



**University of Venda**  
*Creating Future Leaders*

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DEPARTMENT OF ZOOLOGY

**Does observational methods affect the observed impacts of exotic plants on flower visitors in and around Macadamia orchards?**

By

**Ramotjiki Mancha Lorraine**

Student number: 11638633

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**Supervisor: Professor S.H. Foord**

Centre for Invasion Biology  
Department of Zoology  
University of Venda  
School of Mathematical and Natural Sciences

**Co-supervisor: Dr CS Schoeman**

University of Venda  
Department of Zoology  
School of Mathematical and Natural Sciences

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## Declaration

I, the undersigned, hereby declare that the dissertation for the Masters of Science in Zoology is entirely my own work and has not been submitted for a degree at this university or any other university and that all the sources used in this study have been properly acknowledged.

Signed (Student): 

Date: 20 June 2020

## General summary

Pollination of flowering plants is mostly facilitated by insects. However, there is a global decline in the pollination services caused by invasive alien plants (IAPs), excessive use of pesticides, habitat loss, and fragmentation. IAPs affect native plants pollinators and integrate into their network interactions. Habitat loss leads to small remnant patches surrounded by a matrix of mainly agricultural activities, these remnant patches support native vegetation and provide forage to pollinators within and outside the flowering period of agricultural crops. However, these patches are also vulnerable to invasion by IAP's. Macadamia nuts are one of the fastest growing markets in South Africa. Macadamia nut trees (*Macadamia integrifolia*) are mostly pollinated by wild and managed honey bees (*Apis mellifera*), but studies have shown that large densities not only impact negatively on pollination services but affect pollinator communities as a whole.

Here we study plant flower visitation by potential pollinators in a remnant patch, invaded by exotic plants, adjacent to a macadamia orchard in a heavily transformed, commercial agricultural landscape. The impact of IAPs on flower visitor community composition (abundance and species richness) was assessed using two field methods of observation methods (camera trap and visual observation). Relative to visual observation, camera traps recorded smaller species and those that tend to avoid humans, while rarer species were more prevalent in visual observation. Therefore, camera traps observed more species at a sampling point, but less species overall. Observational methods are explained by the largest amounts of conditional variation.

IAPs and native plants were visited by a larger and diverse set of flower visitors than macadamia although macadamia flower densities were twice as high and covered a much larger extent. IAPs were more generalist in the flower visitors they attracted. Native plants were more specialized in the flower visitors they attract with GLMM suggesting although not significantly were associated with more species richness. These plants were also associated with overall visitation of species. More visitation of *Apis mellifera* were on IAP's and native plants which contrast with macadamia with less than 10%. However, this small amount account for 65% of all macadamia flower visitations, the remainder was largely (33%) made up by *Eristalis tenax*. Dominant flower visitors seemed to avoid *A. mellifera* by partitioning their activity in time or

specializing on certain plant species. The importance of IAP's in providing forage to *A. mellifera* before macadamia flowering periods are noted. However, this probably has negatively impacted on other potential native pollinators of macadamias (e.g. *Eristalis tenax*). The removal of IAPs around mass flowering native plants such as *Gymnanthemum myrianthum*, should support and facilitate the second most common flower visitor of macadamia, *E. tenax* and flower visitors in general.

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## Contents

Declaration .....	i
General summary .....	ii
Acknowledgement .....	iv
List of appendices .....	vii
Chapter 1: General introduction .....	1
Importance of pollinator service in the wild and agricultural landscapes.....	1
Habitat fragmentation .....	2
Insect activity .....	3
This study .....	3
Reference .....	5
Chapter 2: Two ways of observing: camera traps versus visual observation of sampling flower visitors in an agricultural landscape.....	10
Abstract.....	10
Introduction .....	10
Methodology.....	13
Study area .....	13
Study design.....	14
Pollinator observations.....	14
Statistical analysis .....	16
Results.....	17
Discussion.....	23
Reference .....	27
Chapter 3: Flower visitor communities in heavily managed and transformed landscapes – the case of <i>Apis mellifera</i> and invaded remnant patches in Macadamia orchards .....	32
Abstract.....	32
Introduction .....	32
Methodology.....	35
Sampling method .....	35
Analysis .....	37
Results.....	39
Discussion.....	50
Reference .....	54
Chapter 4: Thesis synthesis .....	60
Efficacy of sampling methods .....	60

IAP's, native plants and macadamia crop flower visitors. ....	60
Should the IAPs be removed from the agricultural landscape? .....	61
Study limitations, recommendation and future research .....	61
Reference.....	62
Appendix .....	64

## List of appendices

Appendix A: Flowering plants that were sampled by both camera traps and visual observation. Number of samples indicate how many times the plant was sampled throughout 2 years .....**64-65**

Appendix B: Species list of flower visitors and the number of observations for each method. Plants origin; E = IAP, N = native and M= macadamia.....**66-71**

Appendix C: Overall species interactions. Colours indicate the strength of interaction; from high interaction indicated by black to no interaction indicated by white squares.....**72-75**



## Chapter 1: General introduction

### *Importance of pollinator service in the wild and agricultural landscapes*

Pollination is the transfer of pollen from anthers to stigma mostly by animals and is vital for the reproduction of plants (Kevan and Viana 2003, Das et al. 2018). Animals are the greatest important pollinators of most flowering plants and a deficit in them results in decline of plant species (Rodger et al. 2004; Klein et al. 2007; Ojija et al. 2019). They increase the quality, quantity and stability of the production of the world's leading food crops (Grass et al. 2008). By so doing they help in sustaining, conserving and providing a valuable ecosystem service to humankind (Kevan and Viana 2003; Potts et al. 2010). Pollinators plays a very important role in the food industry worldwide; this include the production of fruits and vegetables (Das et al. 2018).

Pollination is an important ecosystem service that is provided mostly by insect pollinators (Maia et al. 2019) These insect pollinators particularly wild insects are at considerable higher risk than other invertebrates guilds (Klein et al. 2007). The crop flowers pollinated by them are more vulnerable because insect diversity and abundance are declining in agricultural landscapes worldwide (Garibaldi et al. 2013). Globally this decrease in the yield of insect pollinated crops is offset by the addition of managed honey bees (Garibaldi et al. 2013). Therefore, the impact of the decline in the wild pollinators on crop yields is currently not known (Garibaldi et al. 2013).

However, a global decline in pollinator services has been observed with significantly more impact in the agricultural communities which could possibly affect both crop production and food security (Potts et al. 2010). There are multiple stressors that are a threat to pollinators and the services they provide such as pesticide usage, introduction of invasive alien pollinators and plants, pests, climate change, diseases, and habitat fragmentation and degradation (Kevan and Viana 2003; Rodger et al. 2004; Goulson et al. 2015; Nicolson and Wright 2017). This in turn affects pollination by decreasing the number of pollinators, changing the resource availability, spatial distribution of floral resources and results in competition of pollinating species (Ghazoul 2004).

### *Habitat fragmentation*

Habitat fragmentation is one of the greatest threats to biodiversity and negatively impacts most taxonomic groups (Fischer and Lindenmayer 2007). It results in the decline of species and modification of native plants and animal communities (Bennett and Saunders 2010). However, habitat loss has a much larger effect on biodiversity than habitat fragmentation per se (Fahrig 2003) although both are primary factors leading to species population decline and extinction in the world (Murphy et al. 2016).

Habitat fragmentation is a process of dividing large habitat into smaller patches (Andren 1994; Fahrig 2003) leading to the destruction and degradation of natural ecosystems (Haddad et al. 2015). This results in the reduction and isolation of patches (Andren 1994) that have a negative effect on species richness and abundance, therefore, resulting in loss of biodiversity at landscape-scale (Fahrig 2003).. Patches become too small to act as viable breeding habitats and maintaining local population of species (Bayne and Hobson 1997; Fahrig 2003; Valdivia and Simonetti 2018). A larger habitat contains more species richness and abundance than small patches (Fahrig 2013).

Habitat fragmentation is one of the most common threats to pollinators affecting their ecological interactions in the ecosystem (Rathcke and Jules 1993; Steffan-Dewenter et al. 2006). Their foraging behaviour is negatively impacted and therefore reducing plants' fitness (Ashworth et al. 2004). Wind pollinated plants are more prone to the immediate and long-term impacts of fragmentation (Rathcke and Jules 1993; Rodger et al. 2004). Habitat fragmentation have greater impacts on specialists than generalist pollinators, as specialists often exist in small patchy populations and are mostly affected (Rathcke and Jules 1993).

Habitat fragmentation can also have positive on species richness and abundance with respect to number and size of patches (Fletcher Jr et al. 2018). These natural patches still play a very important role in agroecosystems. Natural patches can support a diversity of pollinators for many crops although this also depends on the diversity of crops, patterns of landscape and pollinator community (Klein et al. 2007). Although these natural corridors surrounding agricultural landscape support a diversity of pollinators these habitats may also increase the densities of pest species that affects the fruit yield (Hunter 2002).

### *Insect activity*

Plants and insects have coevolved abilities and interactions that enable them to work together in an ecosystem. Insects have sensors for floral rewards whilst plants have signalling mechanisms for temporal variation of rewards to insects (Barp et al. 2011). Different species secrete nectar throughout the day while some don't (Morse and Fritz 1983). Some species also open their flowers at different times of the day though some are always open. Different daily cycle of flower opening helps attract certain flower visitors as not all of them are active throughout the day (Herrera 1990).

The activity of flying insects is affected by extrinsic and intrinsic factors (Herrera 1990). Extrinsic factors such as the abiotic environmental conditions including temperature (Herrera 1990), time of the day and season (Totland 1994), wind speed and light intensity (Herrera 1990; Totland 1994) and biotic factors including predation and competition (Herrera 1990, Azevedo et al. 2018). These factors determine the daily activity window of insects. Weather is an important variable that is considered in determining the activity of insects and it affect their visitation rate (Arroyo et al. 1985; McCall and Primack 1992). Light intensity is positively correlated to visitation rate (Totland 1994). Flower density and other factors such as corolla and pollen production also have an effect on visitation rate (Herrera 1990; Totland 1994; Conner and Rush 1996).

### *This study*

Most agricultural crops worldwide depend on insects for pollination. The most important pollinators that are known to pollinate most agricultural crops are wild and managed honey bees *Apis mellifera* Linnaeus (Rader et al. 2016). Bees are commonly studied pollinators providing ecosystem services to many crops worldwide (Rader et al. 2020). There is a recent study that found other non-bees visiting agricultural crops (Rader et al. 2020). Amongst these insects the most common ones are Lepidoptera, Hymenoptera: wasps, Coleoptera (Rader et al. 2020) and Diptera (Howlett et al. 2015; Rader et al. 2020). Diptera are thought to be less effective pollinators to macadamia crops than other agricultural crops (Howlett et al. 2015). The most common dipterans visiting crops are *Eristalis tenax* (Linnaeus), *Musca domestica* (Linnaeus) and *Calliphora* spp. These insects have been reported as both visitors and pollinators (Rader et al. 2020).

The aim of our study was to compare the efficacy of observational methods (camera traps and visual observation) and assess the impact (negative or positive) that the IAP's play in and around macadamia crop flower visitors. Macadamia is an economically important food crop, widely known for its edible kernel (Heard 1993). Production in South Africa surpassed that of Australia in 2014, making South Africa the world's largest macadamia nut producer despite the crop having its native range in Australia (Department of Agriculture Forestry and Fisheries South Africa, 2015).

The study was conducted in the Luvuvhu area which is the second largest macadamia nut producers in South Africa (Weier et al. 2019). This area is heavily transformed and consists of natural remnant patches at the edge of the macadamia orchards that are predominately invaded by invasive alien plants (IAPs). Due to ongoing research that shows the impacts of IAPs on the pollinator assemblages, it is important to understand to what extent these IAPs affect the macadamia agricultural orchards. The impacts can either be negative (compete) or positive (facilitate) hence this will guide if these IAPs should be eradicated from the remnant patches.

The most common IAPs in the Luvuvhu valley are weeds and they produce more flowers flooding the ecosystem with their own pollen in large amounts (Hansen et al. 2018). This increases their ability to attract pollen dependent insects that visits native flowering plants and the macadamia crop. They then compete with them and attract more pollinators due to their abundance in floral exotic resources (Rathnayake and Wijetunga 2016).

In chapter 2; we will assess the difference in flower visitors' assemblages in the Luvuvhu valley. This will involve the use of two observational methods: camera trap and visual observation to assess if the diversity and complexity differs between methods and if they also differ between plants; IAPs, native and macadamia crop. We will determine whether the observational methods influence the diversity (species richness and abundance) of flower visitors and if this diversity varies between native plants, IAPs and macadamia crop.

In chapter 3; we will make use of flower visitors' network to assess the interaction of flower visitors and plants. This will allow us to quantify if the presence of IAPs in the ecosystem influences the network structure with respect to macadamia flowering period (before and during flowering). Then we will use overlap plots to visualise the

activity pattern on insects throughout the day to determine if species overlap or compete. We will answer the question as to what extent does IAPs affect network structure in space and time across the boundary between macadamia orchards and remnant patches and are the activity pattern and diversity of flower visitors influenced by *A. mellifera*.

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## Chapter 2: Two ways of observing: camera traps versus visual observation of sampling flower visitors in an agricultural landscape.

