

**FIELD MARGIN PLANTS SUPPORT ARTHROPOD NATURAL
ENEMIES IN SMALLHOLDER COMMON BEAN FARMING
SYSTEMS IN NORTHERN TANZANIA**

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ABSTRACT

Field margin plants provide habitats and food resources for natural enemies of pests (NEs), but their potential is poorly understood, particularly in the tropics and on smallholder farms. The experiments were conducted to test the potential of field margin plants on NEs. Sentinel plants were developed to collect *Aphis fabae* and *Maruca vitrata* parasitoids. *Aphidius colemani* was identified as the primary parasitoid of *A. fabae*. The dominant *M. vitrata* larval parasitoids were the Braconidae and Dolichogenidea, while the Platygasteridae were the abundant egg parasitoids. The survey of field margins for plant-NE interactions in bean fields found that NEs most often interacted with *Bidens pilosa* (15.4%) and *Euphorbia heterophylla* (11.3%). Flowering plant species (*Bidens pilosa*, *Lantana camara*, *Euphorbia heterophylla* and *Ageratum conyzoides*) supported NEs out of bean growing season. In cage trials with an aphid-infested bean plant and a single flowering margin plant, the survival of *A. colemani* was more significant in the presence of *E. heterophylla* than *B. pilosa*, *Tagetes minuta* and *Hyptis suaveolens*. UV-fluorescent dye was applied to flowers of specific field margin plant species and NE was sampled from within the bean crop and field margins using sweep-netting and pan-traps, respectively. Captured insects were examined for the presence of the dye, indicative of a prior visit to the margin. Lady beetles and assassin bugs were abundant in plots with *B. pilosa* margins, hoverflies with *T. minuta* and *Parthenium hysterophorus* margins, and lacewings with *T. minuta* and *B. pilosa* margins. NEs were also sampled from high and low plant diversity bean fields using sweep netting and coloured sticky traps, comparing monocropped and intercropped farms. Overall, high-plant diversity fields had higher NEs than low-diversity fields. The field margin had a significantly higher number of NEs than the crop field. However, marginally higher populations of NEs in intercropping than in monocropping were observed, although the effect was not significant. To confirm that NE communities on farms predated *Aphis fabae*, we extracted and amplified *A. fabae* DNA from predatory insects. Fourteen lady beetle larvae, two lacewing larvae, and one assassin bug analyzed were found to contain *A. fabae* DNA, indicating all these groups can regulate the significant aphid pest of beans, *A. fabae*. *A. colemani* was a parasitoid that emerged from several non-aphid species collected from the field margins. Overall, NEs benefitted from field margin plants; those possessing extra floral nectaries had an added advantage. Thus, smallholder farmers protect the field margins for the added benefit of natural pest regulation in their fields and thus for food security and enhancing their livelihoods.

DECLARATION

I, **Baltazar Josephat Ndakidemi**, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

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CERTIFICATION

The undersigned certify that, they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology a thesis titled “*Field margin plants support arthropod natural enemies in smallholder common bean farming systems in northern Tanzania*” in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Life Sciences-Sustainable Agriculture of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
B.P	<i>Bidens pilosa</i>
BCMNV	Bean common mosaic necrosis virus
BCMV	Bean common mosaic virus
BOLD	Barcode of Life Database
Bt	<i>Bacillus thuringiensis</i>
CBC	Conservation Biological Control
CMV	Cucumber mosaic virus
COI	Cytochrome oxidase I
Cont	Control
CREATES	The Africa Centre for Research, Agricultural Advancement, Teaching Excellence and Sustainability
df	Degree of freedom
DNA	Deoxyribonucleic acid
E.H	<i>Euphorbia heterophylla</i>
EPF	Entomopathogenic fungi
FAO	Food and Agriculture Organisation of the United Nations
GDP	Gross domestic product
GLM	Generalised Linear Model
H.S	<i>Hyptis suaveolens</i>
H'	Shannon Diversity Index
ha	Hectare
HIPVs	Herbivore-Induced Plant Volatiles
IITA	International Institute of Tropical Agriculture
IPM	Integrated pest management
MaviMNPV	<i>Maruca vitrata</i> multi-nucleopolyhedrovirus
NCBI	National Center for Biotechnology Information database
NCBI	National Center for Biotechnology Information
NEs	Natural enemies
NM-AIST	Nelson Mandela African Institution of Science and Technology
NPR	Natural pest regulation
OECD	Organisation for Economic Cooperation and Development

P.H	<i>Parthenium hysterophorus</i>
PBPs	Plant-based pesticides
PCR	Polymerase chain reaction
PIPs	Plant-incorporated protectants
RH	Relative humidity
SEM	Standard Error of the Mean
SSA	Sub Saharan Africa
T.M	<i>Tagetes minuta</i>
TPRI	Tanzania Pesticides Research Institute
UV	Ultraviolet radiations
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Agriculture is key to most livelihoods in SSA (Sub-Saharan Africa). Phillips and Throne (2010) estimate that more than 20% of essential food sources are lost globally due to pests in the field and storage. Among the food sources lost are the common beans. The loss of common beans due to pests is very detrimental to human beings. Common beans have great potential to provide food resources, food security, and improved income for African farmers (Broughton *et al.*, 2003; Nedumaran *et al.*, 2015). Therefore, investing in their research is essential to ensure their full utilization for increased food production in Africa. The loss of crops due to pests is a big challenge. Ochilo and Nyamasyo (2011) estimated bean yield loss of up to 100% if pests are uncontrolled. Factors such as lack of knowledge and research and monetary cost limit the smallholder farmers of SSA from applying pest management strategies (Belmain *et al.*, 2013). However, some use synthetic pesticides to combat pests in bean fields (Mkenda *et al.*, 2020).

There is a concern about the harmful effects of synthetic pesticides on the human population and non-target organisms, which has created a need to search for alternative ways of suppressing crop pests (Belmain *et al.*, 2013; Bora *et al.*, 2012). The excessive applications of synthetic pesticides in agricultural fields have disrupted the ecosystem services of pollination and natural pest control through biological control (Bianchi *et al.*, 2006). Most farmers have a limited understanding of the synthetic pesticide handling and spraying practices in agricultural fields, which increases the possibility of getting into non-target areas such as water sources (Ajayi *et al.*, 2007; Keita *et al.*, 2000). More sustainable and environmentally benign practices that are economically feasible for pest management to enhance agricultural product quality for food security in SSA, including Tanzania, have become necessary for most farmers. Natural pest regulation (NPR) through biological control is sustainable and environmentally friendly pest control technique (Abtew *et al.*, 2016).

Conservation biological control is the most accessible form of biological control for smallholder farmers in SSA because it involves affordable field-scale interventions, such as increasing the local plant diversity and abundance, to enhance the natural enemy populations (Girma *et al.*, 2000; Mkenda *et al.*, 2019a; 2019c). Predators and parasitoids are more abundant

in crop fields with sufficient flowering plants as they provide floral resources (nectar and pollen) for their survival (Asif & Saeed, 2010; Colley & Luna, 2000; Hatt *et al.*, 2017a; Hatt *et al.*, 2017b; Martini *et al.*, 2014). However, farmers' knowledge of NEs and how to conserve them is a constraint to using this form of biological control (Mkenda *et al.*, 2020). More studies are required to identify the key roles NEs play in managing important legume pests and their benefits over synthetics (Otieno *et al.*, 2020). NEs interact with field margins for resources and shelter, and this can enhance the biological control of pests in field crops (Mkenda *et al.*, 2019a; 2019c). However, few field studies have investigated the impacts of different plant manipulations on the populations of NEs for suppressing legume pests (Mkenda *et al.*, 2015).

There is a need for more field research on conservation biological control because many of the previous studies have been based on laboratory conditions (Stevenson *et al.*, 2017). The NEs (predators and parasitoids) provide valuable ecosystem services for biological pest control (Potts *et al.*, 2006). This beneficial insect-mediated service is vital for increased crop productivity with minimal synthetic pesticides in agricultural fields. Generating knowledge on different NEs for key pests of a bean crop and establishing their relationships with different habitats for enhancing their densities will prevent bean yield loss and hence improve smallholder farmers' livelihoods. The populations of key NEs such as hymenopterans, syrphids, lacewings, tachinids, and coccinellids are enhanced by flowering plants in crop and non-crop habitats (Asif & Saeed, 2010; Colley & Luna, 2000; Martini *et al.*, 2014). These flowering plant species include *Glebionis segetum* (Corn marigold), *Coriandrum sativum* (Coriander), *Phacelia tanacetifolia* (Phacelia) and *Foeniculum vulgare* (Fennel) (Sievwright *et al.*, 2006). Buckwheat and licorice mint, for instance, are good food sources and enhance the longevity of the adult female of *Microplitis croceipes*, a parasitoid (braconidae) (Nafziger & Fadamiro, 2011). Meena *et al.* (2017) indicated a significant decrease in pest populations in cowpea fields when marigold was used in farmscapping. Despite the importance of non-crop habitats around agricultural systems, few field studies have investigated their impacts in supporting populations of NEs to suppress bean pests. Some of these pests cause enormous bean yield losses. For instance, pod borer (*Maruca vitrata*) and black bean aphid (*Aphis fabae*) cause losses of about 33 to 53% and 37% of beans, respectively in Tanzania (Swaine, 1969; Karel, 1985). Thus, identifying the right plants that enhance the populations of NEs for bean pod borers and aphid control is of utmost importance.

1.2 Statement of the Problem

Numerous constraints limit bean production and storage. Insect pests are challenging for bean production and storage (Hillocks *et al.*, 2006). A study by Ochilo and Nyamasyo (2011) estimated bean yield loss of up to 100% if pests are not controlled. While many smallholder farmers do not apply any control measures because of the high cost and lack of knowledge and research (Belmain *et al.*, 2013; Otieno *et al.*, 2020), others apply synthetic pesticides indiscriminately (Mkenda *et al.*, 2020). This approach is not sustainable and there are potential direct and indirect effects of synthetic pesticides on human health and beneficial insects, including NEs that are biological control agents of insect pests via predation and parasitism (El-Heneidy *et al.*, 2015; Gill & Garg, 2014; Rice *et al.*, 2007). Adopting more sustainable farming practices will benefit the environment and human health. One alternative is conservation biological control which can regulate arthropod NE populations through multitrophic interactions and a balance between pests and their NEs (Tamò *et al.*, 2012). Conserving NEs adapted to the local environment is cost-effective and relatively simple. Thus, this is an important aspect to be included in pest management decisions (Ballal & Verghese, 2015) and strategies such as the engineering of agroecosystems to provide extra resources that would otherwise be limited in field crops (Gurr *et al.*, 2004).

Increasing landscape diversity by manipulating the plants that provide alternative resources to biological control agents such as pollen and nectar is important for natural pest control and pollination (Landis *et al.*, 2000; Saidov & Douglas, 2008). Several plant species, namely *Tagetes erecta*, *Foeniculum vulgare*, *Ocimum basilicum*, *Ziziphora interrupta*, *Foeniculum vulgare*, *Centaurea cyanus*, *Calendula officinalis*, *Anethum graveolens*, *Trida procumbens*, *Sesamum indicum*, and *Emilia sonchifolia* have unveiled their potential in supporting NEs of pests such as parasitic wasps, some predatory bugs, ladybirds and hoverflies (Kopta *et al.*, 2012; Saidov & Douglas, 2008; Zhu *et al.*, 2014). Whilst potential plants and invertebrates have been employed to control pests in other crops, less has been done in bean fields. Thus, this study will identify the key invertebrate taxa of NEs and assess the potential of field margin plants for supporting these NEs of bean pests for crop protection and increased productivity.

1.3 Rationale of the Study

Pest control strategies for most farmers in SSA are limited by factors related to monetary cost, a lack of knowledge, and limited research on different products (Belmain *et al.*, 2013).

Synthetic pesticides are expensive for smallholder farmers; they also negatively impact human health, the environment (Rice *et al.*, 2007), and non-target organisms such as NEs and pollinators (El-Heneidy *et al.*, 2015). Thus, there is a need for effective alternatives to suppress crop pests (Bora *et al.*, 2012). Natural pest regulation (NPR) offers an alternative for pest management in SSA and is an essential component of an integrated pest management system. NPR is an approach to pest management that relies on beneficial insects and biological approaches. It includes sustainable practices best suited to smallholder farmers, such as habitat manipulation to enhance the contribution and use of biological control agents (Gurr *et al.*, 2017; Gurr *et al.*, 2018). Conservation biological control (CBC) has poor application in SSA (Wyckhuys *et al.*, 2013). However, there has been much research on developing this sustainable pest management option. Thus, promoting CBC for sustainability in agriculture is essential, particularly in smallholder farming systems in SSA.

1.4 Research Objectives

1.4.1 General Objective

The contribution of varying field margin plant species on the abundance of NEs and management of bean pests.

1.4.2 Specific Objectives

The study aimed to achieve the following specific objectives:

- (i) To identify the key parasitoids of black bean aphids (*Aphis fabae*) and legume pod borer (*Maruca vitrata*) in bean fields.
- (ii) To determine the effects of field margins on the temporal-spatial distribution of NEs and bean pests.
- (iii) To determine the role of different field margin species on the survival and reproductive success of *A. fabae* parasitoids.
- (iv) To identify the field margin plants that support NEs in field crops.
- (v) To identify floral resources and flowers available during the offseason to support NEs.

- (vi) To identify which non-pest aphids (alternative hosts) exist in field margins during off-season and the parasitoids they support.

1.5 Research Questions

The study intended to answer the following questions:

- (i) What are the key parasitoids of black bean aphids (*Aphis fabae*) and legume pod borer (*Maruca vitrata*)?
- (ii) What is the impact of field margin on the spatial and temporal distribution of NEs?
- (iii) How do different field margin plants influence the survival and parasitism of *A. fabae* parasitoids?
- (iv) Which are the key plant species supporting the key NEs of bean pests?
- (v) What are the floral resources that support NEs outside the bean-growing season?
- (vi) Which non-pest aphids (alternative hosts) exist on margin plants during the off-season? Do they support the parasitoids found in the field crop?

1.6 Significance of the Study

The results will help create more evidence before seeking proper advocacy and recommendations on suitable plant species to incorporate in field margins for the spatial-temporal provision of floral resources. This will improve and sustain NEs' populations in agroecosystems for bean pests' suppression and increase productivity. The findings will also help the biological control researchers, policy makers and agriculture stakeholders to launch awareness and sensitization campaigns on the significance of farmscaping for conserving NEs. The findings will be helpful for smallholder farmers to adopt and implement sustainable pest management.

1.7 Delineation of the Study

This study evaluated the potential of field margin plants to enhance the population of NEs of common bean pests with the following aspects.

The key parasitoids of *Maruca vitrata* and *Aphis fabae* are collected in low and high-plant diversity fields among the smallholder bean-growing farmers in Kwa Sadala Village, in Hai District, Kilimanjaro region, Northern Tanzania.

Trials on investigating the spatiotemporal distribution of natural enemies of bean pests in low and high diversity fields among the smallholder bean growing farmers in Kwa Sadala Village, in Hai District, Kilimanjaro region, Northern Tanzania.

Controlled trial on key field margin plants for enhancing the survival and parasitism of *Aphis fabae* parasitoid, the experiment that was conducted in the controlled setting with cages at the Nelson Mandela African Institution of Science and Technology (NM-AIST).

Field trials on the contribution of flowering field margin plant species on supporting NEs of common bean pests in Kwa Sadala Village, in Hai District, Kilimanjaro region, Northern Tanzania. Experiments on plants available during the offseason to support NEs in Kwa Sadala Village, in Hai District, Kilimanjaro region, Northern Tanzania.

Field survey to determine the non-pest aphids in field margins outside the cropping season and the parasitoids they support in Kwa Sadala Village, in Hai District, Kilimanjaro region, Northern Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The challenge for agriculture today and in the next thirty years is to implement sustainable and ideally carbon-zero farming that is economically viable and resilient to future shocks, including changing climate. This is particularly important in Africa, where 19.1% of the population was undernourished in 2019 (FAO 2020), and farmers are increasingly negatively affected by climate change. Agriculture underpins most livelihoods in SSA (Sub-Saharan Africa) because it provides food security and employment and also contributes to an average of 15% of the total gross domestic product (GDP) (Hillocks *et al.*, 2006; OECD-FAO, 2016). Small-scale farming is common in SSA, but existing studies have neglected smallholder farms (Ricciardi, *et al.*, 2020). It is thought that it will be particularly challenging for smallholders to adopt sustainable intensification as they are vulnerable to production risks such as climate change (Vanlauwe *et al.*, 2014).

Legumes, including common beans (*Phaseolus vulgaris* L.), are frequently grown by smallholder farmers in SSA. Beans are a critically important component of healthy diets in SSA because they are good sources of protein, vitamins, energy, and micronutrients, e.g., iron, zinc, thiamin, and folic acid (Broughton *et al.*, 2003; Nedumaran *et al.*, 2015). Therefore, the common bean and other legumes are a potential crop for increasing food security, improving soil quality, and enhancing livelihoods through increased income in SSA (Katungi, 2010). However, the yield of these grain legumes is constrained and one of the significant challenges is insect pests (Otieno *et al.*, 2020). For example, the common bean has an average yield gap of 2.6 Mg ha⁻¹ across Ethiopia, Kenya, and Tanzania (Loon *et al.*, 2018). Reducing this gap could significantly increase food security in these areas.

The insect pests that cause yield reduction in common beans across SSA include scarab beetles (*Schizonycha* spp.) foliage beetles (*Oothea bennigseni* and *Oothea mutabilis*), black bean aphids (*Aphis fabae* and *Aphis crassivora*), bean stem maggot (*Ophiomyia phaseoli* and *Ophiomyia spencerella*), bean pod borers (*Maruca vitrata* and *Helicoverpa armigera*), silver leaf whitefly (*Bemisia tabaci*) biotype B, the Southern green stink bug (*Nezara viridula*) and the storage pest bean bruchid (*Callosobruchus maculatus*) (Allen *et al.*, 1996; Da-Silva *et al.*, 2019; Muimba-Kankolongo, 2018, Nyamwasa *et al.*, 2018). Control strategies for most

farmers in SSA are limited by factors related to monetary cost, lack of knowledge, and limited research on different products (Belmain *et al.*, 2013). Not only are synthetic pesticides expensive for smallholder farmers, but they can also negatively impact human health, the environment (Rice *et al.*, 2007), and non-target organisms such as NEs and pollinators (El-Heneidy *et al.*, 2015). Thus, there is a need to develop alternative ways of suppressing crop pests (Bora *et al.*, 2012).

Methods of sustainable pest control that are best suited to small holders include habitat manipulation to enhance the biological control agents (Gurr *et al.*, 2017; Gurr *et al.*, 2018), biopesticides including botanical insecticides (Stevenson *et al.*, 2017), breeding for host plant resistance (Anyanga *et al.*, 2017), pheromones for mating disruption of pests (Miller & Gut, 2015), and cultural control (Karungi *et al.*, 2000). Although there has been much research on developing these sustainable pest management options, few field-ready options are targeted or developed for smallholder farmers, especially in SSA, particularly for *M. vitrata* and *A. fabae*, which cause significant yield losses (Table 1). Here is a critical review of the existing methods to control *A. fabae* and *M. vitrata* sustainably using biological control techniques and their compatibility with other sustainable control strategies. Recommendations regarding future directions of research and effective management options for these pests are presented and current challenges in sustainable pest management faced by smallholder farmers, especially in SSA are discussed in the context of available options.

2.2 Selected Common Bean Pests; Bean Pod Borer (*Maruca vitrata*) and Black Bean Aphid (*Aphis fabae*)

Maruca vitrata and *A. fabae* (Plates 1 and 2) are legume pests of economic importance as they account for the major bean yield losses in SSA (Table 1). *Maruca vitrata* is a major Lepidopteran pest of leguminous crops, including the common bean, which causes damage by feeding on bean flowers, buds, and pods and reducing yield (Sharma, 1998). This pest is widely distributed in tropical and subtropical areas and is incredibly destructive in some parts of Africa and Asia (Jayasinghe *et al.*, 2015; Liao & Lin, 2000; Margam *et al.*, 2011). It has previously been recorded as causing 15-53% yield losses in East African countries (Table 1). Another key pest of legumes in SSA is *A. fabae* which causes damage by direct feeding and is also responsible for the spread of several plant diseases, including cucumber mosaic virus (CMV), bean common mosaic necrosis virus (BCMNV) and bean common mosaic virus (BCMV)

(Wamonje *et al.*, 2020). *Aphis fabae* can cause between 37-90% yield losses in East African countries (Table 1). Several host plants are associated with *A. fabae* and *M. vitrata* (Table 2).



Plate 1: *Maruca vitrata* infestation in beans. *Aphis fabae* infestation in beans. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania



Plate 2: *Aphis fabae* infestation in beans. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania

Table 1: The empirically assessed bean yield losses attributed to black bean aphid (*Aphis fabae*) and bean pod borer (*Maruca vitrata*) in East African countries per year

Bean pest	Country	Yield loss%	Reference
<i>A. fabae</i>	Burundi	50	Abate (2000)
<i>A. fabae</i>	Kenya	37-90	Karel and Autrique, (1989)
<i>M. vitrata</i>	Tanzania	33-53	Karel (1985)
<i>A. fabae</i>	Tanzania	37	Swaine (1969)
<i>A. fabae</i>	Uganda	90	Nyiira (1978)
<i>M. vitrata</i>	Kenya	15-25	De-Lima (1983)

Table 2: Host plant species for *Maruca vitrata* and *Aphis fabae*

Bean Pest	Plant Species	Family	References
<i>M. vitrata</i>	<i>Vigna unguiculata</i>	Fabaceae	Abate <i>et al.</i> (2000) and Jackai (1995)
<i>M. vitrata</i> , <i>A. fabae</i>	<i>Phaseolus vulgaris</i>	Fabaceae	Jackai (1995)
<i>M. vitrata</i>	<i>Cajanus cajan</i>	Fabaceae	Sharma (1998)
<i>M. vitrata</i> , <i>A. fabae</i>	<i>Phaseolus lunatus</i>	Fabaceae	Atachi and Djihou (1994), Capinera (2001) and Sharma (1998)
<i>M. vitrata</i>	<i>Sesbania</i> sp.	Fabaceae	Dannon (2010)
<i>M. vitrata</i>	<i>Crotalaria</i> sp.	Fabaceae	Jackai (1983)
<i>M. vitrata</i>	<i>Sesbania pachycarpa</i>	Fabaceae	Baoua <i>et al.</i> (2011)
<i>M. vitrata</i> , <i>A. fabae</i>	<i>Vicia faba</i>	Fabaceae	Larocca <i>et al.</i> (2011) and Sharma (1998)
<i>A. fabae</i>	<i>Beta vulgaris</i>	Amaranthaceae	Adabi <i>et al.</i> (2010) and Ehler <i>et al.</i> (1997)
<i>A. fabae</i>	<i>Solanum tuberosum</i>	Solanaceae	Blackman and Eastop (2007)
<i>A. fabae</i>	<i>Allium cepa</i>	Amaryllidaceae	Capinera (2001)
<i>A. fabae</i>	<i>Lycopersicon esculentum</i>	Solanaceae	Blackman and Eastop (2007)
<i>A. fabae</i>	<i>Dahlia pinnata</i> , <i>Lactuca sativa</i>	Asteraceae	Blackman & Eastop, 2007; Capinera, 2001

2.3 The Use of Biological Control as a Central Focus for *Aphis Fabae* and *Maruca Vitrata* Control

Due to synthetic pesticides' health and environmental hazards, biological control has been proposed as an alternative to control legume pests (Abtew *et al.*, 2016). The NEs can be an important component of integrated pest management (IPM) in agricultural fields and are environmentally benign compared with synthetic pesticides and, in many cases, economically viable (Sampaio *et al.*, 2009). Integrated pest management (IPM) is a decision-based sustainable approach that utilizes all suitable pest management techniques (biological, cultural, physical/mechanical, and chemical methods) to reduce and manage pest populations, diseases, and weeds (Ehler, 2006). Thus, biological control is the process of NPR whereby NEs (predators and parasitoids) control populations of other plants and animals (e.g., insect pests) (Crowder & Jabbour, 2014). These beneficial organisms (also called biocontrol agents) control pests through parasitism, predation, and competition (Nilsson *et al.*, 2016). There are three types of biological controls: conservation, classical, and augmentative (Cock *et al.*, 2010).

