Pollinator diversity, pollination deficits, and pollination efficiency in avocado (*Persea americana*) production across different landscapes in Murang'a county, Kenya

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"Look deep into nature and then you will understand everything better."

Albert Einstein

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Affidavit

Declaration

I, Rose Nyakemiso Sagwe do certify that the thesis entitled " **Pollinator diversity, pollination deficits, and pollination efficiency in avocado (*Persea americana*) production across different landscapes in Murang'a county, Kenya**" as my own work, and the results therein. Equally, I would like to bring to your attension that I did not receive any help or support from any commercial consulting firm and that all sources and materials applied are listed and specified in the thesis. Finally, I do certify that this thesis has not been submitted elsewhere as part of another examination process in similar or dissimilar form.

Hiermit erkläre ich, Rose Nyakemiso Sagwe, an Eides statt, dass ich die Arbeit mit dem Titel **"Community composition, pollinator diversity and pollination efficiency in avocado production across different landscapes in Muranga county, Kenya"** selbstständig verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und die diesen Quellen und Hilfsmitteln wörtlich oder sinngemäß entnommenen Ausführungen als solche kenntlich gemacht habe. Die Arbeit wurde nicht in ähnlicher oder abweichender Form einer anderen Prüfungsbehörde vorgelegt.

Nairobi, 16.12.2021 Signature PhD-student

Summary

Chapter I– General introduction

The decline of pollinator populations poses a significant threat to food and nutrition security and biodiversity conservation. The interaction networks between plants and pollinators make significant contributions to global biodiversity, ecosystem function, ecosystem services to crops, and eventually human nutrition. Furthermore, sustainable agriculture cannot be achieved without proper management practices. The enormous demand for food, especially in sub-Saharan Africa, has led to an unsustainable intensification of crop production with harmful practices that negatively impact ecosystem services. Thus, there is a need for understanding the effect of pollination on crop yield, quality, and marketability and the assessment of pollination deficits of crops. Furthermore, it is necessary to investigate the pollination performance of different pollinator species of certain crops to identify the best management practices to support the ecosystem service to optimize crop pollination. In this study, avocado (*Persea americana* Mill.) was used as a model crop to assess the importance of insect pollination. The study was conducted in Murang'a county, Kenya, classified as a high potential avocado production zone. In this thesis, the focus is on; 1.) pollination deficits in avocado smallholder farms and the assessment of honey bee supplementation on mitigating the pollination deficits in avocado production systems in Kenya (Chapter 2), 2.) the effect of insect pollination on fruit weight, quality, and marketability of avocado fruits (Chapter 3), 3.) the characterization of pollination efficiency of avocado flower insect visitors (Chapter 4), and 4.) general discussion and conclusion (Chapter 5).

Chapter 2: Pollinator supplementation mitigates pollination deficits in smallholder avocado (*Persea americana* Mill.) production systems in Kenya.

The shift from old cereal crop farming to high-value cash crop farming such as fruits and vegetables in sub-Saharan Africa poses challenges for improving and maintaining high yields in terms of quality and

quantity. Among these challenges are the inadequate pollination services due to the declining population and diversity of pollinators caused by habitat loss, land-use changes, monoculture, and indiscriminate use of agricultural chemicals. Therefore, there is a need for adequate pollination services to ensure sustainable food and nutrition security. Avocado is one of Kenya's leading horticultural export fruit, accounting for one-fifth of her total horticultural exports, hence, contributing to the country's economic growth. However, there is scant information on insect pollination in avocado production, especially in sub-Saharan Africa. For this study, normalized difference vegetation index (NDVI) was used to classify landscapes into low, medium, and high based on vegetation productivity. The dependence of avocado fruit set on insect pollination and pollination deficits was evaluated by manipulative pollination experiments (self and wind pollination, self-pollination, insect-pollinated, and hand pollination). The effect of supplementing pollination using the Western honey bee (*Apis mellifera* L.) to address pollination deficits was assessed. Avocado had a high reliance on insect pollination (89.5%), and when the farms were supplemented with the honey bees, an increment of fruit set by 20.7% was recorded, indicating pollination deficits. The fruit retention rate was monitored for two months from the fruit set, and fruit abscission was less on honey bee supplemented farms. The effect of landscape variables (NDVI class, landscape diversity, nearby agricultural habitats, and farm size) on pollination deficits (fruit set) and fruit retention rates were also assessed. The landscape variables had no significant effect on the fruit set. Except for the number of avocados per farm, none of the landscape variables affected fruit retention. The number of avocado trees is inversely correlated with fruit retention percentage. The results revealed that avocado is highly dependent on insect pollinators for fruit set and supplementation of farms with *A. mellifera* offsets pollination deficit resulting in incrementing on avocado fruit yield.

Chapter 3: Insect pollination enhances fruit weight, quality, and marketability of avocado (*Persea americana*)

The contribution of insect pollinators to agricultural production is mainly unknown, especially in terms of human nutrition, particularly in sub-Saharan Africa. Most farmers are improving crop yields through the addition of fertilization, artificial irrigation and pesticides, and clearing of forests for agricultural expansion without recognizing the potential contribution of insect pollination to improved crop yield and quality. Avocado production has experienced significant growth in recent years due to high demand because of awareness of its high nutritional density, particularly monounsaturated oils and other nutrients like protein, fiber, major antioxidants, vitamins, and minerals. Although it is evident that pollination improves the yield of avocado fruits, it is still unclear if pollination has benefits on fruit quality and the nutritional profile, particularly oils. In this study, the contribution of insect pollination to fruit and seed weight, oil, protein, carbohydrate, and phytochemicals contents (flavonoids and phenolics) were quantified, and whether supplementation with pollinators (honey bee) could improve these fruit parameters was assessed. This was through pollinator-manipulative pollination treatments: hand, open, pollinator exclusion experiments. The results showed that avocado fruit weight was significantly higher in open and hand-pollinated than pollinator exclusion treatments, indicating that flower visitors/pollinators contribute to avocado yields and enhance marketability. Furthermore, insect pollination resulted in heavier seeds and higher oil contents, indicating that insect pollination is beneficial for the fruit's high seed yield and quantity of oil. Honey bee supplementation also enhanced the avocado fruit weight by 18% more than in control farms and slightly increased the avocado oil content (3.6%). Contrarily, insect pollination did not influence other assayed fruit quality parameters (protein, carbohydrates, and phytochemicals). These results indicate that insect pollinators are essential for optimizing avocado yields, nutritional quality (oils), and thus marketability, underscoring the value of beehive supplementation to achieve high-quality avocado fruits and improved food security.

Chapter 4: Characterization of pollinator efficiency of avocado (*Persea americana*) flower insect visitors

Not all flower visitors are true pollinators since some visitors are robbers or thieves of pollen or nectar, and they leave without pollinating. Prior studies have shown that honey bees increase avocado's fruit set and yield. However, an avocado flower is being visited by various insect species. Therefore, determining pollination efficiency will allow a comparison of the relative importance of the different insect species to optimize crop pollination for increased fruit set and crop yield and pollinator conservation. The pollination efficiency for avocado flower visitors has rarely been documented, and this study is among the first to report the pollination efficiency of avocado flower visitors in sub-Saharan Africa. In the present study, pollinator efficiency based on pollen deposition after single visits by different pollinator species in avocado flowers was tested, and their frequency was recorded. The estimated pollination efficiency was highest in honey bees (*Apis mellifera*), followed by the hoverfly species (*Phytomia incisa*). These two species had the highest pollen deposition and more pollen grains on their bodies. In addition, honey bees were the most frequent avocado flower visitors, followed by flies. The findings from this study highlight the higher pollination efficiency of honey bees and *Phytomia incisa*. Hence, management practices supporting these species will promote increased avocado fruit yield. Additionally, these results imply that managed honey bees can be maintained to improve avocado pollination, particularly in areas lacking sufficient wild pollinators.

Chapter 5 – General Discussion

This study evaluated the importance of insect pollination in increasing avocado yield and quality and assessed the pollination efficiency of avocado insect flower visitors. This study demonstrated that insect pollination, which is in most cases underestimated, is a vital and economically important determinant of fruit yield, quality, and marketability. The knowledge from this study can be used to increase avocado production in farms managed by smallholder farmers through the introduction of honey bee colonies. Consequently, our comprehensive findings should be transferable to a wide range of crops in improving their quantity and quality. Remarkably, integrating wild-insect species richness with honey bees will benefit farmers. This could be achieved by planting a variety of plants near the avocado orchards to support native pollinator communities and reduce the dependence of avocado pollination on managed honey bees alone.

This would reduce costs for the management of colonies, especially for the sub-Saharan African rural poor, and enhance system resilience.

Zusammenfassung

Kapitel I - Allgemeine Einführung

Der Rückgang der Bestäuberpopulationen stellt eine erhebliche Bedrohung für die Lebensmittel- und Ernährungssicherheit und die Erhaltung der biologischen Vielfalt dar. Die Interaktionsnetze zwischen Pflanzen und Bestäubern leisten einen wichtigen Beitrag zur globalen biologischen Vielfalt, zur Funktion des Ökosystems, zu den Ökosystemleistungen für Nutzpflanzen und schließlich zur menschlichen Ernährung. Darüber hinaus ist eine nachhaltige Landwirtschaft ohne angemessene Bewirtschaftungspraktiken nicht zu erreichen. Die enorme Nachfrage nach Nahrungsmitteln, insbesondere in den afrikanischen Ländern südlich der Sahara, hat zu einer nicht nachhaltigen Intensivierung der Pflanzenproduktion mit schädlichen Praktiken geführt, die sich negativ auf die Ökosystemleistungen auswirken. Daher ist es notwendig, die Auswirkungen der Bestäubung auf den Ertrag, die Qualität und die Marktfähigkeit von Nutzpflanzen zu verstehen und die Bestäubungsdefizite von Nutzpflanzen zu bewerten. Darüber hinaus ist es notwendig, die Bestäubungsleistung verschiedener Bestäuberarten bestimmter Kulturpflanzen zu untersuchen, um die besten Bewirtschaftungspraktiken zur Unterstützung der Ökosystemleistung zu ermitteln und die Bestäubung von Kulturpflanzen zu optimieren. In dieser Studie wurde die Avocado (*Persea americana* Mill.) als Modellpflanze verwendet, um die Bedeutung der Bestäubung durch Insekten zu bewerten. Die Studie wurde im Bezirk Murang'a in Kenia durchgeführt, der als ein Gebiet mit hohem Potenzial für die Avocado-Produktion gilt. In dieser Arbeit liegt der Schwerpunkt auf: 1.) Bestäubungsdefiziten in kleinbäuerlichen Avocadobetrieben und der Bewertung des Einsatzes von Honigbienen, um die Bestäubungsdefizite in Avocadoproduktionssystemen in Kenia zu verringern (Kapitel 2), 2.) den Auswirkungen der Insektenbestäubung auf das Fruchtgewicht, die Qualität und die Marktfähigkeit von Avocadofrüchten (Kapitel 3), 3.) der Charakterisierung der Bestäubungseffizienz von Avocadoblüten-Insektenbesuchern (Kapitel 4), und 4.) der allgemeinen Diskussion und Schlussfolgerung (Kapitel 5).

Kapitel 2: Bestäuberergänzung mildert Bestäubungsdefizite in kleinbäuerlichen Avocado (*Persea americana* Mill.) Produktionssystemen in Kenia.

Die Umstellung vom traditionellen Getreideanbau auf den Anbau von hochwertigen Nutzpflanzen wie Obst und Gemüse in Afrika südlich der Sahara bringt Herausforderungen mit sich, wenn es darum geht, hohe Erträge in Bezug auf Qualität und Quantität zu erhalten und zu verbessern. Zu diesen Herausforderungen gehört die unzureichende Bestäubungsleistung aufgrund des Rückgangs der Bestäuberpopulationen und -vielfalt, der auf den Verlust von Lebensräumen, Landnutzungsänderungen, Monokulturen und den wahllosen Einsatz von Agrarchemikalien zurückzuführen ist. Daher besteht ein Bedarf an angemessenen Bestäubungsleistungen, um eine nachhaltige Lebensmittel- und Ernährungssicherheit zu gewährleisten. Die Avocado ist eine der wichtigsten Exportfrüchte Kenias, die ein Fünftel der gesamten Gartenbauexporte des Landes ausmacht und somit zum Wirtschaftswachstum des Landes beiträgt. Es gibt jedoch nur wenige Informationen über die Bestäubung durch Insekten im Avocadoanbau, insbesondere in den afrikanischen Ländern südlich der Sahara. In dieser Studie wurde der normierte differenzierte Vegetationsindex (Normalized Difference Vegetation Index (NDVI)) verwendet, um Landschaften anhand der Vegetationsproduktivität in niedrig, mittel und hoch zu klassifizieren. Die Abhängigkeit des Fruchtansatzes der Avocado von der Insektenbestäubung und Bestäubungsdefiziten wurde durch manipulative Bestäubungsexperimente (Selbst- und Windbestäubung, Selbstbestäubung, Insektenbestäubung und Handbestäubung) untersucht. Die Wirkung einer zusätzlichen Bestäubung durch die Westliche Honigbiene (*Apis mellifera* L.) zur Behebung von Bestäubungsdefiziten wurde untersucht. Avocado war in hohem Maße von der Bestäubung durch Insekten abhängig (89,5 %), und als die kleinbäuerlichen Betriebe durch Honigbienen ergänzt wurden, wurde ein Anstieg des Fruchtansatzes um 20,7 % verzeichnet, was auf Bestäubungsdefizite hinweist. Die Beibehaltung der Früchte wurde zwei Monate nach dem Fruchtansatz überwacht, und der Fruchtabfall war in den Betrieben mit Honigbienen weniger ausgeprägt. Die Auswirkungen von Landschaftsvariablen (NDVI-Klasse, Landschaftsvielfalt, nahe gelegene landwirtschaftliche Lebensräume und Betriebsgröße) auf Bestäubungsdefizite

(Fruchtansatz) und Fruchterhaltungsraten wurden ebenfalls untersucht. Die Landschaftsvariablen hatten keinen signifikanten Einfluss auf den Fruchtansatz. Mit Ausnahme der Anzahl der Avocadobäume pro Betrieb wirkte sich keine der Landschaftsvariablen auf die Fruchterhaltung aus. Die Anzahl der Avocadobäume ist negativ korreliert mit dem Prozentsatz der Fruchterhaltung. Die Ergebnisse zeigen, dass die Avocado für den Fruchtansatz in hohem Maße von Insektenbestäubern abhängig ist und dass die Ergänzung der Betriebe mit *A. mellifera* das Bestäubungsdefizit ausgleicht, was zu einer Steigerung des Avocado-Fruchtertrags führt.

Kapitel 3: Insektenbestäubung steigert Fruchtgewicht, Qualität und Marktfähigkeit von Avocado (*Persea americana*)

Der Beitrag von Insektenbestäubern zur landwirtschaftlichen Produktion ist weitgehend unbekannt, insbesondere im Hinblick auf die menschliche Ernährung, vor allem in Afrika südlich der Sahara. Die meisten Landwirte verbessern ihre Ernteerträge durch Düngung, künstliche Bewässerung und Pestizide sowie durch die Abholzung von Wäldern für die landwirtschaftliche Expansion, ohne den potenziellen Beitrag der Insektenbestäubung zur Verbesserung der Ernteerträge und -qualität zu erkennen. Die Avocadoproduktion hat in den letzten Jahren aufgrund der hohen Nachfrage und des Bewusstseins für die hohe Nährstoffdichte der Avocado, insbesondere für einfach ungesättigte Öle und andere Nährstoffe wie Proteine, Ballaststoffe, wichtige Antioxidantien, Vitamine und Mineralien, einen erheblichen Anstieg erfahren. Obwohl es offensichtlich ist, dass die Bestäubung den Ertrag von Avocadofrüchten verbessert, ist noch unklar, ob die Bestäubung Vorteile für die Fruchtqualität und das Nährstoffprofil, insbesondere für die Öle, hat. In dieser Studie wurde der Beitrag der Insektenbestäubung zum Frucht- und Samengewicht, zum Öl-, Protein- und Kohlenhydratgehalt sowie zum Gehalt an sekundären Pflanzenstoffen (Flavonoide und Phenole) quantifiziert, und es wurde untersucht, ob eine Ergänzung mit Bestäubern (Honigbienen) diese Fruchtparameter verbessern könnte. Dies geschah mit Hilfe von manipulativen Bestäuberexperimenten: Handbestäubung, offene Bestäubung, Bestäuberausschlussversuche. Die Ergebnisse zeigten, dass das Gewicht der Avocadofrüchte bei offener und manueller Bestäubung signifikant höher war als beim Ausschluss von Bestäubern, was darauf hindeutet, dass Blütenbesucher/Bestäuber zu den Avocadoerträgen beitragen und die Marktfähigkeit verbessern. Darüber hinaus führte die Bestäubung durch Insekten zu schwereren Samen und einem höheren Ölgehalt, was darauf hindeutet, dass die Bestäubung durch Insekten für den hohen Samenertrag und den Ölgehalt der Frucht von Vorteil ist. Der Einsatz von Honigbienen steigerte auch das Gewicht der Avocadofrüchte um 18% im Vergleich zu den Kontrollbetrieben und erhöhte den Ölgehalt der Avocados leicht (3,6%). Im Gegensatz dazu hatte die Insektenbestäubung keinen Einfluss auf andere untersuchte Qualitätsparameter der Früchte (Eiweiße, Kohlenhydrate und sekundäre Pflanzenstoffe). Diese Ergebnisse deuten darauf hin, dass Insektenbestäuber für die Optimierung der Avocadoerträge, der Nährstoffqualität (Öle) und damit der Marktfähigkeit von entscheidender Bedeutung sind, was den Wert der Ergänzung von Bienenstöcken zur Erzielung hochwertiger Avocadofrüchte und einer verbesserten Ernährungssicherheit unterstreicht.

Kapitel 4: Charakterisierung der Bestäubungseffizienz von Blütenbesuchern der Avocado (*Persea americana*) durch Insekten

Nicht alle Blütenbesucher sind echte Bestäuber, denn einige Besucher sind Räuber oder Diebe von Pollen oder Nektar und verlassen die Pflanze, ohne sie zu bestäuben. Frühere Studien haben gezeigt, dass Honigbienen den Fruchtansatz und den Ertrag von Avocados erhöhen. Eine Avocadoblüte wird jedoch von verschiedenen Insektenarten besucht. Die Bestimmung der Bestäubungseffizienz ermöglicht daher einen Vergleich der relativen Bedeutung der verschiedenen Insektenarten, um die Bestäubung der Pflanzen zu optimieren und so den Fruchtansatz und den Ernteertrag zu steigern und die Bestäuber zu schützen. Die Bestäubungseffizienz von Avocadoblütenbesuchern wurde bisher nur selten dokumentiert, und diese Studie ist eine der ersten, die über die Bestäubungseffizienz von Avocadoblütenbesuchern in Afrika südlich der Sahara berichtet. In der vorliegenden Studie wurde die Bestäubungseffizienz auf der Grundlage der

Pollenübertragung nach einzelnen Besuchen verschiedener Bestäuberarten in Avocadoblüten getestet und deren Häufigkeit erfasst. Die geschätzte Bestäubungseffizienz war bei Honigbienen (*Apis mellifera*) am höchsten, gefolgt von einer Schwebfliegenart (*Phytomia incisa*). Diese beiden Arten hatten den höchsten Polleneintrag und mehr Pollenkörner auf ihren Körpern. Darüber hinaus waren Honigbienen die häufigsten Besucher der Avocadoblüten, gefolgt von Fliegen. Die Ergebnisse dieser Studie unterstreichen die höhere Bestäubungseffizienz von Honigbienen und *Phytomia incisa*. Daher werden Bewirtschaftungsmethoden, die diese Arten unterstützen, den Ertrag von Avocadofrüchten steigern. Darüber hinaus deuten diese Ergebnisse darauf hin, dass die Bewirtschaftung von Honigbienen zur Verbesserung der Avocadobestäubung beibehalten werden kann, insbesondere in Gebieten, in denen es nicht genügend Wildbestäuber gibt.

Kapitel 5 - Allgemeine Diskussion

In dieser Studie wurde die Bedeutung der Insektenbestäubung für die Steigerung des Ertrags und der Qualität von Avocadofrüchten untersucht und die Bestäubungseffizienz von Avocado-Insektenblütenbesuchern bewertet. Die Studie hat gezeigt, dass die Insektenbestäubung, die in den meisten Fällen unterschätzt wird, eine entscheidende und wirtschaftlich wichtige Determinante für den Ertrag, die Qualität und die Marktfähigkeit der Früchte ist. Die Erkenntnisse aus dieser Studie können genutzt werden, um die Avocado-Produktion in kleinbäuerlichen Betrieben durch die Einführung von Honigbienenvölkern zu steigern. Folglich sollten unsere umfassenden Erkenntnisse auf eine breite Palette von Kulturpflanzen übertragbar sein, um deren Erträge und die Fruchtqualität zu verbessern. Es ist bemerkenswert, dass die Landwirte von der Integration der Diversität an Wildinsektenarten mit Honigbienen profitieren werden. Dies könnte durch die Anpflanzung einer Vielzahl von Pflanzen in der Nähe der Avocadoplantagen erreicht werden, um einheimische Bestäubergemeinschaften zu unterstützen und die Abhängigkeit der Avocadobestäubung von Honigbienen allein zu verringern. Dies würde die Kosten für die Bewirtschaftung der Bienenvölker senken, insbesondere für die arme Landbevölkerung in Afrika südlich der Sahara, und die Widerstandsfähigkeit des Systems erhöhen.

Chapter 1

General Introduction

This thesis covers three important objectives, which expound on the pollination deficits in avocado smallholder farms, effect of insect pollination on fruit weight, quality, and marketability of avocado and pollination efficiency of avocado flower visitors across different landscapes in Kenya. The first objective evaluated the pollination deficits in avocado smallholder farms and assessed if supplementation could mitigate the deficits in avocado (*Persea americana* Mill.) production systems in Kenya (Chapter 2). This objective was based on the hypothesis that there is no pollination deficit for avocado production systems and pollination supplementation does not mitigate pollination deficits in avocado smallholder farms. The second objective investigated the effect of insect pollination on fruit weight, quality, and marketability of avocado (Chapter 3). The objective tested the hypothesis that insect pollination do not have an affect on fruit weight, quality, and marketability of avocado. The third objective examines the pollination efficiency of avocado flower insect visitors in Kenya (Chapter 4). The objective tested the hypothesis that there is no difference in pollination efficiency in avocado insect flower visitors.

The above objectives and hypotheses were formulated based on the fact that pollination and pollinator decline are raising ecological and social issues that are not sufficiently recognized. The interaction networks between plants and pollinators make significant contributions to global biodiversity; pollinators interact with plants to buttress wider biodiversity, ecosystem function, ecosystem services to agricultural crops and eventually human nutrition. Thus, pollinators are vital to support both natural ecosystems and human food security, which is an exceptional position for such a varied group of organisms. Furthermore, sustainable agriculture cannot be achieved without ecofriendly management practices. The enormous demand for food especially in sub-Saharan African has led to unsustainable intensification of crop production with lethal practices such as overuse of pesticides, herbicides, fertilizers, overgrazing and land clearing which have a negative impact on the environment, affecting the regulating and supporting ecosystem services. Pollinators are among organisms that are very sensitive to disturbance, particularly to human induced activities such as pesticides use, habitat destruction and loss, intensification of land use systems and change in farming practices.

It is therefore vital to understand the extent to which various crops depend on pollinators within a specific region or landscape. This may include the key pollinators, the status of those pollinators, pollinator deficits, the effect of pollination on crop quality and marketability, and pollination efficiency. This will provide valuable information to farmers, conservationists, and decision makers in evaluating the current and future conservation plans, and for the effective development of mitigation measures like pollinator-friendly practices. In this study avocado was used as a model crop to assess the importance of pollination that can help predict possible and real risks of yield and quality losses arising from pollinator decline and identify pollination deficits in order to target interventions.

1.1 Projected global food demand and agricultural intensification

The current world population is estimated to be over 7 billion and is expected to reach 9 billion by the year 2050 (UN, 2017). Therefore, global food production must increase by 70-100 % from the current levels to feed the growing human population (FAO, 2017). Agriculture is expanding and intensifying in many regions of the world to meet the growing demand of human populations leading to absolute loss of most of the forest cover and associated ecosystem services, as well as the various flora and fauna that previously existed (Potschin & Haines-Young, 2016). Biodiversity declining, poverty of people, and pandemics such as COVID-19 are among the world's greatest problems today. Climate change is also putting more stress on world resources and ecosystem services. To mitigate the negative effects of the impending food shortage and depletion of natural resources, strenuous efforts are being employed to increase both food productivity and biodiversity conservation (Brussaard et al., 2010).

Agricultural intensification has been characterized by high level of chemical inputs (pesticides, herbicides, and fertilizers), tillage operations, and landscape homogenization (Flohre et al., 2011). This has led to reduction in abundance and diversity of many taxa (Tscharntke et al., 2012), including pollinators that threatens the productivity of agriculture (Power, 2010). Most agricultural crops depend on insects such as bees, flies, beetles, wasps, and butterflies among others for pollination. Agricultural land intensification has led to destruction and fragmentation numerous natural habitats which act as forage and nesting site for pollinators (Garibaldi et al., 2011). Declines in floral resources resulting from agricultural intensification have negatively impacted the overall health of pollinators, by decreasing their numbers and making them more susceptible to other stressors such as; nutritional stress because of the difficulties in locating of valuable food resources (Di Pasquale et al., 2013). Pollinators require undisturbed habitats for provision of floral resources, nesting sites and refugee areas during the harsh environmental conditions (Nderitu et al., 2007; Holzschuh et al., 2016). The negative consequences of pesticides on pollinators are a serious concern. For instance, it has been reported that 75% of the honey in the world contains traces of insecticides harmful to bees, particularly neonicotinoids (Zhang 2018). Neonicotinoids have been recognized as main contributors to the decline in the number of bees worldwide. Over the past 20 years, pesticide use has increased enormously to 3.5 billion kg/year worth US$ 45 billion annually (Sharma et al., 2020). However, this has not resulted in commensurate yield increases of crops (Khan et al., 2015). Rather, it has negatively influenced the environment, affecting the regulating, and supporting ecosystem services, in addition to the health hazard they pose to humans and animals.

The effects of agriculture intensification through land use modifications on biodiversity has been widely studied (Flohre et al., 2011; Gopel et al., 2020; Raven & Wagner, 2021). In order to conciliate crop production and biodiversity conservation, advocates of wild-friendly farming and eco-agriculture have recently come up with some proposed concepts (e.g. the "land sparing" which suggests that agricultural areas should be intensively farmed so as to maximize yields, and that some areas should be set aside or "spared" for conservation purposes (Fischer et al., 2014). The "land sharing" concept suggests that agriculture and conservation should be synchronized, with agricultural areas being made more biodiversity-friendly (Phalan et al., 2011). Additionally, organic farming which utilize less chemicals than conventional farming has proved to be more valuable for pollinators (Carrié et al., 2018; Fess & Benedito, 2018). Moreover, the heterogeneity of habitats within farmland can benefit biodiversity and pollinators (Wood et al., 2015; Hardman et al., 2016; Martin et al., 2019). Agricultural intensification can negatively affect pollinator faunas (Gossner et al., 2016), with loss of habitat specialists and poor dispersers leaving only the common taxa. Morgen et al. (2016) investigated the impact of crop intensification on diversity of bees and found that crop intensification decreased bee diversity and indicated that this is expected to continue as additional marginal habitats are converted for agricultural production.

Pollinator conservation within agricultural regions is very essential, because crops require pollination by either wild pollinators or managed pollinators especially in areas which are intensively farmed in order to attain maximum yields, quality, and marketability (Lye et al., 2011; Pywell et al., 2015). Some studies have linked crop yield benefits to the proximity of existing pristine habitats (Karp et al., 2013) and others have linked creation of wildlife habitat such as leaving patches of native flowers in large agricultural fields to increased fruit yield (Carvalheiro et al., 2012; Blaauw & Isaacs, 2014). Therefore, effective crop production in intensively agricultural regions require management that work synergistically without excessively constraining crop management or compromising the provision of other ecosystem services.

1.2 Importance of pollinators and pollination

Pollinators plays an important role in the transfer of pollen to wild plants and other numerous crop species. Insects and other animal pollinators play a vital role in the production of healthy crops for food, fibers, edible oils, medicines, biofuels, and construction materials (Ellis et al., 2015). The pollinators help generate significant income to farmers through increased yield and quantity of agricultural community. Although some species rely on abiotic forces, including wind and water for pollen transfer, crop plants accounting for 35% of global food benefit from animal pollination (Klein et al., 2007). Of the world's wild flowering plants, it is estimated that 87.5% of them (approximately 308,000 species) are pollinated by insects and other animals (Ollerton et al., 2011). However, this degree of dependence is thought to be higher in tropical zones although most of the studies have been conducted in Latin American and Asian tropical lowland rainforests. However, there is scanty literature on the degrees of dependence in African tropical forests (IPBES, 2016).

Pollination is also important in human nutrition aspect, including the high quantity and quality of fruits, nuts, and seeds, and their variety (FAO, 2019). Previous studies have estimated pollinators to be responsible for up to 40 percent of the world's supply of nutrients (Ellis et al., 2015). Pollinator-dependent crops provide essential micronutrients (such as vitamin A, iron, and folate) especially to those populations living in areas of the world where micronutrients deficiencies are common. If this trend on pollinator decline continues, nutritious crops like fruits, nuts, and many vegetables will be replaced by staple crops like rice, corn, and potatoes, ultimately resulting in an imbalanced diet (FAO, 2019). Pollinators contribute significantly to the environmental health as they support biodiversity. For instance, it has been reported that in managed ecosystems, using good pollination management practices contributes to improved crop production. Additionally, there has been a high positive correlation between plant diversity and pollinator diversity (Biesmeijer et al., 2006). Integrated pest management practices overall help to maintain pollinator communities in agricultural ecosystems. In unmanaged ecosystems, maintaining natural ecosystems in and around agroecosystems is essential in providing natural habitat and sources of forage for pollinator populations thus increasing and maintaining pollinator diversity and abundance and other biodiversity provided by the natural vegetation (Giovanetti, et al., 2021).

1.3 The influence of landscape configuration on pollinators

The combined effect of landscape degradation caused by land use intensification and climate change is the greatest threat to biodiversity (Hole et al., 2011; Millenium Ecosystem Assessment, 2005; Lee & Jetz, 2008). Global climate change is anticipated to disrupt the overlap in plant flowering time and pollinator emergence/foraging activity, leading to potentially mismatched interactions between both plants and animals (Gérard et al., 2020). Furthermore, these landscape modifications have been considered as leading factor of species endangerment (Potts et al., 2010; Oliver & Morecroft, 2014). Some studies have indicated that proximity to remnant forests is linked to the abundance and diversity of pollinators that visit crop species in agroecosystems (Sagwe et al., 2015; Zou et al., 2018). The diversity of wild pollinators may also be influenced by the distance between forest and crop systems (Klein et al., 2003). Thus, in agroecosystems, and at landscape scale it is very crucial to understand the relationships between temporal and spatial resource distribution, resource use, pollinators and pollination services (Pufal et al., 2017). The agricultural landscape comprises of different annual and perennial crops, semi-natural and natural habitats as well as different land-use systems. Landscape elements around crops or in their vicinity have been known to have an effect on pollination services (Wood et al., 2015; Sardiñas et al., 2016). Thus, to ensure enough and sustainable crop pollination services, both conservationists and farmers need to be taken into consideration (Pufal et al., 2017). There is also need to understand how pollinators utilize landscape and how this affects their diversity and abundance and provision of pollination services as a guide to landscape configuration.

