. J. A., Booij, C. J. H. and Tscharntke, T. (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B: Biological Sciences*. 273(1595), 1715-1727.

Bischoff, A., Pollier, A., Lamarre, E., Salvadori, O., Cortesero, A-M., La Ralec, A., Tricault, Y. and Jaloux, B. (2016). Effects of spontaneous field margin vegetation and surrounding landscape on Brassica oleracea crop herbivory. *Agriculture, Ecosystem & Environment.* 223, 135-143.

Bommarco, R., Kleijn, D. and Potts, S. G. (2013). Ecological intensification: harnessing ecosystem services for food security. *Trends in Ecology & Evolution.* 28(4), 230-238.

Bommarco, R., Miranda, F., Bylund, H. and Björkman, C. (2011). Insecticides suppress natural enemies and increase pest damage in cabbage. *Journal of Economic Entomology.* 104(3), 782-791.

Borenstein, M., Hedges, L. V., Higgins, J. P. T. and Rothstein, H. R. (2009). *Introduction to meta-analysis.* United Kingdom: John Wiley & Sons, Ltd.

Bottrell, D. G. and Schoenly, K. G. (2018). Integrated pest management for resource-limited farmers: challenges for achieving ecological, social and economic sustainability. *The Journal of Agricultural Science.* 156(3), 408-426.

Bras, A., Roy, A., Hackel, D. G., Anderson, P. and Green, K. K. (2022). Pesticide resistance in arthropods: Ecology matters too. *Ecology Letters.* 25(8), 1746-1759.

Brion, G. L. (2015). Swede Midge, *Contarinia nasturtii* (diptera: Cecidomyiidae), Response to Brassica Oleracea in Simulated Intercropping Systems. MSc Thesis. University of Vermont.

Calvo-Agudo, M., González-Cabrera, J., Sadutto, D., Picó, Y., Urbaneya, A., Dicke, M. and Tena, A. (2020). IPM-recommended insecticides harm beneficial insects through contaminated honeydew. *Environmental Pollution*. 267, 115581.

Carvell, C., Isaac, N., Jitlal, M., Peyton, J., Powney, G., Roy, D., Vanbergen, A., O'Connor, R., Jones, C., Kunin, B., Breeze, T., Garratt, M., Potts, S., Harvey, M., Ansine, J., Comont, R., Le, P., Edwards, M., Roberts, S., Morris, R., Musgrove, A., Brereton, T., Hawes, C. and Roy, H. (2016). *Design and testing of a national pollinator and pollination monitoring framework.* Wallingford, NERC/Centre for Ecology & Hydrology. pp 62. (CEH Project no. C05217)

Chaplin-Kramer, R., O'Rourke, M. E., Blitzer, E. J. and Kremen, C. (2011). A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecology Letters*. 14(9), 922-932.

Chen, M. and Shelton, A. M. (2007). Impact of soil type, moisture, and depth on swede midge (Diptera: Cecidomyiidae) pupation and emergence. *Environmental Entomology*. 36(6), 1349-1355.

Chen, M., Li, W. and Shelton, A. M. (2009). Simulated crop rotation systems control swede midge, *Contarinia nasturtii. Entomologia Experimentalis et Applicata*. 133(1), 84-91.

Chen, M., Shelton, A. M., Hallett, R. H., Hoepting, C. A., Kikkert, J. R. and Wang, P. (2011). Swede midge (Diptera: Cecidomyiidae), ten years of invasion of crucifer crops in North America. *Journal of Economic Entomology*. 104 (3), 709-716.

Chinery, M. (1993). *Collins Complete British Insects*. United Kingdom: HarperCollins Publishers.

Cohen, A. L. and Crowder, D. W. (2017). The impacts of spatial and temporal complexity across landscapes on biological control: a review. *Current Opinion in Insect Science*. 20, 13-18.

Collier, R. H., Finch, S., Phelps, K. and Thompson, A. R. (1991). Possible impact of global warming on cabbage root fly (Delia radicum) activity in the UK. *Annals of Applied Biology*. 118(2), 261-271.

Collier, R., Jukes, A., Daniel, C. and Hommes, M. (2016). Ecological selectivity of pesticides and pesticide application methods. Integrated Protection in Field Vegetables. *IOBC-WPRS Bulletin*. 118, 94-98

Collier, R., Mazzi, D., Schjøll, A. F., Schorpp, Q., Thöming, G., Johansen, T. J., Meadow, R., Meyling, N. V., Cortesero, A-M., Vogler, U., Gaffney, M. T. and Hommes, M. (2020). The potential for decision support tools to improve the management of root-feeding fly pests of vegetables in western Europe. *Insects*. 11(6), 369.

Collier, T. and van Steenwyk, R. (2004). A critical evaluation of augmentative biological control. *Biological Control*. 31(2), 245-256.

Cordeau, S., Ryan, M. R., Bohan, D. A., Reboud, X. and Chauvel, B. (2017). Which traits allow weed species to persist in grass margin strips? *Weed Science*. 65(3), 381-394.

Corlay, F., Boivin, G. and Bélair, G. (2007). Efficiency of natural enemies against the swede midge *Contarinia nasturtii* (Diptera: Cecidomyiidae), a new invasive species in North America. Biological Control. 43(2), 195-201.

Cowgill, S. E. Sotherton, N. W. and Wratten, S. D. (1993). The selective use of floral resources by the hoverfly *Episyrphus balteatus* (Diptera: Syrphidae) on farmland. Annals on Applied Biology. 122(2), 223-231.

CropLife Europe. (n.d.). What is IMP? Available at: https://croplifeeurope.eu/what-is-ipm/#:~:text=IPM%20is%20all%20about%20choosing,and%20weeds%20in%20the%20field [Accessed 02/02/2024].

Crowther, L. I., Wilson, K. and Wilby, A. (2023). The impact of field margins on biological pest control: a meta-analysis. *BioControl*. 68, 387-396.

Cruz-Martínez, H., Ruiz-Vega, J., Matadamas-Ortíz, P. T., Cortés-Martínez, C. I. and Rosas-Diaz J. (2017). Formulation of entomopathogenic nematodes for crop pest control – a review. *Plant Protection Science.* 53(1), 15-24.

Dainese, M., Martin, E. A., Aizen, M. A., Albrecht, M., Bartomeus, I., Bommarco, R., Carvalheiro, L. G., Chaplin-Kramer, R., Gagic, V., Garibaldi, L. A., Ghazoul, J., Grab, H., Jonsson, M., Karp, D. S., Kenndey, C. M., Kleijn, D., Kremen, C., Landis, D. A., Letourneau, D. K., Marini, L., Poveda, K., Rader, R., Smith, H. G., Tscharntke, T., Andersson, G. K. S., Badenhausser, I., Baensch, S., Bezerra, A. D. M., Bianchi, F. J. J. A., Boreux, V., Bretagnolle, V., Caballero-Lopez, Cavigliasso, P., Ćetković, A., Chacoff, N. P., Classen, A., Cusser, S., Da Silva E Silva, F. D., De Groot, G. A., Dudenhöffer, J. H., Ekroos, J., Fijen, T., Franck, P., Freitas, B. M., Garratt, M. P. D., Gratton, C., Hipólito, J., Holzschuh, A., Hunt, L., Iverson, A. L., Jha, S., Keasar, T., Kim, T. N., Kishinevsky, M., Klatt, B. K., Klein, A-M., Krewenka, K. M., Krishnan, S., Larsen, A. E., Lavigne, C., Liere, H., Maas, B., Mallinger, R. E., Pachon, E, M., Martínez-Salinas, A., Meehan T, D., Mitchell, M. G. E., Molina, G. A. R., Nesper, M., Nilsson, L., O'Rourke, M. E., Peters, M. K., Plećaš, M., Potts, S. G., Ramos, D. L., Rosenheim, J. A., Rundlöf, M., Rusch, A., Sáez, A., Scheper, J., Schleuning, M., Schmack, J. M., Sciligo, A. R., Seymour, C., Stanley, D. A., Stewart, R., Stout, J. C., Sutter, L., Takada, M. B., Taki, H., Tamburini, G., Tschumi, M., Viana, B. F., Westphal, C., Willcox, B. K., Wratton, S. D., Yoshioka, A., Zaragoza-Trello, C., Zhang, W. and

Steffan-Dewenter, I. (2019). A global synthesis reveals biodiversity-mediated benefits for crop production. *Science Advances*. 5(10), eaax0121.

Deguine, J-P., Aubertot, J-N., Flor, R. J., Lescourret, F., Wyckhuys, K. A. G. and Ratnadass, A. (2012). Integrated pest management: good intentions, hard realities. A review. *Agronomy for Sustainable Development.* 41(38).

Dennis, P. and Fry, G. L. A. (1992). Field margins: can they enhance natural enemy population densities and general arthropod diversity on farmland? *Agriculture, Ecosystems & Environment.* 40 (1-4), 95-115.

Denys, C. and Tscharntke, T. (2002). Plant-insect communities and predator-prey ratios in field margin strips, adjacent crop fields, and fallows. *Oecologia.* 130, 315-324.

Des Marteaux, L. E. and Hallett, R. H. (2019). Swede midge (Diptera: Cecidomyiidae) diapause initiation under stable conditions: not a family affair. *The Canadian Entomologist*. 151(4), 465-474.

Devine, G J. and Furlong, M. J. (2007). Insecticide use: Contexts and ecological consequences. *Agriculture and Human Values.* 24, 281-306.

Ditner, N., Balmer, O., Beck, J., Blick, T., Nagel, P. and Luka, H. (2013). Effects of experimentally planting non-crop flowers into cabbage fields on the abundance and diversity of predators. *Biodiversity and Conservation*. 22, 1049-1061.

Dutky, S. R. (1974). Nematode parasites. In Maxwell, F. G. and Harris, F. A. (eds.), *Proceedings of the Summer Institute on Biological Control of Plant Insects and Diseases*. University Press, Jackson. Pp. 576-590

Ehler, L. (1998). Conservation biological control: past, present and future. In Barbosa, P. *Conservation Biological Control*. Academic Press, California. Pp. 1-8.

Ehler, L. E (2006). Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. *Pest Management Science.* 62(9), 787-789.

Ehlers, R-U. (1996). Current and future use of nematodes in biocontrol: Practice and commercial aspects with regard to regulatory policy issues. *Biocontrol Science and Technology*. 6, 303-316.

Ehlers, R-U. (2001). Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiological Biotechnology*. 56, 623-633.

Emery, S. E., Anderson, P., Carlsson, G., Friberg, H., Larsson, M., Wallenhammar, A-C. and Lundin, O. (2021). The potential of intercropping for multifunctional crop protection in oilseed rape (*Brassica napus* L.). *Frontiers in Agronomy*. 3, 782686.

English-Loeb, G., Villani, M., Martinson, T., Forsline, A. and Console, N. (1999). Use of entomopathogenic nematodes for control of grape Phylloxera (Homoptera: Phylloxeridae): A laboratory evaluation. *Biological Control*. 28(5), 890-894.

Ernoult, A., Vialatte, A., Butet, A., Michel, N., Rantier, Y., Jambon, O. and Burel, F. (2013). Grassy strips in their landscape context, their role as new habitat for biodiversity. *Agriculture, Ecosystems & Environment*. 166, 15-27.

Ester, A., de Putter, H. and van Bilsen, J. G. P. M. (2003). Filmcoating the seed of cabbage (*Brassica oleracea L*. convar. *Capitata* L.) and cauliflower (*Brassica oleracea* L. var. *Botrytis* L.) with imidacloprid and spinosad to control insect pests. *Crop Protection*. 22(5), 761-768.

Evans, B. G. (2017). *Implications of swede midge biology in the development of population management alternatives*. Ph.D. Thesis. University of Guelph.

Evans, B. G. and Hallett, R. H. (2016). Efficacy of biopesticides for management of the swede midge (Diptera: Cecidomyiidae). *Journal of Economic Entomology*. 109(5), 2159-2167.

Evans, B. G., Jordan, K. S., Brownbridge, M. and Hallett, R. H. (2015). Effect of temperature and host life stage on efficacy of soil entomopathogens against the swede midge (Diptera: Cecidomyiidae). *Journal of Economic Entomology*. 108(2), 473-483.

Evenson, R. E. and Gollin, D. (2003). Assessing the Impact of the Green Revolution, 1960 to 2000. *Science*. 300(5620), 758-762.

Federici, B. A. (2007). Bacteria as biological control agents for insects: Economics, engineering, and environmental safety. In: Vurro, M., Gressel, J. (eds) *Novel Biotechnologies for Biocontrol Agent Enhancement and Management*. NATO Security through Science Series. Springer, Dordrecht.

Fife, J. P., Derksen, R. C., Ozkan, H. E. and Grewal, P. S. (2003). Effects of pressure differentials on the viability and infectivity of entomopathogenic nematodes. *Biological Control*. 27(1), 65-72.

Fife, J., Ozkan, H. E., Derksen, R. C., Grewal, P. S. and Krause, C. R. (2005). Viability of a biological pest control agent through hydraulic nozzles. *Transactions of the American Society of Agricultural Engineers.* 48, 45-54.

Finch, S. (1993). Integrated pest management of the cabbage root fly and the carrot fly. *Crop Protection*. 12(2), 423-430.

Finch, S. and Collier, R. (2000). Integrated pest management in field vegetable crops in northern Europe – With focus on two key pests. *Crop Protection.* 19(8-10), 817-824.