Conservation biological control involves human interactions employing field margins to enhance these NEs' populations. For classical biological control, non-native NEs are released into areas where pests are invasive for permanent suppression, while augmentative biological control involves the mass rearing and release of native NEs for controlling pests (Jacas & Urbaneja, 2010; Van-Lenteren 2012; Van-Lenteren *et al.*, 2018). A lack of knowledge about these control methods among farmers in SSA could be responsible for their low adoption (Otieno *et al.*, 2020).

Several NEs have been identified for controlling *M. vitrata* at all developmental stages (Table 3). *Maruca vitrata* adult and larval predators have been investigated for their potential as effective biological control agents. The predators identified for *M. vitrata* include the Araneidae (*Nephila maculata*), Oxypidae (*Oxyopes javanus*), Anthocoridae (*Orius tantillus*), Forficulidae (*Diaperasticus erythrocephala*), and Formicidae (*Camponotus rufoglaucus*) (Okeyo-Owuor *et al.*, 1991). Parasitoids of *M. vitrata* have been identified across a range of crops, including common beans and *Sesbania cannabina*. The hymenopteran larval parasitoids identified in SSA include *Braunsia kriegeri*, *Apanteles taragamae*, *Pristomerus* sp., *Bassus bruesi*, *Testudobracon* sp., *Cadurcia* sp., *Phanerotoma syleptae*, *Dolichogenidea* spp. and *Phanerotoma leucobasis* (Arodokoun, 1996; Arodokoun *et al.*, 2006; Dannon *et al.*, 2010a; 2010b; Huang *et al.*, 2003; Van-Halteren, 1997). The egg parasitoids include *P. syleptae* and *Trichogramma* spp. (Arodokoun, 1996; Souna *et al.*, 2019; van Halteren, 1997; Yule and Srinivasan, 2014). Tachinid flies have also been identified as larval parasitoids (Arodokoun, 1996; Arodokoun *et al.*, 2006).

Table 3: Parasitoids of *Maruca vitrata* larva that have been reported in SSA

Parasitoid Species	Family	References
<i>Apanteles taragamae</i>	Braconidae	Huis and Dicke (2010)
<i>Bassus bruesi</i> , <i>Bracon</i> sp.	Braconidae	Arodokoun <i>et al.</i> (2006)
<i>Braunsia kriegeri</i>	Braconidae	Arodokoun (1996) and Arodokoun <i>et al.</i> (2006)
<i>Cadurcia</i> sp.	Tachinidae	Arodokoun <i>et al.</i> (2006)
<i>Dolichogenidea</i> sp.	Braconidae	Arodokoun <i>et al.</i> (2006) and Huang <i>et al.</i> (2003)
<i>Phanerotoma leucobasis</i>	Braconidae	Arodokoun <i>et al.</i> (2006)

The biological control of aphids is challenging because of their high reproductive rates (Albajes *et al.*, 1999). However, several aphid predators have been reported (Franzman, 2002;

Kontodimas & Stathas, 2005; Farhadi *et al.*, 2010; Farhadi *et al.*, 2011). The predators of *A. fabae* (Table 4) include Coccinellidae, Cantharidae; Diptera (Dolichopodidae, Tachinidae, Syrphidae larvae, and Cecidomyiidae larvae), Staphylinidae, Hymenoptera (Vespidae and Polistinae), Hemiptera (Anthocoridae), and Neuroptera larva (Chrysopidae) (Balog *et al.*, 2013; Banks, 1968; Nordey *et al.*, 2021; Patterson & Ramirez, 2016; Solomon *et al.*, 2000). Coccinellids are particularly effective predators of *A. fabae* (Nyukuri *et al.*, 2012; Twardowski, *et al.*, 2005). *Hippodamia variegata* has been closely associated with *A. fabae* control in Kenya. In contrast, other predatory coccinellids associated with the control of *A. fabae* include *Cheilomenes* spp., *Henosepichna* spp. and *Exochomus* spp. (Nyukuri *et al.*, 2012). Studies on *A. fabae* parasitoids have identified the braconid *Aphidius colemani* as the main primary parasitoid of *A. fabae* in Tanzania, although it is not yet known whether it is also present in other SSA countries (Mkenda *et al.*, 2019b; Nordey *et al.*, 2021).

Table 4: NEs for black bean aphids (*Aphis fabae*), also recorded in SSA

Natural Enemy	Family	References
<i>Aphidius colemani</i>	Aphididae	Mkenda <i>et al.</i> (2019b) and Nordey <i>et al.</i> (2021)
<i>Cheilomenes</i> sp.	Coccinellidae	Nyukuri <i>et al.</i> (2012)
<i>Exochomus</i> spp.	Coccinellidae	Nyukuri <i>et al.</i> (2012)
<i>Henosepichna</i> spp.	Coccinellidae	Nyukuri <i>et al.</i> (2012)
<i>Hippodamia variegata</i>	Coccinellidae	Nyukuri <i>et al.</i> (2012) and Völkl and Stechmann, (1998)

When considering using NEs for pest control, it is crucial to understand how the biotic and abiotic factors influence them. For example, the rate of parasitism by *A. taragamae* decreases as the temperature increases, although this varies depending on the age of the larva. The first and second instars of *M. vitrata* larvae are parasitized to a great extent. In contrast, the older larvae are not, which is likely due to the defensive behaviour in older larvae (Huis & Dicke, 2010). Conversely, the parasitism by *A. colemani* decreases linearly with the temperature (Zamani *et al.*, 2006). The competition for prey also can reduce parasitism (Sampaio *et al.*, 2006). These variables inform how best to deploy NPR in the field.

2.4 Ecological Manipulations for Supporting NEs

Generally, conservation biological control utilizes plant and landscape biodiversity to promote beneficial insect populations by carefully modifying and managing the environment to increase

non-prey resources (Gurr *et al.*, 2004; Landis *et al.*, 2000; Lundgren, 2009; Wäckers & Van-Rijn, 2005; Wäckers & Van-Rijn, 2012). Farmscaping is one term used to describe an ecological approach that enhances biodiversity to augment the presence of beneficial organisms. Terms such as conservation biological control and ecological engineering are also used to describe similar interventions. Conservation biological control seeks to utilize the existing environmental components to support NEs (Gurr *et al.*, 2017). Thus, ecological engineering and farmscaping are the strategies that are used in conservation biological control. Ecological engineering involves practices and interventions that maximise habitat management's benefit in suppressing agricultural pests (Gurr *et al.*, 2017). Farmscaping provides suitable plants to support and attract populations of beneficial insects (Philips *et al.*, 2014). It forms the basis for ecological or sustainable intensification, i.e., maximizing the ability of the system to produce food sustainably (FAO 2009). Farmscaping provides places for insects to overwinter, physical refugia, and forage; it may also act as a habitat for alternative prey and hosts (Gurr *et al.*, 2004; Hatt *et al.*, 2017a; Hatt *et al.*, 2017b; Rahat *et al.*, 2005). Habitat management involving the manipulation of farmland vegetation can exert direct suppressive effects on pests and promote NEs (Gurr *et al.*, 2004). Examples include trap crops, hedgerows, field margins, and cover crops. Habitat disturbance, loss, and fragmentation in agroecosystems may lead to unsuitable environments for NEs (Philips *et al.*, 2014). Increasing landscape diversity by manipulating the plants that provide alternative resources to NEs such as pollen and nectar, is important for augmenting NPR and pollination (Landis *et al.*, 2000; Saidov and Douglas, 2008). A recent meta-analysis demonstrated a positive effect of flower strips on pest control compared to the fields without flower strips (Albrecht *et al.*, 2020). However, farmscaping might support pest populations, as some herbivores depend on sugar and floral resources (Karp *et al.*, 2018; Wäckers *et al.*, 2007; Winkler *et al.*, 2010). For instance, several studies reported no effect of habitat manipulation on either pest or natural enemy abundances (Goncalves & Sousa, 2003; Jado *et al.*, 2019). Therefore, it is vital to understand how NEs and pests interact with plants before assuming that increased diversity might improve conservation biological control.

Several flowering plants found in SSA have been used in farmscaping. For example, *Fagopyrum esculentum*, *Lobularia maritima*, *Mentha piperita*, *Tridax procumbens*, *T. erecta*, *Tagetes minuta*, and *Sesamum indicum* have been found to increase the parasitism rates and longevity of lepidopteran and aphid's NEs. The NEs include *Apanteles ruficrus*, *Cotesia chilonis*, *Cotesia rubecula*, *Trichogramma chilonis*, *Microplitis mediator*, *Diadegma*

semiclausum, and *A. colemani*; thus, they could have the potential for *M. vitrata* and *A. fabae* management (Jado *et al.*, 2019; Pfiffner *et al.*, 2006; Pfiffner *et al.*, 2009; Zhu *et al.*, 2014). However, some species are invasive to SSA, so they should be used cautiously. Crops such as sorghum used as a border in pigeon pea fields increase the abundance of the predators *Coccinella septumpunctata*, *Cheilomenes sexmaculata*, and spiders, with a decrease in *M. vitrata* populations, which eventually leads to increased pigeon pea yields (Sujayanand *et al.*, 2021).

Farmscaping plants can also influence the behaviour of phytophagous and predatory insects through the volatiles they produce (Allmann *et al.*, 2013; Pichersky & Gershenzon, 2002). Volatiles are involved in signalling and therefore play a part in the defence against various pathogens and herbivores and attract beneficial insects (Dudareva *et al.*, 2006). Intercropping some plants that release volatiles when damaged by herbivores will attract NEs of pests to an area. When attacked by herbivores or synthetic equivalents, some semiochemicals produced by plants will repel pests and attract NEs and are known as Herbivore-Induced Plant Volatiles (HIPVs) (Kaplan, 2012; Khan *et al.*, 2008). These can be used to improve conservation biological control. For instance, volatiles released by damaged *M. vitrata* host plants (cowpea and pea bush) attracted the braconid *Therophilus javanus* and the parasitic fly *Nemorilla maculosa* (Agbessenou *et al.*, 2018; Souna *et al.*, 2019). This was also the case for volatile compounds produced by *Vicia faba* damaged by *A. fabae*, which attracted a parasitoid (*Lysiphlebus fabarum*) and a predator (*Orius albidipennis*) (Rashedi *et al.*, 2019). Synthetic HIPVs in attracting *M. vitrata* parasitoids were successful for *A. taragamae* and *P. syleptae* (Osei-Owusu *et al.*, 2020). The mechanism of how HIPV functions could also be necessary for providing the control of *A. fabae*, and this requires further studies. Non-crop habitats such as field margins are essential in providing floral resources to NEs. Therefore, selecting suitable plants to attract/conserves NEs is essential in establishing the effective biological control of pests. Future studies should focus on different host plants and how they influence the abundance of insect pests and NEs and manipulate them for effective biological control.

2.5 Synthetic Chemicals and their Impact on Natural Enemies of *Maruca vitrata* and *Aphis fabae*

Synthetic pesticides can rapidly knock down pests and reduce crop damage and yield losses. Also, they have negative impacts on human health and the environment, which can be a particular problem in SSA, where farmers often use older, more toxic, mislabelled pesticides

that are more likely to be incorrectly handled and sprayed (Ajayi & Aknnifesi, 2007; Nonga *et al.*, 2011). Issues such as mixing different types of chemicals and the increasing frequency and application rates further magnify the problems and create a need for environmentally friendly pest management (Abtew *et al.*, 2016). In the context of biological pest control, pesticides can also kill non-target organisms, such as the NEs of pests and pollinators, which are detrimental to sustainable pest control (Mkenda *et al.*, 2015).

The chemical control of *M. vitrata* is difficult because their feeding sites in floral parts and pods protect the larvae from sprays (Abeeluck *et al.*, 1997; Sharma *et al.*, 1999), so NPR using biological pest control is more appropriate for this pest. Pesticide resistance to cypermethrin and dimethoate has also been reported in *M. vitrata*, making their control more challenging (Ekesi, 1999). Farmers sometimes use doses of synthetic pesticides above the recommended rates to achieve control of *M. vitrata*, which increases non-target impacts and risks and exacerbates the build-up of pesticide resistance (Abeeluck *et al.*, 1997). Where farmers are provided knowledge and support, sustainable pest management strategies and a decreased reliance on synthetic pesticides are feasible (Mkenda *et al.* 2020).

Recent evidence has shown that several NEs of *A. fabae*, including Coccinellidae, Araneidae, Syrphidae, and Chrysopidae, were significantly lower in fields treated with the synthetic pesticide Karate 5 EC (lambda-cyhalothrin) in common bean, cowpea, and pigeon pea field trials compared to those treated with botanicals (Mkenda *et al.*, 2015; Mkindi *et al.*, 2017; Tembo *et al.*, 2018). Other studies have reported lethal and nonlethal effects of synthetic pesticides on arthropod NEs, such as feeding deterrents and mortality (Araya *et al.*, 2010; Liu and Chen, 2001; Munyuli *et al.*, 2007; Schneider *et al.*, 2004; Shoeb, 2010; Thomson *et al.*, 2001).

Natural pest regulation (NPR) that optimizes the services of NEs of *A. fabae* and *M. vitrata* is likely to be the basis of sustainable pest management. Since non-selective synthetic pesticides are incompatible with beneficial insects, they should be used as the last resort in future sustainable farming systems. Thus, in searching for sustainable pest control, biological control is a cornerstone of other sustainable strategies and is favoured for its feasibility (Jackai, 1995), especially for smallholder farmers. However, smallholder farmers require knowledge support of biological control, as knowledge gaps hinder the adoption of conservation biological control (Abtew *et al.*, 2016; Mkenda *et al.*, 2020). Hence, it is important to make proper advocacy of biological control for sustaining ecosystem services offered by the NEs of pests.

2.6 Other Sustainable Alternatives Compatible with NEs for Managing *Maruca vitrata* and *Aphis fabae*

2.6.1 Biopesticides

Biopesticides are natural products or microorganisms that act as alternatives to conventional pesticides and are not persistent in the environment, reducing the harmful effects of conventional pest control that rely on synthetic products (Black, 2013; Isman, 2008; Macías *et al.*, 2000; Moshi & Matoju, 2017; Stevenson *et al.*, 2017). Biopesticides are categorized into three groups: microbial pesticides, plant-incorporated protectants (PIPs), and biosynthetic pesticides or plant-based pesticides (PBPs) (Leahy *et al.*, 2014). Some biopesticides can be used alone, while others can be co-applied with other control methods, particularly the NEs of pests. Biopesticides can interact with other control options, especially NEs, and exhibit additive or antagonistic effects in controlling pests (Srinivasan, 2012).

Microbial biopesticides consist of formulated microorganisms, including bacteria, fungi, protozoa, nematodes, and viruses, that are pathogenic to insects (Black, 2013; Leahy *et al.*, 2014) and have been investigated for the management of *M. vitrata* and *A. fabae*. For example, *Bacillus thuringiensis* (Bt) and its toxins are one of the most widely used and successful microbial biopesticides (Bravo *et al.*, 2011) and have been successfully used to control *M. vitrata* based on the activity of Bt δ -endotoxins (Addae *et al.*, 2020). The commercial Bt product (Bactospeine) causes larval mortality (Kumar *et al.*, 2014). The efficacy of Bt against *M. vitrata* has also been assessed in the field on yard-long beans, where its application in combination with PBPs such as neem is effective in reducing pod damage by *M. vitrata* (Yule & Srinivasan, 2014). However, the effects of these technologies on the NEs of pests are not well-studied and require more attention to determine the compatibility across sustainable approaches to pest management.

Viruses also have efficacy against *M. vitrata*, although most studies have been based in the laboratory (Srinivasan *et al.*, 2015). The baculovirus, *M. vitrata* multi-nucleopolyhedrovirus (MaviMNPV), is a promising candidate as a biopesticide against *M. vitrata* and has been researched for use in SSA in particular. This virus causes significant mortality and reduces the egg viability of *M. vitrata* under laboratory conditions (Traoré, 2019). It has also been demonstrated to be effective against *M. vitrata* larvae in the field and laboratory bioassays. Furthermore, its efficacy can be increased when applied with neem oil and neem, *Azadirachta*

indica, and *Jatropha curcas* extracts (Joelle *et al.*, 2020; Sokame *et al.*, 2015; Srinivasan *et al.*, 2009).

There have been attempts to develop techniques to mass-produce MaviMNPV for smallholder farmers in SSA by the International Institute of Tropical Agriculture (IITA-Benin). Although this has not yet been implemented in smallholder farms on a large scale, it can potentially control *M. vitrata* in a more cost-efficient and sustainable way (Srinivasan *et al.*, 2019). Biopesticides for *A. fabae* and *M. vitrata* are shown in Table 5. The compatibility of microbial biopesticides with the NEs of pests has been reported. For instance, the baculovirus *M. vitrata* multi-nucleopolyhedrovirus (MaviMNPV) showed compatibility with the braconid parasitoid *A. taramagae*, and the parasitoid can transmit the virus between hosts (Srinivasan *et al.*, 2009).

Similarly, the entomopathogenic fungi (EPF) biopesticide *Beauveria bassiana* is compatible with the predatory coccinellids *C. septempunctata* and *H. variegata* (Akmal *et al.*, 2013; Sayed *et al.*, 2021), while *Lecanicillium muscarium*, in combination with the predatory coccinellid *Adalia bipunctata*, showed the possibility of reducing *A. fabae* infestations, although this was not in the field setting, and thus, further field trials are needed (Mohammed, 2018). Likewise, a combination of the EPF *Metarhizium anisopliae* with the PBP pyrethrum does not affect the foraging behaviour of *A. colemani*, a key parasitoid of *A. fabae*, implying likely compatibility. However, when used alone, the EPF exhibited a deterrent effect in *A. colemani*, so more research is needed (Fernández-Grandon *et al.*, 2020).

Table 5: Microbial biopesticides used in the control of *Aphis fabae* and *Maruca vitrata*

Bean Pest	Biopesticide Used	References
<i>M. vitrata</i>	<i>Bacillus thuringiensis</i>	Kumar <i>et al.</i> (2014)
<i>M. vitrata</i>	<i>Beauveria bassiana</i>	Ekesi <i>et al.</i> (2002), Traoré (2019) and Tumuhaise <i>et al.</i> (2015)
<i>M. vitrata</i>	Mavi multi-nucleopolyhedrovirus	Joelle <i>et al.</i> (2020) and Traoré (2019)
<i>M. vitrata</i>	<i>Metarhizium anisopliae</i>	Ekesi <i>et al.</i> (2002) and Tumuhaise <i>et al.</i> (2015)
<i>A. fabae</i>	<i>Lecanicillium muscarium</i>	Saruhan <i>et al.</i> (2015) and Saruhan (2018)
<i>A. fabae</i>	<i>Simplicillium lamellicola</i>	Saruhan <i>et al.</i> (2015) and Saruhan (2018)
<i>A. fabae</i>	<i>Aspergillus flavus</i>	Boni <i>et al.</i> (2020)
<i>M. vitrata</i>	<i>Heterorhabditis</i> sp., <i>Oscheius</i> sp.	Pervez and Rao (2021)

Although biopesticides, including EPFs, *B. thuringiensis*, entomopathogenic nematodes, and baculovirus-based products that target pests other than *M. vitrata* and *A. fabae*, are available commercially in SSA countries such as Kenya (Srinivasan *et al.*, 2019) and Tanzania (Moshi & Matoju, 2017), the knowledge of their production, the high cost of buying them, and their low speed of killing pests have made farmers reluctant to adopt these control options (Moshi & Matoju, 2017). The efficacy of biopesticides may be altered by factors such as humidity, rainfall, temperature, ultraviolet light, leaf surface chemistry, formulation, application method, substrate, and fungal isolates, meaning that their inappropriate use can give poor results (Darbro & Thomas, 2009; Jaronski, 2010). Therefore, all these factors are to be considered in the formulation for increasing the efficacy of biopesticides for the sustainable control of pests. Many EPFs affect specific orders of arthropods differently and may therefore pose a lower risk to NEs than target pests and, so, can be used effectively with NEs (Lacey *et al.*, 1997; Roy and Pell, 2000). Conversely, some biopesticides, such as the EPF *B. bassiana*, infect and kill parasitoids such as *A. colemani* (Shipp *et al.*, 2003), and therefore, toxicity assays are critical in understanding the broader consequences of applying biopesticides for managing pests.

The PBPs exploit naturally occurring entomotoxins of plant origin. They contain metabolites that can inhibit and kill directly, affect reproduction, and alter other metabolic processes in pests (Leahy *et al.*, 2014; Rattan, 2010). The PBPs can be highly toxic but typically have lower environmental impacts than synthetic pesticides, primarily because they do not persist in the environment and are broken down through the actions of sunlight and microorganisms (Mkenda *et al.*, 2015, Mkindi *et al.*, 2017). Thus, the longer-term impacts on NEs and biological control are reduced. The potential of PBPs has been explored in some African countries. However, few of these pesticides have been exploited commercially or in smallholder farms, although some, such as pyrethrum and neem, have been developed into highly successful commercial products (Grzywacz *et al.*, 2014). The PBPs are compatible with NEs and can be used in SSA (Mkindi *et al.*, 2017; Tembo *et al.*, 2018). Many PBPs have significantly reduced the negative impacts on beneficial invertebrates compared to synthetic pesticides (Mkindi *et al.*, 2017; Tembo *et al.*, 2018). For example, extracts of *B. pilosa*, *Lippia javanica*, *Tephrosia vogelii*, *L. camara*, *Vernonia amygdalina*, and *Tithonia diversifolia* were highly effective at controlling insect pests on legume crops. However, their impact on lady beetles, lacewings, spiders, and syrphid flies was reportedly significantly lower than those resulting from exposure to synthetic pesticides (Mkenda *et al.*, 2015; Mkindi, *et al.*, 2017; Stevenson *et al.*, 2017; Tembo *et al.*, 2018). However, several studies outside SSA have

also shown that some PBPs exhibit toxicity to the NEs of pests. For instance, pyrethrum, neem, Chilean plant products, and rotenone caused the mortality of the NEs *Adonia variegata*, *Venturia canescens*, *Orius laevigatus*, and *Encarsia formosa* (Bonsignore & Vacante, 2012; Simmonds *et al.*, 2002; Swaminathan *et al.*, 2010; Tunca *et al.*, 2012).

The PBPs, including commercially available products such as Neem Baan (Azadirachtin), have been found to cause *M. vitrata* larval mortality. However, their efficacy is reduced when applied to the later larval stages (Kumar *et al.*, 2014). Due to the cost of commercial PBPs, it may be more viable for smallholder farmers in SSA to use extracts (Table 6) that are prepared, readily available, and accessible (Belmain & Stevenson, 2001; Isman, 2008). For instance, multiple pesticidal plant extracts from common margin plants and weeds have shown efficacy against *A. fabae*, including *A. indica*, *Allium sativum*, *Eucalyptus* sp., *Swietenia* sp., *Tephrosia vogelii* (Bahar *et al.*, 2007; Kayange *et al.*, 2019), *Matricaria chamomilla* on the broad bean, and tobacco water (*Nicotiana* sp.) on yard-long beans (Biniaś & Gospodarek, 2017). *Annona muricata* and *Piper guineense* extracts significantly reduced *M. vitrata* larval infestations and, thus, increased cowpea yields compared with the synthetic pesticide lambda-cyhalothrin in cowpeas (Ugwu, 2020).

Table 6: Pesticidal plants common to SSA used for *Aphis fabae* and *Maruca vitrata* control

Pesticidal Plant	Plant family	Pest	References
<i>Allium sativum</i>	Amaryllidaceae	<i>Aphis fabae</i>	Bahar <i>et al.</i> (2007)
<i>Annona muricata</i>	Annonaceae	<i>Maruca vitrata</i>	Ugwu (2020)
<i>Azadirachta indica</i>	Meliaceae	<i>Aphis fabae</i>	Bahar <i>et al.</i> (2007)
<i>Eucalyptus</i> sp.	Myrtaceae	<i>Aphis fabae</i>	Bahar <i>et al.</i> (2007)
<i>Matricaria chamomilla</i>	Asteraceae	<i>Aphis fabae</i>	Ugwu (2020)
<i>Piper guineense</i>	Piperaceae	<i>Maruca vitrata</i>	Ugwu (2020)
<i>Swietenia</i> sp.	Meliaceae	<i>Aphis fabae</i>	Bahar <i>et al.</i> (2007)
<i>Tephrosia vogelii</i>	Fabaceae	<i>Aphis fabae</i>	Kayange <i>et al.</i> (2019) and Mkindi <i>et al.</i> (2019)

Many more plant extracts and their compatibility with NEs have not yet been investigated. Additionally, the use of plant products to control *M. vitrata* is practically limited because little research has been conducted on PBPs targeting *M. vitrata*, despite using some of these plants

in managing *A. fabae* (Mkenda *et al.*, 2015; Mkindi *et al.*, 2017; Tembo *et al.*, 2018). As the *M. vitrata* larva feeds inside the pods, flowers, and flower buds and also webs the leaves and flowers, it is protected from spraying with PBPs as much as with synthetics, making foliar sprays challenging to apply effectively (Sharma, 1998). The lack of field trials and field data, particularly for legume crops in Africa, and the toxicity of some PBPs are the drawbacks of effective pest management. Thus, further studies should address these aspects to control bean pests effectively.

