Ecology and management of bat communities to increase pest control in macadamia orchards, Limpopo, South Africa

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requirements for the Degree of

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by

SINA MONIKA WEIER

(Student No. 15015155)

Promoter: Professor Peter J. Taylor,

SARChI Chair on Biodiversity & Change, University of Venda, South Africa

Co-promoters: Professor Teja Tscharntke and Dr. Ingo Grass,

Department of Crop Sciences,

University of Göttingen, Germany

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“The road we have long been traveling is deceptively easy, a smooth superhighway on which we progress with great speed, but at its end lies disaster. The other fork of the road — the one less traveled by — offers our last, our only chance to reach a destination that assures the preservation of the earth.”

— Rachel Carson, Silent Spring

Declaration

I, SINA MONIKA WEIER, hereby declare that the thesis for the degree of Doctor of Philosophy in Mathematical and Natural Sciences at the University of Venda, hereby submitted by me, has not been previously submitted for a degree at this University or any other University, that it is my own work in design and execution and that all referenced material contained therein has been duly acknowledged.

Signature _____________________

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Publication timeline and disclaimer

Please note that chapters' two to four of this thesis were written as stand-alone papers (see below), and therefore some repetition was unavoidable. The following chapters are accepted for publication in journals:

Chapter 2: Weier SM, Grass I, Linden VMG, Tscharntke T, Taylor PJ. 2018. Natural vegetation and bug abundance promote insectivorous bat activity in macadamia orchards, South Africa. Biological Conservation **226**:16-23.

Chapter 3: Weier SM, Linden VMG, Grass I, Tscharntke T, Taylor PJ. The use of bat houses as day roosts in macadamia orchards, South Africa (in press, Peer J).

Chapter 4: Weier SM, Moodley Y, Fraser M, Grass I, Linden VMG, Tscharntke T, Taylor PJ. Insect pest consumption by bats in macadamia orchards established by molecular diet analyses (in press, Global Ecology and Conservation).

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Table of contents

List of Tables

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List of Figures

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List of Appendices

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Overall abstract

An ever growing human population and accelerating land use change is associated with the loss of species and their ecosystem services. Agricultural intensification has led to a worldwide threat of extinction to about one quarter of all bat species, despite the valuable ecosystem service of pest control provided by bats. The decline in bat populations is mainly attributed to the loss or fragmentation of habitats, roost sites and feeding opportunities related to agricultural intensification and land use change. Therefore, proactive management of bat communities in agricultural landscapes is essential. South Africa is the world's largest producer of macadamias and the industry continues to grow.

This study gains insight into the habitat use and foraging behaviour of insectivorous bat species on a temporal and spatial scale, in and around macadamia orchards in order to advise management strategies on how to increase bat activity and, possibly, pest control. It also focuses on the preferences of artificial roost sites used by insectivorous bats in macadamia orchards. The diet of insectivorous bat species is especially difficult to study and the least invasive tool to gain information is the study of bat faecal pellets. In order to provide evidence for the consumption of pest insect species by bats and thus incentive to farmers for a more integrated pest management approach (IPM), this study explored molecular approach to insectivorous bat diet analyses using fragment analysis of bat faecal pellets with fluorescent-labelled species-specific primers (designed for the CO I gene).

This study was conducted in the subtropical fruit growing area of Levubu, Limpopo province, South Africa between the towns Thohoyandou (22°59'03.7 S, 30°27'12.8 E) and Makhado/Louis Trichardt (23°03'03.6 S, 29°55'12.7 E). Levubu also accounts for the second highest production of macadamia in South Africa.

An introduction to the order Chiroptera and into the relevance of insectivorous bat species to agriculture as well as the importance of a more integrated pest management approach (IPM) focusing on bats is provided in **Chapter One**.

Bats were acoustically monitored and light traps were used to catch arthropods during one annual cycle. I sampled five macadamia orchards once a month from September 2015 to August 2016 and used GIS and R to analyse both the general bat activity and foraging bat activity of the two main foraging guilds (open-air/clutter edge guild) in different land use types as well as total bat activity with respect to arthropod abundances. As reported in

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Chapter Two, results show that the overall clutter edge guild activity (number of passes) decreased with macadamia and orchard (all other fruit) cover in the macadamia high season (December to end of May) and increased with bush cover and distance to settlements (potential roosts) in the macadamia low season (June to end of November). Open-air guild activity increased with fallow cover (uncultivated grassland with scattered trees and shrubs) in the high season. Foraging activity (feeding buzzes) of the clutter edge guild increased with bush cover over the whole year. Total activity (both guilds) increased with abundance of true bugs (Hemiptera), including the main macadamia pests, and bush cover. Macadamia cover has a negative effect on the activity of the clutter edge guild in the high season, with low activity in the orchard center (high cover), and activity increasing in a linear way with decreasing orchard cover at the orchard edge (low cover).

These results suggest that the clutter edge guild prefers foraging close to the edges of the orchards rather than in the center, while the open-air guild prefers semi-natural habitats (fallow). When numbers of pest arthropods drop in the macadamia orchards, the natural land use type, bush, becomes a more important foraging habitat and thereby increased the activity of the clutter edge guild.

From June 2016 to July 2017, I scanned 31 bat houses, mounted on poles on six macadamia orchards, for bats or any other occupants such as wasps, birds and bees. Twenty-one multi-chambered bat houses of three slightly different chamber designs were erected on poles, in sets of three. Additionally, five bat houses of the type ‘Rocket box’, four bat houses in sets of two (black and white) and one colony bat house were erected. Bats were counted and visually identified to family or species level. From December 2016 to end of March 2017, three IButtons were installed to record temperature variation between one set of three bat houses. As reported in **Chapter Three**, results show that the central bat house in the set of three and the black bat house in the set of two had a significantly positive effect on bat house occupancy. There was a significant difference in the mean temperature between the houses in the set of three, with a significant difference in temperature of 0.46°C between the central and the first bat house. The three bat houses erected in sets varied slightly in their chamber design, with the central bathouse having the most chambers (six), while the bat houses to either side had less chambers (four), set at an angle or straight. This and the insulation to either side by the other bat houses is assumingly what caused the central bat house to be on average warmer.

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The Yellow-bellied house bat (*Scotophilus dinganii*) was by far the most recorded and the only species observed to co-habitat a bat house with another animal species, in particular honeybees. The study might confirm assumptions in that the microclimate of bat houses, respectively their insulation, sun exposure and color appear to be important factors influencing bat house occupancy. The two preferred bat houses in our study were the black, in the set of black and white, as well as the central, and on average warmest bat house, in the set of three.

I collected bat faecal pellets with two different methods between July 2015 and April 2017 to determine the prevalence of pest insects in faecal pellets. Eighteen of the bat houses (in sets of three) on three different farms and two Egyptian slit-faced bat (*Nycteris thebaica)* roosts were fitted with trays in order to collect pellets from those occupied by bats. I noted occupancy of bat houses to species or family level to keep disturbance minimal. Additionally, I collected pellets from individuals captured by means of mist nets and harp traps. Four of the main pest-insects; the two-spotted stinkbug (Pentatomidae: *Bathycoelia distincta*), the green vegetable bug (Pentatomidae: *Nezara viridula*), the macadamia nut borer (Tortricidae: *Thaumatotibia batrachopa*) and the litchi moth (Tortricidae: *Cryptophlebia peltastica*), were collected from pheromone traps or after scouting for primer development and optimisation. After extracting DNA from the bat faecal samples the target regions were amplified in a multiplex PCR and fluorescently labelled PCR amplicons were analysed and interpreted. In order to verify multiplex analyses results, all samples were amplified with all four sets of primers in plates and those that produced amplicons were purified and sequenced. As reported in **Chapter Four**, results show that fragment analyses yielded a total of 63 out of 103 samples tested positive for pest insect species (61%) with a total of 92 positive fragments. Primer specificity could be confirmed to 100% for the sequences obtained for *Bathycoelia distincta* (26/26) and *Nezara viridula* (12/12) primers but not for all sequences obtained fot *Cryptophlebia peltastica* (18/30) and *Thaumatotibia batrachopa* (1/14) primers. One sample showed no positive fragments but contained a positive sequence for *N. viridula*. Three samples tested positive for one pest-species fragment but contained a positive sequence for a second pest-species (*B. distincta*, *T. batrachopa* and *C. peltastica*). Adding four positive fragments and one additional positive sequence to the data. This means that sequences of pest insect species were obtained from 54 out of the 103 samples (55.6%) with a total of 73 pest insect sequences. For the high season (December to end of May) a total of

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37 positive fragments for the four pest insect species and 24 negative samples were yielded and for the a low season (June to end of November) a total of 36 positive fragments and 15 negative samples. Looking at the pest consumption of the different bat species or families, our results show that all of them foraged on pest insect species. Whereas, all species and families except *Myotis bocagii* and *Rhinolophus simulator* (for which N<2) foraged on both the Lepidopteran and Hemipteran pest species. Therefore, all families of bats of which faecal pellets were analysed for this study (Molossidae, Nycteridae, Rhinolophidae and Vespertilionidae) foraged on one or more of the four pest insect species.

In summary, **Chapter Five** concludes that natural and semi-natural vegetation promote bat activity in macadamia orchards, and potentially bats' provision of the ecosystem service of pest control. In times of accelerating land use change, remnants of natural vegetation are important refuges and need to be maintained or restored to conserve bat species and promote their ecosystem services. The study also shows that bat activity might be improved by adding roosting opportunities to orchards. Warm and well-insulated bat houses mounted freestanding on poles and in sets appeared to work best in northern South Africa. Further research on co-habitation of bat houses and displacement behaviour as well as the potential importance of altitude and distance to water is needed.
All of the species or families of bats from which faecal pellets were collected have been confirmed to forage on at least one of the four pest insects and the bat species have shown to be much more generalist and presumably opportunistic feeders than previously assumed. Thus, this study provides incentive and advice to farmers for a more integrated pest management approach (IPM).

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Chapter 1: The need for integrating insectivorous bats into agroecosystem management

An introduction to the order Chiroptera

Bats (Chiroptera) are the only flying mammals and with about 1200 species of 19 families the most diverse mammal group after the Rodentia (Rodents), accounting for one-fifth of mammalian diversity on the planet (Miller-Butterworth et al., 2007; Teeling et al., 2012). Bats are distributed across all continents, except Antarctica, and can be found on most islands. In short, there range is only limited by the extreme latitudes (Monadjem et al., 2010). Except for the capability of flight, bats also separate themselves from all other mammals in that many bat species have very sophisticated echolocating adaptations (Teeling et al., 2012). According to Miller-Butterworth et al. (2007), molecular data separates the order into two suborders; the Yinpterochiroptera and the Yangochiroptera. The Yinpterochiroptera combines the echolocating superfamily Rhinolophoidea and the non-echolocating family of the Pteropodidae (Miller-Butterworth et al., 2007). Until new fossils of the Pteropodidae can give an elucidating picture, two theories about the evolution of echolocation in bats remain possible. Either echolocation evolved in all bats and got lost in the Pteropodidae or it evolved separately in different lineages (Teeling et al., 2012).