Changes in land use can often cause extinction of some pollinator species at local and regional scales, thereby changing the structure and function of plant-pollinator communities (Burkle et al., 2013). Pollinators differ in their resource requirements and they respond differently to landscape composition and configuration. Overall, specialized pollinator species are more vulnerable to habitat change than generalists (Williams & Osborne, 2009). In addition, the capability to locate and interchange between dispersed resources in different landscapes differs between species (Rader et al., 2011; Carvell et al., 2012). All crop pollinators depend on diverse plants species for food and nesting sites often provided by semi-natural and natural habitats (Garibaldi et al., 2011). Landscape assemblages can influence the temporal and spatial availability of different types of resources required by pollinators (Kremen et al., 2007; Donkersley et al., 2014). Furthermore, diverse pollinator groups respond differently to landscape structures (Sjödin et al., 2008). Therefore, understanding how pollinators respond to landscape fragmentation, habitat loss, and modification, and the effects of these changes by different species is very essential. In order to assess and improve the management of agricultural lands and landscape structure in maintaining of pollination guild (Hipólito et al., 2006).

A study conducted by Moreira et al. (2015), on how local conditions and landscape structure at multiple scales influence plant-pollinator networks indicated that a reduction of habitat quality and landscape heterogeneity can cause species loss and decrease of networks. The pollination success in many agricultural farming systems strongly depends on pollinators diversity and abundance. Pollinator diversity is thus very crucial as it reduces the risk that may arise during the critical flowering period of a crop. In Africa, a few comprehensive valuations of pollinator diversity and pollination assemblies have been done (Melin et al., 2014; Carvalheiro et al., 2011; Carvalheiro et al., 2010). In East Africa considerable research has been done on bee diversity (Chiawo et al., 2011; Grube et al., 2013; Kasina et al., 2009) but information on other pollinators taxa is lacking. The associations between landscape aspects and pollinator community structure have been well established (Steffan-Dewenter et al., 2002; Carré et al., 2009; Tscheulin, et al., 2011); Bartomeus et al., 2014; Martins et al., 2015; Holzschuh et al., 2016). In addition, the relationship between pollinator community assembly and pollination service have been well documented (Jauker & Wolters, 2008; Bommarco et al., 2012; Garibaldi et al., 2013). However, little has been done concerning landscape setting to pollinator community assembly and yield (Zou et al., 2017). Therefore, there is need to identify landscapes sceneries that support ecologically thorough production systems for both productivity and biodiversity conservation (Bommarco et al., 2013). This is true especially for farms dominated with small scale farming landscapes in East Africa.

1.4 Effect of pollination on crop production

There is growing evidence that insufficient pollinators results in low yields and poor fruit sets (Kasina et al., 2009; Willmer, 2011), and affect fruit quality (Gajc-Wolska et al., 2011; Klatt et al., 2014). In addition, some studies have shown that decreased yield losses emerge from fruit deformations and undeveloped fruits caused by pollination deficits, adversely affecting the marketability of fruits (Ariza et al., 2011). Few researchers have contributed to the understanding of the effect of pollination on fruit quality parameters such as Klatt et al. (2013) who demonstrated the effect of pollination in increasing fruit weight and shelf life in strawberries (*Fragaria ananassa*), while Bommarco et al. (2012) indicated that oilseed rape (*Brassica napus*), pollination enhanced seed quality (heavier seeds) and oil content but lowered chlorophyll content. Pollination has also been shown to influence the nutritional composition in almonds (*Prunus dulcis*) whereby fat and vitamin E content of the nuts were highly increased by pollination (Brittain et al., 2014). Furthermore, effective pollination was found to decrease malformations in apples (*Malus domestica*) and strawberries (*Fragaria ananassa*) (Matsumoto et al., 2012; Wietzke et al., 2018), and in oriental melon (*Cucumis melo*), pollination was found to increase flavor-enhancing elements such as sugars and acids (Shin et al., 2007). However, there is data deficiencies on the effect of insect pollination on avocado fruit quality and nutritional composition especially in sub-Saharan Africa. Although, some studies have shown that insect pollination improves avocado fruit set, but it is still unclear if pollinators also enhance fruit quality and the nutritional profile.

Although, various scholars in the world have documented the decline of pollinators and their pollination services they provide, still, there is lack of literature on pollination deficits associated with important crop yield losses in many crops worldwide (Garratt et al., 2021). Thus, there is need to identify and assess pollination deficits in various crops in order to better mitigate and protect against massive crop losses in the event of pollination deficit. Thus, promoting healthy functioning of ecosystems can ensure the resilience of agriculture, while meeting the growing demands for sustainable food production. Especially in Africa, where agricultural practices to improve yields and production in smallholder farms is focusing on applying fertilizers, irrigation, and pesticides without acknowledging pollination deficits as one of the constrains in increasing crop yield (Sawe et al., 2020).

Measuring pollination efficiency has become increasingly important especially in conservation and sustainable agriculture evaluations (Ne'eman et al., 2010). Not all flower visitors of a given plant species are effective pollinators since some could be 'pollen robbers' and some 'nectar robbers' or there could a mismatch of morphological trait (body size) in relation to flower size (Rivest & Forrest, 2020). The behaviour and morphology traits of flower visitors makes them differ in their pollination efficiency (Ornai & Keasar, 2020). This behavioural difference affects the abilities for both pollen export and deposition. Thus, understanding the impact of specific pollinator taxa on certain plant species is essential in order to achieve substantial pollination services. Pollination efficiency is defined as the relative ability of an insect to pollinate effectively as measured by fruit production per some unit of measure (i.e., per visit) (Keys et al., 1995). Pollen load deposition by a single visit to virgin stigma has been considered as a direct measure of pollination efficiency while indirect measures include visitation rates and time of visitation (Spears, 1983). Managed honey bees (*Apis mellifera)* are the most prevalent used species to supplement wild pollinators and may compensate for the loss of pollinators (Aizen and Harder, 2009). However, there is need to understand the contribution of both wild pollinators and honey bees to pollination, and quantify their pollination services. Prior studies have indicated that wild bees are more reliable pollinators than honey bees (Garibaldi et al., 2013; Breeze et al., 2014; Dainese et al., 2019; Martin et al., 2019). This is because of their great adaptability and species diversity associated with a large variation in number of their traits such as; nest location, social behavior, dispersal ability, tongue length, and their body size (Wu et al., 2018).

Sufficient managed honey bees can be maintained in areas where there is less efficient wild pollinator species (Liu et al., 2020). Although, the managed honey bees suffer the dangers associated with predator, parasite and pathogen development (Graystock et al., 2016; Belsky & Joshi, 2019). A meta-analysis conducted by Garibaldi et al. (2013) in Africa on 40 crops across 600 fields found that wild pollinators were as effective as managed honey bees in seeds and fruits production on crops such as; oilseed rape (*Brassica napus*), coffee (*Coffea canephora*), onions (*Allium cepa*), almonds (*Prunus dulcis*), tomatoes (*Solanum* *lycopersicum)*, and strawberries (*Fragaria ananassa)*. They also found that managed honey bee did not substitute wild pollination when it was lost, but only supplemented the pollination that took place. Also, a study conducted by Rader et al. (2016) on the contribution of non-bee insects to global crop pollination they found that fruit set increased with non-bee insects independent of bee visitation rates. This indicated that non-bee insects deliver exceptional benefit that is not provided by bees. Hence, there is need to sought for alternative wild insect pollinators for agricultural systems, and a lot of work is needed to establish their identities and efficiencies, especially in Sub-Saharan countries where identification remains a challenge for many taxa groups (Orr et al. 2021).

Avocado (*Persea americana* Mill.) is an economically-important horticultural crop, grown for local and export market in Kenya. Fruit set is greatly improved by insect pollinators. Prior studies have shown that avocado is being visited by a range of flower visitors and honey bees are the primary pollinators, but there is lack of data especially in East Africa which assess their pollination efficiency. Additionally, a recent meta-analysis global review on avocado pollinators by Dymond et al. (2021) indicated that studies on pollination efficiency for avocado flower visitors have rarely been documented. It is therefore important to determine which are the key avocado pollinators and their efficiency and identify effective ways to improve and protect this ecosystem service. The knowledge is necessary to inform sustainable management of this important crop as well as to help target future research.

1.5 Pollinator population declines and conservation

There is increasing evidence in pollinator population decline for both native and managed pollinators, and the loss of benefits resulting from them is severely felt by the agricultural community (Breeze et al., 2014; Durant & Ponislo, 2021). Likewise, insect pollinated plants are also in decline (Biesmeijer et al., 2006). Human activities such as urbanization have led to fragmentation and loss of habitat. Changes in land use, agricultural practices and overuse of pesticides have interrupted or destroyed the pollinator habitats (Buri et al., 2014). Instantaneous decline in native and managed pollinator populations globally, with evidence in honey bees, bumble bees, and Monarch butterflies, have brought into focus the importance of pollinator conservation (vanEngelsdorp et al., 2009; Pettis & Delaplane, 2010; Cameron et al., 2011; Paudel et al., 2015). Much of this concern comes from well-documented reports on the declines in managed honey bee populations and bumble bees (*Bombus* species) in North America and Europe, (Bartomeus & Winfree, 2013; Bommarco et al., 2012). Native bees, butterflies, and other native pollinators are all reported to be impacted by habitat loss and degradation (Potts et al., 2010; Biesmeijer et al., Archer et al., 2014; Sagwe et al., 2015). To date, most of the information on pollinator decline comes from only five countries (Australia, Brazil, Germany, Spain and the USA), but only 4% of the data comes from Africa with no comprehensive evaluation of the status and trends of pollinators and pollination services (Gemmill-Herren et al., 2014; Melin et al., 2014).

Declines in the health and population of pollinators pose a significant threat to the integrity of biodiversity, human health and food security. Due to the quality of pollinator services decline over time has led to concerns that pollinators will be less resistant to extinction in the future if conservation efforts are not made (Vanbergen et al., 2013). In Africa, little studies have addressed the effects pesticides in the field to pollinators. This can be crucial in obtaining information that can help both conservationists and farmers to choose pesticides that are less toxic to pollinators while still controlling pests. However, there is lack of information on pollinator foraging practices, social structures, and genetics makeups. These distinct differences must be measured when considering pesticide risk valuations and risk mitigation measures. Pollination management should be regarded as a production factor for all crops since it can affect the agronomic yield which include; fruit set and seed set, and fruit quality (Sabbahi et al., 2006). The loss of insect pollinators may affect have both crop yields, and the reproduction of wild plant species and conservation and function of global biodiversity. The sub-Saharan Africa (SSA), a region heavily dependent on agriculture for income, its productivity is severely affected by several insect pests, and this has led to discriminate use of chemical pesticides and insecticides affecting insect pollinators directly. Ecosystem based services such as pollination and integrated pest management (IPM) are key drivers of sustainable productivity.

1.6 Avocado Production

Avocado is a globally significant horticultural crop with a high economic and nutritional value. Presently, avocado has received extensive marketing and wide distribution for many countries with export market value of approximately US$ 6.84 billion globally for 2018 and it is estimated to reach more than US$ 17.9 bn by 2025 (FAO, 2020). Avocado has a great market as a fresh fruit, and oil, cosmetic, soap, and shampoo industry; as well as processed foods derived from it, such as guacamole, frozen products and avocado paste (Margetts, 2009). Avocados are highly nutritious fruits with many health benefits, they are rich in oil (12-30%) , protein (1.5-2.5 %), vitamins and minerals, as well as antioxidants, and fiber, which aid in the lowering of various chronic diseases (Santos *et al*., 2014). For these reasons they have great potential increase in human diets.

Avocado is an economically important crop in Kenya, and currently, it has gained popularity as a healthy superfood due to its nutritional value for human health (Hakizimana & May, 2018). Kenya is currently ranked 6th after Peru, Chile, South Africa, Israel, and Mexico in avocado export volumes (HCDA, 2017). In 2017, Kenya had a total annual avocado production of 233,933 metric tons; 81,098 metric tons worth $90 million were exported, while the remainder (152,835 metric tons) served the local market. In Kenya, avocado is mostly grown by smallholder growers (70%) for subsistence, local markets and for export (Amare et al., 2019). Avocado can thrive in several agro-ecological zones and in Kenya, the main production area is Murang'a. Although several farmers are growing avocado, they are facing pest constraints to avocado productivity and this include: false codling moth (FCM) (*Thamatotibia leucotreta)*, Tephritid fruit flies (*Bactrocera dorsalis and Ceratitis cosyra*), and greenhouse thrips (*Heliothrips haeorhoidalis*) (Odanga et al., 2018) and this has triggered widespread use of insecticides. Smallholder farmers rely heavily on the use of synthetic pesticides for pest management with detrimental effect on pollinators without realizing pollination deficits as a major constraint to crop yield (Oronje, 2012). Although pollinators may contribute substantially to yields at no cost to farmers, many farmers in East Africa handle pollinators with insect pests, and do not manage to conserve them (Martins, 2013). Therefore, in order to obtain high avocado yield and high-quality fruits that meets the international market standards, pollination services are very necessary.

1.7 Avocado Flowering and pollination

There are different cultivars of avocado which are classified by their floral behavior known as protogynous dichogamy (Free, 1993), where the flower has both functional male and female organs in one flower but opens and closes twice over a two-day-period. The first day as functionally female and next day as functionally male. Each opening stage only last about half a day. There are two groups of avocados, A and B. In type A cultivars, the flowers open in the morning as females and in the afternoon of the following day as males. For type B cultivars, the flowers open in the afternoon as females and the following morning as males. This process reassures outcrossing; however, close pollination can occur where avocados are self-fertile (pollination from within the same cultivar or tree) can occur during the daily overlap of male and female flowers (Stout, 1932). Weather conditions play a significant role in flowering synchronization, under cooler temperatures, the length of time for male and female flowers to overlap can increase (Ish-Am & Eisikowitch, 1990; Pattemore et al., 2018). Flowering usually last 5 to 8 weeks (Schaffer et al., 2013). There are many varieties of avocado, and each variety is marked by different traits as well as sizes, forms, and compositions. The leading export varieties from Kenya are Hass, Fuerte and Pinkerton (Me & Arzate-Fernández, 2010).

Avocado production relies on insect pollination (Alcaraz & Hormaza, 2009 ; Garner & Lovatt, 2008 ; Evans et al., 2010 ; Sagwe et al., 2021). Therefore, avocado being among the pollinator-dependent crop, yields may be adversely affected by extensive pollinator declines (Biesmeijer et al., 2006; Potts et al., 2016). Despite the relatively few reported examples on the importance of pollinators in avocado fruit set, there is need for information on the importance of pollination in increasing crop yield and fruit set especially in Kenya (Mulwa et al., 2019). Poor or inadequate pollination during the flowering period will result in poor yields

Honey bees followed by a range of dipteran flies are the key pollinators in East Africa (Mulwa et al., 2019; Okello et al., 2021). Pollination in avocado and bee activity are influenced by temperature with warmest days being favorable to pollination. Therefore, ensuring that there are enough bees in avocado farms when weather conditions are right is key for management of avocado production. Previous studies have revealed that avocado nectar is unattractive to honey bees when compared to alternative blossoms in the surrounding. This is due to the high mineral content of potassium and phosphorous and unique alcohol-sugars (perseitol) that repels honey bees (Afik et al., 2006; Afik et al., 2011).

1.8 Description of the study area

The study was conducted in Murang'a county in central region of Kenya (0° 43' 0.01" N 37° 08 60.00" E). The region is classified as high potential area for avocado production (Amare et al., 2019 (Fig. 1.1). The county occupies a total area of 2,558.8 km^2 with main economic activity in the region being agriculture with the average farm size under large scale holdings of 6.5 hectares. The total population in the region is 1,056,640 according to Kenya's 2019 population and housing census.

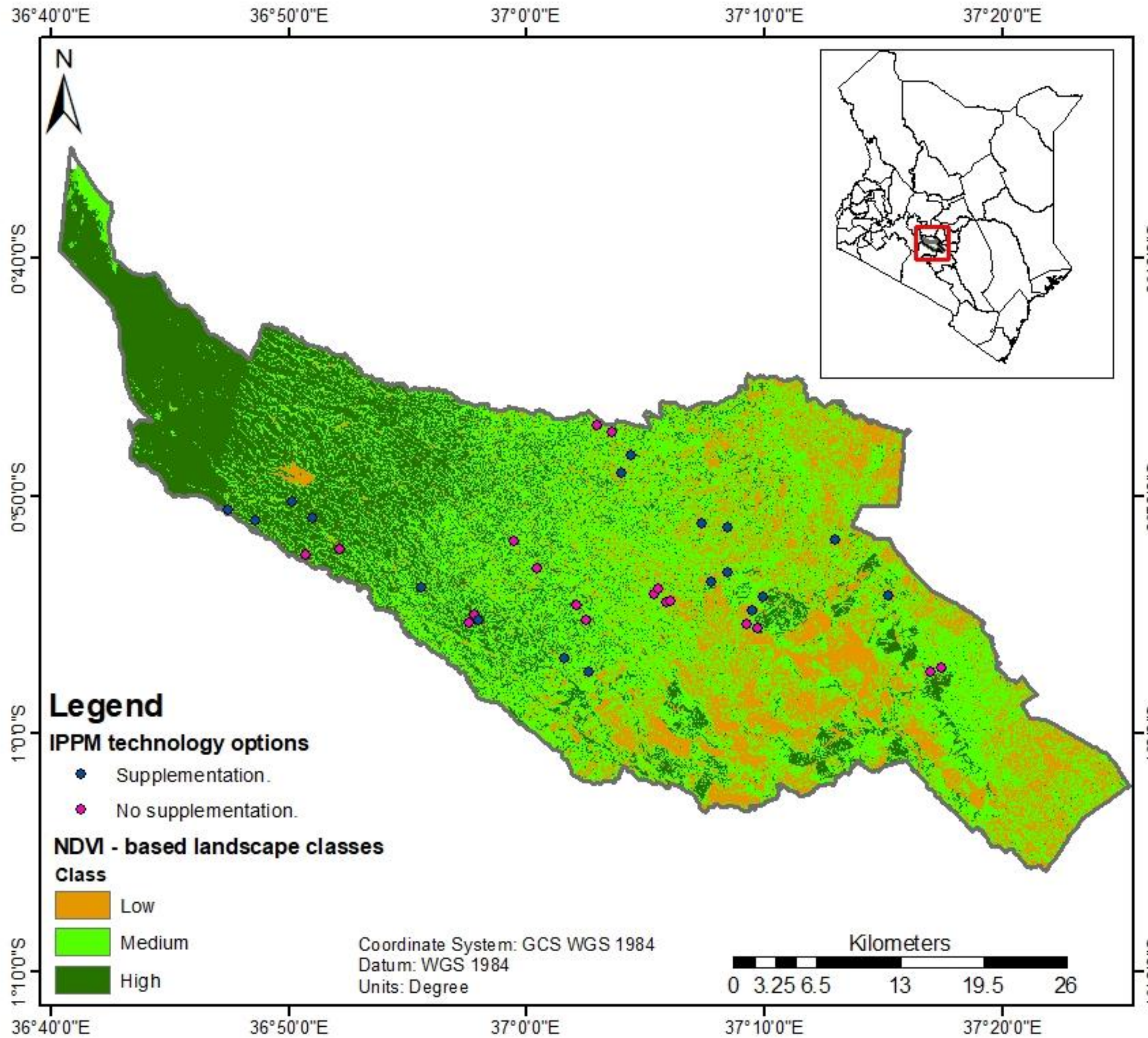

Fig. 1.1. Map of Murang'a County, Kenya, with the location of the 36 study sites (dots). The color pixels with the map indicate the three levels of normalized difference vegetation index (NDVI), a proxy of the net primary productivity of ecosystems. The blue dots represent avocado farms supplemented with two hives of honey bees per farm while the pink dots represent avocado farms without hives. The inlet figure shows the location of Murang'a County within Kenya (adapted from Adan et al., 2021).

The county lies between 914 m above sea level (ASL) in the East and 3,353 m above sea level along the slopes of the Aberdare Mountains in the West. The county is divided into three climatic regions, the

Western region with an equatorial type of climate, the central region with a sub-tropical climate and the Eastern part with semi-arid conditions. The region experiences long and short rains; long rains fall in the months of March, April, and May while the short rains occur during the months of October and November. The county is divided into six agro-ecological zones. The first agro-ecological zone comprises of the highest potential zones where forestry, tea and tourism industry are the major economic activities. The agro-ecological zones two and three are the lowlands east of Aberdares and are suitable for both coffee and dairy farming. Agro-ecological zones 4, 5, and 6 are characterized by arid and semi-arid conditions where crops thrive by irrigation. The geology of the county consists of volcanic rocks of Pleistocene age and basement system rock of Achaean type. Volcanic rocks occupy the western part of the county bordering the Aberdare while rocks of the basement system are in the eastern part.

The major cash crops grown in the region include tea, coffee, avocado, mangoes, macadamia pineapples and horticultural crops. Horticultural crops include tomatoes, cabbage, kales, spinach, and French beans. The food crops grown in the region include maize, beans, sweet potatoes, and cassava. Murang'a county is the leading producer of avocados especially the Hass variety in Kenya, this is because of the increase demand especially in the European market. The total acreage under food crop farming is 1332.4 km^2, while under cash crop farming is 718.9 km^2. Food crop farming is being practiced in all parts of the county while cash crop farming is practiced mostly in the upper zones and in some lower zones of the county. The arable land is 2,135 km^2 while non-arable land is 163.3 km^2, and the gazette forest covers an area of 254.4 km^2. The County has one company ranch owned by Kakuzi limited of approximately 0.86198 km^2, the company grows avocado in large scale (Murang'a County Integrated Development Plan 2013 -2017).

1.8.1 Landscape characterization

The study site was characterized into three vegetation productivity classes using freely available Sentinel-2 (S-2) satellite data which was derived from normalized difference vegetation index (NDVI) as a proxy for vegetation productivity. The NDVI was calculated using multi-date S-2 data of 10 m spatial resolution attained during the dry (December to February) and wet (March to May) seasons in the year 2019. Satellite images were processed and analyzed using the Google Earth Engine platform (GEE) (https://earthengine.google.com, Dublin, Ireland). NDVI was computed as the ratio of the differences between the reflectance at near-infrared (NIR) and red (R) bands and their summation [(NIR − R)/ (NIR + R)]. The K-means unsupervised clustering method was used to differentiate three classes of NDVI, low, medium and high. K-means is a machine learning algorithm that clusters the dataset into K number of clusters, whereby each cluster contains data points close to the cluster's mean value. To cluster the multi-date NDVI imagery, 100 random points were generated using the 'create random points' tool in QGIS version 3.10.2 (https://qgis.org/downloads/). The NDVI values at these 100 points were extracted and put into the Past 3 tool (Hammer *et al*., 2001) to employ the K-means method. The NDVI range values of each of three classes (i.e., low, medium and high) were used as thresholds to re-classify the multi-date NDVI imagery. The NDVI threshold for the low class was −0.425–0.368, while for the medium and high classes, thresholds were 0.368–0.611 and 0.611–0.864, respectively (Adan et al., 2021).

1.8.2 Land use/land cover (LULC) classification

Land use/land cover (LULC) composition was determined in a 3 km radius area around the experimental farms, based on the foraging distance for bees (Visscher & Seeley, 1982) and included the following classes: annual croplands, avocado, built-up, grasslands, perennial croplands, shrublands, tree cover, and water bodies. The altitude of the farm points was recorded using the global positioning system (GPS). The S2 multi-spectral image was used to determine LULC for its high resolution of 10 m in the visible and near-infrared (VNIR) bands, which comes with three red-edge (RE) bands. A composite image was created in GEE S2 level 1C (images top of atmosphere reflectance). The composite image was used in the calculation of two RE vegetation indices (VIs): RE normalized difference vegetation index (RE−NDVI) and RE enhanced vegetation index (RE–EVI). RE–NDVI and RE–EVI were both derived from the S2 RE band with a 20 m resolution. Thus, resampling was conducted on the respective band to 10 m pixel size. The

RE–VIs and reflectance were fused in GEE, and the random forest (RF) algorithm was employed to characterize the landscape. The overall accuracy of the classification was 83.85% with a kappa (total accuracy – random accuracy) / (1 – random accuracy) of 0.7721 (King'ori et al., unpublished data).

1.8.3 Sampling design

Field sampling was conducted from February 2019 to December 2020, this period covered the flowering and harvesting of avocado. Smallholder avocado farms (<0.4 ha) were selected in different villages to maximize the representativity of growers in Murang'a. Within each NDVI landscape class (low, medium, and high), two interventions options, pollinator supplementation (Fig. 1.2a), and no intervention (control) were applied depending on the willingness of the farmer to implement the option. Several criteria were then applied between each farm depending on the treatment: pollinators (supplemented farms) were at least 1.5 −3.5 km away from each other, and pollinator farms were at least 3.5 km apart from the control site which is the normal foraging range for most bee species (Chifflet et al., 2011). Farms with a minimum of seven avocado trees were selected (Adan et al., 2021). On farms, all the experimental trees had a minimum distance of 10 m from the edge of the farm in order to minimize the edge effects. Eighteen farmers were selected for each treatment (pollinators or control), with twelve farms per NDVI class, yielding 36 farms (Fig. 1.1).

Field pollination manipulative experiments were conducted in the 36 farms and in each farm, three trees were randomly selected. Four east-orienting branches (pointing eastwards) on each experimental tree bearing dense and mature flower buds were randomly selected approximately two weeks before flowering, with a total of 432 branches involved in the experiment. Bags for manipulating flower access were put in place 1 – 6 days before flowering. The four pollination treatments were: i) self-pollination (self), using woven bags that were impermeable to wind, allowing only autogamous self-pollination; ii) wind pollination and spontaneous selfing (self + wind), whereby insects were excluded by coarse mesh gauze with 0.8 –1.0 mm openings; iii) open pollination (open), in which all insects had access to flowers (insect pollination);

and iv) hand pollination (hand) (Fig. 1.2.b-e). For hand pollination, pollen was transferred to stigmas with a paint brush and flowers covered with very fine nylon mesh gauze (10 μm), according to Willmer and Stone (1989). Sticky glue was applied on the branch beneath the bagged flowers to eliminate insect contamination, such as ants in bagged experiments. Flowers were tagged and left undisturbed.

Regarding visitation frequency, six smallholder avocado farms were selected for the study. On each farm, three trees spaced 20 m apart were randomly selected for the experiment. Selected trees were spaced a minimum of 10 m from the edge of the farm to minimize edge effects. Flower visitors were observed for a 30 min period and their number was recorded. Pollinator surveys were conducted between 8.00 − 17.00 h, only when weather conditions were suitable for insect sampling. We conducted farm-based flower visitor observations nine times with each farm visited once a month for a period of three months. To measure pollination efficiency single-visit deposition (SVD) method was used for comparison (Ne'eman et al., 2010). Three farms were selected for the study and in each farm 32 panicles per farm were randomly selected and bagged with fine woven bags that were impermeable to wind and excluded pollinators (Fig. 1.3 b). The panicles were unbagged and flowers that had opened were observed until they received their first visitor (Fig. 1.3 c). Pollinators were caught on their first visit as they exited the flowers using sweep nets. (Fig. 1.3 d). The stigmas of visited flowers and control flowers were sampled, put in individual 0.5 mL vials with 70% ethanol and stored in a cooler box at -18°C. Samples were subsequently transported to the laboratory for pollen count analysis and identification (Fig. 1.3 e and f).

Concerning fruit quality analysis all avocado fruits from experimental inflorescences were harvested two weeks before commercial harvest from each of the farms (late April to early May 2020). Avocados were well labelled and bagged individually according to the pollination treatment, tree and farm number, and then transported to the laboratory at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya, to check all the physical quality measures, nutritional parameters and phytochemical contents.

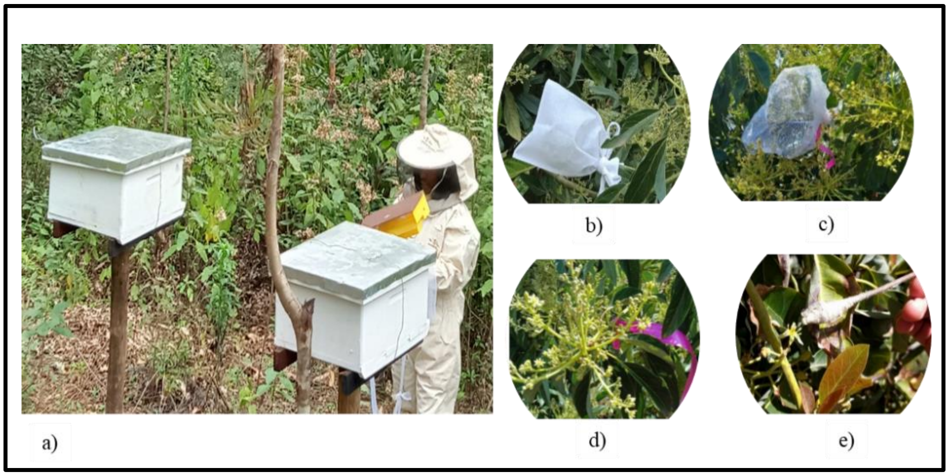

Fig. 1.2. Supplementation of two hives (a) and pollination manipulative experiments ((b)self-, (c), wind self, open(d) and hand pollination(e)) during the avocado blooming season in Murang'a county, Kenya.

Fig. 1.3. Identification of avocado flower buds ready to open (a), bagging of flowers (b), collection of flower visitors using sweep nets (c and e), and pollen staining and identification in the lab (e and f).

Chapter 2

Pollinator supplementation mitigates pollination deficits in smallholder avocado (*Persea americana* Mill.) production systems in Kenya.

Summary

Avocado (*Persea americana* Mill.) is a major horticultural crop that relies on insect mediated pollination. In avocado production, a knowledge gap exists on the importance of insect pollination, especially in East African smallholder farms. In this study, conducted in a leading smallholder avocado production region in Kenya, we assessed the dependence of avocado fruit set on insect pollination and whether current smallholder production systems suffer from a deficit in pollination services. Furthermore, we assessed if supplementation with colonies of the Western honey bee (*Apis mellifera* L.) to farms mitigated potential pollination deficits. Our results revealed a very high reliance of avocado on insect pollinators, with a significantly lower fruit set observed for self- and wind-pollinated (17.4%) or self-pollinated flowers (6.4%) in comparison with insect-pollinated flowers (89.5%). We found a significant pollination deficit across farms, with hand-pollinated flowers on average producing 20.7% more fruits than non-treated open flowers. This pollination deficit could be compensated by the supplementation of farms with *A. mellifera* colonies. Our findings suggest that pollination is limiting fruit set in avocado and that *A. mellifera* supplementation on farms is a potential option to increase fruit yield.

Keywords: Fruit set, Fruit retention, Landscape composition, NDVI, Pollination services, Western honey bee

2.1 Introduction

Pollination is an essential ecosystem service for improved quality and yield of crops, contributing to food security (Klein et al., 2007; Dainese et al., 2019). Currently, the demand for pollination services in most pollinator-dependent crops is increasing (Aizen et al., 2009), and there is a need to incorporate insect pollination in sustainable agriculture systems to enhance food security (Freitas et al., 2016). The economies of most African countries are heavily reliant on agricultural production, but the implementation of sustainable agriculture is a significant challenge, and relatively few studies have explored the relationship between pollination and yields, especially in smallholder farming systems (Freitas et al., 2016).

