Hoverflies (Diptera: Syrphidae) as pollinators of sunflowers in the Waterberg region of Limpopo province, South Africa

by

Xavier Brits

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Written by: Xavier Brits

Supervisors: Prof. Christopher W. Weldon
Associate Professor
Department of Zoology and Entomology
University of Pretoria
South Africa
Email: cwweldon@zoology.up.ac.za

Dr John Midgley
Assistant Director
KwaZulu-Natal Museum
South Africa
Email: jmidgley@nmsa.org.za

Dr Hannelie Human
Research Fellow
Department of Zoology and Entomology
University of Pretoria
South Africa
Email: hhuman@zoology.up.ac.za

I, Xavier Brits, declare that the dissertation, which I hereby submit for the degree of Master of Science (Entomology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: ______________________________

Date: 27/03/2022

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Dissertation summary

Pressure is being placed on the agricultural sector to supply an ever-growing demand for food as the population continues to increase globally. This is further complicated by a continues decrease in pollinators needed for fruit or seed yield due to human activity. One such crop is the economically important sunflower (*Helianthus annuus* L.), prised for its oil and use in animal feed production. Sunflowers are self-pollinated, however, result in a reduced yield without the interaction of pollinators. Most sunflower farms in South Africa rely on wild pollinators or managed honey bees for this service, but the dynamics of these pollinators is poorly known. This study aimed to create a better understanding of the role hover flies have in the pollination of sunflowers, focussing the study on the Lehau, South Africa, sunflower producing region. The two main objectives were (i) to determine hover fly assemblage in and around sunflowers fields during the growing season and (ii) to evaluate their sunflower visitation based on pollen load as well as to quantify their contribution to seed set. Hover fly assemblage was determined sweep net sampling on five sunflower fields and species identification based on taxonomic guides. This showed eleven species present in the area with *Ischiodon aegyptius* being the most abundant species throughout the growing season. Hover flies followed an edge effect with most of the sampled individuals being close to edge of the fields. Pollen load on the hover fly bodies was determined by comparing them to a pre-created pollen atlas from wildflowers found within a 100m radius of sample fields. This revealed that Asteraceae and Solanaceae were the two most prominent wildflowers interactions, with the sunflower containing Asteraceae pollen group being present on almost all of the sampled species. Contribution to seed set was established be enclosing pollinators (Syrphinae, Eristalinae, Muscidae and *Apis mellifera*) in voile bags on sunflower heads for 24 hours and comparing that to heads were pollinators were excluded and heads that was open to all pollinators for 24 hours. This resulted in Muscidae interaction showing the largest contribution to the proportion of pollinated seeds with Syrphinae interaction showing the second highest contribution. This showed that not only do a diverse assemblage of hover flies interact with sunflowers, but they do also contribute to the seed set. *Ischiodon aegyptius* is the most likely candidate for mass rearing as it is a generalist pollinator present throughout the growing season and can interact with a diverse assemblage of wildflowers as alternative food source when sunflower heads are not yet present.

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Background

Worldwide, agriculture is dependent on ecosystem services provided by insect pollinators (Stavert *et al.*, 2018). In 2005 it was estimated that the economic value of wild and managed pollinators was $215 billion (Vanbergen & Initiative, 2013) while Kluser & Peduzzi (2007) estimate the economic value to be £30-70 billion per annum. Approximately 75 to 84% of crop species grown globally depend on pollinators for proper yield or seed production (Klein *et al.*, 2007; Vanbergen & Initiative, 2013). Yield production-dependent plants include apples, stone fruit and cucurbits, while seed production-dependent plants include carrots, onions, legumes, tree nuts and sunflowers (Calderone, 2012; Klein *et al.*, 2007). Many insect-pollinated crops can produce yield without insect assistance although with a much lower yield (Klein *et al.*, 2007).

Pollinator declines

The ecosystem service of pollination needs to be protected to ensure food security for the increasing global human population. However, both honey bees and wild pollinators such as solitary bees, wasps, flies, beetles and butterflies, face a worldwide decline in numbers (Kluser & Peduzzi, 2007; Potts *et al.*, 2010; vanEngelsdorp & Meixner, 2010). This decline can be attributed to habitat loss, misuse of pesticides, exotic pathogen introduction and loss of genetic diversity due to smaller breeding populations, which is a direct result of agriculture expansion (Calderone, 2012; Kluser & Peduzzi, 2007; Stavert *et al.*, 2018). The trend of decline, both in abundance and richness, has been well documented in North America and Europe where the focus has been placed on solitary bees, hover flies, bumblebees, honey bees and butterflies (Potts *et al.*, 2010; Vanbergen & Initiative, 2013; vanEngelsdorp & Meixner, 2010). The trend also shows that migratory species show a lower rate of decline when compared to non-migratory species or species with a restricted host range (Biesmeijer *et al.*, 2006).

To maintain an adequate yield of insect-pollinated crops, many farmers around the world use managed honey bee colonies (Kluser & Peduzzi, 2007). Problematically, honey bees are also declining, with a steady decrease in North America and Europe since 1947 (Calderone, 2012; vanEngelsdorp & Meixner, 2010). Multiple factors are suspected to contribute to losses, including pathogens, pesticides, diseases and environmental changes (Underwood & vanEngelsdorp, 2007). Pathogens include bacteria, *Trypanosoma* Gruby, 1843 and viruses like European foulbrood, Dicistrovirus, Israeli Acute Paralysis virus, Acute Bee Paralysis virus and Sacbrood virus (Evans *et al.*, 2009). Parasites include *Varroa* Oudermans, 1904 mites and honeybee tracheal mites. Another factor that is thought to contribute to honey bee colony losses, which links to disease incidence, is the lowering of honeybee immunity.

Honeybee immunity can be suppressed by pesticides and antibiotics, which have even been detected within the wax located in hives (Evans *et al.*, 2009; Kluser & Peduzzi, 2007; Underwood & vanEngelsdorp, 2007). Exposure to high doses of insecticide will lead to death in bees, however, sub-lethal doses can affect the cognitive functions, physiology and behaviour of bees (Johnson, 2015). Exposure to sub-lethal doses of neonicotinoids negatively affects the ability of *Apis mellifera scutellata* Lepeletier, 1836 to thermoregulate, which can affect the hive duties of individual workers (Tosi *et al.*, 2016).

South Africa has reported colony losses associated with the presence of small hive beetle and *Varroa* mites along with human factors like migratory movement, colony management and agrochemical use (Pirk *et al.*, 2014). Another factor that leads to colony losses in South Africa is the invasion of *A. mellifera capensis* Eschsholtz, 1822 clones into *A. mellifera scutellata* host colonies where the colony will slowly dwindle and die out (Martin *et al.*, 2002; Pirk *et al.*, 2014).

Honey bees are not the only pollinators facing decline. Studies have shown that species of Lepidoptera are also facing global decline with Great Britain showing a 31% decrease in macro moth abundance between 1969 and 2016 (Wagner *et al.*, 2021). Data on Lepidopteran decrease outside of European countries is sparse with only a few data sets from North and South America (Wagner *et al.*, 2021). Pollinating Coleoptera have also decreased in abundance, including a decline in seed and leaf beetles in Central Europe between 1900 and 2017 (Wendorff & Schmitt, 2019). Limited research has been done on the decline of pollinator species outside of Hymenoptera with some research reporting an increase in some species and a decrease in others. Wagner *et al.* (2021) reported that despite the decrease in many Lepidopteran species, some species have shown periodic increases in abundance.

Hover flies as ecosystem service providers

Hover flies (Diptera: Syrphidae) are a diverse family with over 6000 species described worldwide in 210 genera (Janković *et al.*, 2019; Jordaens *et al.*, 2015; Laubertie, 2007; Ssymank *et al.*, 2021). Their common name derives from the characteristic flight of hovering in a single spot followed by rapid, darting flight (Ssymank *et al.*, 2021). Adult flies feed on nectar and pollen, with body length ranging from 5 to 25 mm (Laubertie, 2007; Picker *et al.*, 2019; Ssymank *et al.*, 2021). There are estimated to be 230 species in 38 genera in South Africa (Picker *et al.*, 2019). These genera are divided into three subfamilies: Eristalinae, Microdontinae and Syrphinae (Jordaens *et al.*, 2015). There is, however, a fourth subfamily, Pipizinae, which is not found on the African continent (Mengual *et al.*, 2015). The subfamilies can further be divided into tribes based on larval and adult characteristics (Thompson & Rotheray, 1998). Eristalinae larvae are primarily phytophagous and saprophagous feeders, Microdontinae larvae are primarily myrmecophiles, while Syrphinae larvae are primarily predators of Hemiptera species. However, there are exceptions with some species in the Eristalinae being aquatic predators and some Syrphinae being pollen feeders (Ssymank *et al.*, 2021).

While hover fly taxonomy is relatively well understood, many areas require further research. This gap in taxonomy seems to be due to outdated and incomplete descriptions upon discovery, with only one sex described or based only on morphological characteristics in a family of morphological look-alikes (Jordaens *et al.*, 2015; Ssymank & Jordaens, 2021) as well as the incomplete sampling of poorly studied regions like the Mediterranean and Afrotropics (Chroni *et al.*, 2017). Particularly problematic in this regard is *Eumerus* Meigen, 1822 in Southern Africa and the European region (Chroni *et al.*, 2017), *Meromacrus* Rondani, 1848 in the North American region (Ricarte *et al.*, 2020), and *Merodon* Meigen, 1803 in the Mediterranean region (Ačanski *et al.*, 2016; Vujić *et al.*, 2012) to list but a few. Many of these taxonomic gaps are being filled in, for example by the resampling and redescription of *Meromacroides meromacriformis* Bezzi, 1915 in South Africa in light of newly described morphological characteristics and generic barcoding (Bellingan *et al.*, 2021) which is an expansion on the work done by Curran *et al.* (1927) who erected the genus *Meromacroides* for this species. Another example is the description of female of *Syrittosyrphus opacea* Hull, 1944 by Ssymank & Jordaens (2021). Larvae also provide a valuable source of taxonomic identification, however, the larvae of many Syrphidae species remain undiscovered (Ssymank *et al.*, 2021). Alternatively, DNA barcoding can be used for identification and molecular phylogenetic research is ongoing. In September 2015 a "Syrphidae COI" search in GenBank yielded 2 841 results (Jordaens *et al.*, 2015), this increased to 17 445 results by 14 February 2021.

Hover flies can be potential pollinators of wildflowers and crop species but are more often recognised for their potential as predators (Janković *et al.*, 2019; Laubertie, 2007). In species that frequent flowers, it is suggested that species with a shorter proboscis visit a larger range of flowers than those with a longer proboscis (Ssymank *et al.*, 2021). Predatory larvae are potential biological control agents as their primary diet comprises aphids (Charlet *et al.*, 1997; Janković *et al.*, 2019; Laubertie, 2007) but can also be predators of diamondback moth (*Plutella xylostella* Linnaeus, 1758), African citrus psylla (*Trioza erytreae* Del Guercio, 1918), thrips, and leafhoppers (Prinsloo & Uys, 2015). The effectiveness of being a control agent within the field is still debatable with laboratory-based results showing an up to 80% reduction in pest numbers, but field estimates being largely based on hover fly eggs and adult numbers (Dunn *et al.*, 2020). Hover flies are not the only predatory insects active in agricultural fields and low individual numbers make it hard to quantify their effect (Dunn *et al.*, 2020).

Hover flies are the second-most important group of pollinators in agricultural fields, outdone only by bees, and with Calliphoridae following close behind (Rader *et al.*, 2020). A study comparing hover fly pollination to bumblebee pollination in open flowers showed that hover fly pollination led to lower fruit yield, but higher seed yield (Fontaine *et al.*, 2005). Hover flies are important in agriculture worldwide, visiting 52% of insect-pollinated crop species and 70% of animal-pollinated wildflowers (Doyle *et al.*, 2020). Hover flies can carry pollen up to 400m, with migratory species being able to carry pollen for up to 100km (Doyle *et al.*, 2020; Rader *et al.*, 2011). This allows hover flies to carry pollen to isolated patches, increasing their importance in conservation biology.

Hover flies are generalist pollinators visiting a broad range of flowers. Hover fly adults rely on flowers as a source of energy by consuming nectar and pollen (Picker *et al.*, 2019). Studies in the Holarctic region showed that *Eristalis* Latreille, 1804 species transported pollen from 65 plant taxa, but most individuals only transported one or a few types of pollen at a time (Lucas *et al.*, 2018). A study of three grassland sites in France (Lusignan, Mirecourt and Marcenat) showed that hover flies transported between one and three different types of pollen at a time and carried pollen from grass, dicotyledon flowers, trees and shrubs (Michelot-Antalik *et al.*, 2021). A test on the degree of specialization for specific plant pollination by hover flies in those three sample sites revealed that they were much more generalist pollinators when compared with other Diptera from the samples (Michelot-Antalik *et al.*, 2021). The morphological characteristics of hover flies play a significant role in the effectiveness of their pollination. This includes the length and amount of small hairs on the body and their relatively short proboscis length (Doyle *et al.*, 2020; Rader *et al.*, 2011; van Rijn & Wäckers, 2016). The tiny hairs allow pollen grains to stick to the body while the proboscis length determines which flowers it will visit for nectar consumption.

Although pollinators, in general, are suffering declines in abundance, there is a lower rate of decrease in hover flies, and species diversity has increased in some areas (Biesmeijer *et al.*, 2006; Doyle *et al.*, 2020). Declines can be overcome with the mass rearing with some Eristalinae species being laboratory-reared on cattle manure diets (Nicholas *et al.*, 2018). Nicholas *et al.* (2018) developed a protocol that allows for mass rearing of *Eristalis tenax* Linnaeus, 1758 as the species is widely distributed. This requires the harvesting of wild *E. tenax* adults, larvae and eggs from fields, housing them in climate-controlled environments and providing the hatching larvae with an adequate diet. This comes with its own challenges like maintaining adequate genetic diversity, establishing dietary requirements of both the larvae and adults, and maintaining an optimal environment despite seasonal changes (Nicholas *et al.*, 2018). The success of mass-rearing is also still under investigation as many environmental factors play a role in the abundance of the flies, including wind, rainfall, temperature, pollen availability, predators, prey availability and light intensity (Dunn *et al.*, 2020; Nicholas *et al.*, 2018). Despite these challenges, mass rearing shows promise as reared individuals can be stored as pupae for over 20 days under cold conditions, allowing for a strategic release into agriculture fields when needed (Campoy *et al.*, 2022a). For South Africa it is desirable to locate an endemic species to which these criteria can be applied to prevent the introduction of foreign species that might upset local ecosystem balances.

Hover flies find desired plants using olfactory cues like methyl salicylate and 2-phenylethanol, emitted from some flowering Asteraceae species, and are recognized by chemical receptors in the antennae (Primante & Dötterl, 2010). This allows hover flies to locate desired plants without the use of visual cues. Hover flies are known to use visual cues as well, as demonstrated by attraction to yellow traps. Several studies have also observed their attraction to blue traps and a lack of attraction to white traps (Broughton & Harrison, 2012; Chen *et al.*, 2004). In contrast, field observations have noted that Eristalinae prefers white flowers while Syrphinae showed no specific preference (Dumbardon-Martial, 2016). This contradiction in literature might be due to region or sample timing due to flower availability;

however, it might also hint at chemical and visual cues playing independent roles in attraction. Despite not having a particular preference for a specific flower group, two plant taxa do frequently occur in the literature during wild sampling, the first being grass species (Poaceae) (Dumbardon-Martial, 2016; Michelot-Antalik *et al.*, 2021) and the second being Asteraceae (Michelot-Antalik *et al.*, 2021; Sajjad & Saeed, 2010). Numerous plant families of agricultural important have been reported as well, including Brassicaceae, Cucurbitaceae and Fabaceae (Sajjad & Saeed, 2010).

Other factors also influence hover fly abundance in an area. Grassland density and grass height have a significant effect on hover fly abundance, with longer or denser patches having lower abundance (Michelot-Antalik *et al.*, 2021). Insect interactions can also alter abundance due to predators, like lady beetles, competing for aphid prey, or ants defending aphids (Dunn *et al.*, 2020). Aphid abundance will affect hover fly larval abundance while nectar and pollen availability will also affect adult abundance (Dunn *et al.*, 2020).

Production and pollination of sunflowers

Sunflowers (*Helianthus annuus* Linnaeus, 1753), in the family Asteraceae, are prized for their oil, which is used for producing cooking oil and margarine. After the sunflower oil is extracted the remaining material can be turned into oilcakes for animal feed (DAFF, 2010). This by-product of the sunflower oil industry is high in protein (36%), fibre (18.2%), and low in fat (0.6%), which makes it an ideal addition to animal diets (Rad & Keshavarz, 1976). The sunflower husks, which are typically discarded, can be made into animal food pellets as they are composed primarily of lignin (50%), fibre (21%), lipids (5%) and protein (4%) (Osman *et al.*, 2018).

Sunflowers originated in the Americas, with most varieties originating from North America (Heiser Jr, 1978; Seiler & Rieseberg, 1997). Sunflowers have a robust nature, being able to germinate at 14 to 21°C and grow optimally between 23 to 28°C. However, due to cross-breeding, sunflowers can grow in a wider temperature range of up to 34°C with no yield loss (DAFF, 2010). Crops require approximately 130 days from planting until harvesting is completed (Figure 1.1). The crop requires 500 to 1000mm of rainfall but is also drought and cold resistant (DAFF, 2010). This permits global production of sunflowers, with Ukraine, Russia and Argentina being the highest producing countries in 2019 (Pilorgé, 2020).