Finch, S. Billiald, H. and Collier, R. H. (2003). Companion planting – do aromatic plants disrupt host-plant finding by the cabbage root fly and the onion fly more effectively than non-aromatic plants? *Entomologia Experimentalis et Applicata.* 109(3), 183-195.

Fletcher Jr, R. J., Didham, R. K., Banks-Leite, C., Barlow, J., Ewers, R. M., Rosindell, J., Holt, R. D., Gonzalez, A., Pardini, R., Damschen, E. I., Melo, F. P L., Ries, L., Prevedello, J. A., Tscharntke, T., Laurance, W. F., Lovejoy, T. and Haddad, N. M. (2018). Is habitat fragmentation good for biodiversity? *Biological Conservation.* 226, 9-15.

Frank, S. D., Shewsbury, P. M. and Esiekpe, O. (2008). Spatial and temporal variation in natural enemy assemblages on Maryland native plant species. *Environmental Entomology.* 37(2), 478-486.

Fritch, R. A., Sheridan, H., Finn, J. A., Kirwan, L. and Ó hUallacháin, D. (2011). Methods of enhancing botanical diversity within field margins of intensively managed grassland: a 7-year field experiment. *Journal of Applied Ecology*. 48(3), 551-560.

Frouz, J. and Kindlmann, P. (2015). Source-sink colonization as a possible strategy of insects living in temporary habitats. *PLoS One.* 10(6), e0127743.

Fusser, M. S., Pfister, S. C., Entling, M. H. and Schirmel, J. (2016). Effects of landscape composition on carabids and slugs in herbaceous and woody field margins. *Agriculture, Ecosystem & Environment*. 226, 79-87.

Galimberti, F., Dorati, C., Udias, A. Pistocchi, A. (2020). Estimating pesticide use across the EU. Conference proceedings JRC conference and workshop reports. Available at:

https://op.europa.eu/en/publication-detail/-/publication/120d1fc4-538f-11ea-aece-01aa75ed71a1/language-en [Accessed: 10/01/2024].

Gardner, E., Breeze, T. D., Clough, Y., Smith, H. G., Baldock, K. C. R., Campbell, A., Garratt, M. P. D., Gillespie, M. A. K., Kunin, W. E., McKerchar, M., Potts, S. G., Senapathi, D., Stone, G. N., Wäckers, F., Westbury, D. B., Wilby, A. and Oliver, T. H. (2021). Field boundary features can stabilise bee populations and the pollination of mass-flowering crops in rotational systems. *Journal of Applied Ecology*. 58(10), 2287-2304.

Garland, L. and Wells, M. J. (2020). Native planting versus non-native planting: the state of the debate. In Douglas, I., Anderson, P. M. L., Goode, D. Houck, M. C., Maddox, D., Nagendra, H. and Tan P. Y. *The routledge handbook of urban ecology*. 2nd ed. United Kingdom: Routledge. Pp 1051-1061.

Garriga, A., Morton., García-López, D. and García-del-Pino, F. (2019). Compatibility of entomopathogenic nematodes with natural enemies for horticultural pest control. *Biological Control*. 138, 104050.

Gauger, R., Lewis, E. and Stuart, R. J. (1997). Ecology in the service of biological control: the case of entomopathogenic nematodes. Oecologia. 109, 483-489.

Gaugler, R., Bednarek, A. and Campbell, J. F. (1992). Ultraviolet inactivation of heterorhabditid and steinernematid nematodes. *Journal of Invertebrate Pathology*. 59(2), 155-160.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W. W., Emmerson, M., Morales, M. B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Pärt, T., Bretagnolle, V., Plantegenest, M., Clement, L. W., Dennis, C., Palmer, C., Oñate, J. J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hänke, S., Fischer, C., Goedhart, P. W. and Inchausi, P. (2010). Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology*. 11(2), 97-105.

Geiger, F., Wäckers, F. L. and Biachi, F. J. J. A. (2008). Hibernation of predatory arthropods in semi-natural habitats. *BioControl*. 54, 529-535.

Géneau, C. E., Wäckers, F. L., Luka, H., Daniel, C. and Balmer, O. (2012). Selective flowers to enhance biological control of cabbage pests by parasitoids. *Basic and Applied Ecology*. 13(1), 85-93.

Georgis, R. (1990). Formulation and application technology. In Gaugler, R. (ed). Entomopathogenic Nematology. Oxon, United Kingdom: CABI Publishing.

Georgis, R., Koppenhöfer, A. M., Lacey, L. A., Bélair, G., Duncan, L. W., Grewal, P. S., Samish, M., Tan, L., Torr, P. and van Tol, R. W. H. M. (2006). Successes and failures in the use of parasitic nematodes for pest control. *Biological Control*. 38(1), 103-123.

Giblin-Davis, R. M., Peña, J. E. and Duncan, R. E. (1996). Evaluation of an entomopathogenic nematode and chemical insecticides for control of *Metamasius hemipterus sericeus* (Coleoptera: Curculionidae). *Journal of Entomological Science*. 31(3), 240-251.

Gladbach, D. J., Holzschuh, A., Scherber, C., Thies, C., Dormann, C. F. and Tscharntke, T. (2011). Crop–noncrop spillover: arable fields affect trophic interactions on wild plants in surrounding habitats. *Oecologia*. 166, 433-441.

Glen, D. M. (2000). The effect of cultural measures on cereal pest and their role in integrated pest management. *Integrated Pest Management Reviews*. 5, 25-40.

Godina, G., Vandenbossche, B., Schmidt, M., Sender, A., Tambe A. H., Touceda-González, M. and Ehler, R-U. (2023). Entomopathogenic nematodes for biological control of *Psylliodes chrysocephala* (Coleoptera: Chrysomelidae) in oilseed rape. *Journal of Invertebrate Pathology*. 197, 107894.

Goulson, D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*. 50(4), 977-987.

Gov.uk. (2023). Latest Horticulture Statistics. Available at: https://www.gov.uk/government/statistics/latest-horticulture-statistics [Accessed 04/03/2024].

Gov.uk. (2024a). Statement of reasons for the decision on the application for emergency authorisation for the use of Cruiser SB on sugar beet crops in 2024. Available at: https://www.gov.uk/government/publications/neonicotinoid-product-as-seed-treatment-for-sugar-beet-emergency-authorisation-application/statement-of-reasons-for-the-decision-on-the-application-for-emergency-authorisation-for-the-use-of-cruiser-sb-on-sugar-beet-crops-in-2024 [Accessed: 22/02/2024].

Gov.uk. (2024b). Accredited official statistics: Farm rents in England 2022/23. Available at: https://www.gov.uk/government/statistics/farm-rents/farm-rents-in-england-202223 [Accessed: 01/10/2024].

Grewal, P S., Power, K. T., Grewal, S. K., Suggars, A. and Haupricht, S. (2004). Enhanced consistency in biological control of white grubs (Coleoptera: Scarabaeidae) with new strains of entomopathogenic nematodes. *Biological Control.* 30(1), 73-82.

Grewal, P. S. and Georgis, R. (1999). Entomopathogenic nematodes. In Hall, F. R. and Menn, J. J. *Biopesticides: Use and Delivery*. New Jersey: Human Press. Pp 271-299.

Grunseich, J. M., Aguirre, N. M., Thompson, M. N., Ali, J. G. and Helms, A. M. (2021). Chemical cues from entomopathogenic nematodes vary across three species with different foraging strategies, triggering different behavioural responses in prey and competitors. *Journal of Chemical Ecology*. 47, 822-833.

Gurr, G. M. and Kvedaras, O. L. (2010). Synergizing biological control: Scope for sterile insect technique, induced plant defences and cultural techniques to enhance natural enemy impact. *Biological Control.* 52(3), 198-207.

Haddad, N. M., Brudvig, L. A., Clobert, J., Davis, K. F., Gonzalez, A., Holt, R. D., Lovejoy, T. E., Sexton, J. O., Austin, M. P., Collins, C. D., Cook, W. M., Damschen, E. I., Ewers, R. M., Foster, B. L., Jenkins, C. N., King, A. J., Laurance, W. F., Levey, D. J., Margules, C. R., Melbourne, B. A., Nicholls, A. O., Orrock, J. L., Song, D-X. and Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances.* 1(2), e1500052

Hallett, R. H. (2007). Host plant susceptibility to the swede midge (Diptera: Cecidomyiidae). *Horticultural Entomology*. 100(4), 1335-1343.

Hallett, R. H. and Heal, J. D. (2001). First Nearctic record of the swede midge (Diptera: Cecidomyiidae), a pest of cruciferous crops from Europe. *The Canadian Entomologist*. 133(5), 713-715.

Hallett, R. H., Chen, M., Sears, M. K. and Shelton, A. M. (2009). Insecticide management strategies for control of swede midge (Diptera: Cecidomyiidae) on cole crops. *Journal of Economic Entomology.* 102(6), 2241-2254.

Hallett, R. H., Goodfellow, S. A. and Heal, J. D. (2007). Efficacy of biopesticides for management of the swede midge (Diptera: Cecidomyiidae). *The Canadian Entomologist.* 139(5), 700-712.

Hamman, E. A., Pappalardo, P., Bence, J. R., Peacor, S., D. and Osenberg, C. W. (2018). Bias n meta-analyses using Hedges' *d. Ecosphere*. 9(9), e02419.

Haramoto, E. R. and Gallandt, E. R. (2007). Brassica cover cropping for weed management: A review. *Renewable Agriculture and Food Systems.* 19(4), 187-198.

Harris, D. R. and Fuller, D. Q. (2014). Agriculture: definition and overview. In *Encyclopedia of Global Archaeology.* Smith, C. (ed). Springer, New York. Pp 104-113.

Harris, M., Oetting, R. D. and Gardner, W. A. (1995). Use of entomopathogenic nematodes and a new monitoring technique for control of fungus gnats, *Bradysia coprophila* (Diptera: Sciaridae), in Floriculture. *Biological Control.* 5(3), 412-418.

Hatt, S., Francis, F., Xu, Q., Wang, S. and Osawa, N. (2020). Perennial flowering strips for conservation biological control of insect pests: from picking and mixing flowers to tailored functional diversity. In Gao, Hokkanen, H. M., Menzler-Hokkanen. (eds) *Integrative biological control*. Pp 57-71. Cambridge: Springer.

Hawkes, C. (1972). The estimation of the dispersal rate of the adult cabbage root fly (*Erioischia brassicae* (Bouché)) in the presence of a brassica crop. *Journal of Applied Ecology*. 9, 617-632.

He, X., Kiær, L. P., Jensen, P. M. and Sigsgaard, L. (2021). The effect of floral resources on predator longevity and fecundity: A systematic review and meta-analysis. *Biological Control*. 153, 104476.

Hedges, L. V. and Olkin, I. (1985). *Statistical methods for meta-analysis*. London: Academic Press.

Heimbach, U. and Müller, A. (2013). Incidence of pyrethroid-resistant oilseed rape pests in Germany. *Pest Management Science.* 69(2), 209-216.

Hellmann, J. J., Grundel, R., Hoving, C. and Schuurman, G. W. (2016). A call to insect scientists: challenges and opportunities of managing insect communities under climate change. *Current Opinion in Insect Science*. 17, 92-97.

Herbst, M., Razinger, J., Ugrinović, K., Škof, M., Schroers, H-J., Hommes, M. and Poehling, H-M. (2017). Evaluation of low risk methods for managing *Delia radicum*, cabbage root fly, in broccoli production. *Crop Protection.* 96, 273-280.

Hillocks, R. J. (2012). Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection.* 31(1), 85-93.

Hoarau, C., Campbell, H., Prince, G., Chandler, D. and Pope, T. (2022). Biological control agents against the cabbage stem flea beetle in oilseed rape crops. *Biological Control.* 167, 104844.

Hodgdon, E. A., Chen, Y. A., Hoepting, C. A. and Hallett, R. H. (2017). *Organic management of swede midge*. New York State IPM. Available at: https://ecommons.cornell.edu/handle/1813/55087brion

Højland, D. H., Nauen, R., Foster, S. P., Williamson, M. S. and Kristensen, M. (2015). Incidence, Spread and Mechanisms of Pyrethroid Resistance in European Populations of the Cabbage Stem Flea Beetle, *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae). *PLOS One*. 10(12): e0146045

Hokkanen, H. M. T., Vojinovic, M. Z., Husberg, G.-B., Menzler-Hokkanen, B. W., Klukowski, Z., Luik, A., Nilsson, C., Ulber, B., & Williams, I. (2006). Effectiveness of entomopathogenic nematodes in the control of OSR pests. CD Proc Int Symp Integrated Pest Management in Oilseed Rape, 3–5 April 2006, Goettingen, Germany.

Holka, M. and Kowalska, J. (2023). The potential of adjuvants used with microbiological control of insect pests with emphasis on organic farming. *Agriculture.* 13(9), 1659.

Holland, J. M., Douma, J. C., Crowley, L., James, L., Kor, L., Stevenson, D. R. W. and Smith, B. M. (2017). Semi-natural habitats support biological control, pollination and soil conservation in Europe. A review. *Agronomy for Sustainable Development.* 37(4), 31-54.

Holland, J. M., Oaten, H., Southway, S. and Moreby, S. (2008). The effectiveness of field margin enhancement for cereal aphid control by different natural enemy guilds. *Biological Control.* 47(1), 71-76.

Holland, J.M., Bianchi, F., Entling, M.H., Moonen, A.C., Smith, B.M. and Jeanneret, P. (2016). Structure, function and management of semi-natural habitats for conservation biological control: a review of European studies. *Pest Management Science.* 72(9), 1638-1651.

