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Understanding and Managing Avocado
Pollination in Chile.

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Declaration of original authorship

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

Insect pollinators provide a critical ecosystem service by increasing the yield and quality of many globally important crops, with both managed and wild pollinators playing an important role. The protection of pollinators has gained increasing attention in recent years due to threats to pollination services such as climate change, pesticide usage, and natural habitat loss. However, implementing effective protective measures is challenging, as the level of crop dependency on insect pollination and the specific pollinator species important for different crops are often unknown. Moreover, the adoption of pollinator-friendly land management practices by growers frequently requires external support, which is often not available in many regions. The private sector has a vested interest in safeguarding pollinators given that numerous companies rely on insect-pollinated products within their supply chain and, thus, they have the potential to play a key role in supporting growers. However, at present, only a limited number of companies take action on pollinator protection, largely due to a lack of understanding regarding the risks faced by pollinators and the most effective support mechanisms.

To investigate some of these knowledge gaps, this thesis used avocado (*Persea americana*) as a study crop. Avocado is a globally important insect-pollinated fruit, for which little is known regarding pollination dependency, the contribution of wild pollination services and effective tools for sustainable management of avocado pollination. The first objective was to determine the extent of the contribution of insect pollinators to avocado production, and which insect taxa are the most important pollinators in different growing regions. The second objective was to investigate the impact of proximity to natural habitats on pollinators in avocado orchards, with a focus on the important avocado-growing region of Chile. The final objective was to develop a tool that private sector companies can employ to develop effective strategies for safeguarding pollinators, using an avocado supplier company as a case study.

Chapter 2 involved a literature review and meta-analysis of existing avocado pollination studies and showed that insects contributed greatly to pollination, fruit set, and yield. Honeybees (*Apis mellifera*) were important pollinators in many regions due to their efficiency and high abundance, however, many wild pollinators also visited avocado flowers and were the most frequent visitors in over 50% of studies. Stingless bees (*Meliponini* spp) and blow flies (*Calliphoridae* spp) were identified as effective avocado pollinators, although for the majority of flower visitors' data on pollinator efficiency was lacking.

Chapter 3 reports findings from pollinator surveys and controlled pollination experiments in three avocado orchards in central Chile. The result showed that over 70 different insect species visited avocado flowers and that wild pollinator abundance, visitation rate, diversity, and richness were significantly higher in areas directly adjacent to a natural habitat border. The pollinator exclusion experiments showed that insect pollinators contributed significantly to avocado production, with almost no fruit set when pollinators were excluded. Hoverflies and flies were identified as effective avocado pollinators due to their high flower visitation rate, with fruit set positively correlated with the abundances of these taxa.

Finally, Chapter 4 developed a tool to assist companies in formulating and implementing effective pollinator protection strategies. The tool comprises of seven activities, including new and existing desk-based methodologies, grower surveys, and informant interviews. The activities will help companies to 1) understand the threats to pollinators in different supply regions, 2) recognize the significance of pollinators to their business 3) assess the current implementation of pollinator actions, and 4) identify additional measures to better support pollinators. Application of this tool to an international company sourcing and supplying avocado indicated that increasing knowledge transfer to growers and supporting their participation in environmental certification schemes could serve as effective strategies for pollination protection.

The overall results from this thesis underscore the importance of insect pollinators in avocado production, with wild pollinators and the natural habitats which support them playing significant roles. To optimise yields, growers should implement land management practices that protect and restore natural areas within and around their orchards. Furthermore, the industry tool developed in this thesis provides private sector companies with a means to enhance pollinator protection by providing a mechanism to develop effective safeguarding strategies. Its potential implementation could greatly benefit growers and pollination services worldwide.

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1. Chapter 1: Introduction

1.1. Ecological intensification

In recent years, there has been growing concern around the sustainability of global food production. The rapid growth of the population, coupled with changes in global diets such as an increase in meat and dairy consumption, has led to a significant rise in the demand for food (Bajželj et al., 2014; Springmann et al., 2016; Tilman et al., 2011; Tilman & Clark, 2014). Simultaneously, food production is facing challenges due to issues such as climate change, land degradation, and diminishing land availability (Godfray et al., 2010; Godfray & Garnett, 2014). Furthermore, these challenges are expected to be exacerbated in the future as climate change and land degradation intensify and continued population growth leads to a 35-65% increase in global food demand over the next 30 years (Tilman et al., 2011; Van Dijk et al., 2021).

In many high-income countries, food production is dominated by conventional agriculture. Typically, this is characterized by monocultures and a high reliance on external, often synthetic, inputs like chemical fertilisers, pesticides, and irrigation. Although conventional agriculture is generally high yielding, it is commonly associated with environmental damage (Foley et al., 2005; Garnett et al., 2013; Tilman et al., 2001). For instance, it significantly contributes to climate change as nitrogen-based fertilizers, soil tillage, and agricultural mechanisation release greenhouse gases. Furthermore, practices such as land homogenisation, land fragmentation, and high pesticide use frequently lead to biodiversity loss and the degradation of regulating ecosystem services such as pollination, water regulation, and nutrient cycling (Foley et al., 2005; Ramankutty et al., 2018; Raven & Wagner, 2021; West et al., 2014). Consequently, this can negatively impact yields since agricultural productivity is highly reliant on these services (Dainese et al., 2019; Martin et al., 2019; Power, 2010; Zhang et al., 2007).

In response to these concerns, there has been a call for a global shift in the way we produce food. One proposed alternative to achieve sustainable production is 'ecological intensification' (EI). This promotes the increased utilisation of ecological processes and ecosystem services, such as soil fertility, pest regulation, and pollination, to produce food, instead of relying on external inputs (Bommarco et al., 2013). Some common practices utilised under this system are the enhancement of species diversity, restoring natural areas, and the reduction of synthetic inputs (Garibaldi et al., 2019). Several studies have been conducted to assess the effectiveness of EI and, in general, there is increasing evidence that EI can enhance or maintain agricultural productivity while reducing environmental damage (da Silva et al., 2021; Garibaldi et al., 2017; Kleijn et al., 2019; Power, 2010; Pywell et al., 2015; Redlich et al., 2021). However, the output from EI approaches can vary depending on the practises implemented and the environmental context. Therefore, further research is needed to determine the most effective suite of management practices for particular farm types in different landscape contexts.

1.2. Pollination services

Pollinators provide an important ecosystem service as they significantly increase the yield of many crops. Although the level of pollinator dependence varies by plant species, approximately 75% of the world's leading crops have demonstrated improved production due to animal pollination, with about 35% of total agricultural production relying on pollinators (Klein et al., 2007). Furthermore, pollinators can enhance crop quality. Studies have shown that insect pollinators can significantly improve the quality and marketability of crops such as apples (Garratt et al., 2014), strawberries (Wietzke et al., 2018), oilseed rape, and buckwheat (Bartomeus et al., 2014), leading to positive effects on farmer profit, and land-use efficiency. Additionally, pollinators have a profound effect on human health by contributing substantially to the production of essential vitamin and mineral-rich fruits and vegetables (Chaplin-Kramer et al., 2014; Eilers et al., 2011; Hristov et al., 2020; Potts et al., 2016).

1.2.1. Threats to pollination services

Despite these benefits, pollination services are under threat from various factors. While measuring precise contributions from different causes is complex, it is generally accepted that factors such as land use change, intensive agricultural practices, pesticides, climate change, invasive alien species, and pathogens are all exerting significant negative pressure on pollinator diversity and abundance (Figure 1.1) (IPBES, 2016; Potts et al., 2016). Many of these threats are particularly pronounced in agricultural landscapes, resulting in diminishing pollination services (Garibaldi et al., 2013; Steffan-Dewenter & Westphal, 2008) and thus pollination deficits and reduced yield potential (Garibaldi et al., 2016; Klein et al., 2007; Reilly et al., 2020). Consequently, there is substantial emphasis on enhancing pollination services through either managed or wild pollinators.

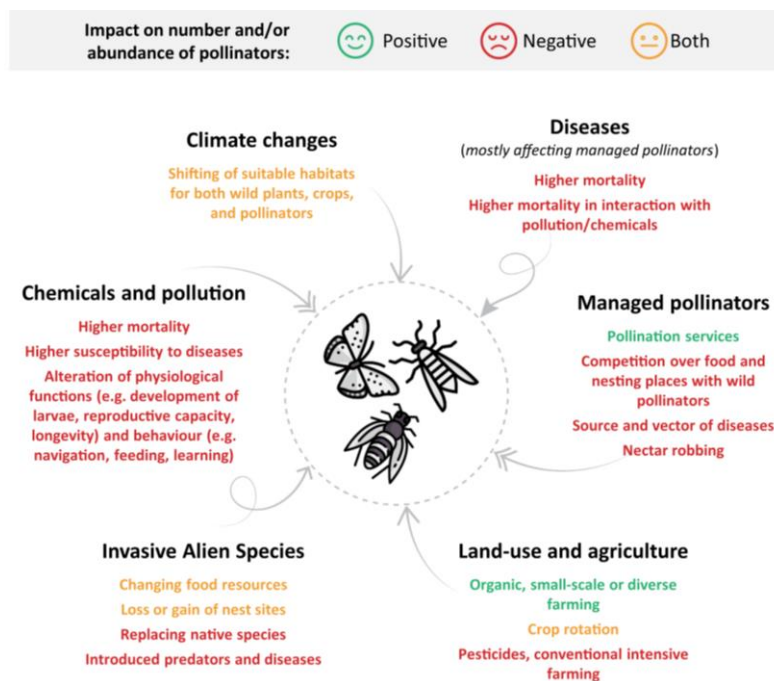


Figure 1.1 Summary of different pressures on pollinators (European Court of Auditors, 2020)

1.2.2. Managed pollinators

In many pollinator-dependent crops, farmers employ managed pollinators. During flowering periods, agricultural fields are stocked with high numbers of beehives to ensure sufficient crop pollination. Worldwide, there are around 20 insect species that are managed for pollination including bumblebees

(*Bombus* spp), stingless bees (*Meliponini* spp), and solitary bees (e.g. *Osmia* spp.) (Mallinger et al., 2017, however, honeybees (*Apis mellifera*) are often the most effective and economical option for growers (Isaacs, et al., 2017) and therefore, are the most widespread globally (DeGrandi-Hoffman, 2003; IPBES, 2016). Honeybees are well-suited to this role due to their ability to forage on a wide range of crops and their natural tendency to live in colonies, providing a large number of pollinators when needed (Delaplane & Mayer, 2000; Free, 1993). Managed honeybees significantly contribute to global food production; estimates suggest they carry out approximately half of the economic value of pollination services in North America and Europe (Kleijn et al., 2015).

There is however a growing concern that managed honeybees are insufficient to supply pollination demand (Breeze et al., 2014). In recent years, there have been significant declines in honeybees in Europe and North America due to bee diseases, commonly spread through varroa mites (IPBES, 2016). Coupled with the increases in pollinator-reliant crops, this has led to demand outstripping the availability of managed pollinators in many regions (Aizen & Harder, 2009; Winfree et al., 2007). Moreover, there are additional benefits that can come only from wild pollinators, such as enhanced production and crop quality; therefore, there is an increasing focus on integrating them into agricultural systems (Garibaldi et al., 2011; Garibaldi et al., 2013; Isaacs, et al., 2017).

1.2.3. Wild pollinators

Wild pollinators encompass a wide range of species and taxa, including bats, birds, mammals, and insects, and there are huge variations in the types of plants they pollinate and their pollination efficiency (Ollerton, 2017). However certain insects, particularly bees, are generally considered the most efficient and abundant pollinators in most agricultural systems (Klein et al., 2007).

1.2.3.1. Contributions from wild pollinators

Pollinators often represent a limiting resource, making higher abundances of wild pollinators crucial for increasing pollen deposition and ultimately, increasing fruit set and fruit size (Blaauw & Isaacs, 2014; Garibaldi et al., 2014; Garibaldi et al., 2016; Garibaldi et al., 2017; Hoehn et al., 2008; Mallinger & Gratton, 2015; Vergara & Badano, 2009). Moreover, pollinator diversity enhances pollination through sampling selection, niche complementarity, and functional facilitation. Sampling selection increases the likelihood of an efficient pollinator being present for a specific crop (Klein et al., 2009); niche complementarity means that different pollinators are available at different times and locations, leading to a more comprehensive service (Blüthgen & Klein, 2011; Hoehn et al., 2008; Senapathi et al., 2021); and functional facilitation occurs when certain wild species displace honeybees, promoting outcrossing and improved pollination (Greenleaf & Kremen, 2006). These mechanisms should significantly increase yield and several studies quantify this. A review by Garibaldi et al. (2013) showed that, across 41 crops, increases in wild pollinators enhanced yield twice as much as an equivalent increase in managed honeybees. Additionally, studies have shown quality improvements in crops due to enhanced pollinator diversity, such as blueberry uniformity and mass (Nicholson & Ricketts, 2019) and improved marketability of leek hybrid seeds (Fijen et al., 2018).

However, a review by Kleijn et al. (2015) highlighted that pollination services are primarily reliant on a small number of wild pollinator species. It is estimated that roughly 2% of bee species are responsible for 80% of visits to cultivated plants in Europe and North America. Whilst this finding suggests some uncertainty on the necessary level of biodiversity, this assessment doesn't account for the spatial and temporal stability and resilience benefits linked with higher biodiversity. Pollination resilience improves as biodiversity increases, as declining species can be supplemented by others filling the same functional role (Blüthgen & Klein, 2011; Winfree & Kremen, 2009). Furthermore, greater diversity provides a more stable pollination system. A recent meta-analysis by Senapathi et al. (2021) showed that in areas with higher pollinator diversity, interannual crop pollinator stability was greater, thus increasing the likelihood of interannual yield stability. Additionally, other studies have

revealed that during extreme weather events like drought and high winds, locations with higher pollinator diversity have significantly higher yields than those with low pollinator diversity, as different species can do the same role under different weather conditions (Brittain et al., 2013; Mukherjee et al., 2019; Rogers et al., 2014). Given future climate predictions of intensified weather extremes, the importance of pollinator diversity is likely to grow. Moreover, climate change is expected to increase temporal mismatches between flowering and pollinator emergence (Gérard et al., 2020), making a broader range of pollinators valuable in mitigating this phenological mismatch (Bartomeus et al., 2013).

1.2.4. Pollinator efficiency

For the majority of crops, a wide diversity of wild and managed pollinators visit their flowers. However, pollination efficiency varies significantly among species due to differences in physical characteristics and behavioural traits that can impact key efficiency variables such as floral resource collection time, visitation rate, and pollen deposition (Rivest & Forrest, 2020). Quantifying the pollinator efficiency of different taxa provides crucial insights into which pollinators are important for different crops. This understanding is vital for effective pollinator management, as it allows for the adaptation of conservation strategies specific to these particular species (Ne'eman et al., 2010). For numerous crops, the most effective pollinator taxa remain unidentified.

1.2.5. Natural Habitats, pollinators, and agricultural yield

Natural and semi-natural habitats (referred to as natural habitats from hereon in) play a critical role for wild pollinators, as they rely on these areas for food, shelter, ovipositional plants, and nesting. Across the globe, natural areas are decreasing, and it is thought that this has contributed to the decline in both the diversity and abundance of wild pollinators (Winfree et al., 2009).

Numerous studies have shown that the proximity of agricultural land to natural areas correlates with increased pollinator biodiversity and abundance, attributed to spillover effects (Gonzalez-Chaves et al., 2020; Hipólito et al., 2018; Hipólito et al., 2019; Klein et al., 2003; Ricketts, 2004; Sritongchuay et al., 2019). However, the extent to which this impacts agricultural yield is under debate. On one hand, it is thought that natural habitats don't uniformly benefit all species, and a few generalist pollinators can thrive in monoculture landscapes, thereby efficiently fulfilling pollination services (Bartomeus & Winfree, 2013; Ghazoul & Koh, 2010). However, only a few studies have substantiated this claim and recent evidence suggests that natural areas are linked with increases in agricultural yield. For instance, a global analysis of 89 studies by Dainese et al. (2019) demonstrated that land simplification leads to species richness declines, negatively affecting yield. Similarly, Martin et al. (2019), using data from many studies, showed that arable landscapes with high edge densities achieved high yields compared to landscapes with low edge densities. These findings underscore the importance of wild pollinators and natural habitats to agricultural yield. Nonetheless, much of this research has been conducted in Europe and North America. Therefore, further research is necessary to understand the significance of natural habitats for wild pollinators and agricultural yield in different growing regions, crops, and climatic environments.

1.3. Pollinator protection

1.3.1. Farm practices

While the preservation and recreation of natural habitats is the most prominent factor in pollinator protection (Dicks et al., 2021; Duque-Trujillo et al., 2023), a range of integrated crop pollination strategies can be employed at the farm level to manage and protect pollinators (Isaacs et al., 2017). For instance, providing feeding and nesting resources, like flora strips (Kovács-Hostyánszki et al., 2017), adopting diversified farming systems, and reducing pesticide application, can all contribute to supporting pollinator populations (IPBES, 2016).

Despite the proven effectiveness of these practices, their adoption among farmers in many parts of the world remains low due to execution challenges, including high implementation costs (Batáry et al., 2015) and limited knowledge of suitable practices (Isaacs et al., 2017). Consequently, in certain countries, governmental support incentivises implementation. For example, agri-environmental schemes in the EU offer financial compensation to growers for implementing various conservation measures. These often include the provision of floral resources beneficial for pollinators (Batáry et al., 2015). However, in many countries, governments lack the financial means and/or political willingness to fund such environmental services. Therefore, other entities such as private sector companies and NGOs can potentially play a pivotal role in providing support to growers (Garibaldi et al., 2019).

1.3.2. Private sector strategies

Many businesses, especially large food companies, have a vested interest in protecting pollinators, as they rely heavily on products that are insect-pollinated throughout their supply chains. The decline of pollinator populations could lead to production instability and potential scarcities of specific goods, disrupting supply chains and elevating purchase costs due to decreased global availability (Murphy et al., 2022; Tremlett et al., 2020). Furthermore, neglecting pollinator protection might expose companies to reputational risks, given that consumers are becoming more aware of pollinator decline issues and might reject products perceived as harmful to pollinator populations (Hoshida et al., 2018).

Companies also possess the power to effect change in this domain, influencing and shaping on-farm practices by implementing pollinator strategies throughout their industry. Currently, a small number of supermarkets and retailers, such as [The Co-operative](#) and [Marks and Spencers](#), have active pollinator strategies, mandating specific conservation practices for their growers. However, overall action among companies remains limited due to an insufficient understanding regarding pollinator dependence for specific crops, uncertainty about vulnerability stemming from pollinator declines, and a lack of evidence demonstrating the return on investment associated with implementing pollinator

strategies (University of Cambridge Institute for Sustainability Leadership et al., 2018). Furthermore, the private sector lacks accessible information on devising effective strategies which diminishes the impetus to take action. The Cambridge Institute for Sustainability et.al. (2018) have developed a high-level process that companies can employ to formulate effective pollinator strategies, but a simplified and tangible tool is necessary to facilitate and incentivise application.

1.4. Avocado production

To enhance our understanding of the role of wild pollinators and natural habitats in commercial agricultural production and to identify effective support mechanisms, this thesis utilises avocado (*Persea americana*) as a study crop. Avocado is a tropical evergreen tree belonging to the Lauraceae family, originally from Central America. It encompasses three distinct landraces; Mexican, Guatemalan, and West Indian (Popenoe, 1920; Popenoe 1934) along with multiple different cultivars (Newett et al., 2002).

Avocados evolved in tropical, high-altitude regions and are well adapted to high rainfall and warm climates (Schaffer & Whiley, 2002). However, over time, cultivars have been developed, enabling avocados to be grown outside of their original climate region. For instance, Mediterranean climates can serve as productive growing regions, although they usually require additional inputs, particularly irrigation (Schaffer & Whiley, 2002). Due to the brittle nature of avocado wood and the fruit's susceptibility to damage, orchards are ideally located in areas with minimal wind exposure (Schaffer & Whiley, 2002). While avocados can be cultivated in a range of soils with modest nutrient demands (Lahav & Kadman, 1980), proper drainage and aeration are vital as the roots are susceptible to phytophthora root rot. Commercial orchards under optimal conditions can yield around 12 tonnes per hectare annually, however, due to alternate bearing, production varies annually, even within the same location (Whiley, 2002).

In recent years, avocados have gained recognition for their high nutritional value (Weschenfelder et al., 2015) leading to an increase in global demand and their economic value. Consequently, avocado production has surged over the past decade. Global production was around 4.2 million tonnes in 2011 and rose to approximately 8.8 million tonnes in 2021 (FAO, 2023). While avocados are produced in several countries including Colombia, Peru, Chile, Dominican Republic, Kenya, and Indonesia, Mexico holds the largest share with around 30% of global production (FAO, 2023).

1.4.1. Avocado flowering and pollination

Avocado flowers possess functional male and female parts that open at different times of the day. Avocado cultivars fall into two categories (Type A and Type B) depending on the timing of their male and female parts' opening. 'A-type' cultivars are female in the morning of the first day and will then open as male in the afternoon of the second day, while 'B-type' cultivars are male in the morning of the second day and female in the afternoon of the first day (Nirody, 1922). This process encourages cross-pollination; however, avocados are self-fertile, allowing for pollination from the same tree or cultivar during the daily flowering overlap (closed pollination) (Nirody, 1922; Stout, 1932). Though self-pollination (within the same flower) is technically possible, it has not been observed to result in successful fertilisation (Sedgley, 1977; Sedgley & Grant, 1983).

Due to this reliance on closed and cross-pollination, insect pollinators are considered essential. Several studies have highlighted a positive relationship between pollinator abundance and yield (Ish-Am & Lahav, 2011; Peña & Carabalí, 2018), and other studies have shown, using controlled pollination trials, that yield, fruit set, and pollination are all significantly higher in insect pollination treatments, in comparison to pollinator exclusion treatments (Figure 1.2) (Cabezas & Cuevas, 2007; Can-Alonzo et al., 2005; Malerbo-Souza et al., 2000; Mulwa et al., 2019; Petersen, 1955; Robbertse & Johannsmeier, 1997; Sagwe et al., 2021). There are some exceptions, with research in Florida and California suggesting that wind plays a dominant role in pollination (Davenport, 2019; Davenport et

al., 1994). This, however, has not been observed in other locations and therefore likely stems from climatic variations and avocado races present in these areas (Wysoki et al., 2002).

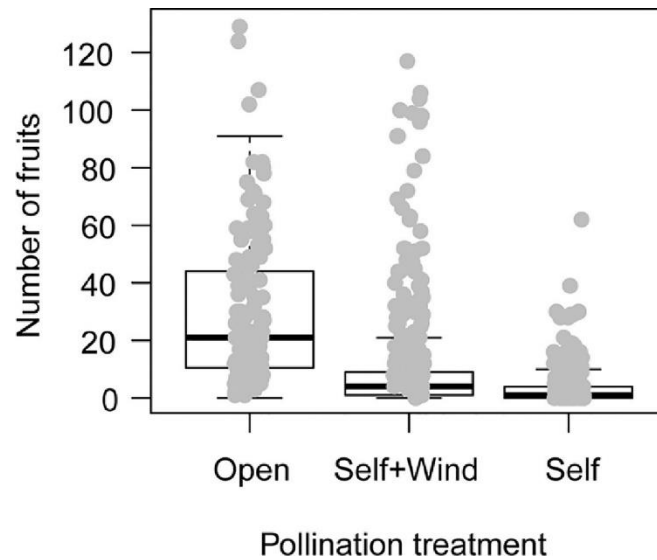


Figure 1.2. The number of avocado fruit set in different pollination treatments (open, self + wind, self). Dots show fruits set per tree. Box and whisker plots show the median, the quartiles and the extreme values (Sagwe et al., 2021).

Given the reliance on insect pollination, many commercial orchards deploy managed honeybees (Perez-Balam et al., 2012). However, it is also known that wild pollinators contribute to pollination and yield improvements (Gazit & Degani, 2002; Sagwe et al., 2021; Vithanage, 1990) with several studies across the globe documenting diverse wild taxa contributing to pollination with many proving to be highly effective (Bushuru, 2015; Can-Alonzo et al., 2005; Carabalí-Banguero et al., 2020; De la Cuadra-Infante, 2007; Evans et al., 2011; Ish-am et al., 1999; Monzón et al., 2020; Okello et al., 2021; Perez-Balam et al., 2012; Vithanage, 1990; Willcox et al., 2019, Figure 1.3). For example, in Kenya, Mexico, and Australia, wild bees have been shown to visit a similar number of flowers and deposit a comparable amount of pollen compared to honeybees (Bushuru, 2015; Can-Alonzo et al., 2005; Perez-Balam et al., 2012; Vithanage, 1990; Willcox et al., 2019). In the majority of key avocado-growing

regions however, little is known about the pollination services provided by wild pollinators, or the contribution from specific species.



Figure 1.3. Examples of wild pollinators visiting avocado flowers. Photos taken by Jaime Martinez-Harm during field work in Chile.

1.4.2. Agronomic inputs to avocado production

1.4.2.1. Soil fertility

The evolution of avocados in mountainous tropical regions makes them adapted to low fertile soils with high amounts of leaf litter and as such, their mineral and nutrient requirements are low compared to other fruit crops (Wolstenholme, 1991). However, avocados can benefit from mulching (Moore-Gordon et al., 1997; Schaffer & Whiley, 2002; Wolstenholme, 1991), and applying fertilisers enhances yield. Fertilisation needs differ based on orchard soil quality, but in general, nitrogen has the greatest impact on tree growth and fruit production, with phosphorus and potassium requiring less frequent application (Lahav & Kadman, 1980; Lahav et al., 2002; Selladurai & Awachare, 2020). Fertilisers are often applied through fertigation, with foliar application used for quicker effects (Lahav et al., 2002).

For mature, fruit-bearing trees, around 200kg of nitrogen per hectare annually is recommended (Lahav et al., 2002).

1.4.2.2. Disease and pest control

Phytophthora root rot is one of the most common diseases affecting avocados. This oomycete attacks the roots, impairing their function and causing wilting, branch dieback, and fruit drop. Selecting well-drained soils helps to prevent this disease, and fungicides can also be used for control (Waite & Martinez Barrera, 2002). Avocado trees are susceptible to various insect and mite pests, with over 30 key species globally (Peña et al., 2013). Pest species and infestation intensity vary by location and cultivar, with thrips and mites being among the most damaging and widespread (Subhagan et al., 2020; Waite & Martinez Barrera, 2002). Pest management approaches differ, but commercial orchards often employ pesticides, particularly where exotic pests are present without natural predators (Peña et al., 2013). Some countries, especially those with strong export markets are moving towards integrated pest management and biological control methods (Peña et al., 2013; Waite & Martinez Barrera, 2002).

1.4.2.3. Irrigation

While water requirements for avocados vary based on the physiological stage and cultivar (Schaffer & Whiley, 2002; Whiley et al., 1988), in general, they require a substantial water supply. Insufficient water can lead to reduced production, higher fruit drop, and fruit deformities (Carr, 2013; Silber et al., 2012). However, avocados are sensitive to waterlogging, which can cause root rot and subsequently reduce yields (Moreno-Ortega et al., 2019; Pegg et al., 2002). Moreover, their shallow root system makes excess water application inefficient. Irrigation is mainly needed in Mediterranean climates and is commonly applied through drip irrigation. The frequency of application can vary from every 1 to 3 days and is determined by evapotranspiration rate, soil moisture, and plant indicators (Lahav et al., 2002).

1.5. Avocado production in Chile

In Chile, avocados are cultivated in the central Mediterranean region, stretching from Petorca and Rancagua (Figure 1. 4). The avocado trees blossom from October to November, the fruit sets between December and January, and harvesting occurs from September to November. Hass is the dominant variety grown in Chile constituting approximately 88% of the total share of production (Gonzalez, 2018). Chile has several factors conducive to production including a warm climate and a low prevalence of pests (Irazabal, 2001) and, consequently, in some areas, production is very high at around 25 tonnes per hectare. However, many areas of Chile encounter challenges such as inadequate irrigation and poor soil quality, resulting in average yields of about 9 tonnes per hectare (Lemus et al., 2005).



Figure 1. 4. Map of Chile, Argentina, and Uruguay. The red box roughly indicates the Mediterranean region of Chile where avocados are grown.

Although some avocados in Chile are consumed domestically, they are primarily grown for international markets. Chile predominately exports to the USA due to trade agreements, but there is a growing trend in exports to Europe. In recent years, Chile has experienced a significant surge in

avocado production. For instance, in the early 2000s, Chile was producing around 98,000 tonnes, compared to around 170,000 tonnes in 2021 (FAO, 2023). Avocado production in Chile holds significant importance in terms of exports and currently, it ranks as the fifth largest global exporter of avocados (Vieli et al., 2021).

1.5.1.Environmental concerns

There are two major environmental concerns associated with avocado production in Chile: the expansion of cultivation into natural areas and the high volume of water necessary for production. Over the past two decades, the area of land utilised for avocado production has doubled (FAO, 2020) with much of this expansion encroaching upon natural areas (Figure 1. 5) (Armesto et al., 2010; Schulz et al., 2010), likely resulting in biodiversity loss. This is particularly alarming given that Chile is considered a world biodiverse hotspot with a high degree of endemic fauna and flora (Henríquez-Piskulich et al., 2021; Myers et al., 2000). Moreover, the irrigation of avocado orchards presents a significant environmental challenge, particularly due to the ongoing “mega drought”. Water reserves are scarce, and avocado plantations are utilising limited water for irrigation. This has led to both social and environmental issues, drawing international concern and influencing consumers’ perceptions of avocados originating from this region (Facchini & Laville, 2018; Milne, 2019).



Figure 1. 5. Photo of an avocado orchard in Chile taken by Juan Luis-Celis Diaz during field work. The hillside towards the back of the photo shows an example of avocado orchards expanding into natural habitats.

These environmental concerns also impact agricultural productivity. The dwindling quantity and quality of water is already adversely affecting yields, and this trend is expected to be exacerbated in the future (Irazabal, 2001; Lemus et al., 2005). Simultaneously, the loss of natural habitat could potentially result in diminished ecosystem services (IPBES, 2019), including pest control and pollination. Given that natural predators help manage various avocado pests (Lemus et al., 2005) and that wild pollinators likely contribute to avocado production (De la Cuadra-Infante, 2007; Monzón et al., 2020), the decline in these services could significantly impact avocado production.