In South Africa, sunflowers are produced for commercial use in four of the nine provinces: Free State, Limpopo, Mpumalanga and North West (DAFF, 2010). The Free State is the highest contributor to production, followed by North West (DAFF, 2020). Limpopo is the third-highest sunflower production area in South Africa, with the Waterberg district being one of the most important production regions. Sunflower production plays an important role in the agriculture economy of South Africa where in the 2018/2019 and 2019/2020 production years it accounted for 4% and 5.3% of the total production value (DAFF, 2020; DAFF, 2021). Currently, South Africa has over 25 varieties of sunflowers available for production with annual production between 500 000 to 700 000 tons, and an average of between 1.2 and 1.8 tons/hectare (DAFF, 2010). In the 2019/2020 production year, sunflower production had a gross value of R4.2 billion (DAFF, 2020).

Production stage	Time between stages
Seed planting to emergence	11 Days
Emergence to head appearance	22 Days
Head appearance to first anthers	27 Days
First anthers to last anthers	8 Days
Last anthers to head maturity	30 Days
Harvest	30-45 Days after bloom
Total	125-130 Days

Fig. 1.1. The time between different sunflower development stages. Source: DAFF, 2010.

The sunflower heads are a composite of 1000-2000 flowers joined at a mutual receptacle. Many sunflower varieties are self-incompatible and thus require insect pollination (DAFF, 2010). The primary pollinators of sunflowers worldwide are honey bees, however, more focus has been placed on alternative pollinators in recent years. Hover flies are frequently represented in data from sunflower field studies around the world, although in low abundance. In India (Andhra Pradesh, Tirupati) and Kenya (Makueni District) only a single hover fly species was recorded in sunflower fields (*Eristalinus quinquestriatus* Fabricius, 1794 and *Phytomia incisa* Wiedemann, 1830 respectively). In contrast, seven species (from the genera *Toxomerus* Macquart, 1855 and *Allograpta* Osten Sacken, 1875) were recorded from multiple sunflower fields across Argentina, and four (*Eristalis tenax*, *Eupeodes valueris* Osten Sacken, 1877, *Metasyrphus americanus* Wiedemann, 1830 and *Toxomerus marginatus* Say, 1823) were collected in multiple southern states in the United States of America (Jadhav *et al.*, 2011; Nderitu *et al.*, 2008; Rogers, 1988; Torretta & Poggio, 2013). None of these records has reported the influence of Syrphidae on seed set.

Study aim

This project aimed to achieve a better understanding of the role of hover flies in the pollination of sunflowers with a particular focus on a sunflower production region in the Waterberg district of Limpopo, South Africa. The two objectives used to address this aim were:

1. Determine the presence and species richness of hover flies in sunflower fields and surrounding vegetation throughout the sunflower growing season.

Hover fly species' richness and abundance in sunflower fields were expected to increase as sunflowers began to flower. Before the flowering of sunflowers, hover flies were expected to be found in surrounding vegetation where adults may forage for nutritional resources, nectar and pollen, and the juvenile stages of predatory species develop on other plants or in aquatic environments.

2. To indirectly evaluate sunflower visitation of hover flies based on pollen load and quantify their contribution to seed set using an enclosure experiment.

I anticipated finding a diverse range of pollen on the bodies of hover flies, given that most hover flies are generalists. In addition, it was also expected that hover flies would provide a significant contribution to sunflower seed set due to their frequent flower visitation for nectar and pollen.

The results of this project will provide information to sunflower farmers on which wildflowers provide suitable resources for hover flies in the absence of sunflowers (outside of the growing season). It will also reveal whether hover flies could represent alternative pollinators for sunflowers should managed honey bee populations continue to decline due to the multiple challenges they face. In particular, I anticipate being able to suggest species that could be targeted for future mass-rearing and release as sunflower pollinators.

Chapter 2: Establishing the species richness and pollen interaction of Syrphidae on sunflower fields in the Lehau region, Waterberg District, Limpopo province of South Africa

Abstract

Sunflowers (Helianthus annuus L.) are an important crop in South Africa, accounting for ~4.5% of the annual agricultural production value. While sunflowers are not reliant on external pollinators for seed set, self-fertilization results in reduced yield. Most sunflower farms in South Africa rely on wild pollinators for this service, but the dynamics of these pollinators is poorly known. Honey bees are the most abundant out of the assemblage of pollinators recorded, which could be explained due to the use of managed honey bee hives in many fields. Pollinators worldwide are seeing a reduction in both abundance and diversity, and bees are no exception. In bees, these declines are also shown to be linked to human-caused factors. This potentially leads to the need to invest in alternative pollinators that can be mass-reared and released into agricultural fields. Five sunflower fields were selected in the Lehau region of the Limpopo Province, South Africa, ranging in size from 4.3 to 19 ha. This study aims to better understand the assemblage of hover fly pollinators in and surrounding sunflower fields as potential mass-rearing candidates as well as seeing their interaction with wildflowers surrounding the fields. Here it was demonstrated that 11 Syrphidae species were present on sunflowers with *Ischiodon aegyptius* being the most abundant species. Most Syrphidae were caught near the field edge and Syrphidae were most abundant during the sunflower's flowering stage. Hover flies showed the highest interaction with Asteraceae, based on the pollen found on their bodies post sampling. Diversity indices indicated that relatively high diversity of species is present in the field, but further sampling is needed to clarify this further. Analysis of the degree of pollen interaction showed that all but two observed hover fly species were deemed as specialist pollinators. This provides evidence that hover flies might be an ideal candidate for future studies in mass-rearing.

Introduction

In a world with an ever-growing population, pressure is being placed on the agriculture sector to produce sufficient food. The majority of crops grown worldwide are pollinator-dependent either for yield, like stone fruit and cucurbits, or for seed production, like carrots and legumes (Calderone, 2012; Vanbergen & Initiative, 2013). Because of this dependence, the decline of many pollinator species; like solitary bees, wasps, flies, beetles and butterflies is of concern (Kluser & Peduzzi, 2007; Vanbergen & Initiative, 2013). The loss of pollinators can be attributed to habitat loss, misuse of agrochemicals, exotic pathogen introduction or loss of genetic diversity (Calderone, 2012; Kluser & Peduzzi, 2007; Stavert *et al.*, 2018). Many farmers rely on managed honey bees to ensure larger yields (Kluser & Peduzzi, 2007). These essential pollinators are also facing a decline worldwide due to the same factors facing other insects with colony losses of up to 84% in some regions (Calderone, 2012; Evans *et al.*, 2009; Klein *et al.*, 2007; Kluser & Peduzzi, 2007; Underwood & vanEngelsdorp, 2007).

Despite the general global decline in pollinators, not all pollinators are equally affected. For instance, some moth species have increased in abundance while others declined (Wagner *et al.*, 2021). Migratory species also seem to be somewhat less susceptible to population decline because they can travel between favourable environments (Biesmeijer *et al.*, 2006). A case in point are some hover flies (Diptera: Syrphidae), which have been recorded travelling over 100 km during their adult stage (Doyle *et al.*, 2020; Rader *et al.*, 2011). Another reason why hover flies may be more resilient to environmental change is the association of many species with human-disturbed habitats. A study on different disturbance levels of forests showed that more disturbed areas had a greater diversity and abundance of Syrphidae compared to more conserved areas (Souza *et al.*, 2014). Urban studies have also revealed a great number of Syrphidae species visiting green roofs, with some plant species showing higher Syrphidae presence than Hymenoptera species (Passaseo *et al.*, 2021; Rewicz *et al.*, 2017). These studies have also come to the conclusion that the restricted diversity and abundance in urban areas and increased diversity and abundance in forest disturbed areas were correlated with the adult and larval food sources (Passaseo *et al.*, 2021; Souza *et al.*, 2014). The larvae of the subfamily Syrphinae are primarily predators of Hemiptera, including important agricultural pests, while Eristalinae larvae are detritus feeders with some inhabiting highly nutrient enriched aquatic systems (Ssymank *et al.*, 2021).

Syrphidae is a diverse family with over 230 recorded species in South Africa, and over 6000 species globally (Kirk-Spriggs & Sinclair, 2021; Picker *et al.*, 2019). They are important in global agriculture, visiting 52% of insect-pollinated crop species and 70% of animal-pollinated wildflowers (Doyle *et al.*, 2020). Adult hover flies have been observed visiting flowers of 157 flowering species, including cherries, blueberries, cranberries, peaches, apples, radishes, and sunflowers (Conner & Rush, 1996; Gervais *et al.*, 2018; Robertson *et al.*, 2020). These pollinators are generalists with some hover fly species carrying pollen from as many as 65 different plant taxa (Lucas *et al.*, 2018). Their capacity for long distance movement also allows them to pollinate non-adjacent fields and isolated patches (Doyle *et al.*, 2020; Rader *et al.*, 2011).

The adults of the subfamilies Eristalinae and Syrphinae feed on pollen and nectar (Janković *et al.*, 2019; Kirk-Spriggs & Sinclair, 2021; Laubertie, 2007). This means they visit flowering crop fields both for the nutritional and energetic needs of the adults and for oviposition on plants infested with Hemiptera pests (for Syrphinae) or manure and water sources (for Eristalinae). During times when adults are not present, larvae have been observed within and surrounding agriculture fields, especially on perennial crops (Robertson *et al.*, 2020). Several studies have determined that hover fly adults are most abundant during Spring, Summer, and early Autumn when flowers are present both in agricultural fields and surrounding vegetation (Chisausky *et al.*, 2020; Robertson *et al.*, 2020). This applies to both the Northern and Southern hemispheres.

Hover fly abundance and diversity are highest on the edge of agricultural fields, like apple, plum, berries and barley, rather than in surrounding vegetation like forests or flower patches (Piekarska-Boniecka *et al.*, 2015; Robertson *et al.*, 2020; Sutherland *et al.*, 2001). Though this pattern is affected by farm practices, like mowing of the field edges, which drives down hover fly abundance (Gervais *et al.*, 2018). It has also been shown that vegetation type surrounding fields is not the driving factor for hover fly abundance but rather the diversity of flowering plant species (Gervais *et al.*, 2018; Piekarska-Boniecka *et al.*, 2015). This is not to say that surrounding vegetation does not play a role, as dense tall vegetation like tall hedges and or dense tree lines can inhibit or divert the movement of low traveling pollinator species like beetles and hover flies (Lövei *et al.*, 1998; Wratten *et al.*, 2003). Wratten and Bowie *et al.* (2003) have also suggested that Syrphidae will fly about 20 centimetres above the ground as it is the optimal height for sensing of pollen and nectar sources. This means that Syrphidae are less likely to cross barriers like roads, open ground or ploughed fields as there is no resources incentive to do so (Lövei *et al.*, 1998), despite being able to migrate large stretches of oceans (Hawkes *et al.*, 2022).

Sunflowers (*Helianthus annuus* Linnaeus, 1753) are an important protein source for animals and humans with the added benefit of providing a cooking oil (Rad & Keshavarz, 1976). In South Africa, sunflowers are produced for commercial use in four of the nine provinces: Free State, Limpopo, Mpumalanga and North West (DAFF, 2010). The Free State is the highest contributor to production, followed by North West (DAFF, 2020). Limpopo is the third-highest sunflower production area in South Africa, with the Waterberg region being one of the most important contributors. Sunflower production plays an important role in the agricultural economy of South Africa where in the 2018/2019 and 2019/2020 production years it accounted for 4% and 5.3% of the total production value respectively (DAFF, 2020; DAFF, 2021). Annual production is between 500 000 to 700 000 tonnes with an average of between 1.2 and 1.8 tonnes/hectare (DAFF, 2010). In the 2019/2020 production year, sunflower production had a gross value of R4.2 billion (DAFF, 2020). Currently, South Africa has multiple varieties of sunflowers available for production with each seed producing company constantly bringing out new strains for optimal production. While many of these are self-pollinating, pollination by honey bees can lead to considerable improvements in yield (Abbasi *et al.*, 2021; Ali *et al.*, 2015; DeGrandi-Hoffman & Watkins, 2000; Nderitu *et al.*, 2008; Oz *et al.*, 2009). In Kenya (Nderitu *et al.*, 2008) (1 species recorded), Pakistan (Ali *et al.*, 2015) (1 species recorded), Islamabad (Rasheed *et al.*, 2015) (1 species recorded) and Germany (Riedinger *et al.*, 2014) (13 species recorded) there is evidence for hover flies also potentially contributing to the pollination of sunflowers, but this information is limited in South Africa. Du Toit (1988) recorded the presence of 10 Diptera species, most of which were Syrphidae from three regions in South Africa. Consequently, there is a need to document the abundance and diversity of hoverflies in and around sunflower fields as a first step towards establishing their importance for pollination and potential improved sunflower production.

The aim of this study was to determine the presence, abundance, and species richness of hover flies in sunflower fields and surrounding vegetation throughout the sunflower growing season. I also investigated interactions with the surrounding floral vegetation by identifying the pollen carried by sampled hover flies. I expected to find a high number of species both within and surrounding the sunflower fields due to the adults being pollen and nectar consumers, and the larvae being associated with aphids or aquatic environments in agriculture landscapes. I also expected to detect little specificity in the pollen found on different hover fly species due to their generalist flower visitation.

Materials and Methods:

Study area

This experiment was conducted in five sunflower fields located within the Lehau area of the Waterberg District (Limpopo province, South Africa) (Figure 2.1). The Lehau area was chosen because it is an area intensively farmed for sunflowers (in rotation with legumes and maize, as observed in field and communication with farmers during the sampling time frame) and due to the willingness of the selected farmers to allow student work on their property. Sunflower fields (Table 2.1) were selected based on having sufficient wild vegetation adjacent to the fields, with the sample field not bordering on another agriculture type. All sample fields were not sprayed with any insecticides for the duration of the study. The smallest sample field was 4,3ha and the largest field was 19ha in size. The vegetation in the Lehau area is classified as Springbokvlakte Thornveld (Mucina *et al.*, 2006), which is dominated by small trees species, like *Vachellia* Wight and Arn, 1834 and *Senegalia* Raf, 1838, or shrubby grassland. Vegetation adjacent to the sunflower fields was primarily one of two types, either long grass (Figure 2.2) or trees and shrubs (Figure 2.3). All the sample sites had non-permanent water sources (deep pits or dry rivers) near the fields that filled during rain. Geographic coordinates were recorded for each field using Google Earth for later mapping of the sampling sites and to ensure that repeated sampling occurred in the same locations.

Table 2.1. Farm location, sunflower cultivar and surrounding vegetation

Farm number	GPS location	Sunflower cultivar	Surrounding vegetation
1	-25.0570543271504, 28.385740194684345	Pannar Clearfield Plus hybrid	Long grass, flowering plants, sparse tree cover
2	-25.059425748763648, 28.473646633086897	Karoo seed Aquara 6	Long grass, flowering plants, no tree cover
3	-25.07187562865518, 28.38055651483737	Pannar Clearfield Plus hybrid	Long grass, flowering plants, dense tree cover
4	-25.056264137670368, 28.432893076253222	Karoo seed Aquara 6	Long grass, flowering plants, sparse tree cover
5	-25.01950142647281, 28.50200772813235	Pannar Clearfield Plus hybrid	Long grass, flowering plants, no tree cover

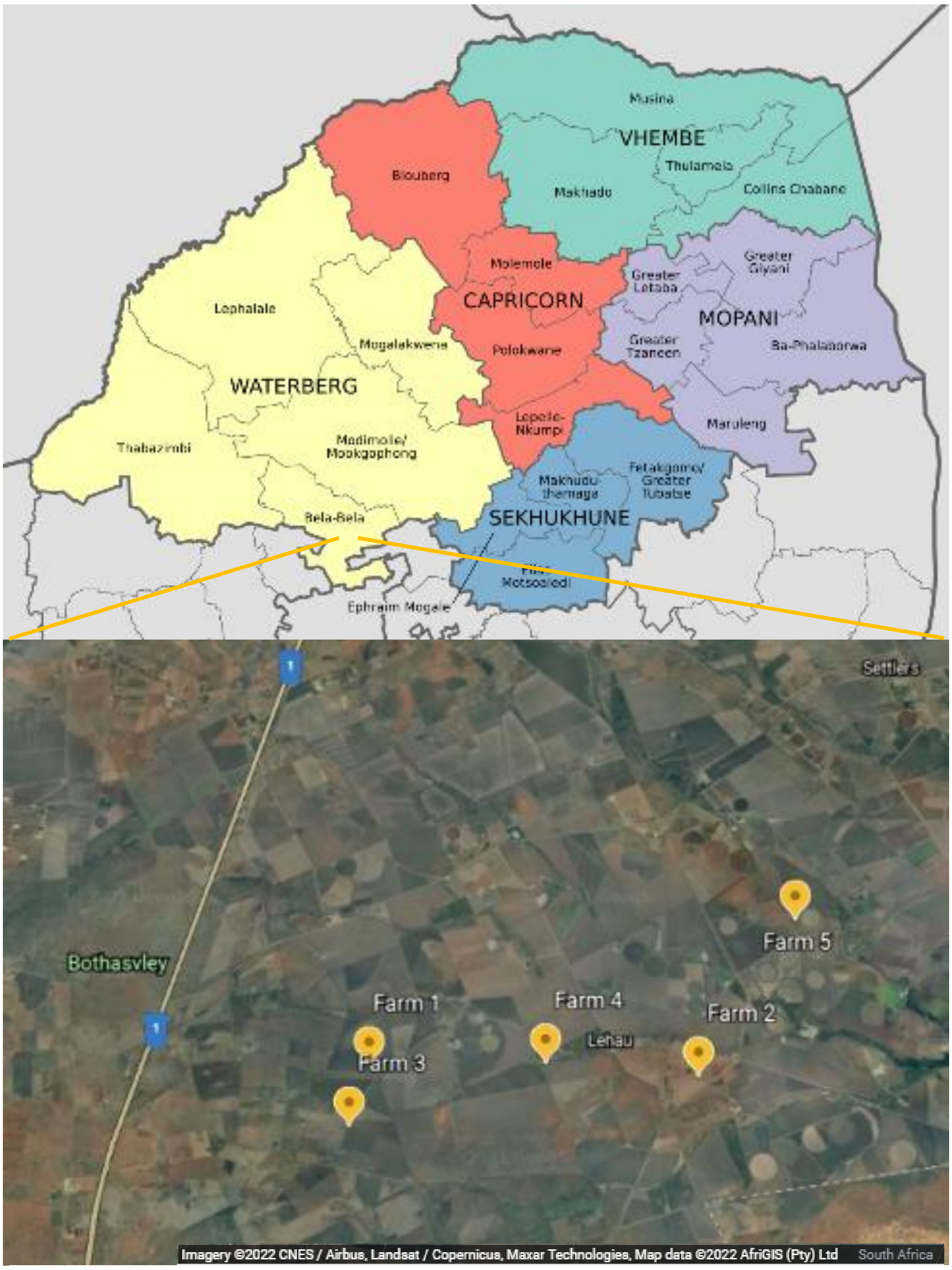

Fig. 2.1. Location of the five study field sites in the Lehau area, Waterberg district of Limpopo. Image was generated using Google Earth and sample site GPS coordinates.