Hou, Y., Pang, X. and Liang, G. (2001). On the partial application of *Steinernema carpocapsae* strain A24 against striped flea beetle *Phyllotreta striolata. Acta Phytopathologica Sinica.* 28, 151-156.

Hoyle, H., Norton, B., Dunnett, N., Richards, J. P., Russell, J. M. and Warren, P. (2018). Plant species or flower colour diversity? Identifying the drivers of public and invertebrate response to designed annual meadows. *Landscape and Urban Planning.* 103-113.

Hsu, G-C., Ou, J-A. and Ho, C-K. (2021). Pest consumption by generalist arthropod predators increases with crop stage in both organic and conventional farms. *Ecosphere*. 12(7), e0325.

Hutchinson, L. A., Oliver, T. H., Breeze, T. D., O’Connor, R. S., Potts, S. G., Roberts, S. P. M. and Garratt, M. P. D. (2021). Inventorying and monitoring crop pollinating bees: Evaluating the effectiveness of common sampling methods. *Insect Conservation and Diversity*. 15(3), 299-311.

Ikemoto, M., Kuramitsu, K., Sueyoshi, M., Seguchi, S. and Yokoi, T. (2021). Relative trapping efficiencies of different types of attraction traps for three insect orders in an agricultural field. *Applied Entomology and Zoology.* 56, 393-405.

Ingrao, A. J., Schmidt, J., Jubenville, J., Grode, A., Komondy, L., VanderZee, D. and Szendrei, Z. (2017). Biocontrol on the edge: Field margin habitats in asparagus fields influence natural enemy-pest interactions. *Agriculture, Ecosystems & Environment.* 243, 47-54.

Inman III, F. L., Singh, S. and Holmes, L. D. (2012). Mass production of the beneficial nematode *Heterorhabditis bacteriophora* and its bacterial symbiont *Photorhabdus luminescens*. *Indian Journal of Microbiology.* 52, 316-324.

Jaffuel, G., Blanco-Pérez, R., Büchi, L., Mäder, P., Fließbach, A., Charles, R., Degen, T., Turlings, T. C. J. and Campos-Herrera, R. (2017). Effects of cover crops on the overwintering success of entomopathogenic nematodes and their antagonists. *Applied Soil Ecology*. 114, 62-73.

James, R. R. and Xu, J. (2012). Mechanisms by which pesticides affect insect immunity. *Journal of Invertebrate Pathology*. 109(2), 175-182.

Joseph, S. V. and Iudice, S. (2020). Evaluation of seedling tray drench of insecticides for cabbage maggot (Diptera: Anthomyiidae) management in broccoli and cauliflower. *Florida Entomologist*. 103(2), 172-179.

Josso, C., Le Ralec, A., Raymond, L., Saulais, J., Baudry, J., Poinsot, D. and Cortesero, A. M. (2013). Effects of field and landscape variables on crop colonization and biological control of the cabbage root fly *Delia radicum*. *Landscape Ecology*. 28, 1697-1715.

Kamali, S., Karimi, J., Hosseini, M., Campos-Herrera, R. and Duncan, L. W. (2013). Biocontrol potential of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* on cucurbit fly, *Dacus ciliates* (Diptera: Tephritidae). *Biocontrol Science and Technology*. 23(11), 1307-1323.

Kapranas, A., Sbaiti, I., Degen, T. and Turlings, T. C. J. (2020). Biological control of cabbage fly *Delia radicum* with entomopathogenic nematodes: Selecting the most effective nematode species and testing a novel application method. *Biological Control*. 144, 104212.

Karp, D. S., Chaplin-Kramer, R., Meehan, T. D., Martin, E. A., DeClerck, F., Grab, H., Gratton, C., Hunt, L., Larsen, A. E., Martínez-Salinas, A., O'Rourke, M. E., Rusch, A., Poveda, K., Jonsson, M., Rosenheim, J. A., Schellhorn, N. A., Tscharntke, T., Wratten, S. D., Zhang, W., Iverson, A. L., Alder, L. S., Albrecht, M., Alignier, A., Angelella, G. M., Anjum, M. M., Avelino, J., Avelino, J., Batáry, P., Baveco, J. M., Biachi, F. J. J. A., Birkhofer, K., Bohnenblust, E. W., Bommarco, R., Brewer, M. J., Caballero-López, B., Carrière, Y., Carvalheiro, L. G., Cayuela, L., Centrella, M., Ćetković, A., Henri, D. C., Chabert, A., Costamagna, A. C., De la Mora, A., de Kraker, J., Desneux, N., Diehl, E., Diekötter, T., Dormann, C. F., Eckberg, J. O., Entling, M. H., Fielder, D., Franck, P., Frank van Veen, F. J., Frank, T., Gagic, V., Garrett, M. P. D., Getachew, A., Gonthier, D. J., Goodell, P. B., Graziosi, I., Groves, R. L., Gur, G. M., Hajin-Forooshani, Z., Heimpel, G. E., Harrmann, J. D., Huseth, A. S., Inclán, D, J., Ingrao, A. J., Iv, P., Jacot, K., Johnson, G. A., Jones, L., Kaiser, M., Kaser, J. M., Keasar, T., Kim, T. N., Kishinevsky, M., Landis, D. A., Lavandero, B., Lavigne, C., Le Ralec, A., Lemessa, D., Letourneau, D. K., Liere, H., Lu, Y., Lubin, Y., Luttermoser, T., Maas, B., Mace, K., Madeira, F., Mader, V., Cortesero, A. M., Marini, L., Martinez, E., Martinson, H. M., Menozzi, P., Mitchell, M. G. E., Miyashita, T., Molina, G. A. R., Molina-Montenegro, M. A., O'Neal, M. E., Opatovsky, I., Ortiz-Martinez, S., Nash, M., Östman, Ö., Ouin, A., Pak, D., Paredes, D., Parsa, S., Parry, H., Perez-Alvarez, R., Perović, D, J., Peterson, J. A., Petit, S., Philpott, S. M., Plantegenest, M., Plećaš, M., Pluess, T., Pons, X., Potts, S. G., Pywell, R. F., Ragsdale, D. W., Rand, T. A., Raymond, L., Ricci, B., Sargent, C., Sarthou, J-P., Saulais, J., Schäckermann, J., Schmidt, N. P., Schneider, G., Schüepp, C., Sivakoff, F. S., Smith, H. G., Whitney, K. S., Stutz, S., Szendrei, Z., Takada, M. B., Taki, H., Tamburini, G., Thomson, L. J., Tricault, Y., Tsafack, N., Tschumi, M., Valantin-Morison, M., van Trinh, M., van der Werf, W., Vierling, K. T., Werling, B. P., Wickens, J. B., Wickens, V. J., Woodcock, B. A., Wyckhuys, K., Xiao, H., Yasuda, M., Yoshioka, A. and Zou, Y. (2018). Crop pests and predators exhibit inconsistent responses to surrounding landscape composition. *PNAS.* 115(33), 7863-7870.

Karssemeijer, P. N., Croijmaans, L., Gajendiran, K., Gols, R., van Apeldoorn, D. F., van Loon, J. J. A., Dicke, M. and Poelman, E. H. (2023). Diverse cropping systems lead to higher larval mortality of the cabbage root fly (*Delia radicum*). Journal of Pest Science. 5, 1-17.

Karungi, J., Adipala, E., Ogenga-Latigo, M. W., Kyamanywa, S. and Oyobo, N. (2000). Pest management in cowpea. Part 1. Influence of planting time and plant density on cowpea field pests infestation in eastern Uganda. *Crop Protection.* 19(4), 231-236.

Kaya, H. K. and Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology*. 38, 181-206.

Kinkler, K., Wäckers, F. L., Termorshuuizen, A J. and van Lenteren, J. C. (2010). Assessing risks and benefits of floral supplements in conservation biological control. *BioControl*. 55, 719-727.

Kirmer, A., Mann, S., Stolle, M., Tischew, S. and Kiehl, K. (2009). Near-natural restoration methods for high nature value areas. Conference: *Salvere* - Regional Workshop in Poland, Poznań.

Koppenhöfer, A. M., Shapiro-Ilan, D. I. and Hiltpold, I. (2020). Entomopathogenic nematodes in sustainable food production. *Frontiers in Sustainable Food Systems*. 4.

Kung, S-P., Gaugler, R. and Kaya, H. K. (1990). Soil type and entomopathogenic nematode persistence. *Journal of Invertebrate Pathology*. 55(3), 401-406.

Kuznetsova, A., Brockhoff, P., B. and Christensen, R., H., B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*. 82(13), 1–26.Lortkipanidze, M. A., Gorgadze, O. A., Kajaia, G. S., Gratiashvili, N. G. and Kuchava, M. A. (2016). Foraging behaviours and virulence of some entomopathogenic nematodes. *Annals of Agrarian Science.* 14(2), 99-103.

Lacey, L. A., Grzywacs, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M. and Goettel. M. S. (2015). Insect pathogens as biological control agents: back to the future. *Journal of Invertebrate Pathology*. 132, 1-41.

Lacey, L. A., Shapiro-Ilan, D. I. and Glenn, G. M. (2010). Post-application of anti-desiccant agents improves efficacy of entomopathogenic nematodes in formulated host cadavers or aqueous suspension against diapausing codling moth larvae (Lepidoptera: Tortricidae). *Biocontrol Science and Technology*. 20(9), 909-921.

Lamberth, C., Jeanmart, S., Luksch, T. and Plant, A. (2013). Current challenges and trends in the discovery of agrochemicals. *Science.* 341(6147), 742-746.

Lamy, F., Dugravot, S., Cortesero, A. M., Chaminade, V., Faloya, V. and Poinsot, D. (2017). One more step toward a push-pull strategy combining both a trap crop and plant volatile organic compounds against the cabbage root fly *Delia radicum. Environmental Science and Pollution Research.* 25, 19868-29879.

Landis, D. A., Wratten, S. D. and Gurr, G. M. (2000). Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology*. 45(1), 175-201.

Laubertie, E. A., Wratten, S. D. and Sedcole, J. R. (2006). The role of odour and visual cues in the pan-trap catching of hoverflies (Diptera: Syrphidae). *Annals of Applied Biology*. 148(2), 173-178.

Lavandero I, B., Wratten, S. D., Didham, R. K. and Gurr, G. (2006). Increasing floral diversity for selective enhancement of biological control agents: A double-edged sward? *Basic and Applied Ecology*. 7(3), 236-243.

Lefebvre, M., Langrell, S. R. H. and Gomez-y-Paloma, S. (2014). Incentives and policies for integrated pest management in Europe: a review. *Agronomy for Sustainable Development.* 35, 27-45.

Letourneau, D. K., Armbrecht, I., Rivera, B. S., Lerma, J. M., Carmona, E. J., Daza, M. C., Escobar, S., Galindo, V., Gutiérrez, C., López, S. D., Mejía, J. L., Rangel, A. M. A., Rangel, J. H., Rivera, L., Saavedra, C. A., Torres, A. M. and Trujillo, A. R. (2011). Does plant diversity benefit agroecosystems? A synthetic review. *Ecological Applications*. 21(1), 9-21.

Lewis, E. E., Campbell, J., Griffin, C., Kaya, H. and Peters, A. (2006). Behavioural ecology of entomopathogenic nematodes. *Biological Control.* 38(1), 66-79.

Lipsey, M. W. and Wilson, D. (2000). *Practical meta-analysis*. SAGE Publications, Inc. California, United States.

Lortkipanidze, M. A., Gorgadze, O. A., Kajaia, G. S., Gratiashvili, N. G. and Kuchava, M. A. (2016). Foraging behaviours and virulence of some entomopathogenic nematodes. *Annals of Agrarian Science.* 14(2), 99-103.

Lowenstein, D. M. and Minor, E. S. (2018). Herbivores and natural enemies of brassica crops in urban agriculture. *Urban Ecosystems.* 21, 519-29.

Lundin, O., Rundlöf, M., Smith, H. G., Fries, I. and Bommarco, R. (2015). Neonicotinoid insecticides and their impacts on bees: A systematic review of research approaches and identification of knowledge gaps. *PloS ONE.* 10(8), e0136928.

MacLeod, A., Wratten, S. D., Sotherton, N. W. and Thomas, M. B. (2004). 'Beetle banks' as refuges for beneficial arthropods in farmland: long-term changes in predator communities and habitat. *Agricultural and Forest Entomology*. 6(2), 147-154.

Mansion-Vaquié, A., Ferrante, M., Cook, S. M., Pell, J. K. and Lövei, G. L. (2017). Manipulating field margins to increase predation intensity in fields of winter wheat (*Triticum aestivum*). *Journal of Applied Entomology*. 141(8), 600-611.

Marshall, E. J. P. (2004). Agricultural landscapes: field margin habitats and their interaction with crop production. *Journal of Crop Improvement.* 12(1), 365-404.

Marshall, E. J. P. and Moonen, A. C. (2002). Field margins in northern Europe: their functions and interactions with agriculture. *Agriculture, Ecosystem & Environment.* 89(1-2), 5-21.

Martin, E. A., Reineking, B., Seo, B. and Steffan-Dewenter, I. (2012). Natural enemy interactions constrain pest control in complex agricultural landscapes. *PNAS.* 110(14), 5534-5539.

Mason, J. M., Matthews, G. A., Wright, D. J. (1998). Appraisal of spinning disc technology for the application of entomopathogenic nematodes. *Crop Protection*. 17(5), 453-461.