### Abstract

Camera trap technology provides an alternate approach to observing pollinator communities. Raising concerns around the impacts of invasive alien plants (IAPs) on pollinators calls for an efficient method to assess the extent of these impacts. This study compares traditional visual observations to automated digital capture of flower visitation in a heavily transformed agricultural landscape. Assessing to what extent flower visitor communities differ between the methods and plant type (native, invasive alien plant (IAP) or crop (macadamia)) using Generalized Linear Mixed Models and model based multivariate analysis. Dipterans, Aves and smaller insect species, were more prevalent in camera trap images while rarer (less abundant) species were recorded more in visual observation. Point sample diversity was higher for camera traps, but overall more species were observed through visual observations. Visual observation also performed better when sampling the mass flowering crop (macadamia). Abundant species were associated with either native plants (Diptera: *Asarkina africana* and Hymenoptera: *Ceratina* sp.) or IAPs (Diptera: *Culicoides* sp.) with none of these having a preference for macadamia. Camera traps efficiency in recording the presence of small insect species must be weighed against its smaller spatial sampling extent.

### Introduction

Sampling methods vary in their efficiency in sampling insect species richness and abundance in ecosystems. Therefore, efficient survey methods are needed to sample biodiversity in both space and time (Darras et al. 2019). Different sampling techniques have been deployed for years to study the interaction between plants and their flower visitors with visual observation been the most commonly used method in the field. Direct observation in the field is labour intensive with a high probability of transcription errors (Steen 2017). It remains however the most traditionally used field method. This method comes with limitations that can affect the detection probability of insects. However, camera traps may well be more efficient than direct observation in recording pollinator activities on flowers.

Although visual observation is a commonly used method in the field this method comes with a limitation to more mobile small insects (Darras et al. 2019). The observer can have an impact on focal species (Caravaggi et al. 2017) disturbing them and resulting in non-detection of insects (Darras et al. 2019). Clothing colour also lowers detection probability. The activity of other species such as birds can be affected by the presence of humans while other curious species can be attracted (Darras et al 2019). With the observer having to recognize and record insect immediately, there is a possibility of missing other insects visiting the plant (Steen 2017). Even with the limitations of the visual observation, this method can provide an insight into plant-pollinators interactions however, given the recent decline in the abundance and diversity of insects, different sampling methods should be used (Lortie et al. 2012).

Camera traps are non-destructive sampling methods used in ecology, automated to capture images or videos of species occurring in a certain area (Villa et al. 2017) providing an alternate approach to observing pollinator communities. They are used for faunal surveys, monitoring population size estimates, activity pattern of animals and their diets (Krauss et al. 2018) and for abundance and habitat preference as well as survey of shy and rare species (Caravaggi et al. 2017; Meredith and Ridout 2018). Up to date about 95% studies on coucted by camera traps their main focus are mostly on mammal species; however, their use in studying pollination has been realized recently (Krauss et al 2018). Recent studies on pollination using camera traps were done mostly on rodents and birds (Melidonis and Peter 2015; Darras et al 2019) however, monitoring insects using camera traps were done using mostly video recording (Lortie et al. 2012; Steen 2017).

Data in camera traps are captured in the form of images that record a group of individual species or different species and can help in quantifying species richness (diversity) and relative abundance (Rovero et al. 2013; Caravaggi et ai. 2017). Unlike visual observation, camera traps have higher material costs although they can be used for years if they sustain little or no damage (Darras et al. 2019), though batteries need to be replaced from time to time. Camera traps data processing is very time consuming because of thousands of pictures captured in one sampling period (Villa et al. 2017). There is currently no automated way of processing and identifying species from camera trap images and some species can be missed whilst working through images. Sometimes due to image resolution, the images can be unclear and only few studies

were able to get 82% accuracy in identifying their species (Villa et al. 2017). Camera traps have high accuracy capture of species that cannot be detected visually (Foster and Harmsen 2012).

Invasive alien plants (IAP's) are amongst the greatest threats to biodiversity worldwide (Hansen et al. 2018) and affect the community structure of plant and animals (Dassonville et al. 2008). They have an adverse impact on the ecosystems they invade and are excellent competitors for resources (Brown and Mitchel 2001; Stout and Tiedeken 2017; Hansen et al. 2018). IAP's have both direct and indirect impacts not only on native plants but also on native pollinators. They affect native pollinator communities (species richness, diversity and composition) and change their network interactions (Stout and Tiedeken 2017). IAP's are promoted by habitat disturbance, largely anthropogenic in origin and can result in the disruption of interaction between species.

The impacts of IAP's can either be beneficial, neutral or detrimental to pollinators (Mack and Lonsdale 2001). IAP's can either support and share pollinators or compete for pollinators with the native plants. This can result in interaction between the plants by supporting pollinator of the other species or it can result in competition (Hansen et al. 2018). The presence of IAP's can result in reduced visitation of pollinators to native plants and/or a decrease in the pollen quality and quantity. Their impact on competition for pollinator services has rarely been considered (Brown and Mitchel 2001).

Generalist pollinators forms the backbone of the pollination networks retarding the extinction of the specialized species when webs are disturbed (Fontaine et al. 2008). The specialist pollinators are more vulnerable to the impacts than the generalists (Ashworth et al. 2004; Fontaine et al. 2008). A slight decrease in the abundance or loss of the pollinator species from the specialist narrow pollinator assemblage (Ashworth et al. 2004).

IAP's can however be beneficial by buffering against the decrease in nectar and pollen under environmental change and decrease the dependence of the native bees on native plants (Potts et al. 2010). IAP's can facilitate the pollination of the native plants by attracting more flower visitors to the area where they coexist (Hansen et al. 2018). IAP's can help support pollinators outside the flowering period of many native or crops.

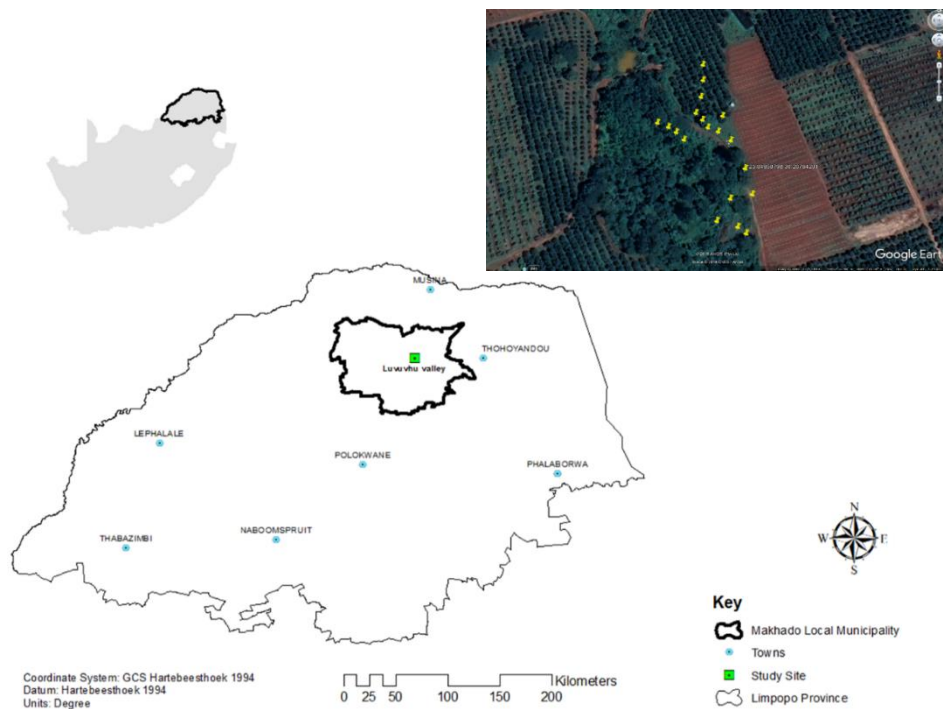
Alien invasive, native and crop plant species could therefore differ significantly in terms of the pollinator communities associated with each of these groups with important implications for crop pollination and the future of remnant patches. Camera trapping could also provide further insights into these differences. This study will therefore aim to answer two questions. (1) Do different methods of observation produce different results in terms of diversity (Species richness and abundance) and (2) does flower visitor diversity vary between native, invasive alien species (IAP) and the crop (macadamia) plants?

## Methodology

### Study area

The study was conducted on the Maluma farm, 42km south-west of Thohoyandou in the Luvuvhu valley, Limpopo province, South Africa (23°02'55.37"S, 30°16'01.29"E). The valley is dominated by extensive monocultures of macadamia (*Macadamia integrifolia*, *M. tetraphylla* and hybrids) and produce the second largest amount of macadamia nuts in the country (Weier et al. 2018). Other monocultures include pecan nuts (*Carya illinoensis*), avocados (*Persea americana*), bananas (*Musa* spp.), pines (*Pinus* spp.) and gum trees (*Eucalyptus* spp.) (Taylor et al, 2013). The orchards are interspersed with remnants of natural vegetation with riparian forests as well as woodland. However, these remnants are invaded by IAP's, predominantly *Chromolaena odorata* (King and Robinson, 1970), *Caesalpinia decapetala* (Roth) Alston, *Solanum mauritianum* (Scopoli) and *Lantana camara* (Lanca). The Luvuvhu valley is also among the most invaded regions in the province of Limpopo (Henderson 2008).

The area is characterized by wet summers and dry winters (Griscom et al. 2009). Daily maximum temperatures in summer are usually between 35°C - 40°C with winter temperatures between 0°C - 25° (Taylor et al 2013). This region receives around 1000mm annual rainfall between November and April (Weier et al. 2019).



**Figure 2.1** Map of study area, inset image of the macadamia orchard and remnant patch (Google earth).

### *Study design*

Repeated observations were done at one site on the Maluma farm over a two-year period before and during the macadamia flowering period. This was largely done because of the logistic and security constraints of using cameras as well as the objectives of this study. The site consists of a macadamia orchard bordered by a patch of remnant vegetation (Figure 2.1). Throughout the Luvuvhu valley, these remnant patches often include a drainage line and are invaded by IAP's. Observations of flower visitor visitation were done for four months; August 2018 and June to August 2019. This coincides with the period just before and during the peak flowering of macadamia (August and September).

### *Pollinator observations*

The number of individual plants observed during each survey was constrained by the number of camera traps ( $n = 14$ ). Focal plants for each survey were proportionally representative of species that dominated floral cover in the study site (> 30% of flower cover) and a grid (10 x 10m) was set out around each focal plant and the percentage cover for each flowering plant species in the grid was estimated (Appendix A).

Data was collected for four consecutive days each survey (month) over a period of 12 hours each day from 06h00 - 18h00 to allow for the capture of temporal variability in flower visitor assemblages (Baldock et al. 2011; Simba et al. 2018). At the start of each survey, floral resources were quantified by counting the total number of flowers in a 2 x 2m plot. As macadamia is a mass flowering plant, the length of three random flower racemes were measured, the number of flowers on each raceme were counted and multiplied with the estimated total number of racemes (with open flowers) in the plot. For other flowering plants, three random flower bunches were selected, open flowers counted and the total number of flower bunches (flowers on individual branch of the plant) in the plot estimated. Camera trap and visual observations were done concurrently.

### Visual observation

Focal plants were each observed four times daily for 10 minutes in a 2 x 2m quadrat with the observer standing 1m away from the focal plant. The four observation periods were evenly distributed 06h00 - 09h00 (morning), 09h00 - 12h00 (before noon), 12h00 – 15h00 (afternoon) and 15h00 – 18h00 (late afternoon). The sequence in which individual focal plants were observed each day was random. Each time an insect visited a flower of the focal plant it was recorded as an individual flower visitation event (Nel et al. 2017). Flower visitors that could not be identified in the field were collected and later identified in the laboratory. Flower visitors were identified to the possible taxonomic level (Appendix B). A reference collection of insects with exclusion of birds is deposited in the University of Venda museum.

### Camera traps

A camera trap (Wingscape BirdCam Pro) was installed next to each focal plant, they were programmed to capture images every minute between 6h00 to 18h00 (Fig 2.2). Image data were processed using DigiKam software (DigiKam Team, 2019). Images were tagged for the presence of flower visitor (an insect that was seen specifically on the flower) and identified up to species level. Total flower visitors were also recorded for each image. Data were extracted through R code for the DigiKam using RSQLite and DBI packages.



**Figure 2.2:** Camera trap mounted on the pole next to the focal plant. Each camera (A) was caged to avoid damage from rain and locked to avoid theft as they were left to run for almost a week whilst (B) is the external battery

### *Statistical analysis*

All statistical analyses were done using R (R Core Team, 2019).

Pollinator visitation data for species richness analysis were pooled within the four three-hour periods for each day and focal plant. This resulted in a total of four periods per day  $\times$  14 focal plants  $\times$  four days  $\times$  four surveys  $\times$  two methods = 1792 pollinator metacommunities. The degree to which species richness varied with observation method, time of day, and whether the focal plant was an IAP, native or macadamia, was explored using Generalized Linear Mixed Effects models. We assumed that pollinator visitations for a focal plant and all the focal plants observed during a particular survey were dependent, this was done by specifying focal plant nested within a survey as a random factor. We did however assume that the different surveys at the site were independent, an assumption that is probably not valid, but considering the constraints of the study as mentioned earlier, could not be avoided. The fact that different focal plants were used during each survey and that the study was done over a two-year period provide some support for this assumption. Time of day was a



categorical variable in the model. An interaction term between the observation methods and the type of plant species were also included. The full model therefore included the following terms:

Species richness ~ method: plant type + time of day + origin + (1|plant/survey)

Flower visitation and species richness were standardized by total floral units/m<sup>2</sup> per plant for both methods. GLMM included a loglink function and Poisson error distributions. The deviance explained by nested models was compared by computing an analysis of deviance table using the 'anova' function. The model with the smallest AIC and that explains a significant amount of additional residual deviance than the next best model, were considered the best model.