Fig. 2.2. Example of grass-dominated vegetation outside of sunflower fields.

Fig. 2.3. Example of shrub-dominated vegetation outside of sunflower fields.

Experimental Design

Sweep net sampling was used in coordination with the different stages of sunflower development. Bird nets (Sponge lined frame cover by fine polycotton) with a 300mm diameter opening and 620mm net length were utilised for sampling (Manufactured by Daro). Sampling was done along the planting lines at a rate of one sweep per step with each sweep touching the top of the sample vegetation. Each field had four plots (10m × 10m in size), two inside and two outside the field that was sampled for 30 min each. Each sampled plot was marked out with poles and white flags that were removed after each sampling day to avoid damage by farm equipment. The plots were located at 5m and 50m in both directions from the field edge (Figure 2.4). Sampling was started two weeks after sowing and was done once for each of the growing phases of the sunflower plants, emergence out of the ground, the emergence of the sunflower head, flowering, mature heads and post-harvest (Figure 2.5) between 28/12/2020 and 06/05/2021. Sampling was done three times a day: two hours after sunrise, midday, and two hours before sunset. Given the time of year when sampling was done, morning and afternoon sampling was started at 08:00 and 18:00 (Emergence), 8:00 and 18:00 (Emergence of sunflower head), 8:30 and 17:00 (Flowering), 9:00 and 16:30 (Mature heads) and 9:00 and 16:00 (Post-harvest) respectively. The repeated sampling throughout the day was done to limit the potential effect of time of day on hover fly activity. Plots were sampled in random order to avoid any bias introduced by sequential sampling. A separate net was used for each plot on a farm and was cleared from any insects after euthanasia (see below) and then tipped inside out and ensured that all remaining debris and plant material was vigorously shaken out between each sampling time to avoid contamination on the next samples. Sample fields were also sampled in random order with no repeating of a field within a sample week and no switching between fields on a sample day.

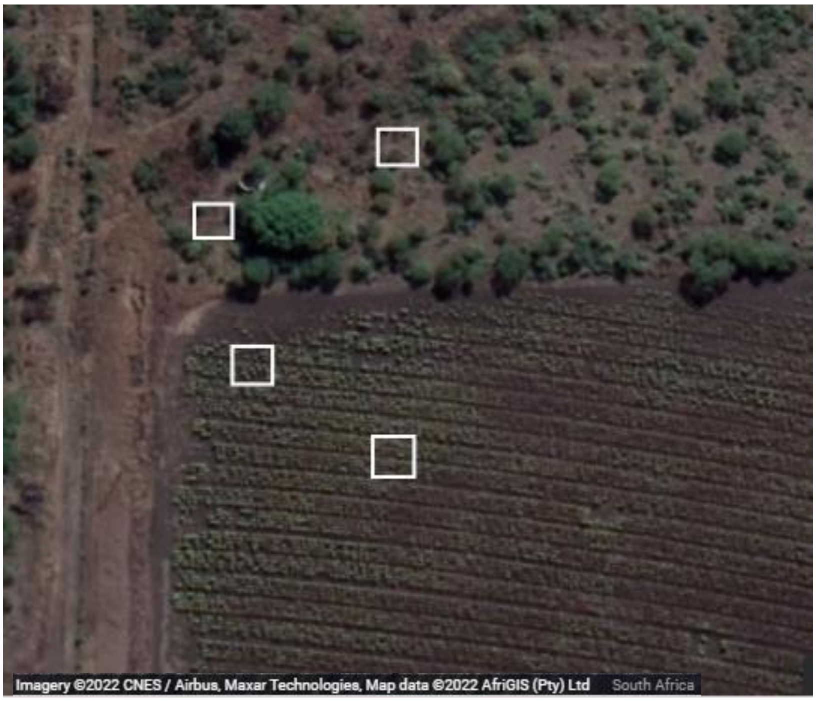

Fig. 2.4. Example of plot layout within a sampling field. Each plot is 10m x 10m, located 5m and 50m into and outside of the sunflower fields. Image was created using Google Earth and sample plot

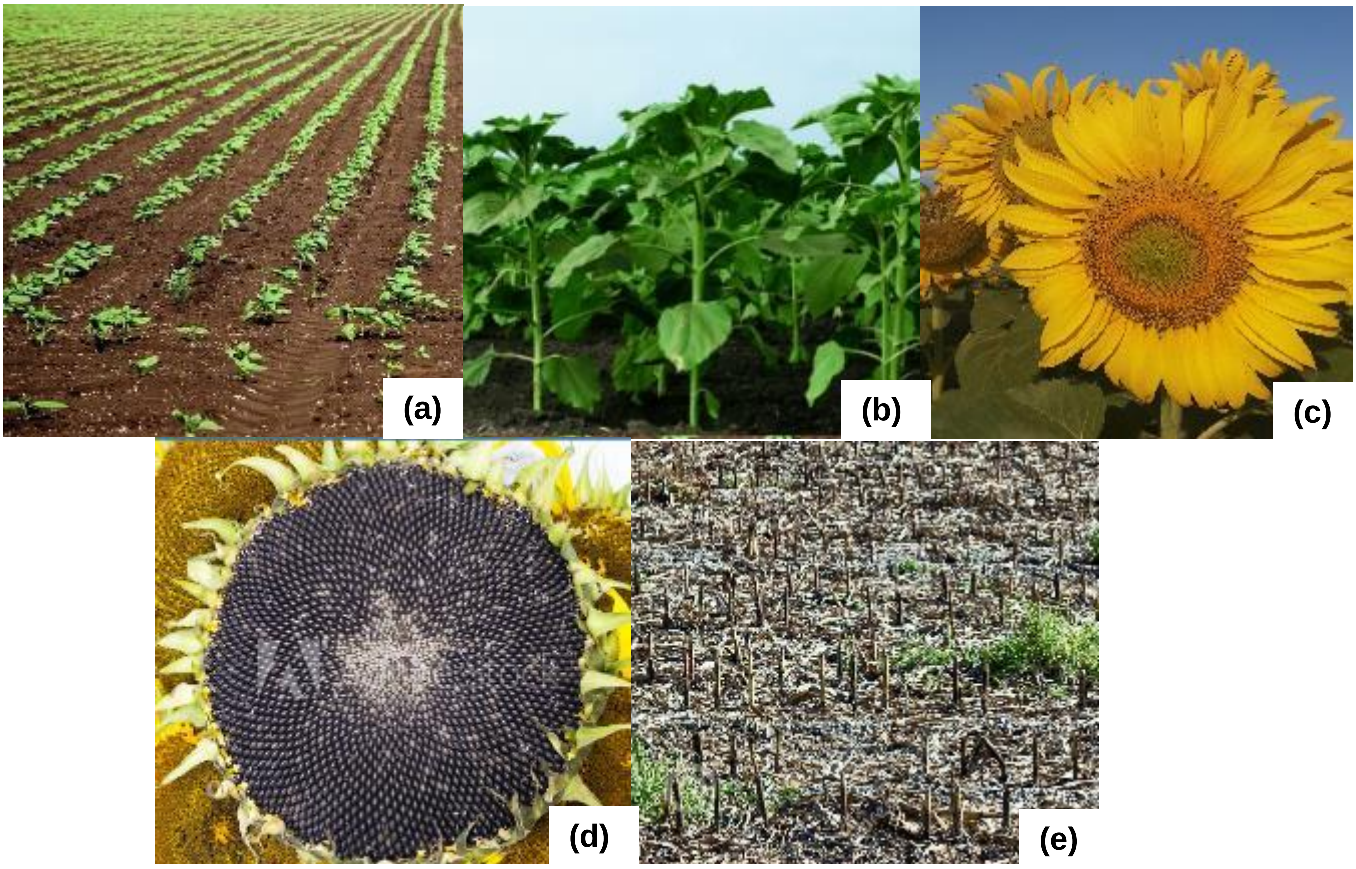

Fig. 2.5. Five development stages of the sunflower plants. Two weeks after sowing (a), emergence of head (b), flowering (c), mature head (d) and post-harvest (e).

Sample processing and identification

Samples collected for each plot were euthanized with ethyl acetate, labelled, and kept on ice until they could be stored in a -20°C freezer for later analysis. All sampled hover fly individuals were isolated from the frozen samples, washed with 95% ethanol to remove all pollen from their bodies, and then pinned and dried for identification. Complete pollen removal was confirmed using 40x magnification on a Wild Leitz GMBH biomed light microscope. Identification to genus was done using the taxonomic key published in the Manual of Afrotropical Diptera Volume 3 (Ssymank *et al.*, 2021). Species level identification was done using individual genus identification keys (Curran, 1939a; Curran, 1939b; De Meyer *et al.*, 2020a; De Meyer *et al.*, 2020b; Smith & Vockeroth, 1980). Specimens will be deposited at the KwaZulu-Natal Museum.

Pollen atlas

During field sampling, pollen was collected from sunflowers and all wildflowers within a 100m radius of the sampled fields. The field-collected pollen was used to create a pollen atlas (Appendix A) against which pollen removed from the bodies of hover flies could be compared. Wildflowers from which pollen was collected (Figure 2.6) were photographed and identified using field guides (Bromilow, 2018; Grabandt, 1985; Manning, 2019). Grass pollen was not collected as it was not expected to stick to the bodies of the hover flies as the pollen of most grasses is not entomophilic (dispersed by insects) but is rather anemophilic (dispersed by wind) (Poppy & Wilkinson, 2005).

Fig. 2.6. Examples of wildflowers sampled within 100m of the sample fields. *Zinnia peruviana* Asteraceae (a), *Parthenium hysterophorus* Asteraceae (b), *Hibiscus trionum* Malvaceae (c) and *Cucumis myriocarpus* Cucurbitaceae (d).

Pollen isolation and acetalization

All collected pollen (both from wildflowers and hover fly bodies) was treated in the same way to ensure uniformity between different samples. Collected pollen from wildflowers was suspended in 1ml 95% ethanol while hover fly body collected pollen was already in an ethanol suspension due to the washing process. The suspension was placed in a centrifuge at 6000 rpm for two minutes to create a pollen pallet that could be isolated for acetalisation according to methods reported by Abu-Asab and Cantino (1989). The acetalisation step is done to remove the outer coating of the pollen, which allows for better identification beneath a microscope. Isolated pollen was treated with 1ml of a 9:1 acetic acid (100%): sulphuric acid (95-97%) blend for an hour. The solution was then heated in boiling water for 5 minutes before being centrifuged at 6000 rpm for 2 minutes. Thereafter the pollen was rinsed three times with 1ml distilled water by centrifuging for 2 minutes for each rinse at 6000 rpm. The pollen was then washed in 1ml of 95% ethanol, followed by centrifugation for 2 minutes. Acetone was added and the sample was heated in an oven at 70°C for 30 minutes. Acetone was then replaced with two drops of 95% ethanol. The two drops were transferred to a clean slide and stained with two drops of Safranin O glycerin solution before a coverslip was added and sealed with clear nail polish. Glycerin stain stock was prepared according to Jones (2012). Safranin O stain stock was made by adding 1g of Safranin crystal powder to 100 ml of 50% Ethanol. Three drops of the Safranin O stain stock were added to 10 ml glycerin and mixed well before being stored on a hot plate at 60°C to prevent solidification of the glycerin solution. The resulting slides were then examined under 40× magnification using a light microscope (Wild Leitz GMBH biomed light microscope). For the pollen atlas, pollen was viewed using a light microscope (Wild Leitz GMBH biomed light microscope) and photographed using an attached digital camera (Canon PowerShot A630).

Data analysis

All data analyses were performed using R statistical software (version 4.2.1) (R Core Team, 2022).

Hover fly presence

Hover fly abundance could not be analysed as counts due to poor model fit resulting from a large number of zeros in the data. Therefore, these data were converted to hover fly presence or absence in each sample. The main effect of distance and development stage on the presence of hover flies was evaluated using a generalized linear mixed model using the "lme4 library (version 1.1-30)" with a binomial family. The random effect in the model was the different sample site to account for repeated sampling over time. This was followed by type III Wald chi-squared test (using the "emmeans library (version 1.8.2)") to calculate p-values for each main effect. Tukey's post-hoc test was used to identify differences between individual growth stages and plot distances from the field edge.

Hover fly diversity

Species accumulation curves were constructed with standard deviation confidence intervals from the observed species richness using the "vegan library (version 2.6-2)" to evaluate whether hover fly species had been sampled to completion. Alpha diversity between plots and overall was analysed with Simpson's diversity index, Shannon-Wiener diversity index and Sorenson's coefficient (Table 2.2). The Simpson's diversity index calculates the probability that two randomly chosen individuals belong to the same species while the Shannon-Wiener calculates the probability that two randomly chosen individuals belong to different species (He & Hu, 2005). Both indices are based on species richness and abundance and are complementary to one another. The maximum value obtainable for the Shannon-Wiener index is calculated by the logarithm of the number of species. Evenness represents to what degree individuals are split between species. Evenness was established from the Shannon-

Wiener index to determine the even spread of species caught within the samples, with higher values indicating a more even spread and 0 indicating only a single species (He & Hu, 2005). Sørensen's coefficient is a beta diversity metric which represents species overlap between sample sites (Vellend, 2001).

Table 2.2. List of diversity indices utilized with their formulas and description

Diversity index	Description
Simpson's diversity index:	$D = \frac{\Sigma(n(n-1))}{N(N-1)}$ Where n is the number of species and N is the total number of individuals caught.
Shannon-Wiener diversity index:	$H = -\Sigma\left(\left(\frac{n}{N}\right) \times Log\frac{n}{N}\right)$ Where n is the number of species and N is the total number of individuals caught.
Evenness:	$E = H/Ln(k)$ Where H is the Shannon-Wiener diversity index and k is number of species.
Sørensen's Coefficient:	$CC = \frac{2C}{S_1 + S_2}$ Where C is the number of species that overlap, and S is the number of species in a site.

Similarity in hover fly assemblages over time and distance from the field edge was visualized by constructing non-metric multidimensional scaling (nMDS) ordinations. Taxon by sample abundance data was used to perform the ordination using the vegan library. The metaMDS procedure was used to perform the ordination, which first involved the calculation of a Chao dissimilarity matrix. A convergent two-dimensional solution was reached after twenties tries. The Chao index of dissimilarity was the most appropriate index to use for the ordination as it takes into account (rare) unseen shared species in the data (Chao *et al.*, 2005). Using randomisation tests (999 permutations), an analysis of similarity (ANOSIM) was performed with Chao dissimilarity to determine whether the macroinvertebrate assemblages sampled using the two methods were significantly different (α = 0.05).

Pollen interaction

Syrphid-pollen interaction was using the bipartite library (version 2.17) and vegan library. The visweb analysis was based on pollen presence on hover fly bodies. The syrphid-pollen interaction was analyzed using the nestedness metric based on overlap and decreasing fill (NODF) (Almeida-Neto *et al.*, 2008) and nestedness temperature calculator (NTC) (Binmatnest) (Rodríguez-Gironés & Santamaría, 2006) methods for nestedness determination. Presence/absence data was chosen to avoid weighted calculations of the parameters due to most of the sampled species being represented by less than five individuals. This results in a score between 0 and 100 with 100 being completely nested in NODF but completely unnested in NTC. NTC is calculated using the squared distance of unexpected absence above the isocline of perfect nestedness and of unexpected presence below the isocline, while NODF is based on decreasing fill (marginal totals of a row (column) further away from most filled row (column) is lower than the one before) and paired overlap.

The interaction presence/absence data was also analysed with the paired differences index (PDI) (Poisot *et al.*, 2012) to determine which hover fly species were classified as generalists or specialists based on pollen interaction. The resulting PDI value is expressed between 0 and 1 with values lower than 0.5 indicating pollination generalists and values higher than 0.5 indicating pollination specialists.

Research ethics statement

This project was granted research ethics approval (NAS338/2020). Written informed consent was obtained from all farm owners prior to sampling on their properties.

Results

Syrphidae presence and abundance

During sweep net sampling a total of 53 individual Syrphidae were caught comprising eleven different species. *Ischiodon aegyptius* (Wiedemann, 1830) was the most commonly caught species representing 58% of all individuals, followed by *Eristalinus tabanoides* (Jaennicke, 1867) comprising 9.4% of all individuals (Table 2.3). Aphidophagous Syrphidae (Syrphinae) was represented by five of the eleven species caught during this experiment but they accounted for 73.6% of all individuals caught during this study. Sampling yielded a greater number of individuals within the sunflower fields (32) than in the surrounding vegetation, with most of the individuals found near the field edge (Figure 2.7). During sampling, it was observed that hover flies had the highest presence during the flowering and mature head life stages of the sunflowers, with no hover flies being present during the sowing and post-harvest life stages (Figure 2.8). Eristalinae species were primarily present when the sunflower heads were in bloom (six of the eleven caught individuals, 77% of which were caught inside of the sunflower field). Only two Eristalinae individuals were caught after the sunflower seed formed. Syrphinae individuals were present from the early growth stages of the sunflowers. During this time frame ten wildflower species were already in bloom, including species from Asteraceae, Cucurbitaceae, Lamiaceae, Malvaceae and Solanaceae.