Mayanglambam, S., Singh, K. D. and Rajashekar, Y. (2021). Current biological approaches for management of crucifer pests. *Scientific Reports.* 11(11831).

Mazoyer, M. and Roudart, L. (2006). *A History of World Agriculture: From the Neolithic Age to the Current Crisis.* NYU Press.

McCabe, E., Loeb, G. and Grab, H. (2017). Responses of crop pests and natural enemies to wildflower borders depends on functional group. *Insects.* 8(3), 73.

McCravy, K. W. and Willand, J. E. (2007). Effects of pitfall trap preservative on collections of Carabid Beetles (Coleoptera: Carabidae). *The Great Lakes Entomologist*. 40(3-4), 154-165.

McGaw, B. A. and Koppenhöfer, A. M. (2008). Evaluation of two endemic and five commercial entomopathogenic nematode species (Rhabditida: Heterorhabditidae and Steinernematidae) against annual bluegrass weevil (Coleoptera: Curculionidae) larvae and adults. *Biological Control*. 46(3), 467-475.

Meek, B., Loxton, D., Sparks, T., Pywell, R., Pickett, H. and Nowakowski, M. (2002). The effect of arable field margin composition on invertebrate biodiversity. *Biological Conservation.* 106(2), 259-271.

Michaud, J. P. (2018). Problems inherent to augmentation of natural enemies in open agriculture. *Neotropical Entomology*. 47, 161-170.

Mkenda, P. A., Ndakidemi, P. A., Mbega, E., Stevenson, P. C., Arnold, S. E. J., Gurr, G. M. and Belmain, S. R. (2019). Multiple ecosystem services from field margin vegetation for ecological sustainability in agriculture: scientific evidence and knowledge gaps. *Peer J.* 7(e8091)

Montgomery, I., Caruso, T. and Reid, N. (2020). Hedgerows as ecosystems: Service delivery, management, and restoration. *Annual Review of Ecology, Evolution, and Systematics.* 51, 81-102.

Morgan, W. H., Thébault, E., Seymour, C. L. and van Veen, F. J. F. (2017). Density dependence and environmental factors affect population stability of an agricultural pest and its specialist parasitoid. *BioControl.* 62, 175-184.

Morris, O. N. (1987). Evaluation of the nematode, *Steinernema feltiae* Filipjev, for the control of the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). The Canadian Entomologist. 119(1), 95-101.

Mrácek, Z. (2002). Use of Entomoparasitic Nematodes (EPANs) in Biological Control. In Upadhyay, R. K. (eds). *Advances in Microbial Control of Insect Pests.* Springer, Boston, MA. pp 235-264.

Muneret, L., Mitchell, M., Seufert, V., Aviron, S., Djoudi, E. A., Pétillon, J., Plantegenest, M., Thiéry, D. and Rusch, A. (2018). *Nature Sustainability*. 1, 361-368.

Naranjo, S. E., Ellsworth, P. C. and Frisvold, G. B. (2015). Economic value of biological control in integrated pest management of managed plant systems. *Annual Review of Entomology*. 60, 621-645.

Ndakidemi, B., Mtei, K. and Ndakidemi, P. A. (2016). Impacts of synthetic and botanical pesticides on beneficial insects. *Agricultural Science.* 7(6)

Nilsson, U. & Gripwall, E. (1999). Influence of application technique on the viability of the biological control agents *Verticillium lecanii* and *Steinernema feltiae*. *Crop Protection*. 18, 53-59.

Noordijk, J., Musters, C. J. M., van Dijk, J. and de Snoo, G. R. (2010). Invertebrates in field margins: taxonomic group diversity and functional group abundance in relation to age. *Biodiversity and Conservation. 19*, 3255-3268.

Noosidum, A., Mangtab, S. and Lewis, E. E. (2021). Biological control potential of entomopathogenic nematodes against the striped flea beetle, *Phyllotreta sinuata* Stephens (Coleoptera: Chrysomelidae). *Crop Protection*. 141, 105448.

Noriega, J. A., Hortal, J., Azcárate, F. M., Berg, M. P., Bonada, N., Briones, M. J. I. del Toro, I., Goulson, D., Ibanez, S., Landis, D. A., Moretti, M., Potts, S. G., Slade, E. M., Stout, J. C., Ulyshen, M. D. Wäckers, F. L., Woodcock, B. A. and Santos, A. M. C. (2018). Research trends in ecosystem services provided by insects. *Basic and Applied Ecology*. 26, 8-23.

Olfert, O., Hallett, R., Weiss, R. M., Soroka, J. and Goodfellow, S. (2006). Potential distribution and relative abundance of swede midge, *Contarinia nasturtii*, an invasive pest in Canada. *Entomologia Experimentalis et Applicata.* 120(3), 221-228.

Olson, D. M. and Wäckers, F. L. (2006). Management of field margins to maximize multiple ecological services. *Journal of Applied Ecology*. 44(1), 13-21.

Orre, G. U. S., Tompkins, J-M., Jonsson, M., Jacometti, M. A. and Wratten, S. D. (2007). Provision of floral resources for biological control restoring an important ecosystem service. *Functional Ecosystems and Communities.* Global Sciences Books: New Zealand.

Ortega-Ramos, P. A., Coston, D. J., Seimandi-Corda, G., Mauchline, A. L. and Cook, S. M. (2021). Integrated pest management strategies for cabbage stem flea beetle (*Psylliodes chrysocephala*) in oilseed rape. *GCB Bioenergy*. 14(3), 267-286.

Parker, M. and Nally, R. M. (2002). Habitat loss and the habitat fragmentation threshold: an experimental evaluation of impacts on richness and total abundances using grassland invertebrates. *Biological Conservation.* 105(2), 217-229.

Pastor, D. A. and Lazowski, R. A. (2017). On the multilevel nature of meta-analysis: a tutorial, comparison of software programs, and discussion of analytic choices. *Multivariate Behavioral Research*. 53(1), 74-89.

Pavlidis, G. and Tsihrintzis, V. A. (2018). Environmental benefits and control of pollution to surface water and groundwater and agroforestry systems: A review. *Water Resources Management.* 32, 1-29.

Pawase, P. P., Nalawade, S. M., Bhanage, G. B., Walunj, A. A., Kadam, P. B., Durgude, A. G. and Patil, M. G. (2023). Variable rate fertilizer application technology for nutrient management: A review. *International Journal of Agricultural and Biological Engineering*. 16(4).

Pearson, D. E. and Callaway. R. M. (2005). Indirect nontarget effects of host-specific biological control agents: Implications for biological control. *Biological Control*. 35(3), 288-298.

Pellissier, M. E. and Jabbour, R. (2018). Herbivore and parasitoid insects respond differently to annual and perennial floral strips in an alfalfa ecosystem. *Biological Control*. 123, 28-35.

Peters, J. L., Sutton, A, J., Jones, D. R., Abrams, K. R. and Rushton, L. (2008). Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *Journal of Clinical Epidemiology*. 61(10), 991-996.

Peterson, R K. and Hunt, T. E. (2003). The probabilistic economic injury level: incorporating uncertainty into pest management decision-making. *Journal of Economic Entomology*. 96(3), 536-542.

Plath, E., Rischen, T., Mohr, T. and Fischer, K. (2021). Biodiversity in agricultural landscapes: Grassy field margins and semi-natural fragments both foster spider diversity and body size. *Agriculture, Ecosystems & Environment.* 316

Pollier, A., Tricaulth, Y., Plantegenest, M. and Bischoff, A. (2018). Sowing of margin strips rich in floral resources improves herbivore control in adjacent crop fields. *Agricultural and Forest Entomology*. 21(1), 119-129.

Portman, S. L., Krishnankutty, S. M. and Reddy, G. V. P. (2016). Entomopathogenic nematodes combined with adjuvants presents a new potential biological control method for managing the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae). *PLoS ONE*. 11(2), e0169022.

Power, A. G. (2010). Ecosystem services and agriculture: tradeoffs and synergies. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 365(1554), 2959–2971.

Prather, C. M., Pelini, S. L., Laws, A., Rivest, E., Woltz, M., Bloch, C. P., del Toro, I., Ho, C-K., Kominoski, J., Newbold, T. A. S., Parsons, S. and, Joern, A. (2013). Invertebrates, ecosystem services and climate change. *Biological Reviews*. 88, 327-348.

Price, C., Campell, H. and Pope, T. (2023). Potential of entomopathogenic nematodes to control the cabbage stem flea beetle *Psylliodes chrysocephala*. *Insects.* 14(7), 665

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

R Core Team. (2022). R: A Language and Environment for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

Racke, K. D. (2006). A reduced risk insecticide for organic agriculture: Spinosad case study. *Crop Protection Products for Organic Agriculture.* 7, 92-108.

Rakow, G. (2004). Species Origin and Economic Importance of Brassica. In Pua, E. C. and Douglas, C. J. (eds). Brassica: Biotechnology in Agriculture and Forestry, vol 54. Pp 3-11. Berlin, Heidelberg: Springer.

Ramsden, M. W., Menéndez, R., Leather, S. L. and Wäckers, F. (2015). Optimizing field margins for biocontrol services: The relative role of aphid abundance, annual floral resources, and overwinter habitat in enhancing aphid natural enemies. *Agriculture, Ecosystems & Environment.* 199, 94-104.

Raymond, B., Johnston, P. R., Nielsen-LeRoux, C., Lereclus, D. and Crickmore, N. (2010). Bacillus thuringiensis: an impotent pathogen? *Trends in Microbiology.* 18(5), 189-194.

Razaq, M., Aslam, M., Amer, M. and Shad, S. A. (2011). Insect pest status of aphids on oilseed brassica crops and need for chemical control. *Crop and Environment*. 2(2), 60-63.

Readshaw, J. L. (1966). The ecology of the swede midge, *Contarinia nasturtii* (Kieff.) (Diptera, Cecidomyiidae). I.—Life-history and influence of temperature and moisture on development. *Bulletin of Entomological Research.* 56(4), 685-700.

Reddy, G. V. P., Tangtrakulwanich, K., Wu, S., Miller, J. H., Ophus, V. L. and Prewett, J. (2014). Sustainable tactics for control of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae) from Quebec, Canada. *The Canadian Entomologist*. 148, 476-478.

Redman, G. (2024). *The John Nix Pocketbook for Farm Management*. 54th ed. Agro Business Consultants Ltd.

Rodenwald, N., Sutcliffe, L. M. E., Leuschner, C. and Batáry, P. (2023). Weak evidence for biocontrol spillover from both flower strips and grassy field margins in conventional cereals. *Agriculture, Ecosystems & Environment*. 355, 108614.

Rosenheim, J. A. (2001). Source-sink dynamics for a generalist insect predator in habitats with strong higher-order predation. *Ecological Monographs.* 71(1), 93-116.

Rundlöf, M., Andersson, G. K. S., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., Jonsson, O., Klatt, B. J., Pedersen, T. R., Yourstone, J. and Smith, H. G. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature.* 521, 77-80.

Rusch, A., Bommarco, R., Jonsson, M., Smith, H. G. and Ekbom, B. (2013). Flow and stability of natural pest control services depend on complexity and crop rotation at the landscape scale. *Journal of Applied Ecology.* 50(2), 345-354.

Russell, M. (2015). A meta-analysis of physiological and behavioral responses of parasitoid wasps to flowers of individual plant species. *Biological Control.* 82, 96-103.

Rust, N. A., Stankovics, P., Jarvis, R. M., Morris-Trainor, Z., de Vries, J. R., Ingram, J., Mills, J., Glikman, J. A., Parkinson, J., Toth, Z., Hansda, R., McMorran R., Glass, J. and Reed, M. S. (2021). *Have farmers had enough of experts?* Environmental Management. 69(31-44).

Sáenz-Aponte, A., Correa-Cuadros, J. P. and Rodríguez-Bocanegra, M. X. (2020). Foliar application of entomopathogenic nematodes and fungi for the management of the diamondback moth in greenhouse and field. *Biological Control.* 142, 104163.

Santolamazza-Carbone, S., Velasco, P. And Cartea, M. E. (2017). Resistance to the cabbage root fly, *Delia radicum* (Diptera, Anthomyiidae), of turnip varieties (*Brassica rapa* subsp. *rapa*). *Euphytica.* 213(27).

Schaub, S., Buber, R., Finger, R. (2020). Tracking societal concerns on pesticides – a Google Trends analysis. *Environmental Research Letters.* 15(8), 084049.

Schellhorn, N. A., Harmon, J. P. and Andow, D. A. (2000). Using cultural practices to enhance insect pest control by natural enemies. In Rachcigl, J. E. and Rachcigl, N. A. *Insect Pests Management: Techniques for Environmental Protection*. Chapter 5. New York, United States: CRC Lewis Publishers.

Schmidt, A., Kirmer, A., Hellwig, N., Kiehl, K. and Tischew, S. (2021). Evaluating CAP wildflower strips: High-quality seed mixtures significantly improve plant diversity and related pollen and nectar resources. *Journal of Applied Ecology*. 59, 860-871.

Schnee, L., Sutcliffe, L. M. E., Leuschner, C. Donath, T. W. (2023). Weed seed banks in intensive farmland and the influence of tillage, field position and sown flower strip. *Land.* 12 (4), 926.

Schoeny, A., Lauvernay, A., Lambion, J., Mazzia, C. and Capowiez, Y. (2019). The beauties and the bugs: A scenario for designing flower strips adapted to aphid management in melon crops. *Biological Control.* 126, 103986.

Schowalter, T. D., Noriega, J. A. and Tscharntke, T. (2018). Insect effects on ecosystem services – Introduction. *Basic and Applied Ecology*. 26, 1-7.