Plant-incorporated protectants (PIPs) are biopesticides such as the gene encoding the Bt toxin that can be introduced into the plant genome allowing the plant to produce this toxin and increasing the resistance of the plant to some pests (Koch *et al.*, 2015). For instance, the Cry proteins expressed in Bt cowpeas have shown a less negative effect on non-target organisms (Ba *et al.*, 2018). Generally, the PIPs available globally for the specific management of *M. vitrata* and *A. fabae* are limited, and equally, their impacts on their NEs have not been studied; thus, more work has to be done to address this gap.

There is a considerable effort for biopesticides to be used more widely in SSA for pest control. However, studies have shown that there is generally a lack of awareness of biopesticide products among smallholder farmers. Across Uganda and Kenya, fewer than 20% of the farmers surveyed had heard of biopesticides (Nyangau *et al.*, 2020). In Kenya, only 10% of the farmers surveyed had used biopesticides on their crops (Constantine *et al.*, 2020). The lack of information could be one of the key factors preventing the broader use of biopesticides across SSA. Other potential barriers to the uptake of biopesticides include the production, cost of buying them, concern over their speed of killing pests, and short persistence. Expertise in product development, packaging, and composition; the knowledge gap among smallholder farmers; and research on the importance and benefits of biopesticides, such as ecological safety and the possession of nutrient supplements advantageous to crops, should be addressed (Isman, 2008).

2.6.2 Compatibility of Pest Resistant Crop Varieties with NEs

The use of resistant varieties is an essential component of IPM which has shown some level of efficacy against legume pests (Adati *et al.*, 2007; Rubiales *et al.*, 2015). Some *M. vitrata*-resistant varieties have been identified (Halder & Srinivasan, 2011; Huesing *et al.*, 2011; Yusuf, 2005). Fewer studies have been conducted on using resistant varieties to reduce *M.*

vitrata infestations in the common bean. Therefore, this requires more work, especially considering the impacts on nontarget species that might be exposed to these toxins through parasitizing or predating pests. For *A. fabae* management, resistant varieties of *P. vulgaris* have been identified within (Mwangi *et al.*, 2008) and outside (Esmaeili-Vardanjani *et al.*, 2013) SSA. Cultivars of *V. faba* and *Beta vulgaris* resistant to *A. fabae* have similarly been identified (Razmjou & Fallahi, 2009; Shannag & Obeidat, 2008). Resistant cultivars have been combined with the NEs of bean pests for better pest control. For example, using partially resistant cultivars of *V. faba* with the coccinellid predator *C. septempunctata* effectively controlled *A. fabae* (Shannag & Obeidat, 2008). However, studies are needed to test the different resistant bean varieties & their compatibility with the NEs of bean pests for maximizing the biological control services in bean fields.

2.6.3 Cultural Control

Control practices such as intercropping systems, variations in planting dates, plant density, and spacing can be effective against *A. fabae* and *M. vitrata*; while are beneficial for NEs (Adipala *et al.*, 2000; Karungi *et al.*, 2000; Munyuli *et al.*, 2007; Nampala, 1998). Some studies observed a low incidence of *M. vitrata* larva and flower pod damage in intercropping systems compared to monocropping systems (Karel, 1993; Omolo *et al.*, 1993). However, several other studies showed no significant reductions in *M. vitrata* when using the intercropping strategy (Bottenberg *et al.*, 1998; Emeasor & Ezueh 1997). Thus, it is important to generate information on the limitations and opportunities of employing intercropping approaches in combating *M. vitrata*, especially in common beans, because currently, there are limited studies on this, significantly where they enhance the natural enemy benefits. Cultural practices may not always benefit pest control. For example, manipulations and variations in planting dates, weeding, and spacing did not help reduce *M. vitrata* populations but increased the pest populations (Adipala *et al.*, 2000; Asiwe *et al.*, 2005). Contrarily, a previous study showed that early cowpea planting reduces the rates of infestations by *M. vitrata* (Ekesi *et al.*, 1996). For *A. fabae*, it was observed that intercropping *P. vulgaris* with *Zea mays* was successful in reducing aphid populations in *P. vulgaris* (Latigo-Ogenga *et al.*, 1993). Limited studies have examined the applicability of different cultural practices for *A. fabae* management in *P. vulgaris*. Therefore, it is important to assess the potential of different cultural practices in combination with NEs and the biological control of bean pests to reduce *A. fabae* infestations in common bean fields.

The compatibility of cultural practices with NEs of *M. vitrata* and *A. fabae* has been reported. The assessment of the intercropping of cowpeas with either green gram or sorghum revealed a high abundance of predators (coccinellids, hoverflies, rove beetles, spiders, praying mantis, ground beetles, predatory mites, big-eyed bugs, dragonflies, damsel bugs, minute pirate bugs, & earwigs) (Munyuli *et al.*, 2007). Conversely, intercropping sorghum with a cowpea crop did not produce significant differences in *M. vitrata* populations, although the parasitism rate by *P. leucobasis* was higher in the intercropping plots (Yusuf, 2005). The coccinellid populations (*H. variegata* and *C. septempunctata*), important NEs of *A. fabae*, were enhanced by intercropping faba beans with the aromatic flowering plant dragonhead (*Dracocephalum* spp.) (Azimi & Amini, 2015). However, limited studies have been conducted to evaluate the compatibility of cultural control practices with the NEs of *A. fabae* and *M. vitrata*, particularly in beans.

2.7 Conclusions and Recommendations

Synthetic pesticides are still the most frequently used strategy to control *M. vitrata* and *A. fabae* by smallholder farmers in bean farming systems. Nevertheless, they are largely incompatible with a biological control dependent on beneficial insects. Here, more sustainable approaches to the pest management of these pests in beans were reviewed and compared their compatibility with other natural pest-regulating options. Ultimately, inadequate evidence indicates how suitable different approaches might be from the existing literature, which needs to be addressed. However, where there is evidence, this strongly supports the notion that more sustainable pest management interventions are more compatible with biological control than synthetic pesticides (Table 7). The deployment of sustainable approaches for *M. vitrata* and *A. fabae* management for biopesticides faces challenges. For example, biopesticides are limited by the lack of field trials. Thus, farmers have a poor perception of their effectiveness and high cost of production challenges facing their registration, and hence, few biopesticides are available commercially. More field trials, increasing awareness, and registering new products at affordable prices would enable farmers in SSA to use more biopesticides.

Additionally, the incorporation of biological control into management strategies for smallholder farmers is hindered by low levels of experience, expertise, and broader knowledge about the NEs and their requirements, especially how landscapes can influence the abundance and diversity of biological control species. In some cases, the species that support beneficial insects may also provide botanical pesticides, such as *Tephrosia vogelii*, *Annona muricata*, and

Piper guineense, and a combination of control options with biological control has shown potential in decreasing *M. vitrata* and *A. fabae* infestations while achieving high yields compared with synthetic pesticides (Adipala *et al.*, 2000; Kayange *et al.*, 2019; Ugwu, 2020). However, the yield data for different management options are also lacking; therefore, it is important to address this gap. The synergistic effects of *M. vitrata* and *A. fabae* control are exhibited when some biopesticides are used with NEs. Therefore, more studies should focus on different biopesticides and their compatibility with NEs for increasing efficacy in managing *A. fabae* and *M. vitrata*. The use of IPM has been advocated in the literature as a solution to sustainably managing pests for years (Agunbiade *et al.*, 2018; Singh *et al.*, 1990), but the implementation still requires work and, in particular, more compatibility studies.

A combination of strategies such as cultural control, application of biopesticides, and using resistant varieties with the efficient monitoring of pests could work together with NEs to effectively manage *M. vitrata*. Some works have reported an increasing control efficiency when a combination of strategies such as resistant cowpea varieties; cultural methods (manipulating the planting date, adjusting planting spacing, and using mixed cropping); and the reduced application of synthetic pesticides with NEs were used in IPM (Ajeigbe & Singh, 2006; Jackai, 1995; Nabirye *et al.*, 2003; Srinivasan, 2012). One challenge limiting cultural control's efficacy is its dependence on seasons and locations. Additionally, the lack of sources of resistance traits has limited the development of some resistant varieties for *M. vitrata* and *A. fabae*. Using resistant varieties and cultural control could help enhance the efficacy of *M. vitrata* and *A. fabae* controls. Thus, more studies should explore the control of these pests by using biological controls with resistant varieties and cultural control efficacy to minimize the losses by *M. vitrata* and *A. fabae*.

This literature review discusses biological control as a feasible IPM strategy for smallholder farmers of SSA. The evidence indicates a considerable potential in employing available plant diversities from farmers' localities to manage pests while enhancing the populations of NEs for *M. vitrata* and *A. fabae* management. This form of NPR is also compatible with other control strategies, such as biopesticides, cultural control, and resistant crop varieties. However, the use of biopesticides requires more investment in their development and efficacy in controlling *M. vitrata* and *A. fabae*. Equally, more work is needed in developing resistant varieties for *M. vitrata* and *A. fabae*; some of the identified varieties for controlling *M. vitrata* and *A. fabae* in the surveyed literature have been found to exhibit moderate resistance to *M.*

vitrata and *A. fabae*. The use of cultural control for *M. vitrata* has shown inconsistent results. Thus, there is a need to generate more knowledge on the limitations and potential of this approach as part of an integrated system. A community-based approach to implementing different management alternatives, such as the growth/preparation of PBPs, the production of EPF/viruses at a community level, or the knowledge exchange among smallholder farmers about NEs, would help to overcome some of these issues (Belmain *et al.*, 2013; Elisante *et al.*, 2019; Mkenda *et al.*, 2020). The training and capacity building of farmers could also facilitate a better understanding of biological control and its compatibility with other control strategies for enhancing the ecosystem services provided by NEs. The information reviewed here informs how biological control is a key component in the sustainable management of *A. fabae* and *M. vitrata* and how it interacts with other sustainable approaches to the management network of key bean pests. More knowledge should be generated on the importance of a system-based ecological approach to increase the understanding of the management options.

Table 7: The compatibility of the *Maruca vitrata* (MV) and *Aphis fabae* (AF) management options with their NEs

Biological Control Agent	Control Method													
	Biopesticides								Resistant Varieties		Cultural Control		Synthetic Pesticides	
	EPF		Bacteria		Viruses		Botanical Pesticides						(-)	
	AF	MV	AF	MV	AF	MV	AF	MV	AF	MV	AF	MV	AF	MV
	(+)						(+)		(+)		(+)	(+)	(-)	
Predators		(*)	(*)	(*)	(*)	(*)		(*)		(*)				(*)
Parasitoids	(+)					(+)	(+)					(+)		
		(*)	(*)	(*)	(*)			(*)	(*)	(*)	(*)		(*)	(*)

Note: (+) shows compatibility; (-) shows non-compatibility; (*) implies the compatibility is less-studied.

CHAPTER THREE

MATERIALS AND METHODS

3.1 To identify the key parasitoids of black bean aphids (*aphis fabae*) and legume pod borer (*maruca vitrata*) in bean fields

3.1.1 Study Areas

For objectives 1, 2, 4, 5 and 6, the field experiments were conducted among farming communities in the selected farms, Kwa Sadala-Hai District, Kilimanjaro region. Kwa Sadala is situated between 3° 10' 0" S and 37° 10' 0" E, with rainfall ranging from 700 mm to 2000 mm per annum and temperature ranges between 15°C to 30°C. The area has a bimodal rainfall pattern, with short rains falling between October and December and long rains between February and May. Farmers depend on subsistence monoculture and mixed farming for their livelihoods. Their crops include maize, beans, sunflower, potatoes, vegetables and tomatoes. Agricultural practices in these areas reflect the wider practice of the region.

For objective number 3, the trial was conducted at the NM-AIST laboratories in Arusha, Tanzania. The molecular works were conducted at NM-AIST and the University of Greenwich in the United Kingdom.

3.1.2 Collection Parasitoids

Thirty-two sites (0.20 ha) with either high (n = 16) or low (n = 16) plant diversity were selected based on the observed number of plant species in non-crop vegetation around each farm using a quadrat method (Ndakidemi *et al.*, 2022) (Plates 3&4). To quantify the diversity of the plant species in our field sites, the Shannon index (H') (Shannon & Weaver, 1963) was used according to the formula below to calculate low diversity fields (H'=1.2) and high diversity fields (H'=2.3).

Shannon Index (H') = $H = -\sum (p_i (\ln p_i))$.

p_i - Proportion (n/N) of individuals of particular species in a whole community:

n - individuals of a particular species

N - total number of individuals found,

Σ - Sum symbol

\ln = natural logarithm to base e.

The field margins were 2.5 m wide. The surrounding composition was similar in all fields (the arable fields, dominated by several flowering weed species) with similar management practices. Fields were located at least 50 m apart.

A further parameter was the inclusion of cropping practice where half of the farmer fields at each level (low and high plant diversity fields) practised monocropping of common beans (*Phaseolus vulgaris*), whereas the other half of farmer fields intercropped beans with maize (*Zea mays*).



Plate 3: High plant diversity fields. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania



Plate 4: Low plant diversity fields. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania

(i) Parasitoids collection

Aphis fabae parasitoids

Sentinel plants (Plate 5) (common beans grown in pots in a screen-house) were infested with 50–60 apterous *Aphis fabae* and used to collect parasitoids, as described by Mkenda *et al.* (2019b). After the infestation of sentinel plants, *Aphis fabae* were left to settle before being placed into fields. Sentinel plants were placed in fields during the fourth and seventh week after seedling emergence for common beans. These times were selected to coincide with the legumes' vegetative and flowering/podding stages in the field to ensure that parasitoids were collected across the cropping season. Two sentinel plants were placed in each field, one in the centre of the crop and one in the field margin, because the abundance and diversity of aphid parasitoid species have been found to vary between crop and field margin habitats (Woolley *et al.*, 2022). Sentinel plants were left in the field for 14 days to allow parasitism of *A. fabae* before being returned to the laboratory, where they were maintained in fine mesh cages for 28 days (Plate 5). All emerging parasitoids were collected and preserved in 95% ethanol for subsequent DNA barcoding.



Plate 5: Sentinel bean plants infested with *Aphis fabae*. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania

Maruca vitrata parasitoids

For monitoring egg parasitoids, *Maruca vitrata* larvae were collected from infested leaves, flowers, and pods in bean farmers' fields and reared on sprouting cowpeas and pods in plastic containers (5×11×18 cm) covered with fine mesh at natural lighting, relative humidity (RH) of 67-69% and the temperature 26–29°C (Plate 6) (Eurekha *et al.*, 2019; Wetrot *et al.*, 2014). Emerging adults were fed using cotton fibres moistened with a 10% glucose solution. Four to five days adults were allowed to mate and then released into wooden cages, each containing a potted bean plant for oviposition (Plate 7) (Dannon *et al.*, 2010b). After 24 h, the potted plants

were transferred into the fields in the fourth and eighth weeks after planting the bean crop, two potted plants per field, one placed within the bean field and the other in the field margin. The plants were left for 48 h (Arodokoun, 1996) and then brought to the laboratory for monitoring the emergence of egg parasitoids. The emerged parasitoids were preserved in 95% ethanol for DNA barcoding.

For monitoring the larval parasitoids, the potted bean plants placed in wooden cages were infested with *M. vitrata* larvae, left for 24 h and then transferred to the field during the flowering and podding stages of the bean crop (Plate 8). They were left in the fields for 48 h (Jayasinghe *et al.*, 2015; Unmole, 2009). The sampled larvae were brought to the laboratory and reared in plastic vials (3.5 cm diameter and 6.6 cm height) covered with moist cotton wools to maintain humidity for monitoring parasitoid emergence. The samples were maintained at room temperature of 25–29° C, with natural lighting and relative humidity (RH) of 66–69% and observed parasitoid emergence. The collected parasitoids were preserved in the 95% alcohol for further identification through DNA barcoding.



Plate 6: *Maruca vitrata* larva rearing. Photo by Ndakidemi B. J, NM-AIST, Arusha, Tanzania

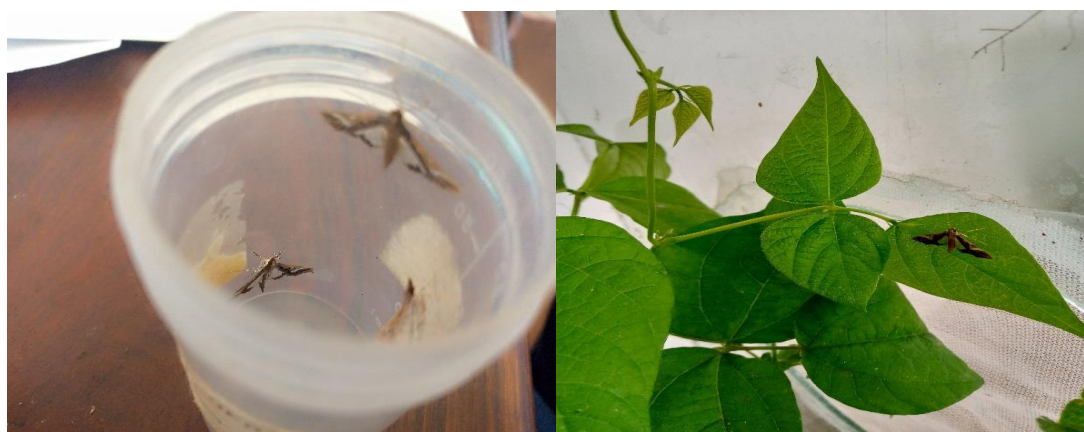


Plate 7: *Maruca vitrata* adults. Photo by Ndakidemi B. J, NM-AIST, Arusha, Tanzania



Plate 8: *Maruca vitrata* larva in bean plant. Photo by Ndakidemi B. J, NM-AIST, Arusha, Tanzania

DNA sequencing

The Chelex method was used to extract DNA from parasitoids. Insects were ground in 25 µl of Chelex buffer (10% w/v Chelex resin (Bio-Rad, Hercules, USA) in TE solution) using a micropestle in a 1.5 ml Eppendorf tube. The micropestle was then rinsed with a further 25 µl of Chelex buffer. A PCR was performed using MyTaq DNA polymerase (Meridian Bioscience, Cincinnati, USA) following the manufacturer's instructions and the primers HCO2198 and LCO1490 (Folmer *et al.*, 1994). The PCR conditions used were: Initial denaturation of 94°C for 3 min, 37 cycles of 94°C for 30 secs, 52°C for 1 min, 72°C for 1 min 30 sec and a final extension step of 72°C for 10 min. If amplification with these primers was unsuccessful, the LepF1/C_ANTMRID (Smith *et al.*, 2008; Hebert *et al.*, 2004) and MLepF1/LepR1 (Hebert *et al.*, 2004; Hajibabaei *et al.*, 2005) primers were used to amplify shorter lengths of DNA as described by Smith *et al.* (2008). PCR products were visualized on a 1.2% agarose gel in 0.5 x TBE stained with GelRed (Biotium, Fremont, USA) using gel electrophoresis. If amplification was successful, PCR products were purified using a GeneJET PCR purification kit (ThermoFisher Scientific, Waltham, USA) following the manufacturer's instructions. The PCR product was sequenced by Eurofins Genomics (Eurofins, Luxembourg) using the forward primer.

DNA Barcoding

All DNA sequences were trimmed using Geneious Prime 2020.0.5 (<https://geneious.com>). Where both LepF1/C_ANTMRID and MLepF1/LepR1 primer pairs produced high-quality sequences, Geneious Prime was used to produce a consensus sequence (full-length COI

barcode) from these shorter sequences. Where amplification with only one of LepF1/C_ANTMRID and MLepF1/LepR1 primer pairs was successful, this short ‘mini-barcode’ (Mkenda *et al.*, 2019b) was used for identification of the sample but not for further analysis. Sequences were compared to published sequences in the Barcode of Life Database (BOLD; <http://www.boldsystems.org/>) for identification. If the specimen sequence gave a close match (greater than 99% for species, greater than 95% for genus and greater than 90% for family) and the sequence clustered with a monophyletic group of this species/genus/family, then the unknown sequence was assigned to this taxon (Hebert *et al.*, 2016).

(ii) Statistical analysis

Parasitoids that emerged from sentinel plants were examined (provisionally identified) morphologically and identified by DNA barcoding. To determine the effect of field margin diversity (high/low) and position of the sentinel plant (margin/centre of the field) on the number of parasitoids, a generalised linear model (GLM) was used in R (RStudio Version 1.2.1335). The GLM had a Poisson distribution with margin diversity and position included as covariates without interactions. Pairwise comparisons were performed with the Holm multiple comparisons test.

3.2 To investigate the effects of field margins on the temporal-spatial distribution of NEs of bean pests

3.2.1 Collection of NEs and pests in the fields

The experiment was conducted among farming communities in the selected farms, Kwa Sadala-Hai District, Kilimanjaro region, as described in 3.1.1. Sweep netting was carried out, one replicate per site per visit, using a standard canvas hand sweep net to sample insects (Plate 9A). Each sweep replicate consisted of three parallel transects in which the net was swept back and forth ten times: transect one along the margin, at least 0.5 m from the crop; transect two in the crop edge, 5 m from the margin, and transect three in the centre of the crop, >15 m away from the margin (Fig. 1). The insects collected by the sweep net were transferred to 95 to 99% ethanol for preservation. This was repeated six times over the growing season, one time at seedling, two times at vegetative and flowering/pod formation, and one time at physiological maturity before pod drying. Yellow sticky traps with glue on both sides measuring 25 x 10 cm (Real IPM, Nairobi, Kenya) were placed in the field margins monthly from May to August, corresponding to the growth stages of the crop (Plate 9B). They were attached approximately

1 m from the ground with a string wire to a wooden cane, two sticky cards in each field margin plant for thirty-two sites. The sticky cards were collected after 48 h (Rebek *et al.*, 2005). Cards were brought to the laboratory to isolate NEs collected (Plate 9C). The cards were examined under a dissecting microscope to record NEs (Sutherland *et al.*, 2001), and then the insects were removed from the traps using soft and thin forceps (Rebek *et al.*, 2005). The insects were preserved in 95 to 99% ethanol.