Table 2.3. The number of individuals from each species caught during sampling within and surrounding sunflower fields during a growing season.

Species	*Caught*	*Within sunflower field*	*Within surrounding vegetation*
Syrphinae			
Ischiodon aegyptius (Wiedemann, 1830)	31	18	13
Eupeodes corollae (Fabricius, 1794)	4	2	2
Allograpta nasuta (Macquart, 1842)	2	2	0
Paragus haemorrhous (Meigen, 1822)	1	1	0
Asarkina sp (Macquart, 1842)	1	1	0
Eristalinae			
Eristalinus tabanoides (Jaennicke, 1867)	5	2	3
Eristalinus taeniops (Wiedemann, 1818)	3	3	0
Eristalinus quinquelineatus (Fabricius, 1781)	1	1	0
Phytomia incisa (Wiedemann, 1830)	1	1	0
Senaspis haemorrhoa (Gerstaecker, 1871)	1	0	1
Syritta flaviventris (Macquart, 1842)	3	1	2
Total caught	53	32	21

A generalized linear mixed model with a binomial family revealed that time of day did not have a significant effect (p value > 0.05 for all sample time slots) on species' presence during sweep net sampling. A separate GLM showed that distance (χ^2 = 10.791, df = 3, p = 0.013) and development stage of the sunflowers (χ^2 = 9.020, df = 3, p =0.029) had significant effects on hover fly presence. Tukey's post hoc tests on distance from the field edge showed a significant difference between 50m outside of field plot and 5m outside of the field (p=0.013) and 5m inside the field (p=0.037) (Figure 2.7). Hover fly presence at 50m inside the field was different from the other distances from the field edge. There was a significant difference between sampling post-sowing and post-harvest and all other developmental stages (Early growth p = 0.046, Flowering p = 0.016, Seed production p = 0.230) (Figure 2.8).

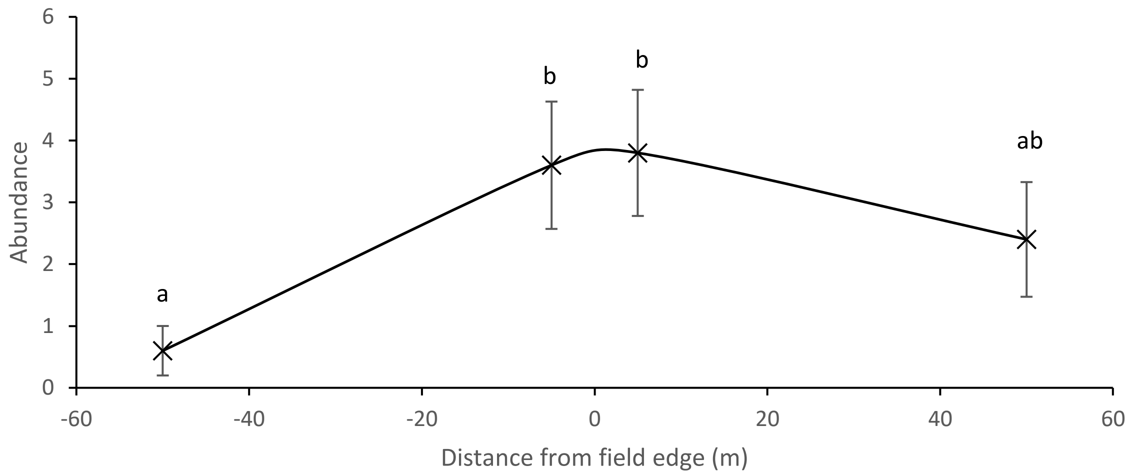

Fig. 2.7. Mean abundance of Syrphidae caught from the field edge over an entire growing season within and around five sunflower fields. Negative x-axis values indicate distance into the sunflower field while positive values indicate into the surrounding vegetation. Error bars indicate the standard error of the mean. Points labelled with the same lowercase letter are not significantly different (Tukey's tests, p>0.05).

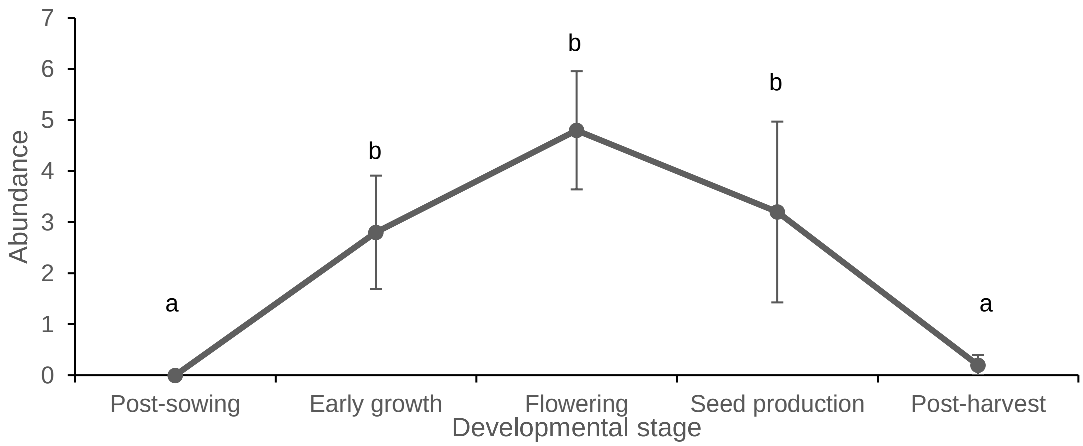

Fig. 2.8. Mean abundance of Syrphidae caught at different sunflower life stages over a single growing period on five sunflower fields. Error bars indicate the standard error of the mean. Points labelled with the same lowercase letter are not significantly different (Tukey's tests, p>0.05).

Hover fly diversity

To determine if all available species within the sampled fields had been collected, a species accumulation graph for the overall area was constructed (Figure 2.9). This revealed that overall, a plateau was almost reached, indicating that most Syrphidae species had been caught with only a few species potentially missing. The individual farms, except Farm 2, did not reach a plateau, so some Syrphidae species may not yet have been sampled from those sites. Farm 2 reached a plateau due to only a single species being present during sampling.

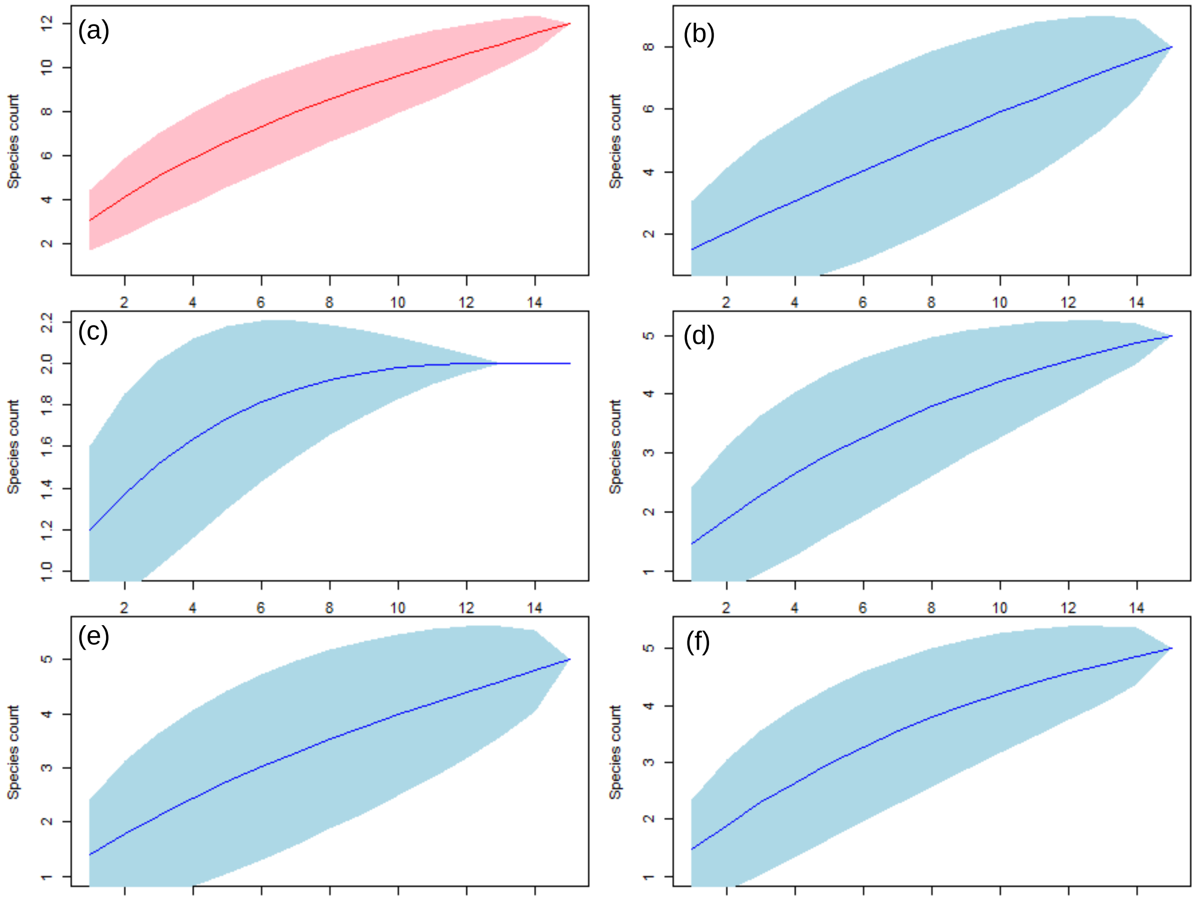

Fig. 2.9. Species accumulation curve showing the number of species collected over time. (a) Overall accumulation curve, (b) Farm 1 accumulation curve, (c) Farm 2 accumulation curve, (d) Farm 3 accumulation curve, (e) Farm 4 accumulation curve, (f) Farm 5 accumulation curve.

The alpha diversity analysis (Simpson's and Shannon-Wiener diversity indices) revealed that Farm 1 had the greatest diversity (12 individuals from 7 species) while Farm 2 had the lowest diversity (4 individuals from a single species) (Table 2.4). The overall alpha diversity of close to 0.5 reveals that diversity is not very high across all sampled sunflower fields. The evenness coefficient supports this with the samples being dominated by a few high abundance species and many low abundance species. When comparing between sample sites (Sørensen's index) there is a high degree of similarity in species between sites (Table 2.4). It also revealed that Farm 1 had the greatest species overlap between all the sampled farms and Farm 2 the lowest.

Table 2.4. Sample location diversity comparison using Simpson's diversity index, Shannon-Wiener diversity index, Evenness and Sørensen's Coefficient.

DIVERSITY INDEX	FARM 1	FARM 2	FARM 3	FARM 4	FARM 5	OVERALL
SIMPSON'S DIVERSITY INDEX	0.106061	1	0.636842	0.25	0.266667	0.376633
SHANNON-WIENER DIVERSITY INDEX	0.726412	0	0.24258	0.415055	0.530103	0.656786
EVENNESS	0.315477	0	0.105351	0.180256	0.230221	0.285239
SØRENSEN'S	**Farm 2**	**Farm 3**	**Farm 4**	**Farm 5**		
FARM 1	0.25	0.363636	0.545455	0.363636		
FARM 2		0.4	0.4	0.4		
FARM 3			0.5	0.5		
FARM 4				0.5		

Nonmetric multidimensional scaling ordination showed a large amount of overlap between different plot locations, indicating that the species composition of sampled plots was very similar (Figure 2.10). This observation was confirmed by means of an analysis of similarity (ANOSIM) using a Chao dissimilarity index, which revealed Syrphidae assemblages did not differ significantly between sampled plots (R = -0.100, p = 0.844). Stress of the nMDS ordination was 0.05. A stress value of zero indicates a perfect fit while 0.2 indicates a poor fit (Kruskal, 1964).

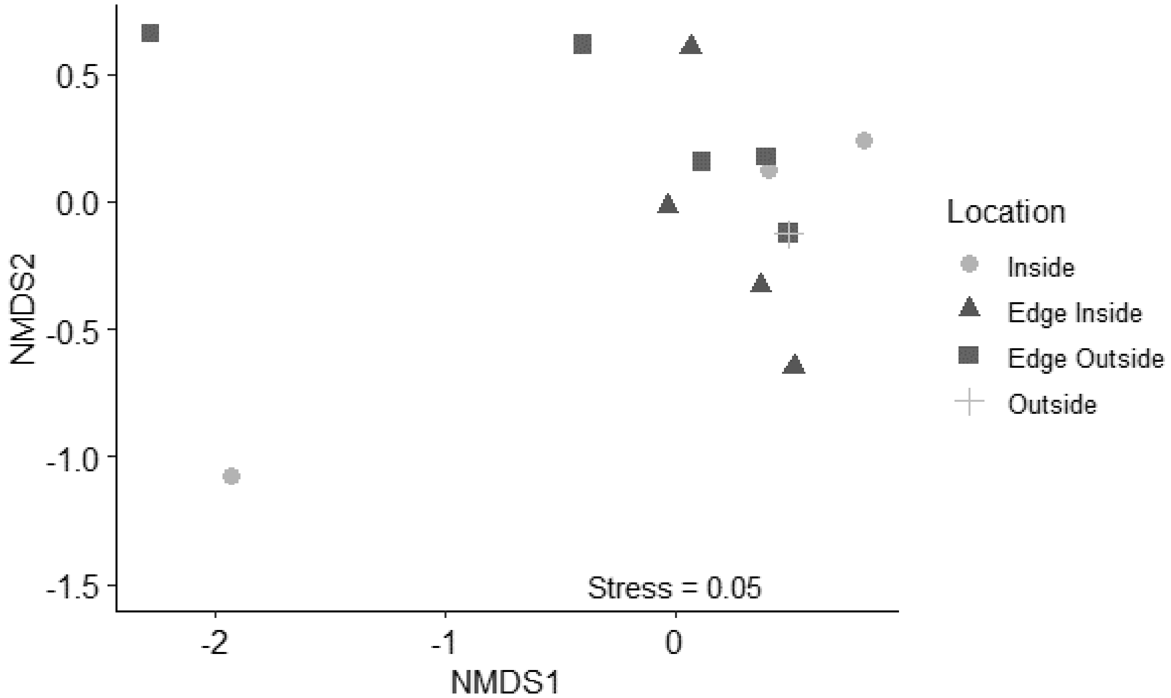

Fig. 2.10. NMDS plot showing the interaction between Syrphidae species assemblage and the four sample plots.

Pollen loads from Syrphidae

Twenty wildflower species were found within 100m of the field boundaries (Table 2.5) and were included in the visual pollen atlas (Appendix A). Asteraceae was the most abundant family with six species, including sunflowers and invasive species like *Zinnia peruviana* (L.). No indigenous Asteraceae were found during sampling. *Zinnia peruviana, Cucumis myriocarpus, Flaveria bidentis* and *Solanum elaeagnifolium* were present around all sampled plots. Asteraceae was divided into three groups based on visual pollen morphologies such as grain size, spike size and presence, shape, and divisions. Similarly, Solanaceae was divided into two groups. Fabaceae was combined into a single group due to sampled species having pollen of similar appearance. Three unidentified pollen grains were found, which were either grass pollen or pollen from flowers outside of the 100-meter sample radius.

Table 2.5. Collected wildflowers included in the pollen atlas.