In southern Africa, about 120 bat species belonging to eight families have been recorded thus far (Monadjem et al., 2010). With the exception of the frugivorous and nectivorous family Pteropodidae and some rare carnivory species (e.g. *Nycteris grandis*), those are insectivorous bat species using echolocation for hunting and orientation (Monadjem et al., 2010). The insectivorous bat species account for about 70% of all bat species circumglobally (Monadjem et al., 2010). According to their ecological adaptations (wing shape and echolocation system), they are separated into Clutter, Clutter edge and Open-air foragers hunting in, between and above vegetation respectively (Monadjem et al., 2010). The species of prey-arthropods preferred largely depends on the size of the bat and its jaw morphology (Monadjem et al., 2010). Large species like the Yellow-bellied house bats (*Scotophilus dinganii*) weighing about 24 grams are, therefore, by nature much more likely to include Coleoptera of moderate size in their diet then the Rusty pipistrelle (*Pipistrellus rusticus*) weighing about 4 gram (Monadjem et al., 2010).

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Ecosystem service provision of insectivorous bat species in agriculture

The concept of 'ecosystem services' has led to a growing appreciation for certain animal species such as bats in regards to the monetary benefits they provide to people (Ghanem 2012; Voigt & Kingston 2015; Wallace 2007; Millennium Ecosystem Assessment 2005). There has also been an increase in studies emphasizing the economic importance of insectivorous bat species for agricultural pest control (Puig-Montserrat et al. 2015; Lopez-Hoffman et al. 2014; Wanger et al. 2014; Maas et al. 2013; Boyles et al. 2011; Kunz et al. 2011; Cleveland et al. 2006). The economic value of insectivorous bats has been suggested to be worth about 22.9$ billion (USD) annually to the agricultural sector of the United States, 21€ (Euro) per hectare in Mediterranean rice plantations and about 730$ (USD) per hectare in tropical cacao plantations (Boyles et al., 2011; Maas et al., 2013; Puig-Montserrat et al., 2015). Additionally, pest control services provided by bats have been suggested to limit the need for pesticide use and increase the value of agricultural products such as cotton (Federico et al., 2008). This decrease in pesticide use also allows for a better long-term control of insect pest species by more stable predator populations such as bats, birds and spiders (Knight & Gurr, 2007; Taylor et al., 2017; Taylor et al., 2018).

In South Africa the ecosystem service insectivorous bat species provide to the macadamia industry, by suppressing stinkbug pest species (Hemiptera: Pentatomidae), is estimated between 59-139$ (USD) per hectare. The role of bats might be equally important in suppressing other major pest species such as certain Lepidoptera (Brown et al., 2015; Taylor et al., 2017; 2018). The presence of insectivorous bats in agroecosystems might, therefore, decrease the need for pesticide use, improve crop quality and increase harvest yields.

Threats to bats

Bats provide a variety of valuable ecosystem services such as pest control (Ducummon, 2000; Fenton, 1997; Kalka et al., 2008; Leelapaibul et al., 2005; McCracken et al., 2012; WilIiams-Guillen et al., 2008). However, about one quarter of all bat species are threatened with extinction and their numbers keep decreasing at an alarming rate (Boyles et al., 2011; Jones et. al., 2003; Mickleburgh et al., 2002). This decline is mainly attributed to the loss and fragmentation of habitats, roost sites and feeding opportunities, with agricultural intensification and related land use changes being by far the most frequent threat listed for bats in some 550 IUCN Red List bat species accounts (see Fig. 1.3 in Voigt & Kingston, 2016). Nevertheless, most African bat species are poorly studied and there is scarce information about their habitat use, foraging ecology or roost site preferences (Monadjem et al., 2009; Taylor, 2000). Likewise, 15% of southern African bat species are listed under Data Deficient by the IUCN (Monadjem et al., 2009; Monadjem et al., 2010; Taylor, 2000). In general, there is particular lack of knowledge on how to conserve bats in conventional (intensive) agricultural systems especially if those are located in biodiversity hotspot areas (Park, 2015). Loss and degradation of natural habitats, on the other hand, is predicted to accelerate until 2050 (Foley et al., 2005; Tilman et al., 2001; Tscharntke et al., 2012).

The South African macadamia industry

With 28,000 ha of land covered by macadamia orchards (SAMAC, 2017), South Africa is the world's leading producer of macadamia nuts since 2014, accounting for an annual production of over 44,000 tons in 2014 and 46,000 tons in 2015 (DAFF, 2016; Taylor et al., 2018). The South African macadamia industry continues to grow with a minimum of 1900 ha planted in 2016 alone (DAFF, 2016). The current annual loss from insect pest damage to macadamia crop has been recently estimated at 15.23 million USD (Taylor et al., 2018; Schoeman, 2009). Pest damage is mainly related to 'unsound kernel', meaning that the macadamia nut in the shell is damaged by pest insect species while ripening on the tree. The major pest species known to the South African macadamia industry are several different stinkbug (Hemiptera: Pentatomidae) and moth (Lepidoptera: Tortricidae) species (De Villiers & Joubert, 2003).

Rationale of this study

Given the accelerating land use change under an ever-growing human population and the ongoing growth of the South African macadamia industry as well as the assumed decline of South African bat populations based on studies from other parts of the world (Voigt & Kingston, 2016), proactive management of bats is indispensable to sustain bat populations and their long-term ecosystem services (Cumming et al., 2014; Taylor et al., 2017; Tuttle et al., 2013). Proactive management of bat population will require to fill existing knowledge gaps about habitat use, foraging ecology and roost site preferences for African bat species and the conservation of bats in intensive agricultural systems in particular (Monadjem et al., 2009; Park, 2015; Taylor, 2000). Therefore, this study aims to provide incentive and advice to farmers on how to include bats into the management of macadamia orchards by providing information about their habitat use (Chapter 2), foraging ecology (Chapters 2 and 4) and roost site preferences (Chapter 3) in this agroecosystem.

Study area

The subtropical fruit growing area of Levubu, Limpopo province, South Africa, is located within the valley of the Levuvhu River and accounts for the second highest production of macadamia in the country (Figures 1 and 2).

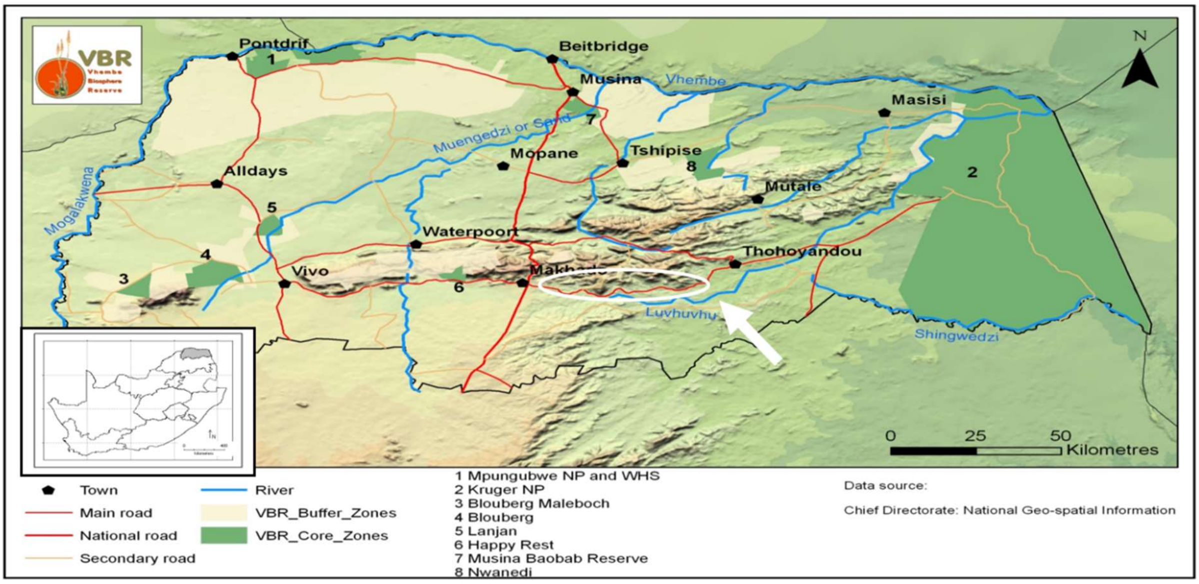

Figure 1 Map of South Africa (left bottom window) showing the location of the study area (white circle) in Levubu, Limpopo, South Africa, within the Vhembe Biosphere Reserve (Map credit: Jabu Linden).

This sub Saharan African region receives its main rain in the summer months between November and April with around 1000 mm of annual rainfall. Apart from macadamia, the main agricultural products farmed in Levubu are pecan, avocado, banana, pine and gum (Taylor et al., 2017). Levubu is part of the UNESCO Vhembe Biosphere Reserve and borders the Soutpansberg (Figure 1), a nationally recognized centre of endemism and biodiversity (Mostert et al., 2008). It also falls within a 'bat species richness hotspot' in northern South Africa (Cooper-Bohannon et al., 2016). Data collection for chapters two to four was conducted in Levubu between the towns of Thohoyandou (22°59'03.7 S, 30°27'12.8 E) and Makhado/Louis Trichardt (23°03'03.6 S, 29°55'12.7 E).

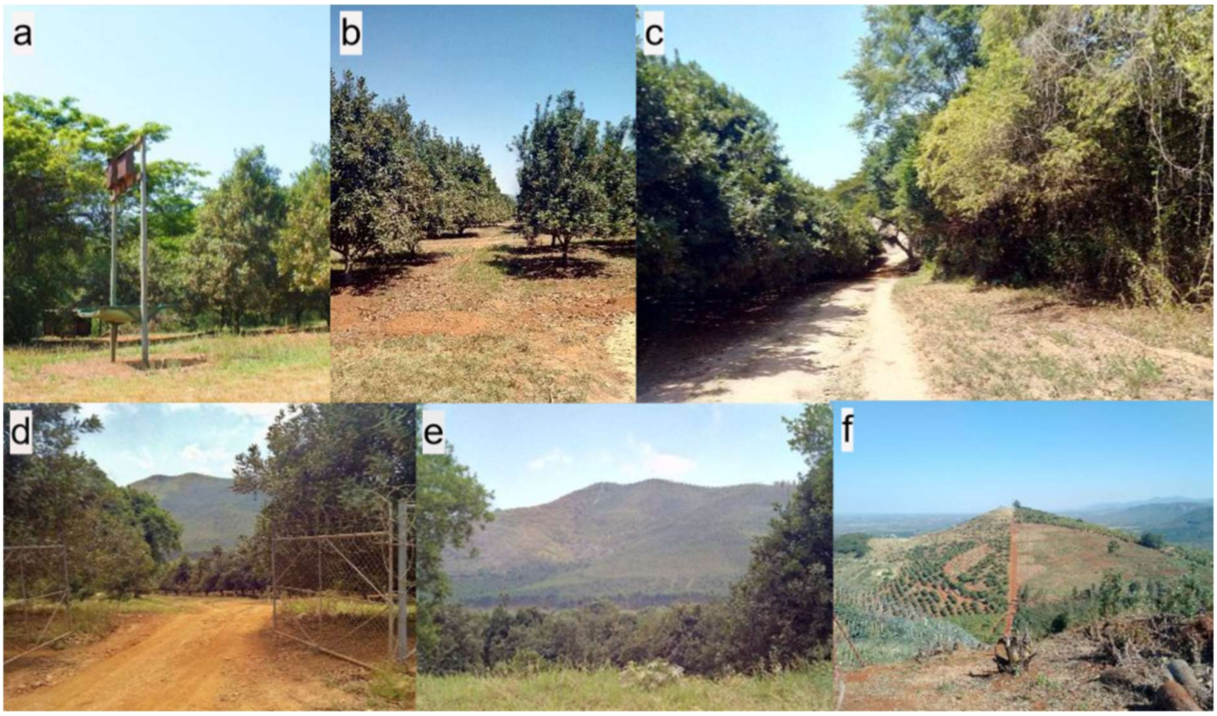

Figure 2 Illustrating the study area in the subtropical fruit growing area Levubu, Limpopo, South Africa. Showing a) a set of three bat houses with a faecal pellet tray underneath, b) a typical way of planting rows of macadamia trees, c) the edge of a macadamia orchard bordering natural vegetation, d) one of the transect roads, e) a macadamia orchard with the Soutpansberg and pine plantations in the background, f) a typical heterogeneous macadamia farming landscape with surrounding patches of natural vegetation and avocado plantings (Photo credit f): Valerie Linden).