To assess the species abundance of flower visitors, we fitted multivariate generalized linear models (GLMs) using the mvabun package (Wang et al. 2012) with the 'manyglm' and 'anova.manyglm' functions. These models allow us to test for the effect of observational methods, and whether a plant is an IAP, native or macadamia on flower visitor species abundance. To account for species correlation, we used Wald statistics with 50 permutations and derived p-values using PIT-residual bootstrap method by resampling 999 rows of the dataset (Warton et al. 2012) and spatial autocorrelation was accounted for by resampling residuals across focal plants within surveys. The surveys were specified in the block argument of the 'anova.manyglm' function (Warton et al. 2012). Marginal and conditional explanatory power of predictors were modelled. Data for focal plants were pooled for this analysis

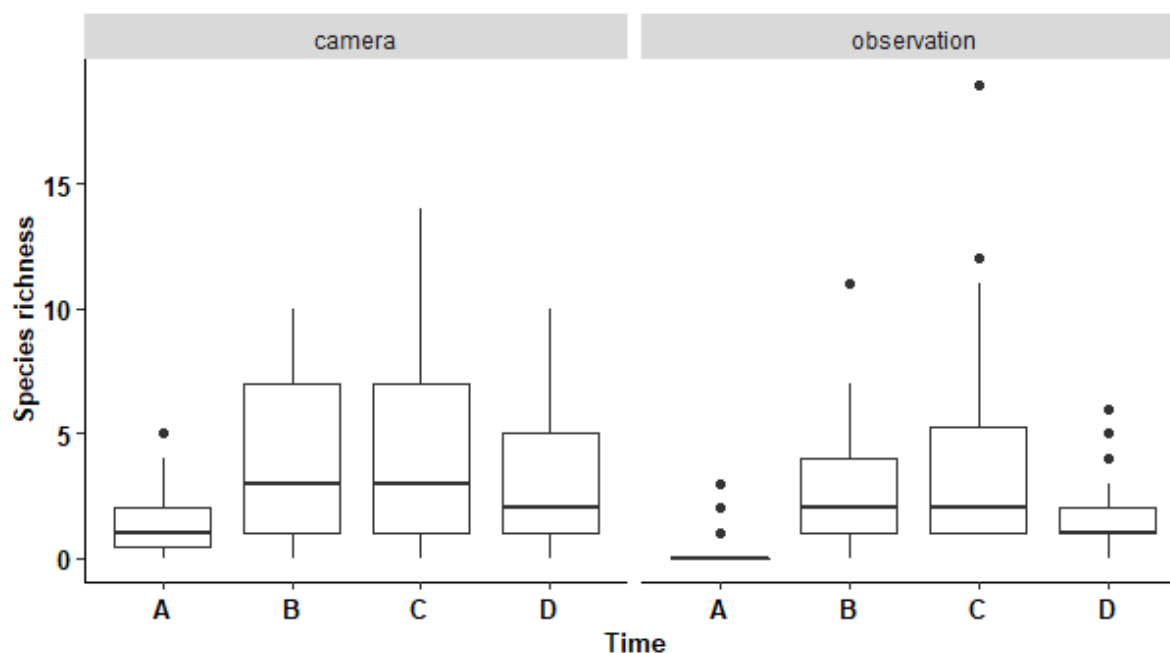
## Results

A total of 8335 visitations were observed by both camera traps and visual observation over the period of this study. Fourteen plant species were included in the study (Appendix A). Camera traps recorded 55 species from six insect orders in contrast to visual observation with 58 species from five insect orders. The most common flower visiting order was Hymenoptera (74%) with *Apis mellifera* accounting for 83% of the Hymenoptera flower visitations. Diptera was the second most diverse order, with 17% of flower visitations and the rest of the orders representing ≤5% of the visitations.

**Table 2.1:** Total number of floral units and percentage of the total for each of the plant types (IAP, Macadamia, Native) over the period of the study

Type	Floral units	Percentage floral units/m <sup>2</sup>	Number of plants
Native	3145	44	13
IAP	2391.5	34	33
Macadamia	1541.25	22	11

The best model included an interaction between method and type of plant species as well as the time of day (Table 2.2). Although there were significantly more species observed using the camera traps, visual observations fared better in the macadamia orchards. There was a significant effect of time of day on species richness, with richness peaking just after midday (Figure 2.3). There was significantly less species on macadamia than on native or IAP plants, but no difference between IAP and native plants. Floral density did not affect the richness of flower visitors (Table 2.2).



**Figure 2.3:** Variation in flower visitor richness observed on plants during the day.



**Table 2.2:** Best Generalized Linear Mixed Models for species richness of floral visitors to a plant based on method of observation, type of plant species (Native, IAP or Macadamia) and time of day.

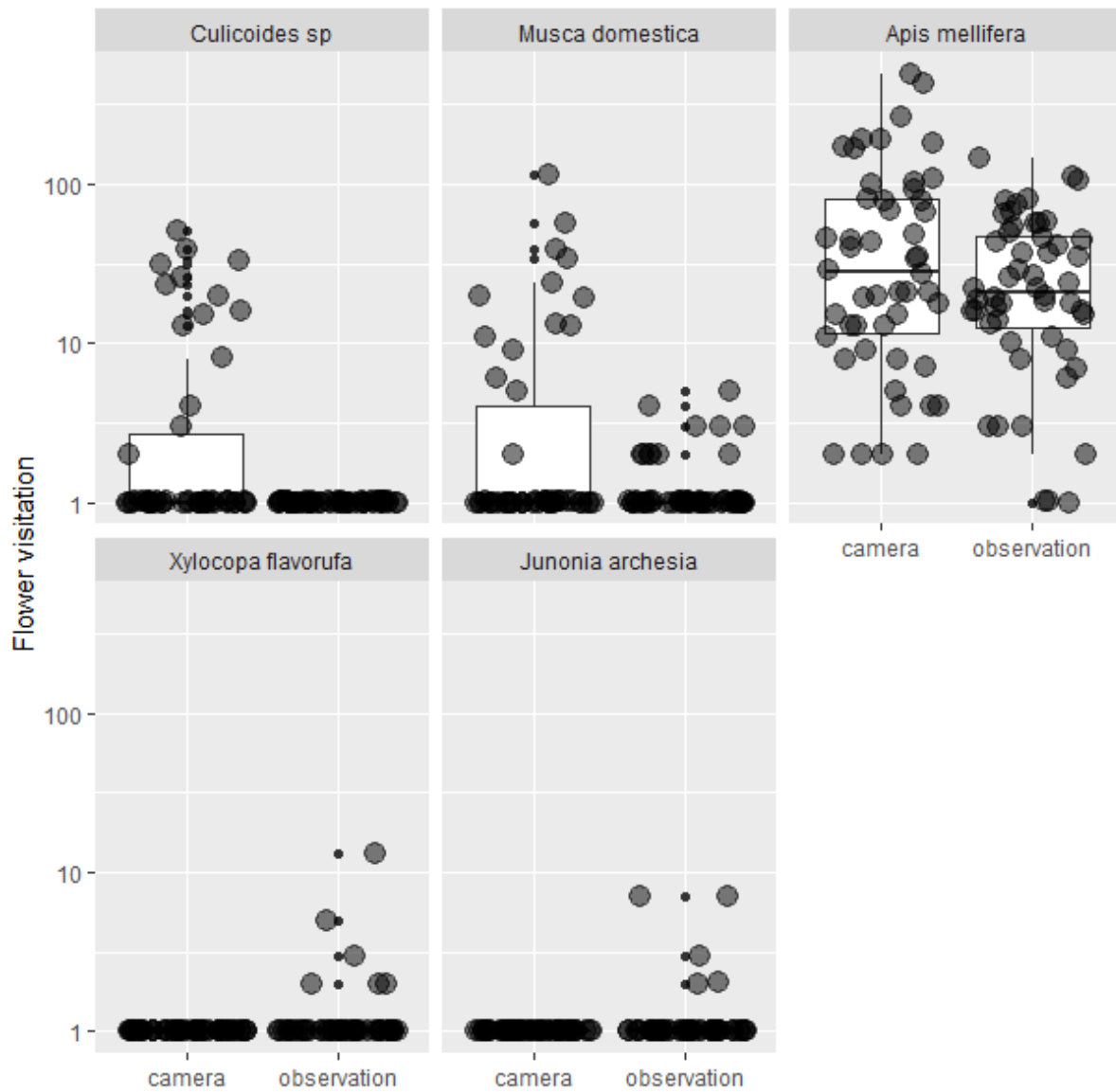
Variables	Visual observation	Macadamia	Native	09h00–12h00	12h00–15h00	15h00–16h00	No. Floral Units/m <sup>2</sup>	Visual Macadamia	x Visual Native	df	AIC	ΔAIC
Model 1 (best model)	-0.4***	-0.85***	0.29	1.7***	2***	1.3***	-	0.7***	-0.03	12	4100	0
Model 2	-0.35***	-0.48*	0.25	1.7***	2***	1.3***	-	-	-	10	4109	9
Model 3	-0.35***	-0.5*	0.25	1.7***	2***	1.3***	0.02	-	-	11	4111	11
Null model										4	4440	340

## Multivariate results

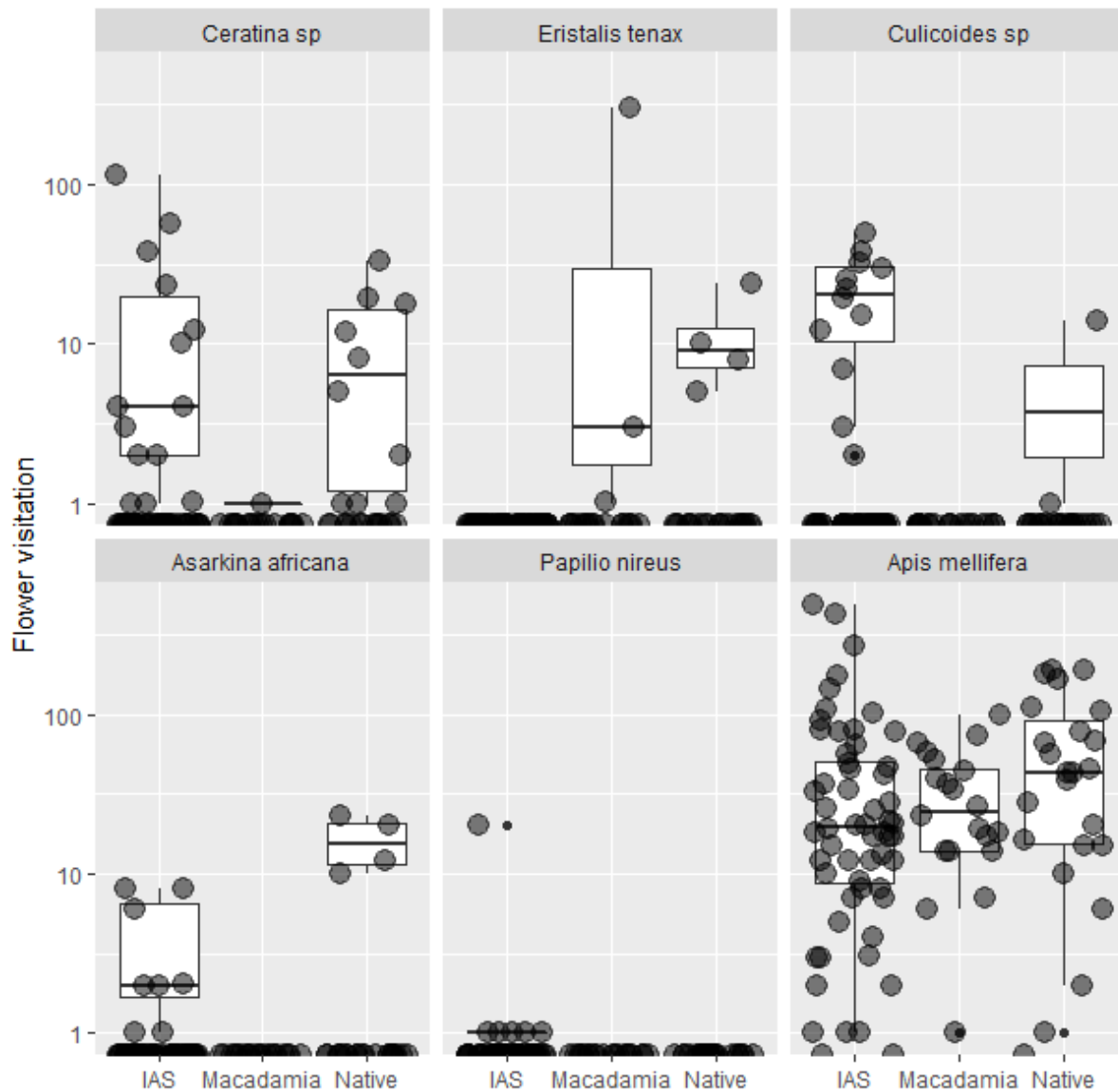
Floral units played a significant role in structuring flower visitor assemblages, but method of observation in particular explained most of the deviance beyond what other predictors explains (Table 2.3). *Musca domestica*, *Culicoides* sp. and *Apis mellifera* were more readily observed using cameras (Figure 2.4) while the rarer species *Xylocopa flavorufa* and *Junonia archesia* (Lepidoptera) are more readily observed through visual methods. The type of plant also affected what kind of species were observed (Figure 2.5). Most of the dominant flower visiting species were either associated native plants (*Asarkina Africana*, *Ceratina* sp.) or IAP (*Culicoides* sp.:Diptera), with none of the species having a preference for macadamia. *A. mellifera* seemed to prefer all the plants although there is a general trend of them preferring more of IAP's.