Family	Species	Grouping
Asteraceae	*Helianthus annuus* (Linnaeus, 1753)	Asteraceae group 1
	Tagetes minuta (Linnaeus, 1753)	
	Tithonia diversifolia (Hemsl. 1883)	
	Zinnia peruviana (Linnaeus, 1759)	
	Parthenium hysterophorus (Linnaeus, 1759)	Asteraceae group 2
	Flaveria bidentis (Linnaeus, 1767)	Asteraceae group 3
Commelinaceae	*Commelina benghalensis* (Linnaeus, 1753)	
Cucurbitaceae	*Cucumis myriocarpus* (Naudin, 1933)	
Fabaceae	*Vicia* sp (Linnaeus, 1753)	Fabaceae group 1
	Sesbania bispinosa (Jacq., 1909)	
	Alysicarpus glumaceus (Vahl., 1825)	
Lamiaceae	*Salvia* sp (Linnaeus, 1753)	
Lophiocarpaceae	*Corbichonia* sp (Scop., 1777)	
Malvaceae	*Hibiscus trionum* (Linnaeus, 1753)	
	Sida acuta (Burm.fil., 1768)	
Nyctaginaceae	*Commicarpus pentandrus* (Burch., 1934)	
Pedaliaceae	*Ceratotheca* sp (Endl., 1832)	
Solanaceae	*Solanum elaeagnifolium* (Cav., 1795)	Solanaceae group 1
	Solanum seaforthianum (Andrews, 1808)	
	Datura ferox (Linnaeus, 1753)	Solanaceae group 2
Zygophyllaceae	*Tribulus terrestris* (Linnaeus, 1753)	

Asteraceae were the most frequently visited flower group with only *Allograpta nasuta* not having Asteraceae pollen on their bodies (Figure 2.11). Both *Z. peruviana* and *F. bidentis* had high association with Syrphinae and Eristalinae species, while *S. elaeagnifolium* had a high association with *Ischiodon*

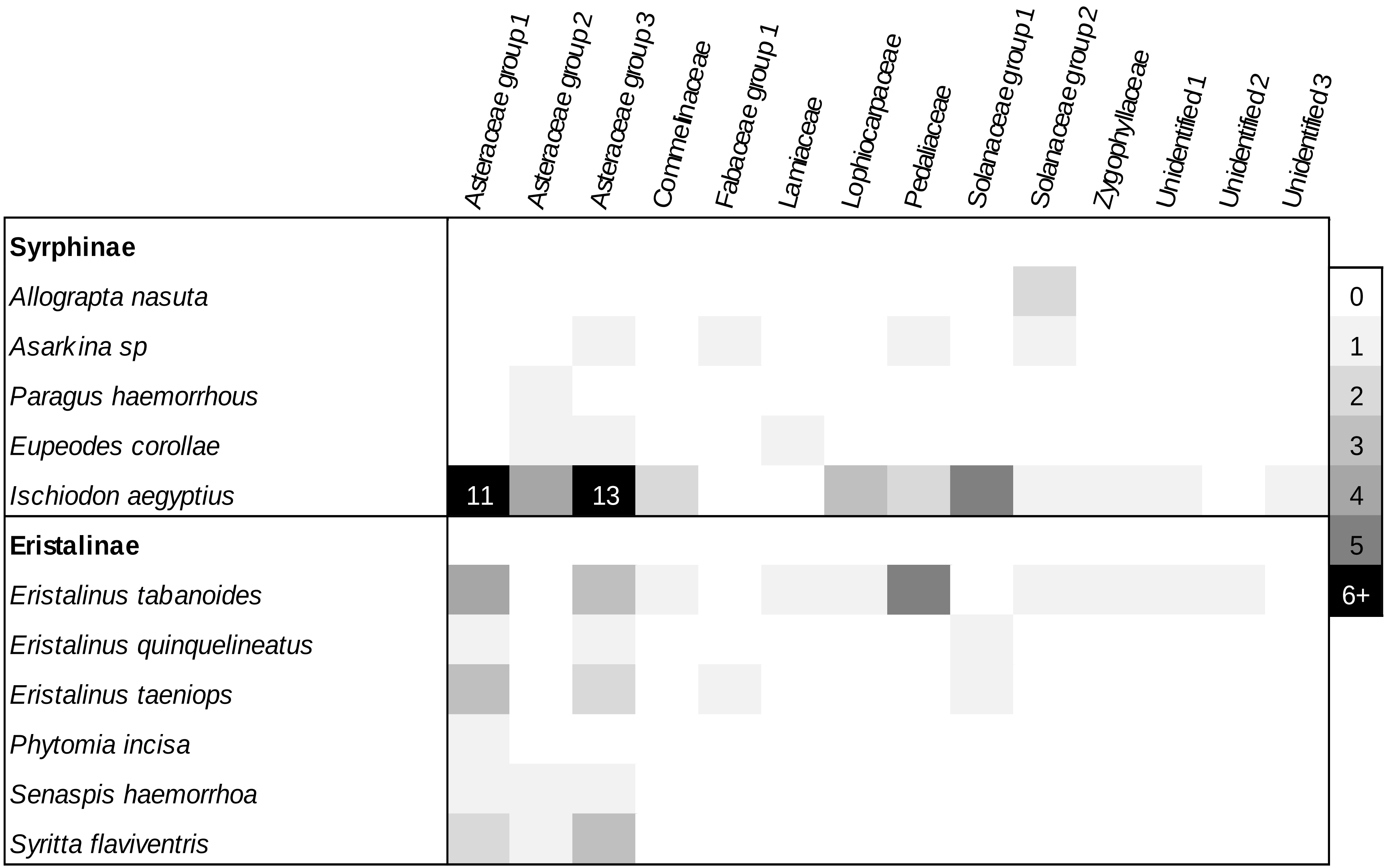

Fig. 2.11. A Visweb interaction matrix of Syrphidae (left) and pollen collected from surrounding flower species (top), showing the amount of caught individuals with pollen on their bodies.

aegyptius. *Cucumis myriocarpus* did not show any association with Syrphidae species and was thus excluded from the Visweb matrix. All Syrphidae that were caught within the sunflower fields had wildflower pollen on their body from the surrounding area except for *Phytomia incisa* which only had Asteraceae group 1 (Sunflower containing) pollen. *Ischiodon aegyptius* was found to visit the most diverse set of flowers, interacting with eleven of the fourteen pollen species found on Syrphidae bodies, while *Allograpta nasuta* only interacted with Solanaceae group 2, *Paragus haemorrhous* only interacted with Asteraceae group 2 and *Phytomia incisa* only interacted with Asteraceae group 1. All other Syrphidae species interacted with at least three pollen groups. *Ischiodon aegyptius* individuals had between zero and three different pollen species on their bodies while *Eristalinus tabanoides* had between three and six pollen species on their bodies (Appendix B).

Nestedness analysis using the NODF method produced a nestedness value of 39.486, indicating a low level of nestedness when data is not ranked based on interaction. The NTC method suggests the contrary with a value of 17.012, which is much closer to 0 indicating a high degree of nestedness (Figure 2.12). The interaction presence/absence was also subjected to a PDI analysis which revealed that all the collected hover flies were specialist pollinators except for two species, one from Syrphinae and one from Eristalinae (Table 2.6). This matches the visweb plot outcome with *Ischiodon aegyptius* (Syrphinae) and *Eristalinus tabanoides* (Eristalinae) being the two species with the highest pollen interaction

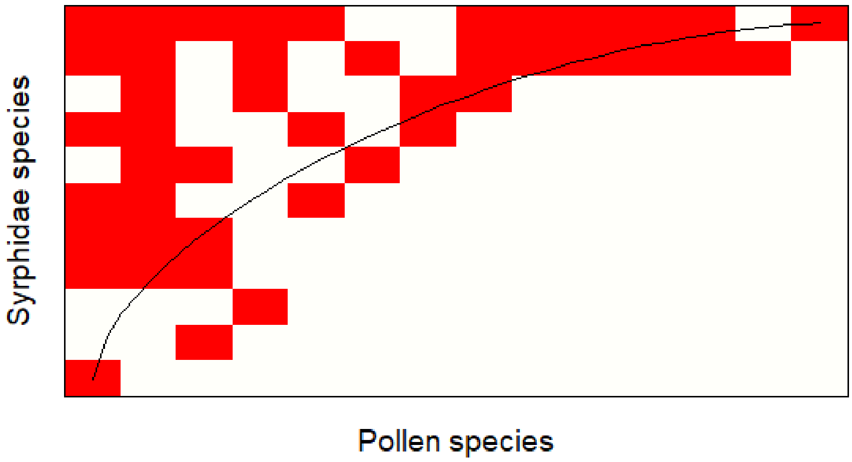

Fig. 2.12. Nestedness temperature calculator (Binmatnest) visweb showing the ranked interaction between Syrphidae species (left) and pollen species (bottom). Red coloured boxes indicate presence of interaction while white boxes indicate no interaction. The black line indicates the Isocline of perfect nestedness.

Table 2.6. Paired differences index indicating generalist and specialist species. Values close to 0 indicate that species are pollen generalists, whereas values of 1 indicate pollen specialists.

Syrphidae species	Pdi value	Specialist/Generalist
Syrphinae		
Allograpta nasuta	1.000	Specialist
Asarkina sp	0.769	Specialist
Eupeodes corollae	0.846	Specialist
Ischiodon aegyptius	0.231	Generalist
Paragus haemorrhous	1.000	Specialist
Eristalinae		
Eristalinus tabanoides	0.308	Generalist
Eristalinus taeniops	0.769	Specialist
Eristalinus quinquelineatus	0.846	Specialist
Phytomia incisa	1.000	Specialist
Senaspis haemorrhoa	0.846	Specialist
Syritta flaviventris	0.846	Specialist

Discussion

Syrphid presence and diversity

In this study hover fly diversity was overall relatively high. When comparing the observed species richness from our study to those obtained from other sunflower trials world-wide, our study had a higher hover fly species richness compared to most others, where only a single species was reported (Ali *et al.*, 2015; Nderitu *et al.*, 2008; Rasheed *et al.*, 2015). Riedinger *et al.* (2014) showed a similar species richness (975 individuals from 13 species) with a higher diversity of aphidophagous species. Du Toit (1998) is the only other South African study that notes hover fly abundance on sunflowers, but the species found were not reported. However, species richness was low when compared with the approximately 230 species present in South Africa (Picker *et al.*, 2019). The species accumulation curves show that most of the species present had been sampled. Unsampled species could have been migratory (Doyle *et al.*, 2020; Rader *et al.*, 2011) and not present during the time of sampling. Weather also played a major role during collection, with moderate wind and rain being present during sampling, which could have reduced hover fly activity. In addition, the species that were sampled in and around sunflower fields may be associated with disturbed areas (Wratten *et al.*, 2003) or relatively uniform environments (Conner & Rush, 1996; Gervais *et al.*, 2018; Robertson *et al.*, 2020).

Field edges in our study had a higher abundance and presence of hover flies, a common pattern in hover fly studies. Many aphidophagous Syrphidae species use field edges (forests, grass fields, flower patches and agriculture fields) more than the fields (Bennewicz, 2011; Söderman *et al.*, 2016; Sutherland *et al.*, 2001). This edge behaviour may be due to edge plants, like shrubs and trees, providing shelter from wind (Söderman *et al.*, 2016) or providing resources like aphids and flowers that might be attractive (Bennewicz, 2011; Söderman *et al.*, 2016). It is also known that while hover flies are attracted to fields with higher plant diversity they will still congregate around field edges, even if the floral diversity is much lower than within the fields (Bennewicz, 2011; Sutherland *et al.*, 2001). In this study, the fields were surrounded by access roads, which had diverse vegetation on the borders of the roads due to the high level of disturbance and movement of farm vehicles. It has been shown that landscape structures could impact pollinator movement between and within fields. One such example is the presence of semi-natural habitat, which provides nesting sites for pollinators, increasing the overall pollinator abundance in agricultural landscapes (Lajos *et al.*, 2021). Wratten *et al.* (2003) showed that crop height and border hedges also have a strong influence on the movement of hover flies between fields. The sunflowers in the sampled plots reached a height of approximately two meters high, which is lower than the hedge barriers tested by Lövei *et al*. (1998) and the same as the fence barriers tested Wratten *et al.* (2003). In both studies the dense high barriers reduced Syrphidae movement and foraging behaviour. Both studies concluded that crop height had an influence on Syrphidae pollination and foraging, with Lövei *et al*. (1998) observing high Syrphidae foraging activity at a height of 75cm.

There was evidence for the presence of hover flies declining by 50m into the sunflower fields. Similarly, Lövei *et al*. (1998) found that hover fly species would only move 20m from attractive flowering plants like those found on the field edge of our sample sites. This is a much lower foraging distance than that of honey bees, which travel an average of 750m from the hive to foraging sites (Bänsch *et al.*, 2020). Solitary bees on the other hand have a maximum foraging range of 150m to 600m from their nesting sites (Gathmann & Tscharntke, 2002). Bee species all have a point to return to and thus maximum travel distances can be recorded while hover flies do not need to return to a nesting site, so it is hard to determine the maximum distance they will travel for foraging. In California, 80% of native bees released in sunflower fields were active within 10m of their release site and no bees activity was found after 50m (Sardiñas *et al.*, 2016). This is similar to the hover fly activity observed within the sunflower fields during our sampling.

Aphid presence can impact hover fly abundance in fields, with studies showing that hover fly females are attracted to aphids for egg laying (Almohamad *et al.*, 2008; Dunn *et al.*, 2020). Aphids are located by hover flies using aphid stress responses (Dunn *et al.*, 2020), honeydew (Söderman *et al.*, 2016) or plant stress responses due to aphid feeding (Almohamad *et al.*, 2008; Almohamad *et al.*, 2007). Aphid colony size has a positive correlation with hover fly presence and is a potential reason for higher presence of hover flies at the field edge. This could also explain the low number of hover flies inside our sampled fields as the sunflowers had no observed aphids present during the study despite no insecticide use for the duration of the sunflower growing period. There was thus no resource attracting the hover flies into the fields, as floral resources were available on the edges. These results were similar to other studies that also showed a higher abundance in aphidiphagous hover flies (generally Syrphinae) compared to other hoverfly species (Söderman *et al.*, 2016).

Very few studies have looked at insect presence during different plant development stages, with most focussing on a single pest species occurrence. Hover flies prefer not to fly over vast barren ground, like ploughed soil or roads (Lövei *et al.*, 1998; Wratten *et al.*, 2003), which explains the lack of hover fly presence inside the sunflower fields during post-sowing and post-harvest. This supports the suggestion to add diverse vegetation that provides resources like nectar, pollen or other food sources (like aphids) could potentially improve pollinator abundance around agriculture fields (Blaauw & Isaacs, 2014; Wratten *et al.*, 2012).

Pollen patterns

Our pollen interaction data shows similar results to those of Klecka *et al.* (2018) who also found high overlap in pollen interaction between species in Syrphinae and Eristalinae. A few unidentified pollen species were also found on the hover fly bodies, which matched the general description of grass (Poaceae) species (Jan *et al.*, 2015). There is also the potential that pollen could have been carried from outside of the 100m radius from which flowers were collected to compile our pollen atlas, with farm homes and other agriculture crops being less than 500m from the sampled sites. Asteraceae pollen was found on all hover fly species except for *Allograpta nasuta*, which only had Solanaceae pollen present on its body. Seven of the hover fly species had pollen from Asteraceae group 1, which indicates that they may interact with sunflowers. Of these, *Ischiodon aegyptius* is the most likely candidate for sunflower interaction based on both pollen interaction, field observation and in which plots they were sampled. Our pollen interaction results indicate that Asteraceae species could be good edge crops to lure hover flies to sunflower fields and provide resources during the post sowing phase. As in other pollen interaction studies, our pollen species had to be divided into morphologically similar groups as it can be impossible to differentiate between pollen from the same genus or even same family when distinguishing pollen using only light microscopy (Jędrzejewska-Szmek & Zych, 2013). The amount of pollen species an individual hover fly carried could be due to morphological characteristics like body size or hairs on the body (Doyle *et al.*, 2020; Rader *et al.*, 2011; van Rijn & Wäckers, 2016).

A high degree of nestedness based on a ranked NTC analysis indicates that a large number of the specialist hover fly species visit a subset of the flowering plants that generalist hover fly species visit. Klecka *et al.,* (2018) found similar results using a ranked nestedness analysis, with few generalists visiting a large number of flower species, while the specialist species mainly visited a subset of those. Most of the species caught in this study were classified as specialists with only two species (*Ischiodon aegyptius* and *Eristalinus tabanoides*) identified as generalists. Generalist species had more than four different pollen species groups they associated with and specialists had four or fewer pollen species groups present on their bodies. As many of the specialist pollinators interact with Asteraceae group 1 (containing sunflowers), it shows that the high degree of nestedness is beneficial in this environment for the interaction and potential pollination of sunflowers. Our results contrast with other studies that suggest hover flies are generalist pollinator species (Michelot-Antalik *et al.*, 2021). This discrepancy could be due to interaction being based on pollen interaction instead of visual flower visits as pollen cannot be identified to species level and is thus group at family level based on morphology. Discrepancy can also be due to sampling occurring when some wildflowers were not in bloom or due to wildflowers hover fly species would interact with were not present between the sunflower fields. Pollen interaction is greatly dependent on the flower composition in the environment, thus having a wider range of flowering plant species present could lead to other sampled hover fly species also being classified as generalists.

Pollinator decline

A recent focus in science is the loss of pollinators both in abundance and diversity. To attempt to mitigate this, many suggestions for agricultural practices have been made and tested. Among these are ecological management approaches such as intercropping, reduced tillage and crop rotation (Kovács-Hostyánszki *et al.*, 2017). Apart from improving soil conditions for better crop performance, these types of interventions increase vegetation diversity to attract various pollinators (Kovács-Hostyánszki *et al.*, 2017). One of the big problems causing pollinator loss is habitat loss and fragmentation which reduces over wintering and nesting sites for many pollinator species (Calderone, 2012; Kluser & Peduzzi, 2007; Stavert *et al.*, 2018). This pattern was also observed surrounding the sampled fields, with large portions of the surrounding vegetation being transformed to agricultural lands and roads. The few remaining patches of natural vegetation are dominated by grasses or tree species with few flowers being observed. To counteract this, it is proposed that semi-natural ecosystems (reservoirs), field borders or buffer strips be planted to improve vegetation diversity and floral resources (Kovács-Hostyánszki *et al.*, 2017). The sampled fields used in this study were between four and sixteen hectare in size, which were on the smaller side compared to the fields present in the area. Based on the observed movement of the hover flies and the field sizes, buffer strips are advised to be more than 100 meters apart to allow for the movement of pollinators into the fields. It has been shown that buffer strips improve pollinator abundance (bumble bees and butterflies) when the correct floral species are present (Cole *et al.*, 2015). This study identified Asteraceae species as an important floral resource if hover fly assemblage is to be improved. Thus, combining agricultural practice with floral preferences can potentially improve crop yield by natural pollinators.

Conclusion

This study highlighted the observed diversity currently present within sunflower fields in South African production region and suggests their interactions with floral resources, including sunflowers. Hover flies in the subfamilies Eristalinae and Syrphinae both had pollen of flowering species of Asteraceae (sunflowers and related species) and Solanaceae on their bodies, which may indicate the value of these plant families as in buffer strips to attract hover flies and other wild pollinators to the agriculture fields. However, much is still unknown and requires further research. Hover fly diversity in other sunflower regions need to be established to be able to select the best hover fly candidate for mass rearing and release. The rearing process will have to be optimized to ensure healthy adult flies can be released to reproduce in the fields.