1.5.2. Avocado pollination in Chile

In Chile, managed honeybees are widely used in avocado production. Orchards are typically stocked with around 10 beehives per hectare during October and November (De la Cuadra-Infante, 1999; Lemus et al., 2005). Research conducted by De la Cuadra-Infante (1999) identified specific hive parameters for optimised pollination, including hive characteristics (e.g., number of frames, bee population, bee health, etc.), and hive placement (timing and placement) and highlighted that increasing the number of beehives per hectare enhances pollination. However, the cost-effectiveness of this approach remains unexplored, warranting further investigation for optimal stocking densities.

Additionally, wild pollinators probably contribute significantly to pollination and production. While honeybees consistently rank as the most prevalent, various other pollinating taxa such as wild bees, hoverflies, and ants have been observed visiting avocado flowers (Celis-Diez et al., 2023; De la Cuadra-Infante, 2007; Monzón et al., 2020; Valdes, 2002). Some studies have evaluated the effectiveness of wild pollinators. Monzón et al. (2020) demonstrated that native bees such as *Colletes cyanescens* and *Cadeguala occidentalis* exhibit similar flower handling times compared to honeybees and Celis-Diez et al. (2021) demonstrated a positive correlation between wild pollinator visits and avocado pollination. However, Valdes (2002) found that honeybees exhibited higher flower visitation rates and more effective pollinator behaviour (e.g., stigma contact) than wild pollinators, leading to the conclusion that honeybees contribute over 80% of pollination in Chile. These contrasting findings highlight the necessity of further research to better understand the effectiveness of different pollinator taxa and their roles in pollination and production.

1.6. Aims of the study

The purpose of this study is to address gaps in the knowledge concerning the contribution of insect pollination to avocado production. It seeks to understand the significance of specific wild pollinator taxa and natural habitats in relation to avocado pollination and production in major avocado-growing regions, with a specific focus on Chile. Additionally, the study aims to help enhance pollinator

protection efforts by developing a tool that private sector companies can utilise to formulate effective pollinator strategies. Specifically, the thesis will address the following research questions:

1. What is the extent of the contribution of insect pollinators to avocado production, and which insect taxa are the most important pollinators in different growing regions?
2. How does the proximity to natural habitats affect pollinators and pollination in avocado orchards in Chile?
3. What approaches can private sector companies employ to develop effective strategies for safeguarding pollinators?

1.7. Thesis structure

Avocado is a globally significant crop, however, the role of wild and managed pollinators in avocado production remains unclear. Chapter 2 of this thesis involved a comprehensive literature review and meta-analysis of existing avocado studies to better understand the extent of insect pollinator contributions to avocado production and to identify the most important insect pollinators across diverse avocado-growing regions.

In Chile, the expansion of avocado orchards into natural habitats has raised concerns about biodiversity loss (Armesto et al., 2010) potentially negatively impacting agricultural production due to declines in pollination services. The role of wild pollinators in avocado production in Chile and their reliance on natural habitats is currently unknown. To address this, in Chapter 3 pollinator observations and controlled pollination trials were employed to quantify the contribution of insect pollinators to avocado production, identify important wild pollinators in this growing region, and determine their reliance on natural habitats.

Preserving pollination services in agricultural landscapes frequently necessitates implementing pollinator protection measures (Kovács-Hostyánszki et al., 2017). However, growers often require

external support to overcome implementation barriers. The private sector offers a potential avenue for providing such support, but currently, businesses lack information on how best to provide support, potentially impeding action. To bridge this gap, Chapter 4 adapts an existing process to create a tool for private companies. This tool enables them to understand the importance of pollinators to their business, recognise threats to pollinators, and to formulate effective preservation strategies. The tool is applied to an avocado supplier company, gauging its applicability, and providing practical insights into its implementation.

Lastly, Chapter 5 provides a synthesis of the major findings of the thesis and discusses their implications within a broader context. This chapter also summarises key pollination management recommendations for growers and private sector companies identified throughout this research and outlines key areas in need of further research.

1.8. Thesis papers

Below is a summary of research papers from this study:

Paper 1 (Chapter 2)

Dymond, K., Celis-Diez, J.L., Potts, S.G., Howlett, B.G., Willcox, B.K. and Garratt, M.P., 2021. The role of insect pollinators in avocado production: A global review. *Journal of Applied Entomology*, 145(5), pp.369-383. <https://doi.org/10.1111/jen.12869>

Paper 2 (Chapter 3)

Dymond, K., Celis-Diez, J.L., Martinez-Harms, J., Rojas-Bravo, V., Potts, S.G. and Garratt, M.P., 2023. Proximity to natural habitat affects wild pollinator compositions and pollination services in avocado orchards. (*Journal of Agriculture, Ecosystems and Environment*) (in preparation).

Paper 3 (Chapter 4)

Dymond, K., Celis-Diez, J.L., Lymna-Dennis, C., Rojas-Bravo, V., Potts, S.G. and Garratt, M.P., 2023. A rapid assessment tool for identifying and managing pollination risks: a case study for the avocado industry. (Conservation Biology) (under review).

I declare that I conducted all the research for these papers and was the principal author. My supervisors, and other co-authors, who also provided data or technical support for data analyses, assisted with editing the content.

2. Chapter 2: The role of insect pollinators in avocado production: a global review

This chapter is from the following publication:

Dymond, K., Celis-Diez, J.L., Potts, S.G., Howlett, B.G., Willcox, B.K. and Garratt, M.P., 2021. The role of insect pollinators in avocado production: A global review. *Journal of Applied Entomology*, 145(5), pp.369-383.

KD and MG conceived research. KD conducted systematic review, analysed data, conducted statistical analyses and wrote the manuscript. MG supported statistical analyses. All authors provided feedback on the manuscript, read, and approved the manuscript.

2.1. Abstract

Insect pollination increases the yield and quality of many crops and therefore, understanding the role of insect pollinators in crop production is necessary to sustainably increase yields. Avocado (*Persea americana*) benefits from insect-pollination however, a better understanding of the role of pollinators and their contribution to the production of this globally important crop is needed. In this study, we carried out a systematic literature review and meta-analysis of studies investigating the pollination ecology of avocado to answer the following questions: 1) Are there any research gaps in terms of geographic location or scientific focus? 2) How much do insect pollinators contribute to pollination and production? 3) Which pollinators are the most abundant and effective and how does this vary across location? 4) How can insect pollination be improved for higher yields? Research from many regions of the globe has been published however, results showed that there is limited information from key avocado producing countries such as Mexico and the Dominican Republic. In most studies, insects were shown to contribute greatly to pollination, fruit set, and yield. Honeybees (*Apis mellifera*) were important pollinators in many regions due to their efficiency and high abundance, however, many wild pollinators also visited avocado flowers and were the most frequent visitors in

over 50% of studies. This study also highlighted the effectiveness of stingless bees (*Meliponini* spp) and blow flies (*Calliphoridae* spp) as avocado pollinators although for the majority of flower visitors there is a lack of data on pollinator efficiency. For optimal yields, growers should ensure a sufficient abundance of pollinators in their orchards either through increasing honeybee hive density or, for a more sustainable approach, by managing wild pollinators through practices that protect or promote natural habitat.

2.2 Introduction

Avocado is one of at least 105 crops that receive yield benefits from animal pollination (Rader et al., 2020), and together, these crops represent approximately 35% of total agricultural production (Klein et al., 2007). Insects are the most important animal pollinator and therefore, to sustainably increase food production and feed a growing population, we need to better understand the role of insect pollinators and how they can be managed effectively in important animal-pollinated crops such as avocado.

Insect pollinators are thought to facilitate avocado pollination and thus increase production, and there is evidence of opportunities to improve this yield through improved pollination service. For example, under normal pollination conditions, fruit set percentage at the tree level is less than 1% whereas, with the addition of hand pollination, fruit set rates have reached 5% at the branch level (Evans et al., 2010; Alcaraz and Hormaza, 2009; Garner and Lovatt, 2008). Furthermore, like many insect-pollinated crops, avocado yields may be adversely affected by widespread pollinator declines (Biesmeijer et al., 2006; Potts et al., 2016).

Optimising avocado yields is increasingly important, as demand for this product is rising with 32.6 million tonnes produced from 1999-2008 and 50.4 million tonnes from 2009-2018 globally (FAO, 2020). Today, avocados are not only a nutritious staple but also an important export crop for many countries (USD 6.84 billion globally for 2018) (FAO, 2020). However, in some avocado growing regions,

expansion is having adverse environmental impacts such as, biodiversity loss and water resource depletion (Magrach and Sanz, 2020) and thus improving sustainable production is crucial.

Avocados have a synchronous dichogamy flowering pattern. Flowers are hermaphroditic (have both male and female parts) but open as female and male separately at different times and this differs between cultivars. In 'A-type' cultivars, flowers commonly open as functionally female in the morning of the first day and functionally male in the afternoon of the second day, whereas, in 'B-type' cultivars, flowers are commonly female in the afternoon of the first day and male in the morning of the second day (Stout, 1932; Nirody, 1922). This process encourages outcrossing; however, avocados are self-fertile and pollination from within the same cultivar or tree (close pollination) can occur during the daily overlap of male and female flowers (Nirody, 1922; Stout, 1932). Daily overlapping is a common occurrence, but weather conditions play an important role in flowering synchronisation and, under cooler temperatures, the length of time for male and female flowers to overlap can significantly increase (Ish-Am and Eisikowitch, 1990; Pattemore et al., 2018). In theory, self-pollination is possible during the male opening, as stigmas can still be receptive (Davenport et al., 1994), however, successful fertilisation during this phase is extremely rare (Sedgley, 1977; Sedgley and Grant, 1983).

Previous studies have explored the effect of insect pollinators on avocado pollination. A few studies have shown no significant difference in pollination rates between open-pollinated treatments with high honeybee-hive density compared with closed-pollination treatments with no access to insect pollinators, therefore it is argued that wind pollination is the dominant pollination mechanism in these systems (Davenport et al., 1994; Davenport, 2019). However, most other controlled pollination experiments have shown that without insect pollinators, pollination (Cabezas and Cuevas, 2007), fruit set (Malerbo-Souza et al., 2000; Can-Alonzo et al., 2005), and yields (Mulwa et al., 2019a; Petersen, 1955; Robbertse and Johannsmeier, 1997) are significantly reduced in comparison to open-pollinated treatments. A better understanding of the role of insect pollinators in avocado production and what factors result in this variation is needed.

Additionally, across the globe, there is growing evidence of the contribution from wild pollinators and natural habitats to pollination services (Dainese et al., 2019; Martin et al., 2019; Garibaldi *et al.*, 2011; Garibaldi et al., 2013). It is therefore important to determine which pollinators are pollinating different crops, including avocado, and identify effective ways to improve and protect this ecosystem service. An updated review of avocado pollination ecology is necessary to inform sustainable management of this important ecosystem services as well as to help target future research.

We build on previous reviews on avocado pollination and reproductive biology by Wysoki et al., (2002), Gazit and Degani, (2002) and Ish-Am, (2005) by providing an updated and systematic analysis of published literature on avocado pollination. The aims of this paper were; 1) to consider the geographic variation and research focus of existing research on avocado pollination, 2) assess the effect of insect pollinators on avocado pollination and production, 3) identify which insect pollinators are the most abundant and effective, and how this varies by geographic location, 4) highlight potential ways to improve insect pollination for higher yields, exploring both wild and managed pollinators and 5) identify evidence gaps and direct future research.

2.3 Methodology

2.3.1 Literature review

Literature was sourced through a systematic review using the following search terms; avocado * AND (pollination* OR pollinators*); "insect pollination*" AND avocado*; "insect pollination*" AND avocado* AND Management; Honeybees* AND avocado*; "Pollination services*" AND avocado*; avocado* AND "improve pollination"*. These terms were used in 2 scientific databases; web of knowledge and google scholar. In web of knowledge, all the returned searches were assessed for suitability, whereas in google scholar, due to the high volume of searches returned, the first 500 most relevant papers were assessed. Google scholar returned a range of sources (e.g. ebooks and grey literature), however, it is possible, that this search methodology missed some wider literature. These

searches provided a total of 4043 papers in which the title and, or abstracts were assessed for suitability. Papers were selected if they had carried out original research which contributed to this review's key aims. This resulted in 36 unique papers and therefore, to increase the sample size and the range of sources, previous avocado pollination reviews were utilised to source additional relevant papers. Paper searches took place from April to June 2020. Searches were carried out in English, and if this returned a paper in another language, it was translated online and assessed for suitability. In total, the search methods produced 41 papers that were subsequently included in this review. All papers were used in the analysis for geographical and research focus and a subset of 35 papers (Appendix 1) provided appropriate quantitative data and were used to assess insect contribution to pollination and pollinator abundance.

2.3.2 Data analysis

2.3.2.1 The contribution of insect pollinators to pollination and production

A meta-analysis was carried out to assess the difference in avocado pollination between open and closed pollinated treatments for pollination and production metrics including the percentage of flowers pollinated, fruit set (data collected between 1 and 3 months' post-flowering and either per branch, inflorescence, or panicle), final fruit count per tree and fruit weight per avocado. Papers that looked at pollination between low and high honeybee density experimental treatments were also included. The mean, standard deviation (SD), and sample size (N) were extracted directly from 3 papers (7 experiments), however, in many studies, this information was not provided. Therefore, studies were either excluded from the analysis or if possible, SD and N were calculated. In 2 cases, SD and N were calculated at the replicate level, however other studies provided only information for different years or different orchards of differing cultivars. In these cases, year and orchard were considered replicates, and the mean and SD were calculated accordingly (Appendix 2 and 3). The initial meta-analysis resulted in high heterogeneity ($I^2 = 95\%$). Therefore, outliers were removed using an influence analysis, and models were sub-categorized based on plausible causes for heterogeneity

including response variable (fruit set, fruit weight, pollination, and yield), climate (humid or dry), cultivar (Hass or other), and experimental scale (branch, tree, site, and year). All meta-analyses were carried out in R version 3.6.1 (R Core Team, 2019) using the 'meta' package (Balduzzi et al., 2019).

In addition to the meta-analysis, to allow the inclusion of studies that had not provided all required data (n, SD, mean), means of open and closed pollination treatments for each variable were summarised graphically. Data was categorised based on the response metric (fruit set per branch, percent of flowers pollinated, and fruit weight) and violin plots were created. A vote count was also implemented for controlled pollination experiments. Studies that had reported statistical significance between pollination treatments were categorised into either Open>Closed, Open<Closed or Non-Significant and tallied. If studies had multiple but conflicting results, either from measuring different variables or applying different treatments (e.g., climate or cultivar) then an overall category was assigned based on which result was most prevalent across the different variables and treatments.

2.3.2.2 Abundance and efficiency of insect pollinators

To compare the relative abundance of different pollinators between studies and regions, data was taken directly from the paper or calculated from data on total observations per species. Sixteen studies provided data on pollinator abundances on avocado. Species were categorised into broad taxonomic groups for comparison. Studies were then grouped by country and total abundances across all studies per country were used to calculate average country abundance. To explore pollinator efficiency, data was taken either directly from the paper or supplementary sources (Appendix 5, 6, and 7). Four studies compared pollen deposition per visit, 5 studies explored the amount of pollen carried by pollinators, and 3 studies looked at the visitation rate between different groups of pollinations. A mean was calculated from the raw data and represented in box and whisker plots. All graphic summaries were produced in R version 3.6.1 (R Core Team, 2019), using the package 'ggplot2' (Wickham, 2016).

2.4 Results

2.4.1 Research focus and geographical spread

The majority of the studies identified by the literature search considered the contribution of insect pollinators to pollination and production (32%) or the abundance and efficiency of different pollinator species (29%) (Figure 2. 1a.). Most of the studies were carried out in the USA (23%) followed by Israel (11%), however, these countries count for only 6.6% of global production (data for 1999-2018) (FAO, 2020). There were less than 5 studies carried out per country for all remaining countries, and for 3 out of the top 6 avocado producing countries, no studies at all were identified by the search (Dominican Republic, Peru, and Indonesia) (Figure 2. 1b).

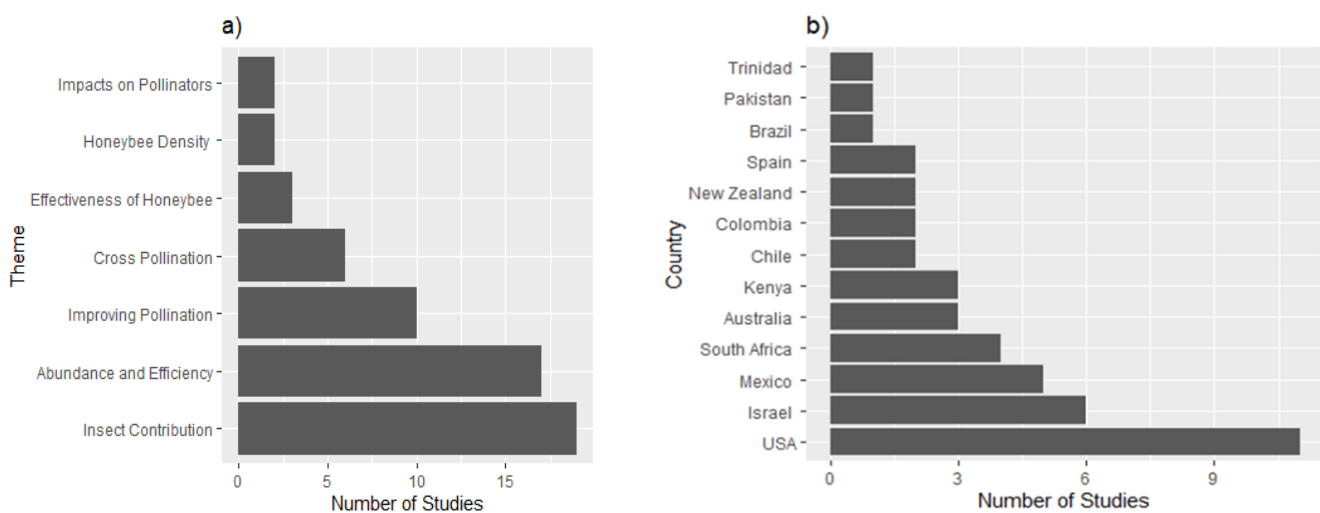


Figure. 2. 1. Number of studies grouped by (a) research theme and (b) country. 41 studies were used for this analysis but, multiple studies contributed to several different themes and 2 studies were based in 2 countries. (a) Research theme code: Impact on pollinators (impacts on pollinators of land management or landscape), Honeybee Density (effect of manipulating honeybee density), Effectiveness of Honeybee (effectiveness of honeybees as avocado pollinators), Improving pollination (ways to improve pollination services), Cross-pollination (cross-pollination contributions to pollination), Abundance and Efficiency (abundance and efficiency of avocado pollinators) and Insect Contribution (insect contribution to avocado pollination).

2.4.2 Contribution of insect pollinators to pollination and production

Results show that overall, pollination, fruit set and yield all increase under open pollination conditions compared to when insect pollinators are excluded, with a standardized mean difference (SMD) of 2.45. However, heterogeneity was high ($I^2 = 77%$) and significant (Figure 2. 2). Following subcategorization by response variable, climate, cultivar, and experimental scale, the mean difference remained higher for open-pollinated treatments compared to closed for all metrics considered, but heterogeneity remained high and significant for all categories indicating that variability between studies was considerable (Appendix 4). These trends were supported by the mean summaries. Nearly all key indicators showed higher values in open treatments compared with closed, and the majority of closed treatments showed close to zero pollination, fruit set, or yield (Figure 2. 3). There was often considerable variation in the results for the open treatments. Similarly, the findings from the vote count showed that most studies had a significantly higher value for pollination and yield in open treatments (Figure 2. 4). Fruit weight was the only pollination variable where the majority of studies did not show a significant positive or negative effect of insect pollination (Figure 2. 4) and the mean summaries and meta-analysis showed only a very small difference (Figure 2. 3 and Appendix 4).

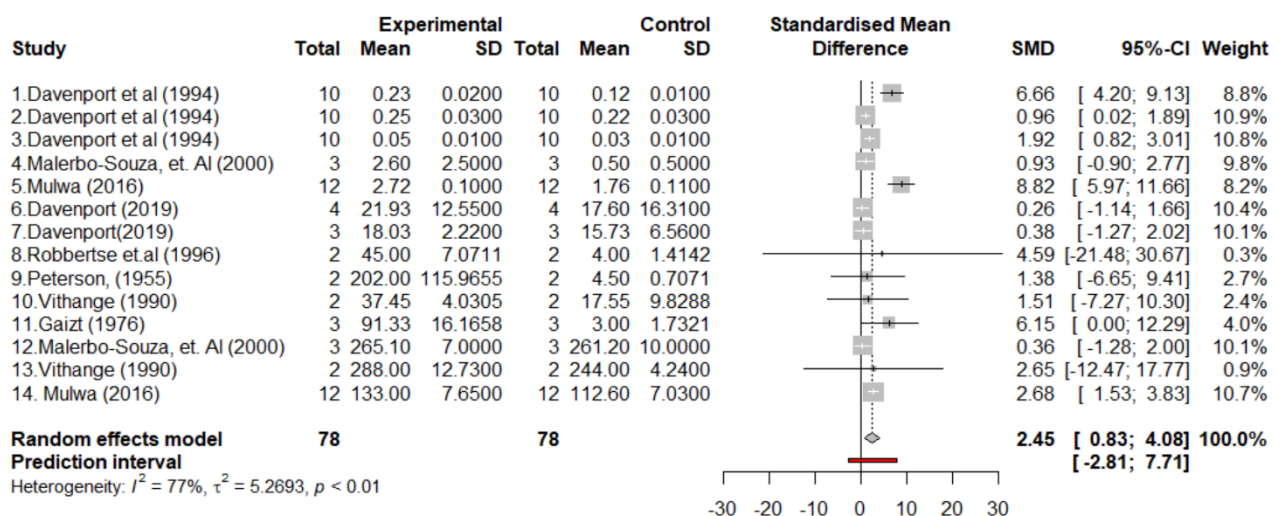


Figure. 2. 2. Forest plot following a randomised meta-analysis to compare pollination and production under insect pollination (Experimental) and no insect pollination (Control) treatments in avocado across multiple studies.

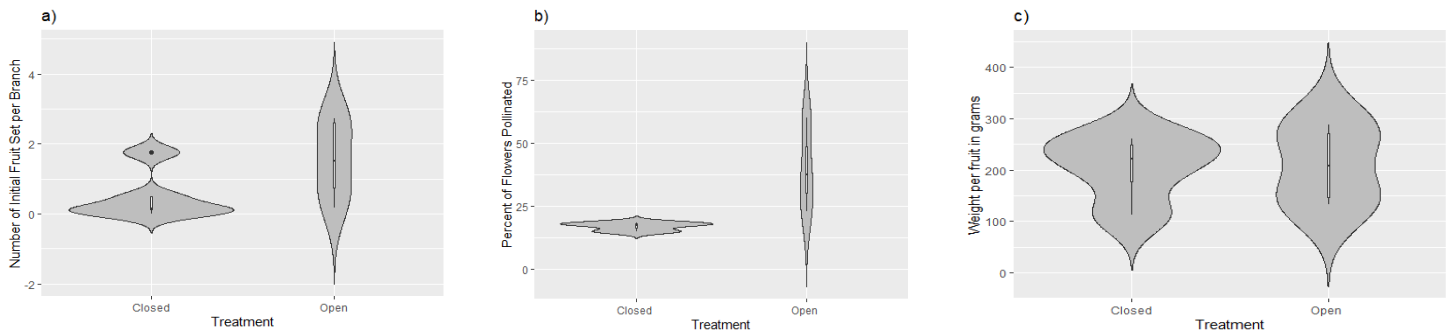


Figure. 2. 3. Summary means for pollination and production variables between insect pollination (Open) and no insect pollination (Closed) collected from multiple studies. (a) Average number of fruits set per branch, N= 5 (b) Percent of flowers pollinated, N= 3 and (c) Average weight per fruit, N=4.

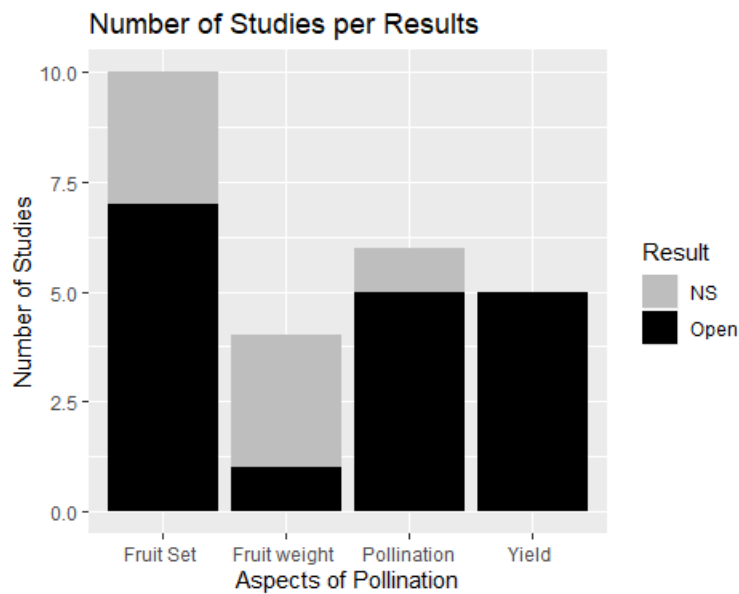


Figure. 2. 4. Summary of significant positive or non-significant (NS) results following a vote-count comparison across multiple studies comparing insect pollinated (Open) and pollinator exclusion treatments (Closed) for the variables fruit set, fruit weight, pollination, and yield.

2.4.3 Abundance and efficiency of insect pollinators

Managed honeybees were the most frequent pollinators overall and were observed in all studies and countries (Figure 2. 5). In 11 out of 16 cases they showed the greatest relative abundance of any single pollinator species, but this did vary considerably between 10% and 92% depending on the study. Hoverflies (*Syrphidae* spp) were also common pollinators with an overall relative abundance of 12%. Stingless bees generally had high abundance in locations where they were found, but they were only observed in 3 studies while conversely, wild bees were seen in 7 studies but had lower abundances (Figure 2. 6). Nine studies measured some aspect of pollinator efficiency, but different metrics and taxa were measured and therefore, there was limited data available to make cross-study comparisons. Honeybees, stingless bees, and blow flies were found to carry and deposit the greatest amount of pollen (Figure. 2. 7). Honeybees are potentially more effective than blow flies due to the higher number of flower visits per minute.

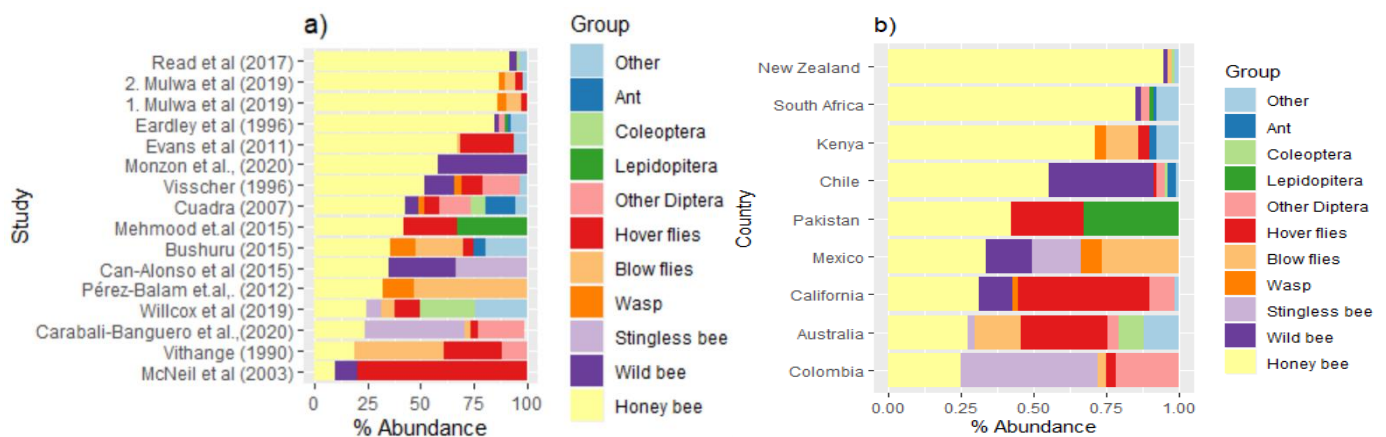


Figure. 2. 5. Relative abundance of pollinators visiting avocado flowers across (a) individual study and (b) grouped by country.

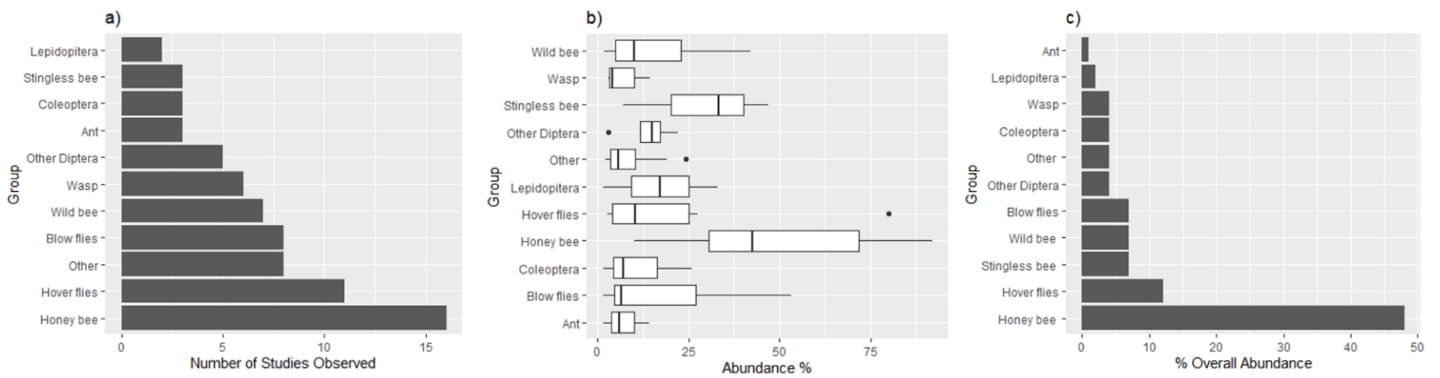


Figure. 2. 6. Insect groups visiting avocado flowers from 16 studies including (a) the number of studies in which each insect group was observed, (b) the total abundance of each insect group and (c) the overall relative abundance of insect groups across all studies.