**Table 2.3:** Conditional and marginal deviance explained in pollinator community composition by predictor variables

<b>Conditional</b>	<b>Deviance</b>	<b>Marginal</b>	<b>Deviance</b>
June 2019 survey	157***	Survey	673
July 2019 survey	104*	Floral units	1043
August 2019 survey	122*	Type of plant	887
Floral units	179***	Method	977
Macadamia	129**		
Native	189**		
Visual observation	271**		



**Figure 2.4:** Species that contribute 30% to differences in flower visitor assemblages observed using cameras and visual observation.



**Figure 2.5:** The five species that contribute most (25%) to differences in flower visitor assemblages observed on native, IAP and Macadamia plant species. *Apis mellifera* was included for illustrative purposes.

## Discussion

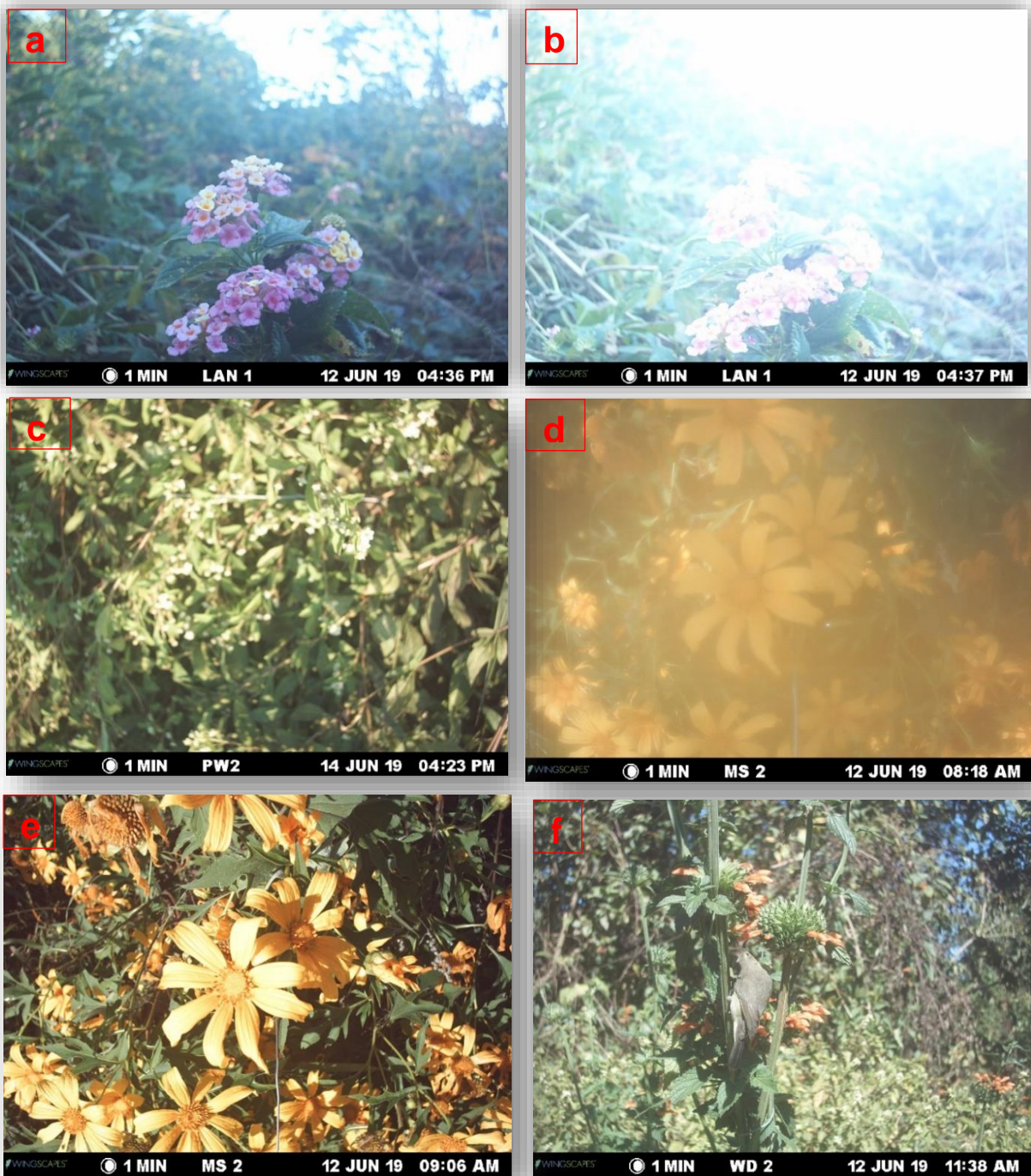
Different methods yielded different species composition that differed between plants and throughout the day. Overall there were more species that were recorded with visual observation but per sample there were more species recorded through camera trap. Visual observation recorded a greater variety of species than camera trap. Species that are relatively small in size (e.g. *Culicoides* sp.) were more evident in

camera trap samples while large conspicuous species were visually observed (e.g. *Xylocopa flavorufa* and *Junonia archesia*) but absent from camera trap samples. As expected, species richness differed across the day. IAP's, native plants and macadamia crops had different floral units. Though macadamia had significantly more floral units than native plants and IAP's, the crop was associated with low species diversity than native plants and IAP's.

Different species were detected by both camera traps and visual observation, camera traps recorded significantly more diverse species assemblages than visual observation. Unlike visual observation, camera trap does not affect the foraging behaviour of insects (Caravaggi et al. 2017) hence there were significantly more *Musca domestica* detected by camera trap. The detection of less *M. domestica* with visual observation than camera trap might have been affected by the presence of observer (human) as Caravaggi et al. (2017) and Darras (2019) found an observer to have an impact on cryptic species that shy away from the presence of humans. There were some species that were only recorded through camera trap such as *Culicoides* sp. *Culicoides* species are nectar feeders and blood suckers (cattle's and human too) and they have a high olfactory sense (van Middelaar et al 2008) hence this might have led to no detection in the presence of human. More rarer species were visually observed than recorded through camera traps although some studies found that the use of camera traps help detect more rarer species (Meredith and Rudout 2018). Camera traps were also associated with records of smaller species such as *Culicoides* sp. whilst bigger species were easily visually observed.

The use of camera traps in our study had limitations similar to the ones of the study done by Villa et al. (2007) such as images showing incomplete species and species captured far from focal point. Our limitations were mostly the environmental conditions: wind made the images blurry and variable light even with pictures taken just a minute apart. These limitations might have affected the probability of the camera capturing an insect leading to decreased records of flower visitation. However, even with the limitation of camera trap from our study (Figure 2.6); camera traps managed to record significantly more species than visual observation alone.





**Figure 2.6:** Camera trap images showing different classification scenarios captured from different plants; (a and b) different illumination though images were taken a minute away from each other, (c) blurry images due to wind, (d) midst from morning made the picture not to be clear, (e and f) the pictures were clear/ideal where on (f) a bird is captured visiting the flowers of native *Leonotis nepetifolia*.

IAP's dominated the macadamia orchards than the native plants. These plants are associated with greater floral rewards that they offer flower visitors than flowering native plants (Bjerknes et al. 2007; Morales and Traveset 2009) hence we found that these plants were visited by more flower visitors than native and macadamia crops. IAP's have the ability to modify the behaviour of native plants flower visitors (Morgles et al. 2017) resulting in more visitation. The presence of IAP'S in the system can affect the visitation to the native plants (Brown and Mitches 2001). The number of floral units does not seem to have an effect on the number of species that visits a flower, but it does affect the composition of those species. Although even with the native plants having significantly lower floral units than macadamia, they have more species visiting them than macadamia marginally but not significantly more than the IAP. Native plants are thought to also support insects, mostly bees, in terms of floral rewards maintaining their population and diversity (Blaauw and Issacs 2014). There were slightly more honey bees that visited native plants than IAP's. Native patches can also support more flower diversity even in the absence of IAP's suggesting the importance they play in the remnant patches supporting flower visitors

The flowering plants within the orchards can influence the abundance of flower visitors (Howlett et al. 2015). Macadamia orchards have lower species richness than native patches and IAP's. In the native range of macadamia crop, honey bees and stingless bees are the most dominant pollinators pollinating the crop (Heard 1994). The crop is also visited by beetles and flies, though their effectiveness as pollinators has not been tested (Howlett et al. 2018). The number of flower visitors that visits macadamia differs within orchards and between seasons (Howlett et al. 2015). *A. mellifera* and *Eristalis tenax* were the dominant flower visitors of macadamia in this study. This is probably the first record of *E. tenax* on macadamia and although flies are thought to be less effective pollinators than bees (Howlet et al. 2015) future studies should explore their role. Other study specified *E. tenax* to be both a flower visitor and a pollinator (Rader et al. 2020).

The use of both methods can give a representation of species richness occurring at a certain area. Even though the methods recorded different species, visual observation alone represented better species richness than camera trap. This method was associated with bigger field of view hence picking up a lot of insects including rare species and bigger insects than camera trap that had smaller field of view. Camera

traps are very difficult to set and have labour and material costs although the method managed to detect small insects and birds that shy away from human presence (Figure 2.6 f) and *Musca domestica*. There were some cameras that were just set up to run for the night and they failed to pick up nocturnal insects even though to our knowledge there are insects visiting some open flowers at night. For future studies both video recording and imaging can be used for insects to also help study the behaviour of insects and capturing time can be reduced to 30sec although this will result in a much larger data to process.

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## Chapter 3: Flower visitor communities in heavily managed and transformed landscapes – the case of *Apis mellifera* and invaded remnant patches in Macadamia orchards

### Abstract

Remnant patches in agricultural landscapes play an important role in supporting flower visitor diversity. However, these remnant patches are often invaded by alien plants (IAPs) which integrate into native plants pollinator network by either competing or facilitating pollination. Managed honey bees are important pollinators in agricultural communities and can coexist with other insects. However, the increase in commercial beehives by beekeepers have disrupted this coexistence. Here we study the impact of IAPs and honey bees, *A. mellifera*, have on the pollinator network structure and activity in and around macadamia orchards. Surveys were conducted before and during the flowering period of macadamia, focusing on the dominant flowering species in the landscape. IAP's are more generalist in flower visitors with macadamia being more specialized. Although flower unit densities were almost twice that of IAPs and native plants, less than 10% of observed *A. mellifera* visitations were on macadamia, while more visitations were on IAP's and native plants. *A. mellifera* temporal activity seem to influence that of the other dominant flower visitors, and in instances where they don't, species become more specialized. This study showed that honey bees prefer IAP's outside the flowering period which could further influence the spread of invasive plants and influence native pollinator communities through competitive interactions.

### Introduction

As natural habitats are cleared and modified for agriculture, species richness and structural diversity are greatly impacted resulting decline of biodiversity (Godefroid and Koedam 2003; Radford and Bennett 2007). Ever-decreasing size of remnant patches from habitat modification can only accommodate small populations of insect pollinators (Adriaens et al. 2009). Patch size is important in conserving species richness and abundance (Godefroid and Koedam 2003). Species occupying remnant patches can indicate the well-being of the ecosystem and a decline in them can be an indicator of change in habitat variables (Pryke et al. 2013).

Many crop plant habitats rely on the surrounding remnant patches for increased production. Natural edge habitat supports a wide array of insect's communities. These



remnant patches provide refuge for some pollinators and birds build their nest there (Blitzer et al. 2012). Natural and seminatural habitats are extensively destroyed by growing population, fragmentation and agricultural land use (Godefroid and Koedam 2003; Steffan-Dewenter 2003) and this in turn results in a major decline in biodiversity (Radford and Bennett 2007) and local extinction of insects species (Steffan-Dewenter 2003) available to pollinate crops.

Invasive alien plants (IAP's), habitat loss and habitat fragmentation are considered globally as the greatest threats to biodiversity (Yates et al. 2004; Bjerknes et al. 2007). Besides the threats that they collectively pose, other studies have shown that habitat loss and fragmentation promote the spread of IAP's (With 2004) whereby they easily establish themselves and disperse.

To understand the impact and extent of invasion in the ecosystems it is important to study the interactions in a multi-species networks (Santos et al. 2012). Networks can be used to infer the effect of IAP's (Grass et al. 2013). Networks are interactions between plants and their flower visitors/pollinators. This interaction between organisms can indicate the strength of the ecosystem, structure and function (Bartomeus et al. 2008; Hansen et al. 2018).

IAP's can affect the structure of the plant-pollinators network (Bartomeus et al. 2008; Hansen et al. 2018). Networks are very sensitive to the introduction of the IAP's (Santos et al. 2012) and interactions between the species in ecosystems might be more vulnerable than the species themselves (Ballantyne et al. 2017).

Not all IAP's have a negative impact (Bartomeus et al. 2008). The impacts do as well differ between plants and between season, sites and time (Ojija et al. 2019). IAP's can integrate with native plant-pollinators and provide ecosystem services to flower visitors within and outside flowering periods (Potts et al 2010). In network matrixes, high nestedness suggest the interaction of generalist and specialists flower visitor (Bosch et al. 2009, Hansen et al. 2018). It indicates that there are core generalist plant and pollinator and other plants and pollinators depend on them (Bartomeus et al. 2008). *A. mellifera* are considered generalist flower visitor and is expected to visit almost all the plants in the plant-visitor network (Santos et al. 2012).