Schroer, S. and Ehlers, R-U. (2005). Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*). *Biological Control.* 33(1), 81-86.

Scott, C. and Bilsborrow, P. E. (2019). The impact of the EU neonicotinoid seed-dressing ban on oilseed rape production in England. *Pest Management Science.* 75(1), 125-133.

Seimandi-Corda, G., Hall, J., Jenkins, T. and Cook, S. M. (2023). Relative efficiency of methods to estimate cabbage stem flea beetle (*Psylliodes chrysocephala*) larval infestation in oilseed rape (*Brassica napus*). *Pest Management Science.* Early view.

Selby, T. P., Lahm, G. P., Stevenson, T. M., Hughes, K. A., Cardova, D., Annan, I. B., Barry, J. D., Benner, E. A., Currie, M. J. and Pahutski, T. F. (2013). Discovery of cyantraniliprole, a potent and selective anthranilic diamide ryanodine receptor activator with cross-spectrum insecticidal activity. *Bioorganic & Medicinal Chemistry Letters.* 23(23), 6341-6345.

Shackelford, G., Steward, P. R., Benton, T. G., Kunin, W. E., Potts, S. G., Biesmeijer, J. C. and Sait, S. M (2013). Comparison of pollinators and natural enemies: a meta-analysis of landscape and local effects on abundance and richness in crops. *Biological Reviews.* 88(4), 1002-1021.

Shah, P. A. and Pell, J. K. (2003). Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology. 61*, 413-423.

Shapiro-Ilan, D. I., and Dolinski, C. (2015). Entomopathogenic Nematode Application Technology. In: Campos-Herrera, R. *Nematode Pathogenesis of Insects and Other Pests*. *Sustainability in Plant and Crop Protection.* Switzerland: Springer Cham.

Shapiro-Ilan, D. I., Gouge, D. H., Piggott, S. J. and Fife, J. P. (2006). Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biological Control.* 38(1), 124-133.

Shapiro-Ilan, D. I., Leite, L. G. and Han, R. (2023). Production of entomopathogenic nematodes. In Moeales-Ramos, J. A., Rojas, M. G. and Shapiro-Ilan, D. I. Second edition. *Mass Production of Beneficial Organisms*. United States: Academic Press (Elsevier). 293-315.

Shapiro-Ilans, D., McCoy, C. W., Fares, A., Obreza, T. and Dou, H. (2000). Effects of soil type on virulence and persistence of entomopathogenic nematodes in relation to control of *Diaprepes abbreviates* (Coleoptera: Curculionidae). *Biological Control*. 29(5), 1083-1087.

Sharmila, R., Subramanian, S. and Poornima, K. (2018). Host range of entomopathogenic nematodes. *Journal of Entomology and Zoology Studies.* 6(3), 1310-1312.

Shrestha, M., Garcia, J. E., Chua, J. H. J., Howard, S. R., Tscheulin, T., Dorin, A., Nielsen, A. and Dyer, A. G. (2019). Fluorescent pan traps affect the capture rate of insect orders in different ways. *Insects*. 10(2), 40.

Smith, A. (2006). Green niches in sustainable development: the case of organic food in the United Kingdom. *Environment and Planning C: Government and Policy*. 24, 439-458.

Smith, J., Potts, S. G., Woodcock, B. A. and Eggleton, P. (2007). Can arable field margins be managed to enhance their biodiversity, conservation, and functional value for soil macrofauna? *Journal of Applied Ecology*. 45(1), 269-278.

Smits, P. H. (1996). Post-application persistence of entomopathogenic nematodes. Biocontrol *Science and Technology*. 6(3), 379-388.

Snyder, W. E. and Evans, E. W. (2006). Ecological effects of invasive arthropod generalist predators. *Annual Review of Ecology, Evolution and Systematics.* 37, 95-122

Snyder, W. E. and Ives, A. R. (2003). Interactions between specialist and generalist natural enemies: parasitoids, predators, and pea aphid biocontrol. *Ecology*. 84(1), 91-107.

Soroka, J., Grenkow, L., Mori, B. and Andreassen, L. (2019). Injury by *Contarinia* (Diptera: Cecidomyiidae) to early-seeded and late-seeded canola in northeastern Saskatchewan, Canada, and assessment of seed treatments for midge control. *The Canadian Entomologist*. 151(2), 219-235.

Southwood, T. R. E. and Henderson, P. A. (2009). *Ecological Methods*. Third edition. Oxford, United Kingdom: Blackwell Science Ltd.

Sparks, T. C. (2015). IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*. 121, 122-128.

Stark, J. D. and Banks, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology*. 48, 505-519.

Stenberg, J. A. (2017). A conceptual framework for integrated pest management. *Trends in Plant Science*. 22(9), 759-769.

Stenberg, J. A., Sundh, I., Becher, P. G., Björkman, C., Dubey, M., Egan, P. A., Friberg, H., Gil, H. F., Jensen, D. F., Jonsson, M., Karlsson, M., Khalil, S., Ninkovic, V., Rehermann, G., Vetukuri, R. R. and Viketoft, M. (2021). When is it biological control? A framework of definitions, mechanisms and classifications. *Journal of Pest Science*. 94, 665-676.

Strand, M. R. and Obrycki, J. J. (1996). Host specificity of insect parasitoids and predators. *BioScience*. 46(6), 422-429.

Stratton, C. A., Hodgdon, E., Rodriguez-Saona, C., Shelton, A. M. and Chen, Y. H. (2019). Odors from phylogenetically-distant plants to Brassicaceae repel an herbivorous Brassica specialist. *Scientific Reports*. 9, 10621.

Susurluk, A. (2008). Potential of the entomopathogenic nematodes *Steinernema feltiae*, *S. weiseri* and *Heterohabditis bacteriophora* for the biological control of the sugar beet weevil *Bothynoderes punctiventris* (Coleoptera: Curculionidae). *Journal of Pest Science*. 81, 221-225.

Susurluk, A. and Ehlers R-U. (2008). Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. *BioControl*. 53, 627-641.

Swinton, S. M., Lupi, F., Robertson, G. P. and Hamilton, S. K. (2007). Ecosystem services and agriculture: Cultivating agricultural ecosystems for diverse benefits. *Ecological Economics*. 64(2), 245-252.

Tang, S. and Cheke, R. (2008). Models for integrated pest control ad their biological implications. *Mathematical Biosciences*. 215(1), 115-125.

Tefera, T. and Vidal, S. (2009). Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. *BioControl*. 54, 663-669.

Thanwisai, A., Muangpat, P., Meesil, W., Janthu, P., Dumidae, A., Subkrasae, C., Ardpairin, J., Tandhavanant, S., Yoshino, T. P. and Vitta, A. (2022). Entomopathogenic nematodes and their symbiotic bacteria from the National Parks of Thailand and larvicidal property of symbiotic bacteria against *Aedes aegypti* and *Culex quinquefasciatus*. *Biology (Basel).* 13(11), 1658.

Thies, C. and Tscharntke, T. (2010). Biological rape pest control in spatio-temporally changing landscapes. In Williams, I. (eds). *Biocontrol-Based Integrated Management of Oilseed Rape Pests*. Springer, Dordrecht. Pp 273-284.

Thompson, H. M. (1996). Interactions between pesticides: a review of reported effects and their implications for wildlife risk assessment. *Ecotoxicology*. 5, 59-81

Tilman, D. (1999). The ecological consequences of changes in biodiversity: A search for general principles. *Ecology.* 80(5), 1455-1474.

Tilman, D., Lehman, C. L. and Bristow, C. E. (1998). Diversity-stability relationships: statistical inevitability or ecological consequence? *The American Naturalist*. 151(3), 277-282.

Toepfer, S., Knuth, P., Glas, M. and Kuhlmann, U. (2012). Successful application of entomopathogenic nematodes for the biological control of western corn rootworm larvae in Europe – a mini review. Proceedings International Conference on the German Diabrotica Research Program.

Török, E., Zieger, S., Rosenthal, J., Földesi, R., Gallé, R., Tscharntke, T. and Batáry, P. (2021). Organic farming supports lower pest infestation, but fewer natural enemies than flower strips. *Journal of Applied Ecology*. 58(10), 2277-2286.

Torretta, J. P. and Poggio, S. L. (2013). Species diversity of entomophilous plants and flower-visiting insects is sustained in the field margins of sunflower crops. *Journal of Natural History*. 47(3-4), 139-165.

Trdan, S., Vidrih, M., Valič, N. and Laznik, Ž. (2008). Impact of entomopathogenic nematodes on adults of *Phyllotreta* spp. (Coleoptera: Chrysomelidae) under laboratory conditions. *Acta Agriculturae Scandinavica Section B-Soil and Plant.* 58(2), 169-175.

Tscharntke, T. Steffan-Dewenter, I., Kruess, A. and Thies, C. (2002). Contribution of small habitat fragments to conservation of insect communities of grassland-cropland landscapes. *Ecological Applications.* 12(2), 354-363.

Tscharntke, T., Grass, I., Wanger, T. C., Westphal, C. and Batáry, P. (2021). Beyond organic farming - harnessing biodiversity-friendly landscape. *Trends in Ecology & Evolution*. 36(10), 919-930.

Ulu, T. C. and Erdoğan, H. (2023). Field application of encapsulated entomopathogenic nematodes using a precision planter. *Biological Control.* 182, 105240

Valantin-Morison, M., Meynard, J. M. and Doré, T. (2007). Effects of crop management and surrounding field environment on insect incidence in organic winter oilseed rape (*Brassica napus* L.). *Crop Protection*. 26(8), 1108– 1120.

van Dijk, J., de Louw, M. D. E., Kalis, L. P. A. and Morgan, E. R. (2009). Ultraviolet light increases mortality of nematode larvae and can explain patterns of larval availability at pasture. *International Journal for Parasitology.* 39(10), 1151-1156.

van Driesche, R. G. and Bellows, T. S. (1996). Pest origins, pesticides, and the history of biological control. In *Biological Control.* Springer, Boston, MA. Pp. 3-20.

van Lenteren, J. C. (2011). The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl.* 57, 1-20.

van Rijn, P. C. L. and Wäckers, F. L. (2015). Nectar accessibility determines fitness, flower choice and abundance of hoverflies that provide natural pest control. *Journal of Applied Ecology*. 53(3), 925-933.

Viechtbauer, W. (2005). Bias and efficiency of meta-analytic variance estimators in the random-effects model. *Journal of Educational and Behavioral Statistics.* 30(3), 261-293.

Viechtbauer,W. (2010). Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*. 36(3), 1–48.

Vrdoljak, S. M. and Samways, M. J. (2012). Optimising coloured pan traps to survey flower visiting insects. *Journal of Insect Conservation.* 16, 345-354.

Wäckers, F. L. and van Rijn, P. C. J. (2012). Pick and mix: selecting flowering plants to meet the requirements of target biological control insects. In Gurr, G. M., Wratten, S. D., Snyder, W. E. and Read, D. M. Y. (eds). *Biodiversity and Insect Pests: Key Issues for Sustainable Management*. Chapter 9. United Kingdom: John Wiley & Sons. Pp 139-165

Wäckers, F. L., Romeis, J. and van Rijn, P. (2007). Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. *Annual Review of Entomology*. 51, 301-323.

Wantulla, M., van Zadelhoff, K., van Loon, J. J. A. and Dicke, M. (2022). The potential of soil amendment with insect exuviae and frass to control the cabbage root fly. *Journal of Applied Entomology*. 147(3), 181-191.

Washuck, N., Hanson, M. and Prosser, R. (2022). Yield to the data: some perspective on crop productivity and pesticides. *Pest Management Science*. 78, 1765-1771.

Wegensteiner, R., Wermelinger, B. And Herrmann, M. (2015). Chapter 7 – Natural enemies of bark beetles: predators, parasitoids, pathogens, and nematodes. In Ranger, C. M. Edited by Vega, F. E. and Hofstetter, R. W. *Bark Beetles: Biology and Ecology of Native and Invasive Species*. Academic Press. Pp 247-304.

Werling, B. P. and Gratton, C. (2008). Influence of field margins and landscape context on ground beetle diversity in Wisconsin (USA) potato fields. *Agriculture, Ecosystems & Environment*. 128(1-2), 104-108.

White, S., Ellis, S., Pickering, F., Leybourne, D., Corkley, I., Kendall, S., Collins, L., Newbert, M., Cotton, L., & Phillips, R. (2020). Project Report No. 623 Integrated pest management of cabbage stem flea beetle in oilseed rape. AHDB Cereals and Oilseeds.

Wietzke, A., Albert, K., Bergmeier, E., Sutcliffe, L. M. E., van Waveren, C-S. and Leuschner, C. (2020). Flower strips, conservation field margins and fallows promote the arable flora in intensively farmed landscapes: Results of a 4-year study. *Agriculture, Ecosystems & Environment*. 304, 107142.

Wilby, A. and Thomas, B. (2002). Natural enemy diversity and pest control: patterns of pest emergence with agricultural intensification. *Ecology Letters*. 5(3), 353-360.

Williams, I. H. (2010). The major insect pests of oilseed rape in Europe and their management: an overview. In *Biocontrol-Based Integrated Management of Oilseed Rape Pests*. Springer, Dordrecht. Pp 1-13.

Willis, C. E., Foster, S. P., Zimmer, C. T., Elias, J., Chang, X., Field, L. M., Williamson, M. S. and Davis, T. G. E. (2020). Investigating the status of pyrethroid resistance in UK populations of the cabbage stem flea beetle (*Psylliodes chrysocephala*). *Crop Protection.* 138, 105316.