Thesis aim and objectives

The aim of this thesis was to gain insight into the habitat use, artificial roost site selection and foraging behaviour of insectivorous bat species on a temporal and spatial scale, in and around macadamia orchards in order to provide incentive to farmers for a more integrated pest management approach (IPM) and to advise management strategies on how to increase bat activity and, possibly, pest control. The objectives for the Chapters two, three and four were as follows:

Chapter Two looks at the habitat use and foraging behaviour of insectivorous bat species on a temporal and spatial scale, in and around macadamia orchards in order to advise management how to increase bat activity and, possibly, pest control. The main research questions of this chapter are: i) How is bat activity and foraging activity related to the

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different land use types, as measured by acoustic surveys? ii) What is the relationship between bat activity and arthropod availability?

Chapter Three aims to provide insight into the preference of artificial roost sites by insectivorous bats in macadamia orchards in South Africa. The main research question of this chapter is; what are key features of occupied artificial roost sites (bat houses)?

Chapter Four aims to determine the level of insectivorous bat species predation on major pest insect species in macadamia orchards with a novel molecular approach, and to assess whether there is a correlation between the macadamia growing cycle and the prevalence of these pest insect species in bat faecal pellets. The chapter also estimates and reviews the efficacy of this molecular approach in assaying species-specific insectivorous bat diets. The main research questions of this chapter are: i) What is the prevalence of pest insect species in bat faecal pellets; ii) how does this differ between the macadamia growing season and the off-peak season? and iii) how effective and accurate is this molecular approach in analysing species-specific insectivorous bat diet?

I hypothesized that habitat use by bats changes over season in relation to prey availability and shifts from the orchards towards the more natural habitat types after the macadamia nut harvest. Furthermore, I hypothesized that bat houses providing a warm microclimate and those erected close to water sources will do particularly well and that pest arthropods will be prevalent in bat faecal pellets especially in the macadamia growing season (>50% of samples).

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Chapter 2: Natural vegetation and bug abundance promote insectivorous bat activity in macadamia orchards, South Africa

Abstract

Accelerating land use change is associated with the loss of species and their ecosystem services. South Africa is the world's largest producer of macadamias and the industry continues to grow. Insectivorous bat species are important for pest control, but bat populations are declining. Therefore, proactive management of bat communities in agricultural landscapes is essential. We acoustically monitored bats and used light traps to catch arthropods during one annual cycle, sampling five macadamia orchards monthly in Limpopo, South Africa. We used GIS and R to analyse both the general bat and foraging bat activity of the two main foraging guilds (open-air/clutter edge guild) in different land use types and total activity with respect to arthropod abundances. Overall clutter edge guild activity (number of passes) decreased with macadamia and orchard (all other fruit) cover in the high season and increased with bush cover and distance to settlements (potential roosts) in the low season. Open-air guild activity increased with fallow cover in the high season. Foraging activity (feeding buzzes) of the clutter edge guild increased with bush cover over the whole year. Total activity (both guilds) increased with abundance of true bugs, including the main macadamia pests, and bush cover.

In conclusion, natural and semi-natural vegetation promote bat activity in macadamia orchards, and potentially bats' provision of the ecosystem service of pest control. In times of accelerating land use change, remnants of natural vegetation are important refuges and need to be maintained or restored. The installation of bat houses might further improve bat activity.

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1. Introduction

The broad appreciation of the ‘ecosystem services’ concept has led to a growing understanding of the monetary benefits provided by wildlife such as bats (Ghanem & Voigt, 2012; Millennium Ecosystem Assessment, 2005; Voigt & Kingston, 2016; Wallace, 2007). Several studies have emphasized the economic importance of insectivorous bat species for agricultural pest control (Boyles et al., 2011; Cleveland et al., 2006; Kunz et al., 2011; Lopez-Hoffman et al., 2014; Maas et al., 2013; Puig-Montserrat et al., 2015; Wanger et al., 2014). For example, Boyles et al. (2011) suggested that the annual value of insectivorous bats to agriculture in the United States is about 22.9$ billion (USD). Similarly, it is proposed that the combined annual value of bats and birds for pest control is about 730$ (USD) per hectare in tropical cacao plantations (Maas et al., 2013). Puig-Montserrat et al. (2015) estimated the value of insectivorous bats in a Mediterranean rice plantation to be no less than 21€ (Euro) per hectare. Furthermore, insectivorous bats can limit the need for pesticide use and, thereby, increase the value of agricultural products such as cotton (Federico et al., 2008). In return, a decrease in pesticide use has been suggested to lead to more stable predator populations such as bats, birds and spiders and, therefore, a better long-term control of pest species (Knight & Gurr, 2007; Taylor et al., 2013b; Taylor et al., 2018). Hence, promoting high bat activity in agricultural landscapes could not only improve the livelihood of farmers but potentially decrease the use of pesticides while maintaining crop yields.

While bats provide a variety of valuable ecosystem services such as pest control (Ducummon, 2000; Fenton, 1997; Kalka et al., 2008; Leelapaibul et al., 2005; McCracken et al., 2012; Williams-Guillen et al., 2008), about one quarter of all bat species are threatened with extinction and their numbers keep decreasing at an alarming rate (Boyles et al., 2011; Jones et. al., 2003; Mickleburgh et al., 2002). This decline is mainly attributed to the loss and fragmentation of habitats, roost sites and feeding opportunities, with agricultural intensification and related land use changes being by far the most frequent threat listed for bats in some 550 IUCN Red List bat species accounts (see Fig. 1.3 in Voigt & Kingston, 2016). Loss and degradation of natural habitats is predicted to accelerate until 2050 (Foley et al., 2005; Tilman et al., 2001; Tscharntke et al., 2012).

With 28000 hectares of land covered by macadamia orchards (SAMAC, 2017), South Africa is the world’s leading producer of macadamia nuts since 2014, accounting for an annual production of over 44 thousand tons in 2014 and 46 thousand tons in 2015 (DAFF, 2016;

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Taylor et al., 2018). The South African macadamia industry continues to grow with a minimum of 1900 ha planted in 2016 alone (DAFF 2016).

The current annual loss from insect pest damage to macadamia crop has been recently estimated at 200 million ZAR or about 15.23 million USD (Taylor et al., 2018; Schoeman, 2009). Pest damage is mainly related to 'unsound kernel', meaning that the macadamia nut in the shell is damaged by pest insect species while ripening on the tree. The major pest species known to the South African macadamia industry are several different stinkbug (Family *Pentatomidae*) and moth (Family *Tortricidae*) species (De Villiers & Joubert, 2003). The recent avoided cost model of Taylor et al. (2018) suggests that the monetary value of insectivorous bat species for the macadamia industry of South Africa, by suppressing stinkbug pest species, is between 59-139$ (USD) per hectare. Looking at the results of the dietary analysis of bat faecal pellets on macadamia orchards by Taylor et al. (2013b; 2018) or pecan orchards by Brown et al. (2015) it is reasonable to assume that the role of bats is equally important in suppressing other major pest species such as certain Lepidoptera. Given the ongoing growth of the South African macadamia industry and the assumed decline of South African bat populations based on studies from other parts of the world (Voigt & Kingston, 2016), proactive management of bats is indispensable to sustain their long-term ecosystem services (Cumming et al., 2014; Taylor et al., 2013b; Tuttle et al., 2013). However, most African bat species are poorly studied and there is scarce information about their habitat use, foraging ecology or roost site preferences (Monadjem et al., 2009; Taylor, 2000). Likewise, 15% of southern African bat species are listed under Data Deficient by the IUCN (Monadjem et al., 2010). In general, there is particular lack of knowledge on how to conserve bats in conventional (intensive) agricultural systems especially if those are located in biodiversity hotspot areas (Park, 2015).

Taylor et al. (2013a) found no significant difference in bat activity between riparian vegetation and macadamia orchards in South Africa, although the preference of bats for this more natural land use type has been established previously (Grindal et al., 1999; Medina et al., 2007; Monadjem & Reside, 2008). Similarly, a radio- tracking study in Swaziland showing that open-air bats prefer foraging on sugarcane fields in comparison with more natural habitats in the vicinity (Noer et al., 2012). This might be linked to seasonal prey availability as Taylor et al. (2013a) showed that, to some degree, there is an overlap in the peak of pest insects and an increased bat activity in macadamia orchards. Likewise, higher bat activity on organic farms as opposed to conventional farms may result from higher food availability and better habitat quality (Park, 2015; Wickramasinghe et al., 2003). Natural habitats in the vicinity of agriculture, providing connectivity, and the presence of water seem to benefit bat foraging activity (Crisol-Martinez et al., 2016; Fuentes-Montemayor et al., 2011; Park, 2015). Although artificial, waterbodies in general, are suggested to be important foraging habitats supporting a much higher bat activity than the land use types around them (Sirami et al., 2013; Stahlschmidt et al., 2012). However, none of these studies have been conducted over a complete annual cycle and most of them in summer season only. Additionally, studies on the habitat selection of insectivorous bats in African agro-ecosystems are lacking. There have been no studies looking at the detailed habitat selection of insectivorous bat species on macadamia orchards in South Africa, despite the rapidly expanding macadamia industry. Therefore, it is essential to take an in-depth look at the habitat selection of insectivorous bat species on macadamia orchards, considering the temporal variation in bat activity, to enable proactive management of bat communities in order to sustain their ecosystem service of pest control.

The objective of this research is to gain insight into the habitat selection and foraging behaviour of insectivorous bat species on a temporal and spatial scale, in and around macadamia orchards in order to advise management how to increase bat activity and, possibly, pest control. The main research questions of this study are: i) How is bat activity (number of passes) and foraging activity (feeding buzzes) related to the different land use types? ii) What is the relationship between bat activity and arthropod availability?