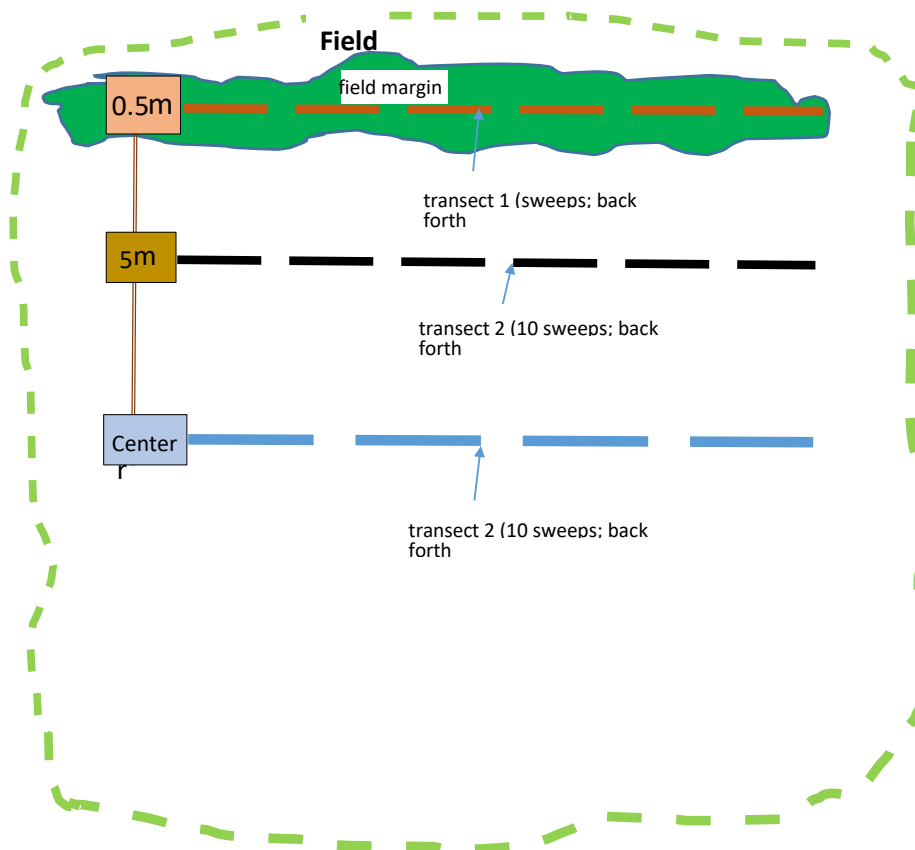


Figure 1: Sweep netting sampling approach used in the field. Three parallel sweep transects were walked, in the margin, near the margin, and in the centre of the crop

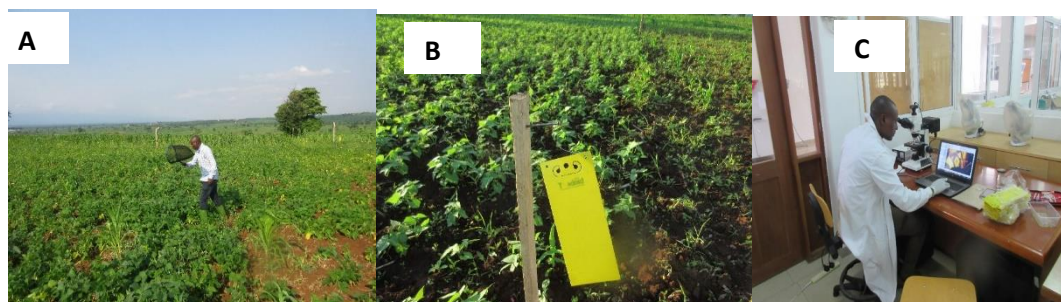


Plate 9: A) Sweep netting B) Sticky trapping C) Identification of NEs Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania

(i) Estimation of aphid severity

The severity of *A. fabae* infestation (Plate 10) was estimated using a visual rating of 1–6, where: 1=no aphids; 2=1–100 aphids; 3=101–300 aphids; 4=301–600; 5=601–1000 and 6>1000 aphids as used previously by Nabirye *et al.* (2003) from ten randomly selected bean plants in each field weekly throughout the crop development stages.



Plate 10: *Aphis fabae* infestation. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania

(ii) *Maruca vitrata* sampling

During the flowering and fruiting stages of bean crop development, *M. vitrata* larvae were sampled from bean plots randomly from 20 pods and 20 flowers/leaves with the sign of infestation (presence of webbing and excreta) and handpicking in each field (Baoua *et al.*, 2011; Jayasinghe *et al.*, 2015; Paulraj & Ignacimuthu, 2008; Traore *et al.*, 2014; Unmole, 2009).

3.2.2 Detection of *A. fabae* from Predators

Predators of *A. fabae* were collected from experimental field sites, including ladybird larvae (collected from the field margin and feeding on *A. fabae* in the crop), lacewing larvae, adult ladybirds, and assassin bugs. A DNA was extracted from the thorax of smaller insects (lacewing larvae, ladybird larvae) and the gut of larger insects (ladybird adults, assassin bugs) using the Chelex method and DNA was amplified using the LCO/HCO primers described in 3.1.2b and the Aph-fab-S106/Aph-fab-A105 primers (Traugott & Symondson, 2008). LCO/HCO primers amplified all insect DNA and were used to check that the DNA extraction had been successful, Aph-fab-S106/Aph-fab-A105 primers amplify *A. fabae* DNA. DNA was then visualized using gel electrophoresis.

Specimens were identified to the highest level of resolution possible but focused on characterising them by life history and functional groups. The Pentatomidae, Carabidae and Syrphidae were categorised into known predators and analysed only these data.

The GLM procedure assuming Poisson distribution with log link function was used to compare the number of NEs (dependent variable) among high and low-diversity fields, location in the fields, months and cropping systems (explanatory variables) (Tena *et al.*, 2015). The Shapiro-Wilk test was used to check for normality were by the data were not normal distributed. Pairwise comparisons were done with the Holm multiple comparisons test in (RStudio Version 1.2.1335) (Lenth *et al.*, 2018).

3.3 To evaluate the role of different field margin plant species on the survival and reproductive success of *A. fabae* parasitoids

Aphidius colemani adults were obtained from *Aphis fabae* mummies collected from bean fields. *A. colemani* was selected for the cage trial because it has been reported as a primary parasitoid of *A. fabae* in SSA (Mkenda *et al.*, 2019b). Moreover, this species is commercially produced for the biological control of many aphid species (Fernandez & Nentwig, 1997; Starý, 2002). They were reared on potted bean plants infested with *A. fabae* in a wooden netted cage 30×30×60 cm (Plate 11). The plants were watered every three days. The *A. fabae* colonies were established from insects collected from farmers' fields at Kwa Sadala village, the location of the field trials. Bean seeds were grown in pots, and then after five weeks, they were infested with 60 *A. fabae* (nymphs and apterous adults) (Adisu *et al.*, 2002; Mkenda *et al.*, 2019b). The seeds from four field margin weeds (*T. minuta*, *H. suaveolens*, *E. heterophylla* and *B. pilosa*) were germinated in pots. The plants were selected because of their benefits to NEs in SSA (Adda *et al.*, 2011; Arnold *et al.*, 2021; Ojija *et al.*, 2019). The experiment consisted of six treatments (*T. minuta*, *H. suaveolens*, *E. heterophylla*, *B. pilosa*, positive control, and negative control), and each treatment was replicated four times (Fig. 2). Each cage contained one of these treatments with a potted bean plant infested with *A. fabae* (Plate 12). The positive control contained a 10% sugar solution (glucose) as often as needed (Dannon *et al.*, 2010b) and a potted bean plant infested with *A. fabae*, while the negative control had only a potted bean plant infested with *A. fabae*. After leaving the aphids in the cage for 24 h to acclimatize, four female and two male parasitoids were introduced to each cage. For the first seven days, the number of live parasitoids and mummies was counted daily to determine the survival of the first generation. Following this, the counting was performed three times a week. The number of

parasitoids that emerged from mummies was recorded. The experiment was carried out for one entire lifecycle of parasitoids (approximately one month). The parasitoids and aphids were maintained under controlled conditions, with an average temperature of 25–27°C, 66–68% R.H., and under natural lighting.

The number of *A. colemani* surviving in cage trials was analyzed over six days using the Kaplan–Meier estimator of survival in R (RStudio Version 1.2.1335) (Kassambara *et al.*, 2017). Pairwise comparisons between treatments were then performed using a log-rank test with Benjamini–Hochberg correction. To assess the parasitism by *A. colemani*, the number of mummies in each cage was analyzed using ANOVA. The normality was assessed using the Shapiro–Wilk test (Charles & Paine, 2016).

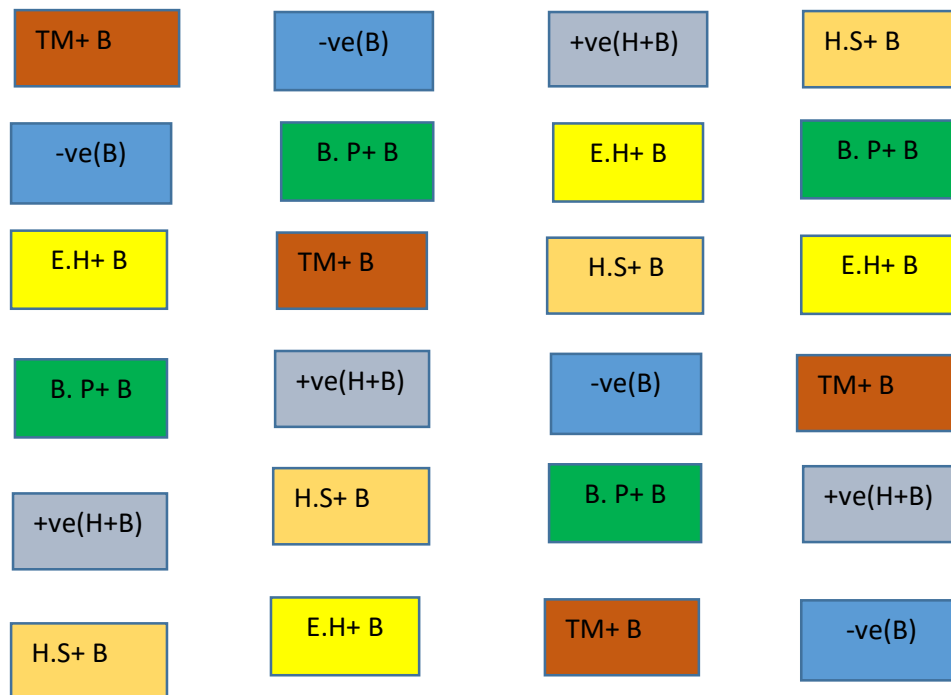


Figure 2: Cage trial experiment where T.M-*Tagetes minuta*; E.H-*Euphorbia heterophylla*; B.P-*Bidens pilosa*; H.S-*Hyptis suaveolens*



Plate 11: Cages used in the experiment. Photo by Ndakidemi B. J, NM-AIST, Arusha, Tanzania



A) *Hyptis suaveolens*



B) *Tagetes minuta*



C) *Bidens pilosa*



D) *Euphorbia heterophylla*

Plate 12: Field margin flowering plants caged with potted bean plants. Photo by Ndakidemi B. J, NM-AIST, Arusha, Tanzania.

3.4 To investigate the field margin plants that support NEs in field crops

Three plant species, *T. minuta*, *B. pilosa*, and *P. hysterothorus*, were cultivated as the field margin. The mentioned plants, whereas they frequently occur in SSA, have previously reported associations with beneficial insects (Adda *et al.*, 2011; Arnold *et al.*, 2021; Ojija *et al.*, 2019) and are therefore straightforward for smallholders to acquire (Hillocks, 1998; Yogesh *et al.*, 2019). The experimental layout was composed of four treatment plots containing common bean (*Phaseolus vulgaris*) with four replications, 0.5 m field margin plants surrounded three plots in each replication, each plot with the specific field margin plant, and the remaining plot was the control without field margin plants: The plots were 15×15 m, with a distance of 15 m between plots (Fig. 3). During the flowering period of beans in the fifth week, powdered UV fluorescent dye (Baker Ross Ltd., Harlow, UK) (Plates 13A, B, C) was applied to the field margin plant flowers using a soft paintbrush. After 24 h, NEs were collected inside the field using sweep nets and then examined using a UV torch (365 nm; UVGear, UK) to detect any fluorescent dye. This allowed the identification of insects that had visited the field margin plants before being caught in the crop fields to indicate the potential value of different species to different NEs. Pan traps were also used to collect NEs in the field margins (Plates 13D) (Mkenda *et al.*, 2019c), two pan traps were placed in the field margin of each plot and NEs were sampled for three months (April-June), coinciding with bean development stages. The collections were preserved in 70% ethanol for further identification.

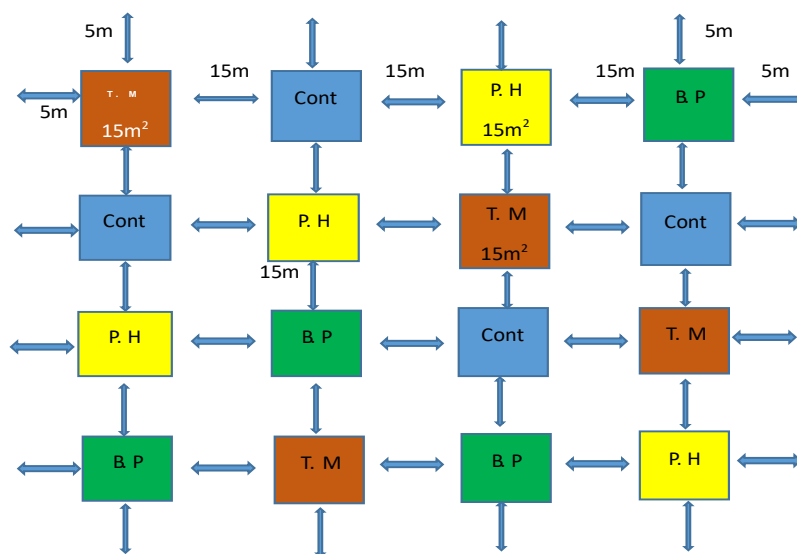


Figure 3: Field margin plants surrounding bean experimental plots for the fluorescent dye trial: T.M-*Tagetes minuta*; Cont-Control; P.H-*Parthenium hysterothorus*; B.P-*Biden pilosa*)



A) *Tagetes minuta*



B) *Parthenium hysterophorus*



C) *Biden pilosa*



D) Control without field margin plants

Plate 13: Field margin plants surrounding bean experimental plots for the fluorescent dye trial. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania

To analyze the number of insects labelled with the fluorescent dye and those captured in the field margin, GLM assuming Poisson distribution with a log link was used, followed by pairwise comparisons and a Holm multiple comparisons test. The normality was assessed using the Shapiro–Wilk test. The GLM with Poisson distribution was selected and this was because there was no normality in our data.

To Identify Floral Field Margin Plants Available Outside the Main Bean Growing Season to Support NEs

This field trial was carried out at Kwa Sadala Village in Hai District, Kilimanjaro, the location of the field trials. Eight sites with a high and eight with a low diversity margin were visited.

The high and low diversity fields were determined by measuring the plant diversity in each farm before selecting the sites described in 3.1.2. A transect walk along one margin, for the length of 60 m, was performed (Plate 14), and the visual observation of the NEs' visits to the specific plant flowers was recorded. The sampling (interactions between natural enemy groups and plant species) were recorded monthly for 12 months (for a year) during transect walks, and this coincided with specific bean crop development stages (Plate 15). Sweep netting was also used to collect NEs monthly for 12 months consecutively, along the transect and each sweep with one replicate per site per visit, sweeping back and forth ten times.

A heatmap in RStudio Version 1.2.1335, constructed an interaction network using the package 'bipartite' (Dormann *et al.*, 2009). This package was also used to calculate the nestedness and connectance of the network. Insects caught in field margins using pan traps were grouped into functional categories of NEs; catch distributions were checked for normality using a Shapiro–Wilk test. GLM with Poisson distribution (RStudio Version 1.2.1335) was selected after this. The GLM assuming Poisson distribution was selected because the data were not normally distributed. The month of sampling and field margin plant species were included in the GLM as covariates without interactions. Pairwise comparisons were performed with the Holm multiple comparisons test in the 'emmeans' package (Length, 2019).



Plate 14: Transects along the field margin. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania



A) *Bidens pilosa*



B) *Lantana camara*



C) *Sphaeranthus suaveolens*



D) *Euphorbia heterophylla*



E) *Ageratum conyzoides*

Plate 15: The NEs interacting with different field margin plants. Photo by Ndakidemi B. J, NM-AIST, Arusha, Tanzania

3.5 To establish which non-pest aphids are existing on non-crop plants in margin out of season and if they support the parasitoids found in the crop

A detailed survey of eight field margins outside the bean cropping season was done, mapping the presence of non-pest aphids in non-crop plants (Plate 16). Sites were visited, 5-10 leaves per plant per species were inspected and aphids were collected and recorded. The mummies from these populations were brought to the laboratory for rearing. The emerging species were identified to establish overlap with crop pest aphid species if they are alternative hosts supporting reservoirs of parasitoids.



A) *Bidens pilosa*

B) *Commellina benghalensis*

C) *Ageratum conyzoides*

Plate 16: Non-pest aphids and mummies in different field margin plants. Photo by Ndakidemi B. J, NM-AIST, Arusha, Tanzania

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Assessment of the status of the key parasitoids of black bean aphids (*Aphis fabae*) and legume pod borer (*Maruca vitrata*) in bean fields

(i) The NEs of black bean aphids (*Aphis fabae*)

Thirty-six (36) parasitoids were identified to species level, 4 to genus, and 67 to family (Fig. 4).

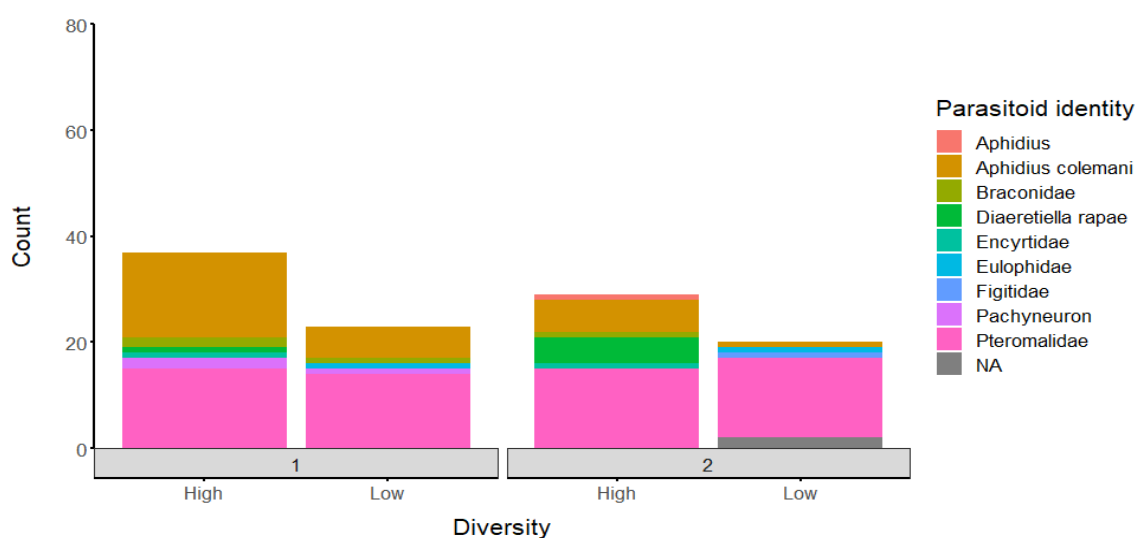


Figure 4: The number of parasitoids that emerged from *Aphis fabae* on sentinel plants in Tanzania. Sentinel plants were placed in fields with either high or low field margin diversity. NA shows where sequencing was unsuccessful or no close (>90%) sequence match was obtained

(ii) Parasitoids of legume pod borer (*Maruca vitrata*)

Sentinel plants infested with *M. vitrata* larvae or eggs were also deployed. The identity of these parasitoids is shown in Figs. 5 and 6. There were significant differences between the number of egg parasitoids that emerged from sentinel plants (Fig. 7) in plots with high or low diversity field margins ($P= 0.001$) and significant differences between the number of parasitoids that emerged from sentinel plants deployed in the centre or margin of the crop ($P= 0.001$). Similarly, field margin diversity ($P=0.005$) and the location of the sentinel plants ($P=0.008$)

had significant effects on the number of *M. vitrata* larval parasitoids (Plate 17) that emerged from sentinel plants (Fig. 8).

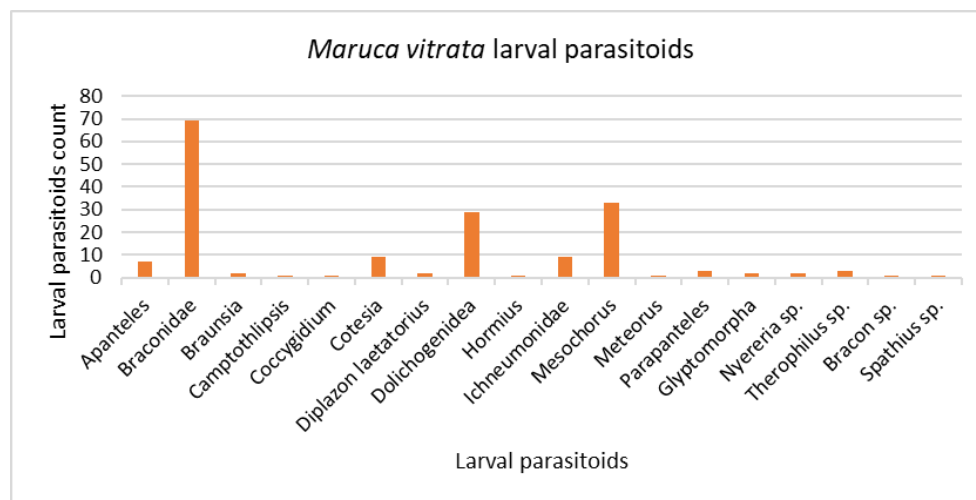


Figure 5: Parasitoids emerged from sentinel plants infested with *M. vitrata* larvae

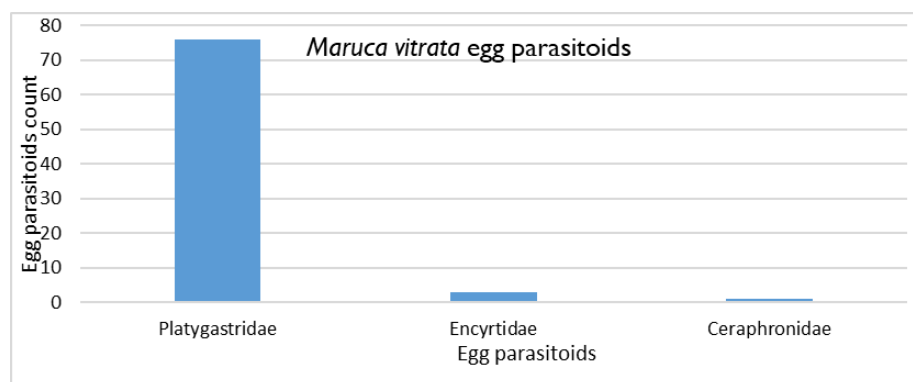


Figure 6: Parasitoids emerged from *M. vitrata* eggs on sentinel plants

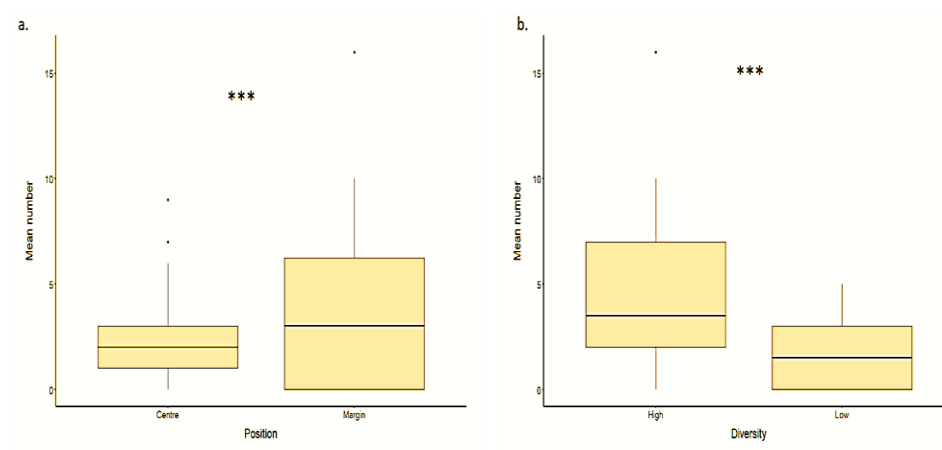


Figure 7: The mean number of *M. vitrata* egg parasitoids that emerged from sentinel plants a) positioned in the centre or at the margin of the bean crop and b) In a field with high or low diversity margins. Significant differences are indicated by * and were calculated using a GLM in RStudio

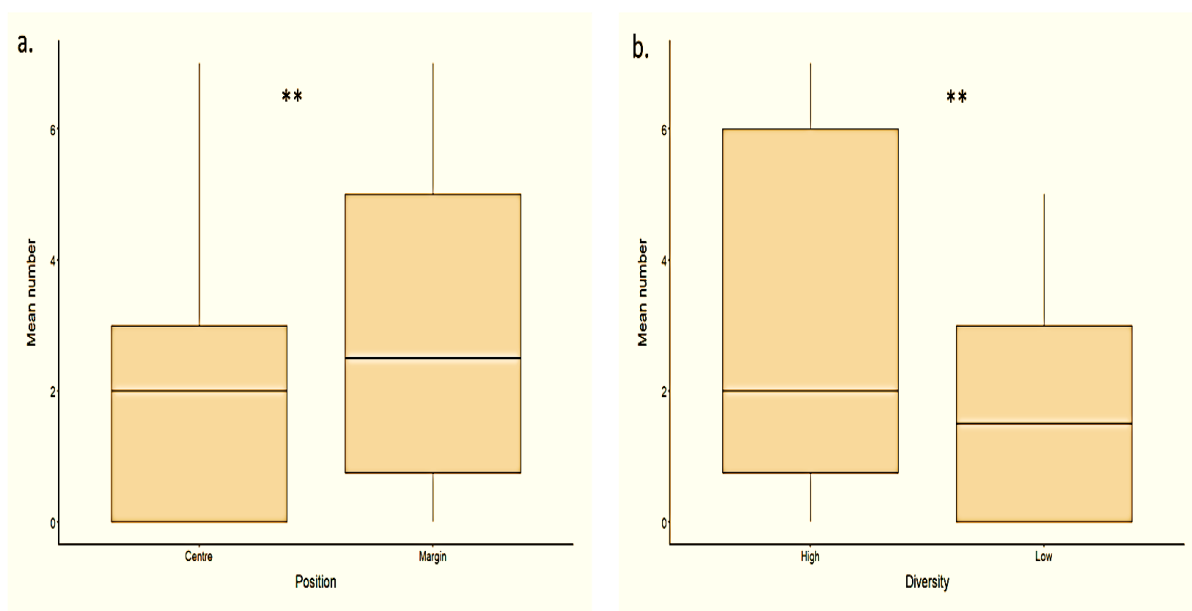


Figure 8: The mean number of *M. vitrata* larval parasitoids that emerged from sentinel plants a) positioned in the centre or at the margin of the bean crop b) In the fields with high or low diversity margins. Significant differences are indicated by * and were calculated using a GLM in RStudio



Plate 17: *Maruca vitrata* larva parasitoids. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania

4.1.2 To investigate the effects of field margins on the temporal-spatial distribution of NEs

(i) Distribution of NEs in bean fields

Insects collected were categorized into taxonomic groups: parasitic wasps (Hymenoptera: Ichneumonidae and Braconidae) including *Aphidius* spp., predatory bugs (Hemiptera: Reduviidae and known predatory Pentatomidae), lady beetles (Coleoptera: Coccinellidae)

including *Cheilomenes lunata*; lacewings (Neuroptera: Chrysopidae) including *Chrysoperla congrua*; parasitic flies (specifically Diptera: Tachinidae), hoverflies (Diptera: known aphidophagous (Syrphinae) Syrphidae only, and excluding Eristalini species with aquatic larvae), predatory flies (specifically Diptera: Dolichopodidae and Asilidae with predatory adults), and all other predatory beetles (Coleoptera: known predatory Carabidae, Lycidae and Staphylinidae) (Plate 18).