Wilson, L. and Barnett, W. (1983). Degree-days: An aid in crop and pest management. *Californian Agriculture.* 37(1), 4-7.

Winkler, K., Wackers, F. L., Termorshuizen, A. J. and van Lenteren, J. C. (2010). Assessing risks and benefits of floral supplements in conservation biological control. *BioControl.* 55, 719-727.

Woodcock, B. A., Bullock, J. M., McCracken, M., Chapman, R. E., Ball, S. L., Edwards, M. E., Nowakowski, M. and Pywell, R. F. (2016). Spill-over of pest control and pollination services into arable crops. *Agriculture, Ecosystems & Environment*. 231, 15-23.

Zamorano, J., Bartomeus, I., Grez, A. A. and Garibaldi, L. A. (2020). Field margin floral enhancements increase pollinator diversity at the field edge but show no consistent spillover into the crop field: a meta-analysis. *Insect Conservation and Diversity*. 13(6), 519-531.

Zehner, G., Gurr, G. M., Kühne, S., Wad, M. R., Wratten, S. D. and Wyss, E. (2007). Arthropod pest management in organic crops. *Annual Review of Entomology*. 52, 57-80.

Zhang, W., Ricketts, T. H., Kremen, C. Carney, K. and Swinton, S. M. (2007). Ecosystem services and dis-services to agriculture. *Ecological Economics.* 64(2), 253-260.

Zhao, Z-H., Hiu, C., Li, Z-H. and Li, B-L. (2015). Habitat heterogeneity stabilizes the spatial and temporal interactions between cereal aphids and parasitic wasps. *Basic and Applied Ecology.* 16(6), 510-518.

Zhu, P., Zheng, X., Xie, G., Chen, G., Lu, Z. and Gurr, G. (2020). Relevance of the ecological traits of parasitoid wasps and nectariferous plants for conservation biological control: a hybrid meta-analysis. *Pest Management Science.* 76(5), 1881-1892.

Zoroa, N., Fernández-Sáez, M. J. and Zoroa, P. (2011). A foraging problem: sit-and-wait versus active predation. *European Journal of Operational Research*. 208(2), 131-141.

Appendices

Appendix 1.

Table A1.1. List of papers included in the meta-analysis. Scores indicate which variables are measured within each paper (1 = included).

Authors (year of publication)	**Natural enemy**		**Herbivorous pest**		**Crop damage/yield**
	Abundance	Diversity	Abundance	Diversity	
Anderson et al. (2013)	1	1	1	1	
Anjum-Zubair et al. (2010)	1	1			
Anjum-Zubair et al. (2015)	1	1	1	1	
Atakan (2010)	1	1			
Balzan and Moonen (2013)	1				
Banks (2000)			1		
Baverstock, Clark & Pell (2008)	1				
Boetzl et al. (2018)	1	1			
Bote and Romero (2012)	1		1		
Campbell et al. (2017)	1	1	1		1
Campbell et al. (2019a)	1	1			
Campbell et al. (2019b)	1		1		
Denys and Tscharntke (2002)	1	1			
Dively, Leslie and Hooks (2020)	1		1		
Fusser et al. (2017)	1	1			
Galle et al. (2020)	1	1			
Grab et al. (2018)	1		1		1
Haenke et al. (2009)	1	1			
Hatt et al. (2017)	1	1			
Meek et al. (2002)	1	1	1		
Middleton and MacRae (2021)			1		
Pecheur et al. (2020)	1	1			
Pywell et al. (2011)	1	1	1	1	1
Pywell et al. (2015)	1	1			
Ramsden et al. (2015)	1		1		
Schmidt-Entling and Dobeli (2009)	1	1			
Segre et al. (2019)	1	1	1		
Serge et al. (2020)	1		1		1
Smith et al. (2008)	1	1	1	1	
Toivonen, Huusela-Veistola and Herzon (2018)	1		1		
Tschumi et al. (2016)	1	1	1		
Tschumi (2015)	1				1
Woodcock et al. (2007)	1		1		
Woodcock et al. (2008)	1	1	1		
Woodcock et al. (2010)	1	1	1		
Woodcock et al. (2016)	1		1		

Table A1.2. Categorical predictor (moderator) variables with descriptions.

Moderator	**Description**
Natural enemy type	Highlights whether a natural enemy is classed as a predator, pathogen, or parasite.
Specialism	Indicates whether a natural enemy is a specialist feeder or generalist feeder. A full list of natural enemies appearing in the included papers and the associated category can be found in Table A.1. If monitored species are specifically classed within the studies as an aphidophagous species, then they have been considered specialists.
Development	Highlights whether pest species development type falls into the endopterygote or exopterygote classification.
Guild	Differentiation of the environmental strata in which a natural enemy resides, feeds, and processes resources; terrestrial/vegetative and aerial. Terrestrial and vegetative guilds were grouped due to a lack of differentiation in the accepted papers.
Margin type	Defines the type(s) of field margins that is compared to the control (grass margin) in each accepted study. Grass margins are most often spontaneous non-copped areas at field edges, dominated by aggressive grasses, and generally have low species diversity. Sown floral margins, described as specifically sown mixes, generally with high floral percentage and little to no grass species included. Naturally-regenerative floral margins (weedy margins), can include naturally colonising annuals, biannual and perennial flora species, and various grass species.

Table A1.3. Natural enemy orders, families and specific species that were featured in included studies, and their classification by dietary specialism and natural enemy type, listed in alphabetical order.

Order (Family/Species)	**Classification (reference for decision)**	
	Dietary specialism	**Natural enemy type**
Araneae		Predator (Ghoneim, 2014; Hambäck et al. 2021).
Araneidae	Generalist (Nyffeler et al. 1989; Snyder & Wise, 2001).	
Gnaphosidae	Generalist (Oelbermann et al. 2007).	
Lycosidae	Generalist (Oelbermann & Scheu, 2002; Oeblermann et al. 2007).	
Thomisidae	Generalist (Morrison III et al. 2016).	
Chilopoda	Generalist (Clark et al. 1993; Bortolin et al. 2018).	Predatory (Bonato et al. 2021).
Coleoptera		Predator (Messina and Hanks; Fonseca et al. 2017).
Carabidae	Generalist (Snyder & Ives, 2001; Brunke et al. 2009).	
Coccinellidae	Generalist (Snyder & Evans, 2006).	
Staphlinidae	Generalist (Brunke et al. 2009).	
Diptera		
Asilidae	Generalist (Symondson et al. 2002; Weseloh & Hare, 2009)	Predator (Samin et al. 2010; Veríssimo et al. 2021).
Syrphidae	Generalist (Hatt et al. 2018).	Predator (Rodríguez-Gasol et al. 2020).
Tachinidae	Generalist (Aldrich, 1995; Stireman, 2003).	Parasitoid (Aldrich, 1995).
Entomophorales		
Pandora neoaphidis	Specialist (Eilenberg et al. 2019).	Pathogen (Ekesi et al. 2005).
Hemiptera		
Anthocoridae	Generalist (Barbosa et al. 2017).	Predator (Wong and Frank, 2012).
Hymenoptera		Parasitoid (Ghahari et al. 2010; Hrcek et al. 2013).
Ichneumonoidea	Specialist (Tschopp et al. 2013).	

Chalcidoidea	Specialist (Symonds & Elgar, 2013).	
Neuroptera		
Chrysopidae	Generalist (Hatt et al. 2018).	Predator (Albuquerque et al. 1994;

References for Table A1.3.

Albuquerque, G. S. Tauber, C. A. and Tauber, M. J. (1994). *Chrysoperla externa* (Neuroptera: Chrysopidae): Life history and potential for biological control in Central and South America. *Biological Control*. 4(1), 8-13.

Aldrich, J. R. (1995). Testing the “new associations” biological control concept with a tachinid parasitoid (*Euclytia flava*). *Journal of Chemical Ecology*. 21, 1031-1042.

Barbosa, P. R. R., Michaud, J. P., Bain, C. L. and Torres, J. B. (2017). Toxicity of three aphicides to the generalist predators *Chrysoperla carnea* (Neuroptera: Chrysopidae) and *Orius insidiosus* (Hemiptera: Anthocoridae). *Ecotoxicology*. 26, 589-599.

Bonato, L., Peretti, E., Sandionigi, A. and Bortolin, F. (2021). The diet of major predators of forest soils: A first analysis on syntopic species of Chilopoda through DNA metabarcoding. *Soil Biology and Biochemistry*. 158, 108264.

Bortolin, F., Fusco G. and Bonato, L. (2018). Comparative analysis of diet in syntopic geophilomorph species (Chilopoda, Geophilomorpha) using a DNA-based approach. *Soil Biology and Biochemistry.* 127, 223-229

Brunke, A. J., Bahlai, C. A., Sears, M. K. and Hallet, R. H. (2001). Generalist predators (Coleoptera: Carabidae, Staphylinidae) associated with millipede populations in sweet potato and carrot fields and implications for millipede management. *Environmental Entomology*. 38(4), 1106-1116

Clark, M. S., Luna, J. M., Stone, N. D. and Youngman, R. R. (1993). Habitat preferences of generalist predators in reduced-tillage corn. *Journal of Entomological Science.* 28(4), 404-416

Eilenberg, J., Saussure, S., Fekih, I. B., Jensen, A. B. and Klingen, I. (2019). Factors driving susceptibility and resistance in aphids that share specialist fungal pathogens. *Current Opinion in Insect Science.* 33, 91-98.

Ekesi, S., Shah, P. A., Clark, S. J. and Pell, J. K. (2005). Conservation biological control with the fungal pathogen *Pandora neoaphidis*: implications of aphid species, host plant and predator foraging. *Agricultural and Forest Entomology*. 7(1), 21-30.

Fonseca, M. M., Lima, E., Lemos, F., Venzon, M. and Janssen, A. (2017). Non-crop plant to attract and conserve an aphid predator (Coleoptera: Coccinellidae) in tomato. *Biological Control.* 115, 129-134.

Ghahari, H., Erdogan, O. C., Šedivý, J. and Ostovan, H. (2010). Survey of the Ichneuomonodea and Chalcidoidea (Hymenoptera) parasitoids of Saturniidae (Lepiodoptera) in Iran. *Efflatounia.* 10, 1-6.

Ghoneim, K. (2014). Predatory insects and arachnids as potential biological control agents against the invasive tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), in perspective and prospective. *Journal of Entomology and Zoology Studies*. 2(2), 52-71.

Hambäck, P. A., Cirtwill, A. R., García, D., Grudzinska-Sterno, M., Miñarro, M., Tasin, M., Yang, X. and Samnegård, U. (2021). More intraguild prey than pest species in arachnid diets may compromise biological control in apple orchards. *Basic and Applied Ecology*. 57, 1-13.

Hatt, S., Uytenbroeck, R., Lopes, T., Mouchon, P., Osawa, N., Piqueray, J., Monty, A. and Francis, F. (2018). Identification of flower functional traits affecting abundance of generalist predators in perennial multiple species wildflower strips. *Arthropod-Plant Interactions*. 13, 127-137.

Hrcek, J., Miller, S. E., Whitfield, J. B., Shima, H. and Novotny, V. (2013). Parasitism rate, parasitoid community composition and host specificity on exposed and semi-concealed caterpillars from a tropical rainforest. *Oecologia.* 173, 521-532.

Messina, F. J. and Hanks, J. B. (1998). Host plant alters the shape of the functional response of an aphid predator (Coleoptera: Coccinellidae). *Environmental Entomology*. 27(5), 1196-1202.

Morrison III, W. R., Mathews, C. R. and Leskey, T. C. (2016). Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. *Biological Control.* 97, 120-130.

Nyffeler, M., Dean, D. A. and Sterling, W. L. (1989). Prey selection and predatory importance of orb-weaving spiders (Araneae: Araneidae, Uloboridae) in Texas cotton. *Environmental Entomology*. 18(3), 373-370.

Oelbermann, K. and Scheu, S. (2002). Effects of prey type and mixed diets on survival, growth and development of a generalist predator, *Pardosa lugubris* (Araneae: Lycosidae). *Basic and Applied Ecology*. 3(3), 285-291

Oelbermann, K., Langel, R. and Scheu, S. (2007). Utilization of prey from the decomposer system by generalist predators of grassland. *Oecologia.* 155, 605-517

Rodríguez-Gasol, N., ALins, G., Veronesi, E. R. and Wratten, S. (2020). The ecology of predatory hoverflies as ecosystem-service providers in agricultural systems. *Biological Control.* 151, 104405.

Samin, N., Sakenin, H., Imani, S. and Shojai, M. (2010). A contribution to the knowledge of robber flies (Diptera: Asilidae) for Tehran province and vicinity, Iran. Journal of *Biological Control*. 24(1), 42-46.

Snyder, W. E. and Evans, E. W. (2006). Ecological effects of invasive arthropod generalist predators. *Annual Review of Ecology, Evolution and Systematics.* 37, 95-122

Snyder, W. E. and Ives, A. R. (2001). Generalist predators disrupt biological control by a specialist parasitoid. *Ecology, Ecological Society of America*. 82(3), 705-716

Synder, W. E. and Wise, D. H. (2001). Contrasting trophic cascades generated by a community of generalist predators. *Ecology.* 82(6), 1571-1583

Stireman III, J. O. (2003). Host location and selection cues in a generalist tachinid parasitoid. *Entomologia Experimentalis et Applicata*. 103(1), 23-34.