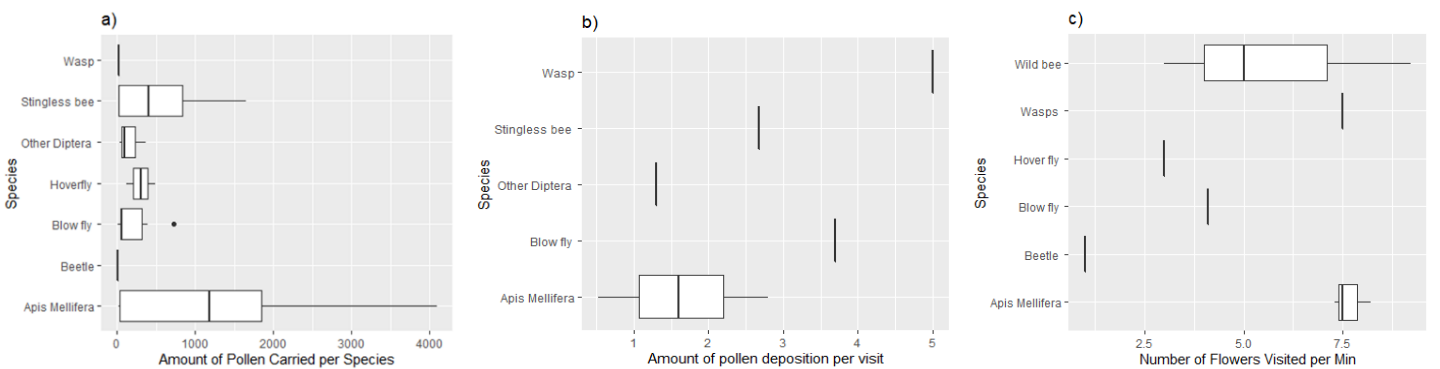


Figure. 2. 7. Pollination efficiency of different insect groups including (a) the amount of pollen carried per insect group, (b) the amount of pollen deposition per visit per insect group and (c) the average number of flowers visited per minute per insect group.

2.4.4 Improving insect pollination

Studies on improving insect pollination have generally focused on three areas; optimising the contribution of honeybees (n=4), utilising other managed species (n= 4), and ways to improve pollination by wild pollinators (n=2) (Table 2. 1). The results showed that increasing honeybee density leads to significantly higher rates of pollination and production. Other managed pollinators assessed

were, the New World Carniolan honeybee (*Apis mellifera carnica* Pollman 1879) (n=2), the buff-tailed bumblebee (*Bombus terrestris* Linnaeus 1758) (n=1), and the western bumblebee (*Bombus occidentalis* Greene 1858) (n=1). Both species of bumblebee were shown to be efficient pollinators, whereas the New World Carniolan honeybee showed no significant difference in visitation rates compared to the Italian honeybee (*Apis mellifera ligustica* Spinola 1806). Two studies looked at wild pollinators and methods to increase their abundance. The results suggest that intensive management practices such as spraying pesticides, removing forested areas, and weeds from the orchard leads to a reduction in pollinator diversity and subsequently, avocado yield.

Table 2. 1. Summary of papers identified during the literature search which considered approaches to improve insect pollination in avocado

Study	Main Theme	Key Points on improving insect pollination
Vithanage (1990)	Honeybee management	The introduction of honeybee hives during flowering led to significantly higher fruit set. Fruit size increased with increased beehive densities.
Ish-Am and Eisikowitch (1998)	Honeybee management	Optimal fruit set required at least 5 honeybees per tree during female flowering. Fruit set was lower when this density was not reached.
Ish-Am et al., (1998)	Other managed pollinators	Pollination rates were higher in treatments using buff-tailed bumblebees in comparison to honeybees. In Etinger avocados, buff-tailed bumblebees increased cross pollination and significantly increased yields whereas in Hass avocados, there was a slight increase in yields mostly due to increases in cross pollination in trees far from hives.
Castaneda-Vildozola et al., (1999)	Wild species	Many native species contributed to pollination. Spraying pesticides reduced the abundance of native pollinators and led to lower yields.
Fetscher et al.,(2000)	Other managed pollinators	Results were not statistically significant but suggest that the New World Carniolan honeybees may have a higher visitation rate to avocado flowers in comparison to Italian honeybees.

McNeil and Pidduck (2003)	Other managed pollinators	Western bumblebees were efficient pollinators and increased avocado yields. Yields increased significantly in rows closest to hives.
Afik et al., (2007)	Other managed pollinators	Trials with the New World Carniolan honeybee showed mixed results. In some locations, this subspecies had a higher avocado visitation rate than the Italian honeybee but in other locations it was lower.
Ish-Am and Lahav (2011)	Honeybee management	There was a strong positive correlation between honeybee density and rates of pollination.
Villamil et al., (2017)	Wild species	Increased forest areas, reduced spraying of pesticides and an increase of weeds in the orchard were positively associated with pollinator biodiversity.
Pena and Carabali (2018)	Honeybee management	High honeybee hive density (4 and 6 hives per hectare) resulted in significantly higher fruit set and yield in comparison to controls (no hive).

2.5 Discussion

Our study has provided an updated review on the role of insects in avocado pollination. It has built on previous reviews by including the analysis of 18 new studies and current topics such as the impacts of land management on wild pollinators. Additionally, we carried out a meta-analysis on the contribution from insect pollinators to pollination and statistically summarised global pollinator abundance and pollination efficiency of key species, thus providing a more nuanced understanding of these issues.

2.5.1 Research focus and geographical spread

The majority of studies were located in the USA and Israel. Only 5 studies were implemented in Mexico despite being the origin of avocados and the world's largest producer (30% of world production, average 1961 to 2018), and no studies were observed in the second-largest producer, the Dominican Republic (7% of world production, average 1961 to 2018)(FAO, 2020). Further work relevant to these countries is needed as factors including local pollinator communities, climate and cultivar are likely to

be unique to each region and currently, the biggest producers are not well represented. Additionally, the contribution from wild pollinators is likely higher in central American countries due to the coevolution of avocados and pollinators in this region (Castañeda-Vildózola et al., 1999; Ish-Am et al., 1999; Brown and Cunningham, 2019) and therefore, the opportunity to better utilise wild pollinators for more sustainable production may be increased (Albrecht et al., 2012; Woodcock et al., 2019; Garibaldi et al., 2015). The majority of studies focused on the contribution of insects to pollination and production (18 papers) and pollinator abundance and efficiency (17 papers), however, the quality of these studies was variable. In some controlled pollination experiments, there was little or no replication and only 9 of the studies on pollinator efficiency provided quantitative data.

2.5.2 Contribution of insect pollinators to pollination and production

Our findings suggest that, in most circumstances, insect pollinators make an important contribution to pollination and avocado production. The meta-analysis showed an SMD of 2.45 for open-pollinated treatments in comparison to closed, and the mean summaries highlighted that there was generally a higher percentage of fruit set and flowers pollinated when visited by insects. The vote count concurred with this, and similar conclusions have been drawn from other studies (excluded from this analysis due to the lack of statistical inference) (Papademetriou, 1976; Lesley and Bringham, 1951; Bergh, 1967) and previous avocado pollination reviews (Ish-Am, 2005; Gazit and Degani, 2002; Wysoki et al., 2002). Furthermore, several aspects of the flower morphology (small stigma, heavy and large pollen grains, the release of a low number of pollen grains, the production of nectar, and the dichogamy flowering pattern) indicate a probable important role for insect pollinators (Gazit and Degani, 2002; Stout, 1932; Vithanage, 1990; Sedgley and Griffin, 1989; Dafni, 1992).

The results also showed a wide variation in the contribution of insect pollinators. The meta-analysis had high and significant heterogeneity and, in the mean summaries, the range for open-pollinated treatments was broad and overlapped with closed pollination treatments. A possible explanation for this variation could be that, in some circumstances, pollinators are thought to contribute little to the

pollination process (Davenport et al., 1994; Ying et al., 2009; Davenport, 2019; Clark, 1923). It is hypothesized that self-pollination is possible if stigmas remain receptive in phase 2 and this is thought to be feasible with specific cultivars (Davenport et al., 1994) and in humid climates (Gazit and Degani, 2002). Additionally, in a recent study, Davenport (2019) argues that regardless of other external factors, self-pollination makes up a major part of avocado pollination. However, this study measures pollen tube growth in the style and does not count the number of pollen tubes to reach the ovule, something which wider evidence suggests does not occur during the male phase (Sedgley and Grant, 1983). Davenport (2019) also suggests that wind pollination is more prominent than insect pollination and shows that there is no difference in the percent of pollinated stigmas between open and closed treatments. This contrasts with several other studies and thus indicates there are likely some varietal or management factors affecting the role of insect pollinators.

Variation in resource availability and pollination services between locations are more probable explanations for the yield disparities in the open-pollinated treatments. Pollinator abundance and, or pollinator efficiency, were seldom reported in controlled pollination studies and this is likely to vary significantly due to differences in; managed bee-hive densities, proximity to natural areas and wild pollinators, and the availability of native avocado pollinators. Variation in resource availability is also likely to be high and this has been shown to impact fruit set. Alcaraz et al. (2013) show that the amount of starch content in the style has a high correlation with the number of flowers that successfully develop into avocado fruits (Alcaraz et al., 2013). Resource availability also likely explains why our results showed that insect pollination did not have a large effect on fruit weight as agronomic factors that influence general tree health (e.g., irrigation), and resource availability are known for having a greater influence on fruit size (Kremer-Köhne and Köhne, 1995). Additionally, the scale at which pollination is assessed can also affect the level of contribution to pollination (Webber et al., 2020; Howlett et al., 2019) and this may explain the significant variation between studies which had measured insect contribution at different scales (inflorescence, branch, tree).

2.5.3 Abundance and efficiency of insect pollinators

Overall, managed honeybees appear to be contributing most to avocado pollination in many regions due to their general efficiency and high abundance. This finding was noted in the majority of studies and has been highlighted in previous reviews (Wysocki et al., 2002; Ish-Am, 2005). However, it is well known that honeybees can be sensitive to wind, rain, and low temperatures (Bushuru, 2015; Can-Alonzo et al., 2005) and often prefer other nectar sources (Ish-Am and Eisikowitch, 1998). Therefore, under poor weather conditions or where other flowers are in bloom at the same time, their contribution to avocado pollination could be reduced, and diverse pollinator communities including both wild and managed pollinators may provide more consistent pollination (Woodcock et al., 2019)

Wild pollinators also played an important role, and in 9 of the 16 studies and 5 of the 9 countries they were more abundant than managed honeybees. Stingless bees and blow flies were highlighted as two important pollinator groups. Stingless bees were shown to carry a comparably high amount of pollen (around 500 grains) and in locations where they were found, they had a high relative abundance. Furthermore, qualitative comments suggest that their body size suits the shape of avocado flowers and they have a preference for avocado nectar (Ish-Am et al., 1999). However, it has been observed that they have a lower visitation rate than honeybees (Can-Alonzo et al., 2005) and, in this analysis, they were only observed in 3 of the 9 countries. Blow flies were also shown to be important pollinators as they deposited a high amount of pollen per visit. This may be attributed to the open structure of the avocado flower making it well suited to fly pollination (Vithanage, 1990). However, in comparison to honeybees, their visitation rate was low, and they were significantly less abundant. The lower abundances of wild pollinators in comparison to honeybees is a key reason why their contribution to pollination is reduced and thus, active management of wild pollinators may be an effective strategy to increase pollination.

Previous reviews have highlighted other potentially important pollinators. In certain locations, wasps are efficient pollinators (Ish-Am et al., 1999; Perez-Balam et al., 2012; Papademetriou, 1976) and some

studies have shown that managed bumblebees can increase pollination (Ish-Am et al., 1998; McNeil and Pidduck, 2003). These pollinator groups were not covered in this analysis due to a lack of quantitative efficiency data and thus, this should be a target for future research. In temperate regions, it is also necessary to consider the potential of nocturnal pollinators, particularly flies and lepidoptera, as lower night temperatures can result in both male and female flowers opening during the night. Pattemore et al., (2018) showed that pollinating insects, some of which were carrying avocado pollen, did visit avocado flowers during the night. However, no other studies have explored this topic and therefore further investigations in this area are also needed.

2.5.4 Improving insect pollination

Tools to better manage pollination in avocado are required given the increasing production of avocados globally (FAO, 2020), the need for more sustainable production systems that make better use of inputs such as insect pollination (Garibaldi et al., 2019), and that pollination deficits in avocado are already in evidence (Evans et al., 2010; Alcaraz and Hormaza, 2009). This study highlighted three key themes to improve pollination: increasing honeybee hives, utilising other managed pollinators, and exploring the potential from wild pollinators.

The results showed that increasing honeybee density and visitation rate led to an increase in pollination and production (Peña and Carabalí, 2018; Ish-Am and Eisikowitch, 1998; Vithanage, 1990; Ish-Am and Lahav, 2011). Similar findings were concluded in a meta-analysis by Rollin and Garibaldi (2019) who showed that, for a range of insect-pollinated crops, production does increase with beehive density, up to a saturation point. However, for avocado production, we still lack evidence for optimal stocking densities and spatial arrangements of hives, particularly for some of the significant global producers.

Four studies focused on utilising other managed pollinators. Trials on the New World Carniolan honeybee showed no significant difference in pollination rates between this race and the Italian

honeybee (Afik et al., 2007; Fetscher et al., 2000). Conversely, bumblebees were effective pollinators, but it is thought that their high cost is currently prohibitive for wide-scale use (Fetscher et al., 2000; Gazit and Degani, 2002) and there are risks associated with introducing managed bumblebees into countries where they are not native (Ings et al., 2005; Goulson, 2010). Stingless bees may also have the potential to be used for avocado pollination, as they are efficient pollinators and can be successfully managed (Ish-Am et al., 1999; Can-Alonzo et al., 2005; Quezada-Euán et al., 2001). However, breeding on a large scale is difficult (Slaa et al., 2006) and therefore may be unfeasible for commercial systems, at least at the present time. In many countries it may not be viable to utilise these managed pollinators and identifying and exploiting alternative pollinators may be promising. In South Africa, Eardley and Mansell (1996) suggest that increasing the abundance of carpenter bees may increase avocado pollination and research done on other crops has highlighted the contribution to pollination services from drone flies (*Eristalis tenax* Linnaeus 1758) (Howlett and Gee, 2019) and blow flies (Cook et al., 2020). These studies suggest the potential for managing a range of different pollinators, but more research is needed to understand which wild pollinators may be beneficial in different locations.

Wild insect species were the most abundant avocado pollinators in many regions and developing approaches that make the most of their contribution may be the most appropriate. This was specifically explored in avocado by two studies identified in the review. The results suggested that management practices such as spraying of pesticides, clearing of weeds, and a reduction in surrounding natural areas can reduce the abundance and diversity of pollinators and that this can decrease avocado yield (Castañeda-Vildózola et al., 1999; Villamil et al., 2017). Similar findings have been observed in many other pollinator reliant crops, and several papers have shown that a reduction in natural habitats or an increase in intensive production leads to a reduction in the abundance and diversity of wild pollinators with negative impacts on yield (Dainese et al., 2019; Martin et al., 2019; Garibaldi et al., 2011; Garibaldi et al., 2013; Reilly et al., 2020). A better understanding of the role of

wild pollinators and how management practices can be best adapted to support in avocado production is required.

2.5.5 Study limitations

The results from this review may have been influenced by publication bias, as studies demonstrating positive effects of insect pollination might have a higher likelihood of being published. Additionally, the meta-analysis results were likely affected by the low number of available studies. For instance, it was necessary to include papers testing a wide range of variables (fruit set, yield, pollination) and utilising a variety of different methods (e.g., open and closed pollination and low and high beehive density) and as such, the data was very heterogeneous. Furthermore, some of these studies provided limited information on the methods used, and often, N and SD were not provided and therefore had to be calculated from the information available. Although publication bias, restricted data availability and the suboptimal quality of some of the studies limited the conclusions that could be made, general findings, research gaps, and recommendations have been identified.

2.6 Conclusions

2.6.1 Key findings and research gaps

- Dominican Republic and Mexico are responsible for 37% of global avocado production but only 12% of studies originated from these countries and therefore, further research in these countries is required.
- In 16 out of 21 studies, insect pollinators contributed significantly to pollination, fruit set, and yield.
- Managed honeybees were identified as the most important pollinators due to their frequency and efficiency. However, further information is needed to optimise local field beehive placement and density.

- The abundance of wild pollinators ranged from 90% to 8% across locations and further research is required to understand their efficiency and contribution to avocado pollination.
- Land management practices affected the abundance and diversity of wild pollinators and this can have negative implications for yield.

2.6.2 Recommendations for growers

- In most situations, growers will benefit from an increased density of pollinators.
- Increasing honeybee hive density will likely increase production but may not be cost-effective in all contexts.
- The utilisation of alternative managed pollinators (e.g., stingless bees) or actively managing for indigenous wild pollinators may be more feasible and sustainable.

3. Chapter 3: Proximity to natural habitat enhances wild pollinator biodiversity and pollination services in avocado orchards.

Dymond, K., Celis-Diez, J.L., Potts, S.G., Martinez-Harms, J., Rojas-Bravo, V., and Garratt, M.P., *Agriculture, ecosystem, and environment* (in preparation).

KD, MG, SP, JCD and JMH conceived research. KD, JCD, JMH and VRB carried out the research. KD analysed data, conducted statistical analyses, and wrote the manuscript. MG supported statistical analyses. All authors provided feedback on the manuscript.

3.1. Abstract

Insect pollination is known to increase avocado yields, with wild pollinators likely playing an important role. In central Chile, the rapid expansion of avocado orchards has resulted in highly diverse natural habitats being replaced by plantations, potentially negatively impacting wild pollinators and thus avocado production. This study aimed to 1) identify what wild pollinators are present in avocado orchards and explore the relationship between pollinator abundance and diversity and proximity to natural habitat, 2) quantify the effectiveness of different insect taxa in providing pollination services to avocados, and 3) measure the contribution to avocado production of insect pollinators and explore to how this varies with proximity to natural habitats. We conducted pollinator observations and controlled pollination trials (open and closed pollination treatments) along a natural habitat gradient in 3 farms in central Chile, across 3 years. The results showed that over 70 different insect species visited avocado flowers, with pollinator abundance, visitation, and richness being significantly higher closer to natural habitats. However, this relationship was non-linear, with wild pollinator abundance and visitation rates approximately 2.55 times higher, pollinator richness around 1.6 times higher and diversity 1.5 times higher at the orchard's edge in comparison to further inside the orchard. The controlled pollination trials confirmed that insect pollinators contribute significantly to avocado production, with almost no fruit set when pollinators were excluded, and higher fruit set at the

orchard edge. Hoverflies and flies were identified as effective avocado pollinators due to their high flower visitation rate and fruit set was positively correlated with the abundances of these taxa. This study demonstrates the importance of natural habitats and wild pollination services in crop production. We recommend that growers implement land management practices that protect and restore natural areas in and around their farms to support wild pollinators.

3.2. Introduction

Pollinators play a crucial role in increasing the quantity and quality of many globally important (Klein et al., 2007) as well as nutritionally valuable crops (Chaplin-Kramer et al., 2014; Eilers et al., 2011). To ensure sufficient production, farmers often introduce managed honeybees (*Apis mellifera*) into their fields and orchards. However, relying exclusively on a single managed species for pollination carries risks, especially considering the combined threats facing honeybees such as disease, pesticides (Kremen et al., 2002), and climate change (Bartomeus et al., 2013). In addition, the escalating global demand for insect-pollinated crops is expected to surpass the supply of managed honeybees (Mashilingi et al., 2022).

Moreover, multiple studies have shown that an increase in the abundance and diversity of wild pollinators can provide a more efficient and comprehensive pollination service compared to managed honeybees (Blüthgen & Klein, 2011; Garibaldi et al., 2013; Hoehn et al., 2008; Klein et al., 2009). Consequently, there has been a growing recognition in recent years of the importance of wild pollinators and protecting or increasing their role in facilitating the transition toward sustainable agriculture (Garibaldi et al., 2014).

Natural habitats provide essential resources for wild pollinators, including food, shelter, and nesting sites, that are often lacking in managed landscapes (Winfree et al., 2009). However, across the world, natural areas are diminishing primarily due to agricultural expansions. For example, in South America, the cover of various terrestrial natural habitat biomes, including grasslands, forests, and the Mediterranean-climate biomes, have decreased by more than 50% (IPBES, 2018). Consequently, this

reduction poses a potential threat to pollination services and, thus, food production (Campbell et al., 2017; IPBES, 2016; Vanbergen et al., 2020). To better understand the relationship between wild pollinators, natural habitats, and agricultural yields, more research is needed across a wider variety of crops and different growing regions.

Avocado (*Persea americana*) relies on insect pollination for optimal fruit production, with many growers employing managed honeybees in avocado orchards to ensure pollination. Avocado exhibits a flowering pattern known as protogynous dichogamy in which the hermaphrodite plants will open as male and female flowers at different times, with different cultivars opening as male and females at different times throughout the day (Nirody, 1922; Stout, 1932). This flowering process limits self-pollination and strongly promotes cross-pollination (Sedgley, 1977), thus increasing the likelihood that insect vectors play a crucial role. Furthermore, controlled pollination experiments have demonstrated that when pollinators are excluded from avocado flowers, fruit set or yield is close to zero (Dymond et al., 2021).

In addition to managed honeybees, wild pollinators contribute to avocado production. Several studies have observed a diverse array of wild pollinators visiting avocado flowers, suggesting that they play a role in the pollination process (Bushuru, 2015; Carabalí-Banguero et al., 2018; Castañeda-Vildózola et al., 1999; Celis-Diez et al., 2023; De la Cuadra-Infante, 2007; Estévez & Martínez, 2020; McNeil & Pidduck, 2003; Monzón et al., 2020; Read et al., 2017; Willcox et al., 2019). Certain species are also known to be effective avocado pollinators. For instance, wild bees have been shown to visit a similar number of flowers and deposit a comparable amount of pollen compared to honeybees (Bushuru, 2015; Can-Alonzo et al., 2005; Perez-Balam et al., 2012; Vithanage, 1990; Willcox et al., 2019).

Chile is a globally significant producer of avocados and currently has the third-largest production area in terms of hectares (FAO, 2022). Avocado orchards are primarily located in the Mediterranean region of central Chile. Within this region, native sclerophyllous forests stand as a biodiversity hotspot due to high levels of endemic fauna and flora (Myers et al., 2000). However, over the past two decades,

the expansion of avocado production has replaced much of this natural habitat (Armesto et al., 2010; Magrach & Sanz, 2020). Numerous studies have evidenced that natural habitats host an increased abundance and diversity of wild pollinators, resulting in a comprehensive and effective pollination service, thereby enhancing crop production in areas adjacent to natural habitats (Dainese et al., 2019; Garibaldi et al., 2011; Martin et al., 2019; Ricketts et al., 2008). As such, it is likely that the expansion of avocado orchards and the increasing isolation from natural habitats has negatively impacted avocado yield in this region. However, direct evidence is needed to test this claim.

This study aimed to address this question using data from field experiments in avocado orchards in Chile to investigate the contribution of natural habitats and wild pollinators to avocado pollination and production. Specifically, the objectives of this study are to: 1) identify the pollinators present in avocado orchards and explore the relationship between pollinator abundance, diversity, and proximity to natural habitat, 2) compare the effectiveness of different insect taxa in providing pollination services to avocados, 3) quantify the contribution of insect pollinators to avocado production and investigate whether this contribution varies with proximity to natural habitats. The findings of this study provide valuable insights for avocado growers regarding land management strategies for enhancing pollination management and achieving sustainable production.

3.3. Methods

3.3.1. Study design

This study was conducted in three Hass variety avocado orchards located in the Mediterranean region of central Chile in the years 2020, 2021, and 2022. To identify the farms, an initial list of commercial avocado orchards was provided from industry partners and other collaborators. Farms from this list were chosen if there was an area of native habitat more than 1km long surrounding the orchard. The selected farms were more than 30km apart, and it was also ensured that they all had similar topography, with plantations situated on the hillside. At all sites', managed honeybees were located throughout the orchard during the flowering season. On each farm, three transects were selected,

extending from the border edge into the centre of the orchard. Each transect was 300m long as, typically, wild bees and other small pollinating insects forage within approximately 100-200m from their nesting site, usually located in natural habitats (Zurbuchen et al., 2010). Two transects were run from the native habitat, and one transect was run from a non-natural habitat border, such as another agricultural crop (e.g., almond orchards) or farm infrastructure (e.g., reservoir), which served as a control.

3.3.2. Pollinator surveys

To collect data on pollinator abundance and diversity, pollinator surveys were conducted along the transects at distances of 0m, 50m, 100m, 200m, and 300m. At each point, two trees were observed for a duration of 5 minutes. The observations took place either 3 or 4 times throughout each flowering season, which occurred from October to December, depending on the year. The observations took place between 10 a.m. and 3 p.m. to coincide with the warmest part of the day when the avocado flowers were open. Observations only took place on warm days with little wind when more than 10% of the flowers on the trees were open. The observation area per tree was selected before the observation started and was identified by placing a 1 metre square quadrat in front of branch and/or branches at eye level. Once the area had been defined, the quadrat was removed to allow for easier observations. Data were recorded on species observed visiting avocado flowers and the number of times that they visited an open flower. If an insect could not be identified to the species level in the field, then the insect was either captured, photographed or a written description was taken, and the insect was identified at a later stage. In cases where identification was still not possible, a broad taxonomic group (e.g., honeybee, wild bee, fly, hoverfly, wasp, beetle) was assigned instead. Data were also recorded on the time of the observation, the stage of the flowers (male or female), the number of open flowers in the observed area, and the prevailing weather conditions (e.g., sunny, cloudy, windy).

3.3.3. Pollinator effectiveness

To assess pollinator effectiveness, flower visitation rate per taxon was used as a proxy, considering its significance as an effectiveness indicator (Rader et al., 2020). In all years, GoPro video cameras were set up in the avocado orchards, in an area close to the natural habitat, as it was hypothesised that these locations would have a greater diversity of pollinators. Two or three video cameras were used every day that pollinator surveys were taking place. Video cameras were focused on a flowering branch and recorded data for 1-2 hrs per day, from around 11 a.m. to 2 p.m. In 2020 and 2021, most recorded observations were of honeybees with limited replication for other taxa. To supplement the data set, in 2022 visitation rate data was also collected through Dictaphone voice recordings as this method allowed for more targeted recordings of less frequently observed taxa such as flies, wasps, and wild bees. To achieve this, the observer actively searched for a pollinator taxon of interest, and once identified, the recording began. The observer recorded when the pollinator arrived on a flower, when it left the flower, and when it landed on a new flower. The observation was continued for 5 minutes or until the pollinator went out of sight.

After the end of each season, the video and Dictaphone recordings were reviewed. The video recordings were watched using a VLC media player as at times it was necessary to use the interactive zoom function available on this software to focus closer on the target branch. After selecting the best view, the videos were watched, and if a pollinator was observed, the taxon, and if possible, the species were identified. However, species identification was not always possible due to the quality of the image. The BORIS software was used to extract observations from the video and Dictaphone recordings (Friard & Gamba, 2016). Using this software, data were recorded on when the pollinator landed on a flower, when it left the flower, when it was moving between flowers, when it landed on a new flower, and when it left the observation area.

3.3.4. Controlled pollination trials

Pollination treatments were established along the same transects used for the pollinator surveys, at distances of 0m, 100m, and 300m. At each distance, five trees and two panicles per tree (on separate branches) were selected and labeled with a brightly colored tag for easy identification. Panicles were chosen as a suitable scale for measuring pollination contribution, considering execution challenges associated with whole tree or branch measurements (Webber et al., 2020). It was ensured that the panicles on the same tree had a similar number of inflorescences and pre-flowering buds, were at a comparable height, and had similar access to light. Each panicle per tree received either a closed or open pollination treatment. Closed treatments involved securely enclosing the panicle with a mesh bag to exclude all pollinators. The bags were placed on the panicles in September before the flowers had opened and remained on the panicles until the end of the experiment in late December. Open pollination served as the control, allowing insect pollinators to access the flowers naturally. To calculate the percentage of fruit set, an estimation of the number of flowers per treatment panicle was conducted in the middle of the flowering season. This estimation involved calculating the average number of flowers per inflorescence by counting the flowers on 100 inflorescences, and then counting the number of inflorescences per treatment panicle. To save time in the field, a photo of the treatment panicle was taken, and then counting was done later, on a computer screen. The average number of flowers per inflorescence (17) was then multiplied by the number of inflorescences per treatment to provide an estimated number of flowers. Six weeks after the end of the flowering season, the number of fruit set per treatment panicle was recorded.

3.3.5. Statistical analysis

3.3.5.1. Pollinator surveys

Given the multiple observations per transects and distances, a mixed-effect model was necessary. We applied a generalised linear mixed model to the dependent variables of wild pollinator abundance, honeybee abundance, pollinator visitation rate, species richness, and species diversity. Species diversity was calculated using the Shannon index and data was combined for both natural habitat

transects at each site and all observation days to calculate the overall value. All models were run using the independent variables of; distance from the edge (categorical variable, 0, 50, 100, 200, 300), habitat type (categorical variable, natural habitat, control), year (categorical variable, 2020, 2021, 2022), the number of open flowers (log-transformed) and all two-way interactions. For most of the models, the random effects were transect nested within site and observation day. However, for pollinator diversity, since the data for natural habitat transects per site and all observation days were combined, only site was used as a random effect. We used a negative binomial family for wild pollinator abundance, honeybee abundance, and wild pollinator visitation rate as a Poisson distribution was highly over-dispersed. For pollinator richness, we used a generalised Poisson distribution with a log link as a Poisson distribution was under-dispersed, and for pollinator diversity, we used a Gamma distribution with a log link. All models were checked for overdispersion, and their assumptions were verified by plotting residuals against fitted values and each covariate in the model. The models were selected for 'best fit' using backwards stepwise deletion based on AIC (Akaike Information Criterion) comparisons and, where necessary, independent variables were dropped from the model. To assess significant differences between all distances at each habitat type, we ran another GLMM model for each dependent variable. The model structure was the same as before, however, a new independent variable was added to combine all possible distances and habitats (e.g., NH0, NH50, etc.). An ANOVA and post hoc Tukey's test were conducted on this model to identify significant differences for each distance and habitat combination.