With sticky trapping, the parasitic wasps were the most abundant taxa, while with the sweep netting, the most abundant taxa were the predatory flies. The high-diversity fields had a significantly higher number of lady beetles, predatory flies, hoverflies, predatory bugs, parasitic flies, other predatory beetles ($P=0.001$) and lacewings ($P=0.005$) caught through sticky trapping used to monitor the field margins NEs than the fields with low diversity. No significant differences were observed in the number of parasitic wasps between high and low-diversity fields (Fig. 9A; Appendix 1).

The NEs populations differed throughout the experiment regarding catches of parasitic wasps ($P=0.004$) and syrphid flies ($P=0.031$) by the sticky trapping. There were no differences in the number of lady beetles, predatory flies, parasitic flies and other predatory beetles in different months. Parasitic wasps and syrphid flies were significantly more numerous in the flowering stage of the crop ($P=0.001$; $P=0.032$), fruiting stage and early maturity stages of the crop ($P=0.005$; 0.008) and late maturity stage of the crop ($P=0.009$; 0.018) than in the late seedling and vegetative stages of the crop, respectively. Predatory bugs were significantly more frequent in the crop's fruiting and early maturity stages ($P=0.032$) than in the flowering stage of the crop (Fig. 10; Appendix 3). More parasitic wasps ($P=0.001$), lacewings ($P=0.006$), syrphid flies ($P=0.009$), parasitic flies ($P=0.001$) and predatory flies ($P=0.001$) were caught via sweep netting from high plant diversity fields compared to fields with low plant diversity in margins, but other insect taxa did not differ in abundance according to margin type (Fig. 9B; Appendix 2).

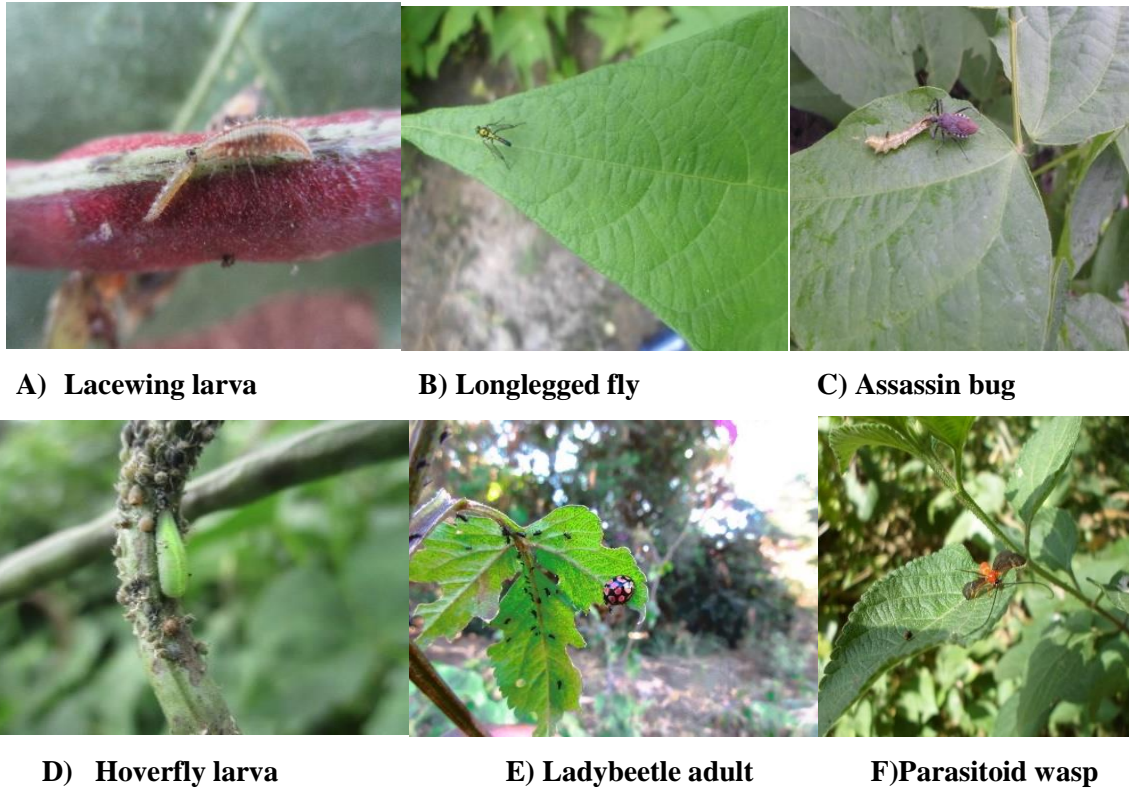


Plate 18: Natural enemies of bean pests. Photo by Ndakidemi B.J, NM-AIST, Arusha, Tanzania

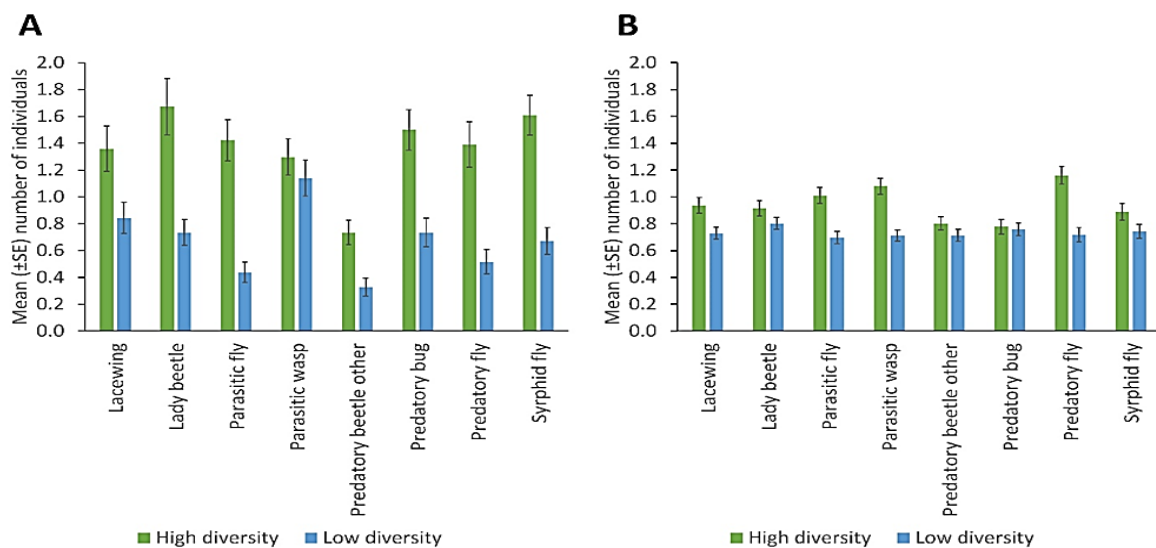


Figure 9: The NEs' abundance in fields with high and low plant diversity margins, collected by (a) sticky trapping and (b) sweep netting (Error bars = s.e.m.)

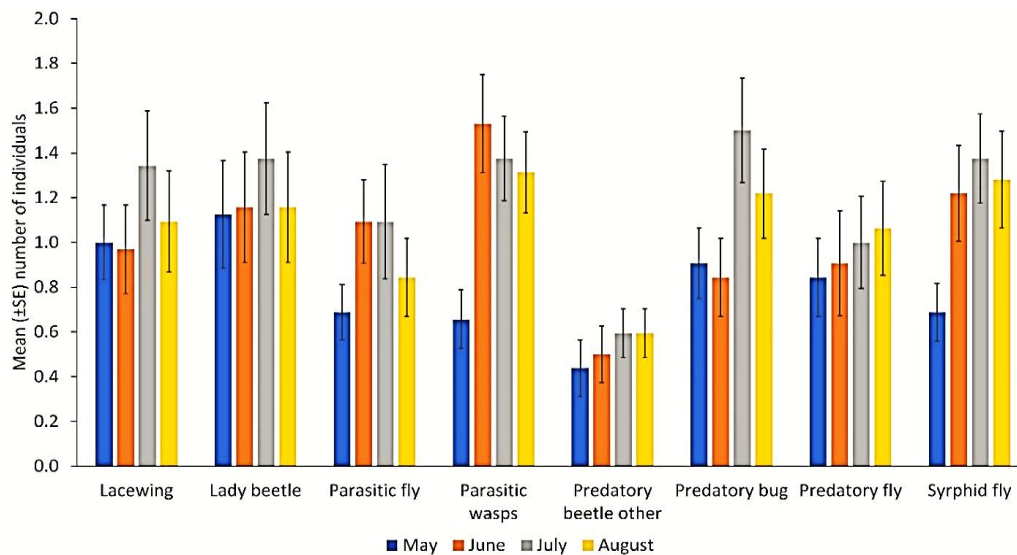


Figure 10: The NEs' distribution in different months collected by sticky trapping. (Error bars = s.e.m.)

There were more NEs in the margin relative to the crop edge (lacewings, $P= 0.046$; parasitic wasps, $P= 0.041$; predatory bugs, $P= 0.004$), except for syrphid flies, other predatory beetles, parasitic flies and predatory flies. Also, there were more insects in the margin relative to the centre of the field (parasitic wasps, $P= 0.001$; syrphid flies, $P= 0.002$; lacewings, $P=0.005$; other predatory beetles, $P= 0.043$) except for parasitic flies, predatory bugs, and predatory flies (Fig. 11). There were few consistent differences in the number of NEs within fields but consistently higher counts from high plant diversity fields were observed (other predatory beetles, $P=0.008$; parasitic wasps, $P=0.001$; predatory fly, $P=0.046$; syrphid fly, $P=0.001$) (Fig. 12).

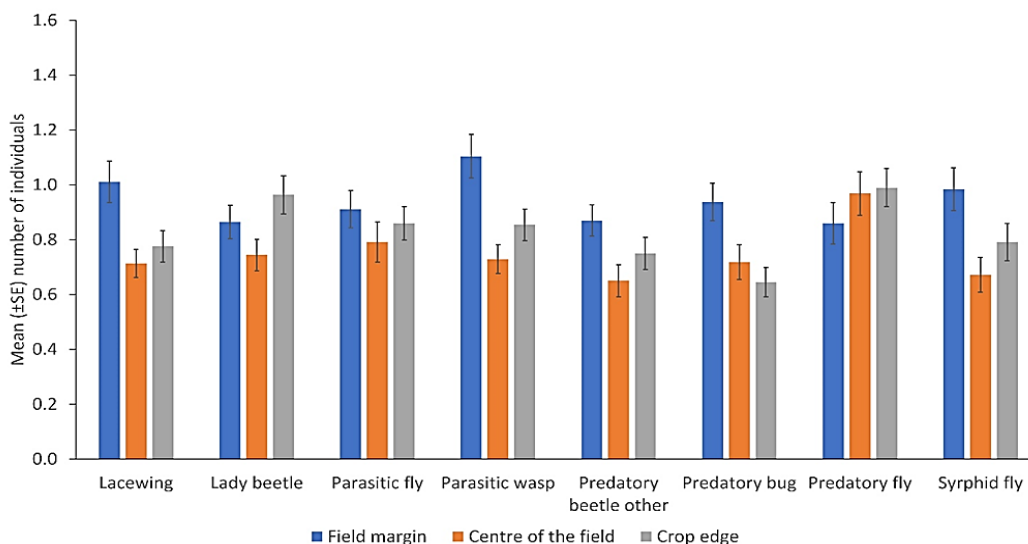


Figure 11: The number of NEs in different field locations collected by sweep netting (Error bars = s.e.m.)

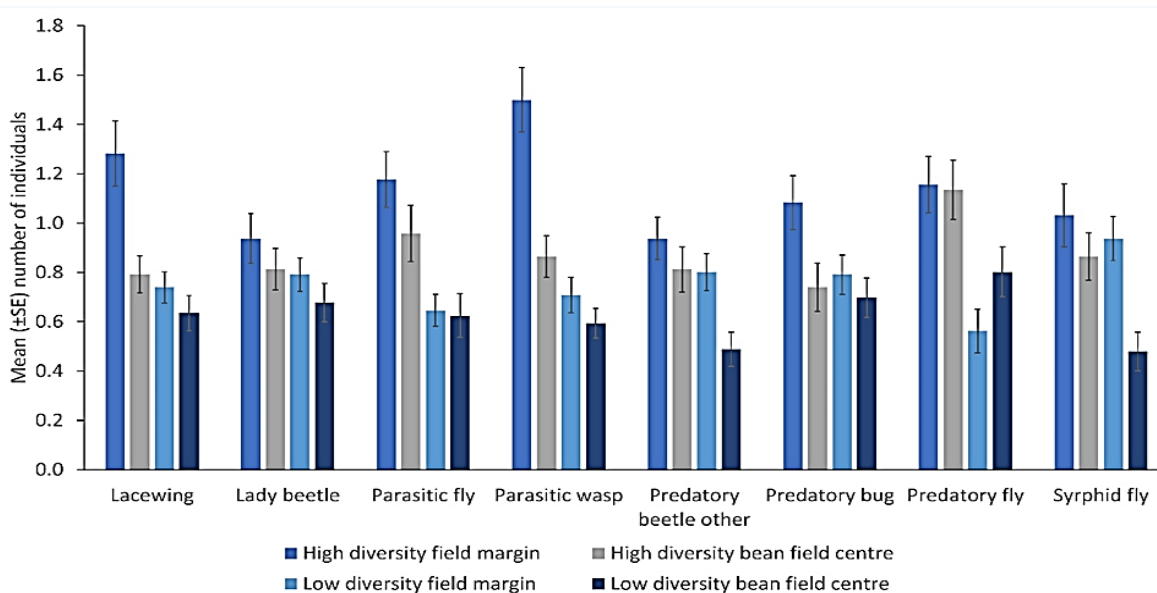


Figure 12: Number of NEs in the field margin and the centre of the fields from low and high plant diversity fields as sampled by sweep nets (Error bars = s.e.m.)

No significant differences were observed between cropping systems (monocropping versus intercropping) for both sticky trap and sweep netting collections (Fig. 13A&B).

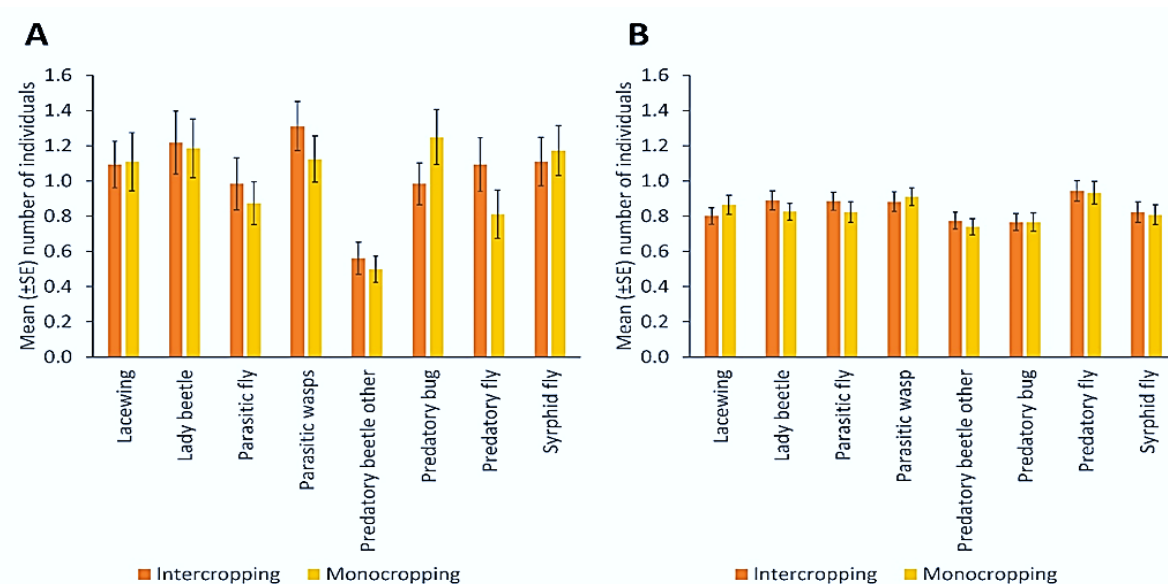


Figure 13: The number of NEs in bean monocropped and intercropped fields, collected by (a) sticky trapping and (b) sweep netting (Error bars = s.e.m.)

Maruca vitrata infestation

Maruca vitrata larvae were significantly numerous in the low diversity fields compared to high diversity fields ($F=21.560$; $df=1$; $P=0.001$) and also their numbers were significantly high in June compared to July ($F=48.280$; $df=1$; $P=0.001$) (Fig. 14).

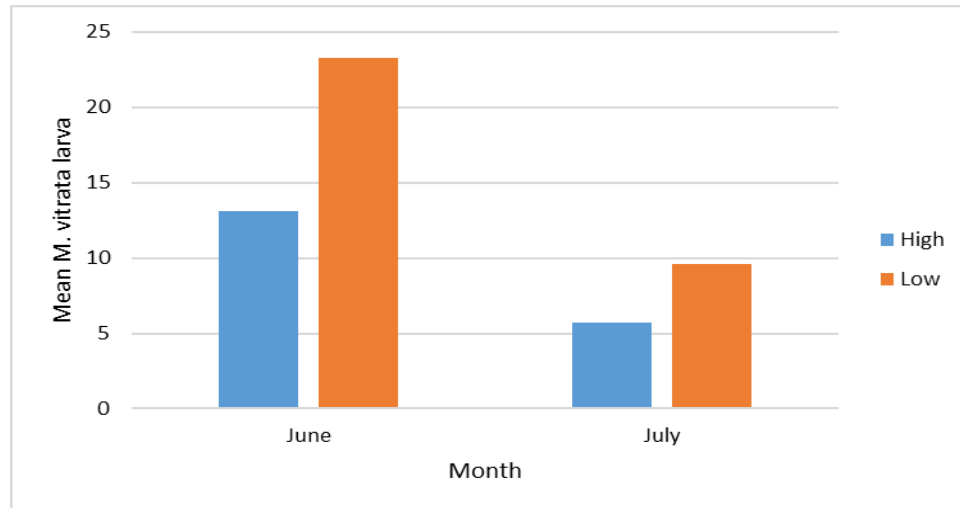


Figure 14: The number of *Maruca vitrata* larvae in the high and low diversity fields *Aphis fabae* severity

No significant differences were observed in the mean number of aphids (*A. fabae*) among different crop development stages and between high and low plant diversity fields. *Aphis fabae* population was high in the flowering stage compared to other crop developmental stages and in low fields compared to the high plant diversity fields (Fig. 15).

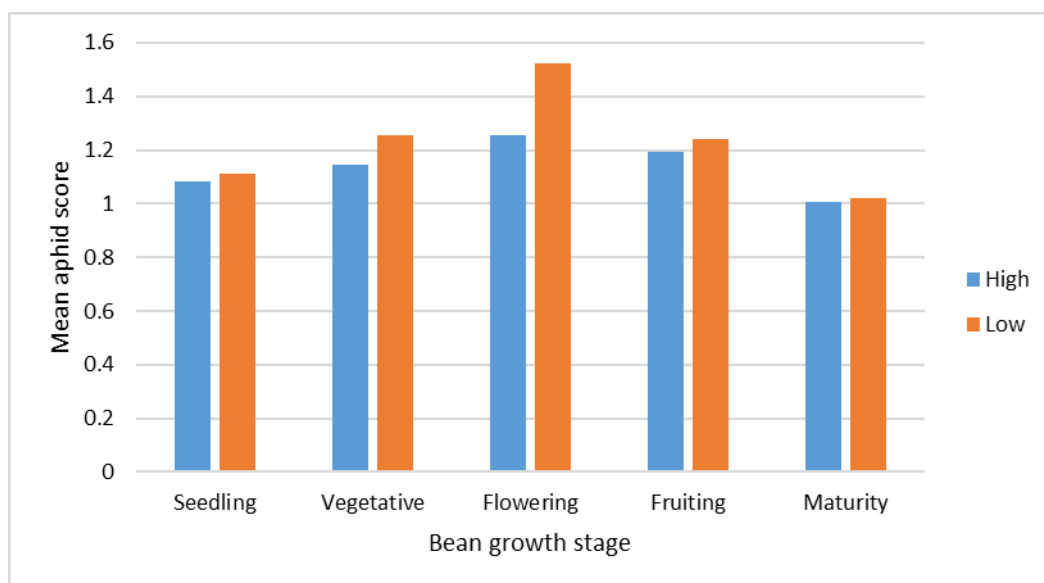


Figure 15: Aphid infestation score, according to margin plant diversity in different bean growth stages

(ii) Identifying gut contents of NEs

Aphis fabae DNA was successfully detected from the gut contents of NEs (Fig. 16). This confirms that detecting prey DNA from gut contents in NEs is possible in our study system.

The NEs were collected from field plots. DNA was successfully amplified and *A. fabae* DNA was detected from some specimens (Fig. 17A&B). Of the 29 lady beetle larvae analysed, 14 contained *A. fabae* DNA. Two lacewing larvae out of seven analysed contained *A. fabae* DNA and one assassin bug out of 20 contained *A. fabae* DNA. None of the adult ladybirds contained *A. fabae* DNA (Fig. 17B).