Using a global analysis based on 29 crops, Dainese et al. (2019) demonstrated increasing evidence of insufficient insect pollination, particularly in regions with high land-use intensity, limiting crop production. Managed colonies of the Western honey bee (*Apis mellifera* L.) have been used in agricultural landscapes to supplement pollination services provided by wild bees and improve the yield and quality of many flowering crops (Geldmann & González-Varo, 2018). It is, however, essential to identify and assess pollination deficits in crops to mitigate and protect against crop losses in the event of pollination deficits. Previous studies have been done on pollination limitation in Africa. For instance, Grass et al. (2018) reported pollination limitation in macadamia (*Macadamia integrifolia*) orchards in South Africa. In another study, Dan et al. (2019) demonstrated the importance of insect pollination as an additional input in enhancing the yield and quality of cowpea (*Vigna unguiculata*) and cucumber (*Cucumis sativus*) in Makueni county, Kenya, while Kasina et al. (2007) revealed the influence of insect diversity on the seed yield of sunflower (*Helianthus annuus*) in Kenya.

Avocado (*Persea americana* M.), a crop that has seen an increase in worldwide production, is highly dependent on pollination services for enabling fruit set (Vithanage, 1990; Altendorf, 2019). Avocado is an economically important crop in Kenya, and since recently, it has gained popularity as a healthy superfood due to its nutritional value for human health, with extensive marketing and wide distribution (Hakizimana

& May, 2018). The first commercial avocado orchard in Kenya was established in 1923 on approximately 80 ha. In 1970, an estimated 23 metric tons of avocado were exported. In 1984, annual production increased to 1,400 metric tons and by 2003, over 20,000 metric tons were being exported (Griesbach, 1985; Griesbach, 2005). In 2017, Kenya had a total annual avocado production of 233,933 metric tons; 81,098 metric tons worth $90 million were exported, while the remainder (152,835 metric tons) served the local market. Kenya is currently ranked 6th after Peru, Chile, South Africa, Israel, and Mexico in avocado export volumes (HCDA, 2017).

Avocado has a complete flower, with an unusual behavior known as protogynous dichogamy, whereby the flower has both functional male and female organs in the same flower but opens and closes twice over two days, the first day as functionally female and the subsequent day as functionally male (Ish-Am & Lahav, 2011). Lack of pollination could be one of the limiting factors in commercial avocado production (Alcaraz & Hormaza, 2009). In Israel, California (USA), and South Africa, which experience Mediterranean climates, wind and spontaneous self-pollination of the avocado flowers were found not to be effective in the absence of pollinating insects (Ish-am et al., 2005). Supplementation of farms with *A. mellifera* effectively enhances fruit production in commercial orchards (Evans et al., 2011). For instance, in New Zealand, the Avocado Industry Council has recommended four to ten hives of honey bees per hectare for avocado pollination (Evans & Goodwin, 2011). In Mexico, stingless bees (Meliponini) and the Mexican honey wasp *Brachygastra mellifica* Say have been identified as the primary avocado pollinators (Ish-am et al., 1999). In Australia, many insect species were found to pollinate avocado flowers, but *A. mellifera* played a leading role (Vithanage, 1990). However, little is known about African avocado pollinators, especially in tropical and sub-tropical habitats dominated by smallholder farming systems (Rapsomanikis, 2015). A study conducted by Mulwa et al. (2019) in Kenya found that honey bees were the most abundant and frequent insect flower visitors of avocado. Other insects that were found to be potential avocado pollinators include the tropical African latrine blowfly *(Chrysomya putoria*), drone fly (*Eristalis tenax),* and polistine wasps (*Polistes* sp.) (Okello et al., 2021).

Natural landscape elements adjacent to crops or in their vicinity can maintain wild pollinator populations and enhance pollination services (Wood et al., 2015; Martin et al., 2019). Various studies have indicated that proximity to remnant forests is positively associated with the abundance and diversity of pollinators that visit crop species in agroecosystems (Ricketts et al., 2008). In addition, there is a substantial decline in the species richness of bee pollinators with decreasing temperature (Classen et al., 2015), which could exaggerate pollination deficits in higher elevation farming systems. Thus, there is a need to understand how different landscape elements, vegetation, farm size (number of avocado trees on the farm), agricultural habitats in the surrounding landscape, and elevation influence pollination services.

Conserving pollinator-supporting habitats within farmlands for increasing pollination services is beneficial to agriculture (Carvalheiro et al., 2010). However, the potential of increasing avocado yield and improving quality by supplementing the fields with managed bees outside of large-scale commercial production systems has not been explored. Understanding the extent to which crops depend on pollinators within a specific region and the occurrence of pollination deficits is crucial to provide valuable information to farmers, and to evaluate the current and future conservation plans, and to develop mitigation measures.

This study used manipulative experiments to quantify the pollination deficit in an African crop system and reports, for the first time, the testing of pollinator (managed *A. mellifera)* supplementation in different landscapes to reduce this deficit.

2.2 Materials and methods

2.2.1 Study area

This study was conducted in Murang'a County, situated in the central region of Kenya (Figure 2.1). The area is located at a latitude of 0°43'0" South and a longitude of 37°9'0" East. The county has a total area of 2,559 km^2 and lies between 914 m above sea level (ASL) in the East and 3,353 m ASL along the slopes of the Aberdare Mountains in the West. The average annual rainfall varies from 1,400 to 2,000 mm, and the mean annual temperature is between 18 and 21°C. The main economic activity in the region is agriculture, with an average farm size under large-scale holdings of 6.5 ha (Murang'a County, 2018). The area is one of the essential avocado-growing regions in Kenya. The county is divided into three climatic regions, the western region with an equatorial climate, the central region with a sub-tropical climate, and the eastern region with semi-arid conditions. The western region is generally wet and humid due to the influence of the Aberdares and Mt. Kenya. The eastern region receives less rain and has a higher mean annual temperature. All regions experience a long (March-May) and short (October-November) rainy season. In Murang'a County, there are two avocado flowering seasons in a year. The major one is from August to October, and the minor one is from February to May.

2.2.2 Landscape productivity

The area covering 1630.50 km^2 was classified into three landscape productivity classes based on low, medium, and high net primary productivity according to the normalized difference vegetation index (NDVI), which was used as a proxy for primary productivity. Freely available multi-date Sentinel-2 (S2) satellite data of 10 m spatial resolution was used to create a composite image with images of wet (March to May) and dry (December to February) seasons in the year 2019. Satellite images were processed and analyzed using the Google Earth Engine (GEE) (https://earth engine.google.com, Dublin, Ireland). NDVI was computed as the ratio of the differences between the reflectance at near-infrared (NIR) and red (R) bands and their summation [(NIR−R)/ (NIR + R)]. The NDVI range values of each of three classes (i.e., low, medium, and high) were used as thresholds to re-classify the multi-date NDVI imagery. The NDVI threshold for the low class was −0.425 –0.368, while for the medium and high classes, thresholds were 0.368 –0.611 and 0.611–0.864, respectively (Adan et al., 2021). For calculating the NDVI, both areas holding avocado trees and those with other vegetation were considered.

2.2.3 Land use/land cover (LULC) classification

Land use/land cover (LULC) composition was determined in a 3 km radius area around the experimental farms, based on the foraging distance for bees (Visscher & Seeley, 1982) and included the following classes: annual croplands, avocado, built-up, grasslands, perennial croplands, shrublands, tree cover, and water bodies. The altitude of the farm points was recorded using the global positioning system (GPS). The S2 multi-spectral image was used to determine LULC for its high resolution of 10 m in the visible and near-infrared (VNIR) bands, which comes with three red-edge (RE) bands. A composite image was created in GEE S2 level 1C (images top of atmosphere reflectance). The composite image was used in the calculation of two RE vegetation indices (VIs): RE normalized difference vegetation index (RE−NDVI) and RE enhanced vegetation index (RE–EVI). RE–NDVI and RE–EVI were both derived from the S2 RE band with a 20 m resolution. Thus, resampling was conducted on the respective band to 10 m pixel size. The RE–VIs and reflectance were fused in GEE, and the random forest (RF) algorithm was employed to characterize the landscape. The overall accuracy of the classification was 83.85% with a kappa (total accuracy – random accuracy) / (1 – random accuracy) of 0.7721 (King'ori *et al*., unpublished data).

2.2.4 Experimental design

To evaluate the effect of *A. mellifera* supplementation on fruit set and fruit retention, we selected 36 smallholder avocado farms (0.2 − 0.4 ha). The avocado varieties Hass and Fuerte were dominating, with 21 farms growing Hass, 12 farms growing Fuerte, and three farms growing both varieties. A multi-stage sampling procedure was used to select sub-counties, wards, and small-scale avocado growers. Smallholder avocado farms were selected in different villages to ensure maximized representation of growers in Murang'a County. In each NDVI landscape class (low, medium, and high), the willingness of the farmer to implement pollinator supplementation during the study was considered, and farmers were classified based on the treatment option they preferred (pollinators and control). Several criteria were then applied between each farm depending on the treatment: pollinators (supplemented farms) were at least 1.5 −3.5

km away from each other, and pollinator farms were at least 3.5 km apart from the control sites. In addition, only farms with at least seven avocado trees were selected (Adan et al., 2021). This led to the selection of farms with between seven to 350 avocado trees, typical for African smallholder farms. On farms, all the experimental trees had a minimum distance of 10 m from the edge of the farm in order to minimize the edge effects. Eighteen farmers were selected for each treatment (pollinators or control), with twelve farms per NDVI class, yielding 36 farms. *Apis mellifera* colonies were supplied as additional pollinators in mid-July 2019, just before avocado blooming. The bees were supplied in standard 10-frame Langstroth hives (African Beekeepers Ltd., Nairobi, Kenya) headed by naturally mated queens at a density of two colonies per farm. All manipulative experiments were performed during the major flowering period.

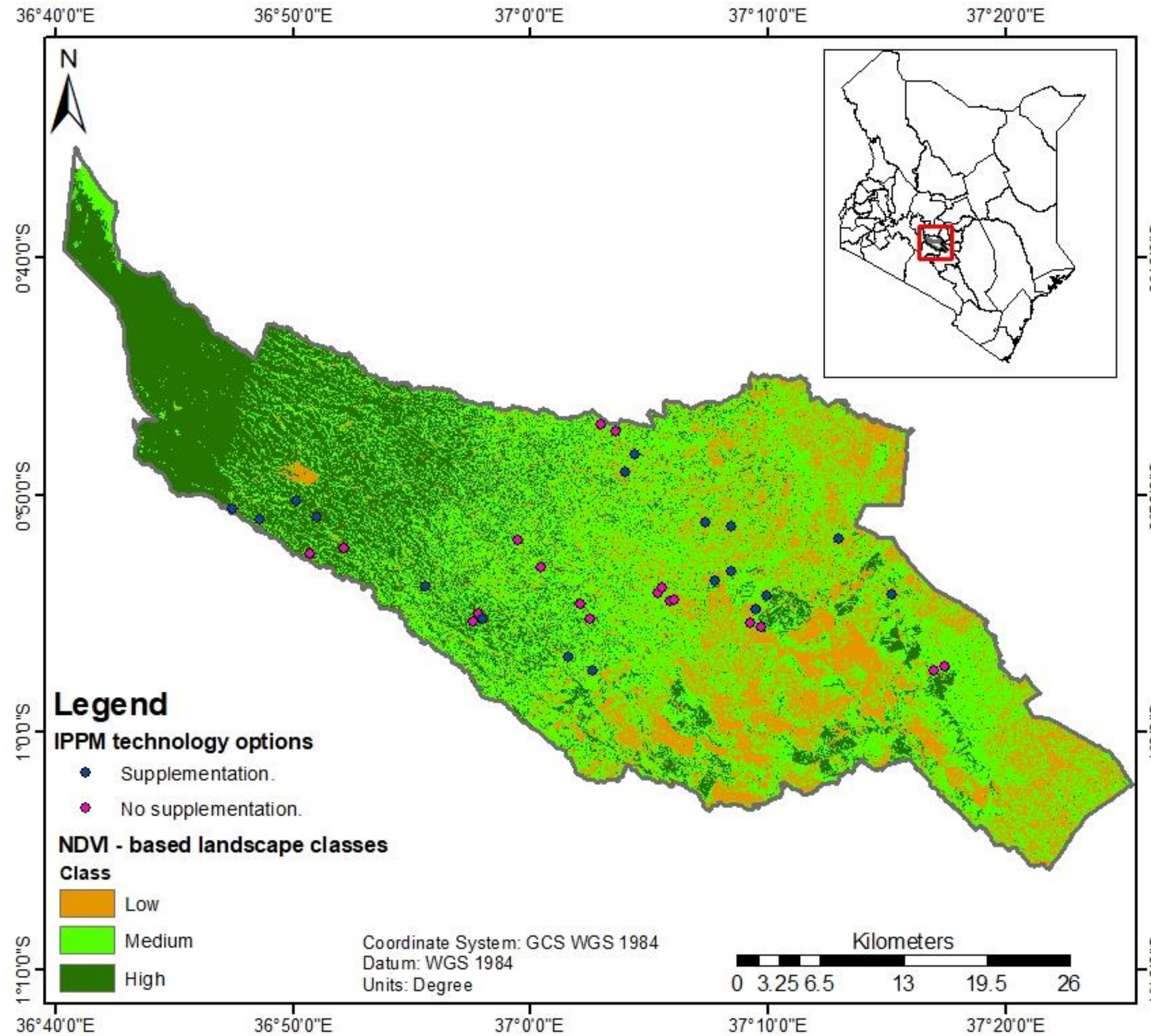

Fig. 2. 1. Map of Murang'a County, Kenya, with the location of the 36 study sites (dots). The color pixels with the map indicate the three levels of normalized difference vegetation index (NDVI), a proxy of the net primary productivity of ecosystems. The blue dots represent avocado farms supplemented with two hives of honey bees per farm while the pink dots represent avocado farms without hives. The inlet figure shows the location of Murang'a County within Kenya (adapted from Adan *et al.*, 2021).

2.2.5 Pollination experiments

Field pollination experiments were conducted from August 2019 to December 2019. At each farm, three trees were randomly selected. Approximately two weeks before flowering, four east-orienting branches (pointing eastwards) on each experimental tree bearing dense and mature flower buds were randomly selected, with a total of 432 branches involved in the experiment. Bags for manipulating flower access were put in place 1 – 6 days before flowering. The four pollination treatments were: i) self-pollination (self), using woven bags that were impermeable to wind, allowing only autogamous self-pollination; ii) wind pollination and spontaneous selfing (self + wind), whereby insects were excluded by coarse mesh gauze with 0.8 –1.0 mm openings; iii) open pollination (open), in which all insects had access to flowers (insect pollination); and iv) hand pollination (hand). For hand pollination, pollen was transferred to stigmas with a paint brush and flowers covered with very fine nylon mesh gauze (10 µm), according to Willmer and Stone (1989). Sticky glue was applied on the branch beneath the bagged flowers to eliminate insect contamination, such as ants in bagged experiments. Flowers were tagged and left undisturbed. Six weeks after the end of the flowering period, bags were removed from flowers, and the number of green fruits per branch was counted for each treatment. In December 2019, two months after the initial fruit set, all fruits retained were counted to assess fruit drops during development.

2.2.6 Data analysis

Generalized linear mixed-effects models (GLMM) as implemented in the R package *lme4* (Bates et al., 2015) were used to investigate the effects of treatments on fruit set, fruit retention, and farm treatment within different landscape classes. We used 'Poisson' distribution in case of count data (i.e., number of fruits) and 'binomial' in case of proportion data (i.e., the proportion of fruits retained after 14 weeks). Pollination treatment was used as a fixed effect to test for differences in the mean number of fruits between open, self + wind, and self-pollination. We tested for a pollination deficit in the control farms (without honey bee supplementation) by comparing if hand-pollinated flowers showed a significantly higher fruit set than open-pollinated flowers. For testing, if the pollination deficit is reduced in farms supplemented with honey bee colonies, we first constructed a model testing for the interactive effect of pollination treatment (hand versus open pollination) and honey bee supplementation (with or without additional honey bee colonies). Second, we analyzed if farms that were supplemented with honey bee colonies exhibited a difference between hand- and open-pollinated flowers in terms of fruit set. If honey bee supplementation reduces the pollination deficit, we would expect a significant interaction term and no significant difference in fruit set in the second model. In all models, we added tree ID nested in farm ID and avocado variety as additive random terms to control for the correlated, hierarchical structure of the data. In the case of over dispersed count data, we added an observation-level random effect to the model (Harrison, 2014). To test if the pollination deficit was dependent on the environment, we added the interaction term pollination treatment (i.e., hand versus open) × environmental variable to the model. We tested five environmental variables, i.e., the influence of elevation (strongly correlated to temperature); the $\log_e$-transformed number of avocado trees on farms (a measure of the farm size); landscape diversity; the proportion of annual crop land in the surrounding landscape; and landscape productivity based on the NDVI classification. Landscape diversity was calculated by applying the Shannon diversity index formula on the proportional contribution of eight land cover types in the study sites' surrounding (within a 3 km distance from the center of the study site). None of the environmental variables were significantly correlated to the farm treatment (supplementation of honey bee colonies or control) (absolute Spearman's rho < 0.35, $P > 0.05$). If the pollination deficit is higher under certain environmental conditions, we would expect the interaction term to be significant.

Regarding the proportion of fruits that were retained on the trees after 14 weeks, we first analyzed which environmental variable significantly explained the probability of fruit retention. We then tested if the effect was modulated by the supplementation of *A. mellifera* colonies on farms by adding an interaction term open × environmental variable (i.e., elevation, the proportion of annual crop land, landscape diversity, the number of avocado trees on farms and the landscape productivity based on the NDVI classification) to the model. All analyses were carried out in R statistical software (v 4.0.2, R Foundation for Statistical Computing, Vienna, Austria).

2.3 Results

2.3.1 Fruit set

Avocado fruit set was significantly higher in the open pollination treatment (89.5%) (mean ± SE fruits produced = 19.89 ± 1.15), where insect pollinators had access to flowers, than in treatments with self + wind pollination (17.4%) (3.71 ± 1.12, $Z = -12.06$, $p < 0.001$) or self-pollination only (6.4%) (1.34 ± 1.17, $Z = -17.36$, $p < 0.001$) (Fig. 2.1).

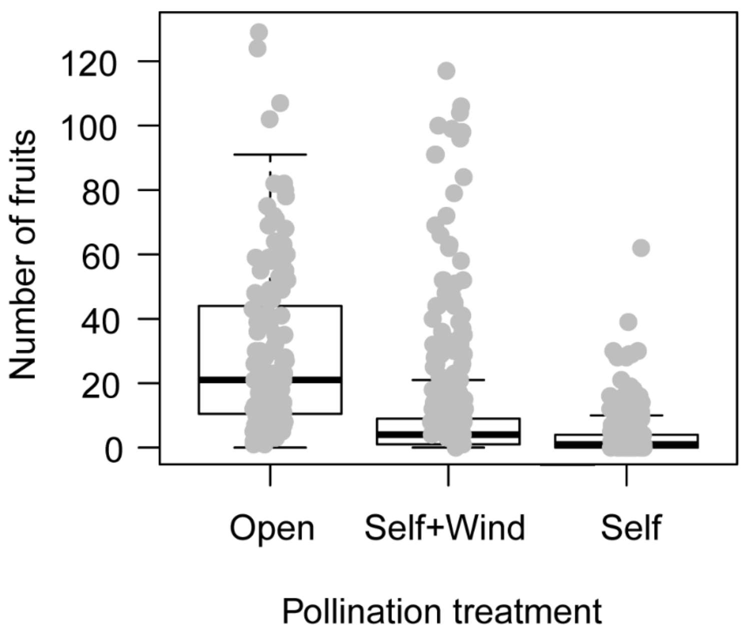

Fig. 2.1. The number of avocado fruits (fruit set) observed in different pollination treatments (open, self + wind, and self-pollination). Dots show the original measures of the number of fruits produced per tree. Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers).

2.3.2 Pollination deficit

On average, there was a 20.7% reduction (Z = -2.749, p = 0.006) in fruit set in avocado trees with open pollination treatment compared with hand pollination (Fig. 2.3A), indicating a pollination deficit. However, the difference between hand and open pollination treatments strongly varied among trees, with some trees even showing a higher fruit set in the open pollination than in the hand pollination treatment (Fig. 2.3A). There was no significant influence of elevation (F = 0.07, p = 0.94), NDVI (F = 0.31, p = 0.73), landscape diversity (F = 0.02, p = 0.88), the proportion of annual croplands (F = 0.00, p = 0.98), and the total number of avocado trees on farms (a measure of the farm size) (F = 0.56, p = 0.46) on the pollination deficit. In farms supplemented with *A. mellifera* colonies, there was no significant difference between the open pollination treatment and the hand pollination treatment (Z = 0.24, p = 0.81) (Fig. 2.3B). A model testing for the interaction between pollination treatments (hand versus open pollination) and the honey bee supplementation treatment revealed a significant interaction effect (Z = 2.06, p = 0.04).

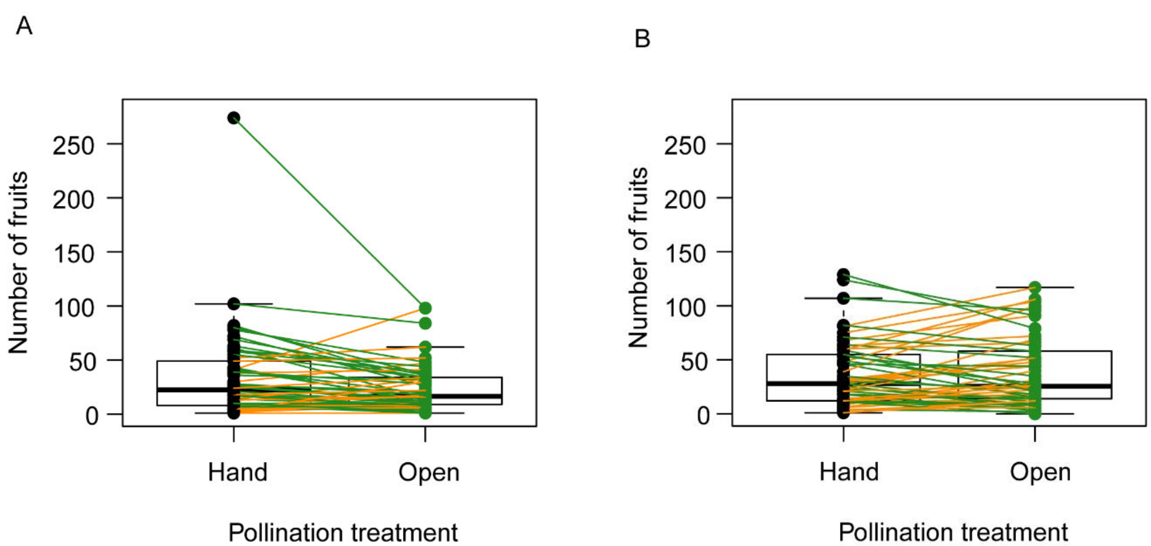

Fig. 2.3. The number of fruits (fruit set) in hand and open pollination treatments on the same tree. Control farms that were not supplemented with honey bees (A). Farms supplemented with two *A. mellifera* colonies per farm (B). Lines connecting the dots between treatments show observations on the same avocado tree. The

green and orange colors indicate lower and higher fruit set, respectively, in open-pollinated flowers in comparison to hand-pollinated flowers. Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers).

2.3.3 Fruit retention rate

We found that the number of fruits counted after six weeks was positively correlated to the number of fruits counted after 14 weeks (Fig. 2.4). On week 14, farms supplemented with honey bees still tended to have a higher fruit set in open-pollinated flowers (Z = 1.90, p = 0.057) than the control farms. Further, the fruit retention rate was dependent on the number of avocado trees on the farm, whereby farms with a higher number of trees (large farm size) were associated with a lower percentage of fruits retained after 14 weeks (Z = –2.10, p = 0.036) (Fig. 2.5). Other environmental variables did not have a significant effect on the fruit retention rate. i.e., elevation, (Z = 1.32, p = 0.19), landscape diversity (Z = – 0.14, p = 0.89), and proportion of agricultural land (Z = –1.71, p = 0.086).

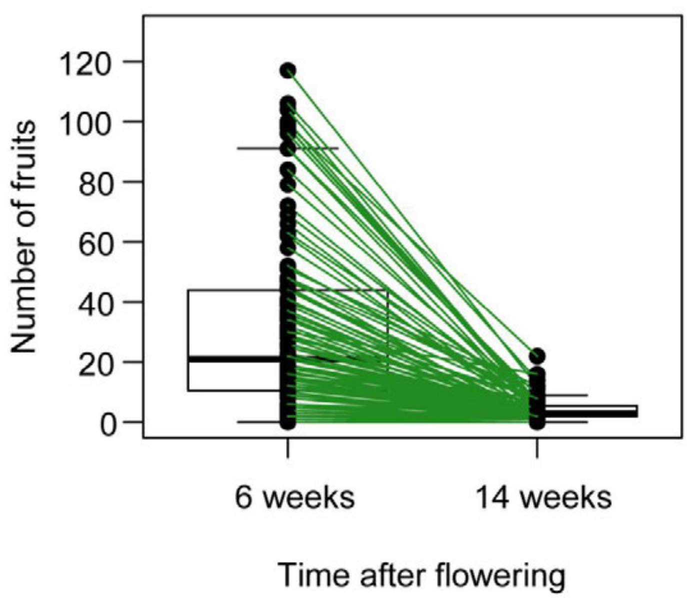

Fig. 2.4. Fruit retention at six and 14 weeks after avocado flowering. The green lines connect observations on the same trees studied.

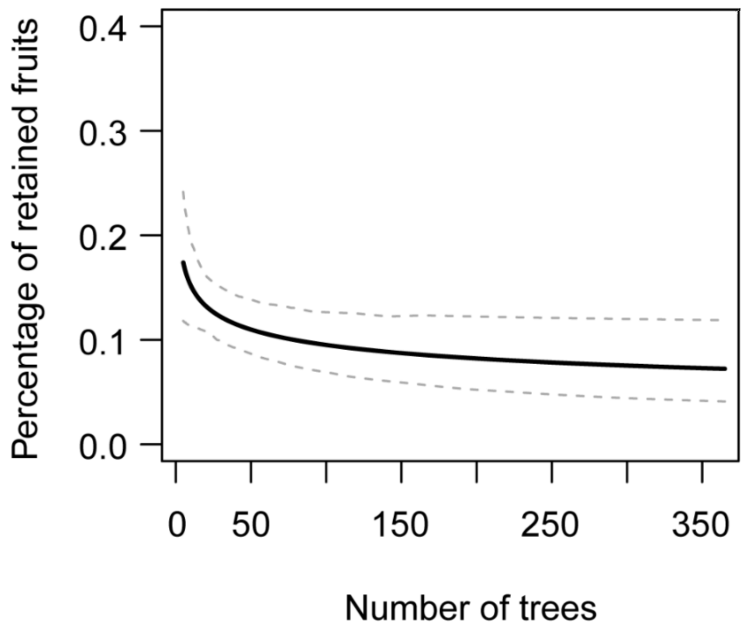

Fig. 2.5. The relationship between the percentages of fruits retained at 14 weeks after flowering and the number of avocado trees per farm. The bold black line indicates the predicted mean fruit retention while the grey upper and lower interrupted lines delimit the 95% confidence interval.

2.4 Discussion

Insect pollination is an essential aspect of crop yield and quality in avocado production. The results from our pollination manipulation experiments show clear evidence of the importance of insect pollinators on avocado fruit set. When pollinators were excluded from the flowers, the initial fruit set (measured six weeks after pollination) was low in the case of self + wind and self-pollination, compared with open pollination in the presence of insect pollinators. Insect pollination has been reported to increase fruit set in avocado significantly (Read et al., 2017). Similar results have been observed by Ish-am (1999) in Mexico, where only a few or no fruit set was reported after caging avocado to prevent pollination by insects. The shaking of flowers could have caused the observed fruit set in self-pollination due to wind motion, gravity (Ish-Am, 2005), or small insects like thrips inside the flowers (Wragg & Johnson, 2011). Even though pollination treatments affected the fruit set aspects of avocado, different unmeasured factors like pruning, mulching, weeding, fertilization regimes, variation in the age of the avocado trees, the availability of mineral elements and nutrients in the soil, pests, and pathogens, weather and other animal interactions in the orchard, may have caused hidden variations in final fruit set levels between open and supplementary hand-pollinated fruits (Samnegård et al., 2019).

In our experiments, we recorded pollination deficits, whereby trees that received supplementary hand-pollination with pollen from other cultivars had a higher fruit set than open pollination treatments. This indicates that the productivity of the studied system is limited by pollinators (Robbertse et al., 1996; Alcaraz & Hormaza, 2009). There was a 20.7% pollination deficit in the orchards without honey bees, indicating that wild pollinators such as tropical African latrine blowfly, drone fly, and polistine wasps (Mulwa et al., 2019; Okello et al., 2021) alone could not attain adequate fruit set and this agrees with our preliminary results (R.S., unpublished data). Our results highlight for the first time that managed pollinator supplementation can resolve pollination deficits in avocado in East Africa. This was evident as farms supplemented with hives did not significantly differ between open and hand pollination. Most individual trees showed an increase in fruit set under open pollination compared to hand pollination, indicating that *A. mellifera* is a required input to ensure sufficient cross-pollination in this agricultural system. *A. mellifera* has been used successfully and almost exclusively for avocado pollination in commercial orchards in which the native pollinators were absent (Davenport, 1998; Ish-am et al., 1999). Our results on smallholder farms imply that the agricultural system is susceptible to a decrease of pollinators and that farmers will experience meager yields if insect pollinators decline in the study area. It is vital to protect wild pollinators effectively and, in the case of pollination limitation, potentially supplement farms with managed pollinators (Rader et al., 2013; Garibaldi et al., 2014) to provide enough and stable pollination services to crops and to fill the short-term pollination deficit.

We expected that the pollination deficit would be smaller on farms characterized by high habitat diversity or structural complexity as they were expected to hold more suitable habitats for pollinators resulting in better pollination services (Dainese et al., 2019). However, there was no significant effect of the landscape variables on the pollination deficit. Study sites characterized by high NDVI class, higher landscape diversity, a lower percentage of agricultural habitat in the surrounding landscape, and smaller farms (with fewer avocado trees) did not differ in the pollination deficit from farms characterized by higher habitat diversity or structural complexity. This may have been attributed to other factors like landscape homogenization which has been reported to reduce the abundance and diversity of many taxa due to low foraging resources and lack of nesting site (Tscharntke et al., 2012).

Additionally, we expected that avocado grown in high elevations (strongly correlated to temperature) to be more pollinator-limited than those at low elevations because hymenopterans are known to be dominant pollinators in low-elevation areas (Marini et al., 2012). We failed to detect a significant elevation effect due to differences in the geographical region, climatic zone, and wide range of elevational gradient. A study conducted by Senapathi et al. (2017) showed that landscape quality could directly impact pollinator communities and influence abundance and richness through the interaction of multiple drivers such as climate change or increased chemical inputs in land management.