Symonds, M. R. and Elgar, M. A. (2013). The evolution of body size, antennal size and host use in parasitoid wasps (Hymenoptera: Chalcidoidea): A phylogenetic comparative analysis. *PLoS ONE.* 8(10), e78297.

Symonson, W. O. C., Sunderland, K. D. and Greenstone, M. H. (2002). Can generalist predators be effective biocontrol agents? *Annual Review of Entomology.* 47, 561-594.

Tschopp, A., Riedel, M., Kropf, C., Nentwig, W. and Klopfstein, S. (2013). The evolution of host associations in the parasitic wasp genus *Ichneumon* (Hymenoptera: Ichneumonidae): convergent adaptations to host pupation sites. *BMC Ecology and Evolution.* 13, 74-87.

Veríssimo, B. A., Auad, A. M., Oliveira, C. M. and Paiva, I. G. (2021). Seasonality of predatory insects (Diptera: Syrphidae and Asilidae) in pasture monoculture and silvopastoral systems from Southeast Brazil. *International Journal of Tropical Insect Science*. 41, 861-872.

Weseloh, R. M. and Hare, J. D. (2009). Chapter 210 - Predation/predatory insects. *Encyclopedia of insects* (Second Edition). United Kingdom: Academic Press. Pp. 839-855.

Wong, S. K. and Franks, S. D. (2012). Influence of banker plants and spiders on biological control by *Orius insidiosus* (Heteroptera: Anthocoridae). *Biological Control*. 63(2), 181-187.

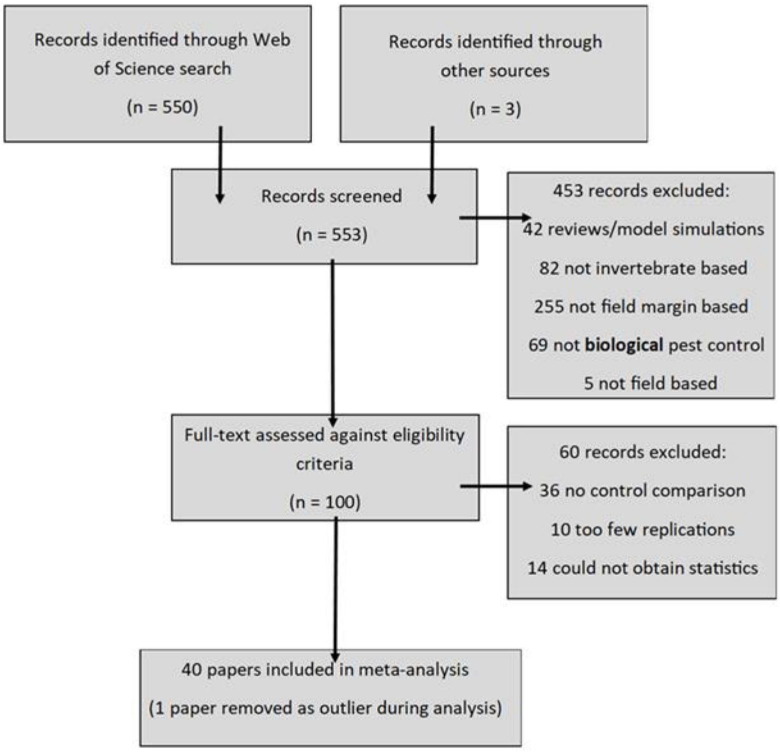

Figure A1.1. PRISMA graph illustrating the reasoning for rejection of papers from the meta-analysis, and how many papers were rejected at each stage.

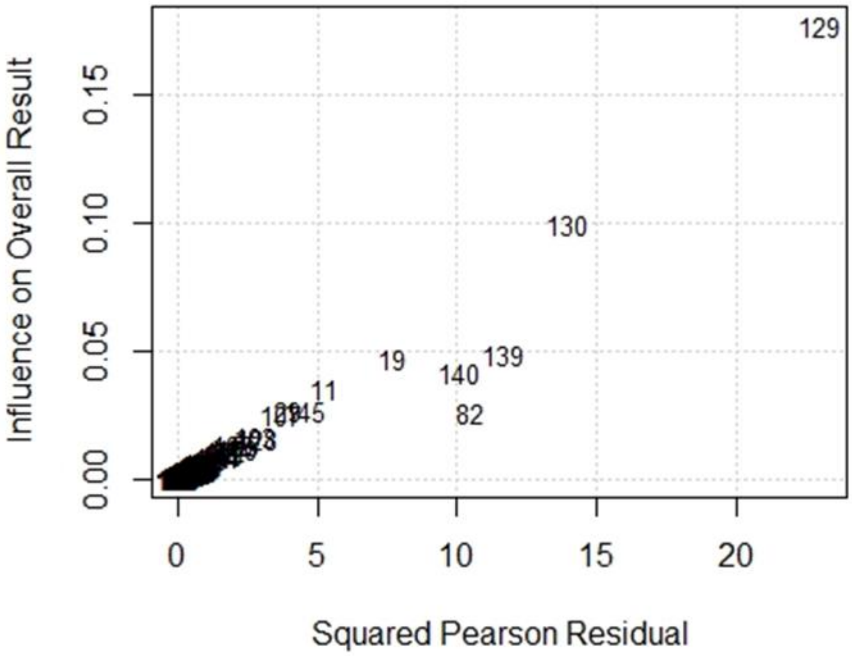

Figure A1.2. Baujat plot to demonstrate which studies overly contributing to heterogeneity. Numbers refer to individual test statistics collected from the included studies. Identified as outliers, test statistics 129 and 130 (Banks, 2000) were removed from data analysis.

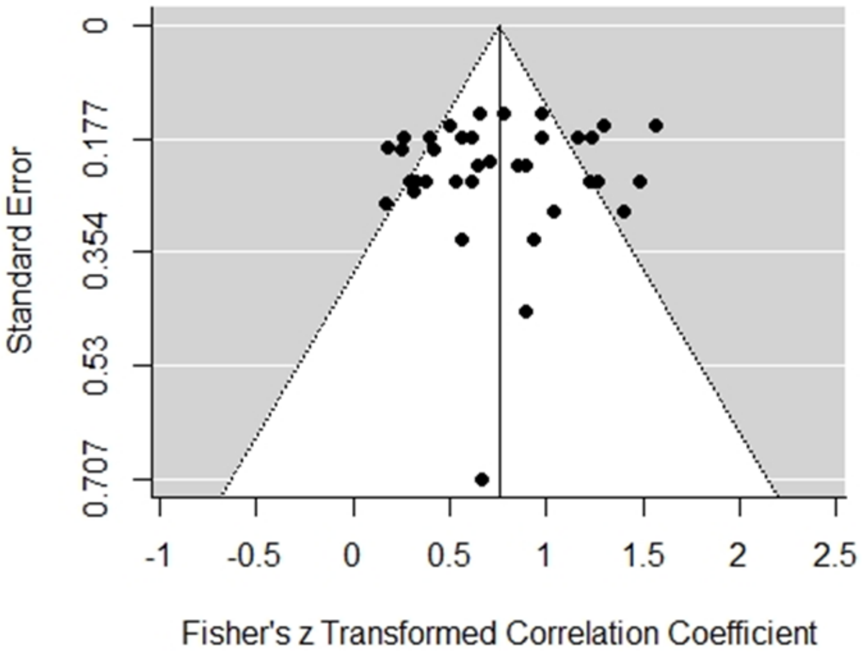

Figure A1.3. Funnel plot to graphically highlight any apparent publication bias in data set.

Appendix 2.

Table A2.1. Feld margin full species list, in alphabetical order by scientific name.

Dicotyledons (broad-leaved) species		**Monocotyledon (narrow-leaved) species**	
Common	**Scientific**	**Common**	**Scientific**
Spear thistle	*Cirsium vulgare*	Common soft brome	*Bromus hordeaceus* subsp. *hordeaceus*
Field horsetail	*Equisetum arvense*	Cocksfoot	*Dactylis glomerata*
Cut-leaved crane's-bill	*Geranium dissectum*	Yorkshire fog	*Holcus lanatus*
Ox-eye daisy	*Leucanthemum vulgare*	Perennial rye	*Lolium perenne*
Black medic	*Medicago lupulina*		
Lucerne	*Medicago sativa*		
Common poppy	*Papaver rhoeas*		
Ribwort plantain	*Plantago lanceolata*		
Meadow buttercup	*Ranunculus acris*		
Bramble	*Rubus fruticosus*		
Broadleaf dock	*Rumex obtusifolius*		
Autumn hawkbit	*Scorzoneroides autumnalis*		
Ragwort	*Senecio spp.*		
Red campion	*Silene dioica*		
Hedge mustard	*Sisymbrium officinale*		
Perennial sow thistle	*Sonchus arvensis*		
White clover	*Trifolium repens*		
Common vetch	*Vicia sativa*		

Appendix 3.

Table A3.1. Contents of the BMG4 Wildflowers and Fine Grasses and Grazing Paddock mix and percentage make-up.

Percentage	**Species** **BGM4**	
25	Chewing’s fescue	*Festuca rubra* subsp. *commutata*
15	Slender creeping red fescue	*Festuca rubra* subsp. *litoralis*
15	Strong creeping red fescue	*Festuca rubra* subsp. *rubra*
15	Hard fescue	*Festuca ovina*
10	Smooth meadow grass	*Poa pratensis*
5	Common bent grass	*Agrostis capillaris*
5	Crested dogstail	*Cynosurus cristatus*
5	Sainfoin	*Onobrychis viciifolia*
1	Birdsfoot trefoil	*Lotus corniculatus*
1	Black medick	*Medicago lupulina*
1	Red clover	*Trifolium pratense*
0.5	Oxeye daisy	*Leucanthemum vulgare*
0.5	Wild carrot	*Daucus carota*
0.5	Black knapweed	*Centaurea nigra*
0.25	Yarrow	*Achillea millefolium*
0.25	Self heal	*Prunella vulgaris*
	Grazing mix	
25	Timothy	*Phleum pratense*
25	Creeping red fescue	*Festuca rubra*
25	Perennial ryegrass	*Lolium perenne*

Table A3.2. Vegetation composition and percentage cover of each species and bare ground within both vegetation treatments for each vegetation strip. Categories based off DOMIN scale; 4a = less than four percent ground cover (i.e. few individuals), 4b = i.e. several individuals, 4c = i.e. many individuals. Highlighted in bold are species contained in the seed mixes.

Species	**Strip 1**		**Strip 2**		**Strip 3**	
	Floral	**Grass**	**Floral**	**Grass**	**Floral**	**Grass**
Bare ground	4-10	4-10	26-33	11-25	11-25	26-33
Bird’s-foot trefoil (*Lotus cornicuatus*)	-	-	<4a	<4a	<4a	<4a
Black bindweed (*Fallopia convolvulus*)	<4a	<4a	<4a	<4a	<4a	<4a
Black medick (*Medicago lupulina*)	-	-	<4a	-	<4a	-
Bristly ox-tongue (*Helminthotheca echioides*)	-	<4a	<4a	-	-	<4a
Charlock (*Sinapis arvensis*)	-	<4a	-	-	-	-
Chickweed (*Stellaria media*)	26-33	26-33	11-25	11-25	4-10	11-25
Common field speedwell (*Veronica*	<4a	<4a	<4a	<4a	<4a	<4a
perscia)	<4a	<4a	<4a	-	<4a	<4a
Common hemp nettle (*Galeopsis tetrahit*)	<4c	<4a	<4a	<4a	<4a	-
Common poppy (*Papaver rhoeas*)	<4a	-	-	-	-	-
Common vetch (*Vicia sativa*)	-	-	-	-	-	<4a
Creeping thistle (*Cirsium arvense*)	<4c	<4b	4-10	<4b	<4b	<4b
Dock (*Rumex obtusifolius* & *R. crispus*)	<4a	<4a	<4b	<4b	<4b	<4a
Fat hen (*Chenopodium album*)	-	-	<4a	<4a	<4a	<4a
Field pansy (*Viola arvensis*)	-	-	-	-	<4a	<4a
Fool’s parsley (*Aethusa cynapium*)	<4a	-	<4a	<4a	-	<4a
Fumitory (*Fumaria officinalis*)	<4a	-	<4a	-	-	<4a
Garlic mustard (*Alliaria petiolate*)	<4a	<4a	<4a	<4a	<4a	<4a
Groundsel (*Senecio vulgaris*)	<4a	<4a	<4a	<4a	<4a	4-10
Hedge mustard (*Sisymbrium officinale*)	<4a	<4a	<4b	<4a	<4b	-
Knot-grass (*Polygonum aviculare*)	<4a	-	<4a	<4a	<4a	<4b
Lucerne (*Medicago sativa*)	-	-	-	<4a	-	-
Nettle (*Urtica dioica*)	<4a	4-10	<4a	<4c	<4a	<4b
Perennial rye grass (*Lolium perenne*)	<4a	-	<4b	<4a	<4a	<4a
Sow-thistle (*Sonchus arvensis* & *S. oleraceus*)	-	-	-	-	-	<4a
Purple tansy (*Phacelia tanacetifolia*)	-	-	-	-	-	<4a
Ragwort (*Senecio* spp.)	-	-	-	-	<4a	-
Red clover (*Trifolium pratense*)	-	<4a	<4a	<4a	<4a	-
Red shank (*Persicaria maculosa*)	<4a	-	<4a	<4b	<4a	<4a
Ribwort plantain (*Plantago lanceolata*)	-	-	<4a	-	<4a	-
Sainfoin (*Onobrychis viciifolia*)	-	-	<4a	<4a	-	-
Scarlet pimpernel (*Anagallis arvensis*)	<4a	<4b	<4a	<4b	<4b	<4a
Scentless mayweed (*Tripleurospermum inodorum*)	<4a	<4a	<4a	<4a	<4a	<4a
Shepherd's purse (*Capsella bursa-pastoris*)	-	-	-	-	<4a	-
Sun spurge (*Euphorbia helioscopia*)	-	-	<4a	<4b	<4a	<4a
Timothy (*Phleum pratense*)	<4a	<4a	<4a	-	-	-
Wheat (volunteer; *Triticum aestivium*)	<4b	<4b	-	-	-	-
Wild oats (*Avena fatua*)	<4b	<4a	<4a	<4a	<4b	<4b
White clover (*Trifolium repens*)	-	-	-	<4a	-	<4a
Yorkshire fog (*Holcus lanatus*)						

Table A3.3. Total counts of pests and natural enemies from the whole-field trial and the counts of the swede midge (*Contarinia nasturtii*), the cabbage stem flea beetle (*Psylliodes chrysocephala*) and cabbage root fly (*Delia radicum*) from the in-crop trials (indicated in italics). Counts are split between vegetation type treatments (grass and floral) and habitat surveyed: crop (cultivated area) and strip (vegetation strip), with the total included in bold. Arthropods are listed alphabetically.