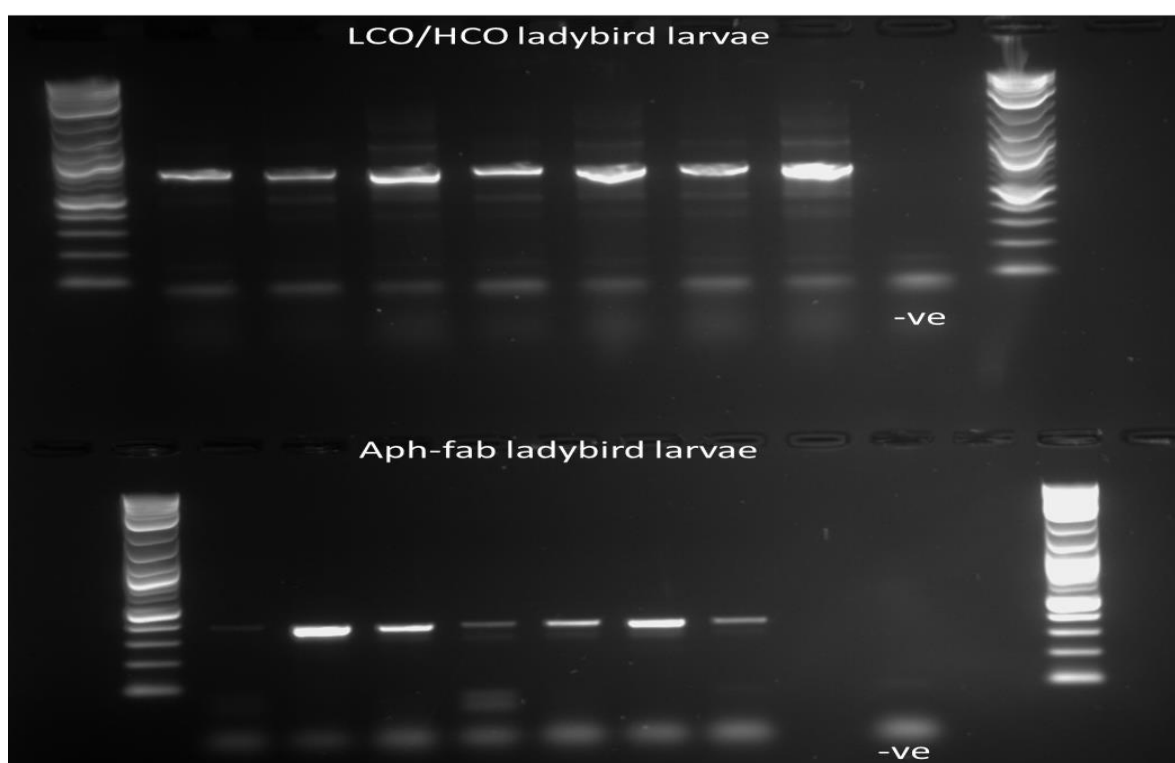
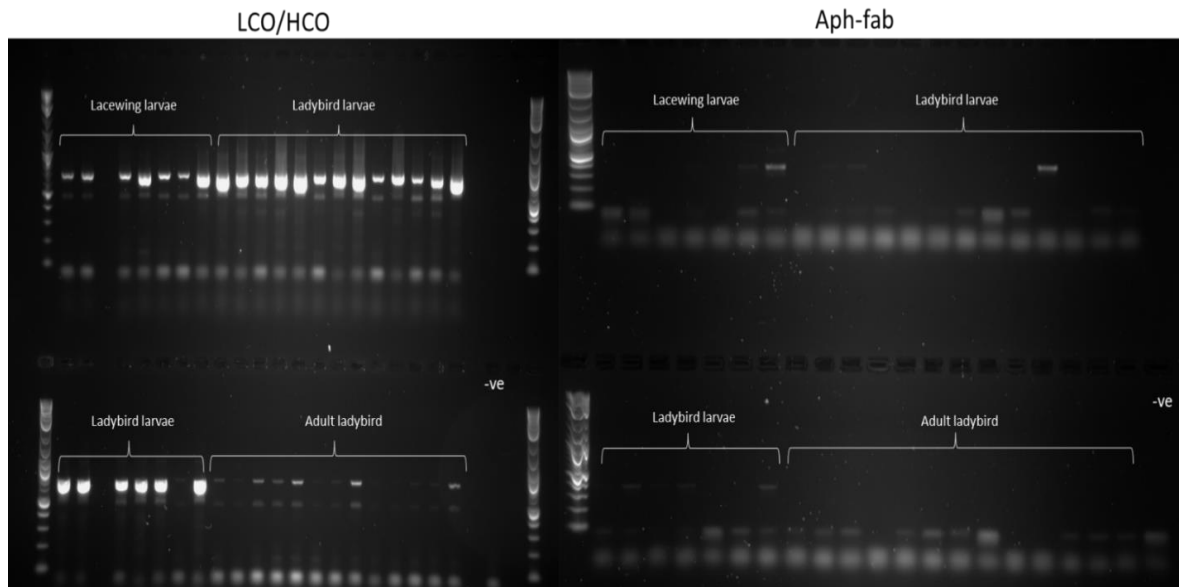


Figure 16: Agarose gel stained with GelRed visualised using a UV transilluminator. Bands show amplification of DNA extracted from ladybird larva feeding on *A. fabae* using HCO/LCO primers (upper row) and Aph-fab-S106/Aph-fab-A105 primers (lower row)

A)



B)

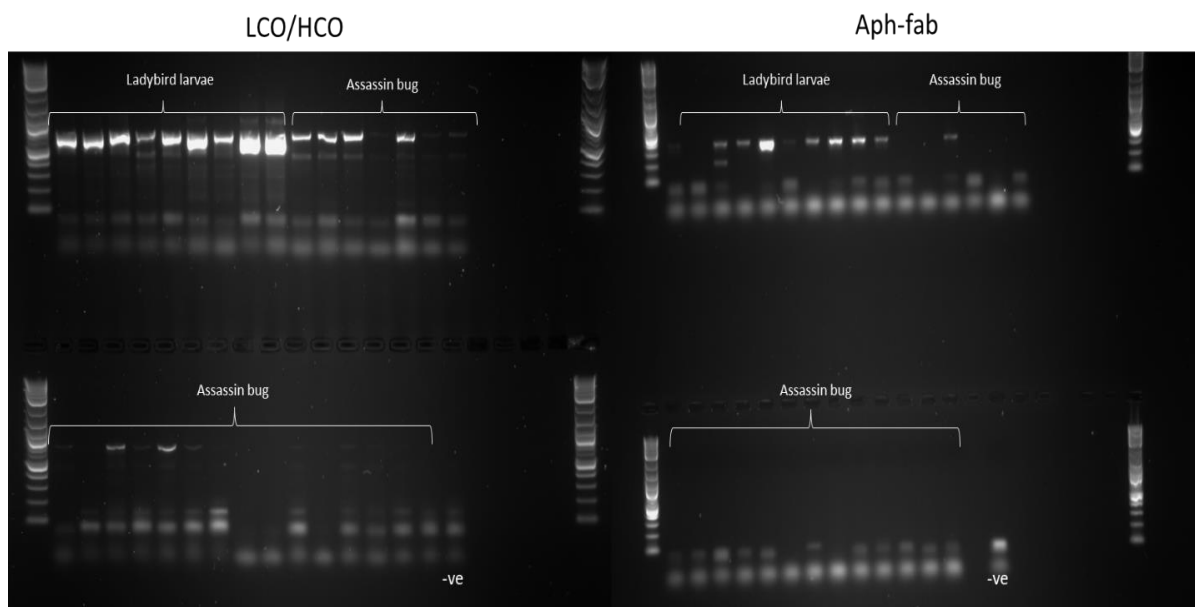


Figure 17: Agarose gels stained with GelRed visualised using a UV transilluminator. Bands show amplification of DNA extracted from NEs captured in the field, using HCO/LCO primers (left) and Aph-fab-S106/Aph-fab-A105 primers (right)

4.1.3 The influence of selected field margin plant species on the survival and reproductive success of parasitoids

When aphid-infested bean plants were caged with *A. colemani* parasitoids and a flowering margin companion plant species, the survival of *A. colemani* differed significantly among the different flower treatments ($P=0.001$; Fig. 18). The only floral resource plant that gave a

significantly different survival of *A. colemani* compared to other floral resource plants and the positive control was *E. heterophylla*. There was no significant difference $F_{4,161.126}$, $P=0.381$) between the number of mummies produced by *A. colemani* given access to floral resource plants or the positive control (Fig. 19).

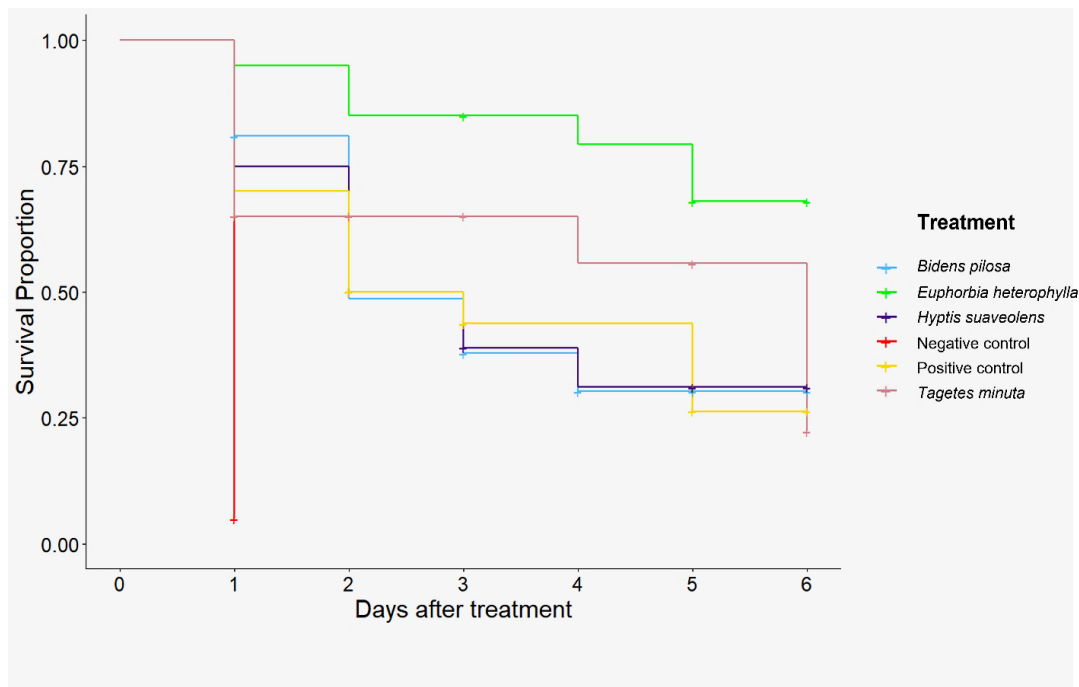


Figure 18: Survival of *A. colemani* when provided different field margin plant species, sugar water (positive control), or only water (negative control)

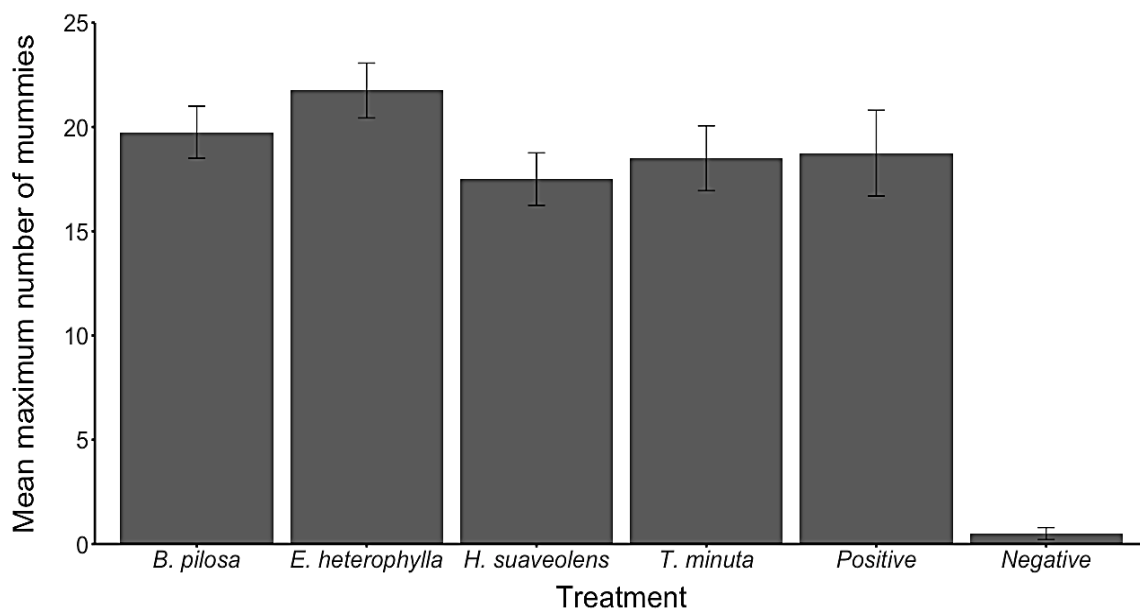


Figure 19: The mean number of *Aphis fabae* mummies produced per cage containing four females and two males of *Aphidius colemani*

4.1.4 To investigate the field margin plants that support NEs in field crops

The NEs collected include lady beetles, hoverflies, assassin bugs, lacewings, parasitoid wasps and long-legged flies. Lady beetles, hoverflies, assassin bugs, and lacewings differed in the number of fluorescent-labelled individuals captured in the crop, according to the surrounding plant species. Lady beetles, lacewings, and assassin bugs with fluorescent dye were particularly numerous in the *B. pilosa*-edged plots (and for lacewings, *T. minuta*), indicating that these species regularly used *B. pilosa* before moving into the crop. Conversely, hoverflies were more numerous in plots surrounded by dye-marked *P. hysterophorus* and *T. minuta*. Plots with *P. hysterophorus* margins were much less frequently used by lady beetles, lacewings, and assassin bugs (Table 8).

The number of NEs caught in pan traps in field margins also varied significantly depending on which field margin plant was present, following similar patterns to the crop. Again, *B. pilosa* plots favoured lady beetles, assassin bugs, lacewings, and parasitoid wasps within the margins, while *T. minuta* and *P. hysterophorus* favoured hoverflies in the margins (Table 9).

Table 8: Mean \pm (SEM) numbers of NEs labelled with UV fluorescent powder within bean crops surrounded by different field margin plant

Treatment	Mean Number of NEs (\pm SEM)					
	Lady Beetle	Hoverfly	Assassin Bug	Lacewing	Parasitoid Wasp	Long-legged Fly
<i>B. pilosa</i>	9.50 \pm 2.02 a	2.50 \pm 0.65 b	5.25 \pm 0.95 a	6.50 \pm 1.04 a	2.00 \pm 0.82 a	1.50 \pm 0.87 a
Control	2.50 \pm 1.04 b	5.50 \pm 2.26 ab	0.75 \pm 0.48 b	2.00 \pm 0.41 b	1.75 \pm 0.63 a	1.75 \pm 0.48 a
<i>P. hysterophorus</i>	4.25 \pm 0.85 b	7.25 \pm 0.48 a	2.25 \pm 0.48 b	2.00 \pm 0.82 b	2.25 \pm 0.75 a	1.75 \pm 0.48 a
<i>T. minuta</i>	6.25 \pm 1.03 ab	8.25 \pm 0.85 a	0.75 \pm 0.48 b	7.00 \pm 1.47 a	3.25 \pm 1.32 a	2.75 \pm 0.48 a

Values followed by the same letters (a and b) within the column are not significantly different ($p < 0.05$).

Table 9: The mean \pm (SEM) number of NEs caught in pan traps in field plots with different field margin plants

Treatment	Mean Number of NEs (\pm SEM)					
	Lady Beetle	Hoverfly	Assassin Bug	Lacewing	Parasitoid Wasp	Long-legged Fly
Control (no plant)	1.83 (\pm 0.63) a	1.92 (\pm 0.54) a	1.25 (\pm 0.70) a	1.50 (\pm 0.86) a	1.75 (\pm 0.49) a	1.75 (\pm 0.63) a
<i>B. pilosa</i>	5.92 (\pm 1.05) b	2.67 (\pm 0.77) ab	3.75 (\pm 0.35) b	3.50 (\pm 0.42) b	5.31 (\pm 1.53) b	1.67 (\pm 0.45) a
<i>P. hysterophorus</i>	2.17 (\pm 1.95) a	4.50 (\pm 0.82) b	2.42 (\pm 0.78) ab	2.42 (\pm 0.86) ab	3.23 (\pm 0.93) b	1.08 (\pm 0.34) a
<i>T. minuta</i>	3.33 (\pm 0.88) a	4.58 (\pm 0.83) b	3.42 (\pm 1.23) b	3.58 (\pm 0.93) b	4.58 (\pm 1.32) b	0.75 (0.22) a

Values followed by the same letters (a and b) within the column are not significantly different ($p < 0.05$).

4.1.5 Identifying floral resource plants and flowers available during the off-season to support NEs

This field trial surveyed the interactions of the field margin plant species with NE in bean fields. When observing NE-plant interactions on transect walks on bean fields, most insect groups investigated had interactions with most species of plant investigated. Overall, 5597 NE-plant interactions were observed, and the greatest number of interactions were recorded with the margin plant *B. pilosa* (861 interactions), followed by *E. heterophylla* (631). Parasitoid wasps were the NE group with the greatest number of observed interactions with field margin plants (724 interactions) (Figs. 20&21)

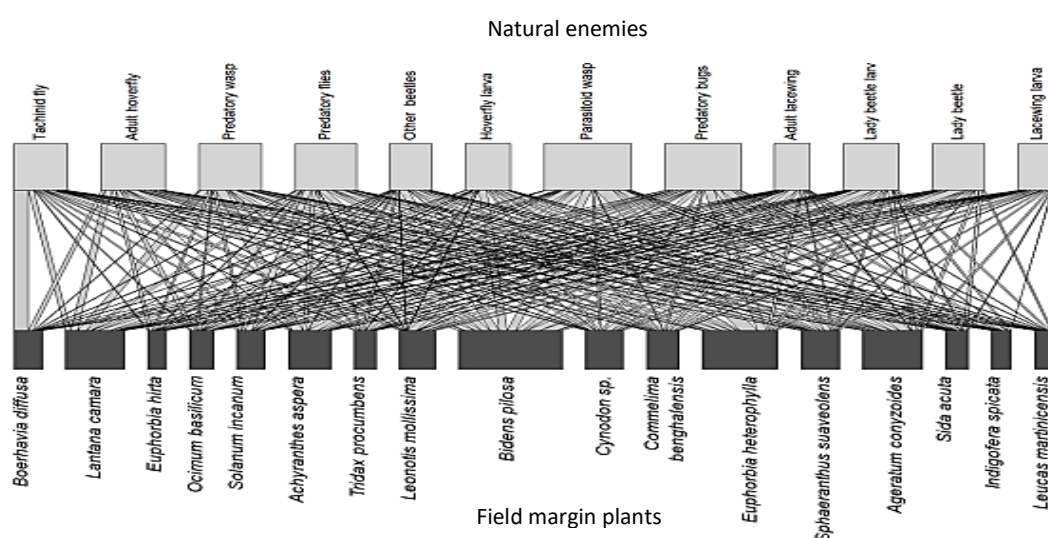


Figure 20: Interactions of NEs with field margin plants observed during transect walks in margins of bean fields over 12 months (throughout the year) through visual observation. The lower row shows plant species present, and the upper row shows the natural enemy guilds; the width of the linking bars indicates the frequency of the interactions observed

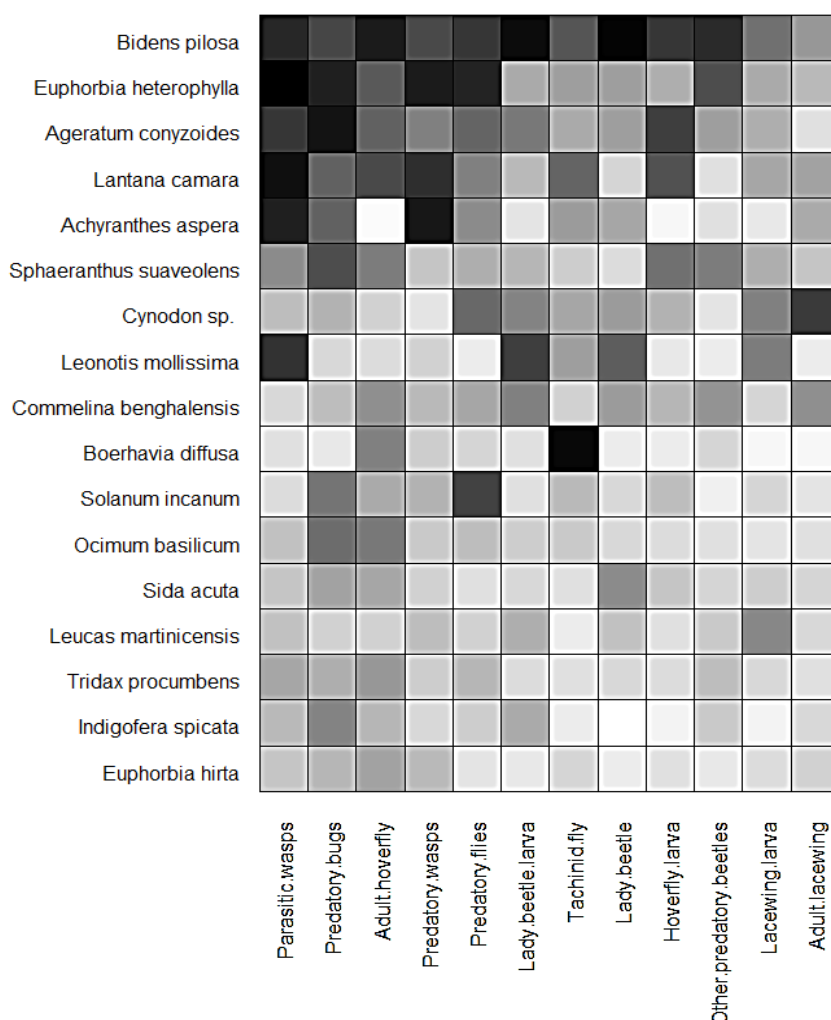


Figure 21: Heatmap showing the interactions between plant species and natural enemy groups in Tanzania. Interactions were observed during transect walks in bean field margins monthly over 12 months. Darker squares indicate a higher number of observed interactions

The NEs were sampled using sweep netting from fields with either high or low diversity margins (Fig. 22). This sampling was done monthly for 12 months. Significantly more ladybird adults, predatory wasps, predatory bugs, parasitoid wasps, predatory flies and tachinid flies were caught in high-diversity field sites compared to low-diversity sites with P values of 0.004, 0.008, 0.001, 0.001, 0.001 and 0.001 respectively. All NEs except lacewing larvae had significant variation in the number of individuals caught each month, with the greatest number of NEs caught in month three after planting the crop.

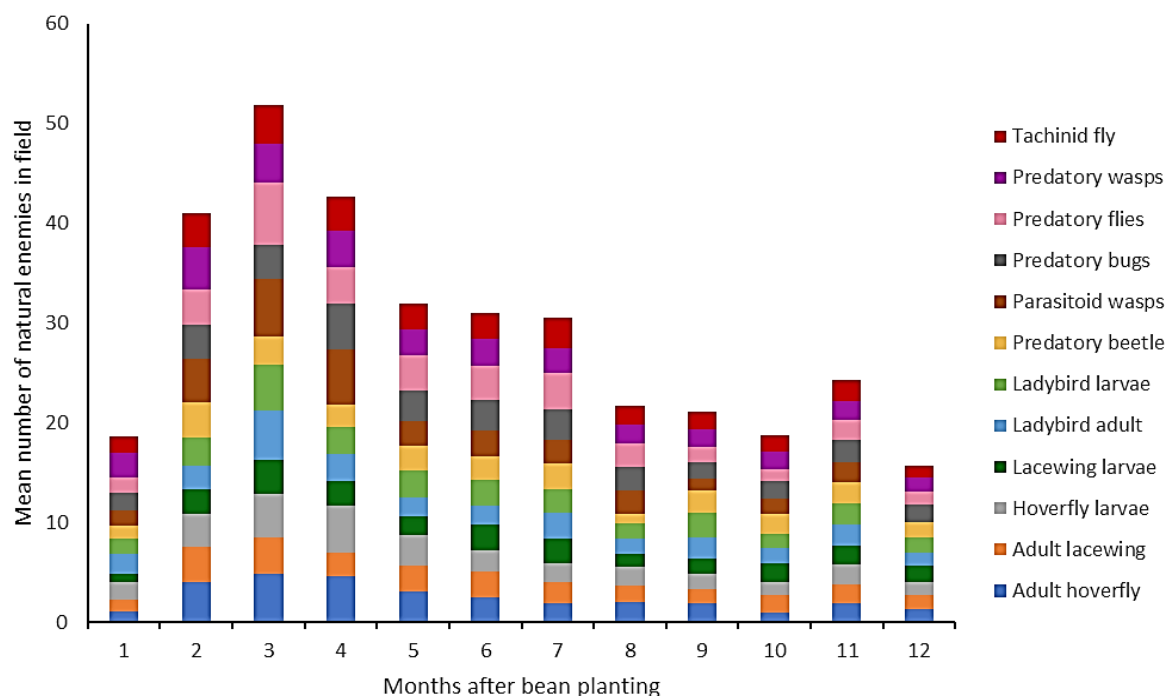


Figure 22: The mean number of NEs caught by sweep netting in bean fields between 1 and 12 months

The flowering plants that supported NEs during offseason include *B. pilosa*, *Achyranthes aspera*, *Sphaeranthus suaveolens*, *Sida acuta*, *Leucas martinicensis*, *Ocimum basilicum*, *Tridax procumbens*, *Solanum incanum*, *E. heterophylla*, *L. camara*, *Boerhavia diffusa*, *Leonotis mollissima*, *Indigofera spicata*, *Euphorbia hirta*, *Ageratum conyzoides*, *Cynodon* sp., and *Commelina benghalensis*. A high number of NEs were associated with *B. pilosa*, *L. camara*, *E. heterophylla* and *A. conyzoides*. *B. pilosa* had a high number of NEs, followed by *L. camara*, *E. heterophylla* and *A. conyzoides* (Fig. 23).