In our study, we observed an early abscission pattern across all the farms. However, less abscission was observed on farms that were supplemented with *A. mellifera* even after two months from the initial fruit set. This suggests that other factors besides lack of pollination contribute to the massive drop of avocado flowers and fruitlets. Among the environmental variables tested, we found that the number of avocado trees in the farm negatively influenced fruit retention, whereby farms with fewer avocado trees showed a higher percentage of fruit retention, than with farms with a high number of trees. This may be associated with reduced resource competition between plants over soil nutrients on smaller farms, better pest control from parasitoids and predators on smaller farms, or a different avocado age structure for smaller farms (Bennett, 2010; Cameron et al., 2007). Trophic interactions within the soil can influence the aboveground community of plants, which may include fruit retention (Cheng & Gershenson, 2007).

2.5 Conclusion

This study shows the existence of pollination deficits in Kenyan smallholder avocado farms that can be resolved by pollinator supplementation. This knowledge can be used to increase avocado production in farms managed by smallholder farmers, which represent most producers in African agriculture. Our results also confirm that bee pollination plays a vital role in avocado production, thus promoting wild colonies of *A. mellifera*, which constitute more than 90% of all colonies in Africa, can be an excellent strategy to ensure enough avocado pollination and hence improved high-quality yields. Notably, protecting or restoring natural habitats in agricultural landscapes could support native pollinator communities and reduce the dependence of avocado pollination on managed honeybees. This would reduce costs for the management of colonies and enhance system resilience. The number of avocado trees on a farm affected fruit retention. Therefore, enhanced fruit retention could be achieved by improved soil management, mixed cropping with other legume crops, and optimizing the number of trees per farm.

Chapter 3

Insect pollination enhances fruit weight, quality, and marketability of avocado (*Persea americana*).

Summary

Avocado is a pollinator-dependent crop rich in fiber, monounsaturated oils, vitamins, and minerals, which is seeing an increase in global demand. Some studies have shown that insect pollination improves avocado fruit set, but it is still unclear if pollinators also enhance fruit quality and the nutritional profile, particularly oils. This study aimed to quantify the contribution of insect pollination to fruit and seed weight, and oil, protein, carbohydrate and phytochemical (flavonoids and phenolics) contents, and assess if the supplementation of pollinators can improve these fruit parameters. The experiment was conducted in 36 smallholder avocado farms where 18 farms were each supplemented with two *Apis mellifera* bee hives while 18 were controls. Four manipulative pollination treatments were conducted: hand, open, self- and wind pollination. We observed that avocado fruit weight was significantly higher (213.7 g) when insects pollinated flowers than wind- (107.8 g) or self-pollination (95.1 g). Heavier fruits (grade 18) are the most preferred in the international market and hence enhance marketability. Furthermore, insect pollination resulted in heavier seeds (29.5 g) as well as higher oil contents, clearly showing that insect pollination is required to reach a high seed yield and quantity of oil. Honey bee supplementation on farms enhanced the avocado fruit weight by 18%, hence the fruits falling under (grade 16) which is also preferred in the international market and increased avocado oil content by 3.6%. By contrast, insect pollination did not influence other assayed fruit quality parameters (protein, carbohydrates and phytochemicals). This study highlights the importance of insect pollination to avocado fruit and nutritional quality (oils), and underscores the value of bee hive supplementation to achieve high-quality avocado fruits.

Keywords: crop pollination, crop yield, ecosystem service, fruit weight, nutritional proximate, pollination deficit.

3.1 Introduction

Pollination is crucial for global food security and human nutrition, contributing to approximately 35% of world food crop production (Klein et al., 2007, Chaplin-Kramer et al., 2014). However, there is increased evidence of pollinator declines for both managed and wild insect pollinators (Potts et al., 2010; Cameron et al., 2011; Dicks et al 2021), which is putting pollination-dependent crops at great risk (Dainese et al. 2019). Pollination-dependent crops such as fruits and vegetables form a substantial component of human food and contribute significantly to a healthy diet by supplying essential nutrients such as vitamins, antioxidants and fiber, which help to reduce micronutrient deficiencies (Eilers et al., 2011). There is lack of sufficient nutrient supply especially in sub-Saharan Africa (Bain et al., 2013). Hence, nutrient supply is not a matter of production quantities alone, but rather depends on the quality of agricultural products, which has become a major challenge. Several factors such as soil quality, pests and diseases, and climatic conditions affect quality in crop production (Liliane and Charles, 2020).

Some studies have shown that decreased yield losses emerge from fruit deformations and undeveloped fruits caused by pollination deficits, adversely affecting the marketability of fruits (Ariza et al., 2011). Few studies have been conducted on the effect of pollination on fruit quality parameters. For instance, pollination increased fruit weight and shelf life in strawberries (*Fragaria ananassa)* (Klatt et al., 2013), while in oilseed rape (*Brassica napus*), pollination enhanced seed quality (heavier seeds) and oil content but lowered chlorophyll content (Bommarco et al., 2012). Pollination also influenced the nutritional composition in almonds (*Prunus dulcis)* whereby fat and vitamin E content of the nuts were highly increased by pollination (Brittain et al, 2014). Furthermore, effective pollination was found to decrease malformations in apples (*Malus domestica*) and strawberries (*Fragaria ananassa)* (Matsumoto et al., 2012; Wietzke et al., 2018), whereas in oriental melon (*Cucumis melo)*, pollination was found to increase flavor-enhancing elements such as sugars and acids (Shin et al., 2007). However, there is scanty literature on the effect of insect pollination on avocado fruit quality and nutritional composition.

Avocado is a unique fruit with high nutritional value. It is a rich source of monounsaturated oleic acid, and it contains important lipid-soluble antioxidants and high levels of phytochemicals such as carotenoids, polyphenols, chlorophylls, tocopherols and phytosterols (Pieterse, 2003; Duarte et al., 2017). The oil content in avocado is a critical quality attribute which affects market acceptance both for industrial and culinary use. Oil content is also used as an index of maturity in avocado fruit. Currently, there is increasing evidence of the health benefits of avocado, which has led to increased production and consumption (Bhuyan et al., 2019). Consumption of avocado fruits aids in promoting cardiovascular health, weight management and healthy aging (Carvajal-Zarrabal et al., 2014; Weschenfelder et al., 2015; Johnny et al., 2019). However, several factors affect avocado production and quality, among them pests and diseases, poor soils, and pollination deficits (Alcaraz & Hormaza, 2009). Avocado requires insects for successful pollination with honey bees being the most common pollinators (Evans et al., 2011). Other potential avocado pollinators include the hoverfly (*Phytomia incisa*), tropical African latrine blowfly (*Chrysomya putoria*), and polistine wasps (*Polistes* sp.) (Mulwa et al., 2019; Okello et al., 2021).

Fruit size and weight are among the most important quality parameters for market evaluation and consumer preference of avocado fruits (Migliore et al., 2018). During the packaging and marketing process, avocados are classified into different grades based on size. For instance, according to OECD and UNECE standards for avocados (OECD 2011; UNECE 2017) and Codex Alimentarius Standards for avocados (CODEX STAN 197-1995), market grading of fresh fruits is classified according to size codes 1 to 30 with a minimum weight of 80 g for avocados of variety Hass. In Europe, the preferred size for Hass avocados is 16 (227 to 274 g) to 18 (203 to 243 g) and for the variety Fuerte, 14 (258 to 313 g) to 16 (227 to 274 g), with bigger fruits generally generating higher market values.

Some studies have been conducted on the dependence of avocado fruit quality on pollination (Can-Alonzo et al., 2005; Altendorf, 2019). Nevertheless, major knowledge gaps exist linking pollination services to fruit quality aspects, which in turn have an economic impact on avocado production. Thus, in-depth knowledge of fruit quality parameters is essential to deliver avocado fruit of excellent quality to the global market. The aim of this study was to quantify the dependence of insect pollination on the avocado fruit quality, specifically the fruit size, proximate composition, and phytochemical contents. Additionally, we assessed if honey bee supplementation would influence fruit quality parameters, nutritional composition, and phytochemical contents.

3.2 Materials and methods

3.2.1 Study site

The study was carried out in smallholder avocado farms in Murang'a County, Kenya (S 0°43'0", E 37°9'0"; Fig. 3.1) during August − September 2019. We selected 36 smallholder avocado farms (0.2 − 0.4 ha) for our study. Half of the farms (18) were selected for pollinator supplementation with two colonies of the *A. mellifera* per farm and the other half (18) were the non-supplemented controls. The treatment option (pollinators and control) depended on the farmer's willingness to implement (Adan et al 2021). To avoid overlapping, the farms with pollination supplementation were at least 1.5 − 3.5 km away from each other informed by the foraging range of the honey bee (Hagler et al., 2011) and at least 3.5 km apart from the control farms. The *A. mellifera* colonies were introduced just before avocado blooming. The experiment included the two dominantly grown avocado varieties, Fuerte and Hass.

3.2.2 Pollination treatments

At each farm, three trees were randomly selected. The trees had a minimum distance of 10 m from the edge of the farm to minimize the edge effects. On each tree, four east-orienting branches (pointing eastwards) at a similar height were randomly selected, leading to a total of 432 branches for the experiment. Bags for manipulating flower access were put in place 1 – 6 days before flowering. Four manipulative pollination experiments were conducted: i) self-pollination (self), using woven bags that were impermeable to wind, allowing only autogamous self-pollination; ii) wind pollination and spontaneous selfing (self + wind), whereby insects were excluded by coarse mesh gauze with 0.8 –1.0

mm openings; iii) open pollination (open), in which all insects had access to flowers (insect pollination); and iv) hand pollination (hand), pollen was transferred to stigmas with a paint brush and flowers covered with very fine nylon mesh gauze (10 μm), according to Willmer and Stone (1989) as a positive control for optimal pollen transfer.

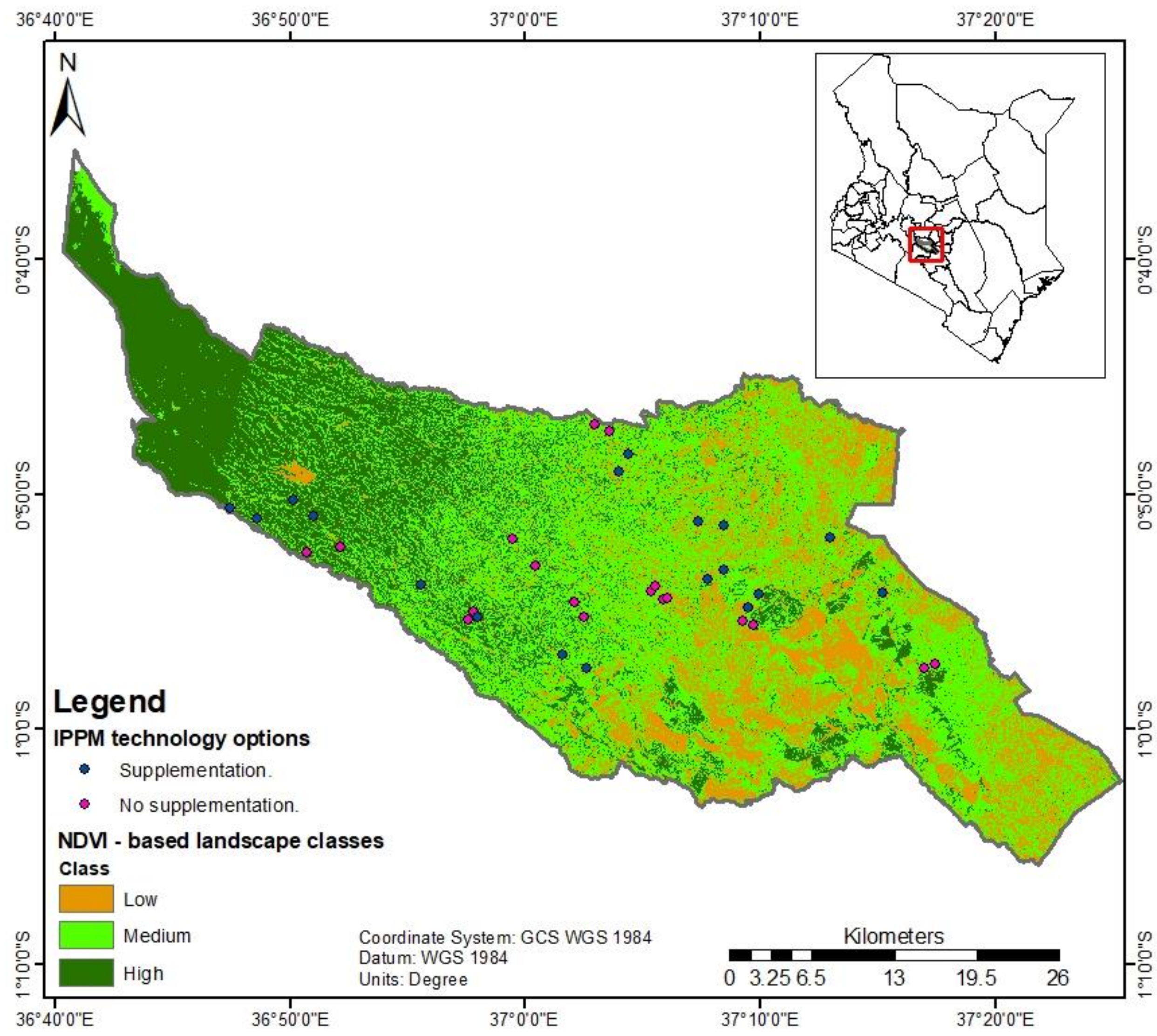

Fig. 3.1. Map of Murang'a County, Kenya, with the location of the 36 study sites (dots). The color pixels with the map indicate the three levels of normalized difference vegetation index (NDVI), a proxy of the net primary productivity of ecosystems. The blue dots represent avocado farms supplemented with two hives of honey bees

per farm while the pink dots represent avocado farms without hives. The inlet figure shows the location of Murang'a County within Kenya (adapted from Adan et al., 2021).

3.2.3 Fruit physical quality parameters

Size and weight

All avocado fruits from experimental inflorescences were harvested two weeks before commercial harvest from each of the farms (late April to early May 2020). Avocados were labelled and bagged individually according to the pollination treatment, tree and farm number, and then transported to the laboratory at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya, for quality assessment. Within 24 h of harvest, we ensured that all physical quality measures had been taken since the fruit moisture content decreases over time. Quality measures included fruit fresh weight taken on an analytical weighing balance sensitive to the nearest 0.0001 g (Mettler Toledo MS403TS/00, Scales Plus LLC, Michigan, United States), maximum width, measured using a tape measure, and height using calipers sensitive to 0.05 mm. After seven days of ripening of the fruits at room temperature, the avocado flesh was removed, and the seed weight was measured.

3.2.4 Fruit nutritional and phytochemical quality parameters

Preparation of avocado fruits (samples)

Seven fruits were sampled from each of four control and supplemented farms, these farms were chosen because they contained most of the fruits from all the pollination treatments during the harvesting stage, except from one supplemented farm and one control farm where either six and eight fruits, respectively, were sampled. Hence, in total 57 fruits were sampled for fruit nutritional and phytochemical quality parameters (30 fruits from four control farms and, 27 fruits from four supplemented farms). Avocado fruit samples were left to ripen naturally in the dark at room temperature (23 – 25℃), for seven days. Each avocado fruit was peeled, and its flesh was cut into small unform pieces that were dried for 12 h at 60℃

using an oven (Memmert AtmoControl, Memmert GmbH, Schwabach, Germany). The dry flesh was then grinded into fine powder and homogenized using a mixer grinder (Philips HL7756/00 Mixer grinder, Philips &Co, Eindhoven, Netherlands).

Moisture content

The moisture content was calculated from the difference in mass between the initial and dried sample as follows:

$$Moisture\ content\ \% \ =\ (wet\ weight - dry\ weight)/wet\ weight\ x\ 100.$$

Oil extraction

From each fruit sample, in three replications, 2 g of avocado powder were placed into a 15 mL falcon tube, then 10 mL of hexane was added and mixed rigorously using a vortex (Vortex-Genie 2 Mixer, Scientific industries, New York, USA) at 2,500 rpm for 5 min. The mixture was filtered using a 0.45 μm microfilter before concentrating the resulting filtrate (extracted lipids) using a concentrator (Eppendorf Concentrator plus, Merck, Hamburg, Germany). The lipid content in the sample was determined by weight difference:

$$Oil\ content\ (\%) = amount\ of\ oil\ extracted (g)/weight\ of\ original\ sample\ (g) \times 100$$

Total carbohydrate content

From each fruit sample, 0.1 g of avocado powder were mixed with 5 mL of 2.5 M hydrochloric acid (HCl) and placed in a boiling water bath for 1 h. Sodium carbonate (Na_2CO_3) was added until effervescence stopped; then 10 mL of distilled water were added. This solution was centrifuged at 4,200 rpm for 5 min. From the solution, 1 mL was placed into a 20 mL test tube, before 1 mL of phenol solution (50% w/v) was added followed by 5 mL of concentrated sulphuric acid (H_2SO_4) in a fume hood. Subsequently, 500 μL of solution was removed into 20 mL test tube and diluted with 3 mL of water. The absorbance was measured at 490 nm against a blank (all reagent minus the sample). The calibration curve was made using a glucose standard at a range of (0 – 0.1 μg mL^{-1}) using five data points that were generated in replicates. This assessment was replicated three times.

Total protein determination test

The total protein (TP) content was determined by the Bradford method with minor modifications (Bradford, 1976), with three replicates. To 0.3 mL avocado fruit solution (5 mg/mL in Tris/EDTA buffer; 15.76 g of Tris and 2.92 g of EDTA in 1 L), 5 mL of Bradford reagent (100 mg of Brilliant Blue G-250 dye, 50 mL of absolute ethanol and 100 mL of 85% phosphoric acid, made up to in 1 L with distilled water) was added. After 2 min, the absorbance was measured at 595 nm against the blank (the reactive solution without the sample) using a spectrophotometer (6850 Jenway, Kobian, Nairobi, Kenya). Bovine serum albumin (BSA) was used to generate the calibration curve (0 –300 μg mL^{-1}). The TP content was calculated and expressed as g of BSA equivalent (E)/100 g or %.

Total flavonoid content

Total flavonoid content (TFC) was determined using a colorimetric method adapted by Zhishen, *et al.* (1999) with minor modifications. Avocado fruit sample (1 g) was diluted with 10 mL of 50% (v/v) methanol. One mL this solution was then mixed with 4 mL distilled water. At the baseline, 0.3 mL of 5% (w/v) $NaNO_2$ was added. After 5 min, 0.3 mL of 10% (w/v) $AlCl_3$ was added, followed by addition of 2 mL 1 M NaOH 6 min later. The final volume of 10 mL was achieved by the addition of 2.4 mL distilled water. The mixture was vortexed to ensure adequate mixing and the absorbance was read at 510 nm. A calibration curve was created using a standard solution of quercetin done in triplicates in the range of (10 – 200 μg mL^{-1}) with five data points. The result was expressed as mg quercetin equivalents (QE)/100 g. This experiment was replicated three times.

Determination of total phenolic content

Total phenolic content was evaluated by the Folin-Cioalteu method as described by *Singleton et al.* (1999) with minor modifications. One g of avocado sample was diluted with 10 mL of 50% methanol, then 1 mL of the solution was mixed with 5 mL of 0.2 N Folin-Ciocalteu reagent for 5 min. After adding 4 mL of 75 g/L sodium carbonate, the mixture was incubated at room temperature for 2 h, then the absorbance of the reaction mixture was measured at 760 nm against a water blank. Gallic acid was used as standard to yield the calibration curve using five data points done in triplicates in the range of (0 – 250 μg mL^{-1}). The total phenolic content was expressed as mg of gallic acid equivalent (mg GAE)/100 g) and this experiment was replicated three times.

3.2.5 Statistical analysis

Linear mixed effect models (LMM) implemented in the lmer function of the *nlme* package (Pinheiro *et al*., 2016) were used to analyze the effects of pollination treatments on the avocado fruit quality (weight, width, and height). Pollination treatment was used as a fixed effect to test for differences in the mean weight of fruits between hand, open, self + wind, and self-pollination. Farm ID and variety were used as random terms. In the control farms (without honey bee supplementation) we compared if there was a significant difference in fruit weight between hand-pollinated and open-pollinated flowers. Furthermore, we analyzed if farms supplemented with honey bee colonies, enhanced fruit weight by comparing hand versus open pollination. The overall effect of pollination treatment on the nutritional quality was assessed using analysis of variance (ANOVA). Pairwise comparison of means were performed using the *lsmeans* package (Lenth, 2016) and with the Tukey method for adjustment of p- values. The differences were summarized using *multcompView* package (Graves *et al*., 2019).

Marketability of fruits for open pollination and pollinator exclusion treatments was evaluated using the market grading classification of UCECE standards for avocados (2017) and Codex Alimentarius Standards for avocado. The economic benefits of pollination services were estimated by comparing the fruit quality in open pollination with pollinator exclusion treatments. The yield gap loss was calculated as the difference between the mean fruit weight in open and self + wind pollination treatments.

All analyses were carried out in R statistical software (v 4.0.2, R Foundation for Statistical Computing, Vienna, Austria).

3.3 Results

3.3.1 Size and weight of avocado fruits

The exclusion of pollinators had a significant effect on the avocado fruit quality parameters (Fig. 3.2). The mean weight of avocado fruits in the open flower treatment (mean ± SE fruit weight = 213.7 ± 7.4 g) was significantly higher ($Z = 4.26$, $p < 0.001$; $Z = 8.63$, $p < 0.001$) than in treatments where pollinators were excluded from flowers [self + wind pollination (107.8 ± 31.8 g) and self-pollination (95.1 ± 27.2 g), respectively]. The same relationship was found in avocado seeds whereby open pollination treatment had significantly larger seeds (mean ± SE seed weight = 29.5 ± 1.6 g) than seeds from pollinator exclusion treatments; self + wind pollination (14.7 ± 3.9 g) and self-pollination only (13.3 ± 3.8 g) (Fig.3.2). No significant differences of avocado fruit weight in control farms were observed between hand and open pollination ($Z = -2.07$, $p = 0.17$) (Fig.3.3) as well as between self and self + wind pollination treatments ($Z = 0.91$, $p = 0.80$). On the contrary, there was a significant difference of avocado fruit weight in supplemented farms between hand and open pollination ($Z = -2.65$, $p = 0.047$) (Fig.3.3).

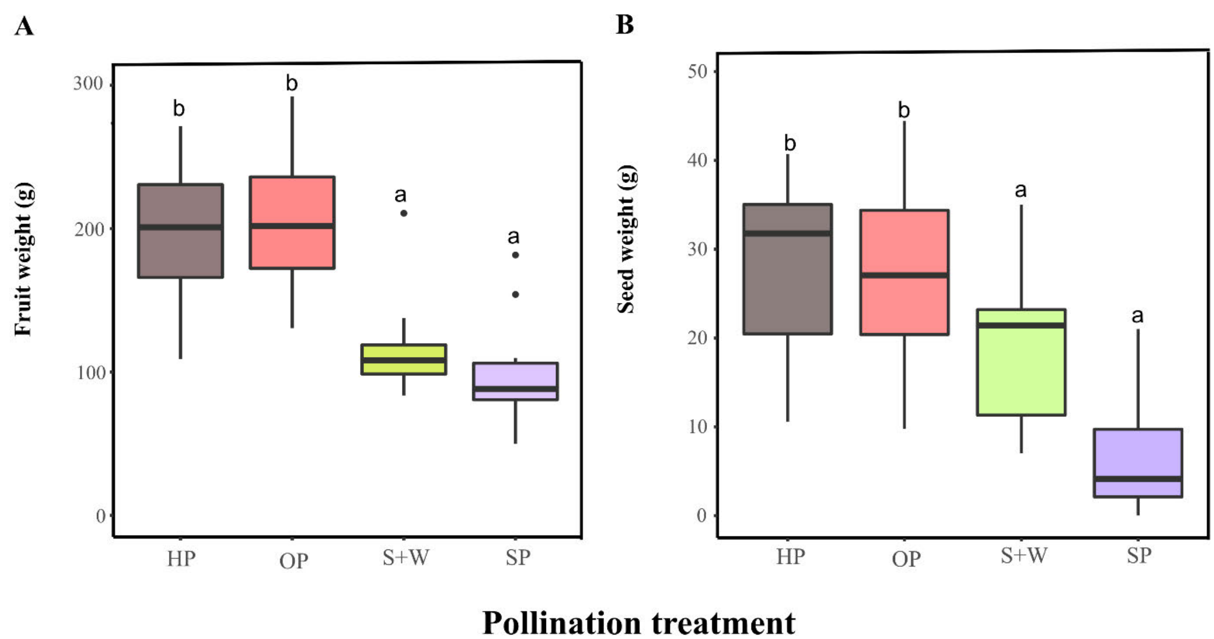

Fig. 3.2. Boxplot showing the effect of pollination treatments (hand-, open-, self + wind-, and self-pollination) on two avocado fruit parameters: (A) fruit weight and (B) seed weight. Means with different letters are significantly different in pairwise comparisons (Tukey's HSD, $P < 0.05$). Dots show the outliers. Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers).

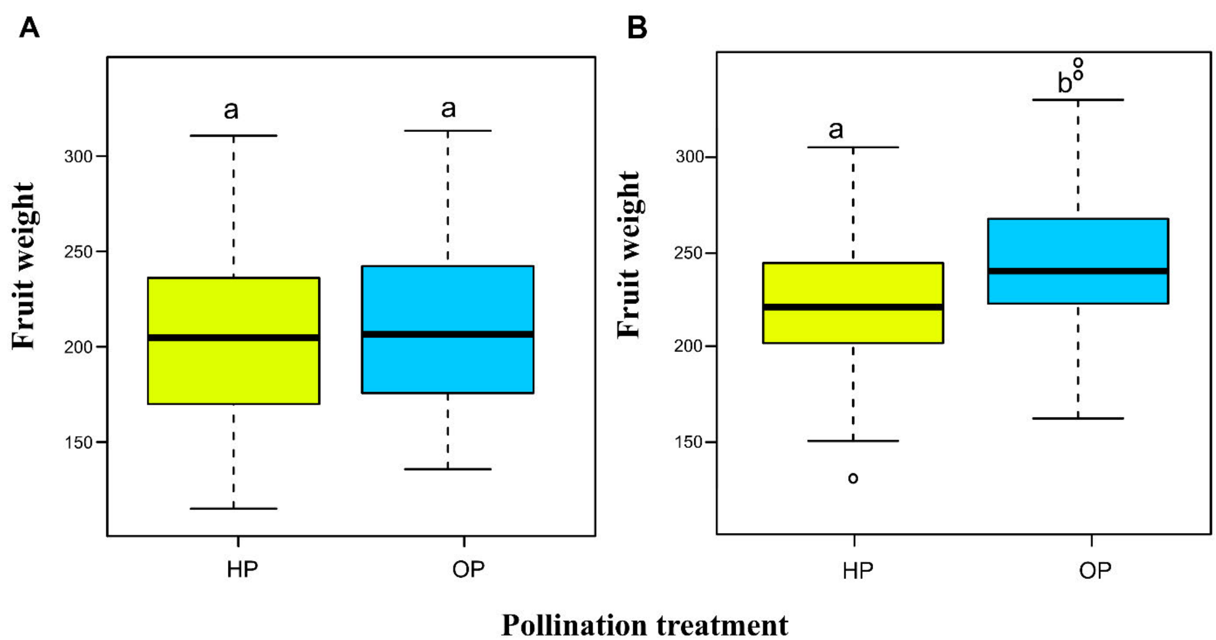

Fig. 3.3. Fruit weight observed in hand (HP) and open (OP) pollination treatments. Control farms that were not supplemented with honey bees (**A**). Farms supplemented with two *A. mellifera* colonies per farm (**B**). Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers).

3.3.2 Avocado nutritional quality

The exclusion of pollinators affected the oil concentration of avocado. (3.S.1, Table 3.1). There was a significant difference in oil concentration in the control farms between open and self + wind pollination ($Z = 4.01$, $p = 0.003$) but no significant effect was observed between open and self-pollination ($Z = 1.08$, $p = 0.70$), hand and open ($Z = 0.44$, $p = 0.97$), and between self and self + wind pollination ($Z = 2.33$, $p = 0.12$) (Fig. 3). The oil concentration was highest in both hand (40.1 ± 1.7 %) and open (38.1 ± 1.9 %) pollination treatments followed by self- (33 ± 6.02 %) and was least in self + wind (20.7 ± 4.3 %) pollination. There was no evidence that insect pollination affected other nutritional parameters; protein ($F= 0.17$, $p = 0.69$), carbohydrates ($F= 0.54$, $p = 0.48$), moisture ($F= 2.08$, $p = 0.19$), flavonoid ($F= 0.18$, $p =0.68$) and phenolic ($F= 3.3$, $p = 0.1$) contents (3.S.1, Table 3.1). Honey bee supplementation increased oil concentration by 3.6% (mean ± SE oil concentration = 41.7 ± 1.9 %), although the increment was not significant ($F=1.56$, $p =0.23$) when compared with control farms (mean ± SE oil concentration = 38.1 ± 1.9 %) in the open treatments. In addition, there was very little variation in the oil content between the two avocado cultivars ($Z = -0.51$, $P = 0.61$), where the average oil percentage in Hass cultivar was 36.0% while it was 34.0% in Fuerte. The avocado moisture and lipid contents were negatively correlated and only marginally significant ($R = -0.24$, $P = 0.08$).

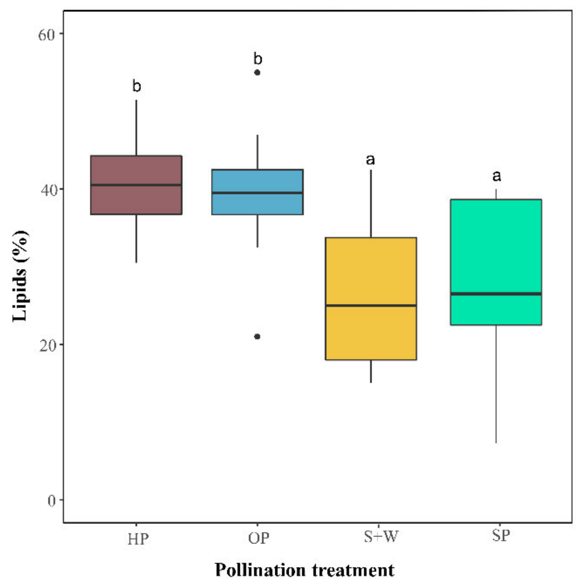

Fig. 3.3. Boxplot showing the effect of pollination treatments (hand-, open-, self + wind-, and self-pollination) on avocado oil. Means with different letters are significantly different (Tukey's HSD, $P < 0.05$). Box and whisker plots show the median (bold line), the quartiles (box) and the extreme values (whiskers).

3.3.3 Economic valuation of pollination services

Avocado fruits from open pollination treatments (mean ± SE fruit weight = 213.7 ± 7.4 g) and hand pollination (200.3 ± 9.8 g) were heavier compared to self + wind (107.8 ± 31.8 g) and self-pollination (95.2 ± 27.2 g). Therefore, fruits from open pollination fall under grade 18 (203-243 g) and those from hand pollination under grade 20 (184-217 g), which are the most preferred market grade for international market according to UNECE standards for avocados and Codex Alimentarius Standards for avocados (UNECE 2017; CODEX STAN 197-1995). While self + wind and self-pollination fall under grade S (less than 123), which are less preferred in the international market. With honey bee supplementation, the mean fruit weight was 253.0 g, which corresponds to grade 16 (227-274 g) according to the UNECE standards for avocados (UNECE 2017). Grade 16 is also a top preferred market grade in the international market.