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2. Methods

2.1 Study area

Our study area was located in Limpopo, the northernmost province of South Africa, which also accounts for the second highest production of macadamia nuts in the country (Figure 3). We conducted the study on five orchards in the subtropical fruit growing area of Levubu in the valley of the Levuvhu river. This sub Saharan African region receives its main rain in the summer months between November and April with around 1000 mm of annual rainfall. Apart from macadamia, the main agricultural products farmed in Levubu are pecan, avocado, banana, pine and gum (Taylor et al., 2013b; Figure 4). The remaining natural vegetation patches (bush) in and around the orchards are classified as 'Soutpansberg Mountain Bushveld' and 'Tzaneen Sour Bushveld' by Mucina and Rutherford (2006).

The study area also borders the Soutpansberg, a nationally recognized center of endemism and biodiversity, and falls within a 'bat species richness hotspot' in northern South Africa (Cooper-Bohannon et al., 2016; Mostert et al., 2008).

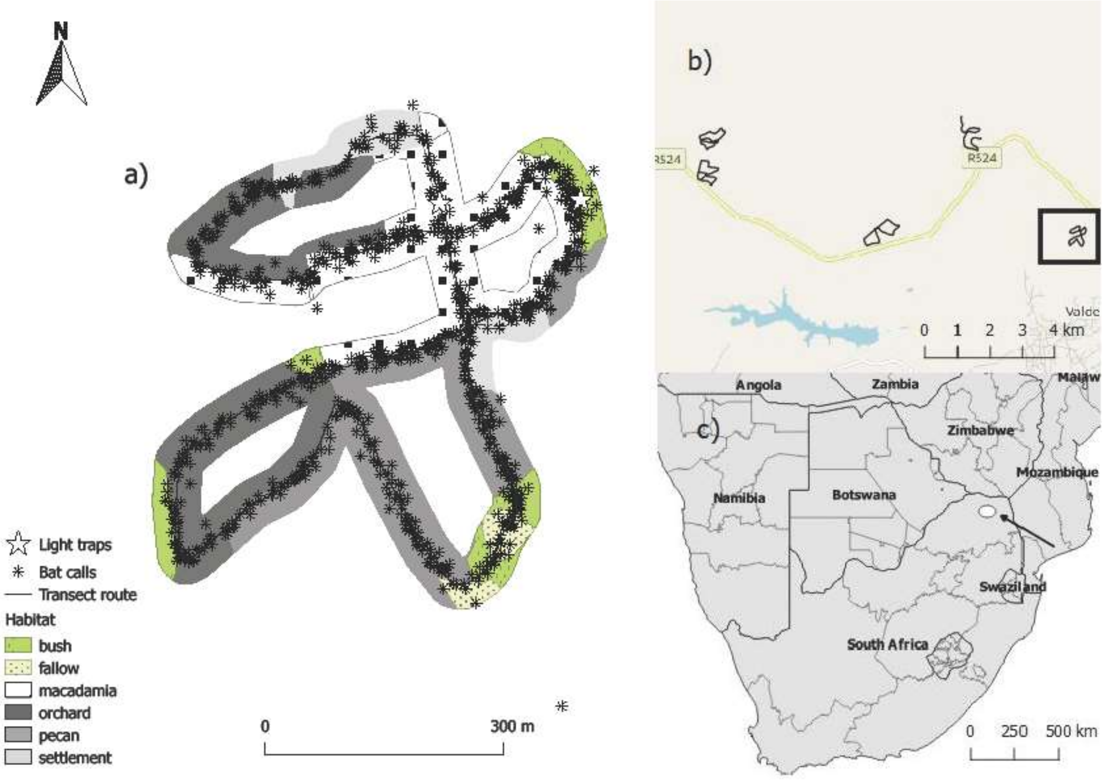

Figure 3 Map of the study area showing a) one of the transects with the different habitat types within the 30 meter buffer and all bat calls (passes) recorded during one annual cycle b) all five transects with the detailed example (rectangle) and c) the location of the study area (white circle) in Levubu, Limpopo, South Africa.

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Figure 4 Picture of a typical landscape in the subtropical fruit growing area Levubu showing a macadamia orchard with surrounding patches of natural vegetation and avocado plantings, Limpopo, South Africa (Photo credit: Valerie Linden).

2.2 Data collection

We conducted standardized, repeated transect drives across five orchards to actively record echolocation calls of insectivorous bats. We chose five orchards in close proximity to each other to avoid variation in climatic conditions through e.g. altitudinal differences (Figure 3). We used an omni-directional microphone at full spectrum (10-130 kHz, FG black microphone) with Batlogger M (Elekon AG). The detector was set to record echolocation calls automatically with the GPS coordinates of the recording. This is the most effective sampling technique for bats in open areas such as the studied macadamia orchards (Fisher-Phelps et al., 2016).

We mounted the microphone onto the car antennae at a 45-degree angle, pointing forward, using a microphone extension cable. Every drive transect took place for three consecutive hours, starting half an hour before sunset to ensure that the expected peak of bat activity,

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right after sunset, is sampled while driving at a speed of 10- 20 km/h (Barlow, 1999; Kunz & Kurta, 1988). We mapped the route on each orchard in advance in order to cover all present land use types. In addition, every route covered the edges of the orchard to sample the surrounding land use types as well as the center. We sampled every orchard once a month for a complete annual cycle, and randomly alternated the starting point for each transect and the monthly order in which we sampled the five orchards. We recorded all five transects using a Garmin Etrex GPS (Garmin Europe Ltd, UK). Fifty-nine transect nights out of 60 were recorded by the principal surveyor (SW) with a total of about 180 hours of sampling time between September 2015 and August 2016. We aborted data collection on very windy or rainy nights (Fischer, 2009). In addition to recording echolocation calls, we also used two light traps to collect arthropods on each transect. We built the light traps, creating a 'hanging' model and powering it with a 12V car battery (Appendix A1). Each light trap was equipped with an 8W super-actinic fluorescent lamp. The light traps were put up right before the transect drives and collected after driving the full three hours, from November 2015 to August 2016. We chose two permanent locations on each orchard, one at a border to natural vegetation and one within the macadamia plantation. We identified all collected arthropods to order level (Picker et al., 2002).

2.3 Call analysis

We used Bat Explorer (Elekon AG, Version 1.10, http://www.elekon.ch) to analyze the recorded calls by comparing them against release calls and by referring to Monadjem et al. (2010) and Taylor et al. (2013c) for call identification. We labelled calls by foraging group according to Monadjem et al. (2010) into the open-air guild (families Molossidae and Emballonuridae) and the clutter edge guild (family Vespertilionidae). In order to obtain bat activity (number of passes) we recorded each orientation call per guild (which we later separated per season or year see 2.5). Foraging activity was identified by so called 'feeding buzzes', which can be clearly distinguished from the regular orientation calls emitted. Hence, we also noted each 'feeding buzz' for each guild.

2.4 GIS analysis

We created digital maps of the sampled land use types using satellite images (Google Earth, 2017) and QGIS (version 2.18.11) with an Albers Equal Area projection for South Africa

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(Fleming, 2018). Land use types were bush (natural vegetation type; Soutpansberg Mountain Bushveld or Tzaneen Sour Bushveld), fallow (uncultivated grassland with scattered trees and shrubs), macadamia, orchard (including all other soft-fruit crops), pecan, settlement, timber and water (Figure 3). We merged all land use types and converted them into a raster file with 1 m resolution. We created Euclidean distance raster files of 1x1 m to settlement (as potential roosts) and (drinking) water according to Rainho and Palmeirim (2011). In order to account for detection distance to either side of the line transect we created a grid with cells of 30x30 m along the transect (Adams et al., 2012; Monadjem et al., 2017). We modelled true absences by adding zero values for absence data, corresponding to our presence data (GPS coordinates of echolocation calls). Given the vast extent and variation in bat home range size, pseudo replication can ordinarily not be completely avoided when using bat call data (Wordley et al., 2015). Therefore, we looked at the activity of bats for our fine scale modelling, without making assumptions on the numbers of individuals passing. We also defined the land use composition within each 30x30 grid cell and calculated land use cover as a percentage value (on a 0-100 % scale).

2.5 Statistical analysis

We split our 12 months data set into a high season (December 2015 to end of May 2016) and a low season (September 2015 to end of November 2015; June 2016 to end of August 2016), according to the macadamia growing cycle and the occurrence of pest insect species (De Villiers & Joubert, 2003). The R-package 'raster' (Hijmans, 2016) was used to model the bat activity (number of passes of each guild) in the high or low season, the total bat activity (number of passes for both guilds) and the foraging activity (feeding buzzes of each guild) for the full annual cycle with the land use and distance raster files. Pecan, water and settlement cover were excluded from further analysis because of their low representation. Models were also tested for multicollinearity (variance inflation factor <5) and timber cover was subsequently removed. We analysed the response variable bat activity for each season (low and high season) for both foraging groups (open-air and clutter edge guild) and as a total. The response variable foraging activity was analysed for the full annual cycle given the much lower number of data points. All models included the cover variables macadamia, orchard, bush and fallow and the distance variables water and settlement. We choose a mixed model approach to allow using the ID of each orchard as a random effect sample because the orchard samples are not independent replicates. After testing all models for normal distribution and constant errors variance, generalized linear mixed models (GLMMs) were applied. As all of the models using the Poisson distribution were overdispersed, we applied GLMMs with a negative binomial distribution (package 'AER' by Kleiber & Zeileis, 2008). As two of the models (for the clutter guild in the low season and for the foraging of the open-air guild) did not converge using GLMMs we used a transformed (log+1) linear mixed model (LMM). All models were fitted using scaled predictor variables and 'orchard' as a random factor.

Additionally, we applied a generalized linear mixed model (GLMM) with a negative binomial distribution using the package 'lme4' (Bates et al., 2015) to evaluate trophic interactions. We analysed the relationship of monthly total bat activity for both guilds per orchard with overall arthropod abundance and the number of Hemiptera and Lepidoptera collected on each orchard and the covariate temperature. All predictor variables were scaled and the variables 'orchard' and 'month' were used as random factors to account for pseudo replication. We selected all final models using the dregde function (package 'MuMIn' by Barton, 2017) based on the lowest values the small-sample size corrected Akaike information criterion (AICc). All statistical analyses were conducted with R (version 3.4, R Core Team, 2017).

3. Results

On 60 transect drives, we recorded a total of 6134 calls in the high season and 3515 in the low season, including one call of a clutter feeder (*Rhinolophus sp*.) in the low season. In total, 4514 (3089 high season/ 1425 low season) calls were recorded for open-air foragers of the Molossidae and Emballonuridae families and 5134 (3045 high season/2089 low season) for clutter edge foragers of the Vespertilionidae family. We recorded a total of 171 foraging calls: 96 for clutter edge and 75 for open-air foragers. In the high season, we caught an average of 180.9 arthropods (55.3 lepidopterans and 29.6 hemipterans) and in the low season an average of 90.9 arthropods (43.6 lepidopterans and 4.5 hemipterans) per night (Appendix A2).

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3.1 Land use types and bat activity

3.1.1 Seasonal response of the guilds

We found that the activity of the clutter edge guild decreased with higher macadamia and orchard cover during the high season, the two variables retained in the final model (Table 1; Figure 5). The activity of the open-air guild increased with higher fallow cover in the high season, the only variable retained in the final model (Table 1; Figure 5).

In the low season, clutter edge guild activity increased with higher bush cover and distance to settlements (Table 1; Figure 5). The final model on the activity of the open-air guild retained the distance to settlements and water, whereas water had only marginal significant coefficients and the coefficients of distance to settlement were non-significant (Table 1; Figure 5).