Chapter 3: Hover fly contribution to sunflower seed set in the Lehau region of the Waterberg District, Limpopo province, South Africa.

Abstract

The human population is continuously growing which places more pressure on the agricultural sector to supply a growing demand for food. One approach to address this demand is the improvement in yield from existing cultivars using better farming practices. Sunflowers are a self-pollinating crop important for oil production for human use and the remaining materials as animal feed. However, sunflower yield can be improved by interaction with Hymenoptera, Diptera and Lepidoptera pollinators. This study quantified the contribution to seed set of different pollinating Diptera (Eristalinae, Syrphinae and *Musca domestica*) in comparison with honey bees (*Apis mellifera scutelata*) in order to find suitable mass-rearing candidates that would benefit sunflower production. The results of an enclosure experiment indicated that *Musca domestica* had the highest contribution to the proportion of pollinated seeds, followed by Open pollinated, Syrphinae and then honey bees, with all trails yielding higher pollination that the no pollinator trail. The total pollinated seeds per head varied greatly with the no pollinator trail yielding the largest amount, however, seed amount in largely dependent on genotypic expression. This indicates that Syrphinae might yield the best candidate for a mass-reared hover fly to benefit sunflower production, however, further studies on an open field environment are needed.

Introduction

Agriculture is an important activity worldwide due to the need for food production to supply a rapidly growing human population. Approximately 75-84% of the crops grown globally, like apples, stone fruit, carrots, legumes and sunflowers, are dependent on pollinators to provide an adequate yield (Calderone, 2012; Klein *et al.*, 2007; Vanbergen & Initiative, 2013). This leads to considerable interest in the pollinators needed for these crops, with some estimating that pollinators have a net worth of £30-70 billion each year (Kluser & Peduzzi, 2007). A diverse group of insects are known to be pollinators of crops globally, with Lepidoptera being the most diverse group, followed by Coleoptera, then Hymenoptera and Diptera (Ollerton, 2017). Among the Hymenoptera, *Apis mellifera* is the most prominent species in agricultural fields (Ollerton, 2017). Many of these pollinators are facing global decline due to habitat loss and pesticide misuse (Calderone, 2012; Kluser & Peduzzi, 2007; Stavert *et al.*, 2018). However, some pollinator species are not exhibiting the same decline. This includes migratory hover flies, which are declining less than their non-migratory counterparts due their relatively wide host and geographic range (Biesmeijer *et al.*, 2006).

Hover flies (Diptera: Syrphidae) are a globally distributed family with over 6000 described species. In South Africa, there are 230 known species (Kirk-Spriggs & Sinclair, 2021; Picker *et al.*, 2019). Within the family, the subfamily Eristalinae and Syrphinae include the most common species occurring in agricultural fields due to their food source location (Kirk-Spriggs & Sinclair, 2021). Syrphinae larvae are generally predators of Hemiptera, especially aphids. The Eristalinae are generally detritus feeders in aquatic habitats, so their presence in agricultural settings may be supported by river sources or storage of water for irrigation (Kirk-Spriggs & Sinclair, 2021). Syrphidae are the most prominent family of Diptera found within agricultural fields and are in many regions only outnumbered by honey bees (Ollerton, 2017; Saunders, 2018). A study done by van Rijn & Wäckers (2016) showed that hover flies were the most abundant pollinator on wild flowers at field edges, outnumbering bees in flower visitation by a ratio of 3:1. *Musca domestica* (Linnaeus, 1758) is another of the underrated Dipteran pollinators. Muscidae species are important pollinators of mango, castor beans and even sunflowers (Douka & Fohouo, 2014; Du Toit, 1990; Kumar *et al.*, 2016). However, sunflower seed set contribution was much lower for *Musca* species than for honey bees (Du Toit, 1990).

In order for pollinators to contribute to seed set, they need to locate their nectar sources. Pollinators make use of various stimuli to locate flowers of interest, including visual cues like the colour and size of flowers (Shi *et al.*, 2009; Sutherland *et al.*, 1999). Male and female *Episyrphus* (Matsumura & Adachi, 1917) hover flies are attracted to yellow and blue artificially coloured flowers (Sutherland *et al.*, 1999). This is largely supported by hover fly wildflower visitation studies showing a wide host range of yellow flowers, but with preference for white flowers over blue flowers (Gervais *et al.*, 2018; Robertson *et al.*, 2020). Flower size also seems to influence hover fly preference. However, literature is divided on flower size preference with some suggesting that a smaller flower size is preferred (Cook *et al.*, 2020; Sutherland *et al.*, 1999), while others show that hover flies are more attracted to larger flowers (Conner & Rush, 1996). What is generally accepted is that wildflower density and diversity have a positive effect on hover fly flower visitation and probing (Conner & Rush, 1996; Gervais *et al.*, 2018). Hover flies also use olfactory cues emitted from flowering plants (Barragán-Fonseca *et al.*, 2020; Primante & Dötterl, 2010) and stress responses due to aphid herbivory (Almohamad *et al.*, 2008) to locate desired hosts.

Apart from floral traits, pollinator morphology also has an impact on pollination. In hover fly species that frequent flowers, it is suggested that species with a shorter proboscis have a larger flower visit range than those with a longer proboscis (Kirk-Spriggs & Sinclair, 2021). It was originally believed that due to the relatively short proboscis of hover flies that they are restricted to visiting shallow simple flowers (Goulson & Wright, 1998; van Rijn & Wäckers, 2016). However, it has been proven that hover flies will visit long spurred flowers, like *Impatiens burtonii* (Hook. fil, 1864) (Vlašánková *et al.*, 2017). Pollination of long spurred flowers will depend on whether the body size of the visiting is sufficient to connect with anthers and stigma (Vlašánková *et al.*, 2017). Hover flies have a relatively small body size when compared to other pollinators (Cook *et al.*, 2020). The body has short hairs that are shorter and less dense than that of many other pollinators. These hairs are still sufficient to carry pollen over great migratory distances (Doyle *et al.*, 2020; Rader *et al.*, 2011). Pollen grains found on hover flies are primarily located on the face and dorsal hairs, which correlates to them feeding on pollen and having small dorsal hairs that trap pollen (Lynn *et al.*, 2020). Hover flies have been found to carry a large diversity of pollen, including from agricultural crops, wildflowers and grass species (Michelot-Antalik *et al.*, 2021). The pollination efficiency of hover flies is generally determined by their contribution to seed set. Hover flies have a positive effect on the seed set of sweet peppers (Jarlan *et al.*, 1997), oilseed rape (Jauker & Wolters, 2008) and onions (Saeed & Masood, 2008). Other studies have implied a contribution to seed set due to the presence of hover flies in the environment, visitation in open fields or pollen found on the bodies, like with Japanese pear orchards, parsley, apples, watermelon, strawberries, mango, carrots, coffee and sunflowers (Burgett, 1980; Rader *et al.*, 2016; Sonoda *et al.*, 2022).

Sunflowers (*Helianthus annuus* Linnaeus, 1753) (Asteraceae). are a globally grown crop prized for their oil and use in animal feed. Sunflowers are predominantly a self-fertilizing crop for which yield can be improved with the addition of pollinators (Tesfay, 2009). The primary pollinators of sunflowers are honey bees due to managed hives being placed within fields, which increases yield even on self-fertile species (Greenleaf & Kremen, 2006; Langridge & Goodman, 1974; Tesfay, 2009). There are, however, numerous wild pollinators that have been recorded visiting sunflower fields, including Hymenoptera, Lepidoptera, Coleoptera and Diptera (Jadhav *et al.*, 2011). One of the important Dipteran families for sunflower pollination is Syrphidae. Globally, hover flies have been recorded to vist sunflower fields, including in Brazil, Hungary, Iran, Yugoslavia and South Africa (de Oliveira *et al.*, 2019; Du Toit & Holm, 1992; Khaghaninia *et al.*, 2010; Lajos *et al.*, 2021; Tesfay, 2009; Thalji, 1992). With the ever-growing problem of pollinator declines, the presence of alternative generalist pollinators may be relied upon should the abundance and efficacy of pollination by a specialist pollinator be limited (Rhodes, 2018). Managed honey bees face a greater threat than most pollinators due to hive vandalism and destruction that have been reported globally (Masehela, 2017).

The objective of this study was to determine the contribution of hover flies to sunflower seed set in comparison with other pollinating species using an enclosure field experiment. A significant contribution to the sunflower seed set by hover flies was expected due to their frequent flower visitation for nectar and pollen. However, due to their bodies being covered in fewer hairs to trap pollen, I anticipated that sunflower seed set induced by hover flies would be less than that by honey bees.

Materials and methods

Study site

Exclusion experiments were conducted on sunflower fields in the Lehau region, Waterberg District, Limpopo province, South Africa (Table 3.1, Figure 3.1). Farm locations were selected based on prior consent given by sunflower farmers (Chapter 2). Large sunflower fields were divided into sub-sections with a 200 m division between sampled blocks. The fields consisted of Pannar Clearfield Plus hybrid sunflower varieties which are a self-pollinating variety.

Table 3.1. Site locations for a pollinator enclosure experiment.

Block number	GPS location	Note
1	-25.0245242, 28.483728	A large farm of 24.6 ha was divided into 3 blocks of 100 × 100 m.
2	-25.0245242, 28.483728	
3	-25.0245242, 28.483728	
4	-25.0415863, 28.3846699	A large farm of 26 ha that was divided into 2 blocks of 100 × 100 m
5	-25.0415863, 28.3846699	

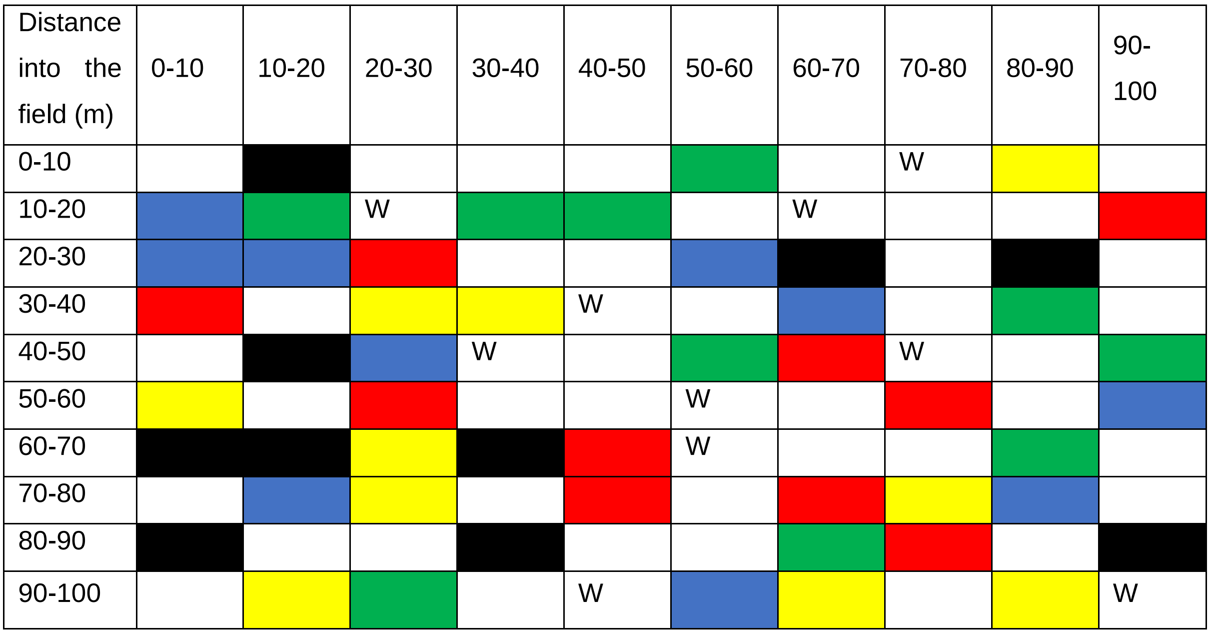

Fig 3.1. Example of a randomly generated field design for a 100 × 100m block divided into 10 x 10 m squares. Six pollinator trails were used: Apis mellifera (yellow), Musca domestica (red), Eristalinae (green), Syrphinae (white; W), no pollination (black) and open to wild pollinators for 24 hours (blue).

Exclusion setup

To determine the contribution that different pollinators had on sunflower seed set, sunflowers needed to be isolated from wild pollinators. To achieve this, sunflower heads that were yet to bloom were enclosed in 300 × 300 mm voile bags and sealed with coloured insulation tape, with different tape colours assigned to the allocated treatments. Five fields were selected with 60 flowers per field used for the experiment. A 100 × 100 m block was selected inside the field and was further divided into 10 × 10 m blocks. The blocks were randomly allocated one of six treatments (10 replicates per treatment), with only one flower head per block enclosed in the voile bag (Figure 3.2). The voile bags (Figure 3.3) ensured that no wild pollinator had access to the sunflower heads during flowering and that the enclosed insect (described below) did not escape. Once the sunflower heads were in bloom, wild-caught insects were inserted into a small slit in the base of the bag and resealed with the treatment's-coloured insulation tape tied around the stem behind the head. The six pollinator treatments were: Syrphinae (*Ischiodon aegyptius*), Eristalinae (*Eristalinus* sp), *Musca domestica* L., *Apis mellifera*, open to wild pollinators, and no pollination as the negative control. Eristalinae consisted of a mix of species as identification could not be done to species level in the field due to the need for a microscope and for specimens to be kept alive. The insects were allowed to pollinate the flowers for 24 hours before being rapidly killed inside the bag by being pinched. By killing the insects inside the bag, it ensured that the bag remained closed and allowed for later identification of the treatment, should the test colours fade. With the open to wild pollinator treatment the voile cover was removed for 24 hours before being replaced onto the colour marked head.

Sunflower heads were allowed to mature, produce seeds and dry before being harvested. The harvested heads were kept in the voile bags in a cool, dry location until the seeds were removed from the heads by hand and placed into clear bags with labelled identification tags. Pollinated and unpollinated seeds were then separated using a JAPS engineering grain cleaner at 30 Hz, which allowed the unpollinated seed to be blown off into a separate collection container. The use of a grain cleaner to sift out impurities like debris or unpollinated seeds has been suggested by grain cleaner patents (Porqueras, 2011) as well as descriptions on the use of such machines (Drincha & Tsench, 2020). The seeds were then counted by hand as unpollinated flowers would form empty seed hulls while pollinated flowers produced endosperms.

Statistical analysis

All data analyses were run using R version 4.2.1 within RStudio version 2022.02.3. Data on seed set was expressed as two data columns representing the number of pollinated and unpollinated seeds. These were then combined as a bivariate response using the 'cbind' function in R. A generalised linear model (GLZ) with quasibinomial distribution was used to determine the effect of pollination treatment on the bivariate seed set response. Similarly, a GLZ with a quasipoisson distribution was used to determine the effect of pollination treatment on the total sunflower seed set. The selected error structures were used for the models because the null and residual deviance were much higher than the degrees of freedom for the binomial or poisson family. Both generalised linear models were summarised using a type II analysis of deviance. Post-hoc pair-wise multiple comparisons were performed using Tukey's tests (using "emmeans library (version 1.8.2)") to determine if there were any significant differences between pollination treatments.

Research ethics statement

This project was granted research ethics approval (NAS338/2020). Written informed consent was obtained from all farm owners prior to sampling on their properties.

Fig 3.2. Location of seed set contribution fields trails on sunflower farms in the Lehau region, Waterberg District, Limpopo province, South Africa. Image was created using Google Earth and exclusion sunflower field GPS coordinates.

Fig 3.3. Examples of voile-covered sunflower heads to prevent unwanted insect pollination.

Results

During the exclusion experiment a total of 193 sunflower heads were assessed for seed set. This reduction from the predetermined sample size was as result of a loss of enclosed heads due to farm activities and a shortage of hover flies due to high winds during collection for the enclosure experiment. This left 20 Eristalinae, 25 Syrphinae, 36 *Apis mellifera*, 39 *Musca domestica*, 34 Open pollination and 39 No pollination heads to analyse.

Sunflower heads varied greatly in the number of seeds (Figure 3.4A) with Eristalinae having the highest average seed count per head (2070 seeds) and *M. domestica* having the lowest average (1642 seeds). Open pollination had the highest variation in total seeds per head while *M. domestica* had the lowest variation in total seeds per head. A GLZ with a quasipoisson distribution revealed a significant positive effect of pollinators on the total sunflower seed set (x^2= 23.025 df= 5, $p < 0.001$). Tukey pairwise post-hoc comparisons revealed that *M. domestica* had a significantly lower total seed count compared to No pollination ($p = 0.001$) and Eristalinae ($p = 0.005$), but a significantly higher seed count compared to *A. mellifera* ($p = 0.046$). All other treatments showed no significant difference in total seed set.