Macadamia belongs to the plant family Proteaceae and the commercially most important species are *Macadamia integrifolia* Maiden & Betche and *M. tetraphylla*

L.A.S. Johnson. It is a small evergreen tree native to the subtropical coastal rainforests of eastern Australia (Heard 1994; Howlett et al. 2015). The nut set in macadamia trees strongly depends on pollination by wild bees and managed honey bees *Apis mellifera* Linnaeus (Heard and Exley 1994). Macadamia is capable of self-fertilization with the recent study depicting 90% of the pollination through cross pollination (Langdon et al. 2019). Other studies demonstrate that high yield cannot be achieved through self-pollination because of early nut drop (Trumann 2013), with recent studies proving 60 – 90% yield through self-fertilization (Richards et al. 2020).

In the native range of macadamia, stingless bees *Trigona carbonaria* Smith and honey bees *A. mellifera* are common pollinators, though they have been also reported to be pollen robbers where their visitation rate is high with little or no pollination (Heard 1994). Honey bee foraging ranges can be impacted by an overabundance of resources from mass flowering crops and they tend to focus on an easily accessible resource only (Grass et al. 2018).

Macadamia nut set is affected by the visitation of bees, the nut set decreases at with high visitation of the honey bees (Grass et al. 2018). The surrounding landscape of the orchards also affect the pollination service of macadamia (Howlett et al. 2015; Grass et al. 2018). Landscape modification i.e. habitat fragmentation and agricultural expansion results in the decline of bee pollination on macadamia (Grass et al. 2018).

Macadamia nut production has been experimentally demonstrated to decline regardless of the abundance of honey bees which might suggest other pollinating insects are also needed to increase the nut set (Grass et al. 2018). However, they found that 99% of pollinators were *A. mellifera* showing that excess use of pesticide (Grass et al. 2018) and habitat fragmentation (Howlett et al. 2015) might be impacting these pollination services. To achieve an increase in the final nut set, management practices that include the removal of invasive plants, plantation of native plants and the reduction of pesticides application should be instigated.

*A. mellifera* is a native pollinator to Africa although it is now found all over the world at different habitats (Carmo et al. 2004; Santos et al. 2012). It is considered the most economically important pollinator in many parts of the world (Iwasa et al. 2004; Zurbuchen et al. 2010). Bees are generally important pollinators in the agricultural community globally and they can enhance crop yields by 96% (Potts et al. 2010).

About 67–74% of bees are polylectic, meaning they can visit many different plants in one geographic region or habitat (Bosch et al. 2009; Goulson and Sparrow 2009).

Honey bees can coexist with other insects; however environmental changes and increased concentration of honey bees by beekeepers potentially disrupt this coexistence (Steffan-Dewenter and Tscharntke 2000). Their presence in the ecosystem changes the foraging pattern of other pollinators and can lower their visitation rate (Dupont et al. 2004). Its introduction in the non-native place may cause a decline of native flower visitors and pollination success (Carmo et al. 2004). Since honey bees occur in large numbers in a region; their abundance can result in competition for floral resources (Nielsen et al. 2017).

Competition for limited resources (pollen and nectar) amongst flower visitors affect the reproductive success and survival of species involved (Steffan-Dewenter and Tscharntke 2000). Honey bees are potential competitors as they can monopolize nectar and pollen for their large colonies and exploit patches of flowering plants. They can visit many flowers and reduce interactions between other flower visitors and plants (Goulson and Sparrow 2009; Santos et al. 2012). To assess the impacts of honey bees on other flower visitors, first there must be an overlap for floral resources of the same plant visited by both honey bee and other flower visitors (Paini and Roberts 2005).

Luvuvhu is heavily invaded by IAPs, large coverage of macadamia orchards, and intensively managed commercial bee hives, representing an ideal site to understand the impacts of IAPs and honey bees on flower visitor assemblages in and around macadamia. This study therefore aims to explore the impact of IAPs and *A. mellifera* on flower visitor communities in a *M. integrifolia* orchard bordered by a remnant patch by asking the following questions: (1) to what extent does IAPs affect the plant-pollinator network structure in space and time across the boundary between a *M. integrifolia* orchard and a remnant patch and (2) are the activity patterns and diversity of flower visitors influenced by *A. mellifera*?

## **Methodology**

### *Sampling method*

This study was done on the farm Maluma, in the Luvuvhu Region of Limpopo Province, over a two-year period. The site consists of a *M. integrifolia* orchard bordered by a

large patch of remnant natural vegetation invaded by IAP's (Fig 3.1 and Appendix C). Observations of flower visitation were done before flowering period (June 2019) and during the macadamia flowering period (late winter – early spring) (July 2019, August 2018 and 2019) for four consecutive days monthly.

On average, 14 individual plants were observed during each survey and the focal plants were species that accounted for >30% in flower cover. Two sets of methods, camera trap and visual observations, were used and data from these two methods were pooled. Visual observation was done for 10-minute periods observing a 2 x 2m area for four sessions 06h00 - 09h00, 09h00 - 12h00, 12h00 – 15h00 and 15h00 – 18h00. Camera traps captured images automatically every minute from 06h00 to 18h00. In total there were 27hours spend for visual observation and 192 hours for camera traps. Floral units per m<sup>2</sup> were quantified for all focal plants for each method separately at the beginning of each sampling period.



**Figure 3.1:** Study area of macadamia orchards (A) bordered by natural vegetation (B) that is invaded mostly by annual flowering IAP's (C) *Lantana camara* and (D) *Solanum mauritianum*.

### *Analysis*

### Networks

Data collected was used to construct flower visitor networks using the Bipartite package (Dormann et al. 2008). Networks were constructed for each sampling month separately. The following network metrics and their derivatives were calculated: connectance, nestedness, number of compartments, nestedrank, linkage density (Table 3.1) for each network (Dormann et al. 2009). A Visweb matrix was also constructed using the bipartite package to visualise the strength of flower visitor interactions (Vázquez et. al, 2009).

**Table 3.1:** Network metrics definitions.

<b>Network metrics</b>	<b>Definition</b>
<b>Connectance (C)</b>	Realised proportion of possible links; mean number of links (L) per species. Connectance shows coevolution of plants and flower visitors (Dormann et al. 2008).
<b>Nestedness (N)</b>	Nestedness is the measure of order in the ecological system.
<b>Weighted nestedness</b>	Nestedness of network considering the weight of interactions. it ranges between 1 (perfect nestedness) and 0 (perfect chaos) (Dormann et al. 2020).
<b>Weighted NODF</b>	Accounts for bias introduced by network size (Stouffer et al. 2014) and is more consistent and better than usual measures of nestedness (Dormann et al. 2020).
<b>Linkage density</b>	Marginal total weighted diversity of interactions per species (Dormann 2020)
<b>Compartments</b>	Compartments are sub-sets of the web which are not connected (through either higher or lower trophic level) to another compartment (Dorman et al. 2020). Compartment is visualised well in a Visweb
<b>Nestedrank (Nr)</b>	Ranks species according to their generality, which is measured as the position in the nestedness matrix. A generalist will interact with more species and have a lower rank, while specialists (and rare species) will interact with few species and have ranks with higher values (Dormann 2020).

### Overlap

Temporal overlap of *A. mellifera* and other dominant flower visiting species were explored using density plots (Meredith and Ridout 2018), calculated as a coefficient of overlap, ranging from 0 (no overlap) to 1 (complete overlap). Overlap is the area lying under the two density curves (Azevedo et al. 2018). The significance of this overlap was tested using 10 000 bootstrap samples with replacement. Bootstraps are used to create a null model which represent predicted activity of a species when there is no interaction. The distributions of the coefficients of the null model is then compared to

the observed coefficient to determine if it was larger or smaller than expected by chance. This was done by calculating the coefficient of overlap ( $\Delta$ ) and compared between the two taxa to see the difference in overlap between two kernel-density estimates (Azevedo et al. 2018). All records used for this analysis were  $>75$  and we therefore used  $\Delta_4$  (Azevedo et al. 2018).

## Results

Flower visitor network structure differed across periods for all four months. The connectance was found to be almost the same before ( $C = 0.28$ ) and during ( $C = 0.27$ ) the flowering period. The network was found to be more nested before the flowering period ( $N = 27.87$ ) than during the flowering ( $N = 23.28$ ), however weighted NODF displays the network during the flowering period to be more nested ( $NODF = 27.18$ ) than before flowering ( $NODF = 24.87$ ). The largest network was found before the flowering period with the highest number of links or interactions ( $L = 112$ ) and highest linkage density (Table 3.2). All the networks had only one compartment. Four most important flower visitors were found visiting more than two plants with *Apis mellifera* being the most common flower visitors visiting all the plants (Table 3.3).

**Table 3.2:** Network statistics for flower visitors at Maluma farm before and during Macadamia flowering

	June 2019	Aug 2018	July 2019	Aug 2019	Average (Mean)
	Before flowering	During flowering			During the flowering
Connectance	0.28	0.36	0.27	0.19	0.27
Nestedness	27.38	24.03	26.07	19.75	23.28
Weighted NODF	24.87	40.59	22.19	18.75	27.18
Weighted nestedness	0.44	0.57	0.43	0.51	0.50
Linkage density	3.54	3.31	3.24	3.60	3.38
Number of compartments	1	1	1	1	1
Links	112	47	53	48	49.33
Plant species	7	5	7	10	7.33
Flower visitors	58	27	29	26	27.33

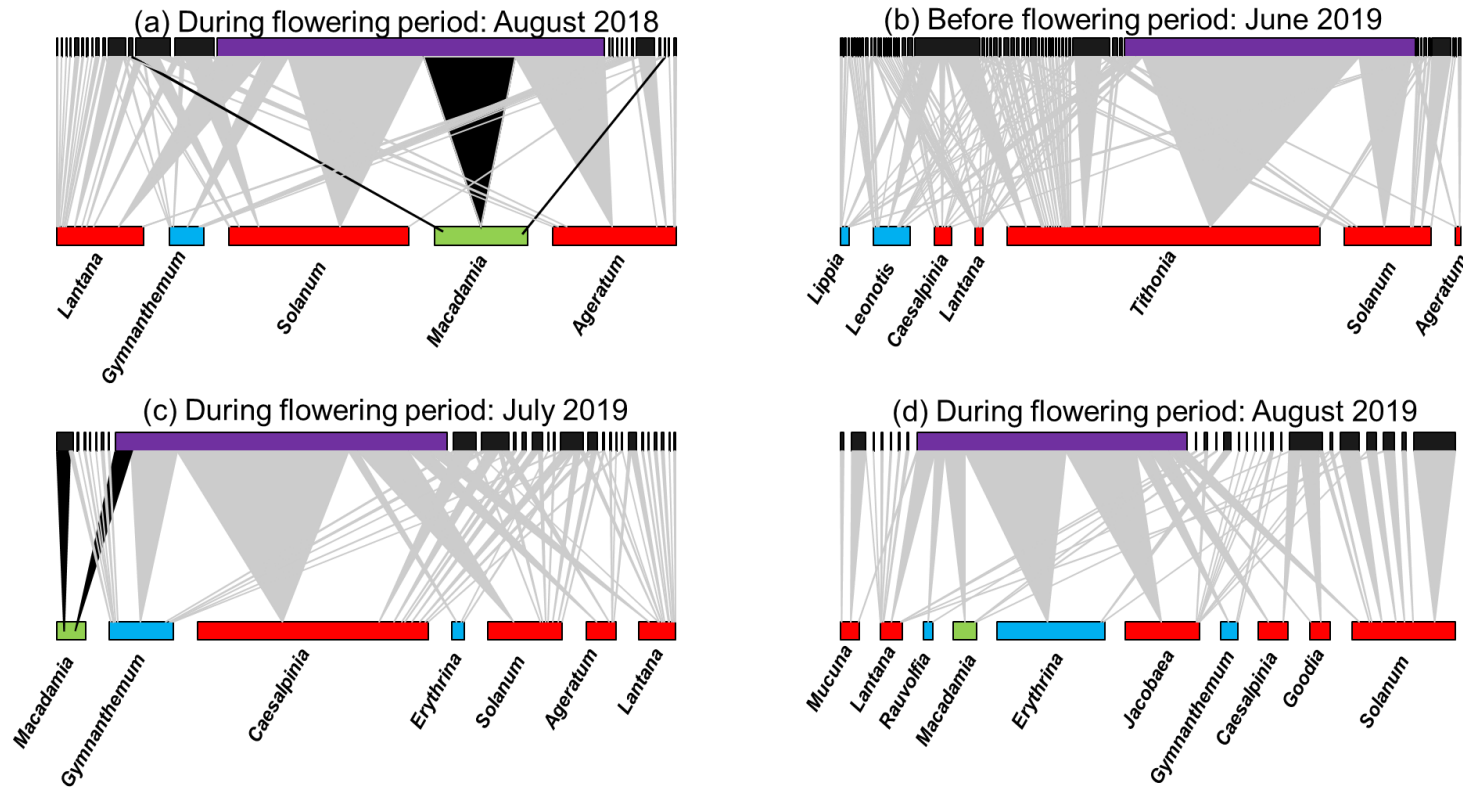


**Table 3.3:** Degree and nestedrank of four most important flower visitors.

Species	Degree	Nestedrank	Degree	Nestedrank
	Before flowering		During flowering (mean)	
<i>Apis mellifera</i>	7	0	7	0
<i>Ceretina</i> sp.	6	0.02	4	0.11
<i>Culicoides</i> sp.	4	0.07	2	0.39
<i>Musca domestica</i>	6	0.04	3	0.19

*A. mellifera* was the most common flower visitor and visited all the plant species (Table 3.3). It was more abundant during the flowering period than before the flowering period and represented 65% of flower visits observed in *M. integrifolia*. Other flower visitors to macadamia include *Xylocopa flavorufa*, *X. caffra* and *Coelioxys* sp. (Hymenoptera), *Musca domestica* and *Eristalis tenax* (Diptera), with *E. tenax* being the second most abundant visitor with 33%. Two-thirds of all honey bee visitations were to IAPs (Table 3.4)