3.3.5.2. Pollinator effectiveness

To determine the visitation rate (flowers visited per minute), we calculated the average time spent on a flower and the average time moving between flowers for each insect observed in the video and Dictaphone recordings and then applied the following formula: $60 / (\text{average time on flower} + \text{average time moving between flowers})$. Since the data was not normally distributed, we performed a Kruskal-Wallis test and post hoc Dunn test to compare between taxa (e.g., honeybees, flies, hoverflies, wild

bees, beetles, and wasps). The data for all years and all observation methods (video and Dictaphone) were combined, as the results from one-way ANOVAs conducted for individual taxa and year, and individual taxa and observation method were non-significant, indicating no difference due to the year observed or the observation method used.

3.3.5.3. Controlled pollination trials

To assess the contribution of natural habitats to fruit set, we applied a generalized linear mixed model with a binomial distribution and logit link to the dependent variable of 'proportion of fruit set'. The independent variables included pollination treatment (categorical variable, open and closed), habitat type (categorical variable, natural habitat and control), distance from the edge (categorical variable, 0m, 100m, and 300m), and all two-way interactions. The random effects were tree nested in transect and transect nested in site, however, transect nested in site was later removed as these effects had no impact on the model and consequently, the model would not converge.

Additionally, we conducted an analysis to explore how the abundance of individual pollinator taxa related to fruit set. For each site, transect, and fruit set distance (e.g 0m, 100m, and 300m only) we calculated the average abundance of each pollinator taxon using the pollinator survey data in 2022, as well as the average fruit set at each distance. We then applied a generalized linear mixed model (binomial family, logit link), using the proportion of fruit set as the dependent variable, the abundance of each pollinator taxon as the independent variables, and transect nested site as the random effect. Models followed the same process of model checking and fitting as before.

Data for all the above analysis were carried out in R version 4.2.3 using the R Core Team (2023) and the package glmmTMB (Brooks et al., 2017).

3.4. Results

3.4.1. Pollinator surveys

Across the three years of surveys and in the three study orchards, a total of 5,340 flower-visiting insects were observed, representing 76 different species. Honeybees accounted for 54% of the observations (n= 2,883), beetles 29.5% (n=1,574), hoverflies 7.4% (n=396), flies 5.8% (n=311), wild bees 1.7% (n= 89), wasps 1.6% (n=85), and Lepidoptera 0.2% (n= 11). All honeybee observations were assumed to be from managed hives, as wild honeybees are not thought to be present in the area.

For the metrics of wild pollinator abundance, visits, richness, and diversity, all independent variables were retained in the model, except for the interaction between distance and year and, in the case of pollinator abundance and wild pollinator visits, the interaction between habitat and year was also dropped. For all wild pollinator models, the interaction between distance and habitat type was significant and the results showed no relationship between control transects and distance to edge, while a negative relationship observed between natural habitat transects and distance to edge (Table 3. 1, Figure 3. 1, and Appendix 8). Furthermore, the results obtained from the ANOVA and Tukey's test demonstrated significantly higher abundance, richness, diversity, and visitation rates at 0m in comparison to nearly all other distances (Appendix 9). For instance, wild pollinator abundance and visitation rates were approximately 2.55 times higher, pollinator richness around 1.6 times higher and diversity 1.5 times higher at the edge compared to all other distances. For honeybee abundance, the number of open flowers, distance from the edge, habitat type, and year were retained in the model; however, only the number of open flowers was found to be significant (Table 3. 2, and Appendix 8).

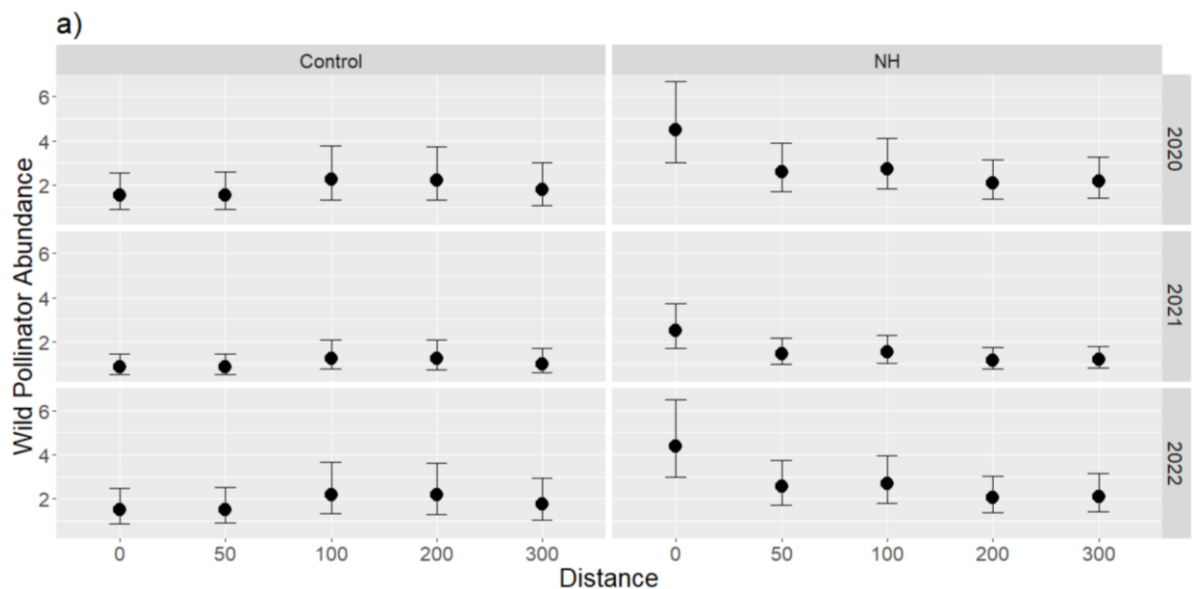
Table 3. 1. Z-values, and *p*-values for the GLMM models of wild pollinator abundance, wild pollinator

		Intercept	Log floral	Year 2021	Year 2022	Natural Habitat: Distance 50	Natural Habitat: Distance 100	Natural Habitat: Distance 200	Natural Habitat: Distance 300	Natural Habitat: Year 2021	Natural Habitat: Year 2022
Wild Abundance	Z value	1.546	5.799	-2.892	-0.257	-2.648	-4.091	-4.998	-4.040	-	-
	P value	0.122	<0.00001	0.004	0.797	0.008	<0.00001	<0.00001	<0.00001	-	-
Wild Visits	Z value	3.094	6.535	-3.267	-0.838	-1.889	-3.074	-3.628	-2.751	-	-
	P value	0.002	<0.00001	0.001	0.402	0.059	0.002	<0.001	0.006	-	-
Richness	Z value	5.076	7.796	-	-	-1.752	-3.561	-3.066	-2.642	2.529	2.919
	P value	<0.00001	<0.00001	-	-	0.019	<0.001	0.002	0.008	0.011	0.004
Diversity	Z value	0.969	-0.038	-	-	-1.752	-2.602	-2.051	-2.356	1.657	0.919
	P value	0.332	0.969	-	-	0.079	0.009	0.040	0.018	0.098	0.358

visits, pollinator richness, and pollinator diversity.

		Intercept	Log floral	Year 2021	Year 2022	Natural Habitat	Distance 50	Distance 100	Distance 200	Distance 300
Honeybee Abundance	Z value	4.534	9.606	-0.727	0.936	0.643	1.100	-0.588	0.214	-0.738
	P value	<0.0001	<0.0001	0.098	0.349	0.521	0.271	0.556	0.831	0.461

Table 3. 2. Z-values, and *p*-values for the GLMM model of honeybee abundance.



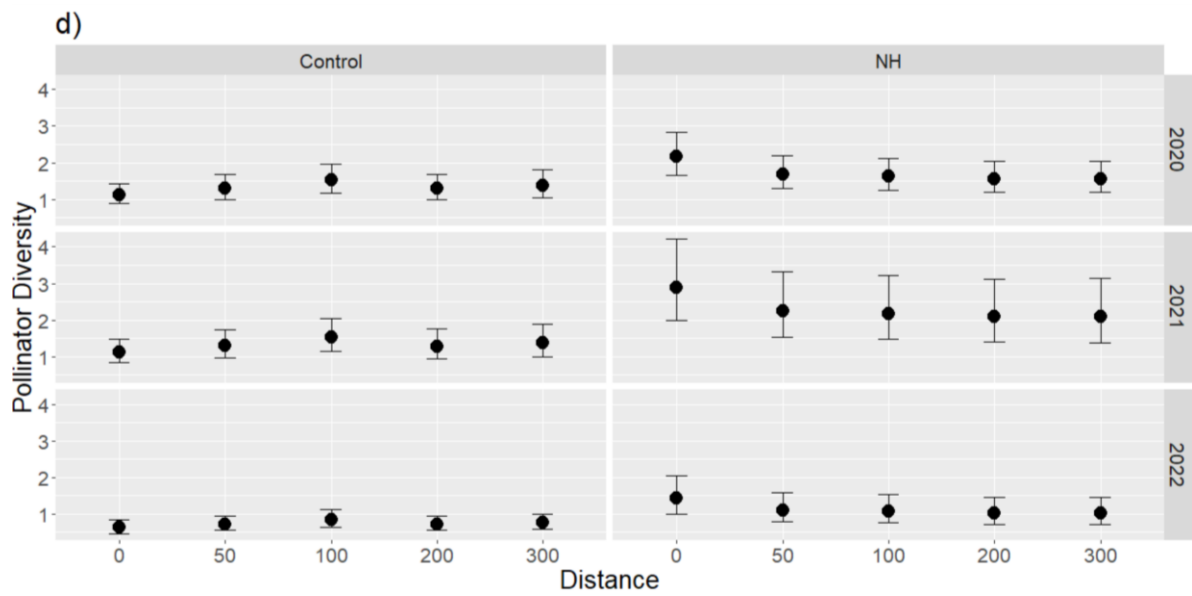
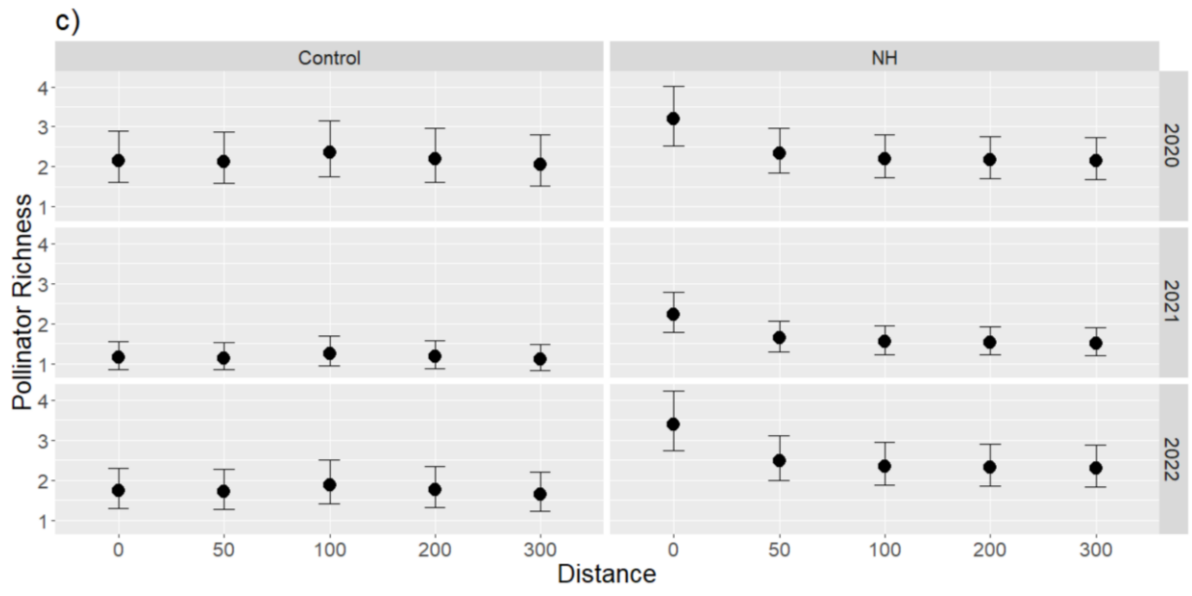
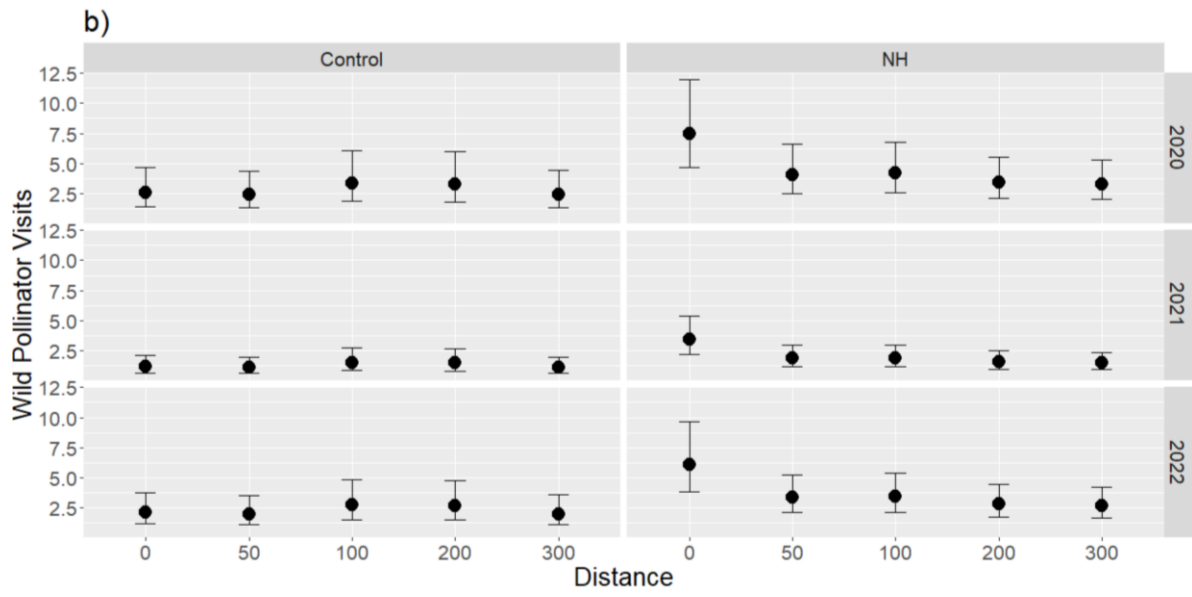


Figure 3. 1. Effects of distance from natural habitat edge and control edge on a) wild pollinator abundance, b) wild pollinator visits, c) pollinator richness and d) wild pollinator diversity. Point denotes the predicted mean for each distance and the bars represent the standard error.

3.4.2. Pollinator effectiveness

The analysis of pollinator visitation rates revealed that honeybees and flies visited the highest number of flowers per minute, averaging 8.5 and 7.9 respectively. In contrast, beetles had the lowest visitation rate with an average of 1.3 visits per minute (Figure 3. 2) with beetles visiting significantly fewer flowers compared to all other taxa (p -value <0.05 for all taxa). Honeybees and flies visited significantly more flowers per minute than hoverflies (p -value for honeybee: hoverfly <0.0001 , p -value for fly: hoverfly 0.001) (Appendix 10).

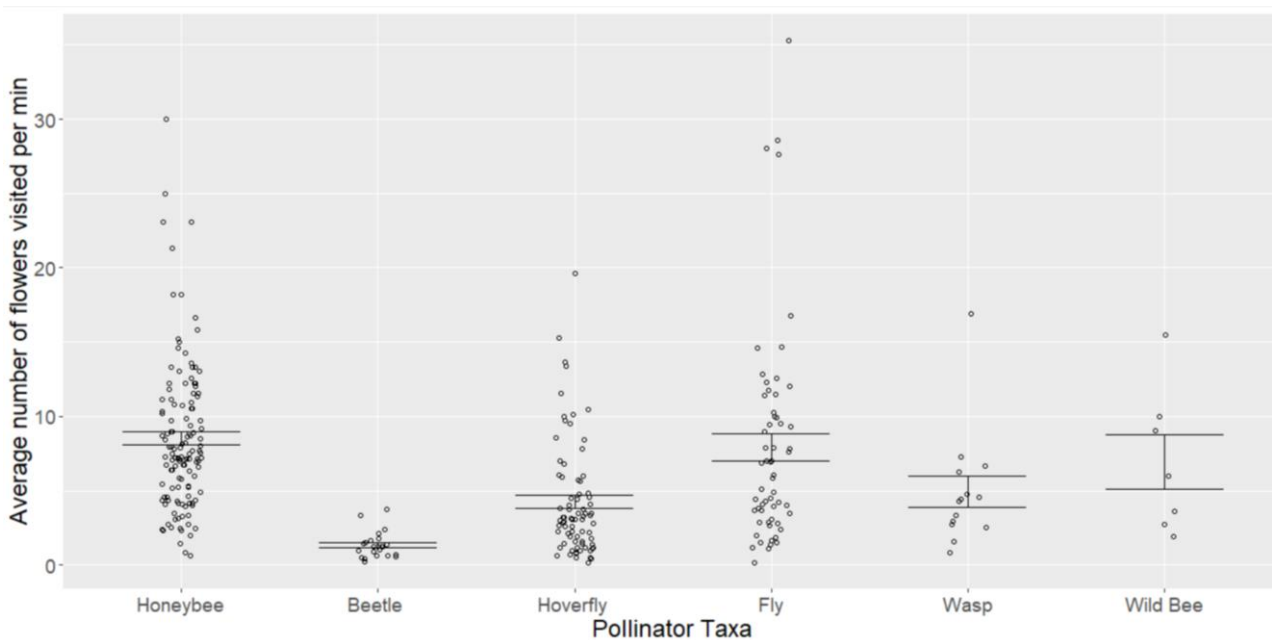


Figure 3. 2. The number of avocado flowers visited per minute for: honeybee ($n = 128$), beetles ($n = 23$), hoverflies ($n = 77$), flies ($n = 60$), wasps ($n = 14$), and wild bees ($n = 7$). Dots show the visitation rate per individual and the bars represent the standard errors. Beetles had a significantly lower visitation

rate compared to all other taxa (p-value <0.05) and honeybees and flies had a higher visitation rate than hoverflies (hoverflies: flies p-value 0.001 and hoverflies: flies p-value <0.0001).

3.4.3. Controlled pollination trials

In the fruit set model, the interaction between distance and habitat, as well as between pollination treatment and habitat were dropped. All other variables were retained in the model and the results showed a significant interaction between pollination treatment and distance, while no effect of habitat type was observed. Specifically, distance from the edge showed a negative linear trend, and the controlled pollination trials showed an average fruit set that was 5.7 times higher in open pollinated treatments compared to closed treatments (Table 3. 3, Figure 3. 3, and Appendix 11).

The analysis of pollinator taxa and fruit set indicated a positive relationship between the abundance of hoverflies and fruit set (p-value 0.003). Although the remaining taxa did not yield statistically significant results, the data suggests a potential positive relationship between fruit set and abundance of flies and wasps, while no such relationship was observed for honeybees and beetles (Figure 3.4).

Table 3. 3. Z-values, and p-values for the GLMM model of proportion fruit set.

	Intercept	Pollination Exclusion	Natural Habitat	Pollination Exclusion: Distance 100m	Pollination Exclusion: Distance 300m
Z Value	-21.413	-9.702	-0.474	4.536	2.333
P Value	<0.0001	<0.0001	0.635	<0.0001	0.019

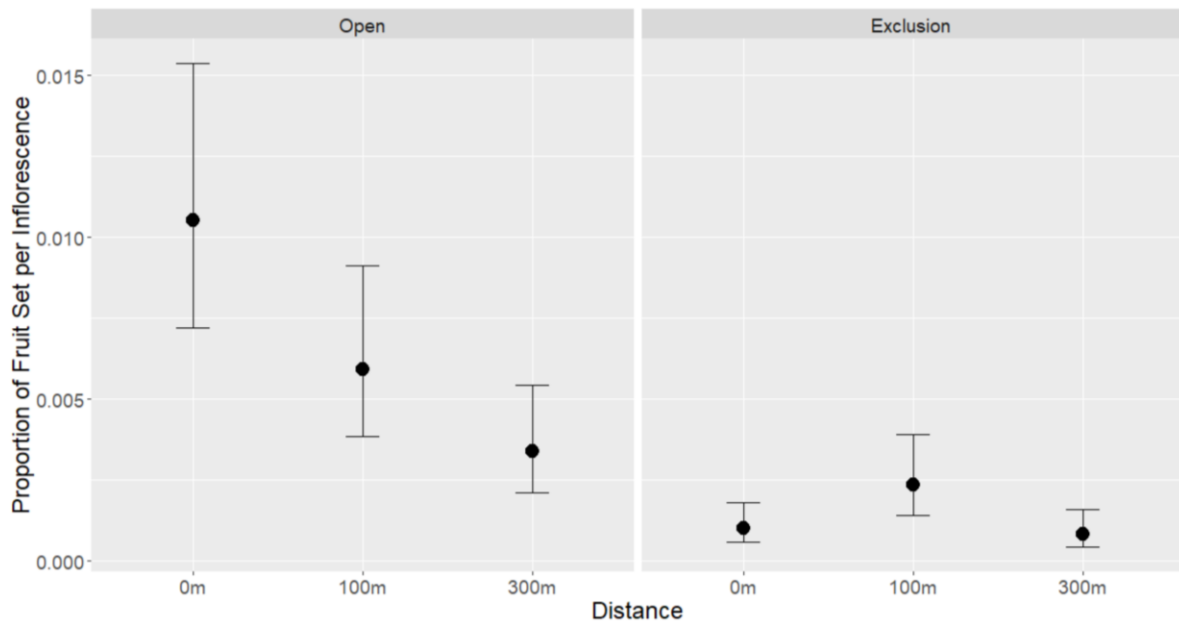


Figure 3. 3. The proportion of fruit set per inflorescence at increasing distances from the edge for open pollinated treatments and exclusion treatments. Dots denote the mean and bars represent the standard errors.

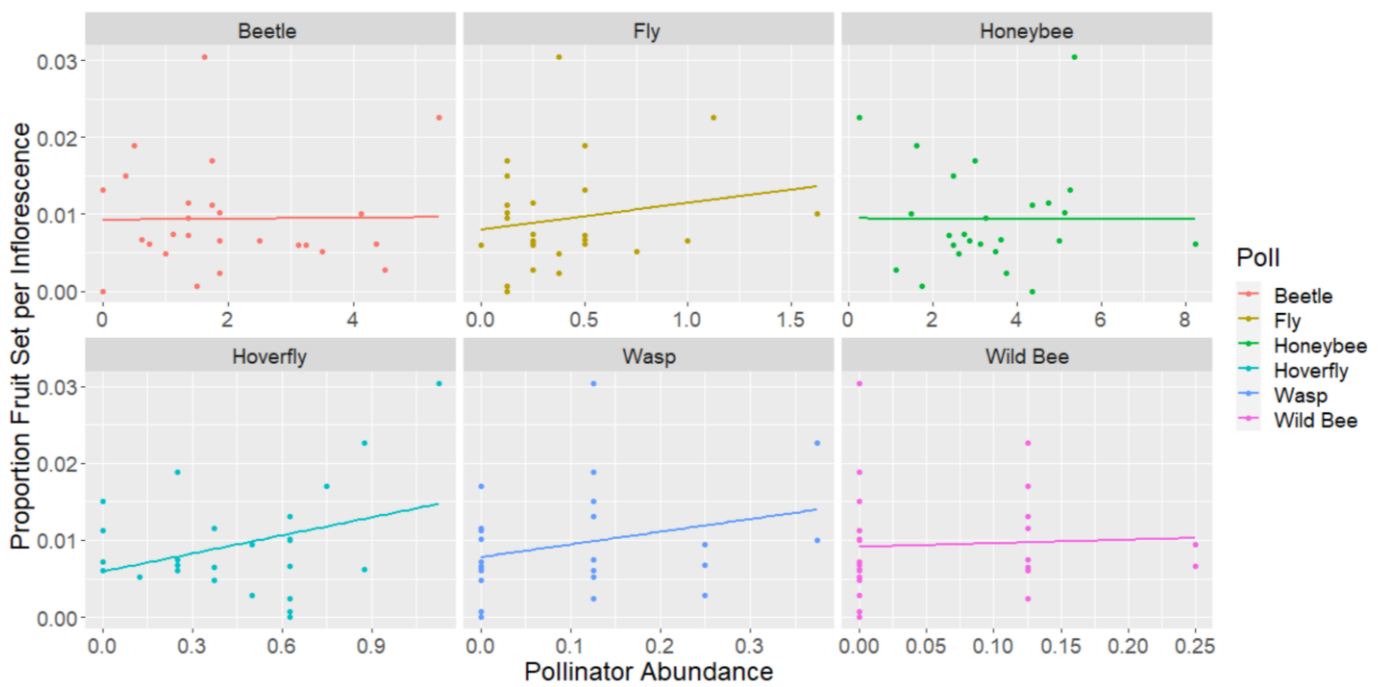


Figure 3. 4. The relationship between proportion of fruit set per inflorescence and average abundance of beetles, flies, honeybees, hoverflies, wasps, and wild bees. Hoverfly showed a significant relationship (p-value 0.003).

3.5. Discussion

Our study revealed that the abundance, diversity, visitation rate, and richness of avocado pollinators were all higher closest to natural habitats. The findings also demonstrated the significant contribution of insect pollinators to avocado production as pollinator exclusion trials yielded almost no fruit set. Wild pollinators were shown to play a crucial role in pollination as we saw higher fruit set in areas nearer the border edges and more specifically, our results emphasised the importance of flies and hoverflies in avocado pollination as they have a high visitation rate and we observed significantly higher fruit set in locations where these taxa were more abundant. Overall, this study contributes to our understanding of the effects of pollination and natural habitats on agricultural practices in Mediterranean central Chile, where robust data, collected over multiple seasons, is limited (Medel et al., 2018).

3.5.1. Pollinator surveys

The abundance, diversity, richness, and visitation rate of pollinators decreased with increasing distances from semi-natural habitats, aligning with findings from other studies and reviews (Bartual et al., 2019; Garibaldi et al., 2011; Klein et al., 2012; Ricketts et al., 2008). This relationship can be attributed to the provision of various resources for pollinators, such as nesting sites and additional food sources, within natural habitats. Consequently, these habitats tend to support higher pollinator abundance and diversity, which spills over into bordering agricultural area (Potts et al., 2005; Öckinger & Smith, 2007).

Additionally, our study highlighted that areas immediately adjacent to natural habitats had higher pollinator abundance and diversity, however, beyond a distance of 50 meters, there were no significant differences observed for greater distances. While a sharp decline in abundance was expected for certain taxa, such as solitary and wild bees that typically nest in natural habitats and have a limited foraging distance (Ricketts et al., 2008; Woodcock et al., 2016), our observations indicated that the majority of wild visitors were flies and hoverflies. These taxa often don't exhibit a strong negative relationship with distance from natural habitat edge, as they are generally not central place foragers and can therefore travel further from natural habitat areas (Rader et al., 2020). One possible explanation for this observation is that when feeding resources are abundant in close proximity to the natural habitat, these taxa are less likely to travel long distances in order to conserve energy (Chacoff & Aizen, 2006).

Furthermore, our results revealed no significant effect of distance from natural habitat on honeybee abundance. This finding is consistent with previous studies, as the abundance of managed honeybees is often influenced by the positioning of the hives in the agricultural landscape rather than natural habitats (Steffan-Dewenter & Kuhn, 2003).

3.5.2. Pollinator effectiveness

Our results showed that honeybees and flies had a higher flower visitation rate per minute compared to other pollinator taxa. Although several factors determine pollinator efficiency, pollination visitation rate is an important indicator of pollination effectiveness (Rader et al., 2020), and, for crops like avocado, which rely on pollen transfer from polliniser trees during the male flowering stage, a high visitation rate is important as it increases the probability that a male flower has been visited and, consequently, that the insect has deposited pollen. This finding supports existing research suggesting the importance of flies as avocado pollinators (Cook et al., 2020; Dymond et al., 2021; Perez-Balam et al., 2012; Vithanage). Future studies should focus on collecting additional metrics, such as single-visit

pollen deposition and flower handling behaviour by different species, to provide a more comprehensive understanding of pollinator effectiveness for avocados (Ne'eman et al., 2010).

3.5.3. Controlled pollination trials

Our results showed that insect pollinators play a vital role in avocado pollination, as we observed close to zero fruit set following pollinator exclusion. To our knowledge, there are only 2 studies that have shown significant avocado pollination in pollinator exclusion trials (Davenport, 2019; Davenport et al., 1994) and this anomaly is generally attributed to thrip pollination within the exclusion bags or humid climatic conditions causing overlapping transitions from the male to female stage on the same flower. Thus, our findings contribute to the existing literature highlighting the significance of pollinators in avocado production (Dymond et al., 2021) and suggest that pollinator contribution could be greater than the 40-90% contribution as identified in the review by Klein et al., (2007). Additionally, this finding underscores the importance of pollinators in a Chilean growing context, for which there is currently limited data.

Our results showed higher fruit set close to the border edges, but no effect of habitat type. One possible explanation for this is that abiotic factors present exclusively at the border edge significantly contribute to fruit set. For example, in our study sites, the border edges had approximately 4-5 metres of space between the orchard edge and the border, thereby increasing the availability of light and other resources. However, another explanation could be that certain pollinator taxa, which are less reliant on semi-natural habitats, contribute more to avocado pollination. Previous studies have shown that flies and hoverflies are effective avocado pollinators (Can-Alonzo et al., 2005; Castañeda-Vildózola et al., 1999; Ish-Am et al., 1999; Perez-Balam et al., 2012; Sagwe et al., 2022; Vithanage, 1990), and our results support this hypothesis, as we observed a positive correlation between fruit set and hoverflies and fly abundance. To investigate this finding further, we attempted to analyse the effect of distance and habitat type on individual pollinator taxa but the sample size for these less

observed taxa was too small to provide robust results. Nonetheless, previous research has shown that flies and hoverflies are less reliant on semi-natural habitats as they are often generalist species (Jauker et al., 2009; Jauker & Wolters, 2008; Rader et al., 2020; Schirmel et al., 2018; Speight, 2014). Consequently, the abundances of these taxa in natural and non-natural edges may be similar, potentially explaining the similar levels of fruit set in areas close to both habitat types. Additionally, such taxa tend to have larger flight distances (Rader et al., 2020) which could account for the strictly linear decline in fruit set, while overall pollinator compositions were higher only in areas immediately adjacent to the natural habitat border. However, it is important to note that our fruit set study was conducted for only one year and measured only initial fruit set. Therefore, further research is needed to fully understand this relationship and future studies should measure final fruit set or yield, as these metrics more accurately reflect production (Webber et al., 2020). Moreover, conducting studies over multiple years is necessary to account for annual fluctuations in production.