Sunflower heads that had no insect pollination for the duration of the experiment showed the highest variation in the proportion of seeds pollinated while those exposed to *M. domestica* showed the least amount of variation in proportion of pollinated seeds (Figure 3.4B). A significant effect of pollinators on the proportion of pollinated sunflower seed set was detected using GLZ with quasibinomial distribution (x^2 = 42.47, df= 5, $p <0.001$). Tukey pairwise post-hoc comparisons revealed that No pollination had a significantly lower seed yield when compared to Open pollination ($p = 0.002$) and Syrphinae ($p = 0.016$). It also showed that *M. domestica* had a significantly higher seed yield compared to No pollination ($p <0.001$), Eristalinae ($p = 0.009$) and *A. mellifera* ($p = 0.016$). All other interactions had no significant differences. Eristalinae showed a non-significant 6.7% increase to the proportion of pollinated seeds when compared to No pollination treatment but a non-significant 6.4% decrease to the proportion of pollinated seeds when compared to Open pollination treatment. Similarly, Syrphinae showed a non-significant 3.2% decrease to the proportion of pollinated seeds when compared to Open pollination treatment. Syrphinae showed a non-significant larger proportion of pollinated seed in comparison to Eristalinae

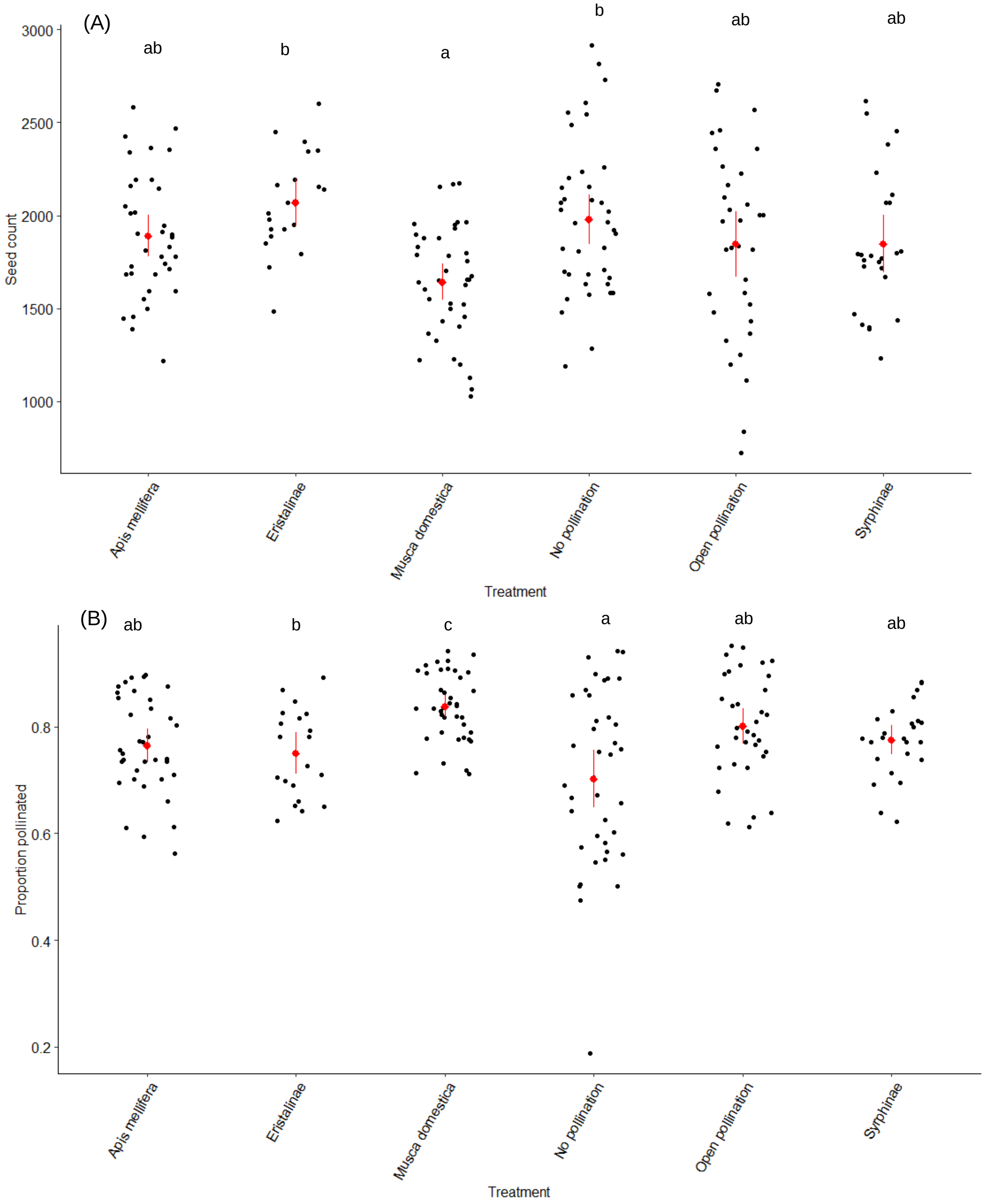

Fig 3.4. Strip plots showing the total seeds per sunflower head due to different pollinator treatments (A) and proportion of pollinated seeds due to different pollinator treatments (B). Significant differences are based on pairwise post-hoc analysis.

Discussion

In this study we aimed to determine the contribution of hover flies to sunflower seed set in comparison to other commonly found pollinators. Our study showed that the sunflowers had an average total seed set of 1978 with 70.24% being pollinated without the interaction of a pollinator. This suggests that pollinator interaction can produce a maximum of 30% increase in yield. The exclusion experiment revealed that Syrphinae had a 6.6% decrease in total seeds per sunflower head when compared to No pollination while Eristalinae had a 4.6% increase compared to No pollination. This would imply that the total seed set of sunflowers are affected by pollinator interaction. However, in sunflower the total seeds (pollinated and unpollinated) per head is based on the plant size, head size and plant genotype rather than pollinator interaction (Golparvar & Dehaghani, 2012; Kaya *et al.*, 2009). Kaya *et al.* (2009) showed that taller sunflower plants, bigger heads and bigger seeds all contributed to increasing seed yield. Golparvar & Dehaghani (2012) observed that plant height had a positive effect on seed yield while flower head size had a negative effect on seed yield. During field work for this experiment, it was noted that sunflower heads used for the enclosure experiment ranged between 150 and 250mm diameter in size as the voile covers were 300mm wide. As random sunflower heads were encased in the voile bags before they bloomed, head size could not be pre-determined or standardized. Unfertilized florets would lead to empty seed hulls forming on the sunflower heads (Dedio & Putt, 1980; Langridge & Goodman, 1981). Thus, it is more accurate to use the proportion of pollinated seeds to determine pollinator contribution under the assumption that no pollinator interaction would lead to a lower proportion of pollinated seeds.

All of the sunflower pollinator interactions led to an increase in proportion of pollinated seeds when compared to the No pollination treatment. This would suggest that hover flies do indeed contribute to sunflower pollination with Syrphinae being the higher contributor. This correlates to the results found in Chapter 2 showing a high proportion of the hover flies caught carrying pollen from sunflowers and other Asteraceae. Although there is limited research done on the effects of hover flies on sunflower pollination, a similar increase in oilseed rape seed set was associated with pollination by Syrphinae (*Episyrphus*) species (Jauker & Wolters, 2008), on sweet peppers seed set by two Syrphinae (*Eupeodes* Osten-Sacken, 1877 and *Sphaerophoria* Lepeletier & Serville, 1828) species (Pekas *et al.*, 2020), and on the number of fertilised strawberry seeds by mixed Syrphinae species (Hodgkiss *et al.*, 2018). Open pollination yielded a higher pollination result than all Syrphidae, suggesting an interaction between pollinators is preferred above a single species. Mallinger *et al.* (2019) also found that during their trials, open-pollinated sunflower heads yielded a 24% higher seed set compared to total pollinator exclusion heads while our study only showed a 14.3% increase in yield. The difference in seed set between our study and others could be due to the self-fertility of the sunflower hybrid used in the fields (Mallinger & Prasifka, 2017; Perrot *et al.*, 2019). Field observations during sampling did reveal both spiders and praying mantes present on the sunflower heads (other than trail heads) when florets were receptive. Apart from predators, hover flies have shown interaction with aphids as prey and competition with other aphidophagous predators (Ingels *et al.*, 2015). Some hover fly species, like *Eristalis tanex*

Linnaeus 1758, have shown territorial behaviour by aggressively interacting with pollinators of different species, like butterflies, bees and wasps (Wellington & Fitzpatrick, 1981). These interactions could lead to a higher movement of pollinators between sunflower heads, taking pollen between heads which could improve pollination to the open sunflower head trail. Interspecies pollination with other Asteraceae can occur in sunflowers which can lead to hybrids or sterile offspring (Christov & Hristova-Cherbadzhi; Kaya *et al.*, 2012). This could lead to increased pollination when pollinators move from the natural environment into sunflower fields.

Among sunflower pollinators, honey bees are seen as one of the most important pollinators due to both being an effective pollinator and the ability to bring large numbers of them to desired fields (Abbasi *et al.*, 2021; Chambó *et al.*, 2011). It was hypothesised that honey bees could potentially be a better pollinator for sunflowers due to its favourable morphological characteristics. When comparing the proportion of pollinated seeds of *A. mellifera* to Syrphinae and Eristalinae (Figure 3.4A) it is observed that Syrphinae resulted in a non-significant higher contribution than *A. mellifera* while Eristalinae resulted in a lower contribution. Thus, the resulting pollinated seed set by other pollinators is equivalent to those produced by Syrphidae species. Similar to these results, Jauker et al. (2012) found that bees produced similar results to hover flies in the pollination of oilseed rape. Another correlation found was that honey bees also showed a lower pollination contribution when compared to the Open pollination treatment just like hover flies did. Improvement to yield can thus be achieved if honey bees are not used alone, but in conjunction with a diverse pollinator community due to behavioural interactions and diversification of flower visits (Carvalheiro *et al.*, 2011; Greenleaf & Kremen, 2006; Mallinger *et al.*, 2019).

Musca domestica was chosen as an out group due to its pollination potential and high abundance within the study area during the initial farm visits. House flies showed a larger increase in pollinated seeds (19.3%) that any other treatment when compared to the No pollination treatment and was the only pollinator that had a higher pollinated seed set result than the Open pollination treatment. A study on onion pollinators in Pakistan showed *M. domestica* had a higher flower visitation rate than the observed bee species (*Apis dorsata* and *Apis florea*) and most of the observed Syrphidae species (Saeed *et al.*, 2008). Saeed *et al*. (2008) also proved that the house flies' pollination effectiveness was not significantly different to the most effective pollinators except for *A. dorsata*. It is assumed that the house fly was such a good pollinator in the enclosed space due to its small size and relative high activity which made it easier to pollinate the flower compared to the flight restrictions for hover flies due to space and the much larger size of the honey bee. Apart from Syrphidae and Muscidae, many other families from Diptera have been observed frequenting agriculture field, with their role in pollination poorly understood. These include Calliphoridae and Bombyllidae which were observed during sweet net sampling (Chapter 2) and hover fly collections for the exclusion experiment. Calliphoridae were in high abundance within the sunflower fields during collection but is also known to visit mangoes (Dag & Gazit, 2000; Saeed *et al.*, 2016), strawberries (Herrmann *et al.*, 2019), carrots (Howlett, 2012) and onions (Clement *et al.*, 2007). Bombyllidae was in relative low abundance within the sunflower fields during collections but are known to visit mangoes (Dag & Gazit, 2000), cashews (Heard *et al.*, 1990) and carrots (Bohart & Nye, 1960). This would suggest that many other potential Dipteran pollinators could be studied to improve our knowledge and understanding of their potential interaction with sunflowers and other crops.

This experiment used individually voile enclosed sunflower heads to exclude any pollination other than the target organisms similar to what was done by other researchers (Astiz *et al.*, 2011; Mallinger *et al.*, 2019). Alternatively, an entire field section could be enclosed in an exclusion material structure like a net dome (Terzić *et al.*, 2010). We chose to use single head enclosing to ensure a pollinator and sunflower head came into contact. Hover fly contribution to the seed set was determined by placing either a Eristalinae (*Eristalinus* sp) or Syrphinae (*Ischiodon aegyptius*) inside the voile bag. Initially, it was thought that the interaction between the bag and the sunflower head could be a potential cause for higher than expected pollination numbers. Mallinger *et al.* (2017) proved this to be incorrect when hand-pollinated sunflower heads both bagged and un-bagged showed similar yields, indicating that the interaction with the bags did not increase yield.

The results from this study would suggest that hover flies, honey bees and house flies are all potentially good pollinators for sunflowers. Sunflowers yield will receive a greater improvement if a diversity of pollinators could reach it instead of relying on just a single species. Thus, the mass rearing and release of hover flies of the correct species could benefit sunflower yield if the hover flies were to remain in the environment. This will require potential alterations in the way farming is done to accommodate the diversity and abundance pollinators needed to reach the maximum yield. It should be noted that care should be taken when interpreting the results with total seed set not being a viable measure for pollination efficiency as it is determined by the sunflower variety, nutrient availability and final head size of the plant rather than the number of florets pollinated. Understanding of the degree to which different farm practices, like strip planting, affect the efficiency of wild pollinators is lacking. A better understanding is still needed on other Diptera pollinator, including many Syrphidae species, for sunflowers and other agriculture crops.

Conclusion

In conclusion, hover fly species do pollinate sunflowers and thus contribute to the sunflowers pollinated seed set. Our data indicate that Syrphinae appears to be a better candidate for sunflower pollination than Eristalinae, however, only a small subset of the known species visiting sunflower fields have been tested. Hover flies showed similar pollination efficiency when compared with honey bees, showing they might be a suitable replacement as a mass released pollinator but cannot yet compete with the benefits of having a hive in a field that will provide continuous insect presence over the flowering period. Alternatively, other Dipteran pollinators, like *M. domestica*, can be utilized as they occur naturally in sunflower environments, can be mass reared, and can lead to a high degree of pollination.

Chapter 4: General discussion and future studies

This dissertation generates better understanding on the role of Syrphidae in the pollination of sunflowers (*Helianthus annuus* Linnaeus, 1753) in the Lehau region of the Waterberg district. Firstly, the species richness and diversity of hover flies within sunflowers fields and their surrounding vegetation was established throughout a growing season. Secondly, the pollen on the bodies of wild hover flies was determined along with the sunflower seed set contribution of two sub-families (Syrphinae and Eristalinae) under enclosed conditions for 24 hours. A literature review on Syrphidae and their interaction with agriculture crops revealed that a large number of pollinator dependent crop species are frequented by Syrphidae species worldwide (Doyle *et al.*, 2020). This review also showed a lack of research on diversity, richness and seed set contribution of hover fly species on sunflowers, especially in South Africa.

In Chapter 2, focus was firstly placed on establishing the species richness and diversity of hover flies within and surrounding sunflower fields throughout a growing season using standardised sweep netting. A total of 53 individuals were caught over five fields with Syrphinae being the predominant sub-family present. Taxonomic keys along with light microscopy was used to identify the caught specimens to species level. Only 11 species of hover flies were observed during sweep netting, however, near plateauing species accumulation curves would suggest that there are potentially only a few more species that could be observed within or surrounding the sunflower fields. Spatial and temporal patterns were also observed with the highest number of individuals caught near the field edge throughout the sampling timeframe, while the highest abundance of hover flies was observed when the sunflowers were in bloom. These spatial patterns match other observations in various agriculture fields and forests (Bennewicz, 2011; Söderman *et al.*, 2016; Sutherland *et al.*, 2001). Syrphid presence, abundance and diversity may have been influenced by the availability of floral resources provided by wild flowers in the disturbed areas surrounding the sunflower fields. This study reports higher species richness than in most reported studies (Ali *et al.*, 2015; Nderitu *et al.*, 2008; Rasheed *et al.*, 2015), but showed a lower abundance than some (Riedinger *et al.*, 2014). With this wide diversity there are many species that can potentially be used for pollination and investigated for mass rearing with *Ischiodon aegyptius* being the most likely candidate for Syrphinae and *Eristalinus tabanoides* for Eristalinae.

Chapter 2 also focused on the identification of wildflower interaction by hover fly species based on pollen found on the caught individuals compared to a pollen atlas created from pollen sampling during the growing season (Appendix A). A total of 21 species of flower pollen from 11 families were collected within a 100m radius of each sampled field, including sunflower pollen to form 15 distinct pollen morphological groups. This comparison showed that the majority of observed hover fly individuals frequented Asteraceae species especially the pollen group containing sunflowers. This would suggest at the very least that hover flies do visit sunflowers to obtain nectar and pollen as a food source as has previously been reported (de Oliveira *et al.*, 2019; Du Toit & Holm, 1992; Khaghaninia *et al.*, 2010; Lajos *et al.*, 2021; Tesfay, 2009; Thalji, 1992). This would also suggest that flowering Asteraceae species would be a good candidate for border cropping to potentially attract a higher abundance of hover flies to agricultural fields. The plant family wth the second highest representation on hover fly bodies was Solanaceae, however, unknown pollen was also discovered on the caught specimens which would suggest potential interaction with grass pollen (Poaceae) or pollen from flowers outside the 100m sampling radius. Analysis of the interaction showed that most hover flies were specialists, which contradicts to previous findings (Michelot-Antalik *et al.*, 2021). These findings create a better understanding of the interactions with wildflowers and their potential use as attractants for pollinators.

In Chapter 3 the aim was to determine the contribution that different pollinator species have on the seed set of sunflowers. This was done by enclosing sunflower heads in voile bags before they started to bloom, thus preventing any pollinator interaction until a single pollinator was introduced for 24 hours. This method for seed set contribution determination has previously been used by researchers successfully (Astiz *et al.*, 2011; Mallinger *et al.*, 2019), however, in contrast with their studies total exclusion of all insects was achieved rather than exclusion of only large insects. This experiment revealed that hover flies contributed between 6.4 and 6.7% to the proportion of pollinated seeds on sunflower heads, which showed that they can potentially hold value on large scale agricultural production. This aligns with other studies showing seed set increases on various agricultural crops due to hover fly pollination (Hodgkiss *et al.*, 2018; Jauker & Wolters, 2008; Pekas *et al.*, 2020). Honey bees showed a similar contribution to the proportion of pollinated seeds as hover flies did, however, both were outdone by the contribution of *Musca domestica*.