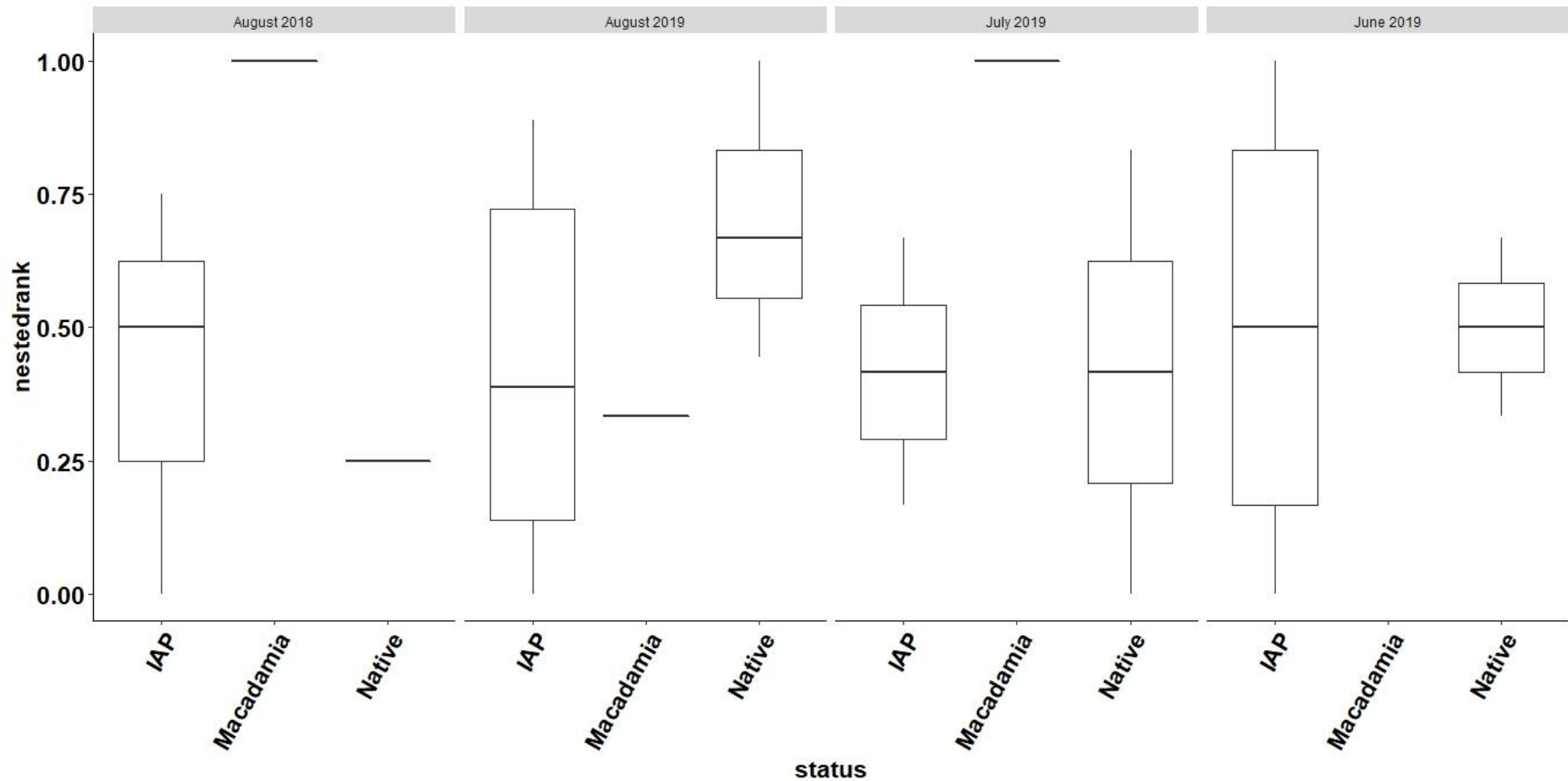
Species richness of macadamia flower visitors were low ( $n = 6$ ) unlike IAPs ( $n = 67$ ) and native plants ( $n = 43$ ). Most IAPs were more nested than native plants and macadamia (Table 3.5). IAPs are more generalist in the flower visitors they attract ( $Nr = 0.44$  and  $SD = 0.33$ ) than native plants ( $Nr = 0.59$  and  $SD = 0.24$ ) and macadamia ( $Nr = 0.78$  and  $SD = 0$ ).



**Figure 3.2:** Flower visitation network for four-month sampling period. Each network rectangle represents (top row) flower visitor species (insects) and the bottom triangle represents the plants. Purple represents *A. mellifera* and at the bottom red (IAP) plants, blue (native) plants and green (Macadamia) crop. Black link represent other *M. integrifolia* crop flower visitors than *A. mellifera*. Species (Flower visitors) names are omitted because they will be too small to read and increasing the font will lead them not being able to see which link they belong to however Visweb (Appendix C) shows the overall species visiting different plants

**Table 3.4:** *A. mellifera* visitation (%) to the plants before and during the flowering period of *M. integrifolia* for visual observations and plants flowering units.

Status	June 2019	Aug 2018	July 2019	Aug 2019	Average (mean): during flowering
	Before	During flowering			
IAP	87%	62%	41%	32%	45%
Native	13%	30%	49%	64%	48%
<i>M. integrifolia</i>	-	8%	10%	4%	7%



**Figure 3.3:** Nestedrank for observation data showing the rank of plant species according to their generality, species that are generalist interact more and thus have a rank closer to 0 whilst species pollinated by a few species will have ranks closer to one. June 2019 is before flowering period and July 2019, August 2018 and 2019 is during the flowering period



**Table 3.5:** Nestedrank which measure the species generality, one representing the plant visited by only few species and zero representing the plant visited by a diversity of flower visitors.

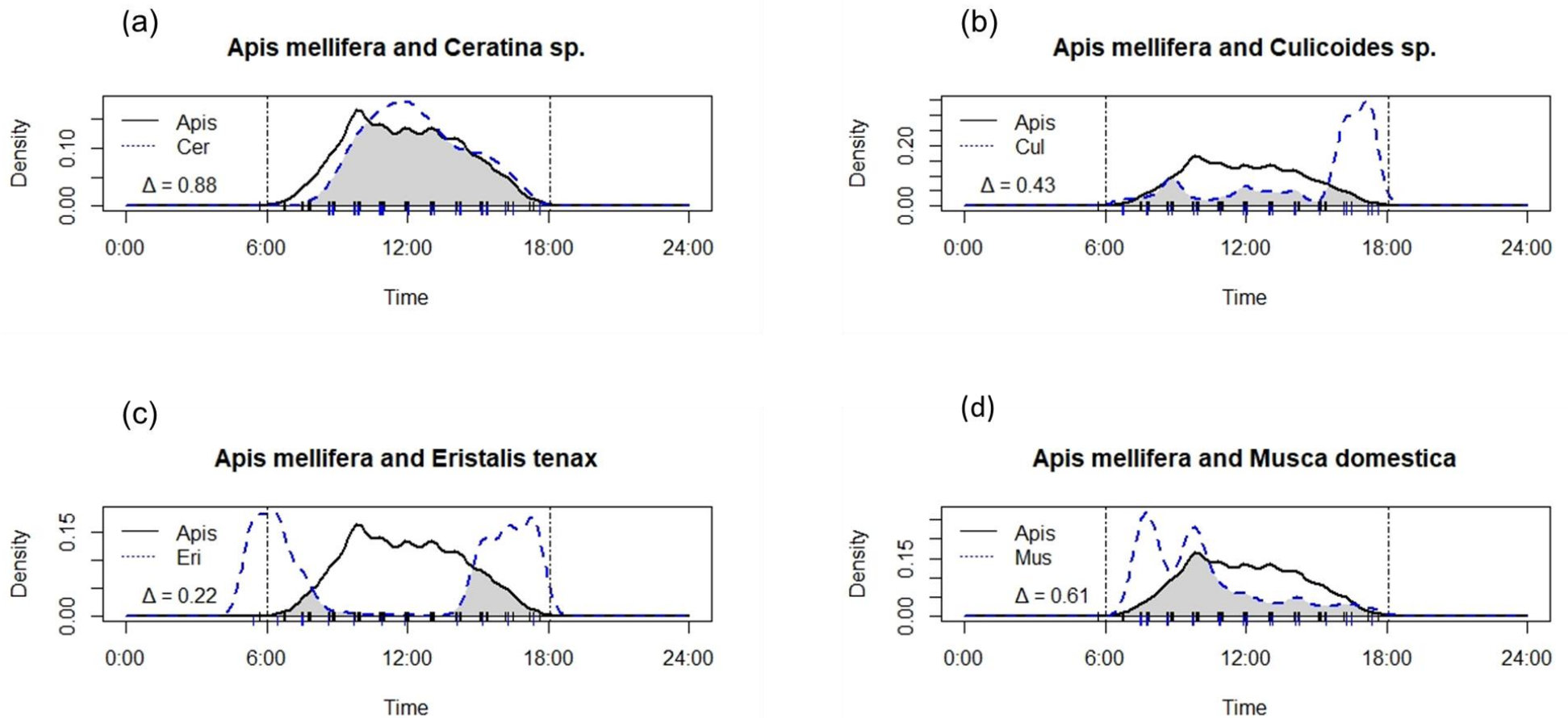
Plant	Origin	Before flowering	During flowering period			Average (mean)	Standard deviation (SD)
		June 2019	Aug 2018	July 2019	Aug 2019		
<i>Tithonia diversifolia</i>	IAP	0	-	-	-	0	0
<i>Lantana camara</i>	IAP	0.17	0	0.17	0	0.09	0.09
<i>Jacobaea vulgaris</i>	IAP	-	-	-	0.11	0.11	0
<i>Gymnanthemum myrianthum</i>	Native	-	0.25	0	0.67	0.31	0.28
<i>Leonotis nepetifolia</i>	Native	0.33	-	-	-	0.33	0
<i>Solanum mauritianum</i>	IAP	0.50	0.75	0.50	0.22	0.49	0.19
<i>Mucuna coriacea</i>	IAP	-	-	-	0.56	0.56	0
<i>Erythrina caffra</i>	Native	-	-	0.83	0.44	0.64	0.20
<i>Caesalpinia decapetala</i>	IAP	0.83	-	0.33	0.78	0.65	0.22
<i>Lippia javanica</i>	Native	0.67	-	-	-	0.67	0
<i>Ageratum conyzoides</i>	IAP	1	0.50	0.67	-	0.72	0.21

<i>Goodia lotifolia</i>	IAP	-	-	-	0.89	0.89	0
<i>Macadamia integrifolia</i>	Macadamia	-	1	1	0.33	0.78	032
<i>Rauvolfia caffra</i>	Native	-	-	-	1	1	0

### Temporal overlap of dominant flower visitor species

The most abundant flower visitor species were *A. mellifera* (58%), *Ceratina* sp. (9%), *M. domestica* (6%), *Culicoides* sp. (5%) and *E. tenax* (5%). There is almost complete overlap between *A. mellifera* and the *Ceratina* sp. ( $\Delta$ : 0.88). Both the species were less active in the morning and late afternoon but *A. mellifera* is more active just before noon whilst *Ceratina* sp. is more active in the early afternoon (Fig 3.4). *M. domestica* ( $\Delta$ : 0.61) and *Culicoides* sp. ( $\Delta$ : 0.43) overlapped less and their activity patterns did not differ significantly from that of *A. mellifera*, although plots suggests that there are some degree of temporal partitioning, with *M. domestica* being largely active during the early morning and *Culicoides* sp. active during the late afternoon (Fig 3.4). *A. mellifera* and *E. tenax* temporal activity ( $\Delta$ : 0.22) differed significantly from each other (Table 3.6). *E. tenax* is more active in the morning and late afternoon compared to *A. mellifera* which is more active during the middle of the day.





**Figure 3.4:** Activity curves for *A. mellifera* with four dominant species. The coefficient of overlapping equals the area below both curves shaded in grey. The black solid line depicts *A. mellifera* and the blue dotted line is for other species.

**Table 3.6:** Density plot analysis estimates of overlapping ( $\Delta$ ) of flower visitors with *A. mellifera*, bootstrap mean and 95% confidence intervals (CI) for camera trap. The difference between bootstrap mean and overlap estimate indicate bootstrap bias.

Species	Bootstrap mean	Estimate	CI
<i>A. mellifera</i> ~ <i>Ceratina</i> sp.	0.88	0.88	0.84 – 0.90
<i>A. mellifera</i> ~ <i>Culicoides</i> sp.	0.45	0.43	0.37 – 0.47
<i>A. mellifera</i> ~ <i>E. tenax</i>	0.26	0.22	0.25 – 0.22
<i>A. mellifera</i> ~ <i>Musca domestica</i>	0.65	0.61	0.57 – 0.65

## Discussion

This study's results suggest that the network structure fluctuates in both space (plants origin) and time (between seasons). Connectance, nestedness, the number of compartments did not differ between networks observed before and during macadamia flowering. Linkage density did not differ between seasons although before the flowering period there were more links than during the flowering period. Macadamia were found to be visited by small proportion of honey bees despite their abundance in the system. Flower visitors nestedrank indicate some insects becoming more specialized in the presence of other flower visitors therefore it is important to highlight the temporal partitioning between these flower visitors. Honey bees and other bees (*Ceratina* sp.) display similar temporal daily activity, but the activity differed between the honey bees and dipteran species: *E. tenax*, *Culicoides* sp. and *M. domestica*. They are active at different times of the day, particularly early morning and late afternoon while honey bees are active before noon and afternoon. The temporal partitioning between honey bees and Dipterans suggest either resource partitioning or competitive interactions.