Furthermore, in line with other avocado pollination studies in Chile (Celis-Diez et al., 2023), our results showed that an increase in honeybee abundance did not have an impact on fruit set. This could suggest that even at low honeybee abundances, there are sufficient honeybee numbers to ensure adequate pollination. However, the average fruit set in this study was around 1% whereas wider studies have shown that under optimal pollination (manual pollination), fruit set can reach up to 5% (Alcaraz & Hormaza, 2009; Evans et al., 2010; Garner & Lovatt, 2008). This suggests a probable pollination deficit in our orchards, and that fruit set rates could be increased with improved pollination services. An alternative hypothesis is that honeybees are not efficient avocado pollinators, potentially due to low pollen deposition or an incompatible shape and size with avocado flowers (Rivest & Forrest, 2020). However, several other studies have shown a positive correlation between honeybee abundance as well as their effective pollen deposition in avocado flowers (Bushuru, 2015; Castañeda-Vildózola et al., 1999; Perez-Balam et al., 2012; Peña & Carabalí, 2018; Sagwe et al., 2022; Vithanage, 1990; Willcox et al., 2019). Therefore, the results likely indicate that pollinator diversity and richness

are beneficial for avocado pollination even when honeybee abundance is high. This has been demonstrated in several other crops (Garibaldi et al., 2013) and is likely due to the complementary pollination services provided by a variety of pollinators (Blüthgen & Klein, 2011; Hoehn et al., 2008) as well as functional facilitation, which occurs when wild pollinators displace honeybees, promoting outcrossing and improved pollination (Greenleaf & Kremen, 2006).

3.5.4. Management implications

Our study highlights the crucial role of insects in avocado pollination and underscores the importance of crop proximity to natural habitats in ensuring pollinator abundance, diversity, and richness. As such, it is recommended that avocado growers protect and enhance natural habitats throughout the agricultural landscape and ensure that crops are located close (ideally <100m) to natural habitat edges. Additionally, our results, along with previous research, indicate the likely importance of flies and hoverflies as key avocado pollinators. These taxa often have a broader foraging range and are not solely reliant on specific plant species that might only be found in natural habitats. Therefore, they can benefit from alternative habitat interventions, such as managed floral plantings within the crop. Several studies have shown that floral strips can improve crop pollination services and they are often considered a cost-effective and relatively easy pollination management strategy (Albrecht et al., 2020; Blaauw & Isaacs, 2014; Krimmer et al., 2019; Lowe et al., 2021; Muñoz et al., 2021; Rundlöf et al., 2018). However, recent reviews have highlighted that the implementation of floral strips can be ineffective without sufficient natural habitat in the landscape (Albrecht et al., 2020; Dainese et al., 2019) and therefore, we recommend that a combination of both management strategies are employed by growers.

Increasing natural habitats in agricultural landscapes can face resistance from growers due to concerns about increased pest prevalence and, in dry regions such as central Chile, the need for increased irrigation to maintain extra vegetation. However, in general, natural habitats have been shown to

reduce pests due to enhanced pest regulation services, leading to further yield improvements (Martin et al., 2019). Additionally, maintaining extra vegetation may not inherently be associated with increased water usage. Results from a recent study on macadamia nuts showed that pollinator plantings could maintain yields even under reduced irrigation rates due to increased pollination rates (Anders et al., 2023). However, further research is needed to understand its relevance to a wider variety of crops, such as avocados.

4. Chapter 4: A rapid assessment tool for identifying and managing pollination risks: a case study for the avocado industry.

Dymond, K., Celis-Diez, J.L., Lymma-Dennis, C., Potts, S.G., Rojas-Bravo, V., and Garratt, M.P., *Conservation Biology* (under review).

KD, MG, CLD, and SP conceived the ideas and designed the methodology. KD collected the data from the literature and interviews. KD and VRB collected the data for the farmer surveys. KD analyzed the data and wrote the manuscript. JCD, SP and MG contributed feedback to the drafts.

4.1. Abstract

Animal pollination plays a vital role in the production of many high-value, globally traded crops. However, pollination services are currently facing threats worldwide. Many companies rely on animal-pollinated crops within their supply chains and yet only a few of them take action to protect pollinators due to a lack of understanding regarding the risks faced by pollinators and the most effective support mechanisms. This study aims to address this issue by developing a tool that businesses can readily employ to better understand pollination services and to create effective strategies for protecting pollinators. To create this tool, we utilized an existing roadmap as a foundational structure and adapted it into a practical tool by conducting a thorough review of the relevant literature and developing specific methodologies. Subsequently, we applied this tool to a case study industry to further refine the methods and gather feedback from a business perspective. The developed tool identifies seven specific activities that industries can implement to achieve the following objectives: 1) understand the threats to pollinators in different regions, 2) recognize the significance of pollinators to their business, 3) assess the current implementation of pollinator actions, and 4) explore additional measures to support pollinators. The methods employed in this process include both new and existing desk-based methodologies, as well as grower surveys and informant interviews. The results obtained

from our industry case study indicate that increasing knowledge transfer to industry growers and supporting them to participate in environmental certification schemes could serve as effective strategies for pollination protection. The tool developed in this study aims to assist companies in identifying effective strategies to safeguard pollinators. Its potential implementation across a wide range of companies could greatly benefit growers and pollination services worldwide.

4.2. Introduction

It is widely recognized that pollinators play a crucial role in enhancing human well-being. Animal pollination is responsible for around 35% of the global food supply (Klein et al., 2007) and this includes some of the most nutritious crops for human health (Chaplin-Kramer et al., 2014; Eilers et al., 2011; Hristov et al., 2020). Additionally, nearly 90% of wild flowering species rely, at least partially, on pollinators (Ollerton et al., 2011) thereby contributing to broader ecosystem functions and biodiversity enhancement (IPBES, 2016). Most pollination services are provided by wild pollinators (Garibaldi et al., 2013; Kleijn et al., 2015), however, evidence suggests a decline in their abundance and diversity across Europe and North America, which is likely indicative of a global trend (Potts et al., 2016). Multiple factors contribute to this decline, including climate change, invasive species, pathogens/diseases, and competition from managed honeybees (IPBES, 2016) however, the most influential threat is agricultural intensification and expansion due to the associated natural habitat loss and fragmentation and the application of pesticides (Campbell et al., 2017; Vanbergen et al., 2020).

It's therefore imperative to develop land management solutions that support pollinators in agricultural landscapes. Various practices, such as creating or conserving pollinator resources on farms, diversifying farming systems, reducing agrochemical inputs, and protecting and restoring remnant natural areas, have proven efficient in promoting pollinator populations (IPBES, 2016). Encouraging landowners to implement these practical measures often requires support such as legal regulations, financial incentives or disincentives, technical advice, and persuasion techniques. While

such initiatives are typically implemented through governmental policies, the private sector can also play a pivotal role (Garibaldi et al., 2019), particularly in cases where government financial capacity or will is lacking.

For many private sector companies, safeguarding pollination services holds significant importance, as declines in wild pollinators can adversely impact the sustainability and profitability of their business. The extent of this threat depends on the company's reliance on insect-pollinated crops and its position within the supply chain (e.g., grower, supplier, processor, or retailer) (Breeze et al., 2022). Growers may experience reduced yield, quality, and production stability, whereas suppliers, processors, and retailers could face supply chain disruption and /or an increase in the purchasing price resulting from global production declines (Murphy et al., 2022; Tremlett et al., 2020). Inaction on pollinator protection also poses reputational risks, as consumers are increasingly aware of the threats to pollinators (Hoshide et al., 2018) and may adapt their purchasing habits based on agricultural production methods (e.g., avoiding products that have used neonicotinoid pesticides in their production).

Similarly, actors within the supply chain have the power to influence pollination services through their actions. Growers directly impact pollinators through their choice of land management practices, while suppliers, processors, and retailers can shape these practices by implementing pollinator-friendly strategies. For instance, supermarkets such as Marks and Spencer, Waitrose, and the Co-operative, encourage or require their growers to plant wildflower seeds and/or reduce their pesticide application (Co-operative, 2009; Marks and Spencer, 2023, Waitrose, 2013) and Jordans, a cereal supplier company, requires that all their growers provided pollinator habitats on their land (Jordans, 2023). However, overall, the implementation of pollinator strategies from agricultural industries remains limited.

In a recent report, the Cambridge Institute for Sustainable Initiatives et al., (2018) highlighted that many companies are reluctant to invest in protecting pollinators due to a perceived lack of accessible evidence demonstrating crop dependencies on pollinators, the absence of an immediate threat to their business, and the lack of evidence of a clear return on investment for pollinator protection strategies. Furthermore, comprehensive information on creating pollinator strategies is not widely available. While there are various resources for growers detailing farm-level pollination practices, and some resources that identify strategies industries could employ to improve biodiversity and nature more broadly (see UK Business and Biodiversity Forum and Get Nature Positive), there is

currently limited guidance specifically focused on actions for pollinators. Consequently, there is a clear need to develop a practical tool in this area.

In their 2018 report, the Cambridge Institute for Sustainable Initiatives et al., proposed a high-level roadmap that industries can follow to understand the importance of pollination services to their business and identify ways to support these services. However, the existing roadmap does not provide a specific and tangible methodology that industries can readily implement. Therefore, the objective of this study is to build upon this roadmap and create a tool that agricultural suppliers, processors, and retailers can use to assess their dependencies on pollinators and identify effective pollinator management strategies in a quick, practical, and cost-effective manner. Firstly, we describe the development of the tool and provide a high-level overview of the assessment process. Secondly, we apply this tool to a real-life case study for an avocado supplier business, providing a detailed demonstration of the approach. Finally, we explore the potential applications and limitations of our

Glossary

- **Pollinator Practices:** A land management technique that is implemented at the ground level to support pollinators and pollination services e.g., planting floral strips.
- **Pollinator Strategies:** A tool that is used by the industry to help support growers implement pollinator practices e.g., providing training on floral strips.

approach and identify potential avenues for further development and adaptation of the tool for wider use.

4.3. Methods

To develop this tool, we used the roadmap outlined in the pollination deficit report as a foundational structure. This roadmap was developed based on research conducted with 27 companies that rely, at least in part, on pollinators. It aims to enable companies to understand pollination services and implement sustainable pollinator management by defining high-level steps, aims, and activities required for the evaluation process (University of Cambridge Institute for Sustainability Leadership et al, 2018) (see Appendix 12 for an extract of the road map).

For our study, we modified this structure to ensure the objectives of our tool were met e.g., an effective, rapid, and cost-effective process that could easily be conducted by non-technical experts. For example, some activities outlined in the original roadmap were considered too time-consuming and complex for our purposes. Additionally, the final step in the roadmap, implement and monitor, was excluded from our process as there was not sufficient time to implement the identified pollinator strategies and monitor their effects. However, we acknowledge the importance of this phase in refining and improving the tool.

Importantly, many of the activities were expanded to provide practical implementation methods. To achieve this, we conducted a thorough review of relevant literature associated with each aim. Initially, the IPBES (2016) report on pollinators served as a comprehensive resource for key pollinator topics, however, if this report did not provide the necessary information, then we conducted detailed searches on Google Scholar. If there were multiple methods available, the one that was most aligned with the aims of this study was selected. In some instances, when existing methodologies were not suitable, we developed new approaches based on a comprehensive review of the broader literature combined with the aims of our study.

To further refine and adapt the approach, we applied the tool to a real-life case study. Once this process had a clear structure, we shared it with the case study business for feedback to ensure its practicality and ability to deliver actionable outputs.

4.3.1. Developing the tool

For each step in the process, we selected one to three aims from the roadmap and adapted them into a specific activity. Subsequently, for each activity, we developed an appropriate method. Figure 4. 1 illustrates the high-level process that was developed, and the case study application section provides details on how to implement the activities and methods.

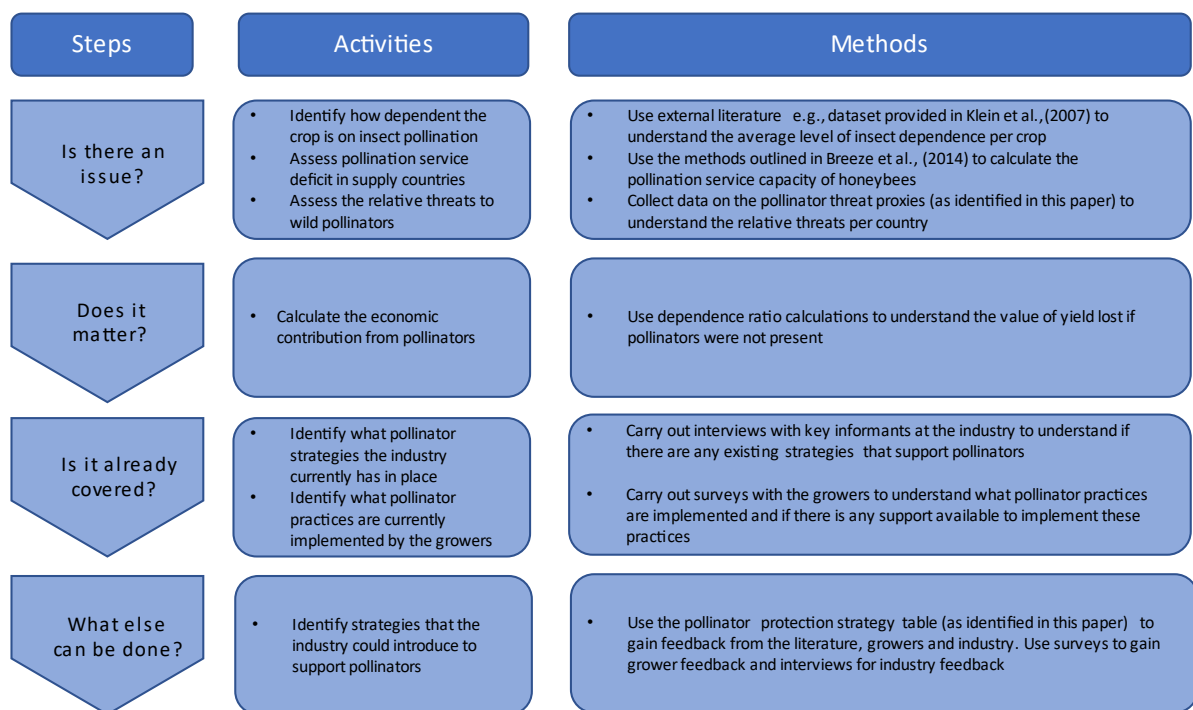


Figure. 4. 1. Overview of the tool that agricultural companies can use to understand the importance of pollinators to their industry and to identify pollination management strategies. Adapted from ‘The Pollination deficit report: Towards supply chain resilience in the face of pollinator decline’ (University of Cambridge Institute for Sustainability Leadership, et.al., 2018).

4.3.2. Case study application

We selected a global agribusiness that specializes in trading avocados (*Persea americana*) as our industry case study. This company was perceived to be a suitable as avocados have a high dependence on insect pollination (40-90% dependence range; (Dymond et al., 2021; Klein et al., 2007)) and additionally, the company is involved in both avocado cultivation on their own farms and sourcing avocados from third party growers and can therefore exert influence on land management practices that directly impact pollinators.

Peru, South Africa, Israel, Chile, and Spain are currently the biggest suppliers of avocados to this industry and so these were considered in our approach. However, the surveys to understand grower pollination practices were implemented only in Chile, where additional avocado research was being carried out and therefore grower surveys could be deployed.

4.3.3. Applying the methods

4.3.3.1. Step 1. Is there an issue?

Activity 1. Identify how dependent the crop is on animal pollination.

To determine the extent of insect pollination dependency, a meta-analysis conducted by Dymond et al. (2021) was reviewed as this paper specifically focuses on the contribution of insect pollinators to avocados. For other crops, different studies or reviews may be available or, alternatively, the review by Klein et al. (2007) offers a comprehensive dataset of pollination reliance of the top 100 globally traded crops.

Activity 2. Assess honeybee pollination deficits in supply countries.

To evaluate honeybee pollination deficits, we adopted the methodology outlined in Breeze et al. (2014). This method calculates the pollination service capacity per country by dividing the supply density of honeybees by the demand density. The supply density is determined by the number of

honeybee hives per country divided by the total area of insect-pollinated crops in that country and the demand density is calculated using the formula: (hectares of insect-pollinated crops per country * (recommended stocking rate (RSR)/2)) / the area of insect-pollinated crops. To account for one movement in honeybee hives per year per country, the RSR is divided by 2, however, the number of movements per season varies per country, and therefore it is recommended to adjust this number based on the specific context. For a more detailed explanation of the methodology, please refer to Breeze et al. (2014).

For this study, we obtained data on the number of honeybee hives per country from the FAO statistic database (FAO, 2022). For Peru, FAO data on hive numbers were not available and therefore this information was taken from the governmental website for the Department of Agriculture. The calculation of 'hectares of insect pollinator crops' included crops where insects were shown to have either a modest, great, or essential contribution to production, as identified by Klein et al. (2007). The total area per country was derived by summing the production hectares for these crops, and data for this calculation was also extracted from the FAO statistic database (FAO, 2022). The RSR for each crop was obtained from various literature sources and to account for variations in recommendations, a range of values (lower, medium, and upper) was provided. In cases where RSR data was unavailable, a rate was assigned based on recommendations for a similar crop or, if recommendations were not available, then an average for all crops was used.

Activity 3. Assess the threats to wild pollinators.

There is limited or no information on the status of wild pollinator trends, especially outside of Europe and North America, and therefore, we developed a method that utilizes easily obtainable proxy data to understand the level of threats to wild pollinators. While this method does not provide a quantitative understanding of how crop pollination might be affected, it does highlight the relative risks for businesses and identifies which supplier countries are most vulnerable to wild pollinator losses.

Initially, we identified key indicators which are known to negatively affect the abundance and diversity of wild pollinators. The selected indicators were land cover and configuration, land management, and pesticide use, as these three factors were identified as highly important in the IPBES pollinator report and further highlighted as the most influential factors, with the highest amount of evidence, in a recent global assessment (Dicks et al., 2021). Although climate change and pests and pathogens were also noted as important in this review, we did not include these factors in our methodology due to the complexity of obtaining accurate data on these indicators. For instance, climate change operates over long periods and may not be representative of a short-term timeframe and limited data is available on how pests and pathogens impact native species in specific regions.

For each of the selected indicators, we used suitable and easily obtainable proxies and sources (see Table 4. 1). The proxy selected for the indicator ‘land cover and configuration’ was ‘predicted loss of suitable habitat’. The data for this information was extracted from a recent global assessment of predicted global habitat loss by Powers and Jets, (2019). Although this paper evaluates future losses in suitable habitat ranges for amphibians, birds, and mammals and does not specifically include insects in its assessment, many studies have demonstrated the importance of natural and semi-natural habitats as predictors of pollinator abundance, diversity, and pollination (Dainese et al., 2019; Martin et al., 2019). Fertilizer use was identified as a proxy for overall land management, as high levels of chemical fertilizer are often used as an indication of intensive agricultural production (Tilman et al., 2011).

Table 4. 1: Proxies and information sources for key indicators that threaten wild pollinators.

Key Indicators	Proxy	Source
Land cover and configuration	Predicted average decadal loss of habitat suitable range (HSR) % per country	Global habitat loss and extinction risk of terrestrial vertebrates under future land-use-change scenarios, Powers and Jets, 2019

Land management	Fertilizer application on agricultural land (kg/hectare) per country. Average taken for the last 4 years	FAO statistics, Data, Land inputs and Sustainability, Input, Fertilizer by Nutrient https://www.fao.org/faostat/en/#data
Pesticide use	Pesticide application on agricultural land (Kg/hectare) per country. Average taken for the last 4 years	FAO statistics, Data, Land inputs and Sustainability, Input, Pesticide Use https://www.fao.org/faostat/en/#data

In this example, data were available for all countries and proxies apart from predicted loss on HSR for Israel, as this paper only assessed countries bigger than 50,000M². Therefore, to obtain data for Israel, an average was calculated using the data from surrounding countries (Jordan, Syria, and Egypt). Once the data for each of these proxies was extracted, a simple ranking system was applied to understand the comparative threats to pollinators across the countries making up the supply base of the case study company. The data for each proxy was split into 5 equal brackets (brackets were determined by dividing the highest score by five) and each country was assigned a number (1-5) based on which bracket that country's data was in. An overall score was then calculated by summing up the ranking figures for all the proxies. The same bracketing principle was then applied to the final score to provide a high, medium, or low relative threat level.

4.3.3.2. Step 2: Does it matter?

Activity 1. Calculate the economic contribution of pollinators.

To calculate the economic contribution of pollinators, we used dependence ratios. These ratios allow us to estimate the economic value of yield lost if pollinators were not present. The formula used is as follows:

$$\text{Economic value of insect pollination (EVIP)} = \text{Quantity of crop} * \text{Selling price of crop} * \text{Dependency value of crop}$$

We obtained the dependence value for avocados from the dataset in Klein et al. (2007), and the midpoint of 65% was used. The quantity of avocados considered was the average amount of avocados (in metric tons) that the industry had exported per country over the last 3 years (2017-2020) and the price used was the current global trading price for avocados. The economic contribution from wild and managed pollinator groups was also calculated, however, due to a lack of data on wild pollinators, the methods and results were excluded from this process (Appendix 13).

4.3.3.3. Step 3. Is it already covered?

Activity 1. Identify what industry strategies currently exist.

To understand the existing pollinator strategies, we conducted key informant interviews with the global head of sustainability at the company and the research and development manager in Chile. These employees were chosen as they hold management positions in the sustainability sector and therefore had a strong understanding of the company's current environmental or sustainability strategies. The interviews were conducted via an online video call at a pre-arranged time between May and September 2021.

To design the interview questions, we reviewed the literature to identify common strategies that promote environmental change. The following areas were identified: advice/ training, regulations, financial incentives, and certification. The questions asked whether the company had strategies in any of these areas to encourage environmental change. The questions were aimed more generally towards environmental protection, as strategies with a broader focus are more likely to be implemented and are expected to benefit pollinators as well. If the initial response was positive, follow-up questions were asked to gather more details, particularly regarding the relevance to pollinators. The interviews followed a semi-structured format to allow for clarification or exploration

of specific points of interest (see Appendix 14 for the complete set of interview questions). Ethical clearance for these interviews and the surveys described in the following section was given by the University of Reading, School of Agriculture, Policy, and Development Ethical Committee 2021 (application reference number 001866).

Activity 2. Identify what pollinator practices are currently being implemented by the growers.

To gain a deeper understanding of the pollinator management practices at the farm level and the available support, a simple survey was conducted with industry growers. Multiple-choice questions were utilized to reduce the time required to complete the survey and encourage maximum participation. The survey began with a general question asking farmers about the pollinator practices implemented on their land e.g., providing native habitat, keeping honeybees, etc. The options presented for pollinator practices were sourced from the IPBES pollinator report (section 6.1.1.1 technical response to restore and protect pollination) and were selected only if the evidence supporting their efficacy was 'well established'. If respondents indicated the implementation of certain practices, follow-up questions were posed to gather further details on the actions. Additionally, participants were asked if they received any support and, if so, the type of support and its source (see Appendix 15, for the survey questions).

The survey was conducted in Chile between June 2022 and May 2023. A list of suitable growers was provided by the case study business and partner organizations (INIA La Cruz: Instituto Nacional de Investigaciones Agropecuarias in Spanish). Farmers were contacted to see if they would be willing to participate in a short survey and, if they responded positively, a date was scheduled. Surveys were conducted via an online call and before the participants gave their consent for the survey to proceed, the purpose of the study, as well as details regarding data storage and usage were explained to the farmers.

4.3.3.4. Step 4. What else can be done?

Activity 1. Identify strategies that the industry could introduce to support pollinators.

To identify suitable strategies, a literature review was conducted to understand existing pollinator strategies. The findings were summarized into three key themes: regulations, economics, and persuasion. For each theme, examples of current strategies for pollination management were compiled, along with suggestions for adapting these strategies for the private sector (Table 4. 2).

Table 4. 2. Pollinator protection strategies and ways in which they can be adapted for the private sector.

Theme	Examples of pollinator management strategies	Adaptation for the private sector
Regulations: legal or mandatory rules.	Bans, regulations, or compulsory labeling on certain pesticides or GMO products. Mandatory inspections and/or registrations for beekeepers. Regulations on the importation and trade of honeybee hives. Prohibited release of nonnative insects. Protected natural areas to maintain or improve biodiversity.	<i>Environmental contract or mandate certification schemes</i> Create regulations on environmental / pollinator management that growers must abide by to sell their produce to the industry or require that growers are part of environmental certification schemes.
Economic: financial incentives for positive behavior or disincentives for negative behavior.	Direct payments to farmers who implement practices that support pollinators. Certification schemes that pay higher prices for products if they are produced in a pollinator-friendly manner e.g., fair to nature.	<i>Certification schemes</i> Increase grower participation in relevant certification schemes. <i>Insurance scheme</i>

	<p>Crop insurance schemes for farmers who participate in certain land management practices e.g., IPM.</p> <p>Inputs provided to farmers for pollination management e.g., wildflower seeds.</p> <p>Taxes or fees for pesticide use.</p>	<p>Provide insurance to growers who make specified environmental changes on their land.</p> <p><i>Environmental inputs</i></p> <p>Provide environmental inputs for pollination management.</p>
<p>Persuasion: Encouraging behavior change and enhancing knowledge.</p>	<p>Training farmers and agronomists on pollinators and pollination management.</p> <p>Community voluntary codes of practice for pollination management.</p> <p>Research on pollinator management/ agroecological farming and increased farmer collaboration in research.</p> <p>Monitoring and evaluation schemes for pollinators on farms</p>	<p><i>Knowledge transfer</i></p> <p>Increase knowledge transfer to growers on environmental/ pollination management.</p> <p><i>Environmental reporting platform</i></p> <p>Create an online platform where growers can report on environmental achievements/ targets.</p> <p><i>Agri-environmental research</i></p> <p>Implement more research in agroecological farming (including pollination management) either on research stations or by encouraging farmer experimentation.</p>

To gain further insights into which of these strategies could be applied to this case study, we collected feedback from various sources, including literature, growers, and industry. We conducted a comprehensive literature review to assess the effectiveness of each strategy (Appendix 16).

Additionally, we conducted online growers' surveys (as described in Step 3, activity 2) to identify the barriers growers face when implementing sustainable land management practices. The survey data was then analyzed to determine the most commonly mentioned barriers and Table 4. 2 was reviewed to identify the strategies that could best address these barriers. Furthermore, we obtained feedback from the industry through key informant interviews (as explained in Step 3, activity 1) where questions were posed regarding the most relevant themes and strategies for their business.

By synthesizing the feedback from all sources, two candidate strategies were identified. The details of these strategies were developed by reviewing relevant literature and current examples in other businesses.

4.4 Results

4.3.4.Step 1. Is there an issue?

Activity 1. Identify how dependent the crop is on insect pollination.

The review by Dymond et al.(2021) shows that avocados have a high reliance on insect pollination. The meta-analysis conducted in this paper revealed significantly higher fruit set in all open-pollinated treatments compared to treatments where pollinators were excluded.

Activity 2. Assess managed honeybee pollination service deficits in supply countries.

The results indicate that managed honeybees can provide sufficient pollination services in Israel with a pollination service capacity greater than 100%. Chile and Spain exhibited low honeybee pollination deficits (approximately 15%), whereas Peru and South Africa had very high deficit levels, with the capacity to provide pollination to less than 20% of all insect-pollinated crops (Table 4. 3). It should be noted that in some countries, for example South Africa, there are high rates of feral honeybees that were not accounted for in this analysis. However, feral honeybee deficits should be considered in the

next activity as they will likely be affected by the same environmental threats that impact other wild pollinators.

Table 4. 3. The pollination service capacity of managed honeybees in supplier countries

Country	Supply Density (available colonies/ha of insect-pollinated crops)	Demand Density (mean colonies required/ha of pollinated crops)	Pollination Service Capacity (% supply of honeybees relative to demand)
South Africa	0.05	1.0	4.66
Chile	1.68	2.04	82.48
Israel	2.83	2.14	132.12
Spain	1.69	1.98	85.52
Peru	0.39	2.15	17.46

Activity 3. Assess the threats to wild pollinators.

When considering the overall threat to wild pollinators, all regions in the study exhibited either a medium or high threat (Table 4. 4). Israel and Chile showed the highest level of threat, as these countries had a high ranking for all proxies. On the other hand, Peru, Spain, and South Africa showed a medium threat level primarily attributed to less intensive agriculture in these countries.

Table 4. 4. The relative threat to pollinators in supplier countries through a proxy assessment.

Proxy Used	South Africa		Spain		Israel		Chile		Peru	
	Result	Rank	Result	Rank	Result	Rank	Result	Rank	Result	Rank
Average decadal habitat suitable range loss %	0.79	5	0.29	2	0.36	3	0.58	4	0.45	3
Fertilizer application (kg/hectare)	63	2	112	3	184	4	273	5	88	2
Pesticide application (kg/hectare)	2.16	1	3.02	2	14.62	5	5.8	3	1.74	1
Total Ranking Score		8		7		12		12		6

4.3.5. Step 2: Does it matter?

Activity 1. Calculate how much pollinators contribute economically to the business.

Across the five study countries, pollinators potentially contributed around 225 M USD in revenue to the industry (Table 4. 5).

Table 4. 5. The contribution from pollinators to the industry revenue.

Country	Total Revenue (USD)	Pollinator Contribution to Revenue (USD)
South Africa	87,780,000	57,057,000
Spain	26,910,000	17,491,500
Chile	72,375,000	47,043,750
Israel	36,270,000	23,575,500
Peru	122,730,000	79,774,500
Total for all pollinators	346,065,00	224,942,250

4.3.6. Step 3. Is it already covered?

Activity 1. Identify what industry strategies currently exist.

The interviews revealed that there are no industry-wide strategies in place to protect pollinators or regulate environmental land management. However, the company emphasized that while they don't have any hard strategies (e.g., mandatory and audited), their core principles are centered around environmental protection, and as such, they exemplify soft strategies (e.g., voluntary and encouraged). On their own farms, they implement environmental best practices, and they believe that leading by example will inspire their suppliers and other growers to adopt similar principles.

Additionally, the company requires all their growers to be part of the global gap certification scheme and they also encourage their suppliers to participate in environmental certification schemes such as the Rainforest Alliance. Although these certification schemes don't specifically focus on pollinators or pollination management, both schemes, particularly the Rainforest Alliance, include requirements that may benefit pollinators such as allocating a percentage of land for natural habitats.