The honey bee supplementation revealed a significant economic impact to avocado producers. Our findings indicated that by introducing honey bee colonies to avocado orchards there was a total yield increment of 18%. Utilizing the method employed by Gallai et al. (2009) to evaluate the economic value of pollination, we are able to determine the surplus obtained by smallholder farmers due to the implementation of honey bee colonies on their farms. We focus exclusively on the benefit due to increased pollination services not taking the production of beehive products into account. We assume based on the data derived on smallholder farms in Murang'a County that the average farm has ten avocado trees, which produce 280 fruits of a total weight of 61.7 kg per tree (Toukem et al., 2021; Sagwe et al., 2021, unpublished data). The production costs of 1 tonne avocado were USD 217.40 (FAOSTAT, 2021). This translates into USD 134.14 of production costs for the whole average smallholder farm (10 trees each 61.7 kg, total of 617 kg). Due to a pollination dependency of 0.4 – 0.9 (mean 0.65) according to Klein et al. (2007) or 0.72 on the smallholder farms in Murang'a county (Sagwe et al., 2021) the total economic value added through pollination is USD 87.19 (pollination dependency (PD) of 0.65) or USD 96.58 (PD 0.72).

Implementing two honey bee colonies on a farm will lead to an increase in fruit weight by 18% which translates to USD 102.88 (PD 0.65) or USD 113.96 (PD 0.72). Due to the implementation of bee colonies farmers might incur a cost of around USD 140 (two hives, bee suite, smoker, and hive tool), while the increase in production costs is at USD 24.14. The benefit through sales depends on the market access (local vs export) and many other factors. Overall, pollination benefits might be much larger as smallholder farms usually plant several different commodities and beehive products can also contribute to additional income. Furthermore, supplementation of honey bees lead to 3.6% increase in oil concentration.

3.4 Discussion

The present study showed that avocado fruit physical parameters were significantly affected by pollination treatments, where insect pollination of avocado flowers increased fruit weights and lipid content significantly. Furthermore, the supplementation of avocado farms with honey bee colonies increased the fruit weight significantly compared to hand and open pollination.

Fruits from open and hand pollination treatments, showed an increase in avocado physical quality through increased fruit and seed weight compared to fruits from pollination exclusion treatments (self- and self + wind pollination), thereby improving the market value. These results confirm findings of other authors, whereby Mulwa et al. (2019) reported a significantly higher fruit yield and larger seeds from unbagged than bagged treatments. Moreover, Ish-Am & Lahav (2011) found that wind contributed only slightly to avocado yield and strongly recommended the use of honey bees for pollination. The low yield observed in self- and self + wind pollination treatments was perhaps a result of lower fertilization success. This result implies that avocado farmers will be experiencing reduced crop yields if insect pollinators are not present. Because avocado is a major horticultural fruit currently making a very significant contribution to the Kenyan income and revenue (Amare et al., 2019), significant yield and income gains could be made by farmers through improved pollinator management.

Further, our results clearly showed that insect pollination contributes significantly to high seed quality (weight and size). Similar results have been reported by Mulwa et al. (2019) in avocado seeds whereby insect pollination treatments produced heavier seed than pollinator exclusion treatments. The seed size has an indication of high seed germination rate to growers. For example, a study conducted by Olorunmaiye et al. (2011) in mango seeds found that heavy seeds produced a greater number of seedlings, seedlings with high vegetation structure, and dry matter accumulation. Moreover, Massini (2018) indicated that large and medium seed-size of barley classes had a significantly higher standard of germination percentages than small size classes. Seed size is an important indicator of physiological quality which affect seed germination and seedling growth especially under stressful conditions (Steiner et al., 2019). This data also supports our early findings showing that insect pollination increases fruit retention since non-viable cause fruit abortion (Sagwe et al., 2021). Further, seed size has been reported to affect crop development and performance in the field (Adebisi et al., 2013). A possible explanation for this could be caused by higher food and other energy reserves, which are essential factors to improve the expression of germination and initial growth of seedlings (Shahi et al., 2015). Therefore, for successful seed germination and crop performance seed size play an important role.

Despite the effect of pollination in enhancing the quality of avocado fruits, other agronomic factors such as irrigation and resource availability influence the general health of the tree which in turn have a greater influence on fruit size (Kremer-Köhne & Köhne, 1995). Besides, it is evident that increasing cross-pollination in orchards needs to be considered because it affected some quality aspects of avocado. To effectively increase cross-pollination in the orchards there is need for high species richness and abundance of pollinators (Samnegård et al., 2019).

In the present study, oil concentration was significantly affected by pollination treatments whereby hand and open pollination treatments had higher oil content compared to pollinator excluded treatments. In addition, the farms that were supplemented with honey bees increased oil concentration by 3.6% although the oil concentration did not differ significantly with the control farms. Similar effects have been reported in other crops, for example, oilseed rape had higher oil and lower chlorophyll contents when adequately pollinated (Bartomeus, 2014). Another study conducted by Fries & Stark (1983) in oilseed rape production indicated that honey bees increased the percentage of oil content of seed. Additionally, Silva et al. (2018) showed that bee pollination in sunflower enhanced unsaturated fatty acids by 0.3 %. Oil in avocado is the most outstanding quality parameter linked to the market price, it also contains healthy fatty acids, which exert many cardiovascular benefits (Forero-Doria et al., 2017). Apart from oil content, none of the nutritional proximate was affected by pollination treatments. Very small quantities of carbohydrates were reported in our study, an indication that avocado has low sugar content making it more beneficial for people with diabetes (Del Toro-Equihua et al., 2016; Tramontin et al., 2020). Both protein and phytochemicals play an essential role in the human diet. For instance, phytochemicals play an important role in cancer prevention, and in scavenging free radicals that have been reported as the root cause of many pathological conditions (Manaf et al., 2018). The oil content was negatively correlated with the moisture content, which was in agreement with other studies such as (Lee et al., 1983; Osuna-García et al., 2010).

Our results confirmed that pollination can make a significant financial contribution to farmers in terms of higher fruit yield. Marketability is one of the most important aspects linked to fruit quality. Our results showed that open pollination treatments produced grade 18 (203 – 243 g) fruits and honey bee supplementation farms produced grade 16 (227 – 274 g) fruits both of which are among the market valued grades in the international market according to UNECE standards for avocados (2017) and Codex Alimentarius Standards for avocados (CODEX STAN 197-1995). Additionally, we examined if the introduction of honey bee colonies to avocado orchard can increase farmer's profit. The results on the economic valuation highlighted a significant increase in profit for farmers. Our findings indicated that by introducing honey bee colonies to avocado orchards there was a total yield increment of 18%. This revealed a higher economic impact when only wild pollinators are present, study conducted by Peña & Carabalí, (2018) showed that farms that were supplemented with honey bee colonies resulted in higher yield than the control farms (without bee colonies). Similar results have been reported by Geslin et al. (2017) in apple fruits whereby, the presence of high-quality honey bee colonies increased farmer's profits by 70%. A study conducted by Gajc-Wolska *et al.* (2011) on cucumber (*Cucumis sativus* L.) indicated that introducing bumblebees resulted in higher fruit yield, firmness, and better quality characteristics. Therefore, introducing honey bees into the field during blooming periods can compensate for the absence of pollinators.

3.5 Conclusion

Our findings suggest that insect pollination is a key driver for both the physical and nutritional quality of avocado, hence higher avocado production. Supplementation with honey bee colonies resulted in significant increase in fruit weight. Furthermore, oil content was higher in open pollination treatments than other treatments. In a scenario of pollination service decline in agricultural landscapes farmers will encounter economic losses. Thus, pollination services are an essential input in avocado management decisions across different landscapes. This will further lead to excellent fruit quality which can compete favorably in the global market. Our results highlight the need to improve pollination services in pollinator dependent crops, because pollination services are at risk from various anthropogenic threats thus advocating the conservation of insect pollinators. Furthermore, our study establishes the need to integrate food quality, nutrition and pollination through international policies and conservation strategies.

Supplementary material

Table 3.S.1. Effect of pollination treatments on avocado nutritional qualities. Shown are parameter estimates (mean ± SE) from linear mixed effects models shown (*n* = 57).

Treatments	oil (%)	protein (%)	carbohydrates (%)	moisture (%)	TFC (mg QE/100 g)	TPC (mg GAE/100 g)
Hand	40.1 ± 1.7^{b}	1.7 ± 0.1^{a}	2.7 ± 0.3^{a}	70.2 ± 1.3^{a}	7.6 ± 2.5^{a}	387.5 ± 38.9^{a}
Open	38.1 ± 1.9^{b}	1.7 ± 0.1^{a}	2.8 ± 0.4^{a}	70.2 ± 0.8^{a}	8.6 ± 3.0^{a}	442.6 ± 48.8^{a}
Self	33.0 ± 6.0^{ab}	1.9 ± 6.0^{a}	2.0 ± 0.2^{a}	72.3 ± 2.3^{a}	4.7 ± 0.9^{a}	482.1 ± 90.0^{a}
Self + wind	20.7 ± 4.2^{a}	1.5 ± 0.1^{a}	1.7 ± 0.2^{a}	72.3 ± 1.5^{a}	7.3 ± 2.0^{a}	331.3 ± 4.7^{a}

The mean values for the fruit parameters within a column with different superscript letters are significantly different ($P < 0.05$) according to Tukey test. TFC (total flavonoid content), TPC (total phenolic contents), QE (quercetin equivalent), GAE (gallic acid equivalent). The nutritional proximate is based on the dry matter content.

Chapter 4

Pollinator efficiency of avocado (*Persea americana*) flower insect visitors

Summary

Pollination services from insects are critical for higher yield and better fruit quality in avocado (*Persea americana*). Measuring pollinator effectiveness is vital for capturing the relative contributions of different insect taxa to pollination services and for identification of the most important pollinators of this globally important crop. In the present study, we tested pollinator efficiency of avocado in Kenya based on pollen deposition after single visits of flowers by different pollinator species and visit frequency. We monitored the pollination frequency during the flowering period replicated across six farms. Three trees were selected per farm, each with five flower panicles. Out of the 14 observed insect flower visitor species, pollination efficiency was highest in the Western honey bee (*Apis mellifera*), followed by the hoverfly species *Phytomia incisa*. These two species had both the highest pollen deposition and pollen grain loads on their bodies. Furthermore, *A. mellifera* was the most frequent avocado flower visitor followed by Diptera except hoverflies. Our results imply that *A. mellifera* can be managed to achieve adequate pollination services for avocado, particularly in areas lacking efficient wild pollinators. Furthermore, we demonstrated that it is possible to estimate the pollination efficiency of flower-visiting insects using a single-visit pollen deposition. This approach can be applied in other crops to determine the pollination efficiency of common flower-visiting insects.

Keywords: Pollination services, pollen deposition, pollination efficiency, visit duration

4.1 Introduction

Insect-mediated pollination is an important ecosystem service for food security, agrobiodiversity and ecosystem resilience (Garibaldi et al., 2014). Pollinator conservation within agricultural regions is essential because crops require wild or managed pollinators, especially in intensive agricultural regions, to attain maximum yields (Lye et al., 2011). However, insect pollination is threatened by several environmental and anthropogenic factors. Concerns have been raised over a looming potential pollinator crisis caused by loss of habitat, climate change and intrusion by alien species (Potts, et al., 2010; Dicks et al., 2021). Therefore, the conservation and understanding of the importance of pollinator diversity is crucial to support the increasing demand for food (Burkle & Alarcón, 2011). Most flowering crops are visited by a variety of insects but their contribution to pollination remain unknown. A flower visitor is considered a pollinator when it transfers and deposits conspecific pollen grains (Fumero-Cabán, 2007). Therefore, distinguishing pollinators from other flower visitors is of utmost importance for conservation of the pollinator species.

Several insects provide pollination services but the Western honey bee (*Apis mellifera*) has been considered as the most important pollinators of many crops worldwide by increasing fruit set, seed production, crop yield and crop quality (Greenleaf & Kremen, 2006; Winfree et al., 2007). Managed *A. mellifera* may partially offset non-managed pollination services and compensate for the loss of wild pollinators (Sagwe et al., 2021). However, it is important to know the actual performance of individual pollinator species for possible selection as candidates for introduction of managed pollinators (Thapa, 2006). Some studies have shown that wild insects can be effective pollinators and cannot be completely substituted by managed pollinators (Jauker et al. 2012). For instance, in oilseed rape (*Brassica napus*), coffee (*Coffea arabica)*, onion (*Allium cepa*), almond (*Prunus dulcis)*, tomato (*Solanum lycopersicum)* and strawberry (*Fragaria ananassa),* wild pollinators have been reported to be more effective than honey bees in terms of fruit set (Klein et al. 2003, Garibaldi et al., 2013; Rader et al., 2016).

Pollination efficiency is the relative ability of an insect to pollinate flowers effectively after a single visit (Keys, et al., 1995). Collection time, visitation time and pollen deposition are key factors for measuring pollination efficiency of insects in crops (Fishbein & Venable, 1996). Understanding how managed bees and wild pollinators differ in pollination efficiency is critical for current and future crop production. Various methods have been used to measure pollination efficiency, for instance, measuring fruit or seed set after flower visitation, measuring pollen load on pollinators and their pollen deposition into stigmas, or measuring plant reproductive success post-pollination (Ne'eman et al., 2010). On the other hand, the abundance of flower visitors has been used as a proxy to estimate pollination services and crop yield (Zou et al., 2017; Dainese et al., 2019). Therefore, it is critical to evaluate the efficiency of different pollinator species to distinguish flower visitors from pollinators, and determine the strength of plant-visitor interactions.

In the present study, we used avocado (*Persea americana*) as a model crop to quantify the pollination efficiency of different insect species in Kenya. In Kenya, avocado is widely grown and has been a major part of horticultural earnings (Amare et al., 2019), with 368,370 metric tonnes produced annually (HCDA, 2017). Kenya is currently ranked 6th in avocado export volumes after Peru, Chile, South Africa, Israel and Mexico (HCDA, 2017). Avocado is an exotic crop in Africa, originating from Southern Mexico. A study by Ish-am et al. (1999) in Mexico identified stingless bees (Meliponini) and the Mexican honey wasp (*Brachygastra mellifica*) as the primary avocado pollinators, while in Southeast Spain and Israel, bumblebee (*Bombus terrestris)* has been reported as an efficient avocado pollinator (Ish-Am and Eisikowitch 1993; Wysoki et al 2002). However, in East Africa where smallholder farming systems are commonly practiced, there is lack of data on pollination efficiency for avocado. Measuring pollinator efficiency of avocado flowers is thus vital for capturing the relative contributions of different insect taxa to pollination services to maximize production.

This study investigated the pollination efficiency of different avocado flower visitors. Specifically, we analysed the performance of flower visitors by determining stigmatic pollen deposition per single visit to assess their efficiency as pollen-transfer agents.

4.2 Materials and methods

4.2.1 Study site

The study was carried out in smallholder avocado farms in Murang'a County, Kenya (S 0°43'0", E 37°9'0"). Two avocado varieties were grown in the orchards: Fuerte and Hass. The experiments were conducted during August – October 2019, which encompasses the major avocado blooming period in Kenya. Six smallholder avocado farms (0.2 – 0.4 ha) were selected for the study.

4.2.2. Visitation frequency

Visitation frequency is the number of pollinators visiting a flower per unit time which is a variable used to assess insect pollination and its contribution to plant reproduction (Bergamo et al., 2016). On each farm, three trees spaced 20 m apart were randomly selected for the experiment. Selected trees were spaced a minimum of 10 m from the edge of the farm to minimize edge effects. On each tree, three branches with at least five flower panicles (clusters) were randomly selected. The flowers surveyed were at a height between 0.5 and 2 m above ground. Flower visitors were observed for a 30 min period and their number was recorded. A 'visit' was recorded if the insect contacted the reproductive structures. Pollinator surveys were conducted between 8.00 – 17.00 h, only when weather conditions were suitable for insect sampling. We conducted farm-based flower visitor observations nine times with each farm visited once a month for a period of three months. Avocado flower insect visitors were grouped into the following taxa: Western honey bee (*Apis mellifera*), hover flies (Syrphidae), other flies (Diptera, except for Syrphidae), wasps (Hymenoptera), butterflies (Lepidoptera), wild bees (Hymenoptera) and beetles (Coleoptera).

4.2.3. Quantifying pollination efficiency: Single-visit pollen deposition

Single-visit deposition (SVD) data was collected for comparison to measure pollinator efficiency (Ne'eman et al., 2010). In the afternoon before the data collection day, 32 panicles per farm in three farms with flower buds were randomly selected and bagged with fine woven bags that were impermeable to wind and excluded pollinators. The bag was carefully removed the following morning from each panicle when the flowers had opened. Virgin stigmas were identified on flowers that had opened overnight using a hand lens and were marked. Individual flowers were observed until they received their first visitor after unbagging the panicles. Pollinators were caught on their first visit as they exited the flowers. The length of stay (flower handling time) was measured using a stopwatch from the moment the insect started to forage on the flower until the moment the insect left it. Insects were always allowed to complete their visit before being captured using a sweep net. Flowers visitors were placed in individual vials for later morphological identification to species level. The survey continued each day until there were no more bagged flowers left to sample or the visitation rate had decreased to a low level (no visits recorded for 1 h) per farm. Control stigmas were removed from newly uncovered virgin flowers before a visit took place to account for pollen found on stigmas due to opening of the flower and/or handling and bagging procedures. The stigmas of visited flowers and control flowers were sampled, put in individual 0.5 mL vials with 70% ethanol and stored in a cooler box at -18°C. Samples were subsequently transported to the laboratory for pollen count analysis.

4.2.4. Palynological analyses

The quantity of pollen carried on insects' bodies was estimated using the method described by Krause & Wilson (1981). In the laboratory, 0.5 mL distilled water was added to the vials with captured insects and vials were shaken vigorously until no more pollen could be seen on their body. The insects were then transferred into vials containing 0.5 mL 70% ethanol for identification. Before washing off the pollen from the insects, the bee corbiculae were removed to avoid sampling of corbicula pollen since they are not available for pollination. The pollen grains were stained using Fuchsin glycerine jelly and were counted using a microscope at 100× magnification.

In the laboratory, the stigmas were crushed on a microscope slide and stained with Fuchsin-glycerine jelly as described by Beattie (1971). All conspecific (avocado pollen grains) and heterospecific (non-compatible with avocado pollen grains) pollen grains deposited were counted under a light microscope.

4.2.5. Statistical analysis

Differences in averages of visitation frequency among pollinators at different times of the day was analysed using linear mixed-effects models (LMM) using the R package *lme4* (Bates et al., 2015). The observational date and farm ID was used as a random factor. Means separation was performed using the *lsmeans* package (Lenth, 2016), and significant differences were generated using the *multcompview* package (Graves et al., 2019). Visitation frequency on different times across the day was considered as a repeated measure. Pollination frequency was the response variable while flower visitors and time were the explanatory variables. SVD for different species was quantified by counting the number of compatible pollen on flowers of avocado, which is a direct measurement of successful pollination per individual visit. Heterospecific pollen was also counted and analyzed separately. Pollination efficiency was taken as the number of compatible pollen grains deposited on the stigma after a single visit. LMM were used to determine the difference in SVD of pollen among the insect species and the amount of compatible pollen on the insect body. In this analysis, sampling date and farm ID was used as a random factor. Likewise, flower handling time data was subjected to LMM to evaluate whether pollinator species differed in flower handling time, observational date and farm ID was used as a random factor. Pearson's correlation analysis based on the means per species was used to establish the linear relationship between pollen on the insect body and pollen deposited on the stigma. Pearson's correlation analysis was also used to evaluate whether there was a relationship between flower handling time, pollen on the insect body and pollen deposited on stigmas. Species that were encountered once were excluded from the analysis. All analyses were performed using R statistical software (v 4.0.2, R Foundation for Statistical Computing, Vienna, Austria).

4.3. Results

4.3.1. Flower visitor's frequency and their visitation time

Four orders (Hymenoptera, Diptera, Lepidoptera, Coleoptera) of avocado flower insect visitors were observed (n = 605), which were grouped into the following seven taxa: Western honey bee (*Apis mellifera*), hoverflies (Syrphidae), other flies (Diptera, except for Syrphidae), wasps (*Vespula vulgaris*), butterflies (Lepidoptera), wild bees (except for *A. mellifera*) and beetles (Coleoptera) (Table 4.1). Western honey bees were the most abundant visitors, followed by other flies, hoverflies and wasps, while beetles, butterflies and wild bees were the least abundant (Table 4.1). The pollinator visitation frequency differed significantly among the different insect groups ($F_{7, 16} = 55.34$, $p < 0.001$). The highest visitation frequency per flower was found for *A. mellifera*, followed by other flies. There was a significant difference observed between visitation time among the different insect taxa ($F_{6, 16} = 1.63$ $p < 0.001$). The visitation time for *A. mellifera* peaked during two-time intervals, namely 10.30 - 11.30 h and 13.30 - 14.30 h. Furthermore, *A. mellif*era visitation was the highest throughout the observation period (9:00 - 17:00 h). Hoverflies were observed to be active late in the afternoon, while other flies were observed mainly at 10.30 - 11.30 h (Fig.A.4.1).

Table 4.1. The relative abundance and visitation frequency (visits/30 min/flower) of flower visitors (n = 605) across smallholder avocado farms in Murang'a County, Kenya.

Order	Flower visitor	Relative abundance (%)	Visitation frequency (visits/30 min)
Hymenoptera	Honey bees	55	2.18 ± 0.22^{c}
Diptera	Diptera, except for Syrphidae (other flies)	16	0.63 ± 0.10^{b}
Diptera	Hover flies	11	0.44 ± 0.07^{ab}
Hymenoptera	Wasps	9	0.33 ± 0.05^{ab}
Hymenoptera	Wild bees	3	0.11 ± 0.03^{a}
Lepidoptera	Butterflies	3	0.09 ± 0.03^{a}
Coleoptera	Beetles	3	0.12 ± 0.02^{a}

The values of visitation frequency are means ± SE of Means with a different superscript letter in a column are significantly different ($P < 0.05$) according to Tukey test.

4.3.2 Pollination efficiency

Different pollinator species showed significantly varying abilities to carry pollen grains ($F_{13,71} = 6.43$, $p < 0.001$). *Apis mellifera* (Fig.4.1E) and *Phytomia incisa* W. (Fig.4.1B) carried relatively more compatible pollen grains of avocado flowers (Fig A.4.2) on their body than other species, while relatively lowest numbers were observed in *Belanogaster griseus* (Fab.) (Fig.4.1G). There was a significant difference in pollen deposition to the stigma i.e., pollination efficiency, among the observed flower-visiting insect species ($F_{13, 71} = 4.54$, $p < 0.001$), with *A. mellifera* and *P. incisa* showing the highest number of pollen depositions per single visit, while the lowest numbers were observed in *Chrysomya chlorophyga*, *Polistes* sp. and *B. griseus* (Table 4.2). There was no significant difference of heterospecific pollen carried on the insect body ($\chi 2 = 14.53$, df = 13, $P = 0.34$) and those deposited in the stigma ($\chi 2 = 0.78$ df = 8, $P = 0.99$) among taxa (Table A.4.1). There was a strong positive correlation between conspecific pollen on the insect body and conspecific pollen deposited in the stigma (Fig. 4.2a). We calculated a ratio of 81.43 between conspecific and heterospecific pollen in stigma, while a ratio of 154.06 was calculated between conspecific and heterospecific pollen on the insect body.

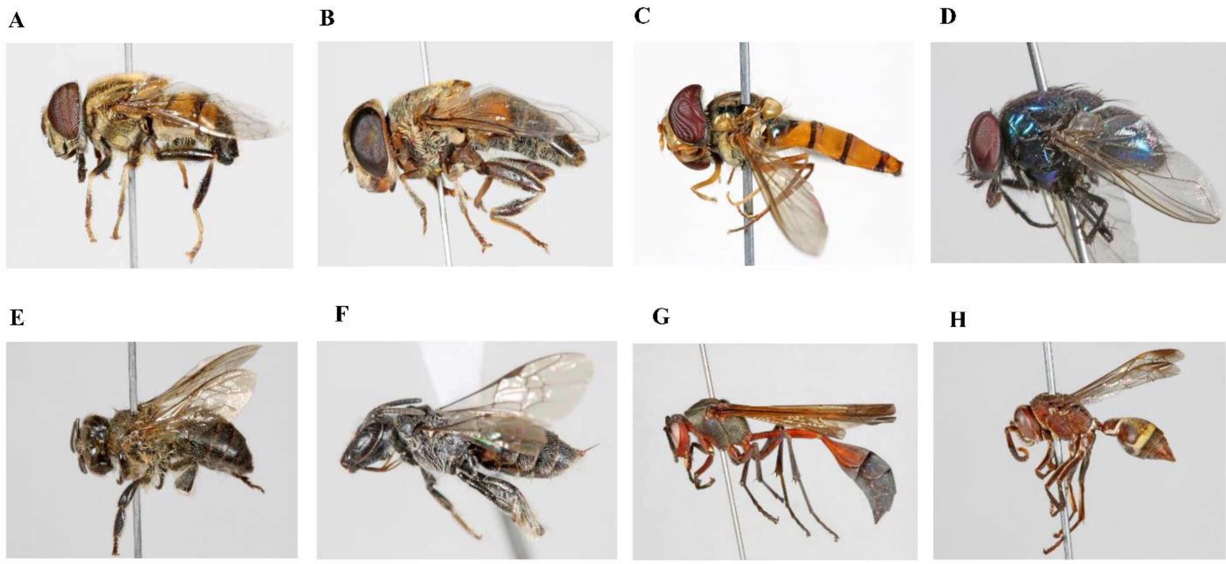

Fig. 4.1. Examples of the avocado flower-visiting species that were evaluated regarding their pollination efficiency in this study. **(A)** Syrphidae *(Eristalinus quinquelineatus)*, **(B)** Syrphidae (*Phytomia incisa*), **(C)** Syrphidae (*Episerphus trisectus*), **(D)** Calliphoridae (*Chrysomya chloropyga*), **(E)** Apidae (*Apis mellifera*), **(F)** Apidae (*Braunsapis faveata*), **(G)** Vespidae (*Belonogaster griseus*), **(H)** Vespidae *(Polistinae* sp.).

Table 4.2. The number of compatible pollen grains carried on the insect body, the number of compatible pollen grains deposited per single visit/pollination efficiency on the stigma of avocado flowers, and per flower handling time (duration) observed across different species in Murang'a county, Kenya.

Species (N)	**Number of pollens on insect**	**Number of pollens deposited/pollination efficiency**	**Flower handling time (s)**
Apis mellifera (41)	282.1 ± 80.8^{d}	30.9 ± 8.5^{c}	9.2 ± 0.8^{ab}
Phytomia incisa (4)	208.3 ± 54.7^{cd}	34.5 ± 12.1^{bc}	8.0 ± 0.0^{ab}
Betasyrphus hirticeps (3)	88.3 ± 40.2^{abcd}	13.0 ± 4.2^{abc}	6. 7 ± 1.2^{ab}
Braunsapis faveata (3)	85.3 ± 9.0^{bcd}	16.3 ± 1.9^{abc}	18.0 ± 9.5^{b}
Eristalis tenax (4)	78.0 ± 23.3^{bcd}	6.1 ± 2.0^{abc}	6.0 ± 0.7^{ab}
Eristalinus quinquelineatus (4)	72.3 ± 24.0^{abcd}	12.8 ± 4.3^{abc}	4.5 ± 0.3^{a}
Eristalinus sp. (3)	39. 7 ± 10.2^{abcd}	9. 7 ± 0. 7^{abc}	6.3 ± 0.3^{ab}
Episerphus trisectus (4)	32.3 ± 7.1^{abcd}	5.5 ± 1.2^{abc}	5.8 ± 0.5^{ab}
Allograpta sp. (5)	19.4 ± 4. 8^{abcd}	3.6 ± 1.0^{abc}	4.4 ± 0.4^{a}
Chrysomya megacephola (4)	18.0 ± 8.5^{abcd}	7.8 ± 5.2^{abc}	3.6 ± 0.5^{a}
Chrysomya chloropyga (7)	15.0± 3.5 ^{abc}	3.4 ± 1.1^{ab}	5.6 ± 1.3^{ab}
Polistes sp. (5)	7.6 ± 2.5^{ab}	1.6 ± 1.4^{a}	4.4 ± 1.1^{a}
Belonogaster junceus (3)	6.0 ± 2.1 ^{ab}	4.0 ± 3.5^{abc}	3.3 ± 0.3^{a}
Belonogaster griseus (3)	3.3 ± 2.4^{a}	1.0 ± 0.6^{a}	4.0 ± 1.4^{ab}

N = the total number of individuals of each species that was evaluated. The values are means ± SE. Means with a different superscript letter within a column are significantly different ($P < 0.05$) according to Tukey test.

4.3.3. Flower handling time

Flower handling time varied significantly among the pollinator species ($F_{13, 71} = 2.63$, $p = 0.004$, Table 4.2). The longest time spent on the flower was observed in *Braunsapis faveata* (Fig. 4.1F), *A. mellifera* (Fig. 4.1E), and *P. incisa* (Fig. 4.1B) (Table 4.2). In addition, there was a positive correlation between the amount of pollen on the insect body and flower handling time and between the amount of pollen deposited and flower handling time (Fig.4.2b and c). Three species were excluded from the analysis because their numbers were less than three *Sceliphron* sp., *Stomoxys* sp., and *Isomyia* sp.

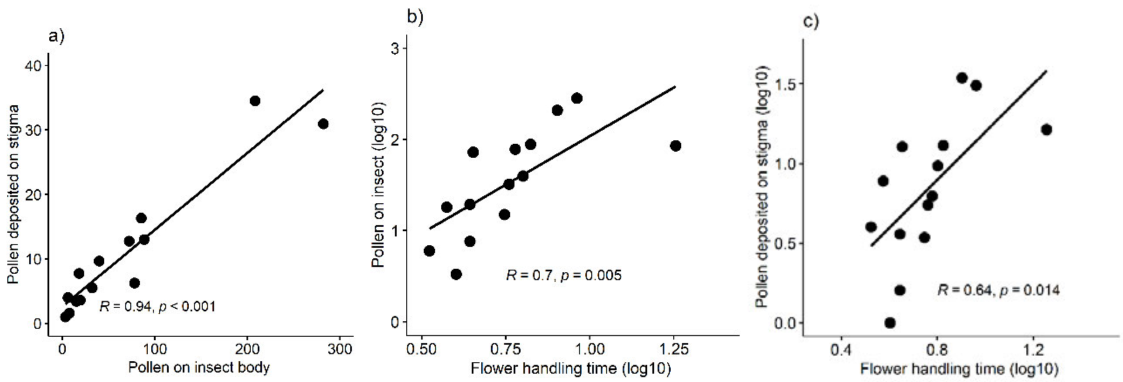

Fig. 4.2. Linear regression showing the relationship between (a) pollen on the stigma and pollen on insect, (b) Pollen on the insect and flower handling time (c) Pollen deposited on the stigma and flower handling time. The *R* and *P*-values are indicated on the graph.

4.4. Discussion

Pollination efficiency, which is the relative ability of an insect to pollinate flowers effectively, is used to rank the importance of different species of flower visitors as pollinators (Ne'eman et al., 2010). After a single flower visit, pollen deposition on the stigma and the number of pollen grains on the insect body have been used previously to estimate pollination efficiency (Ne'eman et al., 2010). However, not all flower visitors of a given plant species are effective pollinators since some could be nectar and pollen robbers, thieves, or there could be a mismatch of the morphological trait (body size) with flower size (Rivest & Forrest, 2020). A recent global meta-analysis on avocado pollinators by Dymond et al. (2021) indicated that pollination efficiency of different avocado flower visitors has rarely been documented. Our study is the first to report the pollination efficiency of avocado flower visitors in sub-Saharan Africa.