IAP's can alter the network structure by creating more connectance and fluctuating linkage density (Stout and Tiedeken 2017). Flower visitor networks that were flooded with more IAP's were more connected that the ones with proportionally less IAP's. High connectance indicate that the network has more generalist flower visitors than

specialists. Connectance decreases with increasing flower visitor diversity and is indirectly proportional to species richness (Memmott and Waser 2002). This was not found, we found that connectance was the same despite the difference in species richness between flowering periods indicating that even with the changes in species composition the network may remain unaffected (Hansen et al. 2018). Other studies found that low connectance can be produced by network having many specialists or as the results of low sampling intensity (Dormann 2020).

Network structures are strongly affected by species richness (Stout and Tiedeken 2017). A network consisting of many species (high richness) produce fewer links than ones with few species (Chacoff et al. 2012). However, before the flowering period the network consisted of the most flower visitors and the highest number of links. The number of links are determined by the number of flower visitors and by the number of bottom species (plants). Linkage density did not differ between seasons although before the flowering period the network was associated with high number of links than other seasons.

The flower visitors' network that is more nested depicts plants that were visited most by generalist flower visitors along with specialist visitors (Lopezaraiza-Mikel et al 2007). Nested network shows that there are core generalist plant and pollinator and there is a dependence of other plants and flower visitors on them (Bartomeus et al. 2008). IAP's are more nested than native plants illustrating that they consist of more generalist flower visitors than specialist flower visitors. Most IAP's are generalist attracting a variety of flower visitors because of their high floral resources therefore can compete for flower visitors with the native (Grass et al. 2013). These IAP's also share most of their flower visitors with native plants and other studies have found that the shared flower visitors amongst plants compete for floral resources (Lopezaraiza-Mikel et al 2007). Nestedness is not affected by the increased connectance so it can either increase or decrease (Bosch et al 2009).

Plant species can share, support or compete for flower visitors (Hansen et al. 2018). All the flowering plants were found to share generalist flower visitors *A. mellifera* before and during the flowering period of macadamia. *A. mellifera* was the most dominant flower visitor across the seasons, with an increase from 54% to 64% during the flowering period. Honey bees are considered a generalist flower visitor (Santos et al.

2012, Grass et al. 2013) and generalist flower visitors are likely to visit more of the IAP's than do specialist or rare flower visitors (Hansen et al. 2018). More visits of honey bees were mostly to the IAP's before the flowering period with about 87% visits of the honey bees.

Though IAP's have undesirable impacts in the ecosystem, they can share flower visitors with the native plants and crop plants and removing them from the system may result in species loss (Carvalho et al. 2008). This study found that the IAP's shared the common abundant flower visitors of macadamia with the native plants. It seems that the IAP's support *A. mellifera* hives outside the flowering season. Although with the abundance *A. mellifera*, the insect does not seem to prefer macadamia. Even with the increase of *A. mellifera* hives to boost the pollination of macadamia, small proportion of the insects visits the crop plant <10% while most of it visits native plants and IAPs. More visits to the IAPs will probably aid invasion process although during the flowering period honey bees visited native plants more. It is not known if this was as the results of increase in flowering of the native plants or if *A. mellifera* was enough for optimal seed set in the native species.

Besides *A. mellifera* there were other flower visitors that visited macadamia; Hymenoptera: *X. flavorufa*, *Coelioxys* sp. and *X. caffra* and Diptera: *M. domestica* and *E. tenax* with the latter being the second most common flower visitor to macadamia. In the native range of macadamia, the crop is commonly visited by stingless bees and honey bees (Heard 1994). There is little to no research that found *E. tenax* visiting macadamia crop though the insect may be considered a potential pollinator for many crops (Howlett and Gee 2019). Other studies found that this insect visits other important crops such as onions, kiwifruit, soybeans, sweet pepper and carrot (Howlett and Gee 2019). Diptera is considered less effective as a pollinator of macadamia than bees and there is lack of studies on their effectiveness (Howlett et al. 2015) but see Rader et al. (2020) macadamia was one of the crops visited by the non-bee insects that included Diptera. *E. tenax* only visited macadamia and the native plant *G. myrianthum* and was not found visiting any of the IAP's. This demonstrate that since *G. myrianthum* flowers at the same time with the macadamia there are no plants found in the study area supporting this flower visitor outside the flowering period of both *G. myrianthum* and macadamia.

Flower visitors have a different ecological niche hence their daily activity pattern differs from organism to organism (Steen 2017). Though competition between bees is always expected (Steffan-Dewenter and Tscharrntke 2000), we found that bees partition for resources. The insects might be foraging on the flowers differently and using resources differently in relation to their body size. Honey bees is larger than *Ceratina* sp. and require more nectar and pollen for their large colonies (Steffan-Dewenter and Tscharrntke 2000). Both *A. mellifera* and *Ceratina* sp. are polylectic (Goulson and Sparrow 2009; Ali et al. 2018).

There was less overlap between *A. mellifera* and Dipteran flower visitors (*M. domestica*, *Culicoides* sp. and *E. tenax*). Diptera are amongst the largest and most diverse group of flower visitors worldwide. They are one of the most important group of pollinators, second only to Hymenoptera (Ssymank et al. 2008). Several Dipterans mimics honey bees with *E. tenax* mimicking the honeybee in both coloration and behaviour (Heal 1982). For our study we found that macadamia attracted a diverse assemblage of Hymenoptera with the Diptera being the second most important flower visitors.

Invasive flower visitors modify the behaviour of native flower visitors (Morgles et al. 2017), *A. mellifera* is native to Africa and Europe and Howlett and Gee (2019) found that *E. tenax* is not native to Africa. The insect is mostly found in Europe, China, Japan and India (Howlett and Gee 2019). There was slight overlap between *A. mellifera* and *E. tenax* and other studies found that bees and flies feed broadly at overlapping periods (Morse 1981). Both the flower visitors seem to have different activity pattern with *E. tenax* being more active in the morning and late afternoon and *A. mellifera* less active during these times. Honey bees are active throughout the day, peaking in their activity between 14h00 and 15h00 (Beer et al. 2016; Acuna et al. 2019). There is little information on the activity of flies (Babael et al. 2018) though *E. tenax* have a diurnal activity pattern (Thyselius and Nordstrom 2016).

Our study found that both the flower visitors depict different activity pattern, *E. tenax* was more active in the morning and late afternoon during which *A. mellifera* was less active. Many insects avoid overheating therefore depicting a binomial daily activity pattern (Totland 1994). *E. tenax* presented a binomial activity pattern. It was not entirely proven if this was because of avoiding heat or because of competition with *A.*

*mellifera*. *A. mellifera* was mostly active before noon and in the afternoon when temperatures were high and less active in the morning and late afternoon when it was cool.

The gaps in research about flies underestimate them as potential pollinators in terms of transferring pollen (Ssymank et al. 2008). *E. tenax* is active throughout the year in all seasons with low activity observed in winter (Howlett and Gee 2019). For future studies based on this study with *E. tenax* having high visitation to macadamia, it will be good to study this insect as an effective pollinator of macadamia probably also in terms of fruit set.

This study was done in winter and it will be good to conduct it in summer outside the flowering period of macadamia to also find other plant species that support this insect outside the flowering period as the other plant found it flowers at the same time with macadamia. Only one study site was sampled with no replication of comparing the difference in flower visitors between the invaded and non-invaded site.

This study identifies the importance that the native plant *G. myrianthum* plays in supporting the flower visitor of macadamia *E. tenax* within the flowering period however it also shows that IAP's support *A. mellifera* outside the flowering period. During the flowering period, honey bees visited more of native plant (48%) and IAP's (45%) which might indicate competition between the plants hence resulting less visits to the macadamia. Natural remnant vegetation does support a great diversity of flower visitors. The abundance of honey bees and more visitation to the IAP's will initiate further spread of the IAP's. The best for such this heavily transformed landscape will be to rather retain the IAP's but clear them around the mass flowering native plants like *G. myrianthum*. The clearing of some part of IAP's can be followed by more plantation of the native plants that will also support the macadamia flower visitors outside the flowering period maintaining their population density.

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## Chapter 4: Thesis synthesis

### *Efficacy of sampling methods*

Different sampling methods to assess the community composition of flower visitors. The use of camera traps to study the diversity and composition of insect pollinators is a rarely implemented method. Even with known limitations (Villa et al. 2017) this method outdid visual observation for sampling points but recorded fewer overall species. Visual observation recorded more species richness and due to bigger field of view the method managed to pick up even larger species easily as compared to smaller insects. Camera traps did well in recording or picking up smaller insects probably because of their smaller field of view. Camera traps and visual observation used together can give a clear representation of species richness occurring at a certain area; both recorded significantly different species, and so we can regard them as being complimentary. The intensive labour that came with setting up the camera traps and the costs of replacing the batteries was not really worthy of the effort for sampling flower visitors. However, this method did well in capturing small insects therefore its efficiency in capturing the presence of small insect species has to be weighed against its smaller spatial sampling extent. Camera traps also helps in sampling species that shy away from the human presence such as *Musca domestica* and birds.

### *IAP's, native plants and macadamia crop flower visitors.*

IAPs mostly have floral display that is associated with attraction of more diversity and abundance of flower visitors (Hansen et al. 2018). These plants together with the native plants were visited by greater diversity of flower visitors than macadamia. Both IAP's and native plants shared most of the flower visitors such as *Ceratina* sp., *Culicoides* sp., *Asarkina africana* and *Stomoxys calcitrans*. None of these species were associated with visitation to macadamia. Though macadamia is capable of self-pollination (Langdon et al. 2019), its nut set is also mostly facilitated by the visitation of wild bees and managed honey bees *Apis mellifera* (Heard and Exley 1994). Honeybee was the most abundant flower before (54%) and during (64%) the flowering period of macadamia.

Even with the honeybee being most abundant in the ecosystem, this insect was only associated with 65% visitations to macadamia. Low visitation might be the results of the impact of IAPs or pesticides. The orchard is heavily sprayed with pesticides and other studies reported pesticides as one of the impacts of pollinators decline (Goulson

et al. 2015; Nicolson and Wright 2017) and low visitation to macadamia (Grass et al. 2018). Beside honey bees there were other insects observed visiting macadamia, Hymenoptera: *Coelioxys* sp., *Xylocopa caffra* and *X. flavorufa* and Diptera: *Eristalis tenax* and *Musca domestica*. Dipteras are associated with both visitation and pollination of different crops (Rader et al. 2020) although they are not regarded as potential pollinators of macadamia crop (Howlett et al. 2015). *E. tenax* made 33% visitations to macadamia and Rader et al. (2020) found this insect to be associated with both visitation and pollination of crops.

This study showed that although not plant species specific, the IAPs did serve an importance in supporting macadamia flower visitors, *Apis mellifera* outside the flowering period (87%).

#### *Should the IAPs be removed from the agricultural landscape?*

Network analysis data can be used as a guide for the eradication of the exotic plants from the system (Howlett et al 2015). It was shown that the IAPs compete with native plant for flower visitors before the flowering period. However, these plants play an important role in supporting the flower visitor of macadamia outside flowering season although it was also associated with competing with the native plants. The removal can only be mitigated only around the mass flowering native plants.

#### *Study limitations, recommendation and future research*

This study was limited to only one site. We couldn't compare our findings obtained from a heavily transformed landscape dominated by IAP's to orchards that contained no IAP's. It would be especially interesting to know how they would differ in terms of flower visitors. Furthermore, for flower visitor networks not much comparison could be done in terms of the impacts of IAP's on the structure (connectance, linkage density, and nestedness) when compared to network with no IAP's. Morphological traits of flowers were not assessed in terms of nectar availability. It was not possible therefore to explain why certain flowers attracted more pollinators than others besides floral density.

The images obtained from camera traps cannot give a clear representation of species abundance as the same insect can be captured more than once. Nevertheless, the maximum number of insects per frame (image) can be used to deduce abundance. The same sampling principle can be applied to for visual observation, as the maximum

number of a species observed on the flower at one time can be used as the total abundance. For future studies both video recordings and imaging can be used. Capturing time can be reduced to 30 seconds although this will result in larger data sets to process.

This study identifies a paucity of recent researches done on macadamia pollination. Also, gaps in research about flies as potential pollinators as they are underestimated in terms of transferring pollen (Szymank et al. 2008) however recent study by Rader et. al (2020) listed some flies associated with both visitation and pollination. For future studies based on this study with *E. tenax* having high visitation to macadamia, it will be good to study this insect as an effective pollinator of macadamia probably also in terms of fruit set. *E. tenax* is active throughout the year in all seasons with low activity observed in winter (Howlett and Gee 2019). This study was done in winter and it will be good to conduct it in summer outside the flowering period of macadamia to also find other plant species that support this insect outside the flowering period as the other plant found visited by this insect flowers at the same time with Macadamia.

This study also identifies native plants associated with more overall visitation depicting the importance that the remnant natural vegetation plays in supporting flower visitors. More visitation to the IAPs will lead to the spread of the plants in the system which will result in increased greater impacts to the local and more diverse pollinator assemblages of the native flowers. The research knowledge we have already about the importance of diverse insect assemblage, it's probably not a bad awareness to the ecosystem to initiate the removal of the IAPs around the mass flowering native plants like *Gymnanthemum myrianthum* in order to maintain the native pollinators. The removal of these plants should be associated with planting of more native plants in the remnant patches. We found that *E. tenax* and *Apis mellifera* were associated with more visitation to the macadamia, planting more of the native plants will conserve not only the pollinators community but also the remnant patches hence preventing the future spread and invasion of IAPs.