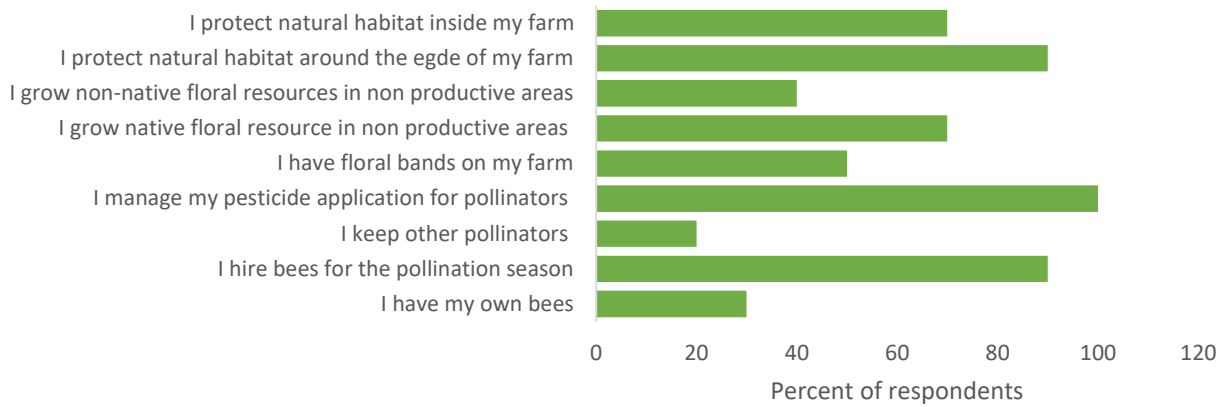
Activity 2. Identify what pollinator practices are currently being implemented.

The surveys showed that the three most common pollinator practices implemented by avocado growers were 'controlling pesticide management to benefit pollinators', 'hiring managed honeybees throughout the pollination season', and 'protecting natural habitat around the edge of the farm' with over 90% of farmers carrying out these practices (Figure 4. 2). In-between 40-70% of farmers actively managed their land for pollinators by either planting floral bands (50%) or restoring non-productive areas of their land with native (70%) or non-native (40%) floral resources. Only a small number of farmers kept their own bees and only 2 out of 10 respondents managed other pollinators such as flies and bumblebees.

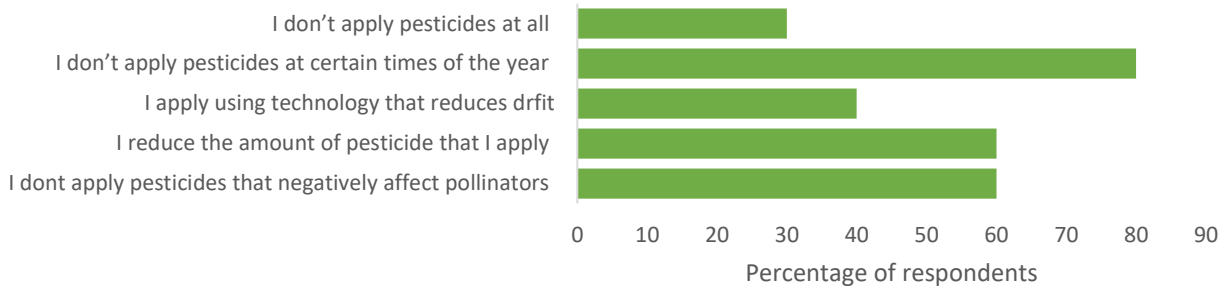
The most common pesticide management practice was to refrain from applying pesticides at certain times of the year, particularly during flowering, with 80% of farmers implementing this practice. When planting floral resources and protecting natural habitats, over 60% of farmers who engaged in these practices allocated more than 5% of their land for this purpose (Figure 4. 2).

All farmers stated that they did not receive any form of support such as training, or financial assistance to implement these pollinator management practices.

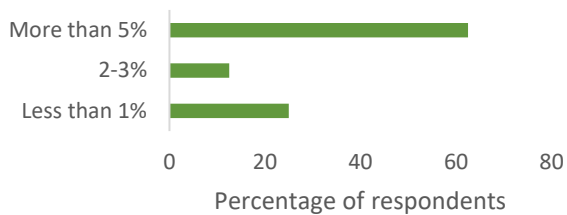
What pollinator management practices do you carry out on your land?



How do you manage pesticide application for pollinators?



How much of your land is dedicated to floral resources?



How much of your land is dedicated to natural habitat?

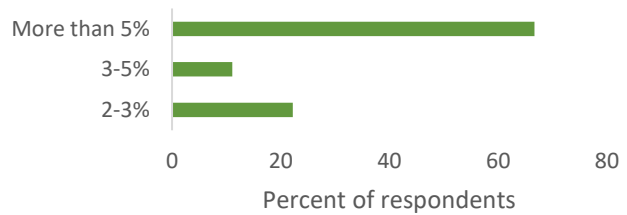


Figure. 4. 2. Questions and responses to grower survey on pollinator management. Surveys were conducted with 10 Chilean avocado farmers who primarily export their avocados to international markets.

4.3.7. Step 4. What else can be done?

Activity 1. Identify strategies that the industry could introduce to support pollinators.

Based on feedback from the growers (Figure 4. 3) and industry, and in conjunction with a literature review (see Appendix 16), the following summary discusses potential strategies to support pollinators.

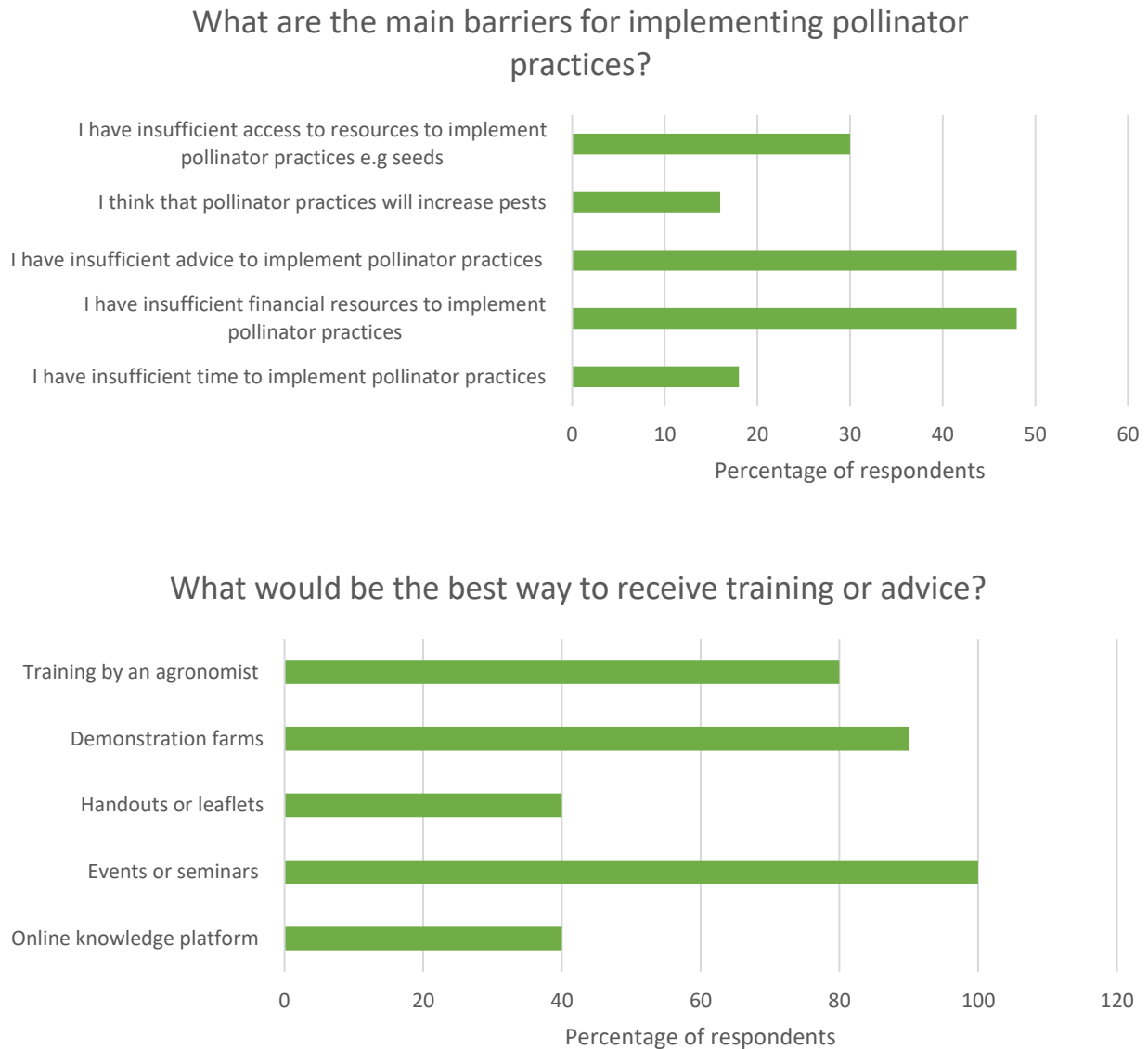


Figure 4. 3 Questions and responses to grower survey on barriers pollinator management and ways to receive training or advice. Surveys were conducted with 10 Chilean avocado farmers who primarily export their avocados to international markets.

Regulations

In this case study, strict regulations are unlikely to be suitable. The literature suggests that environmental regulations are most effective when there is evidence of high public risk (Pannell, 2008), and the industry perspective aligns with this view. For example, one interviewee stated, *“If it is a critical issue, then we can talk about regulations if not, then we talk about principles”*. In relatively low immediate-risk scenarios such as pollinator management, strict regulations may not be effective as they can demotivate landowners and reduce compliance (Mills et al., 2018). Additionally, for regulations to be effective, there needs to be strong enforcement (Steg & Vlek, 2009). The industry perceived this difficult to achieve, as they source around 70% of their produce from external growers and noted that *“ensuring that 3rd party suppliers are meeting certain standards is really tricky”*. It is possible to enforce environmental standards by requiring suppliers to join a certification scheme however, the industry was not keen to mandate this as they perceive their suppliers will sell elsewhere if certification schemes are a requirement.

Economic

Economic strategies to encourage pollinator management are likely to be effective, but only certain types of strategies are feasible in this case study. Economic sanctions are not recommended as growers would be unwilling to sell their products to companies with such regulations, and the literature suggests economic incentives are generally more effective than punishments (Steg and Vlek, 2009). Feedback from growers also supports the implementation of economic incentives as a ‘lack of financial resources’ was identified as one of the biggest barriers to implementing pollinator practices (48% of respondents). Furthermore, the IPBES (2016) pollinator report showed substantial evidence that financial incentives paid to farmers for pollination practices can increase pollinator abundance and diversity, and there was some evidence that certification schemes can promote pollinator conservation. However, direct payments were not seen as a feasible or effective option by the industry. They prefer to encourage change by paying premiums through certification schemes and by

“demonstrating to farmers that doing things (an environmental action) in a certain way will give them more profit”.

Persuasion

For this case study, persuasion techniques are likely to be a suitable strategy. The literature shows that persuasion techniques can enhance intrinsic motivation, thus improving a farmer’s willingness to take action (Mills et al., 2018). The industry perspective agrees with this view, considering persuasion techniques as generally the most effective and appropriate. They believe that education (e.g., using best practice guidelines and potentially demonstration farms), and environmental reporting are the most effective methods to create change. Survey feedback also indicated that persuasion, especially education, would be an effective tool, as a lack of access to necessary advice was identified as another key barrier to implementing pollination practices (48% of respondents).

Overall, the feedback suggests that out of the strategies identified in Table 4. 2, increased participation in certification schemes and enhancing knowledge transfer, with a specific emphasis on the economic benefits of pollinator management, may be appropriate for this industry. Table 4. 6 provides more details on how these recommendations can be effectively implemented.

Table 4. 6. Final strategy recommendations

Recommendation	Implementation methods
<i>Increase participation in certification schemes.</i>	<ol style="list-style-type: none"> 1. Support growers to join certification schemes e.g., providing financial support or connect smaller growers to form cooperatives to make certification more affordable. 2. Increase awareness of additional schemes that benefit pollinators such as fair to nature conservation grade and LEAF Marque.
<i>Increase knowledge transfer on environmental and pollination</i>	<ol style="list-style-type: none"> 1. Create and disseminate best practice guidelines. 2. Provide training on pollination management including monitoring.

<p><i>management tailored for a given farming system.</i></p>	<ol style="list-style-type: none"> 3. Promote collaboration between science and practice such as creating an information sharing platform for growers to provide relevant and up to date research. 4. Initiate pollinator awareness events. 5. Create demonstration farms for showcasing best practices.
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4.4. Discussion

4.4.1. Applying the tool and potential benefits.

Many agricultural companies rely on pollination services to provide products for their supply chains. However, agricultural production itself often contributes to declines in pollinators through natural habitat destruction and agrochemical use (Campbell et al., 2017; Vanbergen et al., 2020). As such, many companies have a strong interest in, and responsibility to, protect pollinators but currently, there is no clear strategy for how they can achieve this. Thus, this paper aims to fill this gap. Combining literature reviews, new and established methods, informant interviews and surveys, a rapid assessment tool was designed that allows agricultural businesses to take actions that safeguard pollinators and pollination in their supply chains in the face of pollinator declines. The tool provides information to industries on the importance of pollinators to their supply chains, understand what the primary risks to pollination are, and allows them to identify effective strategies to secure pollination services.

To develop the methodology and demonstrate its application, the tool was applied to a case study for an avocado supplier company. The results demonstrated that encouraging growers to participate in environmental certification schemes (e.g., through financial support or connecting growers to form a cooperative) could effectively support wild pollinators as in general, environmental certification schemes have been shown to support local biodiversity (IPBES, 2016; Tschardt et al., 2015), and the

industry showed interest in adopting this method. Another identified strategy was to increase knowledge transfer to growers regarding pollinators, especially emphasising the economic contribution of pollinators. Grower surveys revealed that farmers would benefit from enhanced knowledge of pollinator management, and several studies have shown that educating farmers on environmental issues can lead to positive changes (Lobley et al., 2013; Mills et al., 2018; Márquez-García et al., 2018, 2019).

The tool can be applied to other agricultural industries within the supply chain, such as retailers and processes, and can be adapted for different crops and countries with relative ease. Minor adjustments, such as identifying different data sources (e.g., a crop's dependence on pollinators) may be required if the examples used in this paper do not provide information for the crop or country of interest.

The tool's implementation is expected to benefit growers by supporting them to implement pollinator practices, thereby increasing their short-term (Blaauw & Isaacs, 2014; Raderschall et al., 2021) and potentially long-term production (Dainese et al., 2019; Martin et al., 2019). Companies further up the supply chain should also benefit from implementing effective pollinator strategies, as they will ensure sustainable and stable supplies of their trading crop and enhance their sustainability image among the public (Murphy et al., 2022; Tremlett et al., 2020). Widescale implementation of such strategies could have a positive impact on pollinator conservation, considering the significant threat posed by intensive agriculture and the need for targeted actions in these landscapes.

4.4.2. Study limitations and potential solutions

One key limitation of this tool is the use of generalized global data to provide specific and localized outputs. For instance, crop dependency values from external research papers were utilized at various steps in this process but it is well established that a crop's dependency can vary depending on the

cultivar and the country (Breeze et al., 2016). Similarly, data used to calculate managed honeybee deficits relied on global estimates for the RSR which may not represent actual stoking rates.

Additionally, throughout the process, complex assessments were simplified, which poses another key limitation. For instance, the method used to assess threats to wild pollinators considered only three out of several known pollinator threats, employed high-level proxies that may not necessarily indicate a key pollinator threat (e.g., fertilizer application rate), and relied on national overview indicators that may not represent the local scale, where the risk matters most. Furthermore, the method used to create the industry pollinator strategy was developed with limited input from the growers and industry partners, potentially reducing the applicability of the recommendations.

While the limitations listed above were deemed justifiable to ensure efficiency in creating a rapid assessment tool for decision-making, a more detailed approach could overcome some of the challenges. For example, targeted fieldwork, such as exclusion experiments could provide accurate data on crop dependency values (Ratto et al., 2022) and feedback from growers regarding the availability of beehives throughout the pollination season could help obtain localized data on honeybee deficits. To develop a more tailored pollinator strategy, a co-design approach that incorporates recommendations from the growers and the industry could be employed (Berthet et al., 2019; Quinio et al., 2022). To better understand pollinator abundance and diversity in different regions, systematic, high-resolution, and long-term data collection on wild pollinator populations globally is required. However, such data is currently unavailable and therefore improvements to the proxy process developed in this study could be applied. For example, in the future, other threats such as climate change and pests and pathogens could be included in the process especially when we have a better understanding of climate refugia for pollinators or technology that can accurately monitor pathogens in the field.

4.4.3. Future directions

This tool has the potential to be expanded to provide a more comprehensive assessment of pollinator strategies. Conducting a return-on-investment analysis of the recommended pollinator strategies would be of great value for industries, as economic considerations often influence decision-making regarding pollinator declines (University of Cambridge Institute for Sustainability Leadership *et al.*, 2018). Although excluded from this tool due to the perceived challenges and time constraints, such an analysis could be incorporated as part of step 4 (identifying suitable strategies). Future reiterations of the tool should also include a description of the monitoring and evaluation methodology, which is a necessary component of the implementation process but was not covered in this study due to the impracticality of testing it with the case study.

Furthermore, the tool could be adapted to assess other ecosystem services, such as natural pest regulation and soil health. Implementing a single environmental assessment for multiple ecosystem services would be cost-effective and should not complicate the execution, as environmental practices and strategies are often complementary. For instance, pollinator practices can benefit other ecosystem services (e.g., floral strips can be beneficial for pollination and natural pest control (Albrecht *et al.*, 2020; Egan *et al.*, 2020)), and strategies could be easily extended to cover a range of ecosystem services (e.g., knowledge transfer could incorporate several environmental topics). To incorporate this change, significant adaptations would be necessary for steps 1 and 2 as the methods to assess the importance and economic contribution of different ecosystem services are inherently variable. However, steps 3 and 4 could easily be adapted by reviewing the literature on environmental strategies and practices for the ecosystem service of interest and then adapting the interview and survey questions.

This tool aims to encourage agricultural businesses to implement a pollinator strategy by providing a clear process to create effective strategies. However, such methodological tools alone may not guarantee widescale implementation, and therefore, further research is needed to understand effective approaches for encouraging industries to take action. Increased governmental support may

be necessary in some cases, as it is often the preferred choice for the industry (CBI Economics, 2022). However, considering the economic benefits businesses can gain from pollinator protection, softer approaches that raise awareness and highlight the importance of pollinators may also be effective. Additional research in targeted areas, such as quantifying the additional production benefits resulting from pollination (e.g., production stability and waste reduction) and understanding how different actors in the supply chain will be affected by pollinator losses, may also be required to provide further evidence to encourage businesses to act.

5. Chapter 5: Discussion

5.1 Summary

Animal pollination contributes greatly to the quantity and quality of around 75% of the world's leading crops (Klein et al., 2007) and as such, the known declines of wild pollinators (Potts et al., 2016) may pose a significant threat to global food security (Requier et al., 2022). Therefore, protecting wild pollinators, particularly in agricultural landscapes, is crucial. However, designing effective land management practices tailored to specific contexts can be challenging, as for many crops and regions, we do not know the extent of the contribution of insect pollinators to production nor understand the relative contribution of specific pollinator taxa (Rader et al., 2020).

Moreover, growers often face substantial barriers in implementing pollination protection practices, such as insufficient knowledge of which land management practices (e.g., floral strips, natural habitat enhancement, pesticide reduction/adaption of application) are effective for pollinators or insufficient financial resources to implement such techniques. Therefore, growers often require additional support to encourage the adoption of pollination-enhancing practices (Gemmill-Herren et al., 2021). The private sector can play an important role in this process, as many companies rely on insect-pollinated products and therefore have a vested interest in safeguarding pollinators (Breeze et al., 2022; Murphy et al., 2022). However, currently, there is limited information on ways for businesses to implement effective support strategies (University of Cambridge Institute for Sustainability Leadership et al, 2018).

To investigate these knowledge gaps, this thesis focused on avocados as a study crop as this is a globally important insect-pollinated fruit for which there is currently limited knowledge regarding pollination services. In many growing regions, avocado production provides a nutritionally important fruit for local consumption and also contributes significantly to economic growth due to its high export value (Pérez & Gómez, 2022). However, in recent years, the global popularity of avocados has led to

the rapid expansion of avocado orchards and this is consequently destroying natural habitats and causing environmental damage in many of the primary avocado-producing countries (Figure 5.1) (Magrach & Sanz, 2020).



Figure 5.1. Photo of avocado orchard expansion into natural habitat in Central Chile taken by me during field work.

To understand the importance of insect pollinators to avocado production and to investigate which wild pollinators are important in different growing regions, Chapter 2 used a literature review and meta-analysis of existing global avocado pollination studies. The results indicated that insect pollinators contributed significantly to avocado pollination with managed honeybees being the most important pollinators. However, in many regions of the world, wild insects were also highly abundant and effective pollinators and thus likely have a crucial role in pollination.

Chile is one of the largest avocado-growing regions in the world (FAO,2023), and therefore, Chapter 3 aimed to gain a deeper understanding of pollination services in this country. In three avocado

orchards, pollinator observations and exclusion experiments were implemented along transects running from natural habitat edges and non-natural habitat edges into the centre of the orchards. The findings confirmed the contribution of insect pollinators to avocado production in Chile and highlighted the importance of wild pollinators, especially from specific taxa, such as hoverflies and other Diptera. Furthermore, areas closer to natural habitats showed significantly higher abundances and diversity of pollinators, emphasizing the importance of protecting natural habitats to potentially enhance pollination services.

Finally, Chapter 4 developed a tool for businesses to help support growers in implementing pollinator protection practices. The tool provides a straightforward process for businesses to quantify the importance of pollination services to their operations, identify where the risks are most evident throughout their supply chain, and design effective pollinator protection strategies. Additionally, the tool was tested using an avocado supplier. The findings from this case study highlighted that increasing knowledge transfer to growers and supporting them to join environmental certification schemes could be effective strategies to increase pollinator protection.

5.2 Key findings

5.2.1 Insect pollinators and avocado production

The findings from Chapters 2 and 3 demonstrate the crucial role of insect pollinators in avocado production. The field study conducted in Chile showed significantly higher fruit set in open-pollinated treatments compared to exclusion treatments (p -value <0.0001) (Figure 3. 3), and similar findings were observed in Chapter 2, with most studies reporting close to zero fruit set in exclusion treatments (Figure 2. 3). Furthermore, the meta-analysis conducted in Chapter 2 indicated a high contribution across various growing regions, with nearly all studies showing higher pollination and production metrics in open-pollinated treatments compared to exclusion treatments (Figure 2. 2). However, the literature review in Chapter 2 did identify a few studies showing similar levels of pollination between

open and exclusion treatments. For instance, a study by Davenport (2019) found no significant differences in the daily average percentage of avocado flowers pollinated between open and closed pollination treatments. Additionally, approximately 20 % of the exclusion treatments implemented in the field trial in Chile exhibited low levels of initial fruit set. These anomalies could be attributed to challenges in the implementation of the exclusion treatments, such as the potential entry of small pollinating insects like thrips through the mesh bags, or, if the mesh bags were not securely fastened, other insects could have entered and contributed to pollination. Alternatively, in some varieties, wind pollination or self-fertilization may occur. For instance, a study conducted with the varieties Simmonds, Hardee, Tonnage, Tower 2, and Choquette showed successful pollination in pollinator exclusion treatments (Davenport et al., 1994). It has also been suggested that humid weather conditions may enable the overlap of stage 1 and stage 2 of avocado flowering, thus making self-fertilization possible (Gazit & Degani, 2002). However, the overall balance of evidence in this research suggests that this pollination process is not the norm and, that in general, insect pollinators contribute significantly to avocado production, exceeding previous indications in other meta-reviews which identified that avocado insect reliance is between 40-90% (Klein et al., 2007).

Chapter 2 also explored the effect of animal pollination on avocado quality, as this is an important production factor and, for many crops, nutritional value, and commercial attributes have been shown to improve with more diverse and comprehensive pollination services (Gazzea et al., 2023). In Chapter 2, 4 studies were identified that investigated fruit quality. However, these studies only measured one variable of fruit quality (e.g., fruit weight) and the result from the summary means analysis showed only a very small positive effect of insect pollination (Figure 2. 3). However, a recent study conducted in Kenya, which was published after the review, indicates that insect pollination has a measurable impact on avocado quality. This study found that fruit weight, seed weight, and oil content were all significantly higher (approximately 2.2 times) in open pollinated treatments compared to exclusion treatments (Sagwe et al., 2023). This research suggests a potential influence of animal pollination on

avocado quality, but further investigation is needed to explore a wider range of crop quality variables and in different growing regions.

5.1.1 The importance of wild pollinators

The crucial role of managed honeybees as pollinators in various crops, including avocados, has been widely acknowledged in the literature (Ish-Am & Lahav, 2011; Peña & Carabalí, 2018; Sagwe et al., 2022; Willcox et al., 2019). This contribution was confirmed throughout this thesis, as honeybees were shown to be abundant and effective avocado pollinators in the field study, as well as most of the papers reviewed in Chapter 2. However, this study has also identified the importance of wild pollinators in avocado production, for which there has previously been limited research. By using the abundance of flower-visiting pollinators as a proxy to understand pollination contribution, Chapter 2 revealed that wild pollinators were highly abundant in most growing regions (Figure 2. 5), and this observation was similarly evident in the avocado orchards in Chile, where nearly half of all flower visitor observations were wild pollinators.

Furthermore, this research highlighted the relative effectiveness of certain wild taxa, particularly flies and hoverflies, as avocado pollinators. Chapter 3 demonstrated that flies had a comparable flower visitation rate to honeybees, as flies visited an average of 7.9 flowers per minute and honeybees had an average of 8.5 flowers per minute, and this difference was non-significant. Additionally, a study cited in Chapter 2 revealed that there was no significant difference in pollen deposition between flies and honeybees, with both pollinators depositing around 2-5 grains per visit (Perez-Balam et al., 2012). This finding aligns with broader research suggesting the structure of the avocado flower is well-suited to fly pollination (Vithanage, 1990). Additionally, Chapter 3 revealed a significant and positive correlation between the abundance of hoverflies and fruit set (p-value 0.003), implying that hoverflies could potentially be important pollinators. This is further supported by a recent study conducted in

Kenya, which found that hoverflies deposited and carried a similar amount of pollen as honeybees (Sagwe et al., 2022).

Chapter 3 demonstrated that there was no relationship between honeybee abundance and fruit set. This could indicate that avocado orchards in Chile have reached a pollination saturation point, as observed in other crops (Rollin & Garibaldi, 2019). Alternatively, these findings underscore the additional pollination benefits provided exclusively by wild pollinators. Such benefits have been documented in various studies (Garibaldi et al., 2013) and are attributed to a diverse array of pollinators providing complementary and thus more comprehensive pollination services (Blüthgen & Klein, 2011; Garibaldi et al., 2011; Hoehn et al., 2008; Senapathi et al., 2021). Moreover, in the face of climate change, pollinator diversity can ensure a more stable service, as different species can pollinate more effectively under different weather conditions. Consequently, a broader diversity of pollinators enhances the likelihood of effective pollination occurring even under extreme weather conditions (Brittain et al., 2013; Mukherjee et al., 2019).

5.1.2 Protecting wild pollinators

This thesis contributes valuable insights to the well-established body of literature on the importance of natural habitats for wild pollinators. Chapter 3 revealed a significant negative relationship between the distance to a natural habitat border and various wild pollinator parameters, including abundances, visits, richness, and diversity, while no relationship was observed for control transects (Figure 3. 1). Similar results have been documented in various other crops and regions (Bartual et al., 2019; Garibaldi et al., 2011; Klein et al., 2012), and several recent global syntheses have further reinforced these findings, highlighting the critical role of natural habitats in supporting biodiversity and agricultural yield (Dainese et al., 2019; Martin et al., 2019). This relationship is attributed to natural habitats providing enhanced forage and nesting resources for wild pollinators (Potts et al., 2005; Öckinger & Smith, 2007) thereby fostering a higher abundance and diversity of pollinators, which spills

over into agricultural landscapes. Furthermore, this research showed that wild pollinator spillover into the orchard was limited, with wild pollinator abundance and visitation rates approximately 2.55 times higher, pollinator richness around 1.6 times higher, and diversity 1.5 times higher at the orchard's natural habitat edge in comparison to all distances along the transect. Such sharp declines could suggest that the wild pollinators within this region heavily depend on natural habitats and struggle to forage further into the orchard, further emphasising the crucial role of preserving natural habitats.

A scientific understanding of land management practices able to provide the key resources for pollinators is essential to implement effective pollination management practices. However, in many cases, it is often insufficient to ensure implementation due to various barriers associated with their adoption. Therefore, support to growers is often necessary. For instance, in parts of Europe and North America, governmental policies provide training and financial assistance to help growers adopt environmentally friendly land management practices (Batáry et al., 2015). However, the impact of such policies on global pollinator protection is limited since a significant proportion of animal-pollinated crops consumed in these regions are imported from other countries (Breeze et al., 2022). In many countries where animal-pollinated crops are grown mainly for export, agri-environmental policies may not exist due to limited financial resources or political will. Consequently, the private sector could play a crucial role in filling this gap.

Many companies have a vested interest in protecting pollinators, as declines in pollinator populations could disrupt supply chains and lead to increased purchasing prices due to global production declines (Murphy et al., 2022; Tremlett et al., 2020). Furthermore, inaction on pollinator protection may damage their corporate reputation, as consumers and investors are becoming increasingly aware of the threats to pollinators and may adapt their purchasing habits and/or investments accordingly (Hoshide et al., 2018). However, currently, there is limited information available to private companies on how they can support growers most effectively, potentially hindering action. Therefore, Chapter 4

developed a practical tool that businesses can implement to understand the importance of pollination services, assess current pollinator threats, and develop effective actions. To test the feasibility, the tool was applied to an avocado supplier industry; however, the process can easily be applied to other industries and crops.