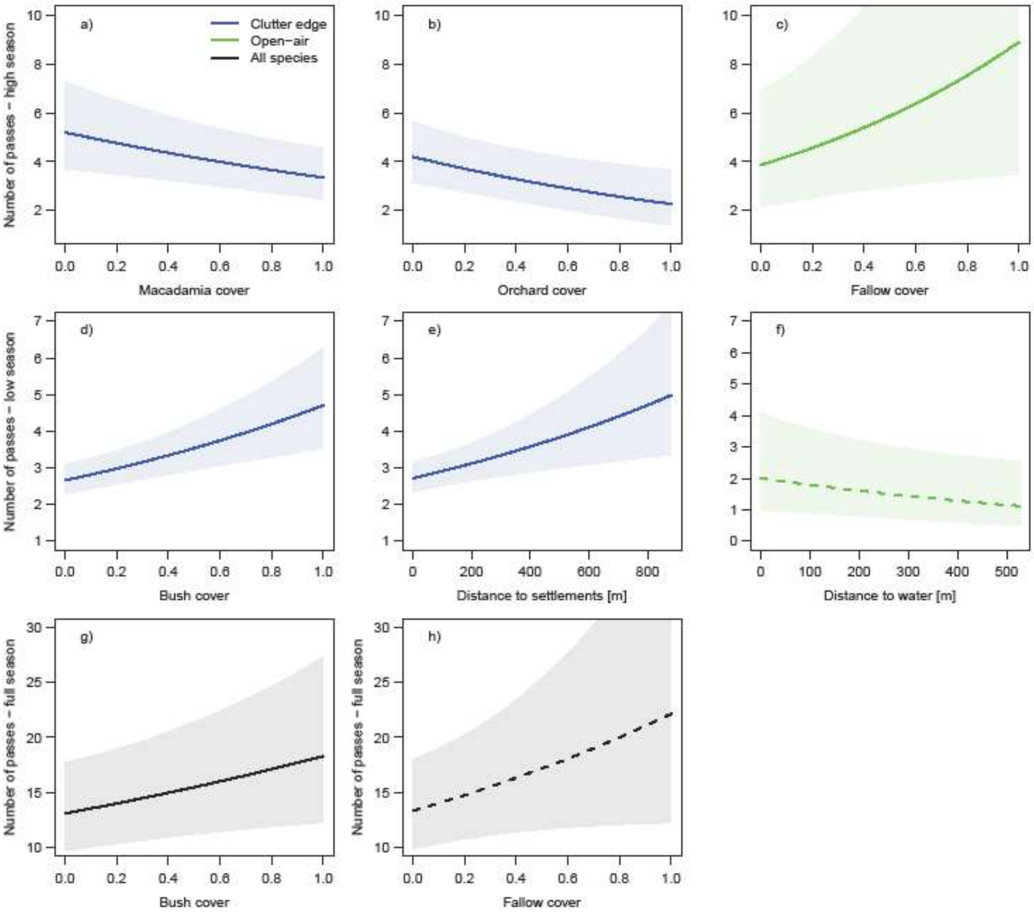

Figure 5 Final models showing the relationships between bat activity (number of passes) for both bat guilds (clutter edge and open-air), in the low and high season and the total bat activity over one year with the habitat cover variables ('macadamia, orchard, bush, fallow') and the Euclidean distance to water and settlement in macadamia orchards, Levubu, South Africa (solid line = $p<0.05$ and dashed line = $0.10> p > 0.05$).

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Table 1 Final models testing the relationship between bat activity (number of passes) and foraging activity (feeding buzzes) with the four cover variables (‘macadamia, orchard, bush, fallow’) and the Euclidean distance variables water and settlement in macadamia orchards, Levubu, South Africa (significance level p< 0.05).

Clutter edge guild

Bat activity	***Estimate***	***SE***	***Z***	***p- value***	**AICc**
High season					[a]2937.581
Macadamia cover	**-0.176**	**0.056**	**-3.135**	**0.001**	**2928.896**
Orchard cover	**-0.166**	**0.058**	**-2.818**	**0.004**	
Low season					[a]1387.777
Bush cover	**0.139**	**0.033**	**4.166**	**<0.001**	**1376.117**
Settlement distance	**0.113**	**0.036**	**3.109**	**0.002**	
Foraging activity					[a]463.0647
Bush cover	**0.304**	**0.089**	**3.39**	**<0.001**	**455.235**

Open-air guild

Bat activity	**Estimate**	**SE**	**Z**	**p- value**	**AICc**
High season					[a]2800.404
Fallow cover	**0.108**	**0.050**	**2.16**	**0.030**	**2797.143**
Low season					[a]2094.89
Settlement distance	-0.125	0.078	-1.595	0.110	**2093.862**
Water distance	-0.131	0.070	-1.869	0.062	
Foraging activity					[a] -166.4643

Total bat activity (both guilds)

All year					[a]4137.079
Bush cover	**0.081**	**0.036**	**2.265**	**0.023**	**4132.066**
Fallow cover	0.065	0.034	1.905	0.056	

[a] *null models*

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3.1.2 Total bat activity

We found that, looking at total bat activity (both guilds combined) over the full annual cycle, the higher cover of the natural vegetation type bush and the semi-natural vegetation type fallow increased bat activity. These were the only two variables retained in the final model, whereas fallow cover showed only marginal significance of coefficients (Table 1; Figure 5).

3.2 Foraging activity

The final model on foraging activity of the clutter edge guild retained the variable bush cover. We found that the foraging activity of the clutter edge guild increased with higher bush cover over the full annual cycle (Table 1; Figure 6). Model selection for the open-air guild retained only the null model.

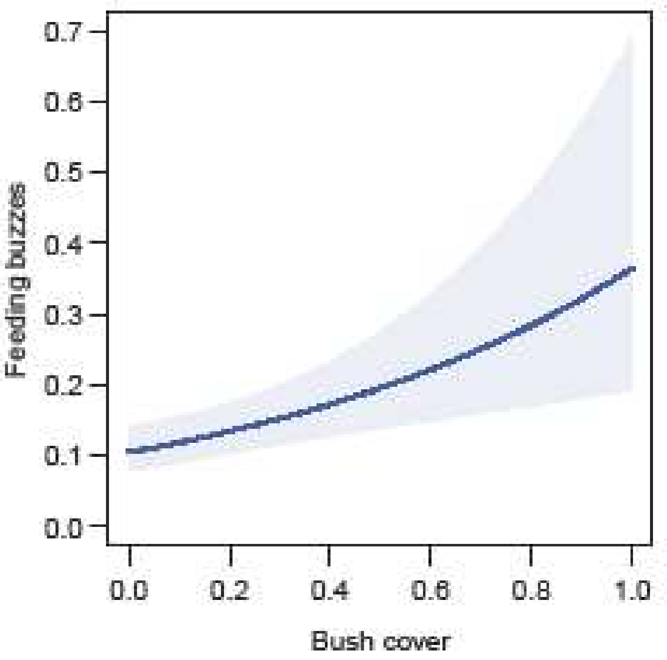

Figure 6 Final model showing the relationship between foraging activity (feeding buzzes) for the clutter edge guild throughout the year, increasing with the habitat cover variable bush' in macadamia orchards, Levubu, South Africa.

3.3 Prey availability and bat activity

Looking at the full annual cycle, bat activity of both guilds combined increased with the number of true bugs (Hemipteran) caught in light traps (ß= 0.32, p = 0.022; Figure 7). On the contrary, neither the overall arthropod abundance nor the number of moths were retained in the final model.

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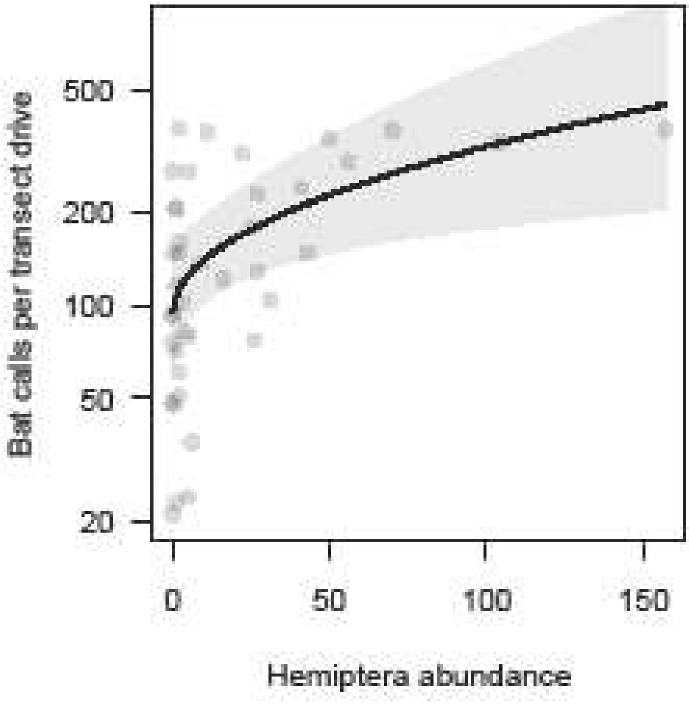

Figure 7 Final model showing the increase of total bat activity (number of passes for both guilds per transect) with increasing Hemiptera abundance in macadamia orchards, Levubu, South Africa.

4. Discussion

In accordance with the different niches the two foraging guilds (clutter edge and open-air) occupy, their dissimilar response to the different land use types was not surprising (Monadjem et al., 2010). While the activity of the clutter edge guild decreased with macadamia and orchard cover in the high season, it increased with bush cover as well as distance to settlements in the low season. The same applied for foraging activity, which increased with bush cover for the clutter edge guild looking at the full annual cycle. The foraging activity of the open-air guild was not affected by any of the fine-scale land use variables in this study. General activity of the open-air guild was increased by higher fallow cover in the high season and showed a marginally significant decrease to distance to water in the low season. Overall bat activity (number of passes for both guilds) increased with

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numbers of true bugs across both seasons. The importance of natural (bush) and semi-natural vegetation (fallow) was supported by looking at the total activity over the full year. Increasing macadamia cover, often at the expense of natural vegetation, reduced the activity for the clutter edge guild in the high season. In contrast, in the low season bush cover promoted activity of the clutter edge guild. We suggest that macadamia cover has a negative effect in the high season because the clutter edge guild increased their activity in the orchards, which provide higher prey availability during peaks in pest insect species, while they prefer foraging close to the edge of the orchards rather than in the center (Crisol-Martinez et al., 2016; Ewers & Didham, 2006). When numbers of pest arthropods drop in the macadamia orchards (Appendix A3), the natural land use type, bush, becomes a more important foraging habitat and thereby increased the activity of the clutter edge guild. The importance of natural and semi-natural land use types as foraging habitat for bats has been highlighted previously (Crisol-Martinez et al., 2016; Fuentes-Montemayor et al., 2011).