In terms of frequency and abundance, the contribution of *A. mellifera* to pollination was much higher than other species in our study site. Our results concur with previous studies, that *A. mellifera* is an important avocado pollinator due to its high flower visitation frequency and abundance (Wysoki et al., 2002; Ish-Am, 2005). The findings by Read et al. (2017) in New Zealand reported that Western honey bees were the dominant flower visitors (92.9%) in all avocado orchards surveyed. Evans et al. (2011) studied the role of insect pollinators in avocado in New Zealand and reported that 97.4% of all flower visitors were honey bees, while in Australia, 37.9% were honey bees. In our study, honey bees were the most active flower visitors since they visited avocado flowers between 09:00 and 17:00 h, with the peak time being 11:00 h. The high numbers recorded for this species could be due to their large social colonies and efficient foraging behaviour that could outcompete solitary bees and other insects (Balfour et al., 2015). However, previous studies revealed that *A. mellifera* can abandon avocado flowers when there are alternative blooms in the vicinity (Afik et al., 2006; Afik et al., 2014). Our result showed that flies were also regular avocado flower visitors with an overall abundance of 11% hoverflies (Syrphidae) and 16% of other flies. Similar findings of relative abundance of 12% hover flies have been reported by in avocado crop Dymond et al. (2021). Also, a study conducted by Evans et al. (2011) reported that 49.7% of the visitors were hover flies, and 12.4% of the visits were from other insects (flies other than hover flies and native bees). The result of the present study also shows that the contribution of wasps especially, *Polistes* sp. was low because of their lower numbers.

Wild and managed pollinators can be essential to supplement honey bees during poor weather conditions or when other flowers are in bloom at the same time in order to reach substantial pollination services. Previous studies highlighted the importance of conserving wild pollinators at the landscape scale (Require et al. 2019). Therefore, maintaining heterogeneous natural habitats can be an excellent strategy to conserve the abundance of wild pollinators around the orchards (Woodcock et al., 2019; Klein et al., 2012; Potts et al., 2016).

Our result suggests that *A. mellifera* and *P. incisa* are efficient pollinators of avocado after a single visit. This was evident because they showed many pollen grains on their body and had high pollen deposition. The higher the number of pollen grains deposited, the higher the chance of pollination success (Alcaraz & Hormaza, 2021). It has been shown that pollen deposited during a single visit is a more direct and practical method in assessing pollination efficiency (King et al., 2013). This study agrees with previous findings by Vithanage (1990) in New South Wales, which indicated that *A. mellifera* played a leading role in pollen transfer for avocado. Similar findings have also been reported by Wysoki et al. (2002) and Peña (2003) where they found *A. mellifera* as the primary avocado pollinator even though *Bombus terrestris* L. has been reported as an efficient avocado pollinator in Southeast Spain and Israel (Ish-Am and Eisikowitch 1993; Wysoki et al 2002). A study conducted by Ish-Am, (2005) in three different avocado varieties (Hass, Ettinger, and Reed) in Israel indicated that twenty or more pollen grains are required to reach the stigma for adequate fertilization to take place. Therefore, *A. mellifera* and *P. incisa* can achieve adequate fertilization with a single visit deposition hence, they are supposed to be efficient avocado flower pollinators.

The high efficiency of *A. mellifera* and *P. incisa* in pollination might be explained by the hairiness of their bodies and their large sizes, which result in more contact with the stigma. Hairiness is considered an essential trait in pollinators as it is involved in pollen collection and transfer (Thorp, 2000; Amador et al., 2017; Roquer-Beni et al., 2020). A study conducted by Stavert et al. (2016) demonstrated that pollinator hairiness is strongly linked to pollination. Furthermore, recent studies have found a positive relationship between body size and amount of pollen deposited per visit in oilseed rape *Brassica napus* L. (Phillips et al., 2018) and watermelon *Citrullus lanatus* T. but (Bartomeus *et al*., 2018). With greater body size, pollinators such as *A. mellifera and P. incisa* can carry larger pollen loads and deposit more pollen (Goulnik et al., 2020). In our study, wasps were less important pollinators, perhaps because of a body integument with low pollen adhesion capacity limiting their potential as avocado pollinators (de Vega and Gomez 2014). This is contrary to some studies that have reported wasps as efficient pollinators (Ish-am et al., 1999; Pérez-Balam et al., 2012). In our study site, *A. mellifera* was found to transfer more heterospecific pollen than other species, indicating that they visited other flowers in the vicinity than avocado. Heterospecific pollen transfer occurs in nature and does not result in fruit production (Morales and Traveset, 2008; Mitchell et al., 2009).

Our result showed a significant difference among the flower insect visitors in terms of flower handling time. The longest flower handling time was recorded in *B. foveata,* followed by *A. mellifera*. Engel & Irwin (2003) indicated that the time spent in the flower might affect floral resource exploitation and consequently the pollination of a given plant species. This agrees with our study, where we found a moderate positive correlation between flower handling time and pollen on the insect body and pollen deposited, indicating that time spent on the flower may play a role in pollination efficiency.

4.5. Conclusion

This study provides important insights into the pollination effectiveness of avocado. We observed that flower-visiting species had different capabilities in transferring compatible pollen grains in avocado, demonstrating the importance of species behaviour and morphological traits in determining pollination efficiency. To achieve optimum pollination services for avocado, managed honey bees or conservation habitats of wild living colonies can be valuable tools for growers in improving the yield for avocado, which can help to supplement wild pollinator species. This study is vital in guiding the farmers to know the necessary measures to implement within and around the orchards to support the pollinator species and their corresponding pollination services. Understanding the specific effectiveness of different pollinators that visit avocado flowers will help determine which species are critical to its production. Pollination services can be measured by quantifying each species' pollination effectiveness as well as their abundance.

In optimizing crop pollination, it is crucial to investigate the contribution of different flower-visiting insects.

4.6. Appendices

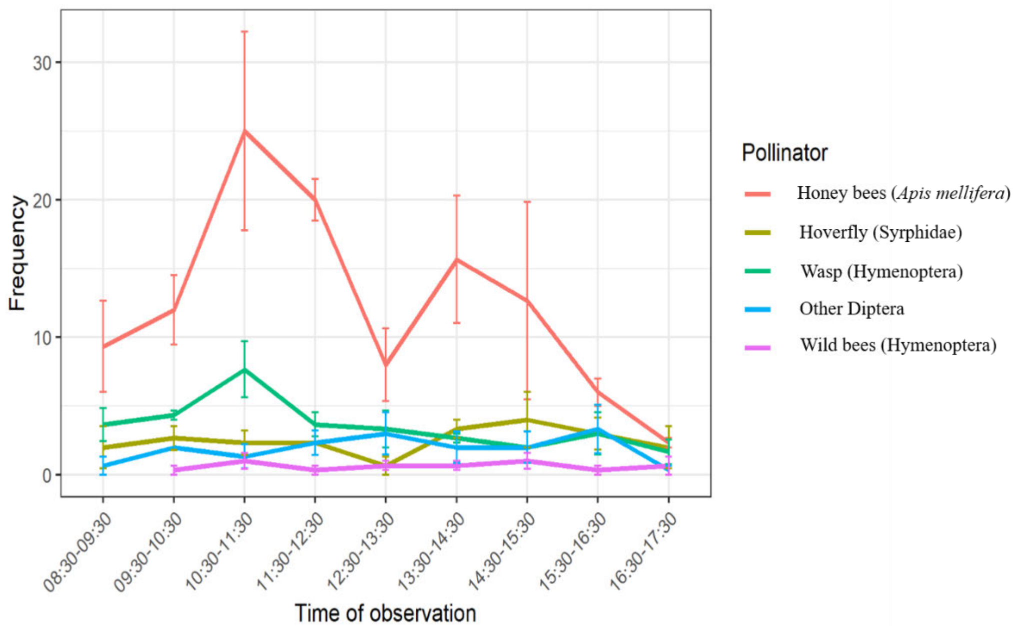

Fig. A.4.1. Visitation frequency of the most abundant insect groups during the avocado blooming period in Murang'a county, Kenya.

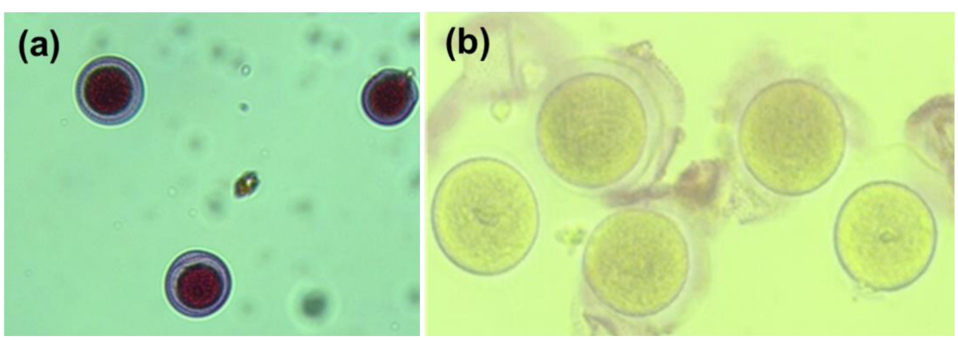

Fig. A.4.2. Avocado pollen grains with and without stain taken under a microscope at 100× magnification. (a) General view of pollen grains with stain and (b) pollen without stain.

Table A.4.1. Percentage of heterospecific pollen on the insect and heterospecific pollen deposited on the stigma of avocado flowers across smallholder avocado farms in Murang'a County, Kenya.

Species	**Heterospecific pollen on the insect (%)**	**Heterospecific pollen on the stigma (%)**
Allograpta sp.	3.19	0.00
Belonogaster griseus	2.13	9.52
Belonogaster junceus	0.00	0.00
Betasyrphus hirticeps	5.32	0.00
Braunsapis faveata	1.06	0.00
Chrysomya chloropyga	3.19	9.52
Chrysomya megacephola	10.64	4.76
Episerphus trisectus	2.13	4.76
Eristalinus quinquelineatus	2.13	0.00
Apis mellifera	51.06	47.62
Eristalinus sp.	5.32	14.29
Phytomia incisa	2.13	4.76
Eristalis tenax	6.38	4.76
Polistes sp.	5.31	0.00

Chapter 5

General Discussion

Plants rely on pollen vectors to transfer their pollen to another plant. It has been reported that between 60% and 90% of plant species require animal pollinators, and nearly 75% of globally significant agricultural crops rely on arthropod pollination to produce quality yields (Abrol, 2011). Several groups of insects (wild and managed) are important as pollinators including bees, flies, beetles, butterflies, and moths (FAO, 2016). However, the relationship of pollinator diversity and crop production in terms of increased yield have received little attention, particularly, in Sub-Saharan Africa (Amit et al., 2015). Moreover, little work has been done on their role as pollinators in line with yield increase, quality, and thus marketability. (Valiente-Banuet et al., 2015). Pollinator conservation within agricultural regions is critical to maximize yields (Lye et al., 2011). It is therefore important to develop strategies for managing pollinators such as bees and other wild pollinators in the ever-changing landscapes without threatening agricultural production. Previous studies have shown that horticultural crops such as fruits, vegetables and edible oils are more vulnerable to loss of pollinators than staple food crops (Novais et al., 2018). It is also predicted that in most regions of the world, there will be a decline in agricultural production due to losses in pollinating insects (Koh et al., 2016). In this study, pollination deficits in avocado smallholder farms, which is a typical representation of African agriculture was investigated and whether honey bee supplementation can boost pollination levels was evaluated (Chapter 2). In temperate regions, avocado has been reported to suffer from inadequate pollination in commercial orchards, resulting to low fruit yields (Vithanage, 1990; Gazit and Degani, 2002). Thus, avocado farmers in those regions often supplement their farms with honey bee colonies to boost pollination services.

There has been evidence that pollination services increase fruit and seed set, especially, that of vegetables and fruits (Klein et al., 2007). However, there is still limited studies linking fruit quality aspects and pollination. Therefore, there is need for research to evaluate how different pollination treatments affect fruit quality (Chapter 3). Previous studies have indicated that avocado benefit from a wide range of insects such as stingless bees, honey bees and social wasps (Gazit and Degani, 2002; Can-Alonzo et al., 2005; Afik *et al*., 2006). But yet still, no studies have been conducted in East Africa to assess pollination efficiency of flower visitors in avocado orchards (Chapter 4). In this study single-visit pollen deposition (SVD) approach was applied to investigate the pollen transfer efficiency of different flower visitors, which is commonly used as a direct measure of pollination efficiency (Ne'eman et al., 2010). In summary, these findings highlight the importance of insect pollination, as one of the strategies in curbing the current food and nutritional security in sub-Saharan Africa.

5.2 Pollinator supplementation mitigates pollination deficits in smallholder avocado (*Persea americana* Mill.) production systems in Kenya.

5.2. 1 Fruit set and pollination deficits

Pollination is an important measure for fruit set in avocado production. The main aim of this study was to determine the effect of different pollination treatments and their effectiveness for successful fruit set and assess if smallholder avocado farms suffer from pollination deficits. From the study, open pollination gave greater fruit set than pollinator exclusion treatments, indicating that avocado is a pollinator-dependent crop. Furthermore, my results suggest that the smallholder avocado agricultural systems are threatened by decreased pollination services and that farmers will experience yield losses if insect pollinators decline in the study area. Therefore, the need to explore the extent of pollination deficits where maximum yield is not being achieved and whether crops are pollinator dependent, is of outmost importance (Garrat et al., 2021). It is vital to protect wild pollinators effectively and, in case of pollination limitation, potentially supplement farms with managed pollinators (Rader et al., 2013; Garibaldi et al., 2014). Although this study identified the yield deficits due to insufficient pollination by insects, other unmeasured factors like farm management regimes like pruning, mulching, weeding, fertilization, variation in the age of the avocado trees, the availability of mineral elements and nutrients in the soil, pests, and pathogens, weather and other animal interactions in the orchards, may have caused hidden variations in final fruit set levels between open and hand-pollinated fruits (Samnegård et al., 2019).

Previous studies conducted in other crops linking pollination deficits and production yield have indicated that the yield deficits could be addressed through habitat management (Foldesi et al., 2016; Sutter et al., 2018) and through effective use of managed pollinators (Geslin et al., 2017). Methods used in this study can be employed in other crops to assess levels of pollination services and deficits (i.e., by supplementary and pollinator exclusion techniques), which will help to make informed management decisions (Garratt et al., 2019). This study highlights an opportunity for farmers to supplement their orchards with managed honey bees to increase pollination services, thus minimizing yield loss. Studies conducted by Ish-Am & Eisikowitch (1998), Ish-Am & Lahav (2011) and Peña & Carabalí (2018) showed that increasing honey bee density led to an increase in pollination and production of avocado. Implementation of honey bee colony is thus likely to increase production but may not be cost-effective in all circumstances especially in the sub-Saharan African, thus the utilization and management of alternative pollinators (wild pollinators) may also be applicable in some circumstances.

5.2.2 Fruit retention rate

Determining the cause(s) of fruit abscission is important for the development of strategies to increase fruit set and yield (Garner & Lovatt, 2016). Fruit abscission can occur as a result of many factors, including temperature extremes, nutritional deficiencies, and genetic factors (Garner & Lovatt, 2008). In addition, avocado abscission is a natural process to relieve a tree of too much fruit, but stress especially lack of fertilization and pests can also cause excessive fruit loss (Garner & Lovatt, 2016). In this study, fruit abscission was observed two months after the initial fruit set, and it occurred across all the farms, but tended to be less on the farms that were supplemented with *A. mellifera*. This clearly indicated that fruit abscission occurred mostly due to lack of fertilization. This is in line with a study conducted by Garner & Lovatt (2016), where they found a strong evidence of avocado fruit abscission in Hass variety due to lack of fertilization, indicating that to some degree, fruit retention depends on pollination. In my study area, habitat diversity, NDVI, elevation, and agricultural land did not affect pollination deficits or fruit retention. This may have been attributed to other factors like landscape homogenization which has been reported to reduce the abundance and diversity of many taxa due to low foraging resources and lack of nesting site (Tscharntke et al., 2012). Therefore, this result highlights the importance of promoting existing semi-natural habitats and encouraging landscape crop diversity to provide pollinators with sufficient food and nesting resources. In this study, it was found that the number of avocado trees in the farm negatively influenced fruit retention, with farms having fewer avocado trees showing a higher percentage of fruit retention than farms with a high number of trees. This may be associated with reduced resource competition between plants over soil nutrients on smaller farms, better pest control from parasitoids and predators on smaller farms, or a different avocado age structure for smaller farms (Bennett, 2010; Cameron *et al*., 2007). Trophic interactions within the soil can also influence the aboveground community of plants, which may include fruit retention (Cheng & Gershenson, 2007).

5.3 Insect pollination enhances fruit weight, quality, and marketability of avocado (*Persea americana*).

5.3.1 Effect of pollination on physical fruit quality

Pollination is the most important ecosystem service performed mostly by insects, and it is vital for increasing fruit quality hence improving the socio-economic status of farmers. My result revealed that pollination had pronounced effect on fruit weight. I noted that insect pollinated avocado fruits were heavier than fruits from pollinator exclusion treatments. Furthermore, the supplementation of avocado farms with honey bee colonies increased the fruit weight significantly compared to control farms, thereby producing commercially more acceptable fruits that meets the international market standards. These results confirm the findings of Mulwa et al. (2019), where they reported a significantly higher fruit yield and larger seeds from unbagged fruits than bagged fruit treatments. The low yield observed in pollinator exclusion treatments was perhaps as a result of lower fertilization success. This result implies that avocado farmers will be experiencing reduced crop yields if insect pollinators are not present. Further, the other results clearly showed that insect pollination contributed significantly to high seed quality (weight and size). Similar results have been reported by Mulwa et al. (2019) in avocado seeds whereby insect pollination treatments produced heavier seed than pollinator exclusion treatments. The seed size has been reported to have an indication of high seed germination rate. For instance, a previous study by Olorunmaiye et al. (2011) in mango seeds, reported that heavy seeds produced a greater number of seedlings with high vegetation structure, and high dry matter accumulation. The size of the seed has also been shown to have a significant influence on crop development and performance in the field (Adebisi et al., 2013). The above scenario could be as a result of higher food and other energy reserves, which are among the major factors required to improve the germination rate (Shahi et al., 2015). Therefore, pollination is critical in determining seed size that plays a substantial role for successful seed germination and crop performance.

5.3.2 Effect of pollination on fruit nutritional quality

The avocado fruit is today extensively regarded as an important fruit because of its nutritional values for human health. Avocado fruit contain a variety of essential nutrients and important phytochemicals (Dreher & Davenport, 2013). For instance, avocado oil is the most outstanding quality parameter linked to the market price and it contains predominantly monounsaturated oleic acid, which has been found to reduce the harmful low-density lipoprotein cholesterol, consequently exerting many cardiovascular benefits (Forero-Doria et al., 2017). Thus, there is need for improving the avocado quality, which will result to high marketability. The result from this study indicated that oil concentration was significantly affected by pollination treatments, whereby hand and open pollination treatments had higher oil content compared to pollinator exclusion treatments. In addition, honey bee supplementation increased oil concentration in avocado by 3.6% though this did not differ significantly with the concentration of oil in avocados from the control farms. Comparable to my results, insect pollination has been reported to increase quality and the nutritional composition in some fruits and seeds, reduce malformations, and increase higher commercial grades and shelf lifetime of several globally important crops (Klatt et al., 2011; Matsumoto et al., 2012; Wietzke et al., 2018; Bommarco *et al*., 2012; Brittain et al., 2014). Additionally, Silva et al. (2018) showed that bee pollination in sunflower enhanced unsaturated fatty acids by 0.3 %. However, other nutritional parameters and phytochemical contents were not affected by pollination treatments. Low concentrations of carbohydrates were found in our study, an indication that avocado has low sugar content, hence can be consumed by patients with diabetes (Del Toro-Equihua et al., 2016; Tramontin et al., 2020). A negative correlation was observed between the oil and moisture content, which agreed with previous studies (Lee et al., 1983; Osuna-García et al., 2010). Thus, the current study highlights the importance of insect pollinators especially bees in improving nutritional quality of avocado and provides useful information for future evaluations of the effects of pollinators on human diets and health.

5.3.3 Economic benefits of insect pollination

Pollination can make a significant financial contribution to farmers in terms of high fruit yield and quality. Marketability is one of the most important aspects linked to fruit quality, as quality fruits fetch high prices. My results showed that open pollination treatments produced fruits of good grades, 18 (203 – 243 g) in control farms, and honey bee supplementation farms produced grade 16 (227 – 274 g) fruits. These grades are among the commercially valued in the international market. Additionally, the introduction of honey bee colonies to avocado orchard increased the total yield by 18%, which translates to an increase in sales of USD 24.14 per farm of 10 avocado trees. Without honey bee supplementation, the sales stands at USD 134.14 per a smallholder farm (10 trees each 61.7 kg, total of 617 kg; sale at 1 tonne for USD 217.40 as per FAOSTAT 2021).

This revealed a high economic impact as compared to when only wild pollinators are present. This loss could be even greater in avocado farmers where there are two harvest seasons per year. A study conducted by Peña & Carabalí, (2018) showed that avocado farms that were supplemented with honey bee colonies resulted in higher yield than the control farms (without bee colonies). Similar results have been reported by Geslin et al. (2017) in apple fruits whereby, the presence of high-quality honey bee colonies increased farmer's profits by 70%. Therefore, introducing honey bees into the field during blooming periods can compensate for the absence of pollinators. Thus, pollination services and landscape management to conserve pollinating insects should be a major consideration in drafting agricultural policy, to enhance food and nutritional security. Although average values of pollinator benefits are generally assumed, there is potential for large spatial variation among crop species and varieties or among pollinator management strategies, even within the same region and year. Therefore, insect pollination is an important aspect for global food production in general and human nutrition, and this study underscores the critical role pollinators play in the economy.

5.4 Characterization of pollinator effectiveness of avocado (*Persea americana*) flower insect visitors

5.4.1 Flower visitor's frequency

The relative abundance of different pollinator species varied in my study, with honey bees recorded as the most frequent avocado flower visitors followed by flies. Comparable results have been reported in previous studies, where it was observed that *A. mellifera* was an important avocado pollinator due to their high flower visitation frequency and abundance (Wysoki et al., 2002; Ish-Am, 2005; Evans et al. 2011; Read et al., 2017). In the present study, the highest number of honey bees was observed between 10:30 - 11:30 h. The high numbers recorded for *A. mellifera* could be due to their aggressive foraging behaviour that outcompetes other solitary bees and other insects (Balfour et al., 2015). However, a previous study had revealed that *A. mellifera* often, can abandon avocado flowers when there are alternative blooms in the vicinity (Afik et al., 2006; Afik et al., 2014). Therefore, wild pollinators such as hoverflies and calliphoridae, which were also observed in relatively large numbers, can be important to supplement honey bees when other flowers are in bloom at the same time in order to reach substantial pollination services. Moreover, many wild species other than honey bees have been shown to visit crops (Delaplane & Mayer, 2000) and have been recognized for their role in increasing and stabilizing crop-pollination services (Garibaldi et al., 2011; Garibaldi et al., 2013).

5.4.2 Pollination efficiency

To assess pollinating species and their ability to pollinate avocado flowers, I determined the flower-visiting species and their pollination efficiency in terms of single pollen deposition. In the present study, I recorded honey bees (*A. mellifera*) and hoverfly species (*Phytomia incisa*) as efficient avocado pollinators after a single visit pollen deposition. For successful pollination to occur the number of pollens deposited by insect pollinators is the most important determinant factor. Previous studies have indicated that avocado flower require more than twenty pollen grains for successful pollination (Ish-Am, 2005). In my study, honey bees (*A. mellifera*) and hover fly species (*P. incisa*) deposited on average thirty pollen grains after a single flower visit. These results are in agreement with the findings of other studies (Vithanage, 1990; Wysoki et al., 2002; Peña, 2003), where it was noted that honey bees are the most efficient avocado pollinators. The efficiency assays revealed that not all pollinators were equally efficient. The body size of an insect have been reported to account for insect species' efficiency as pollinators (Phillips *et al.*, 2018; Bartomeus et al., 2018; Goulnik et al., 2020). Additionally, pollinator hairiness have been strongly linked to pollination efficiency (Thorp, 2000; Amador et al., 2017; Roquer-Beni et al., 2020; Stavert et al., 2016).

The longest flower handling time was recorded in *Braunsapis faveata*, and it was also observed that there was a positive correlation between the amount of pollen on the insect body and flower handling time and between the amount of pollen deposited and flower handling time. A positive correlation has been reported between time spent in the flower and floral resource exploitation. Engel & Irwin (2003) indicated that the time spent in the flower may affect floral resource exploitation and consequently the pollination of a given plant species. For farmers to obtain optimal yields, it is necessary to ensure a sufficient abundance of pollinators in their avocado orchards either through managed bees or by managing wild pollinators through practices that protect or promote natural habitats. My study highlights the need to integrate honey bees with wild species so as to significantly increase the crop yields as well as farmers' profit. Previous studies highlighted the importance of conserving wild pollinators through maintaining heterogeneous natural habitats, which can be an excellent strategy to conserve the abundance of wild pollinators around orchards (Woodcock et al., 2019; Klein et al., 2012; Potts et al., 2016). Although my focus was on avocado, this study has important application value for other crops, as there is still limited data estimating the pollination efficiency of pollinators for different crops (Rader et al., 2009; Artz and Nault, 2011; Jauker et al., 2012; Nurul et al., 2015). Therefore, more studies are needed to investigate pollination efficiency for the common flower visiting species and on different crops.

5. 5 General conclusion

Based on the findings of this study, it can be concluded that avocado is an insect-depended crop and pollination is limiting its fruit set. Furthermore, my results highlight the major importance of insect pollination for improved avocado fruit quality and thus marketability. Therefore, for optimal yields and increased quality, farmers should ensure sufficient abundance of pollinators in their orchards, through *A. mellifera* supplementation on farms, which is a potential option to increase fruit yield. Additionally, more sustainable approaches such as, managing wild pollinators through maintaining natural and semi-natural habitats will ensure persistence of pollinators required by avocado, and by providing both sufficient floral and nesting sites in and around the avocado orchards. Quality improvements of crops can greatly affect marketability and contribute to food security. Under the current situation of rapid increase in human population and global food demand, achieving high quality and quantity of crops is a pressing issue. My study suggests that comprehensive analyses of the benefits of pollination for insect-dependent crops, may clearly increase the economic value of this ecosystem service. Pollination appears to be economically much more important than previously recognized and needs better support through adequate agricultural management and appropriate policies. This study also highlighted the high efficiency of honey bees (*A. mellifera*) and hoverfly species (*Phytomia* *incisa*) as efficient avocado pollinators. Therefore, management to support these species will promote avocado crop pollination, hence increased avocado productivity. This study recommends that the assessments of pollination service and deficits in crops can be used to quantify the supply and demand for pollinators, and hence help the targeted local farmers to address these deficits.

References

Abrol, D. P. (2011). Foraging. In *Honeybees of Asia* (pp. 257-292). Springer, Berlin, Heidelberg.

Adan, M., Abdel-Rahman, E. M., Gachoki, S., Muriithi, B. W., Lattorff, H. M. G., Kerubo, V., ... & Dubois, T. (2021). Use of earth observation satellite data to guide the implementation of integrated pest and pollinator management (IPPM) technologies in an avocado production system. *Remote Sensing Applications: Society and Environment*, 100566. https://doi.org/10.1016/j.rsase.2021.100566.

Adebisi, M. A., Okelola, F. S., Ajala, M. O., Kehinde, T. O., Daniel, I. O., & Ajani, O. O. (2013). Evaluation of variations in seed vigour characters of West African rice (*Oryza sativa* L.) genotypes using multivariate technique. *American Journal of Plant Sciences*, 4, 356-363

Afik, O., Dag, A., Kerem, Z., & Shafir, S. (2006). Analyses of avocado (Persea americana) nectar properties and their perception by honey bees (*Apis mellifera*). *Journal of Chemical Ecology*, *32*(9), 1949-1963. https://doi.org/10.1007/s10886-006-9120-1

Afik, Ohad, Delaplane, K. S., Shafir, S., Moo-Valle, H., & Quezada-Euán, J. J. G. (2014). Nectar Minerals as Regulators of Flower Visitation in Stingless Bees and Nectar Hoarding Wasps. *Journal of Chemical Ecology, 40*(5), 476-483. https://doi.org/10.1007/s10886-014-0455-8

Aizen, M. A., Garibaldi, L. A., Cunningham, S. A., & Klein, A. M. (2009). How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Annals of Botany*, *103*(9), 1579–1588. https://doi.org/10.1093/aob/mcp076

Aizen, M. A., & Harder, L. D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Current biology*, *19*(11), 915-918.

Alexandratos, N. (2009). World food and agriculture to 2030/50. In *Highlights and views from MID-2009: Paper for the Expert Meeting on "How to Feed the World in* (Vol. 2050).

Alcaraz, M. L., & Hormaza, J. I. (2009). Avocado Pollination and Fruit Set – A Perspective from Spain. *California Avocado Society 2009 Yearbook*, *92*, 113–135.

Alcaraz, María L., & Hormaza, J. I. (2021). Fruit Set in Avocado: Pollen Limitation, Pollen Load Size, and Selective Fruit Abortion. *Agronomy*, *11*(8), 1603. https://doi.org/10.3390/agronomy11081603

Altendorf, S. (2019). Major Tropical Fruits Market Review 2017. FAO, Ed. FAO, Rome.Italy, pp. 1–10.

Amador, G. J., Matherne, M., Waller, D., Mathews, M., Gorb, S. N., & Hu, D. L. (2017). Honey bee hairs and pollenkitt are essential for pollen capture and removal. *Bioinspiration and Biomimetics*, *12*(2). https://doi.org/10.1088/1748-3190/aa5c6e

Amare, M., Mariara, J., Oostendorp, R., & Pradhan, M. (2019). The impact of smallholder farmers' participation in avocado export markets on the labor market, farm yields, sales prices, and incomes in Kenya. *Land Use Policy*, *88*, 104168. https://doi.org/10.1016/j.landusepol.

Amit, J., & Sreedevi, K. (2015). Impact of abiotic factors on foraging behaviour of major pollinators in sunflower ecosystem. *Asian Journal of Environmental Science*, *10*(1), 1-6.

Ariza, M. T., Soria, C., Medina, J. J., & Martínez-Ferri, E. (2011). Fruit misshapen in strawberry cultivars (Fragaria × ananassa) is related to achenes functionality. *Annals of Applied Biology*, *158*(1), 130–138. https://doi.org/10.1111/j.1744-7348.2010.00451.x

Archer, C. R., Pirk, C. W. W., Carvalheiro, L. G., & Nicolson, S. W. (2014). Economic and ecological implications of geographic bias in pollinator ecology in the light of pollinator declines. *Oikos*, *123*(4), 401-407.