5.2 Recommendations

5.2.1 Growers

This study underscores the crucial role of insect pollinators in avocado production, emphasizing the need for growers to actively manage pollination services to ensure optimal yields. Several methods can be employed to achieve this, with the choice depending on specific circumstances, such as the local environment and the grower's economic situation (Isaacs et al., 2017). One potential approach is to increase the use of managed honeybees. However, for some farmers, this may not be economically viable due to the cost of hiring managed honeybees or it may not be logistically feasible because of limited honeybee availability during the pollination season. Moreover, relying solely on one species for pollination services presents risks, especially considering the numerous threats to honeybees, such as diseases spread through varroa mites (IPBES, 2016). Furthermore, both this research and broader studies suggest that higher densities of honeybees may not further improve yields in all cases, as a broader diversity of pollinators is likely to provide additional pollination benefits (Garibaldi et al., 2013). Consequently, in many circumstances, increasing wild pollinators may be a more suitable and effective approach to pollination management.

This research underscores the importance of natural habitats in providing wild pollination services to avocado orchards, as demonstrated by the significantly higher abundance and diversity of wild pollinators in areas of avocado orchards adjacent to natural habitat borders, in contrast, to control borders. Therefore, growers are advised to maintain and protect high-quality natural habitats that are known to provide resources for pollinators around their property. Furthermore, given the non-linear

relationship of natural habitat effects on pollinators along transects, with wild pollinator compositions showing significantly higher rates at 0m compared to all other distances further into the orchard, increasing natural habitats within orchards is expected to further enhance pollination services and yield.

Additionally, Chapters 2 and 3 of this study highlighted the likely significant contribution of flies and hoverflies to avocado pollination. Wider research has shown that these insects are not so reliant on natural habitats and can benefit from other habitat interventions, such as floral plantings (Jauker et al., 2009; Rader et al., 2020). Moreover, research in avocado orchards in Chile showed that native flower strips increased visitation by non-bee insects and subsequently increased avocado yield (Muñoz et al., 2021). Therefore, implementing more floral resources, for example within the orchard rows or around the orchard edge, is also likely to be beneficial. However, other studies note that hoverflies are highly dependent on natural habitats, as they have been observed in higher abundance and diversity in areas closer to natural habitats (Schirmel et al., 2018). Similarly, several reviews have demonstrated that even in landscapes where smaller habitats are in place e.g., floral bands, natural habitats are still vital in maintaining a greater diversity and abundance of pollinators (Albrecht et al., 2020; Dainese et al., 2019; Scheper et al., 2015). Therefore, it is recommended that landscape-scale natural habitat protection is maintained alongside the implementation of smaller natural habitats and floral resources within the crop.

Studies identified in Chapter 2 demonstrated the importance of reduced pesticide application and less-intensive agricultural practices in avocado orchards to encourage a high diversity and abundance of pollinators (Castañeda-Vildózola et al., 1999; Villamil et al., 2017). Consequently, the findings from these studies, combined with the data collected for this thesis, suggest that agroecological farming approaches that reduce chemical inputs and increase ecosystem services could help effectively promote pollination services. Moreover, such pollination practices are known to be multi-functional,

potentially supporting a range of other ecosystem services such as pest regulation and soil fertility and therefore, could further enhance avocado production (Blaauw & Isaacs, 2014; Campbell et al., 2017; Shackelford et al., 2013; Tschumi et al., 2016).

5.2.2 The private sector

The tool designed in Chapter 4 aims to encourage the private sector to implement more pollinator protection strategies. For companies uncertain of the importance of pollinators, or not fully aware of the severity of pollinator threats, it is recommended that they implement all steps in the process to understand their necessity to act. However, companies already possessing a strong understanding of the challenges and a high motivation to act, could directly implement some of the strategies identified in Table 5.1. The research undertaken in Chapter 4 highlighted that facilitating knowledge transfer to growers could help increase pollinator protection for the case study industry. Such programs could be implemented through ‘Farmer Field Schools’, which have been shown to increase beneficial land management practices such as IPM (Waddington et al., 2014) or through online knowledge resource platforms that provide relevant and targeted information (e.g., CABI Plantwise). Additionally, for the company in Chapter 4, encouraging participation in certification schemes, such as the Rainforest Alliance or LEAF, was suggested as an effective strategy. These approaches are likely applicable to a range of businesses since they are known to be effective in promoting environmental change (IPBES, 2016; Lobley et al., 2013; Mills et al., 2018; Márquez-García et al., 2018). Moreover, they can also address other environmental aims within the business, making implementation easier and more attractive. For instance, knowledge transfer could cover a variety of topics and certification schemes generally audit a range of environmental and social targets.

Table 5.1. Pollinator protection strategies for the private sector as identified in Chapter 4.

Theme	Adaptation for the private sector
Regulations: legal or mandatory rules.	<i>Environmental contract or mandate certification schemes</i>

	<p>Create regulations on environmental / pollinator management that growers must abide by to sell their produce to the industry or require that growers are part of environmental certification schemes.</p>
<p>Economic: financial <i>incentives</i> for positive behavior or <i>disincentives</i> for negative behavior.</p>	<p><i>Certification schemes</i></p> <p>Increase grower participation in relevant certification schemes.</p> <p><i>Insurance scheme</i></p> <p>Provide insurance to growers who make specified environmental changes on their land.</p> <p><i>Environmental inputs</i></p> <p>Provide inputs for pollination management e.g., native seeds/ plants.</p>
<p>Persuasion: Encouraging behavior change and enhancing knowledge.</p>	<p><i>Knowledge transfer</i></p> <p>Increase knowledge transfer to growers on environmental/ pollination management.</p> <p><i>Environmental reporting platform</i></p> <p>Create an online platform where growers can report on environmental achievements/ targets.</p> <p><i>Agri-environmental research</i></p> <p>Implement more research in agroecological farming (including pollination management) either on research stations or by encouraging farmer experimentation.</p>

5.3 Future research

5.3.1 The contribution of insect pollinators to avocado production

Additional research is required to understand the contribution of pollinators to avocado production. While this study and several other papers have investigated the contribution of insect pollinators to initial fruit set, percent of flowers pollinated, or final fruit weight (Can-Alonzo et al., 2005; Davenport, 2019; Davenport et al., 1994; Malerbo-Souza et al., 2000; Mulwa et al., 2019), no studies have been identified that measure the contribution to final fruit set or yield, which is the most important indicator, especially for growers. Furthermore, most studies have focused on a single growing season, not providing information to growers on yield stability, another crucial production variable. Therefore, future studies should be implemented over several years to account for seasonal variation and thus achieve a more accurate measure of pollination contribution to a range of yield and quality characteristics.

Additionally, further research should include an analysis of the contribution of pollinators to fruit quality. Insect pollination has been shown to enhance marketability, quality, and nutritional value for several other crops (Silva et al., 2023; Fijen et al., 2018; Gazzea et al., 2023; Klatt et al., 2014; Nicholson & Ricketts, 2019; Wietzke et al., 2018), however, limited research has been conducted in this area for avocado production.

5.3.2 The effectiveness of avocado pollinators

To understand pollination effectiveness, ideally, experiments should measure the number of pollen grains deposited on a receptive stigma per visit and the visit frequency for specific pollinating species (Ne'eman et al., 2010). While the experiments conducted in Chapter 3 and several of the papers identified in Chapter 2 measured certain aspects of pollinator effectiveness, typically only one variable was assessed, such as visitation rate, thereby reducing the reliability of these findings (Can-Alonzo et

al., 2005; Castañeda-Vildózola et al., 1999). Chapter 2 did identify some studies that included a more comprehensive measure of pollinator effectiveness (Perez-Balam et al., 2012; Sagwe et al., 2022; Willcox et al., 2019), however, these focused on a small number of species and specific growing regions. Therefore, there is a need for further research encompassing a wider variety of pollinating insects, countries, and longer time scales.

5.3.3 Wider research on pollinator protection measures

Most research on pollination practices has been conducted in Europe and North America, which raises concerns about the applicability of recommendations to areas in different climatic regions or agricultural systems. For instance, floral bands planted in agricultural landscapes are a common and effective pollinator protection practice that is implemented in many areas in Europe and North America (Albrecht et al., 2020). However, in drier regions, it may be unfeasible to maintain extra vegetation, due to low annual rainfall and the high cost of irrigation. Consequently, growers in these regions may be unwilling to implement such practices. To address this limitation, future studies should investigate practices that are more suitable for different climatic zones and for a variety of crops and growing systems, for example, the effectiveness of native drought-tolerant plant species in floral strips should be a key area for future studies. Additionally, a recent study in almond orchards showed that intercropping trees with beneficial pollinator plants and the adoption of no-tillage increased water retention and availability in the Mediterranean (Almagro et al., 2023). Such practices may be applicable for more arid regions and a wider variety of crops, but further research is needed in this area.

Many pollinator practices focus solely on improving habitats and food resources that benefit bees. This research, along with multiple other studies, highlights that a wide variety of insects play crucial roles as pollinators (Garibaldi et al., 2013; Mallinger & Gratton, 2015; Rader et al., 2016). Such non-bee species often require different floral resources and nesting and reproductive habitats (Rader et

al., 2020). For example, studies have shown that certain species of hoverflies rely on organic matter from semiaquatic habitats in the early stages of their life (Speight, 2011), and hoverfly abundance has been shown to increase with the presence of pond habitats in agricultural landscapes (Stewart et al., 2017). Given the likely contribution of hoverflies to avocado production, as identified in Chapter 3, the management of semi-aquatic habitats may be an effective intervention for avocado pollination. However, to date, relatively little is known about habitats and resources for many species of non-bee insects, and therefore, further research is needed to understand which plants and land management techniques would be beneficial to increase the abundance and diversity of such species (Duque-Trujillo et al., 2023; Rader et al., 2020).

Similarly, research into pollinator protection strategies has focused narrowly on policies that can be implemented at the governmental level and by richer nations e.g., agri-environmental schemes in which governments pay landowners to implement certain environmentally/pollinator friendly practices (Gemmill-Herren et al., 2021). In many parts of the world however, governments lack the financial resources or public backing to implement such policies. Therefore, more research should focus on support mechanisms applicable in these circumstances and implementable through a variety of avenues. Chapter 4 explored one way that this could be achieved by creating a tool to support the private sector in selecting effective pollinator strategies. To encourage wide-scale implementation, future research should investigate how to expand the tool to incorporate other ecosystem services. Agricultural production relies on a range of natural processes e.g., pest control and water provision, and as such, businesses have a range of environmental protection priorities. A more comprehensive tool would allow them to understand the contribution of different ecosystem services, compare threats to these services in different regions, and design effective protection strategies. Consequently, this should be more attractive to businesses, allowing them to priorities the most impactful environmental actions in the most critical regions. Additionally, a better understanding of how global

pollinator declines will impact different actors in the private sector should incentives action, as it would clarify the importance of pollinator and environmental protection to the industry.

5.4 Concluding remarks

This thesis has demonstrated the vital contribution of insect pollinators to avocado pollination and production, with particular emphasis on the substantial role played by wild pollinators. The research further underscored the significance of natural habitats in enhancing wild pollinator abundance and diversity. This adds to the growing evidence of the crucial role these habitats play in enhancing agricultural production. This study has also delivered a valuable tool for industries – a means to identify effective strategies to support pollinator management. Such a tool holds the potential to play an important role in protecting pollinators, particularly in regions where other support mechanisms are limited.

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7. Appendixes

7.1 Appendix 1. Papers used in the literature review (Chapter 2).

This appendix contains information on the papers used in the literature review and meta-analysis in Chapter 2.

Study	Location	Variable Studied	Main Finding
Davenport (2019)	USA	The contribution to pollination from pollinators and wind	Most pollination took place in stage 2 and this was primarily self-pollination. Wind contributed more than pollinators. This was shown in humid and dry environments.
Pena and Carabali (2018)	Colombia	The effect of honeybee density on yield	Higher honeybee density led to higher fruit set. Distance from the hives did not influence fruit set. Honey bees were more efficient pollinators than other species.
Ish-Am and Lahav (2011)	Israel	Contribution to pollination from pollinators and wind	Bee density was positively correlated with fruit set and yield. Wind did not contribute much to pollination.
Davenport et al. (1994)	USA	Contribution to pollination from pollinators and wind	There was no significant difference in the rate of pollination for open and closed treatments. Most pollination is self-pollination during stage 2.
Malerbo-Souza et al. (2000)	Brazil	Contribution to pollination from pollinators	Crop yield was reduced by 81% without pollinators. Open pollination produced 2.5 fruits per branch and closed 0.5 fruits per branch
Johannsmeier et al. (1997)	South Africa	Contribution to pollination from honeybees and polliniser trees	Closed treatments and treatments without polliniser trees had significantly lower yields than open treatments and treatments with polliniser trees
Mulwa et al. (2019a)	Kenya	Contribution to pollination from pollinators and the abundance and variety of pollinators	Pollinators lead to a 330% increase in yield. The major pollinators observed were: honey bees, wasps, drone fly and blow fly.
Bezuidenhout et al. (2016)	South Africa	Contribution to pollination from pollinators and different pollinizer trees	Honeybees led to significantly higher yields. Did see an increase with polliniser trees, but not clear if it cost effective.

Cabezas and Cuevas, (2007)	Spain	Contribution to pollination from pollinators and wind.	Honeybees and bumblebees significantly increased fruit set. Wind was not significant.
Petersen, (1955)	USA	Contribution to pollination from pollinators	There was a significantly higher yield in cages with bees and very limited fruit set in cages with no bees.
Alcaraz and Hormaza, (2009)	Spain	Yield limitations due to pollination and contribution to pollination from pollinizer trees	Hand pollination was significantly higher than open pollination. Most of the final fruits were the result of outcrossing. Different pollinizer trees were more effective than others.
Robbertse et al. (1996)	South Africa	Contribution to pollination from pollinators and pollinizer trees	Open pollinated treatments had significantly higher fruit set than closed treatments. Etinger pollen contributed significantly. Distance from polliniser tree showed mixed results across years
Gazit, 1976		Contribution to pollination from pollinators and polliniser trees	Self-pollination can occur but yields increase with bees and polliniser trees
Willcox et al. (2019)	Australia	Abundance and effectiveness of pollinators contributing to 3 key crops, including avocado	Variety of species present on avocado flowers but most common were Apis mellifera, Tetragon ula spp and Stomarmhina discolor. Apis mellifera was the main contributor.
Vithanage (1990)	Australia	Abundance and effectiveness of different avocado pollinators. Contribution to yield from different bee hive densities	A wide variety of insects contributed to pollination but honeybees were the most important. Yields significantly increased when hives were added and fruit size increased at high bee density.
Read et al. (2017)	New Zealand	Abundance of pollinators	Honey bees were the most abundant but there was a high variation in different sites. Beetles and bumblebees were also commonly observed.
Eardley and Mansell (1996)	South Africa	Abundance of pollinators	The honeybee was the most abundant and significant pollinator. Other species were effective but had low abundance.
Mulwa et al.(2019b)	Kenya	Abundance of pollinators	Honey bees, blow flies, hoverflies and wasps were the major avocado flower visitor.
Castaneda-Vildozola et al. (1999)	Mexico	Abundance and effectiveness of avocado pollinators	Honeybees were the main pollinators but there were many native species that contributed.

Bushuru (2015)	Kenya	Abundance and effectiveness of avocado pollinators	A range of insects found on avocados but honeybees were the most efficient as they carried the most pollen and were the most abundant.
Mehmood et al. (2015)	Pakistan	Abundance of pollinator	Hymenopteran pollinators were higher in numbers (42%) followed by Lepidopterans (33%) and Dipterans (25%)
Can-Alonzo et al. (2005)	Mexico	Abundance and effectiveness of avocado pollinators	Stingless and honeybees are the most efficient pollinators. Flies and wasps were also observed but these were not as abundant. No self-pollination occurred.
Perez-Balam et al. (2012)	Mexico	Abundance and effectiveness of avocado pollinators	Honeybees and flies were more effective pollinators for avocados than wasps. All deposited a similar amount of pollen but honeybees and flies were more abundant.
Visscher (1997)	USA	Abundance of pollinators	Honeybees were the most abundant pollinators but wild bees were also observed
Evans et al. (2011)	New Zealand and Australia	Abundance of pollinators	Range of pollinators in Australia but not so diverse in New Zealand. The amount of pollen deposition and fertilisation was low in both countries.
De la Cuadra (2007)	Chile	Abundance and efficiency of pollinators	Range of pollinators observed, but only a few contribute. Honeybees contributed the most.
Castaneda-Vildoza et al. (1999)	Mexico	Abundance and efficiency of pollinators	Honeybee was the main pollinator but there were many native species that contributed. Biodiversity of pollinators was reduced in areas that were sprayed with pesticides.
Carabali-Banguero et al. (2020)	Colombia	Abundance of pollinators and analysis of pollen loads	Several different pollinators with avocado pollen. All species also carried pollen from different botanical families.
Monzon et al. (2020)	Chile	Abundance and efficiency of pollinators	In total 6 pollinator species were observed but the honeybee was the most abundant. Honeybees and wild bees had similar visitation rates.

Villamil et al. (2017)	Mexico	Impact on diversity of pollinators from landscape and land management.	Intensive management influences the variety for flowers and biodiversity. The key factors were insecticides, removal of weeds and forested areas.
Afik et al. (2007)	Israel	Exploring honeybees behaviour around the avocado bloom and reasons for avoidance	High levels of mineral contents (Potassium and Phosphate) present in nectar deter honeybees. Different races and breeds of bees do have different preferences for these minerals.
Ish-Am et al. (1998)	Israel	Efficiency of bumblebees and honeybees as avocado pollinators	In Etinger, Bumblebees increased cross pollination and significantly increased yields. In Hass there was a slight increase in yields mostly due to increases in cross pollination in trees far from hives.
Ish-Am and Eisikowitch (1998)	Israel	The contribution and effectiveness of honeybees in avocado pollination.	During late blooming cultivars, fewer non avocado flowers were blooming and therefore avocado flowers received more pollinators and had a higher fruit set in comparison to early blooming cultivars. There was a negative correlation between bee density and pollination.
Fetscher et al. (2000)	Israel and USA	Contribution from different races of honey bees and different pollinizer trees to avocado yield.	Results were not significant but suggest that New World Carniolan (NWC) race of bees might be more effective than Italian honeybees as they carried more pollen back to the hive and visited more flowers. Yield increased with closeness to polliniser tree.
McNeil and Pidduck (2003)	USA	Effectiveness of Bumblebees as avocado pollinators	Bumblebee, honeybees and syrphid flies were all observed on the flowers. Bumblebees were efficient pollinators and could increase yield.

7.2 Appendix 2: Data used in the meta-analysis (Chapter 2).

This appendix shows the raw data used in the meta-analysis in Chapter 2.

Author	Me	Se	Mc	Sc	Ne	Nc	Subgroup_variable	Subgroup_cultivar	Subgroup_climate	Subgroup_size	Calculated Data
1.Davenport et al (1994)	0.2	0.0	0.1	0.0	10.0	10.0	Fruitset	Other	Humid	Branch	Taken from Paper
2.Davenport et al (1994)	0.3	0.0	0.2	0.0	10.0	10.0	Fruitset	Other	Humid	Branch	Taken from Paper
3.Davenport et al (1994)	0.1	0.0	0.0	0.0	10.0	10.0	Fruitset	Other	Humid	Branch	Taken from Paper
4.Malerbo-Souza, et. Al (2000)	2.6	2.5	0.5	0.5	3.0	3.0	Fruitset	Hass	Dry	Branch	Taken from Paper
5.Mulwa (2016)	2.7	0.1	1.8	0.1	12.0	12.0	Fruitset	Hass	Dry	Site	Taken from Paper
6.Davenport (2019)	21.9	12.6	17.6	16.3	4.0	4.0	Pollination	Hass	Humid	Year	Calculated
7.Davenport(2019)	18.0	2.2	15.7	6.6	3.0	3.0	Pollination	Hass	Dry	Year	Calculated
8.Robbertse et.al (1996)	45.0	7.1	4.0	1.4	2.0	2.0	Yield	Hass	Dry	Tree	Calculated
9.Peterson, (1955)	202.0	116.0	4.5	0.7	2.0	2.0	Yield	Mix	Dry	Tree	Calculated
10.Vithange (1990)	37.5	4.0	17.6	9.8	2.0	2.0	Pollination	Hass	Dry	Year	Calculated
11.Gaizt (1976)	91.3	16.2	3.0	1.7	3.0	3.0	Yield	Mix	Dry	Site	Calculated
12.Malerbo-Souza, et. Al (2000)	265.1	7.0	261.2	10.0	3.0	3.0	Fruitweight	Hass	Dry	Branch	Taken from Paper
13.Vithange (1990)	288.0	12.7	244.0	4.2	2.0	2.0	Fruitweight	Hass	Dry	Site	Calculated
14. Mulwa (2016)	133.0	7.7	112.6	7.0	12.0	12.0	Fruitweight	Hass	Dry	Site	Taken from Paper

7.3 Appendix 3: SD and Mean used in the meta-analysis (Chapter 2).

This appendix shows the data used to calculate the SD and Mean used in the meta-analysis in Chapter

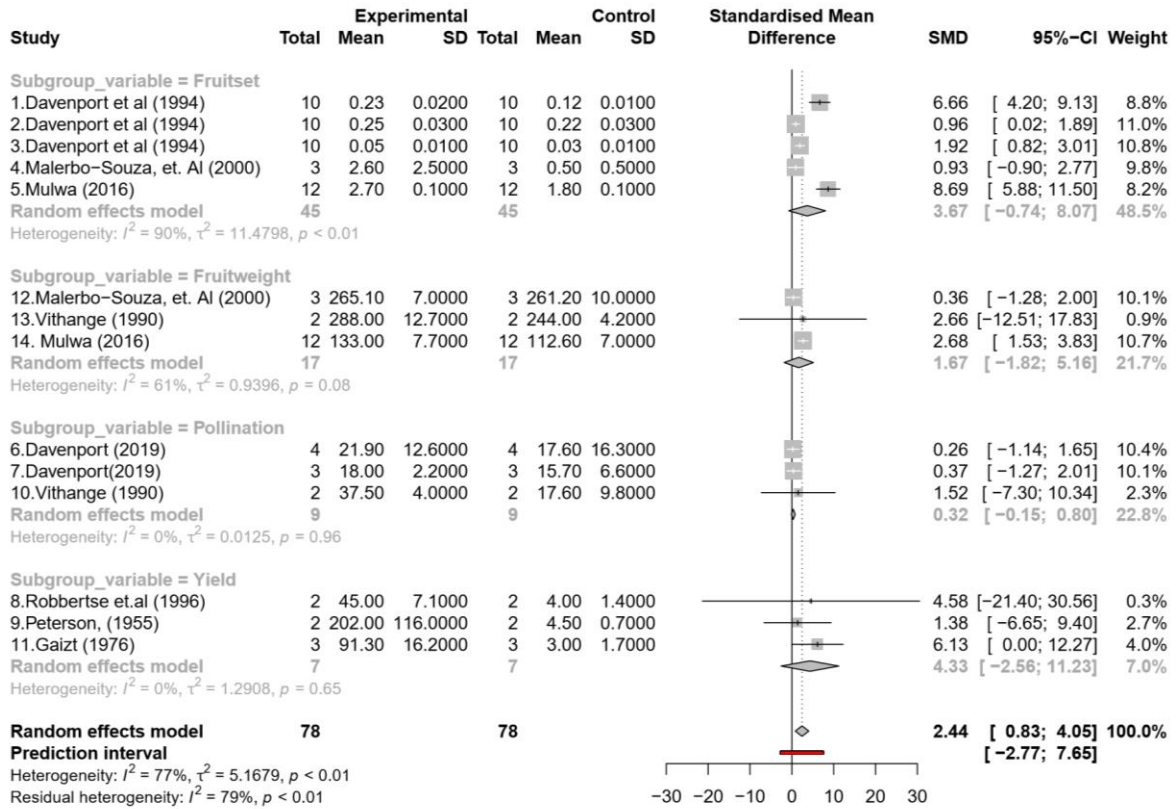
2.

Study	Replication Level	Treatment	Data				Mean	SD
6.Davenport (2019)	Year	Open	18.6	38.7	8.5	21.9	21.9	12.6
6.Davenport (2019)	Year	Closed	18.2	40.1	3	8.7	17.5	16.3
7.Davenport(2019)	Year	Open	16.7	16.8	20.6		18.0	2.2
7.Davenport(2019)	Year	Closed	18.9	20.1	8.2		15.7	6.6
8.Robbertse et.al (1996)	Site	Open	50	40			45.0	7.1
8.Robbertse et.al (1996)	Site	Closed	3	5			4.0	1.4
9.Peterson, (1955)	Cultivar	Open	284	120			202.0	116.0
9.Peterson, (1955)	Cultivar	Closed	5	4			4.5	0.7
10.Vithange (1990)	Year	Open	40.3	34.6			37.5	4.0
10.Vithange (1990)	Year	Closed	24.5	10.6			17.6	9.8
11.Gaizt (1976)	Cultivar	Open	82	82	110		91.3	16.2
11.Gaizt (1976)	Cultivar	Closed	2	5	2		3.0	1.7
13.Vithange (1990)	Site	Open	279	297			288.0	12.7
13.Vithange (1990)	Site	Closed	241	247			244.0	4.2

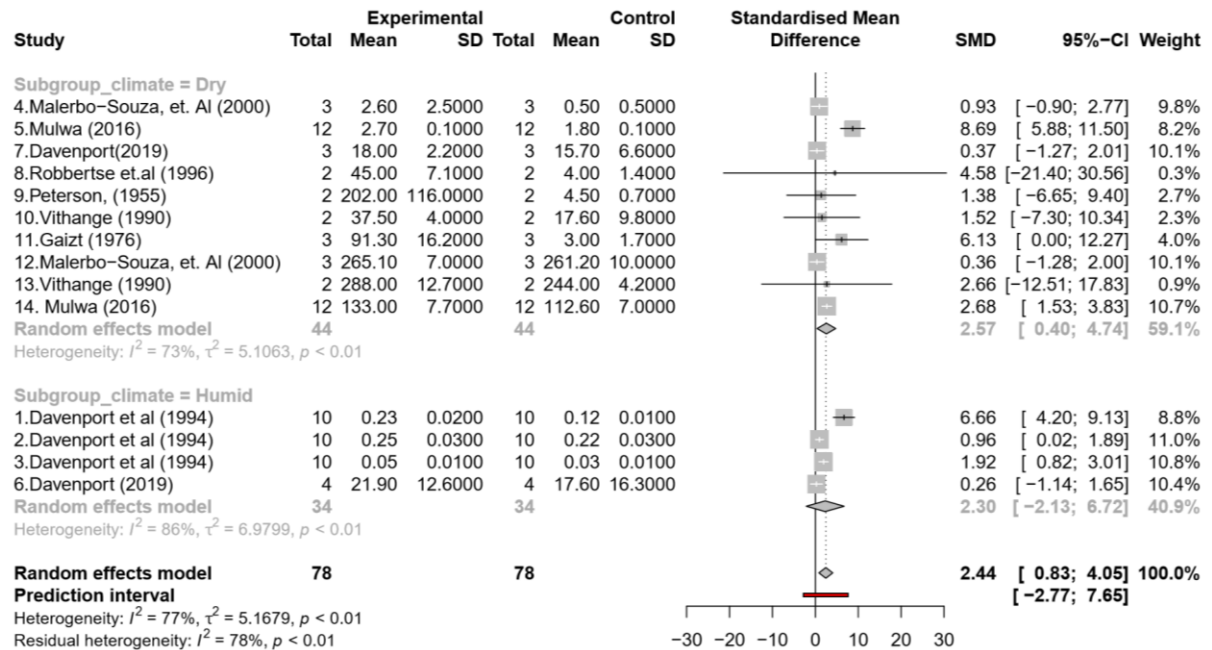
7.4 Appendix 4: Subcategorised Forest plots (Chapter 2).

This appendix shows the forest plots following a randomised meta-analysis to compare effects of pollination and production under open (Experimental) and closed (Control) pollination treatments in avocado across multiple studies subcategorised by (A) variable, (B) climate, (C) cultivar and (D) experimental scale.