Our analysis of the foraging activity of bats, based on feeding buzzes, corroborates the conclusion that the natural vegetation, bush, positively influences the activity of the clutter edge guild, and hence potentially biocontrol of macadamia pests. We assume that the preference for the natural vegetation type (bush) by the clutter edge guild and the semi-natural vegetation type (fallow) by the open-air guild is explained by their wing morphology. While open-air feeders have a ‘high wing-loading’ for flying fast above vegetation, they prefer open areas without obstacles as the classification ‘open-air’ suggests. The clutter edge guild has a lower wing-loading and intermediate wing size (compared to the open-air and clutter guilds) and a preference to hunt at the ‘edges’ of vegetation such as forests (Monadjem et al., 2010). In addition, we found a positive relationship between bat activity (number of passes for both groups) in the macadamia orchards and abundances of Hemipteran collected in light traps across the season. These important findings support our assumption that bats play an important role in pest control by limiting stinkbug species and efficiently tracking the major pests of macadamia (Taylor et al., 2017; Taylor et al., 2013a; McCracken et al., 2012).

The distance to water, on the other hand, affected only the open-air guild and results were only marginally significant in the low season. It seems reasonable that drinking water becomes scarcer in the low (dry) season but also that prey abundances are higher around water sources (Stahlschmidt et al., 2012). Not all the orchards in this study had permanent

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water features present and we propose to get further insight into the use of water features (both artificial and natural) on macadamia orchards and the surrounding landscape, by placing stationary bat detector at water and in equal distance(s) to water (Sirami et al., 2013; Stahlschmidt et al., 2012).

In the low season, the clutter edge guild responded with an increase in bat activity with distance from settlements. The settlements in the study area are farm houses, most of which house bat roosts in their roof spaces, whereas the macadamia trees do not provide any roosting opportunities such as tree hollows (Taylor et al., 2013b). Therefore, the settlements appeared to provide roosting opportunities for maternity colonies. Based on our species list for the study area obtained from recordings, live captures and bat house observations, the majority of bat species give birth and raise their young during the macadamia high season (Appendix A4). Supported by our own local observations, at least one clutter edge bat (*Scotophilus dinganii*) is known to roost in farmhouses (Appendix A4). As previously shown, home range and foraging distance decreases significantly while bats are lactating (Clark et al., 1993; Henry et al., 2002; O'Donnell, 2001), which coincides with our high season (Appendix A4) and might explain why the activity of the clutter edge guild is less dependent on the vicinity of settlements during low season.

Additionally, the higher pest species abundance provides a higher prey availability during the high season, with all the settlements located in the immediate vicinity of the macadamia orchards (Taylor et al., 2017; Taylor et al., 2013a). If our assumptions are correct, the results on settlement distance suggest that the clutter edge guild in the orchards included a high ratio of female bats. As mentioned by Park (2015), it would be desirable to get in depth information on the proportion of male and female bats using the orchards. This is important, as females are much more selective in their habitat use and dependence on suitable maternity roosts. Our results also suggest that bat houses, in particular colony houses, might further improve bat activity on macadamia orchards given the decrease of natural roost sites (such as mature trees) and the desire of excluding bats from houses by most farmers. A study on rice plantations in Catalonia, Spain, showed a colonization of bat boxes by 3500 individuals within a period of under 10 years, resulting in a significant increase of the local bat population (Puig-Montserrat et al., 2015).

Our results show that bat activity in the high season (December- end of May) was nearly double that of the low season (June- end of November). This might be influenced by prey

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availability but we suggest that it is also a consequence of a generally lower bat activity in austral winter when Molossidae and Vespertilionidae become torpid more frequently (Monadjem et al., 2010).

In conclusion, our study supports the findings of other studies (Crisol-Martinez et al., 2016; Fuentes-Montemayor et al., 2011; Park, 2015) that natural and semi-natural vegetation are promoting bat activity and potentially biological control of major crop pests in agricultural landscapes. Our study highlights that the conservation of bat species and the promotion of their ecosystem services requires farmers to keep natural and semi-natural vegetation patches intact and that bat activity might be improved by adding roosting opportunities to orchards. Both foraging guilds rely on natural vegetation and various roosting opportunities are important especially in the breeding season.

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Chapter 3: The use of bat houses as day roosts in macadamia orchards, South Africa

Abstract

Despite the valuable ecosystem service of pest control provided by bats, an ever growing human population and agricultural intensification has led to a worldwide threat of extinction to about one quarter of all bat species. The loss of roost sites is one of the major drivers of this decline, while the roost site preference and artificial roost site use by African bat species is little understood. In this study, we focus on the preference of artificial roost sites by insectivorous bats in macadamia orchards in South Africa (Levubu, Limpopo).

From June 2016 to July 2017 we scanned 31 bat houses, mounted on poles on six macadamia orchards, for bats or any other occupants such as wasps, birds and bees. Twenty-one multi-chambered bat houses of three different designs (6-Chambered, Nursery and Old George bat houses) were erected on poles, in sets of three. Additionally, five Rocket boxes, four bat houses, in sets of two (black and white) and one colony bat house were erected.

Bats were counted and visually identified to family or species level. From December 2016 to end of March 2017, three IButtons were installed to record temperature variation between one set of three bat houses. We related the occupancy of bat houses in the different types of houses to the environmental variables distance to water, altitude and height of bat house as well as the difference in mean temperature between the set of three bat houses. The central bat house in the set of three and the black bat house in the set of two had a significantly positive effect on bat house occupancy. There was a significant difference in the mean temperature between the houses in the set of three, with a significant difference in temperature of 0.46°C between the central and the first bat house. The Yellow-bellied house bat (*Scotophilus dinganii*) was by far the most recorded and the only species observed to co-habitat a bat house with other animal species, in particular honeybees. Our study might confirm previous assumptions in that the microclimate of bat houses, respectively their insulation, sun exposure and color appear to be important factors, positively influencing bat house occupancy. The two preferred bat houses in our study were the black, in the set of black and white, as well as the central house, in the set of three. In conclusion from the different bat houses tested in this study, the designs we assume the warmest and best insulated, which were mounted freestanding on poles and in sets worked best to attract bat house occupancy in northern South Africa. Further research

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on the preferred microclimate of bat species especially on the response to temperature variation within bat houses, co-habitation of bat houses and displacement behavior as well as the potential importance of altitude and distance to water is needed.

1. Introduction

In Europe, artificial bat roosts have long been tested and reviewed to compensate for a loss of natural roost sites, particularly in silviculture (Bäumler 1988; Issel&Issel 1955; Natuschke 1960; Schwenke 1983). More recently, the value of insectivorous bats for agriculture and the use of bat houses in agricultural landscapes has received growing attention (Boyles et al. 2011; Cleveland et al. 2006; Flaquer et al. 2006; Kunz et al. 2011; Lopez-Hoffman et al. 2014; Maas et al. 2013; Puig-Montserrat et al. 2015; Taylor et al., 2018; Wanger et al. 2014). Nevertheless, an ever growing human population and related ongoing land use change, especially agricultural intensification, has led to a worldwide threat of extinction to about one quarter of all bat species (see Fig. 1.3 in Voigt & Kingston 2016; Mickleburgh et al. 2002, Tilman et al. 2011; Tscharntke et al. 2012). The loss of roost sites is one of the major drivers of this decline (Mickleburgh et al. 2002; Park, 2015) and there is a particular lack of knowledge regarding the roost site preferences of African bat species (Monadjem et al. 2009; Monadjem et al. 2010a; Taylor 2000). Given the accelerating land use change from natural to agricultural landscapes especially in the third world and the assumed decline in South African bat populations (Voigt & Kingston, 2016), proactive management of bat populations is indispensable to sustain their long-term ecosystem services (Cumming et al., 2014; Taylor et al., 2017; Tuttle et al., 2013). Proactive management of bat population will require to fill existing knowledge gaps on roost site preferences for African bat species and the means of successful conservation of bats in intensive agricultural systems in particular (Monadjem et al., 2009; Park, 2015; Taylor, 2000).

Peer-reviewed studies focusing on artificial roost site use by African bat species are literally non-existent and by far the most studies have been conducted in Europe followed by North America and Australia (Rueegger, 2016).

Summarizing the studies conducted globally so far, bat species seem to have a general preference for bat houses with a large volume, multi-compartments and those mounted on poles or houses compared to bat houses mounted on trees (Rueegger, 2016). There also seems to be a preference for bat houses build from ‘woodcement’ (Dodds& Bilston 2013;

Gerell 1985; Haensel& Tismer; 1999), although these studies are all limited to one climatic region in Europe (‘mild temperate/fully humid/warm summers’), according to the Köppen climate classification. Generally, the microclimate of bat houses, respectively insulation, sun exposure and color seems to be an important factor influencing bat house occupancy (Fukui et al. 2010; Rueegger, 2016; Shek et al. 2012). Looking at different designs and colors of bat houses, studies suggest that preferences also vary greatly depending on the reproductive state of females (Baranauskas 2009; Fukui et al. 2010; Flaquer et al. 2006; Kerth et al. 2001). Furthermore, many bat species seem to be sensitive to competition for bat houses by other species, mostly by birds and social bees, ants and wasps (Baranauskas 2009; Dodds& Bilston 2013; Meddings et al. 2011).

There is certainly a great need for research on artificial roost site use, especially in Africa, and this is the first peer-reviewed study looking at the occupancy of bat houses in South Africa. Successful bat house design and deployment seem to relate mostly to the climatic region and bat species targeted, while the pressure of land use change and loss of roost sites is increasing. The objective of this study was, therefore, to gain insight into the preference of artificial roost sites by insectivorous bats in macadamia orchards in South Africa. The main research question was, what are key features of occupied artificial roost sites (bat houses)?

We hypothesized that bat houses providing a warm microclimate and those erected close to water sources will do particularly well.

2. Methods

2.1 Study area and bat house design

The study was conducted in the fruit growing area of Levubu, Limpopo, South Africa, which accounts for the second highest production of macadamia in the country (see Figure 8). The climate in the study area is subtropical with around 1000 mm of annual rainfall. Other dominant land use types other than macadamia are pecan, avocado and banana orchards as well as pine and gum plantations (Taylor et al. 2017).

The Subtropical Fruit Association of South Africa (Subtrop) arranged for twenty-one multi-chambered bat houses to be mounted on poles, in sets of three, on four macadamia orchards in the Levuvhu river Valley, Limpopo, South Africa, in 2014. Every set of three

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houses compromised one 6-Chamber bat house in the middle as well as an Old George bat house and a Nursery bat house to either side (see Figure 9).