Artz, D. R., & Nault, B. A. (2011). Performance of Apis mellifera, Bombus impatiens, and Peponapis pruinosa (Hymenoptera: Apidae) as pollinators of pumpkin. *Journal of Economic Entomology*, *104*(4), 1153-1161

Assessment, M. E. (2005). *Ecosystems and human well-being* (Vol. 5, p. 563). United States of America: Island press

Bain, L. E., Awah, P. K., Geraldine, N., Kindong, N. P., Sigal, Y., Bernard, N., & Tanjeko, A. T. (2013). Malnutrition in Sub - Saharan Africa: Burden, causes and prospects. *Pan African Medical Journal*, *15*, 1–9. https://doi.org/10.11604/pamj.2013.15.120.2535

Balfour, N. J., Gandy, S., & Ratnieks, F. L. W. (2015). Exploitative competition alters bee foraging and flower choice. *Behavioral Ecology and Sociobiology*, *69*(10), 1731–1738. https://doi.org/10.1007/s00265-015-1985-y

Bartomeus, I., Potts, S. G., Steffan-Dewenter, I., Vaissière, B. E., Woyciechowski, M., Krewenka, K. M., Tscheulin, T., Roberts, S. P. M., Szentgyörgyi, H., Westphal, C., & Bommarco, R. (2014). Contribution of insect pollinators to crop yield and quality varies with agricultural intensification. *PeerJ, 2*, e328. https://doi.org/10.7717/peerj.

Bartomeus, I., & Winfree, R. (2013). Pollinator declines: reconciling scales and implications for ecosystem services. *F1000Research,2*. https://doi.org/10.12688/f1000research.2-146.v1

Bartomeus, I., Cariveau, D. P., Harrison, T., & Winfree, R. (2018). On the inconsistency of pollinator species traits for predicting either response to land-use change or functional contribution. *Oikos*, *127*(2), 306-315.

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Beattie, A. J. (1971). A technique for the study of insect-borne pollen. *The Pan-Pacific Entomologist*, *47*(1), 82

Belsky, J., & Joshi, N. K. (2019). Impact of biotic and abiotic stressors on managed and feral bees. *Insects*, *10*(8). https://doi.org/10.3390/insects10080233

Bennett, A. (2010). The role of soil community biodiversity in insect biodiversity. *Insect Conservation and*

Diversity, *3*(3), 157–171. https://doi.org/10.1111/j.1752-4598.2010.00086.x

Bélanger, J., & Pilling, D. (2019). *The state of the world's biodiversity for food and agriculture*. Food and Agriculture Organization of the United Nations (FAO)

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science, 313*(5785), 351-354. https://doi.org/10.1126/science.1127863

Biesmeijer, J. C., Sorensen, P. B., & Carvalheiro, L. G. (2011). How pollination ecology research can help answer important questions. *Journal of Pollination Ecology*, *4*(9), 68-73.

Bommarco, R., Kleijn, D., & Potts, S. G. (2013). Ecological intensification: Harnessing ecosystem services for food security. In *Trends in Ecology and Evolution, 28*(4), 230-238. https://doi.org/10.1016/j.tree.2012.10.012

Bommarco, R., Marini, L., & Vaissière, B. E. (2012). Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia, 169*(4), 1025-1032. https://doi.org/10.1007/s00442-012-2271-6

Bhuyan, D. J., Alsherbiny, M. A., Perera, S., Low, M., Basu, A., Devi, O. A., Barooah, M. S., Li, C. G., & Papoutsis, K. (2019). The odyssey of bioactive compounds in Avocado (Persea Americana) and their health benefits. *Antioxidants*, *8*(10). https://doi.org/10.3390/antiox8100426

Blaauw, B. R., & Isaacs, R. (2014). Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. *Journal of Applied Ecology*, *51*(4), 890–898. https://doi.org/10.1111/1365-2664.12257

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.

https://doi.org/10.1016/0003-2697(76)90527-3

Breeze, T. D., Bailey, A. P., Balcombe, K. G., & Potts, S. G. (2014). Costing conservation: An expert appraisal of the pollinator habitat benefits of England's entry level stewardship. *Biodiversity and Conservation*, *23*(5), 1193–1214. https://doi.org/10.1007/s10531-014-0660-3

Brittain, C., Williams, N., Kremen, C., & Klein, A. M. (2013). Synergistic effects of non-Apis bees and honey bees for pollination services. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1754). https://doi.org/10.1098/rspb.2012.2767

Brussaard, L., Caron, P., Campbell, B., Lipper, L., Mainka, S., Rabbinge, R., Babin, D., & Pulleman, M. (2010). Reconciling biodiversity conservation and food security: Scientific challenges for a new agriculture. *Current Opinion in Environmental Sustainability*, *2*(1–2), 34–42. https://doi.org/10.1016/j.cosust.2010.03.007

Buri, P., Humbert, J. Y., & Arlettaz, R. (2014). Promoting pollinating insects in intensive agricultural matrices: Field-scale experimental manipulation of hay-meadow mowing regimes and its effects on bees. *PloS one*, *9*(1), e85635. https://doi.org/10.1371/journal.pone.0085635

Burkle, L. A., & Alarcón, R. (2011). The future of plant-pollinator diversity: Understanding interaction networks acrosss time, space, and global change. *American Journal of Botany*, *98*(3), 528–538. https://doi.org/10.3732/ajb.1000391

Burkle, L. A., Marlin, J. C., & Knight, T. M. (2013). Plant-pollinator interactions over 120 years: Loss of species, co-occurrence, and function. *Science*, *339*(6127), 1611-1615. https://doi.org/10.1126/science.1232728

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences, 108*(2), 662. https://doi.org/10.1073/pnas.1014743108

Cameron, T. C., Wearing, H. J., Rohani, P., & Sait, S. M. (2007). Two-species asymmetric competition: Effects of age structure on intra- and interspecific interactions. *Journal of Animal Ecology*, *76*(1), 83–93. https://doi.org/10.1111/j.1365-2656.2006.01185.x

Can-Alonzo, C., Quezada-Euán, J. J. G., Xiu-Ancona, P., Moo-Valle, H., Valdovinos-Nunez, G. R., & Medina-Peralta, S. (2005). Pollination of 'criollo' avocados (*Persea americana*) and the behaviour of associated bees in subtropical Mexico. *Journal of Apicultural Research*, *44*(1), 3–8. https://doi.org/10.1080/00218839.2005.11101138

Carré, G., Roche, P., Chifflet, R., Morison, N., Bommarco, R., Harrison-Cripps, J., Krewenka, K., Potts, S. G., Roberts, S. P. M., Rodet, G., Settele, J., Steffan-Dewenter, I., Szentgyörgyi, H., Tscheulin, T., Westphal, C., Woyciechowski, M., & Vaissière, B. E. (2009). Landscape context and habitat type as drivers of bee diversity in European annual crops. *Agriculture, Ecosystems and Environment*, *133*(1-2), 40-47. https://doi.org/10.1016/j.agee.2009.05.001

Carrié, R., Ekroos, J., & Smith, H. G. (2018). Organic farming supports spatiotemporal stability in species richness of bumblebees and butterflies. *Biological Conservation*, *227*, 48–55. https://doi.org/10.1016/j.biocon.2018.08.022

Carvajal-Zarrabal, O., Nolasco-Hipolito, C., Aguilar-Uscanga, M. G., Melo-Santiesteban, G., Hayward-Jones, P. M., & Barradas-Dermitz, D. M. (2014). Avocado oil supplementation modifies cardiovascular risk profile markers in a rat model of sucrose-induced metabolic changes. *Disease Markers*, *2014*. https://doi.org/10.1155/2014/386425

Carvalheiro, Luísa G., Seymour, C. L., Nicolson, S. W., & Veldtman, R. (2012). Creating patches of native flowers facilitates crop pollination in large agricultural fields: Mango as a case study. *Journal of Applied Ecology*, *49*(6), 1373–1383. https://doi.org/10.1111/j.1365-2664.2012.02217.x

Carvalheiro, Luísa G., Seymour, C. L., Veldtman, R., & Nicolson, S. W. (2010). Pollination services decline with distance from natural habitat even in biodiversity-rich areas. *Journal of Applied Ecology*, *47*, 810–820. https://doi.org/10.1111/j.1365-2664.2010.01829.x

Carvalheiro, Luísa Gigante, Veldtman, R., Shenkute, A. G., Tesfay, G. B., Pirk, C. W. W., Donaldson, J. S., & Nicolson, S. W. (2011). Natural and within-farmland biodiversity enhances crop productivity. *Ecology Letters*, *14*(3), 251-259. https://doi.org/10.1111/j.1461-0248.2010.01579.x

Carvell, C., Jordan, W. C., Bourke, A. F. G., Pickles, R., Redhead, J. W., & Heard, M. S. (2012). Molecular and spatial analyses reveal links between colony-specific foraging distance and landscape-level resource availability in two bumblebee species. *Oikos*, *121*(5), 734-742. https://doi.org/10.1111/j.1600-0706.2011.19832.x

Codex Stan 197-1995 standards for avocado (available at http://www.fao.org/input/download/standards/321/CXS_197e.pdf. June, 2021)

Chaplin-Kramer, R., Dombeck, E., Gerber, J., Knuth, K. A., Mueller, N. D., Mueller, M., Ziv, G., & Klein, A. M. (2014). Global malnutrition overlaps with pollinator-dependent micronutrient production. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1794). https://doi.org/10.1098/rspb.2014.1799

Chen, H., Morrell, P. L., Ashworth, V. E. T. M., De La Cruz, M., & Clegg, M. T. (2009). Tracing the geographic origins of major avocado cultivars. *Journal of Heredity*, *100*(1), 56-65. https://doi.org/10.1093/jhered/esn068

Cheng, W., & Gershenson, A. (2007). Carbon Fluxes in the Rhizosphere. In *The Rhizosphere* (pp. 31-56). Academic Press. https://doi.org/10.1016/B978-012088775-0/50004-5

Chiawo, D. O., Kioko, E. N., Ogol, C. K., & Gikungu, M. W. (2011). Bee diversity and floral resources along a disturbance gradient in Kaya Muhaka forest and surrounding farmlands of coastal Kenya.

Chifflet, R., Klein, E. K., Lavigne, C., Le Féon, V., Ricroch, A. E., Lecomte, J., & Vaissière, B. E. (2011). Spatial scale of insect-mediated pollen dispersal in oilseed rape in an open agricultural landscape.

Journal of Applied Ecology, *48*(3), 689-696. https://doi.org/10.1111/j.1365-2664.2010.01904.x

Classen, A., Peters, M. K., Ferger, S. W., Helbig-Bonitz, M., Schmack, J. M., Maassen, G., Schleuning, M., Kalko, E. K. V., Böhning-Gaese, K., & Steffan-Dewenter, I. (2014). Complementary ecosystem services provided by pest predators and pollinators increase quantity and quality of coffee yields. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1779). https://doi.org/10.1098/rspb.2013.3148

Classen, A., Peters, M. K., Kindeketa, W. J., Appelhans, T., Eardley, C. D., Gikungu, M. W., Hemp, A., Nauss, T., & Steffan-Dewenter, I. (2015). Temperature versus resource constraints: Which factors determine bee diversity on Mount Kilimanjaro, Tanzania? *Global Ecology and Biogeography*, *24*(6), 642–652. https://doi.org/10.1111/geb.12286

Dainese, M., Martin, E. A., Aizen, M. A., Albrecht, M., Bommarco, R., Carvalheiro, L. G., Chaplin-kramer, R., Garibaldi, L. A., Ghazoul, J., Grab, H., Jonsson, M., Daniel, S., Andersson, G. K. S., Badenhausser, I., Baensch, S., Ekroos, J., Fijen, T., Franck, P., Freitas, B. M., … Björn, K. (2019). *A global synthesis reveals biodiversity-mediated benefits for crop production*. *5*(10), 1–19.

Dan, S., Murungi, L. K., & Kioko, E. (2019). Diversity and abundance of insect pollinators and their effect on yield and quality of cowpea and cucumber in Makueni, Kenya. *African Journal of Horticultural Science*, *16*, 43-54.

Davenport, T. L. (2011). Avocado Flowering. *Horticultural Reviews*, *8*(6668), 257–289. https://doi.org/10.1002/9781118060810.ch7

Davenport, T.L. (1998). What if there is no pollinator? *Subtrop. Fruit News*, 6, pp.15-17

Delaplane, K. S., Mayer, D. R., & Mayer, D. F. (2000). *Crop pollination by bees*. Cabi

Del Toro-Equihua, M., Velasco-Rodríguez, R., López-Ascencio, R., & Vásquez, C. (2016). Effect of an avocado oil-enhanced diet (Persea americana) on sucrose-induced insulin resistance in Wistar rats. *Journal of Food and Drug Analysis*, *24*(2), 350–357. https://doi.org/10.1016/j.jfda.2015.11.005

De Vega, C., Herrera, C. M., & Dötterl, S. (2014). Floral volatiles play a key role in specialized ant pollination. *Perspectives in Plant Ecology, Evolution and Systematics*, *16*(1), 32-42

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J. L., & Alaux, C. (2013). Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? *PloS one*, *8*(8), e72016. https://doi.org/10.1371/journal.pone.0072016

Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., & Wilson, K. (2014). Honeybee nutrition is linked to landscape composition. *Ecology and Evolution*, *4*(21), 4195-4206. https://doi.org/10.1002/ece3.1293

Dos Santos, M. A. Z., Alicieo, T. V. R., Pereira, C. M. P., Ramis-Ramos, G., & Mendonça, C. R. B. (2014). Profile of bioactive compounds in avocado pulp oil: Influence of the drying processes and extraction methods. *JAOCS, Journal of the American Oil Chemists' Society*, *91*(1), 19-27. https://doi.org/10.1007/s11746-013-2289-x

Dicks, L., Breeze, T., Ngo, H., Senapathi, D., An, J., Aizen, M., ... & Potts, S. (2020). A global assessment of drivers and risks associated with pollinator decline

Duarte, P. F., Chaves, M. A., Borges, C. D., & Mendonça, C. R. B. (2017). Avocado: Characteristics, health benefits, and uses. *International News on Fats, Oils and Related Materials*, *28*(3), 28–32. https://doi.org/10.1590/0103-8478cr20141516

Durant, J. L., & Ponisio, L. C. (2021). A regional, honey bee-centered approach is needed to incentivize grower adoption of bee-friendly practices in the almond industry. *Frontiers in Sustainable Food Systems*, 261.

Dreher, M. L., & Davenport, A. J. (2013). Hass avocado composition and potential health effects. *Critical reviews in food science and nutrition*, *53*(7), 738-750

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

Eilers, E. J., Kremen, C., Greenleaf, S. S., Garber, A. K., & Klein, A. M. (2011). Contribution of pollinator-mediated crops to nutrients in the human food supply. *PLoS ONE*, *6*(6). https://doi.org/10.1371/journal.pone.0021363

Ellis, A. M., Myers, S. S., & Ricketts, T. H. (2015). Do pollinators contribute to nutritional health? *PLoS One*, *10*(1), e114805. https://doi.org/10.1371/journal.pone.0114805

Engel, E. C., & Irwin, R. E. (2003). Linking pollinator visitation rate and pollen receipt. *American Journal of Botany*, *90*(11), 1612–1618. https://doi.org/10.3732/ajb.90.11.1612

Evans, L.J. & Goodwin, R.M. (2011). The role of insect pollinators in avocado (*Persea americana*) pollination in New Zealand and Australia. In *Proceedings. VII World Avocado Congress*. (5 –9 September) pp. 7 Cairns, Australia.

Evans, L. J., Goodwin, R. M., & Howlett, B. G. (2011). The role of insect pollinators in avocado (*Persea americana*) pollination in New Zealand and Australia. In *Proceedings VII World Avocado Congress* (p. 7).

FAO, F. (2017). The future of food and agriculture–Trends and challenges. *Annual Report*, *296*.

FAO (2020). Major tropical fruits - Preliminary market results 2019. Rome, 24

FAOSTAT. (2021). Food and Agriculture Organization of the United Nations (FAO). Food and Agriculture Organization. https://www.fao.org/faostat/en/#home

Fess, T. L., & Benedito, V. A. (2018). Organic versus conventional cropping sustainability: A comparative system analysis. *Sustainability (Switzerland)*, *10*(1). https://doi.org/10.3390/su10010272

Fischer, J., Abson, D. J., Butsic, V., Chappell, M. J., Ekroos, J., Hanspach, J., Kuemmerle, T., Smith, H.

G., & von Wehrden, H. (2014). Land sparing versus land sharing: Moving forward. In *Conservation Letters*, *7*(3), 149-157. https://doi.org/10.1111/conl.12084

Fishbein, M., & Venable, D. L. (1996). Diversity and temporal change in the effective pollinators of Asclepias tuberosa. *Ecology*, *77*(4), 1061-1073

Forero-Doria, O., García, M. F., Vergara, C. E., & Guzman, L. (2017). Thermal analysis and antioxidant activity of oil extracted from pulp of ripe avocados. *Journal of Thermal Analysis and Calorimetry*, *130*(2), 959–966. https://doi.org/10.1007/s10973-017-6488-9

Földesi, R., Kovács-Hostyánszki, A., Kőrösi, Á., Somay, L., Elek, Z., Markó, V., ... & Báldi, A. (2016). Relationships between wild bees, hoverflies and pollination success in apple orchards with different landscape contexts. *Agricultural and Forest Entomology*, *18*(1), 68-75

Fumero-Cabán, J. J., & Meléndez-Ackerman, E. J. (2007). Relative pollination effectiveness of floral visitors of Pitcairnia angustifolia (Bromeliaceae). *American Journal of Botany*, *94*(3), 419-424

Flohre, A., Fischer, C., Aavik, T., Bengtsson, J., Berendse, F., Bommarco, R., Ceryngier, P., Clement, L. W., Dennis, C., Eggers, S., Emmerson, M., Geiger, F., Guerrero, I., Hawro, V., Inchausti, P., Liira, J., Morales, M. B., Oñate, J. J., Pärt, T., … Tscharntke, T. (2011). Agricultural intensification and biodiversity partitioning in European landscapes comparing plants, carabids, and birds. *Ecological Applications*, *21*(5), 1772–1781. https://doi.org/10.1890/10-0645.1

Freitas, B.M., Vaissiére, B.E., Saraiva, A.M., Carvalheiro, L.G., Garibaldi, L.A., Ngo, H. (2016). Identifying and assessing pollination deficits in crops. in: Gemmill-Herren, B. (Ed.), Pollination services to agriculture. FAO with Routledge, New York, USA. pp. 17–33. https://doi.org/10.4324/9781315696355

Free, J. B. (1993). Insect pollination of crops 2nd edAcademic Press. *San Diego, California*.

Fries, I., & Stark, J. (1983). Measuring the importance of honeybees in rape seed production. *Journal of*

Apicultural Research, *22*(4), 272–276. https://doi.org/10.1080/00218839.1983.11100599

Gajc-Wolska, J., Kowalczyk, K., Mikas, J., & Drajski, R. (2011). Efficiency of cucumber (*Cucumis sativus* L.) pollination by bumblebees (*Bombus terrestris*). *Acta Sci. Pol*, *10*, 159-169

Gallai, N., Salles, J. M., Settele, J., & Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, *68*(3), 810-821. https://doi.org/10.1016/j.ecolecon.2008.06.014

Garibaldi, L. A., Carvalheiro, L. G., Leonhardt, S. D., Aizen, M. A., Blaauw, B. R., Isaacs, R., ... & Winfree, R. (2014). From research to action: enhancing crop yield through wild pollinators. *Frontiers in Ecology and the Environment*, *12*(8), 439-447. https://doi.org/10.1890/130330

Garibaldi, L. A., Steffan-Dewenter, I., Kremen, C., Morales, J. M., Bommarco, R., Cunningham, S. A., ... & Klein, A. M. (2011). Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecology letters*, *14*(10), 1062-1072. https://doi.org/10.1111/j.1461-0248.2011.01669.x

Garibaldi, L. A., Aizen, M. A., Klein, A. M., Cunningham, S. A., & Harder, L. D. (2011). Global growth and stability of agricultural yield decrease with pollinator dependence. *Proceedings of the National Academy of Sciences*, *108*(14), 5909-5914. https://doi.org/10.1073/pnas.1012431108

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., Kremen, C., Carvalheiro, L. G., Harder, L. D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N. P., Dudenhöffer, J. H., Freitas, B. M., Ghazoul, J., Greenleaf, S., … Klein, A. M. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, *340*(6127), 1608–1611. https://doi.org/10.1126/science.1230200

Garner, L. C., & Lovatt, C. J. (2008). The relationship between flower and fruit abscission and alternate bearing of 'Hass' avocado. *Journal of the American Society for Horticultural Science*, *133*(1), 3-10.

Garner, L. C., & Lovatt, C. J. (2016). Physiological factors affecting flower and fruit abscission of "Hass" avocado. *Scientia Horticulturae*, *199*, 32–40. https://doi.org/10.1016/j.scienta.2015.12.009

Garratt, M. P., de Groot, G. A., Albrecht, M., Bosch, J., Breeze, T. D., Fountain, M. T., ... & Zhusupbaeva, A. (2021). Opportunities to reduce pollination deficits and address production shortfalls in an important insect pollinated crop. *Ecological Applications*, e02445.

Gazit, S., & Degani, C. (2002). Reproductive biology. *The avocado: Botany, production and uses*, 101-133.

Geslin, B., Aizen, M. A., Garcia, N., Pereira, A. J., Vaissière, B. E., & Garibaldi, L. A. (2017). The impact of honey bee colony quality on crop yield and farmers' profit in apples and pears. *Agriculture, ecosystems & environment*, *248*, 153-161. https://doi.org/10.1016/j.agee.2017.07.035

Geldmann, J., & González-Varo, J. P. (2018). Conserving honey bees does not help wildlife. *Science*, *359*(6374), 392–393. https://doi.org/10.1126/science.aar2269

Gemmill-Herren, B., Aidoo, K., Kwapong, P., Martins, D., Kinuthia, W., Gikungu, M., & Eardley, C. (2014). Priorities for research and development in the management of pollination services for agriculture in Africa. *Journal of Pollination Ecology*, *12*

Gérard, M., Vanderplanck, M., Wood, T., & Michez, D. (2020). Global warming and plant–pollinator mismatches. *Emerging topics in life sciences*, *4*(1), 77-86.

Giovanetti, M., Albertazzi, S., Flaminio, S., Ranalli, R., Bortolotti, L., & Quaranta, M. (2021). Pollination in agroecosystems: A review of the conceptual framework with a view to sound monitoring. *Land*, *10*(5), 540

Gossner, M. M., Lewinsohn, T. M., Kahl, T., Grassein, F., Boch, S., Prati, D., Birkhofer, K., Renner, S. C., Sikorski, J., Wubet, T., Arndt, H., Baumgartner, V., Blaser, S., Blüthgen, N., Börschig, C., Buscot, F., DIekötter, T., Jorge, L. R., Jung, K., … Allan, E. (2016). Land-use intensification causes multitrophic homogenization of grassland communities. *Nature*, *540*(7632), 266–269.

https://doi.org/10.1038/nature20575

Goulnik, J., Plantureux, S., Van Reeth, C., Baude, M., Mesbahi, G., & Michelot-Antalik, A. (2020). Facial area and hairiness of pollinators visiting semi-natural grassland wild plants predict their facial pollen load. *Ecological Entomology*, *45*(6), 1296-1306

Göpel, J., Schüngel, J., Stuch, B., & Schaldach, R. (2020). Assessing the effects of agricultural intensification on natural habitats and biodiversity in Southern Amazonia. *PloS one*, *15*(11), e0225914. https://doi.org/10.1371/journal.pone.0225914

Grass, I., Meyer, S., Taylor, P. J., Foord, S. H., Hajek, P., & Tscharntke, T. (2018). Pollination limitation despite managed honeybees in South African macadamia orchards. *Agriculture, Ecosystems & Environment*, *260*, 11-18. https://doi.org/10.1016/j.agee.2018.03.010

Graystock, P., Blane, E. J., McFrederick, Q. S., Goulson, D., & Hughes, W. O. H. (2016). Do managed bees drive parasite spread and emergence in wild bees? *International Journal for Parasitology: Parasites and Wildlife*, *5*(1), 64–75. https://doi.org/10.1016/j.ijppaw.2015.10.001

Greenleaf, S. S., & Kremen, C. (2006). Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences*, *103*(37), 13890-13895

Griesbach, J., 1985. The avocado industry in Kenya. In X African Symposium on horticultural crops 158 Jan 16 (pp. 87-92) Addis Ababa, Ethiopia. Doi: 10.17660/ActaHortic.1985.158.9

Griesbach, J., 2005. Avocado growing in Kenya. World Agroforestry Centre (ICRAF). Kul Graphics Ltd. Nairobi.

Gruber, K., Schöning, C., Otte, M., Kinuthia, W., & Hasselmann, M. (2013). Distinct subspecies or phenotypic plasticity? Genetic and morphological differentiation of mountain honey bees in East Africa. *Ecology and evolution*, *3*(10), 3204-3218. https://doi.org/10.1002/ece3.711

Hagler, J. R., Mueller, S., Teuber, L. R., MacHtley, S. A., & Van Deynze, A. (2011). Foraging range of honey bees, Apis mellifera, in alfalfa seed production fields. *Journal of Insect Science*, *11*(144), 1–12. https://doi.org/10.1673/031.011.14401

Hakizimana, C., & May, J. (2018). Can smallholder avocado production reduce poverty and improve food security through internal markets? The case of Giheta, Burundi. *Forests Trees and Livelihoods*, *27*(4), 203–216. https://doi.org/10.1080/14728028.2018.1515041

Hammer, o., Harper, d. a., & Ryan, p. d. (2001). Palaeontological statistics software package for education and data analysis. *Palaeontol. Electron*, *4*(9)

Hardman, C. J., Harrison, D. P. G., Shaw, P. J., Nevard, T. D., Hughes, B., Potts, S. G., & Norris, K. (2016). Supporting local diversity of habitats and species on farmland: A comparison of three wildlife-friendly schemes. *Journal of Applied Ecology*, *53*(1), 171–180. https://doi.org/10.1111/1365-2664.12557

Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, 2, e616. https://doi.org/10.7717/peerj.616

Hole, D. G., Huntley, B., Arinaitwe, J., Butchart, S. H., Collingham, Y. C., Fishpool, L. D., ... & Willis, S. G. (2011). Toward a management framework for networks of protected areas in the face of climate change. *Conservation Biology*, *25*(2), 305-315. https://doi.org/10.1111/j.1523-1739.2010.01633.x

Holzschuh, A., Dainese, M., González-Varo, J. P., Mudri-Stojnić, S., Riedinger, V., Rundlöf, M., ... & Steffan-Dewenter, I. (2016). Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecology letters*, *19*(10), 1228-1236. https://doi.org/10.1111/ele.12657

Hipólito, J., Boscolo, D., & Viana, B. F. (2018). Landscape and crop management strategies to conserve pollination services and increase yields in tropical coffee farms. *Agriculture, Ecosystems & Environment*, *256*, 218-225

HCDA, 2017. Horticultural Crops Directorate Validated Report 2016 –2017. *Agriculture and Food Authority (AFA),* Nairoi, Kenya

Ish-am, G., Barrientos-Priego, F., Castaneda-Vildozola, A., & Gazit, S. (1999). Avocado (Persea americana Mill.) pollinators in its region of origin. *Revista Chapingo Serie Horticultura*, *5*, 137–143

Ish-am, G., Barrientos-Priego, F., Castaneda-Vildozola, A., & Gazit, S. (2005). Avocado (*Persea americana* Mill.) pollinators in its region of origin. *Revista Chapingo Serie Horticultura*

Ish-Am, G., & Lahav, E. (2011). Evidence for a major role of honeybees (*Apis mellifera*) rather than wind during avocado (*Persea americana* Mill.) pollination. *Journal of Horticultural Science and Biotechnology*, *86*(6), 589–594. https://doi.org/10.1080/14620316.2011.11512808

Ish-Am, G. (2005). Avocado pollination: A review. *New Zealand*. https://doi.org/10.1016/0921-5093(93)90309-3

Ish-Am, G., & Eisikowitch, D. (1990). Possible routes of avocado tree pollination by honeybees. In *VI International Symposium on Pollination 288* (pp. 225-233)

Ish-Am, G., & Eisikowitch, D. (1993). The behaviour of honey bees (*Apis mellifera*) visiting avocado (*Persea americana*) flowers and their contribution to its pollination. *Journal of Apicultural Research*, *32*(3-4), 175-186

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., ... & Vanbergen, A. J. (2016). *The assessment report on pollinators, pollination and food production: summary for policymakers*. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services

Jauker, F., & Wolters, V. (2008). Hover flies are efficient pollinators of oilseed rape. *Oecologia*, *156*(4), 819-823. https://doi.org/10.1007/s00442-008-1034-x

Jauker F, Bondarenko B, Becker HC, Steffan-Dewenter I (2012) Pollination efficiency of wild bees and hoverflies provided to oilseed rape. Agricultural and Forest Entomology 14: 81-87

Jeliazkov, A., Mimet, A., Chargé, R., Jiguet, F., Devictor, V., & Chiron, F. (2016). Impacts of agricultural intensification on bird communities: New insights from a multi-level and multi-facet approach of biodiversity. *Agriculture, Ecosystems & Environment*, *216*, 9-22. https://doi.org/10.1016/j.agee.2015.09.017

Johnny, E. G., Mariara, J. K., Mulwa, R., & Ruigu, G. M. (2019). Smallholder avocado contract farming in Kenya: determinants and differentials in outcomes. *African Journal of Economic Review*, *7*(2), 91-112. https://www.ajol.info/index.php/ajer/article/view/188379

Karp, D. S., Mendenhall, C. D., Sandí, R. F., Chaumont, N., Ehrlich, P. R., Hadly, E. A., & Daily, G. C. (2013). Forest bolsters bird abundance, pest control and coffee yield. *Ecology Letters*, *16*(11), 1339–1347. https://doi.org/10.1111/ele.12173

Kasina, M, Nderitu, J., Nyamasyo, G., & Oronje, M. (2007). Sunflower pollinators in Kenya: Does diversity influence seed yield? *African Crop Science Conference Proceedings*, *8*, 1149–1153. http://www.cabi.org/ISC/FullTextPDF/2010/20103268420.pdf

Kasina, M., Kraemer, M., Martius, C., & Wittmann, D. (2009). Diversity and activity density of bees visiting crop flowers in Kakamega, Western Kenya. *Journal of Apicultural Research*, *48*(2), 134-139. https://doi.org/10.3896/ibra.1.48.2.08

Kenya National Bureau of Statistics. (2019). 2019 Kenya Population and Housing Census Volume II: Distribution of Population by Administrative Units

Keys, R. N., Buchmann, S. L., & Smith, S. E. (1995). Pollination effectiveness and pollination efficiency of insects foraging Prosopis velutina in south-eastern Arizona. *Journal of Applied Ecology*, 519-527

King, C., Ballantyne, G., & Willmer, P. G. (2013). Why flower visitation is a poor proxy for pollination: Measuring single-visit pollen deposition, with implications for pollination networks and conservation.

Methods in Ecology and Evolution, *4*(9), 811–818. https://doi.org/10.1111/2041-210X.12074

Koh, I., Lonsdorf, E. V., Williams, N. M., Brittain, C., Isaacs, R., Gibbs, J., & Ricketts, T. H. (2016). Modeling the status, trends, and impacts of wild bee abundance in the United States. *Proceedings of the National Academy of Sciences*, *113*(1), 140-145

Khan, M., Mahmood, H. Z., & Damalas, C. A. (2015). Pesticide use and risk perceptions among farmers in the cotton belt of Punjab, Pakistan. *Crop Protection*, *67*, 184-190. https://doi.org/10.1016/j.cropro.2014.10.013

Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., & Tscharntke, T. (2013). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1775). https://doi.org/10.1098/rspb.2013.2440

Klein, A. M., Steffan-Dewenter, I., & Tscharntke, T. (2003). Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology*, *40*(5), 837-845.https://doi.org/10.1046/j.1365-2664.2003.00847.x

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Klein, A. M., Brittain, C., Hendrix, S. D., Thorp, R., Williams, N., & Kremen, C. (2012). Wild pollination services to California almond rely on semi-natural habitat. *Journal of Applied Ecology*, *49*(3), 723-732.