The highest number of NEs was collected in the fifth month after the emergence of the bean crop, followed by the sixth month after the emergence. There was a decreasing trend in the number of NEs collected from the fifth month towards the tenth month and, after that, an increasing trend.

Significant differences in months after the emergence of beans on the abundance of NEs were observed for *B. pilosa* ($F=2.29$, $df=7$, $P=0.025$); *S. incanum* ($F=2.17$, $df=7$, $P=.034$); *E. heterophylla* ($F=6.10$, $df=7$, $P=0.001$); *L. camara* ($F=3.93$, $df=7$, $P=0.001$); *B. diffusa* ($F=3.21$, $df=7$, $P=0.002$); *L. mollissima* ($F=2.26$, $df=7$, $P=0.028$); *E. hirta* ($F=2.09$, $df=7$, $P=0.042$); *A. conyzoides* ($F=3.30$, $df=7$, $P=0.002$).

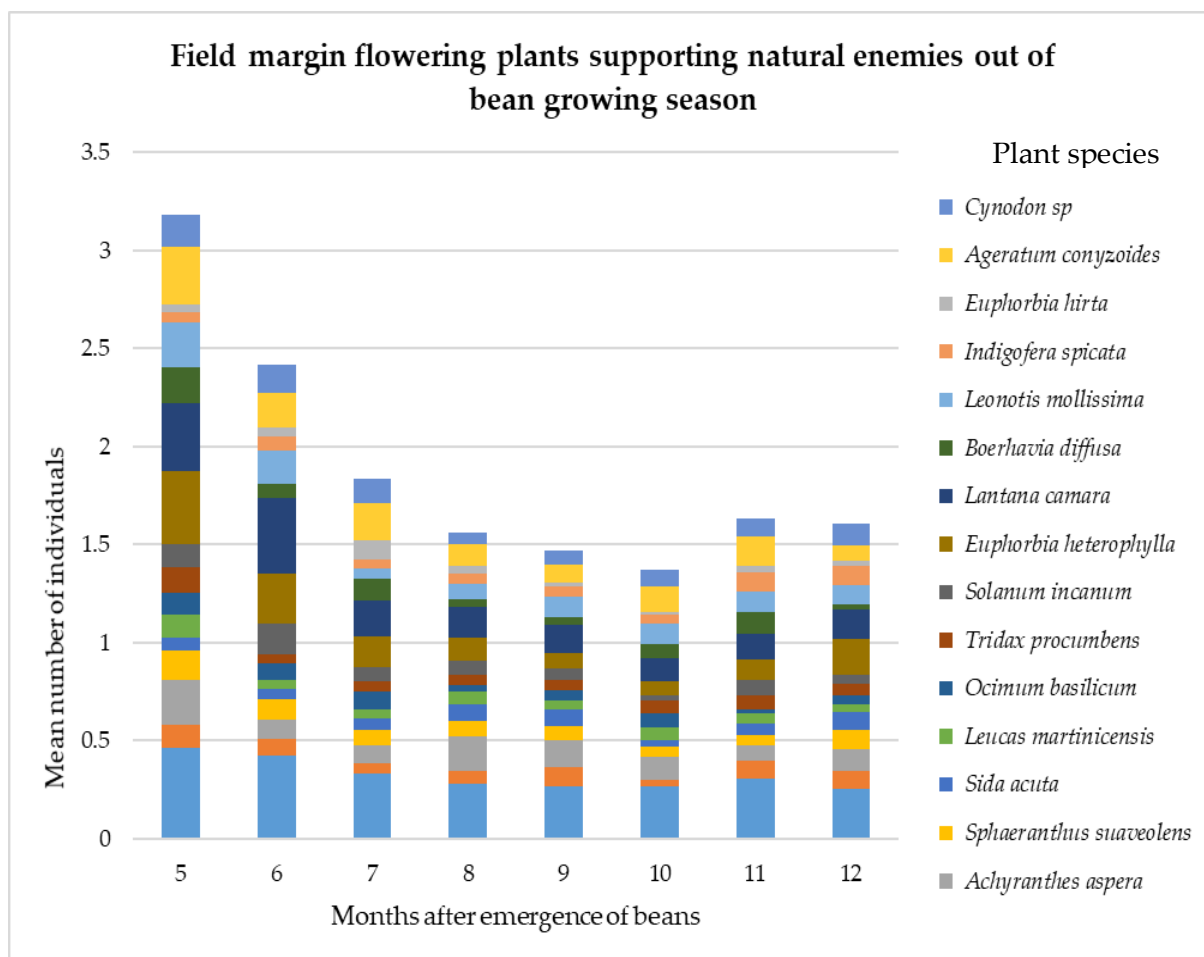


Figure 23: The mean number of NEs caught by sweep netting in bean fields between 5 and 12 months (offseason sampling)

4.1.6 To establish which non-pest aphids are existing on non-crop plants in margins outside of the cropping season and the parasitoids they support

(i) Aphids from field margin plants

The following aphid species were identified from field margin plants: *Uroleucon* sp., *Cryptomyzus ribis*, *Aphis coreopsidis*, *Hyperomyzus carduellinus*, *Uroleucon sonchi*, *Aphis* sp., *Aphis celastrii* and *Sitobion avenae*.

(ii) Parasitoids from non-pest aphids

Parasitoids from non-pest aphids collected from field margin plants were identified by DNA barcoding as explained in 3.1.2, and all sequences were >99.70% similar to *A. colemani* sequences from BOLD. Therefore, they are most likely to be *A. colemani*. The abundance of parasitoids (*A. colemani*) identified was affected significantly by the time of sampling;

$F=52.118$, $df=2$; $P=0.001$. A significantly high number of parasitoids emerged in August compared to September and October ($P=0.001$). No significant differences were observed between September and October (Fig. 24).

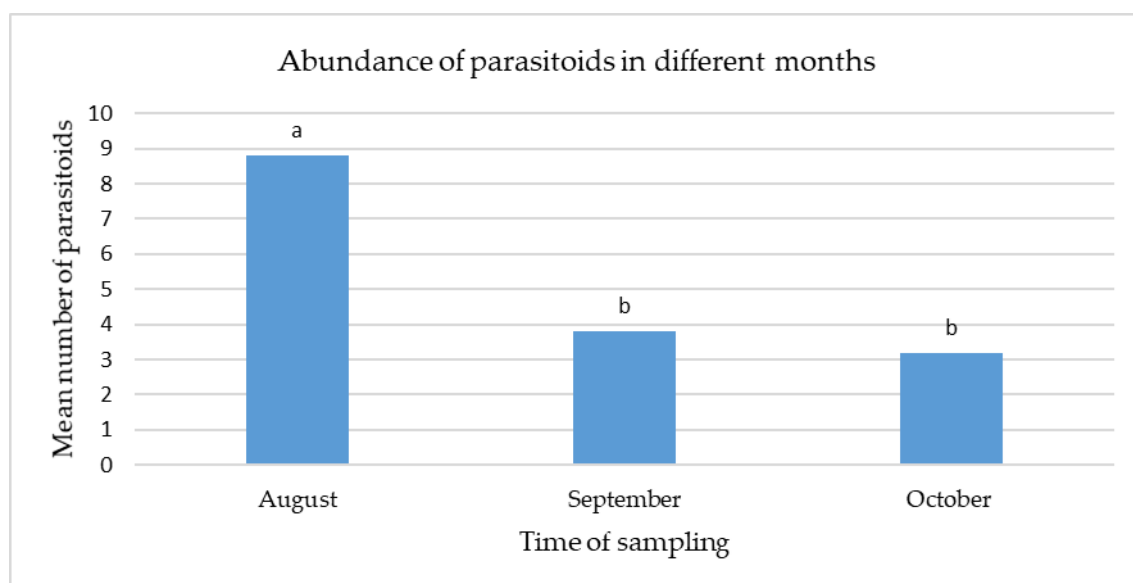


Figure 24: The mean \pm (SEM) number of parasitoids that emerged from aphids at different sampling times

4.2 Discussion

Maruca vitrata and *Aphis fabae* are pests that account to yield losses of 15-53% and 37-90%, respectively (Abate, 2000; De Lima, 1983; Karel & Autrique, 1989; Karel, 1985; Swaine, 1969). *Aphis fabae* also transmits plant diseases, including bean common mosaic necrosis virus (BCMNV), bean common mosaic virus (BCMV) and cucumber mosaic virus (CMV) (Wamonje *et al.*, 2020). The use of biological control agents such as parasitoids is known to be a more sustainable and environmentally friendly strategy (Arodokoun, 1996; Arodokoun *et al.*, 2006; Boivin *et al.*, 2012; Dannon *et al.*, 2012; Huang *et al.*, 2003; Van-Halteren, 1997).

Aphidius colemani was identified as an important parasitoid for the control of *A. fabae*. *Aphidius colemani* is a hymenopteran polyphagous solitary endoparasitoid that belongs to the family braconidae (Charles & Paine, 2016). This is commercially produced for the biological control of many aphid pests (Fernandez & Nentwig, 1997; Starý, 2002). It has been reported that *A. colemani* parasitises more than 40 aphid species (Fernández & Nentwig, 1997; Messing & Rabasse, 1995). Various host aphid species have been reported for *A. colemani*. The species are the Russian wheat aphid, *Diuraphis noxia*; greenbug aphid, *Schizaphis graminum*; bird cherry-oat aphid, *Rhopalosiphum padi*; corn leaf aphid, *Rhopalosiphum maidis*; Oleander

aphid, *Aphis nerii*; cotton aphid, *Aphis gossypii*; cabbage aphid, *Brevicoryne brassicae*; turnip aphid, *Lipaphis erysimi* and soybean aphid, *Aphis helianthi* (Elliott *et al.*, 1994). In Tanzania, *A. colemani* has been an important agent in the biological control of the black bean aphid (*Aphis fabae*) (Mkenda *et al.*, 2019b). The primary parasitoid *Diaeretiella rapae* was present in low numbers in high-diversity areas in this study *D. rapae* has a host range of at least 60 aphid species, but only 5-6 of these species are commonly attacked (Ayal, 1987; Baer *et al.*, 2004; Kant *et al.*, 2008). It is possible that *D. rapae* was isolated from a few sites near Brassica species (crops or wild species) infested with *B. brassicae*, and *A. fabae* was attacked as a secondary host species. *Aphidius platensis* was also present in low numbers and is associated with the green peach aphid, *Myzus persicae* as its host (Khaki *et al.*, 2022), while *Lipolexis oregmae* has been associated with melon/cotton aphid (*Aphis gossypii*) as its hosts (Akhtar *et al.* 2011). The Braconidae also emerged from sentinel plants in this study. These did not show sequence similarity with any known aphid parasitoids and may result from contamination of the sentinel plant with a second pest species.

Secondary parasitoids from sentinel aphids were identified, with a high proportion at both high and low-diversity sites. Identifying most of these secondary parasitoids at the family level was only possible, the most common being Pteromalidae. This family is one of the most important groups of secondary aphid parasitoids, they typically parasitize hosts that are already mummified and as a result, they are often polyphagous (Müller & Godfray, 1999). A small number of *Pachyneuron* sp., a member of the family Pteromalidae, were identified at the genus level. Species in this genus have been reported to be secondary parasitoids of *A. fabae* and *Aphidius* sp. (Hübner, 2001; Nagasaka *et al.*, 2010).

Several parasitoids of *M. vitrata* were identified, with the Braconidae and Dolichogenidea being the dominant group of parasitoids. Hymenopteran *M. vitrata* larval parasitoids have been identified in SSA, which include *Apanteles* spp., *Pristomerus* sp., *Bassus bruesi*, *Chelonus* sp., *Bracon* sp., *Braunsia kriegeri*, *Testudobracon* sp., *Cadurcia* sp., *Phanerotoma leucobasis*, *Phanerotoma syleptae* and *Dolichogenidea* spp. (Arodokoun, 1996; Arodokoun *et al.*, 2006; Dannon *et al.*, 2010a; 2010b; Huang *et al.*, 2003; Okeyo-Owuor *et al.*, 1991; Van-Halteren, 1997). *Cotesia* sp. has also been identified as *M. vitrata* parasitoid (Shepard and Barrion, 1998). Other parasitoids identified in other studies were *Antrocephalus* sp. *Tetrastichus sesamiae*, *Braunsia* sp., *Bracon* sp. (Okeyo-Owuor *et al.*, 1991). *Mesochorus* spp., a group of ichneumonids (Hymenoptera: Ichneumonidae), was identified in our studies. These

hyperparasitoids will parasitise the pests only after parasitisation by the primary parasitoid (Wahl, 1993; Sullivan, 1987), so they have low potential as biological control agents.

Hymenopteran parasitoids from the family Platygasteridae were identified as egg parasitoids. The parasitoid has been reported to parasitize the eggs of other Crambidae (Can & Kamuran, 2016). Species from the family Platygasteridae are the egg parasitoids of several species of the order Lepidoptera which comprise a group of pests with global importance, including *Spodoptera frugiperda* and *Chilo partellus* (Cave, 2000; Cengiz *et al.*, 2016; Marquis *et al.*, 2019; Pomari-Fernandes *et al.*, 2015). The family comprises two subfamilies; Platygasterinae and Sceliotrachelinae (Austin *et al.*, 2005). Sceliotrachelines parasitise several immature stages of orders Coleoptera, Hymenoptera, and Hymenoptera (Chen *et al.* 2021), while Platygasterinae is a parasitoid of gall midges (Diptera, Cecidomyiidae) (Austin *et al.* 2005). Parasitoids from the families Ceraphronidae and Encyrtidae were also collected. The parasitoids from these families are hyperparasites (Polaszek & Lasalle, 1995) with less importance in biological control.

In most cases, the high-diversity fields had more parasitoids than the low-diversity fields. Also, the field margin margins had high numbers of parasitoids than the crop. The NEs benefit from non-crop plants in agricultural systems through the provision of shelter, nectar, and pollen for effective biological control (Arnold *et al.*, 2021; Kishinevsky *et al.*, 2017; Mkenda *et al.*, 2019a; 2019c). For instance, important NEs such as parasitoids use nectar to fulfil their nutrition requirements at some stages of their development. It has been found that populations of NEs in the field crops decline as the distance from the field margins increases and this demonstrates the essential function of field margins in maintaining NEs (Jacobsen *et al.*, 2022). Non-crop habitats such as field margins are important during crop senescence as NEs move from field crops for resources (Rand *et al.*, 2006).

The classic assumptions of conservation biological control are that flowering plants and an abundance of non-crop habitat near a crop will enhance populations of NEs, that the NEs will move into the crop from this habitat, and that those NEs will eat or parasitise pests of the crop, resulting in better pest management and ultimately reduced yield losses. To test these assumptions, smallholder farms with different levels of plant diversity in field margins in terms of the populations of NEs they support were compared and then evaluated whether those predatory NEs controlled key pests within the cropping area.

The NEs collected in bean fields included predatory bugs, lacewings, predatory flies, parasitic flies, parasitic wasps, lady beetles, and diverse predatory beetles. Per the expectations of conservation biocontrol theories, plant diversity in field margins positively impacted the number of NEs. Notably, this showed up more in the sticky trap data than in the sweep netting data. This suggests that either method alone may not give an accurate indication of NEs; sticky traps may oversample volant insects relative to non-volant insects (including larvae), indicating that whether or not populations were higher in the rich margins, flight activity (implying perhaps movement within the crop) may benefit from richer margins. In no cases did the rich margins reduce populations of any NE taxa.

Our findings that field margins promote NE activity and populations concur with other studies on smallholder farms and studies such as Arnold *et al.* (2021) and Mkenda *et al.* (2019c), which found a strong association between flower strips and plant-rich patches with NE communities. As reported by Rebek *et al.* (2005), we found that parasitic wasps were the most abundant of the groups studied and that highly mobile individuals such as parasitic flies, syrphid flies, parasitic flies and lady beetles were caught in large numbers by sticky cards (Rebek *et al.*, 2005). With the yellow sticky traps, different insect behaviours might have affected the number of NEs caught (Sutherland *et al.*, 2001). For example, yellow traps are more likely to trap Hymenoptera and Diptera, whereas blue is favoured by Thysanoptera (Pobozniak *et al.*, 2020; Tang *et al.*, 2016). There was no significant difference in the number of parasitic wasps collected through sticky trapping, and no significant differences were observed in the number of lady beetles, predatory bugs, and other predatory beetles collected through sweep netting between high and low plant diversity fields. These NEs might have been influenced by other factors like the host (aphids) in the field crop (Barbosa, 1998). Other between-site differences in NE populations, even where the plant abundance was similar, may be explained by wider differences in field management (Martin *et al.*, 2019). Disturbances, such as pesticide applications and cutting, impact the activities of NEs and could affect prey populations for NEs (Vandereycken *et al.*, 2015).

Higher numbers of parasitic wasps (from both sweep netting and sticky trapping), syrphid flies (sweep netting), syrphid flies (sticky trapping), and predatory bugs in July mainly corresponded to the bean flowering stage and changes in the frequency of catching of lacewings (sweep netting) might be due to the biotic and abiotic factors contributing to seasonal dynamics in arthropod abundance (Khodeir *et al.*, 2020; Pinheiro *et al.*, 2002). NE communities respond to

environmental factors differently (Kataira & Kumar, 2017). The variations may also be explained by changing prevailing environmental conditions, for example, an increase in floral resources towards the flowering stage of the bean crop. Our work adds to existing research findings showing that a high abundance of NEs is associated with the provision of floral resources from plants (Bommarco *et al.*, 2012; Khodeir *et al.*, 2020; Lundgren, 2009a; 2009b; Narbona & Dirzo, 2010; Ramsden *et al.*, 2014; Rebek *et al.*, 2005; Tallamy *et al.*, 2004). Including a mixture of plants in agricultural systems can provide varied and complementary resources that play specific roles to NEs (Parolin *et al.*, 2012). NEs depend on other local and landscape characteristics such as fertilizer and pesticide application, crop rotations, tillage practices and composition of the field surroundings (Bakker *et al.*, 2022; Jacobsen *et al.*, 2022; Muneret *et al.*, 2019; Redlich *et al.*, 2018). Some studies have shown the harmful effects of synthetic pesticide application on the NEs of pests. Thus field margins can be used to mitigate the adverse effects of insecticides on populations of NEs (Araya *et al.*, 2010; Bakker *et al.*, 2021; Golmohammadi *et al.*, 2021; Liu & Chen, 2001; Mkenda *et al.*, 2015; Mkindi *et al.*, 2017; Schneider *et al.*, 2004; Shoeb, 2010; Tembo *et al.*, 2018; Thomson *et al.*, 2001). Lethal and non-lethal effects such as mortality and feeding deterrents on NEs have been associated with the application of synthetic pesticides (Araya *et al.*, 2010; Golmohammadi *et al.*, 2021; Liu & Chen, 2001; Schneider *et al.*, 2004; Shoeb, 2010; Thomson *et al.*, 2001). Generally, there was an increase in mean aphid populations around the flowering stage of the bean crop and this might have corresponded to the availability of quality host plants. However, no significant differences were observed among different crop stages. High populations of aphids have been observed in the flowering stage by Azimi and Amini (2015). The study by Birch (1985) found the lowest *A. fabae* populations in the crop maturity stage, probably due to older plants that are lower quality hosts and increased predation/parasitism by NEs. The survival and reproduction of aphids depend on high-quality hosts for food sources (Karley *et al.*, 2004).

Then high numbers of *M. vibrata* in low diversity fields compared to the high diversity fields was possibly due to the presence of NEs (e.g. predatory bugs) in the plant-rich field margins, which might have moved into the field crop in search of prey or hosts and thus leading to the decrease in their numbers in high diversity fields. The flowering field margins have positive effects in sustaining populations of beneficial insects for effective biological control of insect pests (Rand & Tscharntke, 2006; Sanchez *et al.*, 2003). The low numbers in July compared with June also might be associated with high predation rates by predatory bugs as their numbers

were also high in July. Also, these might be associated with low-quality host plants towards July (Karley *et al.*, 2004).

For most insect taxa, consistently higher numbers in the margin relative to the crop were observed. This agreed with most other studies, showing limited movement into the crop of insects with margin-based communities (Caballero-López *et al.*, 2012; Clem & Harmon-Threatt, 2021; Lavandero *et al.*, 2006; Mkenda *et al.*, 2019a; 2019c; Nilsson *et al.*, 2021; Samanarayake & Costamagna, 2019). However, a few taxa also occurred in high numbers in the centre of the field, including other predatory beetles, parasitic wasps, predatory flies and syrphid flies; as these readily enter the crop, they could be an ideal focus for future biocontrol research.

There was a subtle effect of intercropping versus monocropping on the natural enemy numbers. While overall populations were higher in intercropped systems, no individual taxon was more abundant in intercropped fields. Parasitic wasps, for instance, come out higher in intercrops on sticky traps, but their numbers are high in monocrops with sweep nets. Thus, with no consistent patterns, the effects of mono v intercropping were not significant. A few studies have found populations of NEs enhanced through intercropping (Azimi & Amini, 2015; Tiroesele *et al.*, 2019). However, based on our evidence intercropping alone as a method to support NE populations may not yield improved pest management benefits and needs to be combined with other agroecological interventions. The NEs have been associated with the field margins and non-crop habitats for resources such as pollen and nectar. Also, these habitats may offer alternative prey, corridors for their dispersal and places for overwintering and reproduction (Arnold *et al.*, 2021; Blaauw & Isaac, 2015; Clem & Harmon-Threatt, 2021; Dover, 2019; Li *et al.*, 2020; Marshall & Moonen, 2002; Mkenda *et al.*, 2019c; Pywell *et al.*, 2015; Rebek *et al.*, 2005). Thus, with habitat disturbances and loss due to agricultural intensification, field margins could play a key role in conserving NE communities and enhancing biological control of pests in bean fields, especially for resource-constrained smallholder farmers (Amoabeng *et al.*, 2014).

In addressing whether natural predators do consume the major crop pest, barcoding of gut extracts from different NEs to detect *A. fabae* DNA was used and found this in lady beetle and lacewing larvae and a few predatory bugs tested, but not in adult lady beetles. The advantage of this DNA approach is that it provides evidence of predation even if the behaviour is not recorded. It can be asserted that NE groups have the potential to control pests in fields. Two

of the groups sampled were in larval stages and thus will be relatively slow-moving and non-volant; this is an important consideration given that it was also demonstrated that numbers primarily build in the crop, as the farms may need additional interventions to encourage these insects to move in among crop plants where their services are most helpful.