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## Appendix

**Appendix A:** Flowering plants that were sampled by both camera traps and visual observation. Number of samples indicate how many times the plant was sampled throughout 2 years.

Scientific name	Family	Growth form	Native/IAP	Mean Plant cover (%)	Floral units/m <sup>2</sup>	Sampled months	Number of samples	
							Camera trap	Visual observation
<i>Ageratum conyzoides</i>	Asteraceae	Herb	IAP	63	670.25	Aug 2018, June and July 2019	6	7
<i>Caesalpinia decapetala</i>	Fabaceae	Shrub	IAP	54	70.50	June and July 2019	4	3
<i>Erythrina caffra</i>	Fabaceae	Tree	Native	60	62.00	July and Aug 2019	3	2
<i>Goodia lotifolia</i>	Fabaceae	Shrub	IAP	55	5.00	Aug 2019	1	1
<i>Gymnanthemum myrianthum</i>	Asteraceae	Shrub/ small tree	Native	80	1935.25	Aug 2018, July and Aug 2019	4	4
<i>Jacobaea vulgaris</i>	Asteraceae	weed	IAP	90	277.00	Aug 2019	2	2



<i>Lantana camara</i>	Verbenaceae	Shrub	IAP	86	1196.00	Aug 2018, June, July and Aug 2019	7	8
<i>Leonotis nepetifolia</i>	Lamiaceae	Herb	Native	63	132.50	June 2019	2	2
<i>Lippia javanica</i>	Verbenaceae	Herb/shrub	Native	85	718.75	June 2019	2	2
<i>Macadamia integrifolia</i>	Proteaceae	Tree	Macadamia	91	1541.25	Aug 2019, July and Aug 2019	9	9
<i>Mucuna coriacea</i>	Fabaceae	Herb	IAP	50	14.00	Aug 2019	-	1
<i>Rauvolfia caffra</i>	Apocynaceae	Tree/shrub	Native	85	296.50	Aug 2019	1	1
<i>Solanum mauritianum</i>	Solanaceae	Tree/shrub	IAP	75	129.50	Aug 2018, June, July and Aug 2019	7	7
<i>Tithonia diversifolia</i>	Asteraceae	Subshrub, Herb	IAP	80	29.25	June 2019	2	2

**Appendix B:** Species list of flower visitors and the number of observations for each method. Plants origin; E = IAP, N = native and M= macadamia.

Species	Plant/s	Visitation per observational methods		Total visitation
		Camera trap	Visual observation	
<b>Araneae</b>				46
<i>Salticidae</i> sp.	E	41		
<i>Araneae</i> sp.2	E	5		
<b>Aves</b>				19
<i>Aves</i> sp.1	N	7		
<i>Aves</i> sp.2	N	1	2	
<i>Aves</i> sp.3	N	2	1	
<i>Aves</i> sp.4	N	4	2	
<b>Diptera</b>				1381
<i>Asarkina africana</i>	E, N	49	46	
<i>Atriadops vespertilio</i>	E		1	
<i>Bombomyia discoidea</i>	E, N	17	5	
<i>Chrysomya chloropyga</i>	E, N	2	2	

<i>Chrysomya marginalis</i>	E		1	
<i>Chrysomya albiceps</i>	N		1	
<i>Culicoides</i> sp.	E, N	271		
Diptera sp.1	N		14	
Diptera sp.2	N		3	
Diptera sp.3	N		14	
<i>Eristalis tenax</i>	N, M	314	38	
<i>Hermyia diabolus</i>	E	3		
<i>Musca domestica</i>	E, N, M	339	19	
<i>Stomoxys calcitrans</i>	E, N	230	12	
<b>Hemiptera</b>				
<i>Acanthocoris</i> sp.	N	5		
<i>Cletus</i> sp.	N		1	
<i>Deraeocoris</i> sp.	E	2		
<i>Encosternum delegorguei</i>	N	27		

Hemiptera sp.1	E	4		
Hemiptera sp.2	E		1	
<i>Leptoglossus membranaceus</i>	N	6		
<i>Mirperus jaculus</i>	E	30		
<i>Phonoctonus</i> sp.	N	54		
<i>Rhinocoris segmentarius</i>	E, N	30		
<b>Hymenoptera</b>				6078
<i>Allodapula variegata</i>	E		1	
<i>Amegilla caelestina</i>	E	17	2	
<i>Apis mellifera</i>	E, N, M	3352	1675	
<i>Batozonellus fuliginosus</i>	E	1		
<i>Ceratina</i> sp.	E, N	536	340	
<i>Ceratina</i> spp.	E		3	
<i>Coelioxys</i> sp.	E, N, M	9	4	
<i>Delta emarginatum</i>	E, N	3		

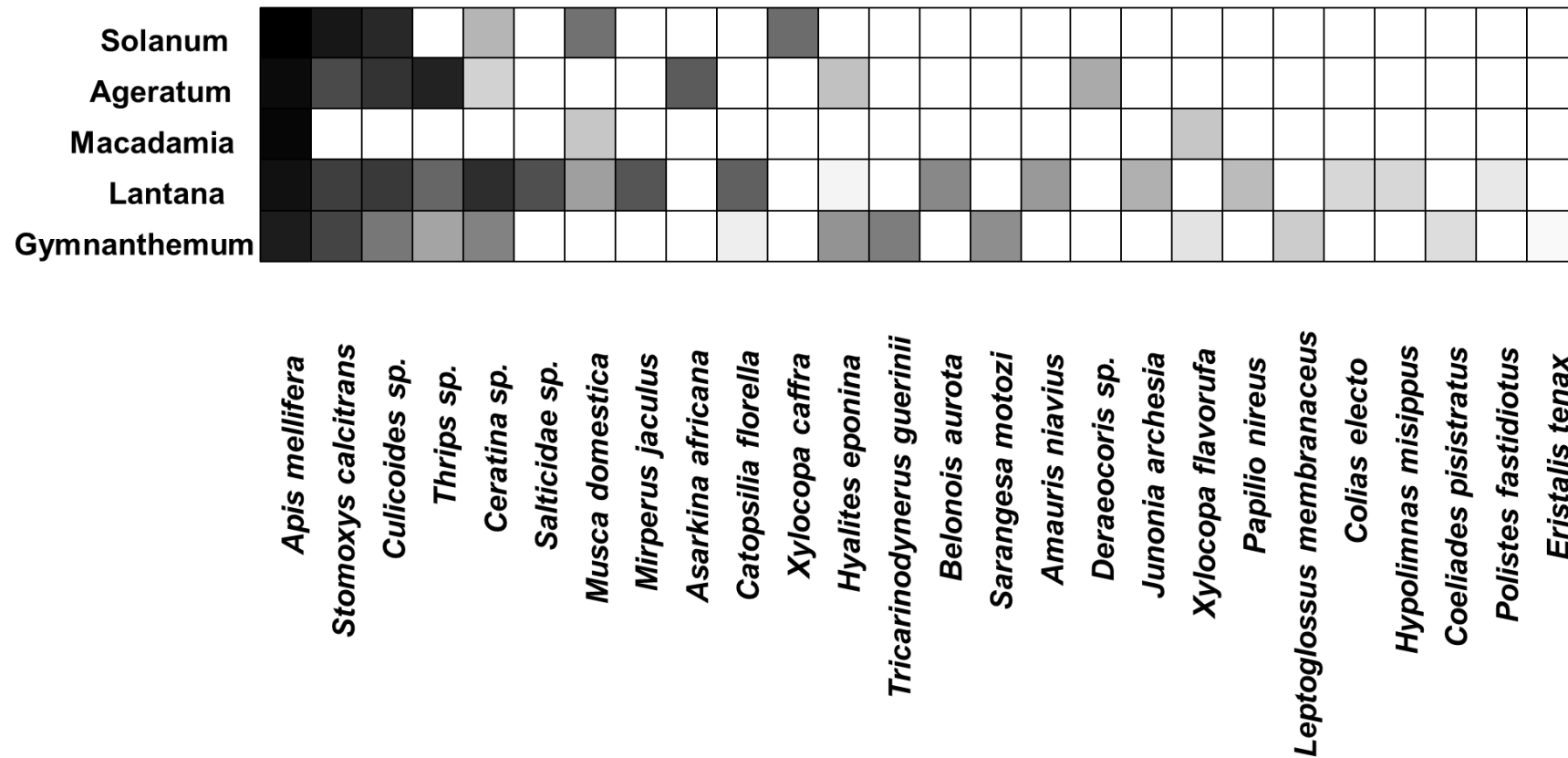
<i>Delta hottentottum</i>	E	3	1	
<i>Episyron histrio</i>	E	1		
Hymenoptera sp.1	E	1		
Hymenoptera sp.2	E, N	20	2	
<i>Megachile combusta</i>	E, N	1	5	
<i>Nomia amabilis</i>	E, N	8	4	
<i>Polistes fastidiosus</i>	E, N	16	6	
<i>Pteromalus puparum</i>	E		1	
<i>Tachytes observabilis</i>	E	1		
<i>Tricarinodynerus guerinii</i>	E, N	15	2	
<i>Xylocopa caffra</i>	E, M	19	9	
<i>Xylocopa flavorufa</i>	E, N, M		21	
<b>Lepidoptera</b>				
<i>Acraea natalica</i>	E	7	3	
<i>Amauris niavius</i>	E		2	
<i>Anthene definita</i>	N		1	

<i>Belonois aurota</i>	E		2	
<i>Catopsilia florella</i>	E, N	11	33	
<i>Cynthia cardui</i>	E, N		2	
<i>Coeliades pisistratus</i>	E, N	11	15	
<i>Colias electo</i>	E		2	
<i>Colotis auxo</i>	E		4	
<i>Cynthia cardui</i>	E, N	15	15	
<i>Eurema brigitta</i>	E		1	
<i>Hamanumida daedalus</i>	E, N	43		
<i>Hyalites eponina</i>	E, N	6	18	
<i>Hyalites esebria</i>	E	1	2	
<i>Hypolimnas misippus</i>	E		1	
<i>Junonia archesia</i>	E		16	
<i>Junonia hierta</i>	E		1	
<i>Junonia oenone</i>	E, N	9	2	
Lepidoptera sp.1	E		1	

Lepidoptera sp.2	E, N	47	15	
<i>Mylothris agathina</i>	E		2	
<i>Papilio demodocus</i>	E	1		
<i>Papilio domocus</i>	E		3	
<i>Papilio nireus</i>	E	1	24	
<i>Phalanta phalantha</i>	E		3	
<i>Sarangesa motozi</i>	E, N	34		
<i>Spialia sp.</i>	E, N	57	2	
<b>Orthoptera</b>				48
Orthoptera sp.1	E	37		
Orthoptera sp.2	E	11		
<b>Thysanoptera</b>				98
Thysanoptera sp.	E, N	69	29	

**Appendix C:** Overall species interactions. Colours indicate the strength of interaction; from high interaction indicated by black to no interaction indicated by white squares.

### August 2018: During the flowering period







## July 2019: During flowering period

	<i>Apis mellifera</i>	<i>Asarkina africana</i>	<i>Ceratina sp.</i>	<i>Musca domestica</i>	<i>Eristalis tenax</i>	<i>Amegilla caelestina</i>	<i>Xylocopa caffra</i>	<i>Culicoides sp.</i>	<i>Ceratina spp.</i>	<i>Catopsilia florella</i>	<i>Sarangesa motozi</i>	<i>Polistes fastidiosus</i>	<i>Delta emarginatum</i>	<i>Hyalites eponina</i>	<i>Polistes Fastidiosus</i>	<i>Tricarindynerus guerinii</i>	<i>Junonia archesia</i>	<i>Diptera sp.3</i>	<i>Eurema brigitta</i>	<i>Spialia sp.</i>	<i>Areneae sp.1</i>	<i>Cynthia cardui</i>	<i>Aves sp.3</i>	<i>Diptera sp.2</i>	<i>Coeliades pistratus</i>	<i>Papilio domocus</i>	<i>Thrips sp.</i>	<i>Junonia oenone</i>	
Caesalpinia	■		■	■		■	■		■				■			■													
Solanum		■	■	■		■	■					■			■														
Gymnanthemum		■	■	■	■						■							■				■		■					
Lantana	■		■	■			■	■		■				■		■	■		■							■	■		
Ageratum	■	■						■														■						■	
Macadamia	■				■																								
Erythrina	■			■																			■						

## August 2019: During flowering period

Erythrina																									
Solanum																									
Jacobaea																									
Caesalpinia																									
Macadamia																									
Lantana																									
Goodia																									
Mucuna																									
Gymnanthemum																									
Rauvolfia																									
	<i>Apis mellifera</i>	<i>Orthoptera sp.1</i>	<i>Xylocopa flavorufa</i>	<i>Hymenoptera sp.2</i>	<i>Lepidoptera sp.2</i>	<i>Orthoptera sp.2</i>	<i>Amegilla caelestina</i>	<i>Aves sp.4</i>	<i>Ceratina sp.</i>	<i>Polistes fastidiosus</i>	<i>Tricarinosdenerus guerinii</i>	<i>Coelioxys sp.</i>	<i>Musca domestica</i>	<i>Papilio nireus</i>	<i>Catopsilia florella</i>	<i>Xylocopa caffra</i>	<i>Junonia archesia</i>	<i>Eristalis tenax</i>	<i>Hemiptera sp.1</i>	<i>Culicoides sp.</i>	<i>Leptoglossus membranaceus</i>	<i>Aves sp.2</i>	<i>Hymenoptera sp.1</i>	<i>Tachytes observabilis</i>	<i>Papilio demodocus</i>