Krause, G. L., & Wilson, W. T. (1981). Honey bee pollination and visitation patterns on hybrid oilseed sunflowers in central Wyoming (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 75-82

Kremen, C., Williams, N. M., Aizen, M. A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., ... & Ricketts, T. H. (2007). Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology letters*, *10*(4), 299-314. https://doi.org/10.1111/j.1461-0248.2007.01018.x

Kremer-Köhne, S., & Köhne, J. S. (1995). Approaches to solving the Hass small fruit problem: progress report. *South African Avocado Growers' Association Yearbook*, *18*, 59-60

Lee, S. K., Young, R. E., Schiffman, P. M., & Coggins, C. W. (1983). Maturity Studies of Avocado Fruit Based on Picking Dates and Dry Weight. *J. Amer. Soc. Hort. Sci*, *108*(3), 390–394

Lee, T. M., & Jetz, W. (2008). Future battlegrounds for conservation under global change. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1640), 1261-1270. https://doi.org/10.1098/rspb.2007.1732

Lenth, R. V. (2016). Least-squares means: The R package lsmeans. *Journal of Statistical Software*, *69*(1). https://doi.org/10.18637/jss.v069.i01

Liu, R., Chen, D., Luo, S., Xu, S., Xu, H., Shi, X., & Zou, Y. (2020). Quantifying pollination efficiency of flower-visiting insects and its application in estimating pollination services for common buckwheat. *Agriculture, Ecosystems and Environment*, *301*, 107011. https://doi.org/10.1016/j.agee.2020.107011

Liliane, T. N., & Charles, M. S. (2020). Factors affecting yield of crops. *Agronomy-Climate Change & Food Security*, 9

Lye, G. C., Jennings, S. N., Osborne, J. L., & Goulson, D. (2011). Impacts of the Use of Nonnative Commercial Bumble Bees for Pollinator Supplementation in Raspberry. *Journal of Economic Entomology*, *104*(1), 107-114. https://doi.org/10.1603/EC10092

Manaf, Y. N., Rahardjo, A. P., Yusof, Y. A., Desa, M. N. M., & Nusantoro, B. P. (2018). Lipid characteristics and tocopherol content of the oils of native avocado cultivars grown in Indonesia. *International Journal of Food Properties*, *21*(1), 2758–2771. https://doi.org/10.1080/10942912.2018.1564761

Margetts, J. (2009). *Market Opportunities for Avocados Beyond Fresh Fruit Sales*. Horticulture Australia

Marini, L., Quaranta, M., Fontana, P., Biesmeijer, J. C., & Bommarco, R. (2012). Landscape context and elevation affect pollinator communities in intensive apple orchards. *Basic and Applied Ecology*, *13*(8), 681–689. https://doi.org/10.1016/j.baae.2012.09.003

Martins, D. J. (2013). People, plants and pollinators: uniting conservation, food security, and sustainable agriculture in East Africa. *Conservation biology: voices from the tropics*, *27*, 232-238.

Martins, K. T., Gonzalez, A., & Lechowicz, M. J. (2015). Pollination services are mediated by bee functional diversity and landscape context. *Agriculture, Ecosystems and Environment.*, *200*, 12-20. https://doi.org/10.1016/j.agee.2014.10.018

Martin, E. A., Dainese, M., Clough, Y., Báldi, A., Bommarco, R., Gagic, V., Garratt, M. P. D., Holzschuh, A., Kleijn, D., Kovács-Hostyánszki, A., Marini, L., Potts, S. G., Smith, H. G., Al Hassan, D., Albrecht, M., Andersson, G. K. S., Asís, J. D., Aviron, S., Balzan, M. V., … Steffan-Dewenter, I. (2019). The interplay of landscape composition and configuration: new pathways to manage functional biodiversity and agroecosystem services across Europe. *Ecology Letters*, *22*(7), 1083–1094. https://doi.org/10.1111/ele.13265

Massimi, M. (2018). Impact of seed size on seeds viability, vigor and storability of Hordeum vulgare (L.). *Agricultural Science Digest-A Research Journal*, *38*(1), 62-64

Matsumoto, S., Soejima, J., & Maejima, T. (2012). Influence of repeated pollination on seed number and fruit shape of "Fuji" apples. *Scientia Horticulturae*, *137*, 131–137. https://doi.org/10.1016/j.scienta.2012.01.033

Me, G. T., & Arzate-Fernández, A. M. (2010). West Indian avocado: where did it originate?. *Phyton*, *79*, 203

Melin, A., Rouget, M., Midgley, J. J., & Donaldson, J. S. (2014). Pollination ecosystem services in South African agricultural systems. *South African Journal of Science*, *110*(11-12), 01-09. https://doi.org/10.1590/sajs.2014/20140078

Migliore, G., Farina, V., Dara Guccione, G., & Schifani, G. (2018). Quality determinants of avocado fruit consumption in italy. Implications for small farms. *Quality - Access to Success*, *19*(163), 148–153

Millenium Ecosystem Assessment. (2005). Ecosystems and Human Well-Being: Biodiversity Synthesis. In *British Cataloguing*. https://doi.org/10.1057/9780230625600

Mitchell, R. J., Flanagan, R. J., Brown, B. J., Waser, N. M., & Karron, J. D. (2009). New frontiers in competition for pollination. *Annals of botany*, *103*(9), 1403-1413

Morales, C. L., & Traveset, A. (2008). Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences*, *27*(4), 221-238

Moreira, E. F., Boscolo, D., & Viana, B. F. (2015). Spatial heterogeneity regulates plant-pollinator networks across multiple landscape scales. *PloS one*, *10*(4), e0123628. https://doi.org/10.1371/journal.pone.0123628

Mogren, C. L., Rand, T. A., Fausti, S. W., & Lundgren, J. G. (2016). The effects of crop intensification on the diversity of native pollinator communities. *Environmental entomology*, *45*(4), 865-872

Mulwa, J., Kahuthia-Gathu, R., & Kasina, M. (2019). Avocado (*Persea americana*) yield as influenced by pollinators in Murang'a County, Kenya. *Journal of Agricultural Research Advances*, *1*(3), 34-41

Murang'a. County., 2018. Murang'a County Integrated Development Plan 2018–2022, Muranga.county, Muranga, Kenya

National Research Council. (2007). *Status of pollinators in North America*. National Academies Press. https://doi.org/10.17226/11761

Ne'eman, G., Jürgens, A., Newstrom-Lloyd, L., Potts, S. G., & Dafni, A. (2010). A framework for comparing pollinator performance: Effectiveness and efficiency. *Biological Reviews*, *85*(3), 435–451. https://doi.org/10.1111/j.1469-185X.2009.00108

Novais, S. M., Nunes, C. A., Santos, N. B., DAmico, A. R., Fernandes, G. W., Quesada, M., ... & Neves, A. C. O. (2018). Correction: Effects of a Possible Pollinator Crisis on Food Crop Production in Brazil. *Plos one*, *13*(5), e0197396

Nderitu, J., Kasina, M., Nyamasyo, G., & Oronje, M. L. (2007). Effects of insecticide applications on sunflower (*Helianthus annuus* L.) pollination in Eastern Kenya. *World Journal of Agricultural Sciences*, *3*(6), 731-734

Odanga, J. J., Mohamed, S., Mwalusepo, S., Olubayo, F., Nyankanga, R., Khamis, F., ... & Ekesi, S. (2018). Spatial distribution of Bactrocera dorsalis and Thaumatotibia leucotreta in smallholder avocado orchards along altitudinal gradient of Taita Hills and Mount Kilimanjaro. *Insects*, *9*(2), 71. https://doi.org/10.3390/insects9020071

OECD, 2011. (2011). *International Standards for Fruit and Vegetables*. 1–105. http://www.oecd-ilibrary.org/agriculture-and-food/international-standards-for-fruit-and-vegetables_9789264116351-en-fr

Okello, E.N., Amugune, N.O., Mukiama, T.K., Lattorff, H.M.G., 2021. Abundance and Community Composition of Flower Visiting Insects of Avocado (*Persea americana*) in the East African Region. Int. J. Trop. Ins. Sci., https://doi.org/10.1007/s42690-021-00463-1

Oliver, T. H., & Morecroft, M. D. (2014). Interactions between climate change and land use change on biodiversity: attribution problems, risks, and opportunities. *Wiley Interdisciplinary Reviews: Climate Change*, *5*(3), 317-335. https://doi.org/10.1002/wcc.271

Olorunmaiye, K. S., Fatoba, P. O., C OreOluwa, A., & Olorunmaiye, P. M. (2011). Seed weight and

Morpho-physiological parameters in Mango (*Mangifera indica*) seedlings. *Pomologia Croatica: Glasilo Hrvatskog agronomskog društva*, *17*(3-4), 77-86

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals?. *Oikos*, *120*(3), 321-326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Oronje, M. L., Hagen, M., Gikungu, M., Kasina, M., & Kraemer, M. (2012). Pollinator diversity, behaviour and limitation on yield of karela (Momordica charantia L. Cucurbitaceae) in Western Kenya. *African Journal of Agricultural Research*, *7*(11), 1629-1638. https://doi.org/10.5897/ajar11.725

Ornai, A., & Keasar, T. (2020). Floral complexity traits as predictors of plant-bee interactions in a mediterranean pollination web. *Plants*, *9*(11), 1–12. https://doi.org/10.3390/plants9111432

Orr, M. C., Hughes, A. C., Chesters, D., Pickering, J., Zhu, C. D., & Ascher, J. S. (2021). Global patterns and drivers of bee distribution. *Current Biology*, *31*(3), 451-458

Osuna-García, J. A., Doyon, G., Salazar-García, S., Goenaga, R., & González-Durán, I. J. L. (2010). Effect of harvest date and ripening degree on quality and shelf life of Hass avocado in Mexico. *Fruits*, *65*(6), 367–375. https://doi.org/10.1051/fruits/2010031

Pattemore, D., Buxton, M. N., Cutting, B. T., McBrydie, H., Goodwin, M., & Dag, A. (2018). low overnight temperatures associated with a delay in'hass'avocado (*persea americana*) female flower opening, leading to nocturnal flowering. *Journal of Pollination Ecology*, *23*

Paudel, Y. P., Mackereth, R., Hanley, R., & Qin, W. (2015). Honey bees (*Apis mellifera* L.) and pollination issues: Current status, impacts, and potential drivers of decline. *Journal of Agricultural Science*, *7*(6), 93

Peña, J. F., & Carabalí, A. (2018). Effect of honey bee (Apis Mellifera L.) density on pollination and fruit set of avocado (Persea Americana Mill.) cv. Hass. *Journal of Apicultural Science*, *62*(1), 5–14. https://doi.org/10.2478/JAS-2018-0001

Pérez-Balam, J., Quezada-Euán, J. J. G., Alfaro-Bates, R., Medina, S., McKendrick, L., Soro, A., & Paxton, R. J. (2012). The contribution of honey bees, flies and wasps to avocado (Persea americana) pollination in southern Mexico. *Journal of Pollination Ecology*, *8,* 42–47

Pettis, J. S., & Delaplane, K. S. (2010). Coordinated responses to honey bee decline in the USA. *Apidologie*, *41*(3), 256-263. https://doi.org/10.1051/apido/2010013

Pieterse, Z. (2003). Avocados (monounsaturated fatty acids), weight loss and serum lipids. *Energy (kJ)*, *26*, 65-71

Potschin, M., & Haines-Young, R. (2016). Defining and measuring ecosystem services. *Routledge handbook of ecosystem services*, 25-44. https://doi.org/10.1017/CBO9781107415324.004

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in ecology & evolution*, *25*(6), 345-353. https://doi.org/10.1016/j.tree.2010.01.007

Potts, S. G., Roberts, S. P. M., Dean, R., Marris, G., Brown, M. A., Jones, R., Neumann, P., & Settele, J. (2010). Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research*, *49*(1), 15–22. https://doi.org/10.3896/IBRA.1.49.1.02

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., ... & Vanbergen, A. J. (2016). Safeguarding pollinators and their values to human well-being. *Nature*, *540*(7632), 220-229

Power, A. G. (2010). Ecosystem services and agriculture: Tradeoffs and synergies. In *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2959-2971. https://doi.org/10.1098/rstb.2010.0143

Pufal, G., Steffan-Dewenter, I., & Klein, A. M. (2017). Crop pollination services at the landscape scale. *Current Opinion in Insect Science*, *21*, 91–97. https://doi.org/10.1016/j.cois.2017.05.021

Phalan, B., Onial, M., Balmford, A., & Green, R. E. (2011). Reconciling food production and biodiversity conservation: land sharing and land sparing compared. *Science*, *333*(6047), 1289-1291. https://doi.org/10.1126/science.1208742

Phillips, B. B., Williams, A., Osborne, J. L., & Shaw, R. F. (2018). Shared traits make flies and bees effective pollinators of oilseed rape (*Brassica napus* L.). *Basic and Applied Ecology*, *32*(2017), 66–76. https://doi.org/10.1016/j.baae.2018.06.004

Pywell, R. F., Heard, M. S., Woodcock, B. A., Hinsley, S., Ridding, L., Nowakowski, M., & Bullock, J. M. (2015). Wildlife-friendly farming increases crop yield: Evidence for ecological intensification. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1816). https://doi.org/10.1098/rspb.2015.1740

Rader, R., Bartomeus, I., Garibaldi, L. A., Garratt, M. P. D., Howlett, B. G., Winfree, R., Cunningham, S. A., Mayfield, M. M., Arthur, A. D., Andersson, G. K. S., Bommarco, R., Brittain, C., Carvalheiro, L. G., Chacoff, N. P., Entling, M. H., Foully, B., Freitas, B. M., Gemmill-Herren, B., Ghazoul, J., … Woyciechowski, M. (2016). Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences*, *113*(1), 146–151. https://doi.org/10.1073/pnas.1517092112

Rader, R., Edwards, W., Westcott, D. A., Cunningham, S. A., & Howlett, B. G. (2011). Pollen transport differs among bees and flies in a human-modified landscape. *Diversity and Distributions*, *17*(3), 519-529. https://doi.org/10.1111/j.1472-4642.2011.00757.x

Rader, R., Reilly, J., Bartomeus, I., & Winfree, R. (2013). Native bees buffer the negative impact of climate warming on honey bee pollination of watermelon crops. *Global Change Biology*, *19*(10), 3103–3110. https://doi.org/10.1111/gcb.12264

Rapsomanikis, G. (2015). The economic lives of smallholder farmers: An analysis based on household data from nine countries. FAO. United Nations, Rome. pp. 1–48

Raven, P. H., & Wagner, D. L. (2021). Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(2), 1–6. https://doi.org/10.1073/PNAS.2002548117

Read, S. F. J., Howlett, B. G., Jesson, L. K., & Pattemore, D. E. (2017). Insect visitors to avocado flowers in the Bay of Plenty, New Zealand. *New Zealand Plant Protection*, *70*, 38–44. https://doi.org/10.30843/nzpp.2017.70.25

Requier, F., Garnery, L., Kohl, P. L., Njovu, H. K., Pirk, C. W., Crewe, R. M., & Steffan-Dewenter, I. (2019). The conservation of native honey bees is crucial. *Trends in ecology & evolution*, *34*(9), 789-798

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng', A., & Viana, B. F. (2008). Landscape effects on crop pollination services: Are there general patterns? *Ecology Letters*, *11*(5), 499–515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Rivest, S., & Forrest, J. R. K. (2020). Defence compounds in pollen: why do they occur and how do they affect the ecology and evolution of bees? *New Phytologist*, *225*(3), 1053–1064. https://doi.org/10.1111/nph.16230

Robbertse, P. J., Coetzer, L. A., Johannsmeier, M. F., & Swart, D. J. (1996). Hass Yield and Fruit Size as Influenced by Pollination and Pollen Donor — a Joint Progress Report. *South African Avocado Growers' Association Yearbook*, *19*, 63–67

Roquer-Beni, L., Rodrigo, A., Arnan, X., Klein, A. M., Fornoff, F., Boreux, V., & Bosch, J. (2020). A novel method to measure hairiness in bees and other insect pollinators. *Journal of Ecology and evolution*, *10*(6), 2979-2990. https://doi.org/10.1016/j.tree.2019.04.008

Sabbahi, R., De Oliveira, D., & Marceau, J. (2006). Does the honeybee (Hymenoptera: Apidae) reduce the blooming period of canola?. *Journal of Agronomy and Crop Science*, *192*(3), 233-237.

https://doi.org/10.1111/j.1439-037X.2006.00206.x

Sagwe, R. N., Muya, S. M., & Maranga, R. (2015). Effects of land use patterns on the diversity and conservation status of butterflies in Kisii highlands, Kenya. *Journal of insect conservation*, *19*(6), 1119-1127

Sagwe, R. N., Peters, M. K., Dubois, T., Steffan-Dewenter, I., & Lattorff, H. M. G. (2021). Pollinator supplementation mitigates pollination deficits in smallholder avocado (Persea americana Mill.) production systems in Kenya. *Basic and Applied Ecology*, *56*, 392-400

Samnegård, U., Hambäck, P. A., & Smith, H. G. (2019). Pollination treatment affects fruit set and modifies marketable and storable fruit quality of commercial apples. *Royal Society Open Science*, *6*(12). https://doi.org/10.1098/rsos.190326

Sardiñas, H. S., Tom, K., Ponisio, L. C., Rominger, A., & Kremen, C. (2016). Sunflower (Helianthus annuus) pollination in California's Central Valley is limited by native bee nest site location. *Ecological Applications*, *26*(2), 438-447. https://doi.org/10.1890/15-0033

Sawe, T., Eldegard, K., Totland, Ø., Macrice, S., & Nielsen, A. (2020). Enhancing pollination is more effective than increased conventional agriculture inputs for improving watermelon yields. *Ecology and Evolution*, *10*(12), 5343–5353. https://doi.org/10.1002/ece3.6278

Senapathi, D., Goddard, M. A., Kunin, W. E., & Baldock, K. C. R. (2017). Landscape impacts on pollinator communities in temperate systems: evidence and knowledge gaps. *Functional Ecology*, *31*(1), 26–37. https://doi.org/10.1111/1365-2435.12809

Silva, C. A. S., Godoy, W. A. C., Jacob, C. R. O., Thomas, G., Câmara, G. M. S., & Alves, D. A. (2018). Bee pollination highly improves oil quality in sunflower. *Sociobiology*, *65*(4), 583–590. https://doi.org/10.13102/sociobiology.v65i4.3367

Singleton, Vernon L., Rudolf Orthofer, and R. M. L.-R. (1999). Analysis of Total Phenols and Other

Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, *299*, 152–178

Sutter, L., Albrecht, M., & Jeanneret, P. (2018). Landscape greening and local creation of wildflower strips and hedgerows promote multiple ecosystem services. *Journal of applied ecology*, *55*(2), 612-620

Scaven, V. L., & Rafferty, N. E. (2013). Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Current zoology*, *59*(3), 418-426.

Shahi, C., Kiran Bargali, V., & Bargali, S. S. (2015). How seed size and water stress effect the seed germination and seedling growth in wheat varieties. *Current Agriculture Research Journal*, *3*(1), 60-68. https://doi.org/10.12944/carj.3.1.08

Sharma, A., Shukla, A., Attri, K., Kumar, M., Kumar, P., Suttee, A., ... & Singla, N. (2020). Global trends in pesticides: A looming threat and viable alternatives. *Ecotoxicology and Environmental Safety*, *201*, 110812. https://doi.org/10.1016/j.ecoenv.2020.110812

Shin, Y. S., Park, S. D., & Kim, J. H. (2007). Influence of pollination methods on fruit development and sugar contents of oriental melon (Cucumis melo L. cv. Sagyejeol-Ggul). *Scientia Horticulturae*, *112*(4), 388–392. https://doi.org/10.1016/j.scienta.2007.01.025

Sjödin, N. E., Bengtsson, J., & Ekbom, B. (2008). The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *Journal of Applied Ecology*, *45*(3), 763-772. https://doi.org/10.1111/j.1365-2664.2007.01443.x

Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C., & Tscharntke, T. (2002). Scale-dependent effects of landscape context on three pollinator guilds. *Ecology*, *83*(5), 1421-1432.

Stavert, J. R., Liñán-Cembrano, G., Beggs, J. R., Howlett, B. G., Pattemore, D. E., & Bartomeus, I. (2016). Hairiness: the missing link between pollinators and pollination. *PeerJ*, *4*, e2779

Steiner, F., Zuffo, A. M., Busch, A., De Oliveira Sousa, T., & Zoz, T. (2019). Does seed size affect the germination rate and seedling growth of peanut under salinity and water stress? *Pesquisa Agropecuaria Tropical*, *49*(e54353), 1–9. https://doi.org/10.1590/1983-40632019v4954353

Stout, A. B. (1932). Sex in avocados and pollination. *Sex in avocados and pollination.*

Spears, E. E. (1983). A direct measure of pollinator effectiveness. *Oecologia*, *57*(1-2), 196-199

Thapa, R. B. (2006). Honeybees and other insect pollinators of cultivated plants: a review. *Journal of the Institute of Agriculture and Animal Science*, *27*, 1-23

Thorp, R. W. (2000). The collection of pollen by bees. In *Pollen and pollination* (pp. 211-223). Springer, Vienna

Tramontin, N. dos S., Luciano, T. F., Marques, S. de O., de Souza, C. T., & Muller, A. P. (2020). Ginger and avocado as nutraceuticals for obesity and its comorbidities. *Phytotherapy Research*, *34*(6), 1282–1290. https://doi.org/10.1002/ptr.6619

Tscharntke, T., Clough, Y., Wanger, T. C., Jackson, L., Motzke, I., Perfecto, I., ... & Whitbread, A. (2012). Global food security, biodiversity conservation and the future of agricultural intensification. *Biological conservation*, *151*(1), 53-59. https://doi.org/10.1016/j.biocon.2012.01.068

Tscheulin, T., Neokosmidis, L., Petanidou, T., & Settele, J. (2011). Influence of landscape context on the abundance and diversity of bees in Mediterranean olive groves. *Bulletin of Entomological Research*, *101*(5), 557-564. https://doi.org/10.1017/s0007485311000149

UNECE, 2017. United Nations Economic Commission for Europe. UNECE STANDARD FFV-42, Concerning the marketing and commercial quality control of avocados. United Nations, New York and Geneva

UN, U. (2017). Department of Economic and Social Affairs. *Population Division*

Vaissière, B., Freitas, B. M., & Gemmill-Herren, B. (2011). *Protocol to detect and assess pollination deficits in crops:*

a handbook for its use (pp. 81-p). FAO

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., ... & Zamora, R. (2015). Beyond species loss: the extinction of ecological interactions in a changing world. *Functional Ecology*, *29*(3), 299-307

Van Swaay, C., Van Strien, A., Aghababyan, K., Astrom, S., Botham, M., Brereton, T., ... & Warren, M. (2015). The European Butterfly Indicator for Grassland species: 1990-2013. http://nora.nerc.ac.uk/id/eprint/511714/

Vanbergen, A. J., Garratt, M. P., Vanbergen, A. J., Baude, M., Biesmeijer, J. C., Britton, N. F., Brown, M. J. F., Brown, M., Bryden, J., Budge, G. E., Bull, J. C., Carvell, C., Challinor, A. J., Connolly, C. N., Evans, D. J., Feil, E. J., Garratt, M. P., Greco, M. K., Heard, M. S., … Wright, G. A. (2013). Threats to an ecosystem service: Pressures on pollinators. *Frontiers in Ecology and the Environment*, *11*(5), 251–259. https://doi.org/10.1890/120126

van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). Environmental opportunities for insect rearing for food and feed. *Edible insects–Future prospects of feed and food security. Roma: Food and Agriculture Organization of the United Nations*.

VanEngelsdorp, D., Evans, J. D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B. K., ... & Pettis, J. S. (2009). Colony collapse disorder: a descriptive study. *PloS one*, *4*(8), e6481. https://doi.org/10.1371/journal.pone.0006481

Van Swaay, C., Van Strien, A., Aghababyan, K., Astrom, S., Botham, M., Brereton, T., ... & Warren, M. (2015). The European Butterfly Indicator for Grassland species: 1990-2013.

Visscher, P. K., & Seeley, T. D. (1982). Foraging Strategy of Honeybee Colonies in a Temperate Deciduous Forest. *Ecology*, *63*(6), 1790. https://doi.org/10.2307/1940121

Vithanage, V. (1990). The role of the European honeybee (Apis mellifera L.) in avocado pollination. *Journal* *of Horticultural Science*, *65*(1), 81-86. https://doi.org/10.1080/00221589.1990.11516033

Wasilwa, L. A., Njuguna, J. K., Okoko, E. N., & Watani, G. W. (2004). Status of avocado production in Kenya. *Kenya Agricultural Research Institute, Nairobi, Kenya*

Willmer, P. (2011). *Pollination and floral ecology*. Princeton University Press

Weschenfelder, C., dos Santos, J. L., de Souza, P. A. L., de Campos, V. P., & Marcadenti, A. (2015). Avocado and Cardiovascular Health. *Open Journal of Endocrine and Metabolic Diseases*, *05*(07), 77–83. https://doi.org/10.4236/ojemd.2015.57010

Wietzke, A., Westphal, C., Gras, P., Kraft, M., Pfohl, K., Karlovsky, P., ... & Smit, I. (2018). Insect pollination as a key factor for strawberry physiology and marketable fruit quality. *Agriculture, Ecosystems & Environment*, *258*, 197-204. https://doi.org/10.1016/j.agee.2018.01.03

Williams, P. H., & Osborne, J. L. (2009). Bumblebee vulnerability and conservation world-wide. *Apidologie*, *40*(3), 367-387. https://doi.org/10.1051/apido/2009025

Willmer, P. G., & Stone, G. N. (1989). Incidence of entomophilous pollination of lowland coffee (Coffea canephora); the role of leaf cutter bees in Papua New Guinea. *Entomologia Experimentalis et Applicata*, *50*(2), 113–124. https://doi.org/10.1111/j.1570-7458.1989.tb02380.x

Winfree, R., Williams, N. M., Dushoff, J., & Kremen, C. (2007). Native bees provide insurance against ongoing honey bee losses. *Ecology letters*, *10*(11), 1105-1113.

Wood, T. J., Holland, J. M., & Goulson, D. (2015). Pollinator-friendly management does not increase the diversity of farmland bees and wasps. *Biological Conservation*, *187*, 120–126. https://doi.org/10.1016/j.biocon.2015.04.022

Woodcock, B. A., Garratt, M. P. D., Powney, G. D., Shaw, R. F., Osborne, J. L., Soroka, J., ... & Pywell, R. F. (2019). Meta-analysis reveals that pollinator functional diversity and abundance enhance crop pollination and yield. *Nature Communications*, *10*(1), 1-10. https://doi.org/10.1038/s41467-019-

09393-6

Wu, P., Axmacher, J. C., Song, X., Zhang, X., Xu, H., Chen, C., ... & Liu, Y. (2018). Effects of plant diversity, vegetation composition, and habitat type on different functional trait groups of wild bees in rural Beijing. *Journal of Insect Science*, *18*(4), 1.

Wragg, P. D., & Johnson, S. D. (2011). Transition from wind pollination to insect pollination in sedges: Experimental evidence and functional traits. *New Phytologist*, *191*(4), 1128–1140. https://doi.org/10.1111/j.1469-8137.2011.03762.x

Wysoki, M., Van Den Berg, M. A., Ish-Am, G., Gazit, S., Peña, J. E., & Waite, G. K. (2002). Pests and pollinators of avocado. *Tropical fruit pests and pollinators: biology, economic importance, natural enemies and control*, 223-293

Zou, Y., Bianchi, F. J., Jauker, F., Xiao, H., Chen, J., Cresswell, J., ... & van der Werf, W. (2017). Landscape effects on pollinator communities and pollination services in small-holder agroecosystems. *Agriculture, ecosystems & environment*, *246*, 109-116. https://doi.org/10.1016/j.agee.2017.05.035

Zhang, W. (2018). Global pesticide use: Profile, trend, cost/benefit and more. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *8*(1), 1.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, *64*(4), 555–559

Authors' Contribution

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Author contribution	Author Initials Responsibility decreasing from left to right		
Study design and methodology	RNS/HMGI		
Data collection	RNS		
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Data analysis and interpretation	RNS/ MKP		
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Author contribution	Author Initials Responsibility decreasing from left to right		
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Author contribution	Author Initials Responsibility decreasing from left to right		
Study design and methodology	RNS/HMGI	MKP	ISD
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Data processing	RNS		
Data analysis and interpretation	RNS/ MKP		
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Publication List

Publications as part of the doctoral thesis

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Additional publications

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Presentations and poster

Rose Nyakemiso Sagwe, Marcell K. Peters, Thomas Dubois Ingolf Steffan-Dewenter, H. Michael G. Lattorff. Pollinator supplementation mitigates pollination deficits in smallholder avocado (*Persea americana* Mill.) production systems in Kenya. The Society of Environmental Toxicology and Chemistry (SETAC) 10th Biennial Conference (virtual conference) held on 20 - 22 September 2021.

Rose Nyakemiso Sagwe, Marcell K. Peters, Thomas Dubois Ingolf Steffan-Dewenter, H. Michael G. Lattorff**.** Pollinator supplementation mitigates pollination deficits in smallholder avocado (*Persea americana* Mill.) production systems in Kenya. African Association of Insect Scientists (AAIS), Addis Ababa University, Ethiopia to be held on 22nd - 26th February 2022. 24th AAIS Meeting and Scientific Conference, Under "Migratory Pests and Invasive Species: Early Warning System, Monitoring, Control and their Impact on Food Security, Environment and Livelihoods during COVID-19 pandemic."

Thomas Dubois, Nadia Toukem, H. Michael G. Lattorff, **Rose N. Sagwe**, Abdullahi A. Yusuf, Elfatih M. Abdel-Rahman, Marian Salim Adan, Beatrice Muriithi. Integrated pest and pollinator management (IPPM) as a novel tool to merge ecosystem services: lessons learnt from avocado in Kenya" for presentation at Tropentag 2021, held on September 15-17 as virtual conference "Towards shifting paradigms in agriculture for a healthy and sustainable future".

Rose Nyakemiso Sagwe, Marcell K. Peters, Thomas Dubois Ingolf Steffan-Dewenter, H. Michael G. Lattorff**.** Importance of pollination and pollination deficits in different crops. Talk at the workshop series of farmstead empowerment network, Nigeria held virtually on September 8th April 2021.

Awards

A ward of Excellence by *icipe's* Governing Council 18th November 2021. **Best published science paper Second Runner Up (Certificate & Cash Prize – 100 US $).** Title: Pollinator supplementation mitigates pollination deficits in smallholder avocado (*Persea americana* Mill.) production systems in Kenya. Basic and Applied Ecology, 56, 392-400.https://doi.org/10.1016/j.baae.2021.08.013 .

A ward of Excellence by *icipe's* Governing Council 18th November 2021. **Best Science Poster,** (**First Runner Up Certificate & Cash Prize – US$ 75**). Title: on Pollination efficiency and visitation frequency of avocado (Persea americana) flower insect visitors.

Dedication

This thesis is dedicated to my beloved husband Dr. John Nduko Masani and to my two lovely sons Dylan and Jammie who have sincerely, unconditionally and truly supported me through their patience, love, encouragement and prayers made it possible to complete this study.

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