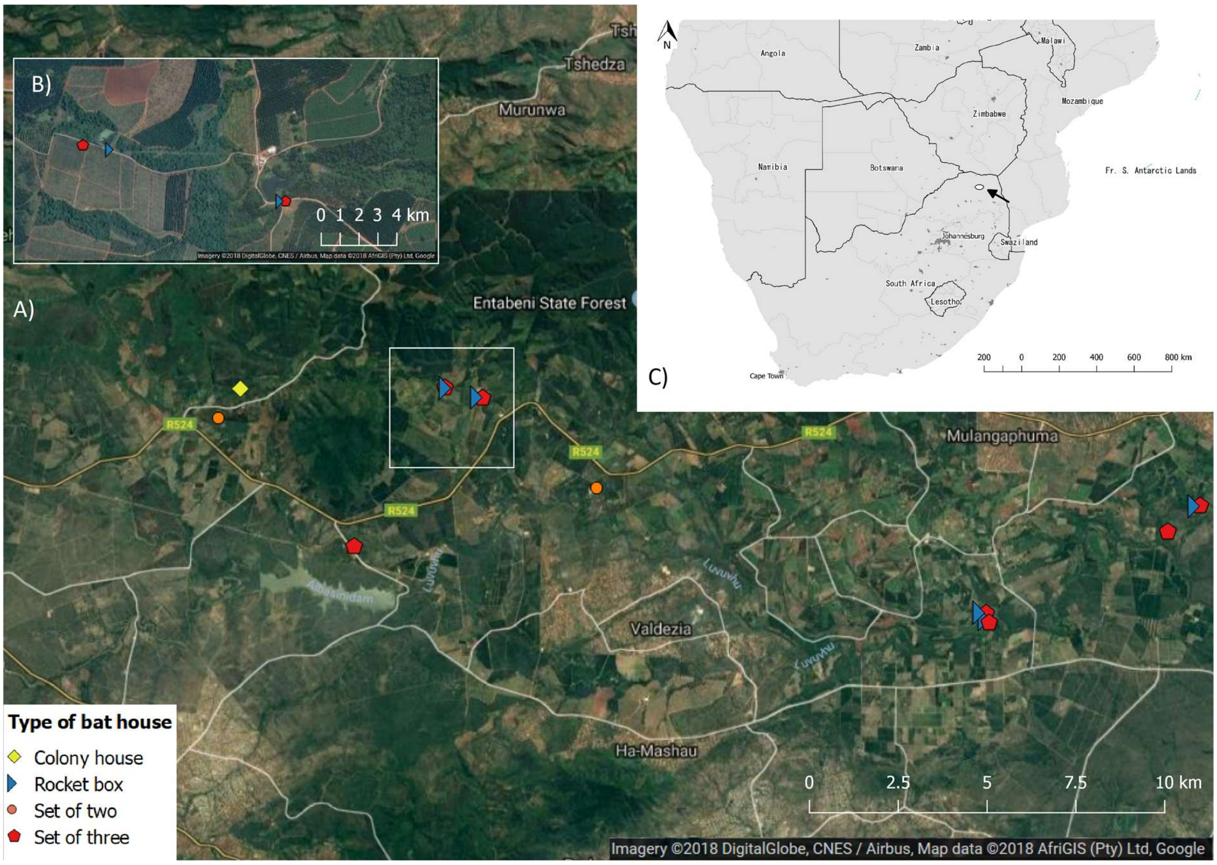

Figure 8 Map showing the aerial image of the study area with the location of the different bat houses and the study area in Levubu, Limpopo, South Africa (Google Maps 2018; QGIS version 2.18.11). A) Study area with the location of each bat house B) showing a detailed example of one study site and C) the location of the study area (white circle) in Levubu, Limpopo, South Africa.

Both the Nursery bat house and the Old George bat house vary slightly in their chamber design. The Old George model is different from the other designs, in that several of the spacers between the different chambers are set at an angle (Freed & Falxa, 2010). Whereas, the Nursery bat house has four chambers getting shorter in length towards the back of the house (see Figure 9). These bat houses were maintained in May 2016 and five two-chambered Rocket boxes (see Figure 9) were put up close to all but one set of three bat houses. The freestanding rocket boxes allow the bats to move around in 360° degrees within the house and choose from a range of temperatures respectively sun exposure. An additional four four-chambered bat houses, in sets of two (painted black and white in order to provide different microclimates), also mounted on poles and one colony bat houses were erected in March 2016 on two additional macadamia orchards (see Figure 9). All bat houses are constructed of wood and mounted on pine poles, also see Appendix (Table A5) for detailed information.

As proposed for warm climates by the 'North American bat house research project', all bat houses erected for this study have open bottoms (Kiser & Kiser 2004). They are also placed near water sources and natural vegetation, wherever possible. Except for the colony bat house, several bat houses were erected at each location, which is proposed to positively influence occupancy by providing different microclimates (Sedgeley, 2001). Alternating between different bat houses might also be necessary for bats to avoid predators and high ectoparasite loads (Lewis, 1995; Reckardt & Kerth, 2006).

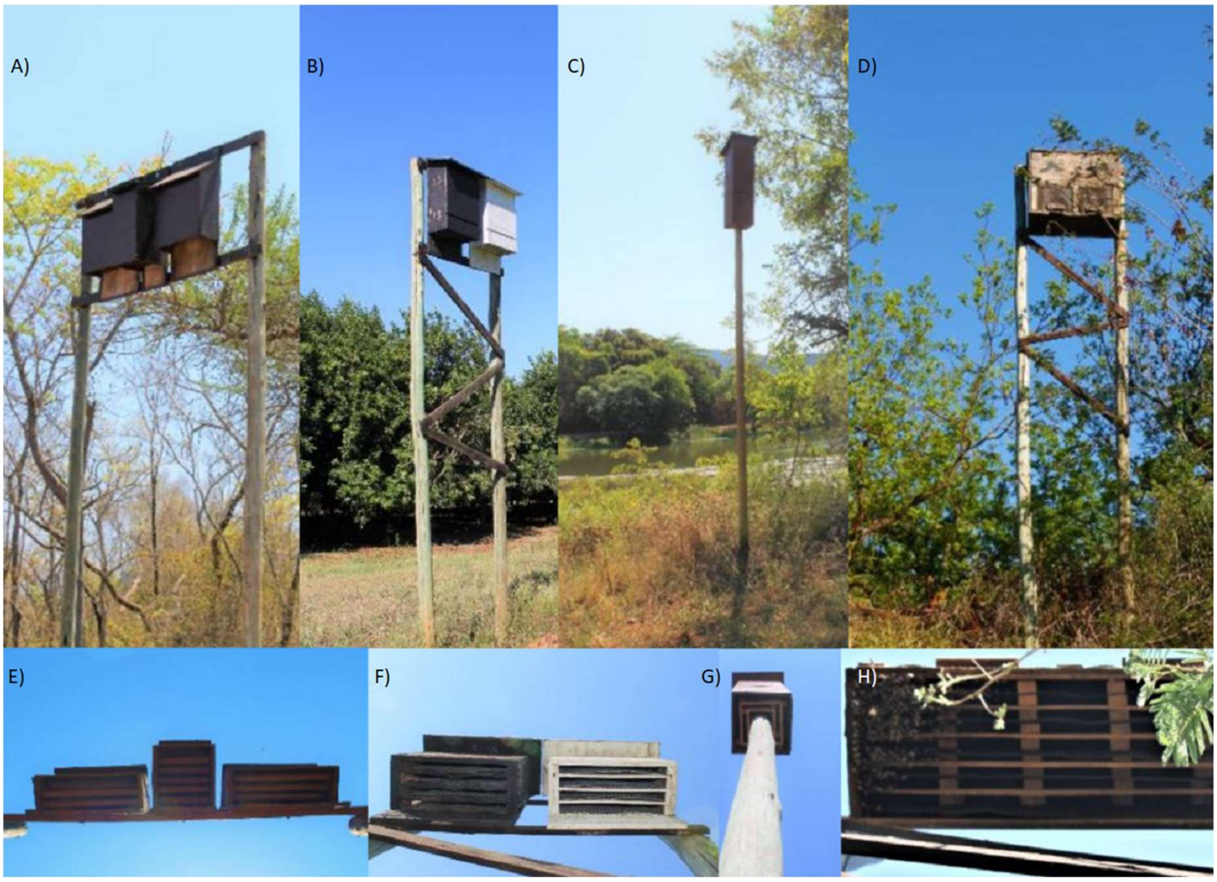

Figure 9 Showing the different bat house designs from the front and below, erected in the study area Levubu, Limpopo, South Africa. A) showing the set of three houses B) showing the set of black and white bat houses C) showing the Rocket box design and D) showing the Colony bat house. The below view showing E) the Old George, the 6-Chamber and the Nursery bat house (left to right) F) black and white house G) Rocket box H) Colony bat house.

2.2 Bat house occupancy

All bat houses were scanned monthly from June 2016 to July 2017 for bats or any other occupants such as wasps, birds and bees unless weather prevented access, by reflecting sunlight with mirrors into the houses. This process was kept as short as possible to avoid any major disturbance to occupant bats. Bats were counted and identified visually to family or species level referring to Monadjem et al. (2010b) and species records of Weier et al. (2018). Bat houses solely occupied by wasps or bees were cleared during maintenance in May 2016. We are working under the ongoing permit (No. 001-CPM403-00010) for research on small mammals from the Limpopo Department of Economic Development, Environment and Tourism. During 1st of December to 20th of March 2017, three IButtons (Thermochron, DS1921G-F5) were successfully installed with a tongue and sticky tape at each of the three entrances of one set of three bat houses to record temperature variation between the bat houses 1.5 hourly. While scanning the houses and fitting the IButtons we tried to avoid touching the bat house or the poles close to the house to keep disturbance to a minimum (Tuttle et al. 2013).

Additionally, we recorded the distance to the closest water source (Google Earth), the cardinal direction facing (Digital Compass, Axiomatic Inc.), the altitude above sea level (GPS Waypoints, Bluecover Technologies) and the height on poles of each bat house.

2.3 Data analyses

All statistical analyses was conducted with R (version 3.4, R Core Team 2017). The bat house of the type ‘colony’ was not used for analyses, as there was only one bat house of this type. The relatively small sample size of 31 bat houses (with 21 of them erected in sets of three) lead to a correlation between the type of bat house and the cardinal direction the different houses are facing. Additionally, not all possible combinations of the different bat house types and cardinal directions are available for statistical analyses. Therefore, we fitted a model to analyze the relationship of the response variable ‘presence or absence of bats’ and the predictor variables ‘type of bat house’, ‘altitude’, ‘distance to water’ and ‘height of the bat house’.

After testing the model for normal distribution and constant errors variance, we applied a generalized linear mixed model (GLMM) with a binomial distribution (package ‘lme4’ by Bates et al. 2015). The variables ‘farm’ and ‘month’ were used as random factors to account for pseudo replication and all the numeric predictor variables were scaled. We used the

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dregde function (package ‘MuMIn’ by Barton 2017) based on the lowest values of the Akaike information criterion (AICc), corrected for a small-sample size, to select final models.

In order to analyze differences in temperature recorded between one of the sets of three houses we fitted an ANOVA and used the Tukey test for multiple comparison (package ‘stats’ by R Core Team 2017). Additionally, we used the summary statistics base function ‘tapply’ to look at differences in range and mean of temperature (version 3.4, R Core Team 2017).

3. Results

We recorded a total of 166 individual bats within the 31 bat houses, from June 2016 to July 2017, with a maximum of 5 individual bats in one bat house. The maximum number of occupied bat houses was recorded in May 2017 with nine houses occupied by bats. The highest average number of bats was recorded in March and May (on average 0.53 bats per bat house), whereas the lowest average numbers were recorded in August and November (0.3). We recorded Yellow-bellied house bats (*Scotophilus dinganii*) on 43 occasions, small Plain-faced bats (*Vespertilionidae*) on nine occasions, Free-tailed bats (*Molossidae*) on 21 occasions and Mauritian tomb bat (*Taphozous mauritianus*) on three occasions (Table A6).