	Grass			**Floral**		
	Crop	**Strip**	**Total**	**Crop**	**Strip**	**Total**
Pests						
Coleoptera						
Brassicogethes aeneus	198	176	**374**	247	182	**429**
Phyllotreta striolata	826	27	**853**	739	22	**761**
Psylliodes chrysocephala	5088	366	**5454**	3952	352	**4304**
	2035			*1878*		
Diptera						
Anthomyiidae (incl. *Delia radicum)*	2946	3093	**6039**	3038	3810	**6848**
	1094			*1090*		
Cecidomiidae (incl. *Contarinia*	2912	1703	**4615**	2473	1534	**4007**
nasturtii)	*3090*			*2424*		
	4	9	**13**	2	8	**10**
Tipulidae						
Hemiptera	1152	351	**1503**	1793	338	**2131**
Aphidoidea						
Hymenoptera	4	17	**21**	4	18	**22**
Athalia rosae						
Lepidoptera	5	15	**20**	9	23	**34**
All larvae	0	1	**1**	0	0	**0**
Mamesta brassicae	14	20	**34**	33	25	**58**
Pieris brassicae & *rapae*	1	0	**1**	1	0	**1**
Plutella xylostella						
Natural enemies						
Arachinda						
Acari	1	3	**4**	0	4	**4**
Linyphiidae	35	60	**95**	37	119	**156**
Lycosidae	42	128	**170**	35	120	**155**
Opiliones	14	17	**31**	9	14	**23**
Chilopoda	2	2	**4**	1	4	**5**
Coleoptera						
Cantharoidae	8	34	**42**	4	20	**24**
Carabidae	150	224	**374**	166	218	**384**
Collinellidae	7	37	**44**	15	22	**37**
Staphylinoidae	327	166	**493**	254	185	**439**
Neuroptera						
Chrysopidae	2	1	**3**	3	3	**6**

Diptera						
Asilidae	10	11	**21**	5	13	**18**
Dolichopodidae	149	109	**258**	139	120	**259**
Empididae	92	79	**171**	102	84	**186**
Hybotidae	10	13	**23**	8	9	**17**
Scathophadidae	39	230	**269**	63	188	**251**
Syrphidae	37	63	**100**	58	53	**111**
Hemiptera						
Anthocoridae	14	61	**75**	15	52	**67**
Reduviidae	34	124	**158**	64	108	**172**
Nabidae	13	32	**45**	6	33	**39**
Hymenoptera						
Braconidae	57	73	**130**	83	66	**149**
Ceraphronoidea	4	7	**11**	3	5	**8**
Chalcidoidea	212	265	**477**	193	286	**479**
Chrysididae	6	4	**10**	3	4	**7**
Cynipoidea	324	258	**582**	301	245	**546**
Ichneumonidae	711	807	**1518**	715	809	**1524**
Platygastridae	1073	1273	**2346**	1189	1067	**2256**
Proctotrupoidea	4	10	**14**	5	6	**11**
Sphecidae	37	144	**181**	60	74	**134**
Vespula	23	56	**79**	18	53	**71**

Table A3.4. Results of Generalised Linear Mixed Models on vegetation strip control data, with normal error distribution. Describing the effect of the variables: vegetation type, planting, habitat (location of survey; vegetation strip or cultivated area) date of survey, and key interactions. Significant variables are shown in bold.

Measure	**Variable**	**df**	**F val**	**P**
Swede midge abundance	Vegetation type	1,48	0.08	0.78
	Planting	2,48	22.92	**<0.001**
	Habitat	1,48	0.01	0.92
	Date	3,48	15.65	**<0.001**
	Veg type x planting	2,48	0.4	0.69
	Habitat x planting	2,48	16.75	**<0.001**
Cabbage stem flea beetle abundance	Vegetation type	1,48	0.29	0.6
	Planting	2,48	20.66	**<0.001**
	Habitat	1,48	80.98	**<0.001**
	Date	3,48	5.72	**0.002**
	Veg type x planting	2,48	0.63	0.54
	Habitat x planting	2,48	47.14	**<0.001**
Cabbage root fly abundance	Vegetation type	1,48	3.05	0.08
	Planting	2,48	27.37	**<0.001**
	Habitat	1,48	15.68	**<0.001**
	Date	3,48	30.18	**<0.001**
	Veg type x planting	2,48	1.24	0.29
	Habitat x planting	2,48	20.96	**<0.001**

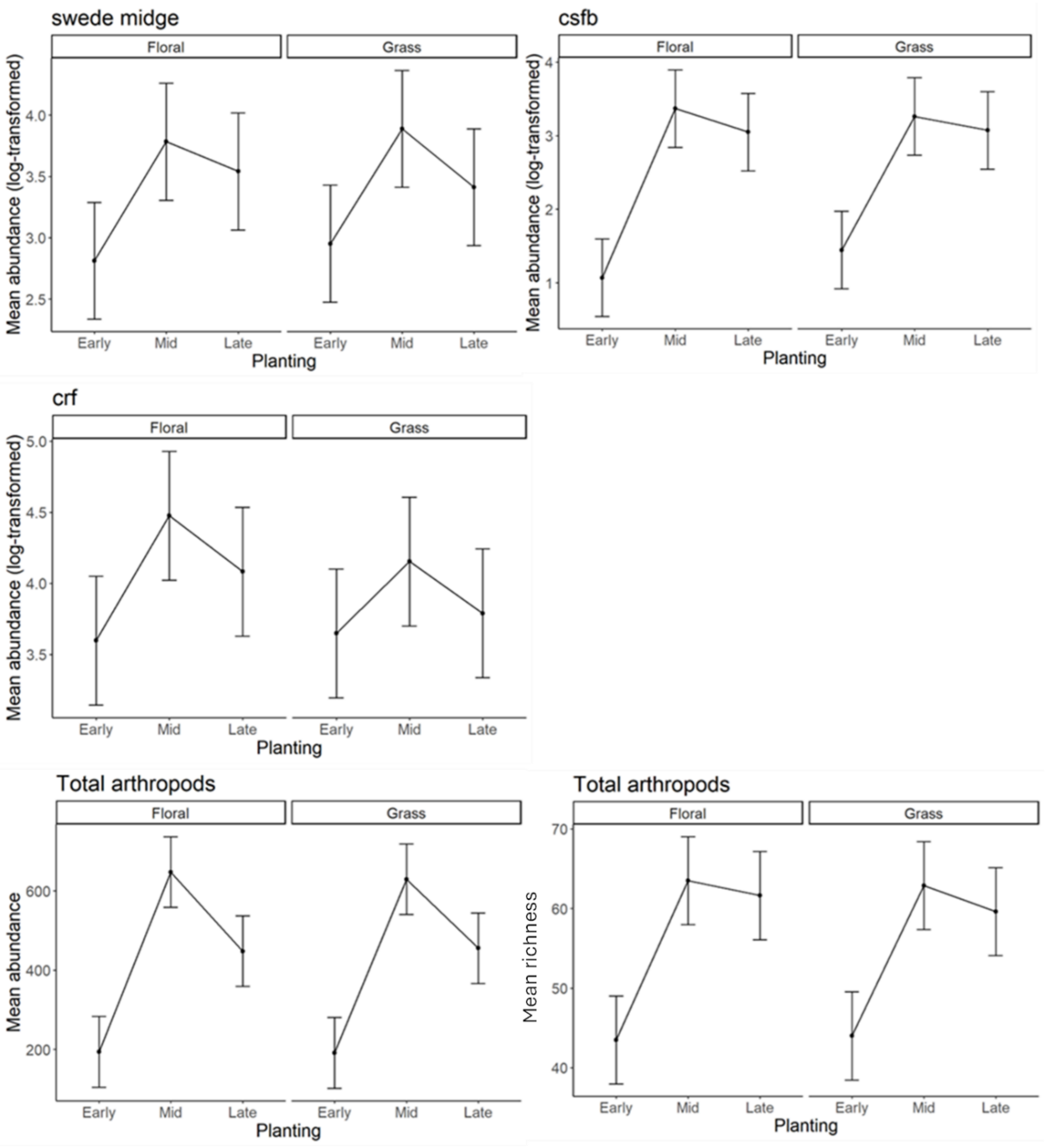

Figure A3.1. Mean abundance and species richness of all arthropods from the vegetation strips and cultivated area. Counts were grouped by vegetation strip treatments, floral and grass. Error bars indicate 95% confidence intervals.

Appendix 4.

Table A4.1. Species and percentages of seed mixes used for the two vegetation strip treatments.

Percentage	**Species**	
	AB8 traditional grasses and wildflower mix	
40	Strong creeping red fescue	*Festuca rubra rubra*
25	Chewings fescue	*Festuca rubra commutata*
15	Hard fescue	*Festuca trachyphylla*
5	Smooth stalk meadow grass	*Poa pratensis*
3.75	Sainfoin	*Onobrychis viciifolia*
3.75	Common vetch	*Vicia sativa*
3	Crimson clover	*Trifolium incarnatum*
2.5	Red clover	*Trifolium pratense*
0.5	Bird's-foot trefoil	*Lotus corniculatus*
0.5	Ribwort plantain	*Plantago lanceolata*
0.25	Lucerne	*Medicago sativa*
0.25	Alsike clover	*Trifolium hybridum*
0.25	Yarrow	*Achillea millefolium*
0.25	White clover	*Trifolium repens*
	Grazing mix	
25	Timothy	*Phleum pratense*
25	Creeping red fescue	*Festuca rubra*
50	Perennial ryegrass	*Lolium perenne*

Table A4.2. Results of Generalised Linear Mixed Model, for the measures adult swede midge abundance, adult cabbage root fly abundance and total pest abundance and diversity. Describing the interactions between the variables: *vegetation type, pesticide*, *field*, *crop age*.

Measure	**Explanatory variable**	**df**	**F**	**P**
Swede midge	Vegetation*pesticide	4	0.6767	0.6085
	Vegetation*field	2	0.9656	0.382
	Pesticide*field	8	0.2362	0.2362
	Vegetation*age	3	1.9971	0.1141
	Pesticide*age	12	0.5267	0.8972
Cabbage root fly	Vegetation*pesticide	4	1.4279	0.2242
	Vegetation*field	2	0.2522	0.7773
	Pesticide*field	8	0.9175	0.5018
	Vegetation*age	3	2.2355	0.0838
	Pesticide*age	12	0.5512	0.8802
Pest abundance	Vegetation*pesticide	4	0.3644	0.8339
	Vegetation*field	2	0.5444	0.5807
	Pesticide*field	8	0.7692	0.6302
	Vegetation*age	3	0.3823	0.7658
	Pesticide*age	12	0.4044	0.9617
Pest richness	Vegetation*pesticide	4	0.7144	0.5825
	Vegetation*field	2	0.0187	0.9815
	Pesticide*field	8	0.7889	0.6127
	Vegetation*age	3	0.7522	0.5216
	Pesticide*age	12	0.5787	0.8594

Table A4.3. Results of Generalised Linear Mixed Model, for the measures swede midge-associated crop damage and crop yield of sprouting broccoli. Describing the interactions between the variables: *vegetation type, pesticide* and *field*.

Measure	**Explanatory variable**	**df**	**F**	**P**
Crop damage	Vegetation*pesticide	4	0.123	0.9738
	Vegetation*field	2	0.3351	0.7162
	Pesticide*field	8	1.8336	0.0827
Crop yield	Vegetation*pesticide	4	3.0209	0.1592
	Vegetation*field	2	0.9932	0.3218
	Pesticide*field	8	1.8131	0.0864

Table A4.4. Results of Generalised Linear Mixed Model, for the measures natural enemy abundance and diversity. Describing the interactions between the variables: *vegetation type, pesticide* and *field*.

Measure	**Explanatory variable**	**df**	**F**	**P**
Natural enemy abundance	Vegetation*pesticide	4	0.0819	0.9879
	Vegetation*field	2	0.7563	0.4709
	Pesticide*field	8	0.1937	0.9915
Natural enemy richness	Vegetation*pesticide	4	0.3805	0.8224
	Vegetation*field	2	2.3919	0.0944
	Pesticide*field	8	1.016	0.4256