The composition of plants for a field margin that effectively supports NEs may require different plant communities than pollinators. However, some plants may provide nectar and pollen to NEs and pollinators (Carrié *et al.*, 2012). The species that support NEs most effectively in East Africa are still poorly understood. Some common field margin species, such as *E. heterophylla* (Euphorbiaceae), *P. hyterophorus*, *B. pilosa*, *T. minuta* (Asteraceae), and *H. suaveolens* (Laminaceae), are invasive to SSA. However, their potential has been explored for pest control, pollination, and medicinal activities (Adda *et al.*, 2011; Arnold *et al.*, 2021; Hillocks, 1998; Mkenda *et al.*, 2019a; Ojija *et al.*, 2019). Our study aimed to determine which field margin plant species in SSA benefited NEs in smallholder bean farms.

This was demonstrated by using a cage trial experiment, which showed that all plants, i.e., *B. pilosa*, *H. suaveolens*, *T. minuta*, and *E. heterophylla*, resulted in improved *A. colemani* survival, and showed similar results to the positive control, which provided an in-cage carbohydrate food supply. Survival of parasitoids on *E. heterophylla* was greater than the positive control and all other plants, suggesting that this species provided a greater nutritional benefit to *A. colemani*. Our results concur with similar studies, showing that access to flowers prolongs the lifespan and increases parasitism by *A. colemani*. For instance, studies on *A. colemani* and *Diadegma insulare* have shown improved performance compared with controls both in the field and when caged with flowering plant species such as *Fagopyrum esculentum*, *Conium maculatum*, *Photinia × fraseri*, *Brassica kaber*, *Barbarea vulgaris*, *Salvia apiana*, *Ligustrum japonicum*, *L. camara*, *Eriogonum fasciculatum*, *Daucus carota*, and *Thlaspi arvense* (Charles & Paine, 2016; Idris & Grafius, 1995; Lee & Heimpel, 2008; Munir *et al.*, 2018).

One of the reasons that *B. pilosa*, *H. suaveolens* and *E. heterophylla* supported *A. colemani* survival during the cage trial could be the presence of extrafloral nectaries on these species (Díaz-Castelazo *et al.*, 2004; Hernandez *et al.*, 2013; Koptur *et al.*, 2010; Patt, 2018). Extrafloral nectaries are easily accessible, and the nectar composition differs from floral nectar and may be secreted differently (Mizell, 2001). Extrafloral nectar sugars are typically more concentrated than floral nectar and usually present in larger volumes and secreted for a more

extended period (Lu *et al.*, 2014; Mizell, 2001). Plants with extrafloral nectaries can be particularly important for NEs, as well as attractive to parasitoids (Patt & Rohrig, 2017) because these insects have mouthparts that are not suited to feeding on floral corollas; hence, they depend on plants with extrafloral or otherwise exposed flower nectaries (González-Teuber & Heij, 2009; Patt *et al.*, 1997; Wäckers *et al.*, 2008). Indeed, *E. heterophylla* produces extrafloral nectar up to fruit maturation, possibly providing food resources to attract NEs of seed and fruit pests (Patt, 2018).

Although *T. minuta* does not have extrafloral nectaries, it supported *A. colemani* survival in cage trials and plots. This concurs with the previous report that *T. minuta* increased the longevity of the egg parasitoid *Trichogramma minutum*, which enhanced the parasitism of the *Grapholita molesta* eggs (Shearer & Atanassoy, 2004). Other species of *Tagetes*, including *T. erecta*, increased the longevity of *Cyrtorhinus lividipennis*, a NE of rice brown planthopper (*Nilaparvata lugens*) (Zhu *et al.*, 2014). The extrafloral resources from the field margin plants could benefit NEs and support biological control. Incorporating those field margins with extrafloral resources could positively affect pest control in bean fields.

In station trials, it was found that plots surrounded by *B. pilosa* margins were used frequently by lady beetles, parasitoids, and assassin bugs; *T. minuta* field margins were associated with catches of hoverflies, assassin bugs, lacewings, and parasitoids; and *P. hysterothorus* only with higher numbers of hoverflies and parasitoids. Furthermore, NEs caught inside the field crops with fluorescent dye indicated that the insects visited the flowers (possibly consuming nectar and pollen) before moving into the crop, where they can provide pest control benefits. Relatively few long-legged flies and parasitoids were captured with the fluorescent dye, but this could be due to their small size rather than a lack of interaction with field margin species. Previous studies have shown the importance of *B. pilosa*, *T. minuta*, and *P. hysterothorus* in supporting NEs (Fonseca *et al.*, 2017; Grombone-Guaratini *et al.*, 2004; Ojija *et al.*, 2019; Ouispe *et al.*, 2017; Shearer & Atanassoy, 2004). Floral resources from non-crop habitats are expected to support NPR by NEs (Dufour, 2000; Gurr *et al.*, 2004; Heimoana *et al.*, 2017; Jervis *et al.*, 2004; Mitsunaga *et al.*, 2006; Rahat *et al.*, 2005; Shearer & Atanassoy, 2004). Thus, selecting suitable plants for NEs is an important component of agricultural landscaping, as some plants will be better at supporting NEs. For instance, providing adult hoverflies with floral resources can enhance biological control by their larvae (Hogg *et al.*, 2011; Sutherland

et al., 2001). Moreover, pollen from some plants is superior to others in enhancing the performance of NEs (Lu *et al.*, 2014).

More parasitoids were recorded in plots with field margin plants than in the control (without field margin plants). This could suggest that parasitoids are a NE group for which field margin plants are particularly important, providing carbohydrates, amino acids, and vitamins in nectar that enhance their pest-controlling activities and optimize their metabolism (Gurr *et al.*, 2017; Jonsson *et al.*, 2008; Wäckers *et al.*, 2005). Flowering plants provide food and shelter for NEs and can promote natural pest regulation in crops. Our study highlights the potential of field margin plants in supporting populations of NEs in smallholder farms. It shows that conservation biological control could promote NEs in these agroecosystems. Certain plant species appear to be preferred by different NE groups and provide different benefits. In addition, *B. pilosa*, *T. minuta*, and *P. hystrophorus* supported different groups of NEs when planted as a field margin. However, it is important to consider the wider implications of using these plants in conservation biological control. For example, *P. hystrophorus* is toxic (Patel, 2011; Ramos *et al.*, 2002), and other plants may be invasive to the area and present a challenge as weeds. Some field margin plant species might also provide food and shelter for specific pests, and therefore it is crucial to study the biology of the host plants and how they interact with pests.

This field trial surveyed the interactions of the field margin plant species with NE in bean fields. When observing NE-plant interactions on transect walks on bean fields, most insect groups investigated had interactions with most species of plant investigated. The highest number of NEs were observed interacting with *B. pilosa* and *E. heterophylla*. NE groups were shown to interact with the flowers of these field margin plants, suggesting that the provision of nectar and pollen supports them. The transect walk showed that NEs interact with multiple field margin plant species, although certain species had more interactions (*B. pilosa* and *E. heterophylla*). NEs depend on pollen and nectar from the plants, and plants provide alternative hosts in the absence of crops (Bianchi & Van-der-werf, 2004; Gurr *et al.*, 2017; Jonsson *et al.*, 2008; Landis *et al.*, 2000; Lundgren, 2009a; 2009b; Mitsunaga *et al.*, 2004; Tylianakis *et al.*, 2004; Wäckers *et al.*, 2005). Similar results were found in a recent study by Arnold *et al.* (2021) which showed that *B. pilosa* and *Euphorbia* sp. were preferred by NEs and pollinators in SSA. Using *B. pilosa* and *E. heterophylla* by NEs could indicate that they provide valuable food resources or habitats. The observed interactions of NEs with *E. heterophylla* concur with the

study by Patt (2018), showing that this species provided nectar for lady beetles (*Coelophora inaequalis*, *Cryptolaemus montrouzieri*, and *Harmonia axyridis*). Similarly, *B. pilosa* is effective in attracting populations of lady beetles (*Cycloneda sanguinea*) and hoverflies (*Pseudodoros* sp.) (Fonseca *et al.*, 2017; Grombone-Guaratini *et al.*, 2004). In addition, chemical cues from *B. pilosa* play a role in attracting NEs (Ouispe *et al.*, 2017).

It is possible to optimize the pest management contribution of NEs by managing field margin plants. However, to maximize this benefit, it is necessary to understand the specific advantages of each flowering plant to NE to improve natural pest regulation (NPR) and increase crop productivity. For instance, some flowers are tubular and lack nectaries and these are not suitable for NEs (Géneau *et al.*, 2012; Patt *et al.*, 1997; Sivinski *et al.*, 2011; Tschumi *et al.*, 2014; Wäckers *et al.*, 2012).

High-diversity fields had significantly more ladybird adults, predatory wasps, predatory bugs, parasitoids, predatory flies and tachinid flies than low-diversity sites. A high number of NEs were caught in month three after bean planting. This can be associated with the richness and diversity of floral resource base in high diversity fields and month three after bean planting. Non-crop habitats are rich in plant biodiversity such as field margins offer nectar, pollen, shelter and alternative hosts to NE communities, and thus provide support to enhance their populations and enhance sustainable agricultural benefits (Bommarco *et al.*, 2012; Khodeir *et al.*, 2020; Lundgren, 2009 a & b; Narbona & Dirzo, 2010; Ransden *et al.*, 2014; Rebek *et al.*, 2005; Tallamy *et al.*, 2004). The floral resources are important for the fecundity and longevity of parasitoids and predators of pests (Lavandero & Tylianakis, 2003). The presence of non-crop habitats surrounding or within arable land has been associated with increasing arthropod NEs of pests by providing floral resources and sustaining their populations (Chaplin-Kramer *et al.*, 2011; González *et al.*, 2022). For instance, spiders, lady beetles, rove beetles, syrphid flies, true bugs and lacewings use non-crop habitats to provide them refuge, alternative hosts, pupation and overwintering sites (Baggen & Gurr, 1998; Bianchi *et al.*, 2006; Chaplin-Kramer *et al.*, 2011; González *et al.*, 2022; Landis *et al.*, 2000; Long *et al.*, 1998; Liu *et al.*, 2022; Nicholls *et al.*, 2001; Olson & Wäckers, 2007; Pywell *et al.*, 2005; Thies & Tscharnkte, 1999; Thomson & Hoffman, 2009; Tylianakis *et al.*, 2004). However, floral resources provide different benefits to specific taxa of NEs (Wäckers, 2004) and thus NEs would respond differently to the proportions of non-crop habitats.

Thus, semi-natural habitats at a landscape scale offer benefits to NEs (Bartual *et al.*, 2019; Kovács *et al.*, 2019), while establishing non-crop habitats such as field margins will benefit NEs at a local scale (Tschumi *et al.*, 2016), to enhance the ecosystem service of biological control of pests in the agricultural fields. The off-season samples also highlighted the highest numbers of NEs associated with more NEs associated with *B. pilosa*, *L. camara*, *E. heterophylla* and *A. conyzoides*. A combination of colours and persistent blooming by *L. camara* might have contributed to attracting more NEs (Swarbrick *et al.*, 1998; Parsons *et al.*, 2001; Carrión-Tacuri *et al.*, 2012). It is reported that floral colour, morphology and blooming time affect how specific plants support NEs of pests in agricultural fields (Anderson & Dobson, 2003; Begum *et al.*, 2004). Also, *L. camara* phytochemical compounds such as α -humulene and β -caryophyllene might play a role in attracting NEs (Nelson & Jackson, 2013). The aromatic weed *A. conyzoides* produces several phytochemicals that include coumarins, alkaloids, resins, saponins, tannins, glycosides, and flavonoids (Amadi *et al.*, 2012; Kamboj & Saluja, 2008; Sultana *et al.*, 2012) and this might have contributed to attracting NEs. *A. conyzoides* and *L. camara* have attracted populations of adults *Coccinella* sp. (Ngatimin, 2020). The chemical cues from *B. pilosa* and the extra floral nectar from *E. heterophylla* are essential in attracting NEs (Koptur *et al.*, 2010; Patt, 2018; Ouispe *et al.*, 2017). Several groups of predators and parasitoids belonging to the families Reduviidae, Vespidae, Syrphidae, Braconidae and Ichneumonidae have been attracted by *A. conyzoides* while Miridae, Asilidae, also Vespidae and Reduviidae have been attracted by *L. camara* (Mexzón & Chinchilla, 1998).

Several species of aphids from the field margins were identified. Several parasitoids and host plants have been reported for the identified aphids. For instance, the host plants of the genus *Cryptomyzus* are from the family Lamiaceae (Bašilova & Rakauskas, 2007). The *Aphidius ribis* has been reported as the parasitoid of *Cryptomyzus ribis* (Alaoğlu 1994; Uysal *et al.* 2004). *Uroleucon* sp., including *Uroleucon sonchi* are associated with Asteraceae hosts such as *Conyza* spp; *Sonchus oleraceus* (Barjadze & Kanturski, 2020; Brumley, 2020). The braconid parasitoids *Aphidius ervi*, *Aphidius rosae*, *Aphidius sonchi*, and *Praon volucre* parasitize aphids from this genus (Carver, 1999; Laamari *et al.*, 2011; Mescheloff & Rosen, 1990; Tomanović *et al.*, 2009). Also, *Hyperomyzus carduellinus* is associated with Asteraceae hosts such as *Sonchus* and *Reichardia* spp. and is known to transmit Lettuce Necrotic Yellows Virus (LNYV) (Randles & Carver, 1971). The aphid *Sitobion avenae* is a cereal crop pest and accounts for great crop losses (Vereijken, 1979). The braconids *Lysiphlebus fabarum* and *Praon volucre* have been reported to parasitize this aphid (Tomanović *et al.*, 2009) while

Lysiphlebus fabarum parasitise *Aphis celastrii* (Tomanović *et al.*, 2009). *A. colemani* parasitizes several groups of aphids from the genus *Aphis* including *Aphis craccivora*, *Aphis nasturtii*, *Aphis umbrellae*, *Aphis verbasci*, *Aphis gossypii*, *Aphis intybi*, *Aphis nerii*, *Aphis epilobiaria*, *Aphis fabae*, *Aphis chloris* (Mescheloff & Rosen, 1990).

In our study, *A. colemani* was the dominant species identified from the non-pest aphid species from mummies collected from the field margins. The braconid, *A. colemani* has been identified as the primary parasitoid for the black bean aphid (*A. fabae*) (Mkenda *et al.*, 2019b). *A. colemani* is produced commercially to control aphids (Boivin *et al.*, 2012; Grasswitz, 1998). *A. colemani* is an important parasitoid as it parasitizes economically damaging aphids including *Aphis craccivora*, *Aphis fabae*, *Myzus persicae*, *Sitobion avenae*, *Rhopalosiphum padi* (Adisu *et al.*, 2002; Benelli *et al.*, 2014). Its widespread distribution is likely due to its escape from glasshouses or intentional release for biological control (Adisu *et al.*, 2002; Starý, 2002) and is most widely thought to originate from India or Pakistan (Adisu *et al.*, 2002; Messing and Rabasse, 1995). It has also been reported that the commercial strain (Koppert) of *A. colemani* originated from East Africa before being reared in Pakistan and transported to Europe (Adisu *et al.*, 2002).

There was a decreasing trend in the mean number of parasitoids from August to October. Host abundance, the type of plant, and the flowering stage affect the abundance of NEs at a particular period (Kishinevsky & Keasar, 2017). Thus, there is limited nectar availability and accessibility towards October as the quality of the flowers is low due to drying up. This also limits the availability of hosts and, thus, low numbers of NEs (Birch 1985; Karley *et al.*, 2003).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

5.1.1 Parasitoids of *Aphis fabae* and *Maruca vitrata*

Flowering plants provide food and shelter for NEs and can promote NPR in crops. Several parasitoids for *A. fabae* and *M. vitrata* were identified. *A. colemani* was the key primary parasitoid for *A. fabae*. Key larval parasitoids of *M. vitrata* include Braconidae and Dolichogenidea. These parasitoids have been reported in other SSA but not in Tanzania. The only dominant group of *M. vitrata* primary egg parasitoid was Platygasteridae. This is being reported to parasitise other genera from Crambidae. Hyperparasitoids for *A. fabae* and *M. vitrata* were also found, including Mesochorus sp. for *M. vitrata* and species from Pteromalidae for *A. fabae*. These are of low importance in biological control as they parasitise the hosts that primary parasitoids have already parasitised.

5.1.2 Effects of field margin on temporal-spatial distribution of NEs

In most cases, high plant diversity fields and field margins supported populations of NEs. Field margins are valuable in minimizing the negative impacts of agricultural intensification on NE populations, bringing resilience at local and landscape scales. The abundance of plants within field margins can provide a wide range of seasonal resources to NEs.

5.1.3 Field margin plants enhancing the survival and reproductive success of *A. fabae* parasitoid

Resources from flowering plants enhance NEs' survival, longevity, and fecundity and, in turn, facilitate them in providing pest suppression. The *E. heterophylla*, for example, supported the survival of *A. colemani* and, thus, an important plant for NEs. Generally, plants offer resources for survival and parasitism of *A. colemani* in the same way as the positive control.

5.1.4 The established field margin plants that supporting NEs in field crops

Specific plant species have been found to provide different benefits to NEs. This was observed in fluorescent dye trials. The *B. pilosa*, *T. minuta* and *P. hysterophorus* have been associated with key NEs, including the ladybeetle, hoverflies, assassin bugs, and lacewings. They support

NEs by enhancing their longevity and fecundity by providing resources such as nectar and pollen. However, some plants, such as *P. hysterothorus* are invasive and noxious and present great challenges as weeds and thus should be treated with great caution.

5.1.5 Flowering plants available which support NEs during the offseason

In transect walks, several NEs interact with field margin plants, with the parasitoid wasps interacting with numerous margin plants. Also, plants that had a high number of interactions included *B. pilosa* and *Euphorbia heterophylla*. These plants supported populations of NEs during the offseason together with *L. camara* and *A. conyzoides*. The long-life span plants such as *L. camara* are essential in sustaining populations of NEs. Hence they are of crucial importance.

5.1.6 Alternative *A. fabae* parasitoids to sustain NEs during offseason

During a field margin survey in the offseason, several field margin aphid species have been found associated with it. Therefore, these aphids that are not bean pests support *A. colemani* as hosts when bean pests are in the field. Thus, alternative hosts are an important aspect to consider when planning for effective biological control services.

5.2 Recommendations

- (i) Our results have identified several parasitoid species of *M. vitrata* and *A. fabae*. The egg parasitoids from the Platygasteridae are less studied for *M. vitrata*. Thus, future studies should focus on species from this family to understand the biology and biotic and abiotic factors that influence the species.
- (ii) This study has highlighted the importance of flower-rich field margins to support arthropod NEs of pests in bean fields for enhancing NPR and crop protection. This calls for farmers to protect their field margins for the added advantage of ecosystem service NEs offer for pest control. The limitation for this is the lack of knowledge by smallholder farmers on the benefits of the field margin; hence, some would clear them and use them as animal fodders. Implementing optimal plant diversification in farmers' fields will optimise the resources such as pollen and nectar for NEs for pest suppression. Also, the distance from the non-crop habitats may affect the abundance of the NEs in

the field crops. Therefore, it is important to critically analyse this aspect to enhance the populations of NEs in the field crop.

- (iii) Training, sensitisation and capacity-building programs are important for raising awareness among the smallholder farmers on the importance of landscapes and plant diversity in fields; how these aspects contribute to NPR through enhancing populations of NEs and, thus, ecosystem services that NEs offer.
- (iv) It is also important to critically analyse the vegetation in non-crop habitats. These habitats might also harbour potential pests depending on the type of vegetation in the non-crop habitats (field margins). Therefore, identifying plants in the non-crop vegetation that would benefit the NEs but not pests is of utmost importance. Selecting suitable plants that would play a part in conservation biological control is essential in establishing effective control strategies.
- (v) Research efforts are required for assessing plants from field margins that have a long life span for sustaining and supporting populations of NEs. Few studies have been conducted specifically for assessing these plants out of the bean growing season for sustaining their populations throughout and hence effective biological control.
- (vi) Also, the results have identified several aphid species from the field margins as alternative hosts that sustain the populations of parasitoids when there is no bean crop in the fields. This also should be the focus of future research, investigating different non-crop pests that will sustain populations of NEs in bean fields for sustainable pest management options.

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APPENDICES

Appendix 1: Mean \pm (SEM) numbers of NEs in fields collected by sticky traps

Diversity	Mean Number of NEs (\pm SEM)							
	Lady Beetle	Syrphid Fly	Lacewing	Parasitic Wasp	Predatory Fly	Parasitic Fly	Other Predatory Beetles	Predatory bug
High	1.67 \pm 0.21a	1.61 \pm 0.17a	1.36 \pm 0.17a	1.30 \pm 0.14a	1.39 \pm 0.17a	1.42 \pm 0.15a	0.73 \pm 0.09a	1.50 \pm 0.15a
Low	0.73 \pm 0.96b	0.67 \pm 0.09b	0.84 \pm 0.12b	1.14 \pm 0.14a	0.51 \pm 0.09b	0.44 \pm 0.08b	0.33 \pm 0.07b	0.73 \pm 0.12b

Values followed by the same letters (a and b) within the column are not significantly different ($p < 0.05$).

Appendix 2: Mean \pm (SEM) numbers of NEs in fields collected by sweep nets

Diversity	Mean Number of NEs (\pmSEM)							
	Lady Beetle	Syrphid Fly	Lacewing	Parasitic Wasp	Predatory Fly	Parasitic Fly	Other Predatory Beetles	Predatory bug
High	0.90 \pm 0.06a	0.89 \pm 0.06a	0.90 \pm 0.06a	1.08 \pm 0.06a	1.16 \pm 0.17a	1.01 \pm 0.06a	0.80 \pm 0.05a	0.78 \pm 0.05a
Low	0.80 \pm 0.04a	0.74 \pm 0.05b	0.73 \pm 0.04b	0.71 \pm 0.04b	0.72 \pm 0.05b	0.70 \pm 0.05b	0.71 \pm 0.05a	0.76 \pm 0.05a

Values followed by the same letters (a and b) within the column are not significantly different ($p < 0.05$).

Appendix 3: Mean \pm (SEM) numbers of NEs in fields collected by sticky traps

Month	Mean Number of NEs (\pm SEM)							
	Lady Beetle	Syrphid Fly	Lacewing	Parasitic Wasp	Predatory Fly	Parasitic Fly	Other Predatory Beetles	Predatory bug
May	1.22 \pm 0.24a	0.69 \pm 0.13a	1.00 \pm 0.17a	0.66 \pm 0.12a	0.84 \pm 0.18a	0.69 \pm 0.12a	0.44 \pm 0.13a	0.91 \pm 0.16ab
June	1.16 \pm 0.25a	1.22 \pm 0.21b	0.97 \pm 0.20a	1.53 \pm 0.22b	0.91 \pm 0.24a	1.09 \pm 0.19a	0.50 \pm 0.13a	0.84 \pm 0.18a
July	1.38 \pm 0.25a	1.38 \pm 0.20b	1.34 \pm 0.24a	1.38 \pm 0.19b	1.00 \pm 0.21a	1.09 \pm 0.26a	0.59 \pm 0.11a	1.50 \pm 0.23b
August	1.16 \pm 0.25a	1.28 \pm 0.22b	1.09 \pm 0.23a	1.31 \pm 0.19b	1.06 \pm 0.21a	0.84 \pm 0.18a	0.59 \pm 0.11a	1.22 \pm 0.20ab

Values followed by the same letters (a and b) within the column are not significantly different ($p < 0.05$)

May corresponds to the late seedling and vegetative stage; June corresponds to the flowering stage; July corresponds to the fruiting stage and early maturity stages and August corresponds to the late maturity stage of the crop near harvest

RESEARCH OUTPUTS

(i) Research Papers

Ndakidemi, B. J., Mbega, E. R., Ndakidemi, P. A., Stevenson, P. C., Belmain, S. R., Arnold, S. E., & Woolley, V. C. (2021). Natural pest regulation and its compatibility with other crop protection practices in smallholder bean farming systems. *Biology*, 10(8), 805.

Ndakidemi, B. J., Mbega, E. R., Ndakidemi, P. A., Belmain, S. R., Arnold, S. E., Woolley, V. C., & Stevenson, P. C. (2022). Field margin plants support natural enemies in sub-Saharan African smallholder common bean farming systems. *Plants*, 11(7), 898.

Ndakidemi, B. J., Mbega, E. R., Ndakidemi, P. A., Belmain, S. R., Arnold, S. E., Woolley, V. C., & Stevenson, P. C. (2022). Plant-rich field margins influence natural predators of aphids more than intercropping in common bean. *Insects*, 13(7), 569.

(ii) Poster Presentation

Appendix 4: Poster Presentation