Mass-rearing of hover flies

This study shows that agriculture may benefit from hover flies as potential commercial pollinators that can be released into the fields alongside natural pollinators and honey bees to increase the pollination and yield of crops. Many crops rely heavily on the use of honey bees due to many natural pollinators declining in diversity and abundance. Laboratory rearing of different hover fly species has been successful, with adult flies being maintained on artificial diets or fresh pollen and a sugar source like raw sugar or honey (Campoy *et al.*, 2022b; Francuski *et al.*, 2014). Mated adult Eristalinae are also supplied with an oviposition medium like soaked cereal (Francuski *et al.*, 2014), spent brewers grain (Campoy *et al.*, 2022b), or water-soaked organic debris (Holloway & Ottenheim, 1994). This allows the emerging larvae to immediately start feeding. Larval rearing substrates vary between experiments with some preferring manure (Holloway & Ottenheim, 1994) while others used soaked grain mediums (Campoy *et al.*, 2022b; Francuski *et al.*, 2014) or decaying plant matter (Marcos-García & Pérez-Bañón, 2002). Gravid Syrphinae (aphidophagus) adults are provided with aphid-infested leaves or plants (Bellefeuille *et al.*, 2019; Fraser, 1972). Laboratory conditions are kept between 20 – 26 °C under 12 hours light: 12 hours dark conditions maintained at 50 – 80% relative humidity (Campoy *et al.*, 2022b; Francuski *et al.*, 2014; Fraser, 1972). Larval development is species dependent. For example, Syrphinae larvae have been shown to pupate after 14 days (Fraser, 1972) and emerge from the pupae after 21-28 days (Fraser, 1972). Eristalinae larvae pupated after 11-30 days (Campoy *et al.*, 2022b;

Holloway & Ottenheim, 1994), while pupae emergence occurred after 5-12 days (Campoy *et al.*, 2022b; Holloway & Ottenheim, 1994). Survival and performance of the individuals is also diet dependent for each species, suggesting that a lot of research will have to go into creating an optimal diet for larvae that will not necessarily be useable for other species.

There are, however, a few matters that need to be considered when trying to mass-rear these pollinators. First is that laboratory reared populations are susceptible to genetic changes due to inbreeding and bottleneck effects (Francuski *et al.*, 2014). This can lead to changes in morphology such as wing size, wing shape, colour and body size, which can negatively affect their performance in the field (Francuski *et al.*, 2014). These changes can also potentially affect survivability and mating with wild populations, as shown in other mass-reared flies (Economopoulos *et al.*, 1978; Pereira *et al.*, 2007; Weldon, 2005; Weldon & Taylor, 2011). One way to combat this genetic shift is to frequently bring in new individuals from wild populations. This ties in with the concern that released individuals perform as good or better in pollination as their wild counterparts. Some greenhouse trials demonstrated positive results with Syrphinae adults, with successful crop interactions such as aphid predation (Bellefeuille *et al.*, 2021; Pineda & Marcos-Garcia, 2008). However, Bellefeuille *et al.* (2021) also observed that released adults in a greenhouse would immediately fly to the top of the ceiling and bump into the glass instead of exploring the surrounding vegetation, bringing into question how well released flies will perform under open field conditions. Field trials of released adults showed that the adults were only present in the fields for up to 8 days (Pineda & Marcos-Garcia, 2008), which is not a problem if the crop's flowering time is short but it will require multiple releases if a crop's flowering time is over a long time period. This does suggest that hover flies still hold value as a mass-reared and released pollinator, however, careful consideration will need to be given to which native species is used, what they are reared on and how they are released.

Agricultural practices for wild pollinators

A general consensus in pollinator research is that improved wild pollinator diversity and abundance leads to a greater benefit in yield for crops due to the potential synergy between and different activities of pollinators. However, to achieve this increase in ecosystem services, changes in agricultural practice might need to be implemented. These changes can be divided into two categories: off-field practices and on-field practices (Garibaldi *et al.*, 2014). The most known off-field practice is the use of reservoirs in natural habitats that can provide nesting locations and various resources (Garibaldi *et al.*, 2014). Tested reservoirs consist mainly of unaltered wild landscapes or planted patches of various wild flowers near crop fields (Carvalheiro *et al.*, 2012; Venturini *et al.*, 2017). Many studies reveal that the size and diversity of these reservoirs has a positive effect on the pollinator diversity present, however, distance from the field also plays a significant role in the amount of pollinators present and distance into the field they were present (Carvalheiro *et al.*, 2012; Garibaldi *et al.*, 2011; Venturini *et al.*, 2017). This suggests that reservoirs need to be close to target fields. Another off-field practice is the planting of flowering plants as border crops (Garibaldi *et al.*, 2014), which has a similar purpose as reservoirs in providing resources that pollinators require and to facilitate further movement into the fields by pollinators.

Both of these off-field practices require the use of the correct flowering species to attract the desired pollinators. In this study, Asteraceae and Solanaceae were found to be the flowering families with pollen most often encountered on hover fly bodies. However, interactions with many more flowering plant families were found. The optimal way to use wildflowers is by understanding the native diversity of flowering plants that desired pollinators interact with, and utilising different flowering times (Isaacs *et al.*, 2009). This allows for a design where there are always resources available during the time that the crop requiring pollinators will bloom (Isaacs *et al.*, 2009). This also diversifies the resources available, attracting a wide range of pollinators (Garibaldi *et al.*, 2014).

On-field practices requires alteration to how the crop area is designed or an alteration to what is applied to the fields. One such alteration is how insecticides are applied to the field. Insecticides should only be applied, if necessary, outside of the flowering times. This will reduce the chance that beneficial insects are exposed (Garibaldi *et al.*, 2014). However, when considering Syrphinae larvae as a biocontrol agent, insecticides need to be avoided as this will also kill the beneficial larvae that are present prior to crops blooming. Another suggested alteration is the reduction of field sizes or field widths. This is done to allow floral resource strips to be placed along or in between crops, reducing the distance pollinators are required to travel (Garibaldi *et al.*, 2014). This is done as it has been found that pollinators rarely travel further than one kilometre from nesting sites (Garibaldi *et al.*, 2011), which is further supported by my findings showing that some pollinators, like hover flies, do not travel long distances into monoculture sunflower fields, but carried pollen from more than 100m in natural habitats. These suggested options for improving wild pollinator abundance need to be weighed using the costs and benefits for the farmer, especially as some of these practices will not show results immediately but over time. It is, however, important to note that in order to improve the chances for mass-rearing to have a positive effect, it needs to be integrated with agricultural practices to create the optimal environment for success.

Future Studies

This dissertation shows that despite the global pollinator decline and threat to sufficient production, there is promise in the mass-rearing of hover flies to add diversity to natural pollinators and relieving the need of honey bees as sole pollinator in many crops. It is still unclear as to which species would be the most ideal to mass-rear with Syrphinae species like *I. aegyptius’* larvae being aphidiphagous, thus making lab rearing more complicated, while many of the Eristalinae larvae being detritus feeders’ eases lab mass rearing. This also revealed that other Dipteran species might be worth investigating for the potential in agriculture pollination. To effectively choose a species to mass-rear for sunflower fields, further investigation is needed in diversity and interaction of hover flies in other sunflower regions of South Africa.

Conclusion

This study showed that a diverse assemblage of hover flies may visit sunflowers, with Syrphinae showing higher visitation than Eristalinae. When sunflower heads are not yet presents, hover flies would frequent mostly Asteraceae species followed by Solanceae species based on the pollen present on sampled individuals. Syrphinae and Eristalinae also produce similar pollination contribution as seen with honey bees and Muscidae, indicating their potential for improved crop yield. Alterations to agricultural practice like buffer strips or natural habitat reservoirs with a diverse vegetation could improve the natural assemblage of wild pollinators, including hover flies. Alternatively, mass-rear and release of pollinators, like *Ischiodon aegyptius* (Syrphinae), can hold benefit for increasing crop yield, not only on sunflowers but many fruiting pollinator dependent crops. Further research still needs to be done on how effective mass-rearing would be with individual species and how to rear them with minimal to no negative effects on performance. A better understanding of hover fly diversity in other sunflower regions of South Africa is also needed before the best candidate can be chosen.

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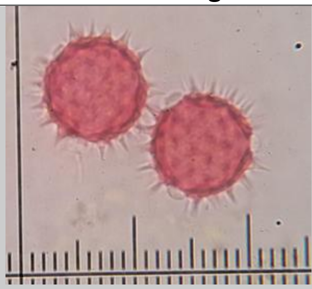
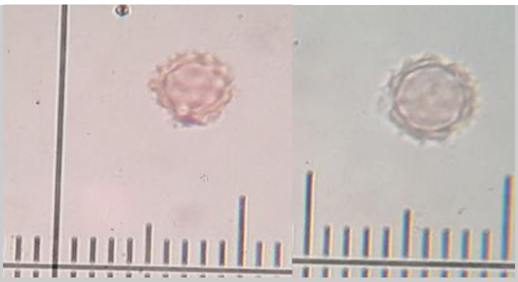
Appendix A: Pollen atlas with flower photos taken in the field and pollen photos taken at 40× magnification using a light microscope (Wild Leitz GMBH biomed light microscope).

Asteraceae Group 1	**Pollen at 40x magnification**



Asteraceae Group 2

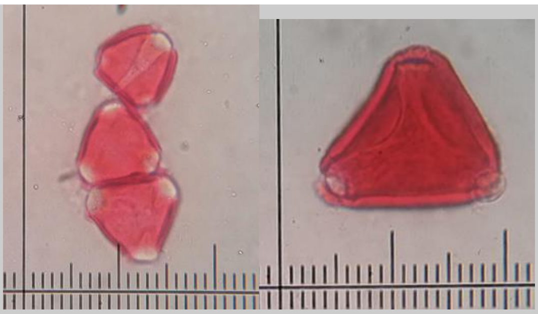


Asteraceae Group 3

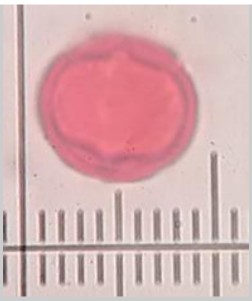
Commelina benghalensis

Cucumis myriocarpus

Fabaceae group 1

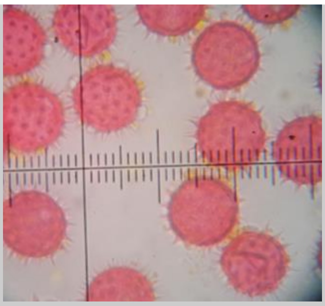


Salvia sp

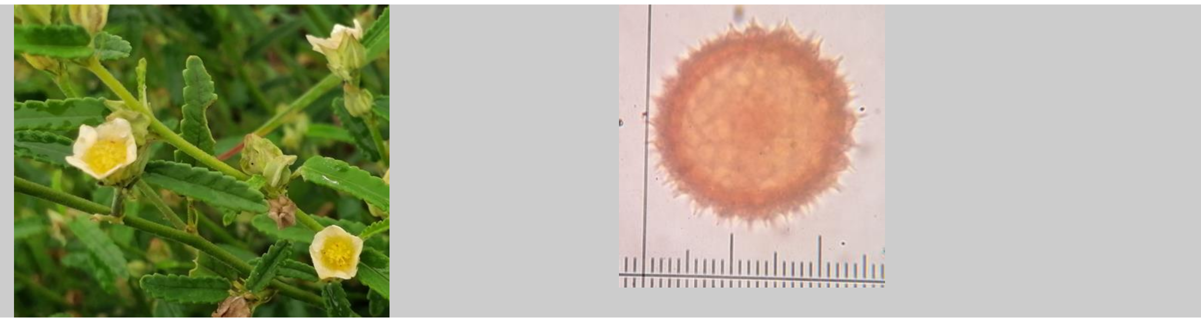


Corbichonia sp

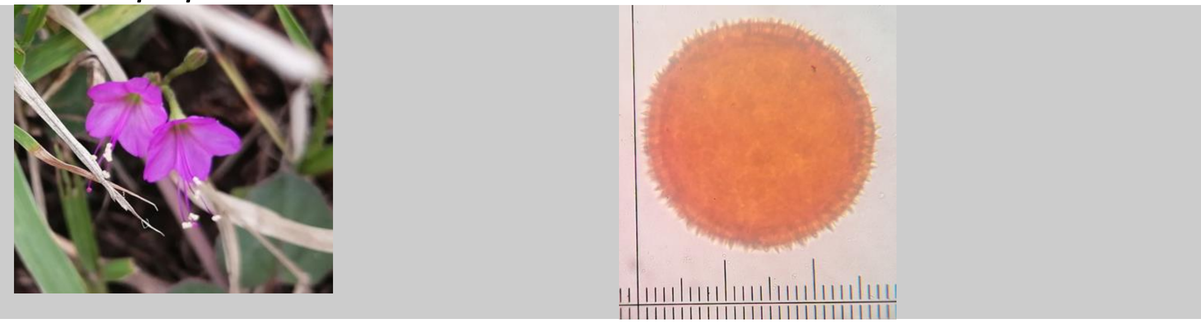
Hibiscus trionum



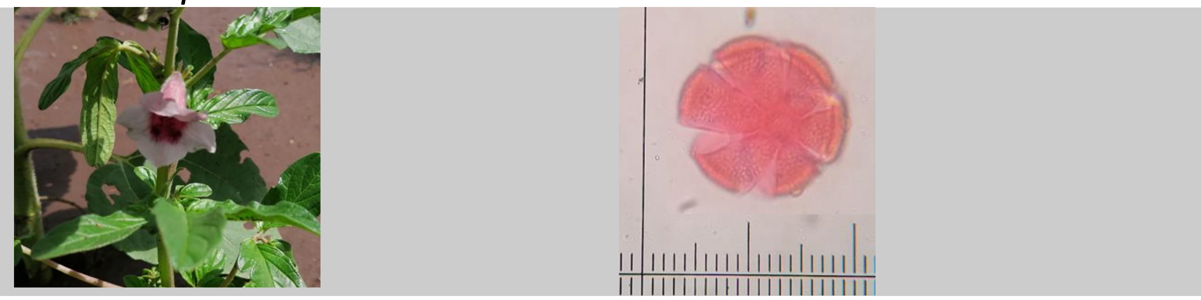
Sida acuta



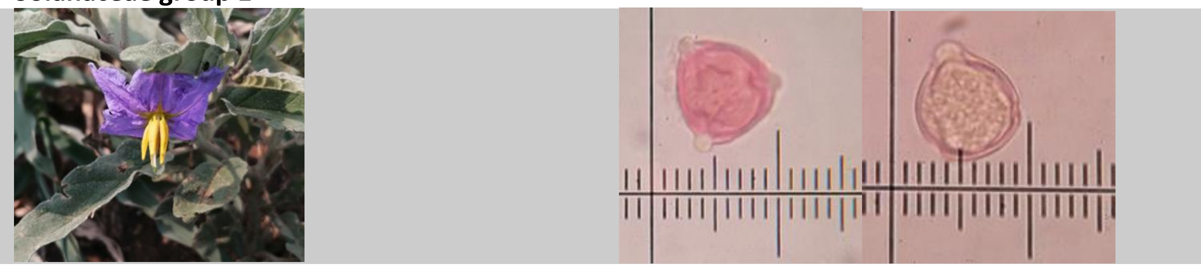
Commicarpus pentandrus



Ceratotheca sp



Solanaceae group 1



Solanaceae group 2

Tribulus terrestris

Appendix B: Individual sampled species' interaction with identified pollen.

ID	Asteraceae group 1	Asteraceae group 2	Asteraceae group 3	Commelinaceae	Fabaceae	Lamiaceae	Lophiocarpaceae	Pedaliaceae	Solanaceae group 1	Solanaceae group 2	Zygophyllaceae	Unidentified group 1	Unidentified group 2	Unidentified group 3	
Syrphinae															
Allograpta nasuta										■					
Allograpta nasuta	■									■					
Asarkina sp			■		■			■		■					
Eupeodes corollae															No pollen
Eupeodes corollae			■												
Eupeodes corollae		■				■									
Eupeodes corollae															No pollen
Ischiodon aegiptius	■														
Ischiodon aegiptius	■		■				■	■							
Ischiodon aegiptius															No pollen
Ischiodon aegiptius															No pollen
Ischiodon aegiptius				■											
Ischiodon aegiptius							■				■				
Ischiodon aegiptius															No pollen
Ischiodon aegiptius		■					■								
Ischiodon aegiptius		■													
Ischiodon aegiptius	■		■												
Ischiodon aegiptius	■								■						
Ischiodon aegiptius	■		■						■						
Ischiodon aegiptius	■		■												
Ischiodon aegiptius	■														
Ischiodon aegiptius	■														
Ischiodon aegiptius			■					■				■			
Ischiodon aegiptius	■	■												■	
Ischiodon aegiptius															No pollen
Ischiodon aegiptius			■												
Ischiodon aegiptius			■												
Ischiodon aegiptius	■		■												
Ischiodon aegiptius	■		■							■					
Ischiodon aegiptius			■	■					■						
Ischiodon aegiptius			■												
Ischiodon aegiptius															No pollen
Ischiodon aegiptius			■						■						
Ischiodon aegiptius		■													
Ischiodon aegiptius															No pollen
Ischiodon aegiptius									■						
Ischiodon aegiptius			■												
Ischiodon aegiptius															No pollen
Pandasyopthalmus haemorrhous	■	■													
Eristalinae															
Eristalinus quinwuelineatus	■		■						■						
Eristalinus tabanoides	■		■					■							
Eristalinus tabanoides	■							■			■				
Eristalinus tabanoides	■		■	■		■		■				■			
Eristalinus tabanoides	■		■					■					■		
Eristalinus tabanoides							■	■		■					
Eristalinus taeniops	■														
Eristalinus taeniops	■		■												
Eristalinus taeniops	■		■		■				■						
Phytomia incisa	■														
Senaspis haemorrhoa	■	■	■												
Syritta flaviventris	■	■	■												
Syritta flaviventris	■		■												
Syritta flaviventris			■												