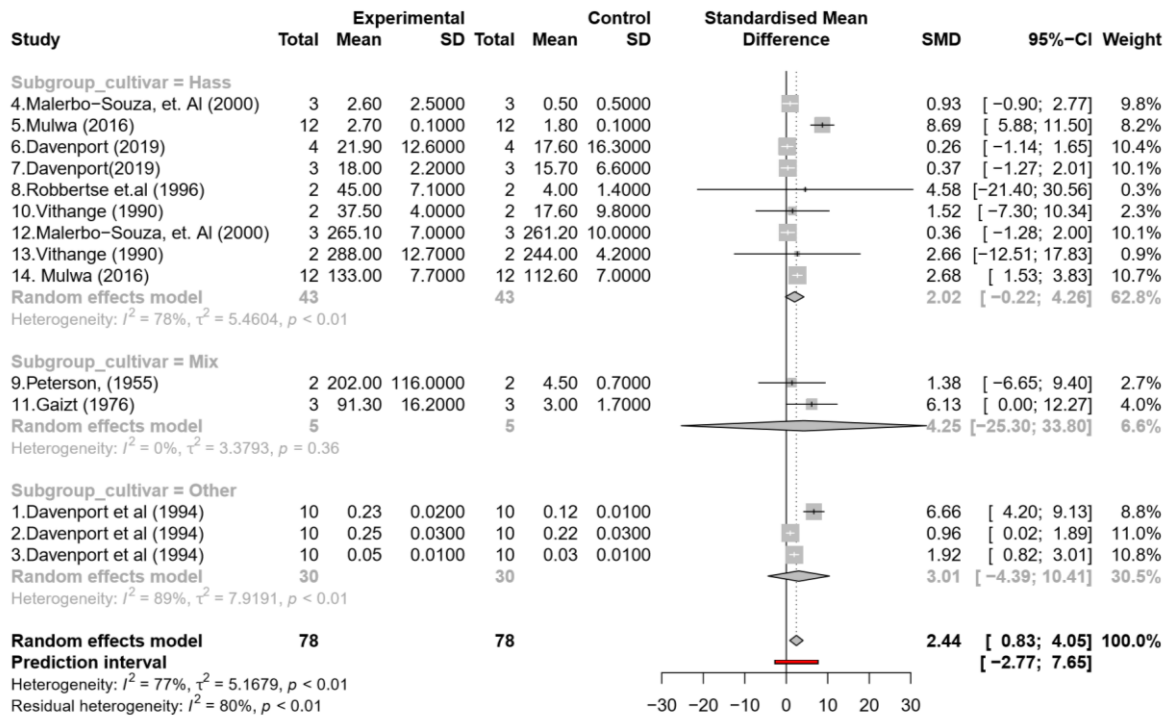
A)



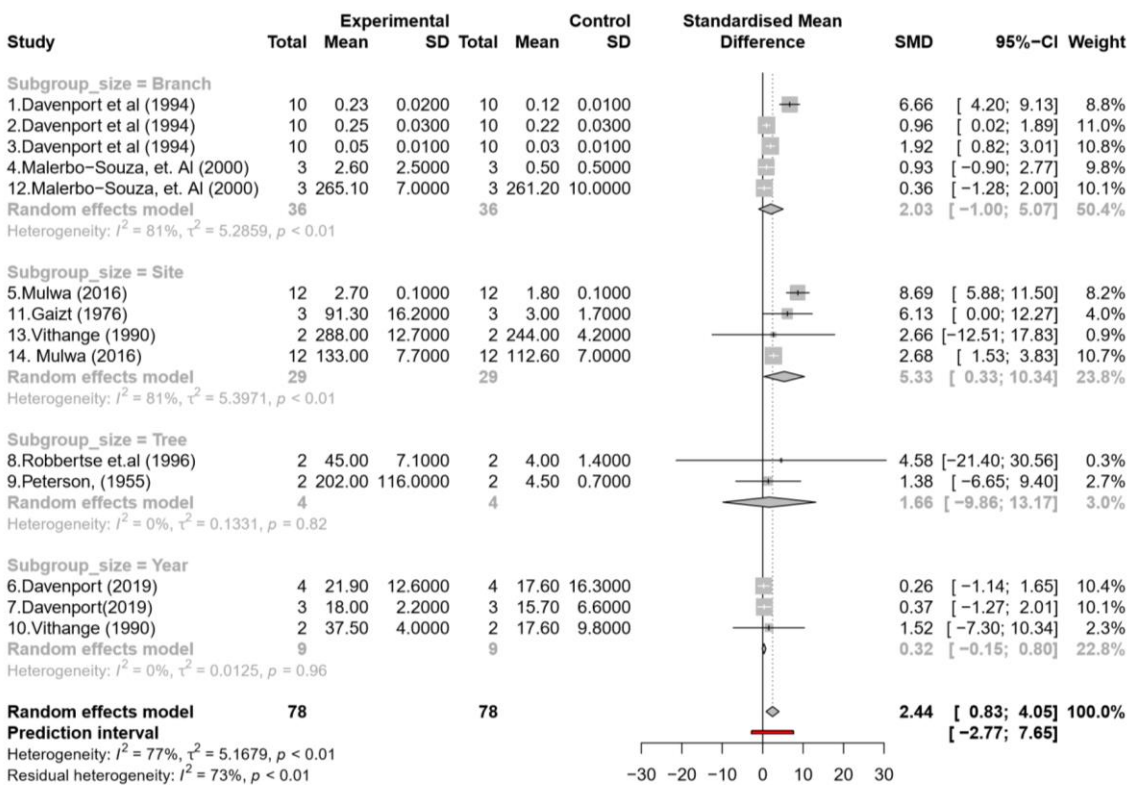
B)



C)



D)



7.5 Appendix 5: Data to calculate the amount of pollen carried (Chapter2).

This appendix shows the raw data used to calculate the amount of pollen carried by different pollinators.

Study	Species	Pollen
Vithange (1990)	Apis Mellifera	4090
Vithange (1990)	Blow fly	722
Vithange (1990)	Blow fly	65
Vithange (1990)	Blow fly	395
Vithange (1990)	Hoverfly	483
Vithange (1990)	Other Diptera	369
Bushuru (2015)	Apis Mellifera	37
Bushuru (2015)	Blow fly	5
Bushuru (2015)	Beetle	2
Can-alonzo et. al (2005)	Apis Mellifera	1842
Can-alonzo et. al (2005)	Stingless bee	1645
Can-alonzo et. al (2005)	Stingless bee	831
Pérez-Balam, et.al,. (2012)	Apis Mellifera	21
Pérez-Balam, et.al,. (2012)	Blow fly	45
Pérez-Balam, et.al,. (2012)	Wasp	24
Carabali-Banguero et al.,(2020)	Apis Mellifera	1179
Carabali-Banguero et al.,(2020)	Stingless bee	21
Carabali-Banguero et al.,(2020)	Stingless bee	406
Carabali-Banguero et al.,(2020)	Stingless bee	15
Carabali-Banguero et al.,(2020)	Other Diptera	27
Carabali-Banguero et al.,(2020)	Blow fly	53
Carabali-Banguero et al.,(2020)	Other Diptera	88
Carabali-Banguero et al.,(2020)	Hoverfly	119

7.6 Appendix 6: Data to calculate pollen deposition (Chapter 2).

This appendix shows the raw data used to calculate the amount of pollen deposited per visit by different species.

Study	Species	SVD
Willcox, et al (2019)	Apis Mellifera	1.6
Pérez-Balam, et.al,. (2012)	Apis Mellifera	2.8
Evans, et al (2011)	Apis Mellifera	0.53
Willcox, et al (2019)	Stingless bee	2.67
Willcox, et al (2019)	Other Diptera	1.3
Pérez-Balam, et.al,. (2012)	Blow fly	3.7
Pérez-Balam, et.al,. (2012)	Wasp	5

7.7 Appendix 7: Data to calculate flowers visited per min (Chapter 2).

This appendix shows the raw data used to calculate the number of flowers visited per min for different species.

Study	Species	Flower_min
Pérez-Balam, et.al., (2012)	Apis Mellifera	8.2
De la Cuadra (2007)	Apis Mellifera	7.3
Pérez-Balam, et.al., (2012)	Blow fly	4.1
Pérez-Balam, et.al., (2012)	Wasps	7.5
De la Cuadra (2007)	Wild bee	5
De la Cuadra (2007)	Hover fly	3
De la Cuadra (2007)	Beetle	1
De la Cuadra (2007)	Wild bee	3
Monzon et al., (2020)	Wild bee	9.2
Monzon et al., (2020)	Apis Mellifera	7.5

7.8 Appendix 8. Results from GLMM models for wild pollinator variables (Chapter 3).

This appendix shows the full results from the GLMM models of wild pollinator abundance, wild pollinator visits, pollinator richness, pollinator diversity, and honeybee abundance, including Estimated regression parameter, standard errors, z-values, and *p*-values.

	Estimate	Std. error	Z value	P value
<i>Wild Pollinator Abundance</i>				
Intercept	0.412	0.267	1.546	0.122
Natural Habitat	1.219	0.273	4.466	<0.00001
Distance 50	0.014	0.221	0.065	0.948
Distance 100	0.407	0.214	1.902	0.057
Distance 200	0.398	0.218	1.830	0.067
Distance 300	0.183	0.217	0.844	0.399
Log floral	0.263	0.045	5.799	<0.00001
Year 2021	-0.572	0.198	-2.892	0.004
Year 2022	-0.05	0.195	-0.257	0.797
Natural Habitat: Distance 50	-0.688	0.259	-2/648	0.008
Natural Habitat: Distance 100	-1.031	0.252	-4.091	<0.00001
Natural Habitat: Distance 200	-1.287	0.257	-4.998	<0.00001
Natural Habitat: Distance 300	-1.038	0.257	-4.040	<0.00001
<i>Wild Pollinator Visits</i>				

Intercept	0.933	0.302	3.094	0.002
Natural Habitat	1.066	0.305	3.500	<0.001
Distance 50	-0.071	0.234	-0.304	0.761
Distance 100	0.266	0.230	1.155	0.248
Distance 200	0.243	0.235	1.030	0.303
Distance 300	-0.049	0.236	-0.211	0.833
Log floral	0.334	0.051	6.535	<0.00001
Year 2021	-0.784	0.239	-3.267	0.001
Year 2022	-0.199	0.238	-0.838	0.402
Natural Habitat: Distance 50	-0.531	0.281	-1.889	0.059
Natural Habitat: Distance 100	-0.845	0.275	-3.074	0.002
Natural Habitat: Distance 200	-1.021	0.282	-3.628	<0.001
Natural Habitat: Distance 300	-0.776	0.282	-2.751	0.006
<i>Pollinator Richness</i>				
Intercept	0.764	0.150	5.076	<0.00001
Natural Habitat	0.389	0.145	2.678	0.007
Distance 50	-0.012	0.110	-0.113	0.909
Distance 100	0.084	0.108	0.777	0.437
Distance 200	0.015	0.114	0.132	0.895
Distance 300	-0.045	0.114	-0.397	0.691
Log floral	0.183	0.024	7.796	<0.00001
Year 2021	-0.629	0.150	-4.191	<0.00001
Year 2022	-0.224	0.146	-1.536	0.125
Natural Habitat: Distance 50	-0.298	0.128	-2.328	0.019
Natural Habitat: Distance 100	-0.451	0.127	-3.561	<0.001
Natural Habitat: Distance 200	-0.395	0.128	-3.066	0.002
Natural Habitat: Distance 300	-0.348	0.132	-2.642	0.008
Natural Habitat: Year 2021	0.271	0.108	2.529	0.011
Natural Habitat: Year 2022	0.287	0.098	2.919	0.004
<i>Pollinator Diversity</i>				
Intercept	0.129	0.133	0.969	0.332
Natural Habitat	0.657	0.189	3.472	<0.001
Distance 50	0.145	0.161	0.901	0.368
Distance 100	0.305	0.160	1.904	0.057
Distance 200	0.138	0.162	0.849	0.396
Distance 300	0.205	0.163	1.264	0.206
Log floral	-0.002	0.047	-0.38	0.969
Year 2021	-0.581	0.141	-4.113	<0.00001
Year 2022	-0.138	0.143	-0.968	0.333
Natural Habitat: Distance 50	-0.399	0.228	-1.752	0.079
Natural Habitat: Distance 100	-0.591	0.227	-2.602	0.009
Natural Habitat: Distance 200	-0.463	0.226	-2.051	0.040

Natural Habitat: Distance 300	-0.533	0.226	-2.356	0.018
Natural Habitat: Year 2021	0.294	0.178	1.657	0.098
Natural Habitat: Year 2022	0.168	0.182	0.919	0.358
<i>Honeybee Abundance</i>				
Intercept	0.877	0.193	4.534	<0.0001
Natural Habitat	0.079	0.124	0.643	0.521
Distance 50	0.108	0.099	1.100	0.271
Distance 100	-0.059	0.101	-0.588	0.556
Distance 200	0.021	0.009	0.214	0.831
Distance 300	-0.075	0.101	-0.738	0.461
Log floral	0.341	0.036	9.606	<0.0001
Year 2021	-0.145	0.199	-0.727	0.467
Year 2022	0.185	0.198	0.936	0.349

7.9 Appendix 9. Results of the Tukeys test (Chapter 3).

This appendix shows the Z-Value and P-Value for the results of the post hoc Tukeys test for differences in distances a) wild pollinator abundance, b) wild pollinator visits, c) pollinator richness, and d) pollinator diversity (Chapter 3).

a) Wild pollinator abundance

Comparison	Z-Value	P-Value
Control 100 – Control 0	1.776	0.71590
Control 200 – Control 0	1.684	0.77469
Control 300 – Control 0	0.728	0.99915
Control 50 – Control 0	-0.017	1.00000
NH 0 – Control 0	3.907	0.00309
NH 100 – Control 0	2.069	0.50697
NH 200 – Control 0	1.126	0.97774
NH 300 – Control 0	1.218	0.96263
NH 50 – Control 0	1.921	0.61465

Control 200 – Control 100	-0.061	1.00000
Control 300 – Control 100	-1.049	0.98633
Control 50 – Control 100	-1.794	0.70380
NH 0 – Control 100	2.594	0.19096
NH 100 – Control 100	0.735	0.99907
NH 200 – Control 100	-0.218	1.00000
NH 300 – Control 100	-0.124	1.00000
NH 50 – Control 100	0.585	0.99986
Control 300 – Control 200	-0.976	0.99184
Control 50 – Control 200	-1.712	0.75747
NH 0 – Control 200	2.618	0.18071
NH 100 – Control 200	0.776	0.99858
NH 200 – Control 200	-0.170	1.00000
NH 300- Control 200	-0.077	1.00000
NH 50 – Control 200	0.627	0.99975
Control 50 – Control 300	-0.747	0.99895
NH 0 – Control 300	3.359	0.02265
NH 100 – Control 300	1.518	0.86460
NH 200 – Control 300	0.572	0.99988
NH 300 – Control 300	0.666	0.99958
NH 50 – Control 300	1.370	0.92321
NH 0 – Control 50	3.921	0.00288
NH 100 – Control 50	2.084	0.49585
NH 200 – Control 50	1.141	0.97576
NH 300 – Control 50	1.232	0.95976

NH 50 – Control 50	1.933	0.60536
NH 100- NH 0	-3.734	0.00560
NH 200 – NH 0	-5.532	< 0.001
NH 300 – NH 0	-5.327	< 0.001
NH 50 – NH 0	-4.016	0.00180
NH200 – NH 100	-1.838	0.67365
NH 300 – NH 100	-1.651	0.79473
NH 50 – NH 100	-0.294	1.00000
NH 300 – NH 200	0.178	1.00000
NH 50 – NH 200	1.538	0.85516
NH 50 – NH 300	1.361	0.92615

b) Wild pollinator visits

Comparison	Z-Value	P-Value
Control 100 – Control 0	1.158	0.9732
Control 200 – Control 0	1.035	0.9876
Control 300 – Control 0	-0.211	1.0000
Control 50 – Control 0	-0.304	1.0000
NH 0 – Control 0	3.503	0.0134
NH 100 – Control 0	1.583	0.8322
NH 200 – Control 0	0.934	0.9941
NH 300 – Control 0	0.780	0.9985
NH 50 – Control 0	1.507	0.8697
Control 200 – Control 100	-0.99	1.0000
Control 300 – Control 100	-1.362	0.9258

Control 50 – Control 100	-1.464	0.8887
NH 0 – Control 100	2.644	0.1707
NH 100 – Control 100	0.723	0.9991
NH 200 – Control 100	0.069	1.0000
NH 300 – Control 100	-0.084	1.0000
NH 50 – Control 100	0.648	0.9997
Control 300 – Control 200	-1.251	0.9558
Control 50 – Control 200	-1.349	0.9299
NH 0 – Control 200	2.699	0.1493
NH 100 – Control 200	0.790	0.9984
NH 200 – Control 200	0.143	1.0000
NH 300- Control 200	-0.009	1.0000
NH 50 – Control 200	0.717	0.9992
Control 50 – Control 300	-0.093	1.0000
NH 0 – Control 300	3.648	0.0082
NH 100 – Control 300	1.740	0.7404
NH 200 – Control 300	1.093	0.9819
NH 300 – Control 300	0.939	0.9938
NH 50 – Control 300	1.666	0.7853
NH 0 – Control 50	3.742	0.0058
NH 100 – Control 50	1.821	0.6853
NH 200 – Control 50	1.167	0.9718
NH 300 – Control 50	1.012	0.9895
NH 50 – Control 50	1.737	0.7417
NH 100- NH 0	-3.792	0.0046

NH 200 – NH 0	-5.044	<0.001
NH 300 – NH 0	-5.259	<0.001
NH 50 – NH 0	-3.897	0.0032
NH200 – NH 100	-1.259	0.9539
NH 300 – NH 100	-1.543	0.8528
NH 50 – NH 100	-0.143	1.0000
NH 300 – NH 200	-0.291	1.0000
NH 50 – NH 200	1.104	0.9805
NH 50 – NH 300	1.394	0.9151

c) Pollinator richness

Comparison	Z-Value	P-Value
Control 100 – Control 0	0.696	0.9994
Control 200 – Control 0	-0.041	1.0000
Control 300 – Control 0	-0.545	0.9999
Control 50 – Control 0	-0.201	1.0000
NH 0 – Control 0	4.206	<0.001
NH 100 – Control 0	1.437	0.9000
NH 200 – Control 0	1.341	0.9325
NH 300 – Control 0	1.250	0.9562
NH 50 – Control 0	1.861	0.6593
Control 200 – Control 100	-0.730	0.9991
Control 300 – Control 100	-1.221	0.9625
Control 50 – Control 100	-0.892	0.9958
NH 0 – Control 100	3.675	0.0072

NH 100 – Control 100	0.901	0.9955
NH 200 – Control 100	0.804	0.9981
NH 300 – Control 100	0.714	0.9993
NH 50 – Control 100	1.325	0.9375
Control 300 – Control 200	-0.501	1.0000
Control 50 – Control 200	-0.158	1.0000
NH 0 – Control 200	4.210	<0.001
NH 100 – Control 200	1.463	0.8897
NH 200 – Control 200	1.368	0.9248
NH 300- Control 200	1.278	0.9499
NH 50 – Control 200	1.884	0.6422
Control 50 – Control 300	0.348	1.0000
NH 0 – Control 300	4.548	<0.001
NH 100 – Control 300	1.846	0.6696
NH 200 – Control 300	1.751	0.7337
NH 300 – Control 300	1.663	0.7888
NH 50 – Control 300	2.261	0.3751
NH 0 – Control 50	4.345	<0.001
NH 100 – Control 50	1.589	0.8300
NH 200 – Control 50	1.494	0.8761
NH 300 – Control 50	1.403	0.9127
NH 50 – Control 50	2.011	0.5508
NH 100- NH 0	-5.513	<0.001
NH 200 – NH 0	-5.730	<0.001
NH 300 – NH 0	-5.833	<0.001

NH 50 – NH 0	-4.764	<0.001
NH200 – NH 100	-0.184	1.0000
NH 300 – NH 100	-0.346	1.0000
NH 50 – NH 100	0.799	0.9982
NH 300 – NH 200	-0.164	1.0000
NH 50 – NH 200	0.986	0.0013
NH 50 – NH 300	1.144	0.9755

d) Pollinator diversity

Comparison	Z-Value	P-Value
Control 100 – Control 0	1.773	0.7526
Control 200 – Control 0	0.695	0.9995
Control 300 – Control 0	1.061	0.9882
Control 50 – Control 0	0.751	0.9991
NH 0 – Control 0	4.976	<0.001
NH 100 – Control 0	3.112	0.0585
NH 200 – Control 0	2.814	0.1312
NH 300 – Control 0	2.765	0.1479
NH 50 – Control 0	3.331	0.0295
Control 200 – Control 100	-1.062	0.9882
Control 300 – Control 100	-0.685	0.9996
Control 50 – Control 100	-1.021	0.9911
NH 0 – Control 100	3.219	0.0420
NH 100 – Control 100	1.362	0.9384
NH 200 – Control 100	1.060	0.9883

NH 300 – Control 100	1.035	0.9902
NH 50 – Control 100	1.582	0.8572
Control 300 – Control 200	0.375	1.0000
Control 50 – Control 200	0.050	1.0000
NH 0 – Control 200	4.224	<0.001
NH 100 – Control 200	2.441	0.3012
NH 200 – Control 200	2.139	0.4991
NH 300- Control 200	2.123	0.5103
NH 50 – Control 200	2.660	0.1899
Control 50 – Control 300	-0.321	1.0000
NH 0 – Control 300	3.818	0.0054
NH 100 – Control 300	2.055	0.5599
NH 200 – Control 300	1.755	0.7636
NH 300 – Control 300	1.744	0.7700
NH 50 – Control 300	2.271	0.4079
NH 0 – Control 50	4.234	<0.001
NH 100 – Control 50	2.372	0.3426
NH 200 – Control 50	2.070	0.5491
NH 300 – Control 50	2.029	0.5777
NH 50 – Control 50	2.593	0.2208
NH 100- NH 0	-1.842	0.7079
NH 200 – NH 0	-2.138	0.5000
NH 300 – NH 0	-2.119	0.5141
NH 50 – NH 0	-1.628	0.8344
NH200 – NH 100	-0.307	1.0000

NH 300 – NH 100	-0.318	1.0000
NH 50 – NH 100	0.218	1.0000
NH 300 – NH 200	-0.011	1.0000
NH 50 – NH 200	0.525	0.9999
NH 50 – NH 300	0.534	0.9999

7.10 Appendix 10. Results for the post hoc Dunn test for visitation rate (Chapter 3).

This appendix shows the z-values and p-values for the post hoc Dunn test for flower visitation rate and pollinator taxa.

Comparison	Z-Value	P-Value
Beetle – Fly	-6.1	<0.0001
Beetle – Honeybee	-7.9	<0.0001
Fly – Honeybee	-2.0	0.7
Beetle – Hoverfly	-3.5	<0.001
Fly- Hoverfly	3.9	0.001
Honeybee – Hoverfly	6.8	<0.0001
Beetle-Wasp	-3.1	<0.05
Fly – Wasp	1.5	1
Honeybee – Wasp	2.7	0.1
Hoverfly – Wasp	-0.8	1
Beetle – Wild Bee	-3.4	<0.001
Fly – Wild Bee	0.1	1
Honeybee – Wild Bee	0.8	1

Hoverfly – Wild Bee	-1.7	1
Wasp – Wild Bee	-0.9	1

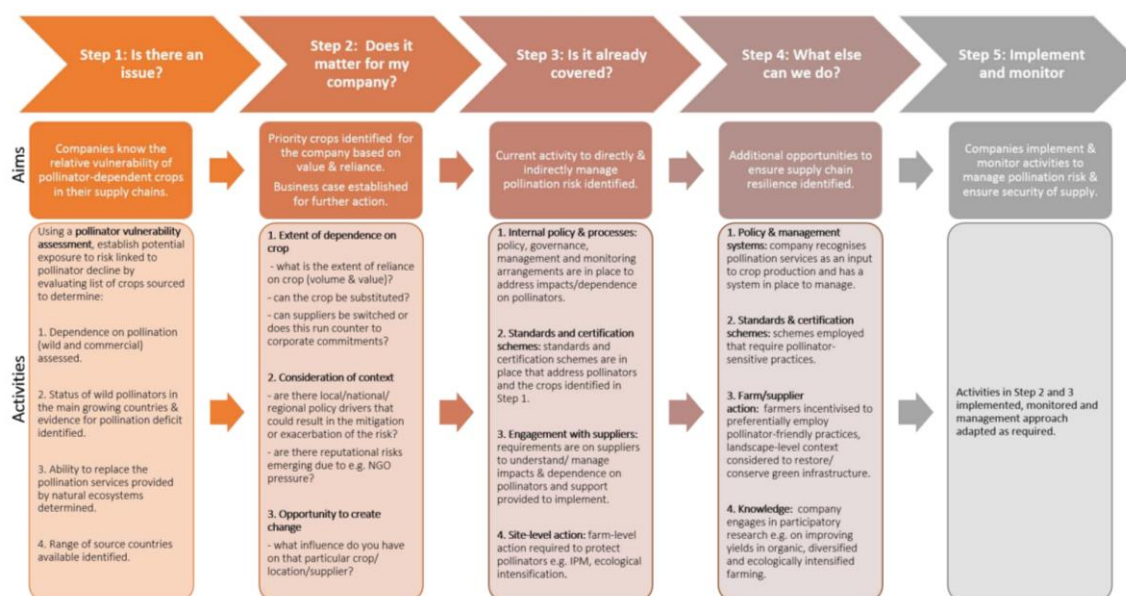
7.11 Appendix 11. Results for the GLMM model of proportion fruit set (Chapter 3).

This appendix shows the full results for the GLMM model of proportion fruit set, including estimated regression parameter, standard errors, z-values, and p-values.

	Estimate	Std. error	Z value	P value
Intercept	-4.506	0.210	-21.413	<0.0001
Pollination Exclusion	-2.331	0.240	-9.702	<0.0001
Natural Habitat	-0.099	0.211	-0.474	0.635
Distance 100m	-0.574	0.243	-2.363	0.018
Distance 300m	-1.130	0.257	-4.399	<0.0001
Pollination Exclusion: Distance 100m	1.407	0.310	4.536	<0.0001
Pollination Exclusion: Distance 300m	0.909	0.389	2.333	0.019

7.12 Appendix 12. Roadmap identified in the Pollination deficit report (Chapter 4).

This figure shows the roadmap towards pollination management in the private sector as outlined in the Pollination deficit report: Towards supply chain resilience in the face of pollinator declines.



7.13 Appendix 13. The economic contribution from wild pollinators (Chapter 4).

This information outlines the method and results to calculate the economic contribution from wild pollinators.

To calculate the proportional economic contribution from wild and managed pollinators an analysis on pollinator efficiency and abundance was carried out. Pollinator efficiency was calculated by taking the average value for key pollination efficiency characteristics (amount of pollen carried, amount of pollen deposited, and the number of flowers visited per min) from the supplementary information in the Dymond et al., (2021) review on avocado insect pollinators. This value was divided by the total average contribution from both wild and managed pollinators, to give a proportional contribution. This was repeated for each efficiency characteristic and a final proportional efficiency value was calculated by dividing the total efficiency value for each pollinator group by the total for both groups. Pollinator abundance values were taken from the same review as mentioned above and the abundance values and efficiency values per group were summed together. This figure was then divided by the total abundance and efficiency value for both pollinator groups to give a proportional contribution and finally, this figure was multiplied by the level of dependency.

Data on abundance and efficiency of wild pollinator was only available for South Africa and Chile and the results showed that wild pollinators contributed significantly more to Chile (~37%) in comparison to South Africa (~10%) see the table below for more details.

Table to show the economic contribution to industry revenue from wild and managed pollinators in South Africa and Chile.

Country	Pollinator Group	Total Revenue (USD)	Pollinator Contribution to Revenue (USD)
South Africa	All	27,735,000.00	18,027,750.00
	Managed		16,145,126.99
	Wild		1,882,623.01
Chile	All	16,963,500.00	11,026,275.00

	Managed		6,952,408.27
	Wild		4,073,866.73

7.14 Appendix 14. Interview questions with key informants (Chapter 4).

The list below highlights the key questions asked during the interviews. Depending on the response from the interviewee, further follow up questions were asked, however these questions have not been outlined here as they varied between interviews. Questions 1-5 were designed to understand if there are any industry strategies that support pollinators and question 6 was designed to gain feedback on potential strategies.

1. Are there any regulations the growers must follow in order sell their produce to Westfalia?
2. Does Westfalia provide any agricultural or sustainability training or guideline to its growers?
3. Does Westfalia provide any incentives to growers for produce that has been grown in a more sustainable manner?
4. Do growers have to be part of any certification schemes to sell their produce to Westfalia? If so, which schemes?
5. Does Westfalia carry out any agri- environmental research at its research stations?

The themes as outlined in table 2 (regulations, financial incentives, and persuasion techniques) and potential pollinator strategies were explained to the key informants and for each of these themes the following questions were asked.

6. Do you think this measure could be implemented by Westfalia?
 - a. Why or why not?
 - b. If yes, what would be the best way to implement this measure?

7.15 Appendix 15. Farmer survey questions (Chapter 4).

The information below shows the key questions in the farmer survey to understand what practices are currently being implemented (question 1- 8), if there is any support available (question 9), and the barriers to implementing pollination practices (questions 10-11. Not all survey questions have been outlined here as this survey was part of wider research and therefore only the relevant questions for this study have been included.

1. How likely would you be to implement the following pollination management practices

	I would never do this practice	Very unlikely	Neither likely nor unlikely	Very likely	I currently do this practice
Keep my own honeybees					
Hire honeybees during the pollination season					
Keep other pollinators					
Careful application of pesticides					
Include floral bands on my land					
Restore non-productive areas with native plants					
Restore non- productive areas with non- native plants					
Protect natural areas around the orchard					
Protect natural areas inside the orchard e.g riverbanks, roadsides					

2. Do you implement any other pollination management practices on your land?

The following questions were only asked if the grower said that they implemented a certain pollination management practice.

3. How many beehives do you have?

4. How many beehives do you hire per season?
5. What other pollinators do you keep?
6. How do you manage your application of pesticides in relation to pollination management?
 - a. I don't apply pesticides at all.
 - b. I don't apply pesticides that negatively affect pollinators.
 - c. I reduce the amount of pesticides that I apply.
 - d. I don't apply pesticides at certain times of the year for example during flowering.
 - e. I apply the pesticides using technology that reduces drift.
 - f. Other, please specify.
7. On average how much of your land is dedicated to floral bands?
 - a. Less than 1%
 - b. 2-3%
 - c. 3-5%
 - d. More than 5%
8. On average, how much of your land is dedicated to natural areas?
 - a. Less than 1%
 - b. 2-3%
 - c. 3-5%
 - d. More than 5%
9. Do you receive any advice or financial support to help you implement these pollination practices?
 - a. Yes, I receive training.
 - b. Yes, I receive financial support.
 - c. No, I receive neither financial support nor training.
10. Indicate to what extent you agree with the following statements

	Completely disagree	Disagree	Neither disagree nor agree	Agree	Strongly agree
I have access to the necessary resources to implement pollination management (seeds or plants).					
Natural areas and flower bands increase the number of pests					
I do not have the necessary advice to implement pollination management					
I do not have sufficient finances to implement pollination management					
I do not have time resources to implement pollination management practices					

11. What would be the best way to receive information on pollination management? Please select

all valid answers

- a. Training by an agronomist
- b. Demonstration farms
- c. Handouts or leaflets
- d. Events or seminars
- e. Online knowledge platform
- f. Other, please specify

7.16 Appendix 16. Effectiveness of pollinator strategy theme (Chapter 4).

This table outlines the effectiveness of each pollinator strategy theme.

Theme	Effectiveness
<p>Regulations: legal or mandatory rules.</p>	<p>Regulations work well when there is evidence of high public risk (Pannell, 2008) and when enforcement is carried out effectively (Steg & Vlek, 2009) e.g. restrictions on pesticide use (IPBES, 2016). However, the process can be bureaucratic, expensive, difficult to monitor, and can demotivate individuals leading to a lack of compliance (Mills et al., 2018).</p>
<p>Economic: financial <i>incentives</i> for positive behavior or <i>disincentives</i> for negative behavior.</p>	<p>Economic incentives are more effective than punishments and financial incentives can increase environmental action (Steg & Vlek, 2009). There is some evidence that financial incentives for pollinator practices increase pollinators (substantial evidence for payments, some evidence for certification, and little evidence for insurance schemes) (IPBES, 2016). However, financial incentives can weaken intrinsic motivation resulting in low adoption rates/ compliance and are often associated with high costs (Kremen & Merenlender, 2018; Mills et al., 2018).</p>
<p>Persuasion: Encouraging behavior change and enhancing knowledge.</p>	<p>Persuasion techniques are generally cost-effective, and they often improve intrinsic motivation, thus enhancing the farmer's willingness to act and create long-term change (Mills et al., 2018). However, some studies have shown limited benefits as the success can depend on the situation (e.g., barriers to implementation and intervention complexity), and therefore persuasion techniques may be most effective when implemented with other interventions (Marselle et al., 2021).</p>

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