3.1 Type of bat house

The first five models selected by the function ‘dredge’, analyzing the relationship of the response variable ‘presence or absence of bats’ with the predictor variable(s) were all within a delta AICc of <2. After testing all five models, which each retained ‘type of bat house’ as the only significant variable, we decided for the simplest final model (presence of bats~type of bat house). The bat house occupancy on the macadamia orchards was influenced by the type of bat house, in that the central bat house ($\beta=1.43$, SE= 0.41, $p<0.001$) in the set of three and the black bat house in the set of two ($\beta=2.30$, SE=0.56, $p<0.001$) had a significantly positive effect on the presence of bats (see Table 2 and Figure 10).

3.2 Temperature

The ANOVA showed that there was a significant difference in the mean temperature values between the houses in the set of three ($F_{(2,5682)} = 4.34$, $p=0.0139$), in the summer months December 2016 to April 2017 (DatasetS2). A post hoc Tukey test showed that there was a significant difference in temperature of 0.46°C between the second, 6-Chamber bat house, and the first bat house ($p=0.016$, a Nursery bat house, and a marginal significant difference

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between the third, Old George bat house, and the second house (p=0.055). The temperature range was between a minimum of 12.5 and maximum of 40.5°C in the Nursery bat house and between min. 12 and max. 41°C in the second and third bat house (6- Chamber and Old George house). The mean temperature was the warmest in the second (central) bat house with 23.52°C compared to 23.06°C in the first bat house and 23.13°C in the third bat house. The ambient temperature recorded in Ratombo (about 7 km as the crow flies from the Ibuttons used in this study) over the last 30 years, from December to end of March, shows a mean daily minimum of 18-19°C and a mean daily maximum of 27-28°C with peaks of up to 35°C in December (Meteoblue, 2018).

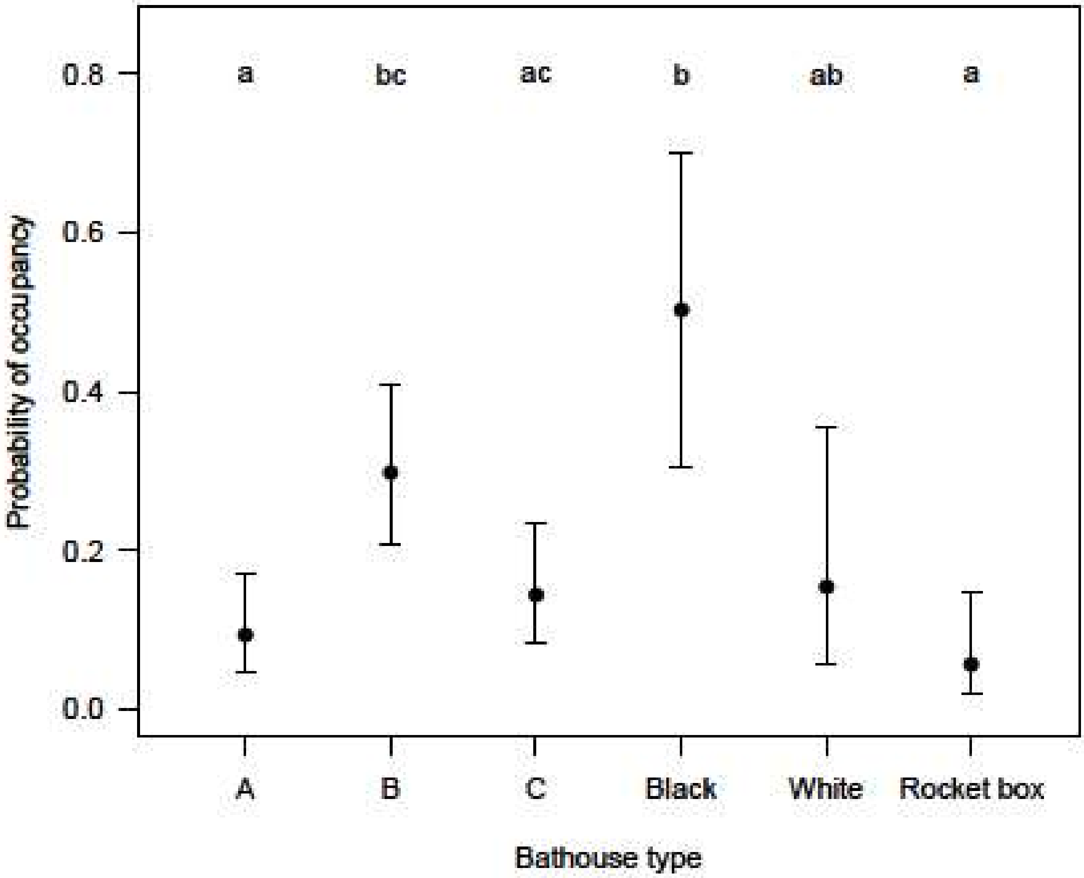

Figure 10 Showing the probability of a certain type of bat house being occupied with (95% confidence intervals) in the study area Levubu, Limpopo, South Africa. Whereas, bat house A (=Old George), bat house B (=6-Chamber) and bat house C (=Nursery) are always set up in sets of three with the 6-Chamber bat house in the middle. The black and white pained bat houses are also erected in sets. Annotated letters show same probability of occupancy (same letter) or a significant difference in occupancy levels (different letter).

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Table 2 Final model testing the relationship between the occupancy of bat houses and the different types of bat houses in macadamia orchards, Levubu, South Africa (significance level of bold $p < 0.05$).

Variable	**Estimate**	**SE**	**Z**	**p-Value**	**AICc**
Null model					388.2
Type of bat house					**364.6**
6-Chamber	**1.43**	**0.41**	**3.45**	**0.000**	
Black	**2.30**	**0.56**	**4.08**	**0.000**	
Nursery box	0.50	0.45	1.10	0.268	
Rocket box	-0.50	0.62	-0.81	0.415	
White	0.57	0.67	0.85	0.394	

3.3 Other bathouse occupants

We observed a number of other animals occupying the bat houses in the presence and absence of bats. Namely, we encountered Lesser Galago (*Galago moholi*), Tree squirrel (*Paraxerus cepapi*) and nests, which could also belong to Dormouse (*Myoxidae*), Lizards (*Lacertilia*), social wasps (*Vespidae*) and Honeybees (*Apis*). We twice (May 2017 and June 2016) observed Yellow-bellied house bats (*Scotophilus dinganii*) sharing a bat house with an active honeybee hive.

4. Discussion

From 166 individual bats observed during our study, the highest average number of bats were recorded in March and May, whereas the lowest average numbers were recorded in August and November. The bat house type was a significant variable influencing bat house occupancy, with a preference for the black house, from the set black and white, and the 6-Chamber model, the central house in the set of three. We assume that these are the two warmest houses, because of color (black) and insulation (central house of three and 6-Chambered) and are, therefore, the preferred types of bat houses occupied by bats.

The highest (March and May) and lowest (November and August) average numbers of bats recorded during our study matches the high (December to end of May) and the low (June to end of November) season of pest insect species occurrence on macadamia orchards, according to Weier et al. (2018). The study of Weier et al. (2018), conducted in the same study area, shows that not only bat activity is nearly doubled in the high season but also that bat activity increases with Hemipteran abundance. This also supports the assumption that

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insectivorous bat species track outbreaks of insect pest species such as stinkbugs (Taylor et al. 2013; Taylor et al. 2017; McCracken et al. 2012). We, therefore, suggest that colonization of bat houses in and around macadamia orchards might be highest in times of high prey availability of pest insect species particularly stinkbugs (see Figure 11).

Figure 11 A Common Slit-faced Bat (*Nycteris thebaica*) foraging on a green vegetable stinkbug (*Nezara viridula*) in the study area Levubu, Limpopo, South Africa (Photo credit: Merlin Tuttle).

Our study might confirm previous assumptions in that the microclimate of bat houses respectively insulation, sun exposure and color are important factors influencing bat house occupancy (Fukui et al. 2010; Kerth et al. 2001; Lourenço & Palmeirim 2004; Sedgeley, 2001; Shek et al. 2012).

Bats generally preferred the black houses, in the set of black and white, and 6-Chamber models, the central bat house in the set of three. The 6-Chamber models were mounted flanked either side by other houses and had the most chambers of all erected bat houses, which provided additional insulation (see Figure 9). We suggest that this insulation affected the preference by bats rather than the bat house design. It would be worth investigating how

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occupancy changes if this central bat house, the 6-Chamber model, is erected individually. However, we suggest that the insulation to either side of the 6-Chamber bat house by an additional bat house and the amount of chambers had a significant effect on bat house occupancy respectively microclimate. It should be noted, however, that only one set of three houses was measured with Ibuttons in our study and temperature deductions are based on this data. Future studies should aim at a higher sample size in order to investigate possible temperature variances caused by environmental factors. Because black colors absorb wavelength and therefore energy and white colors reflect them, it is reasonable to assume that the black bat houses in the sets of two are significantly warmer than the white bat houses. However, precise information on the differences in temperature would provide further insight into the preferred microclimate of bat houses by different bat species.
The colony bat house remained unoccupied throughout this study. In order to distinguish if this is an effect of the location or the type of bat house, several colony bat houses need to be erected and monitored. We also suggest that the colony bat house might have remained unoccupied because it did not provide any other artificial roost sites in close vicinity so alternating between different bat houses was not possible. The Rocket box design did particularly well in the United States and Canada with 62% overall occupancy (Kiser&Kiser, 2004), therefore, occupancy of the Rocket boxes might still increase with time (Agnelli et al., 2011). However, one component, which both the Rocket box and the Colony bat house are missing compared to the other designs erected, is a landing patch (see Figure 10).

Yellow-bellied house bats were by far the most recorded (42 times compared to nine observations of small plain-faced bats). This species is naturally tree cavity roosting but well known to utilize anthropogenic structures particularly rooftops (Monadjem et al. 2010a). Given the large size of Yellow-bellied house bats (weighing up to 37 grams), they might have a competitive advantage over smaller bat species (Monadjem et al. 2010a). However, displacement behaviour between different bat species can only be confirmed by for example fitting cameras to bat houses, such as in the study of Kerth et al. (2001). Furthermore, it is not yet known what effect the installation of artificial roosts have on the local community composition of bats and if it might lead to displacement behaviour of rare species by common species (Russo& Ancillotto, 2015).
The distance to water, the altitude above sea level and the height of bat houses did not significantly influence bat house occupancy in our study. While water availability is known to

influence bat activity (Crisol-Martínez et al. 2016; Grindal et al. 1999; Rainho & Palmeirim 2011), the bat houses in this study were erected within 2 to 680 meters from the closest water source, all within the known home range of even small plain-faced bats (Monadjem et al. 2010b). We also suggest that the distance to water might become more significant in regions that are more arid than our subtropical study area. There might also be a difference in this response during the dry season, which we suggest should be analyzed once a large data set becomes available. The altitude above sea level did range from 607 to 932 meters in this study and did, therefore, not provide a great variation in temperature.

While we observed a number of other animals in the bat houses during this study, the present bat house design did not seem to attract birds such as reported by Dodds& Bilston (2013). Interestingly, while there are contrasting observations on whether wasps displace bats from bat houses (Rueegger 2016), we did not observe co-habitation with wasps but with active beehives. However, tree squirrel and/or dormice might be able to displace bats as we only found a bat house occupied by bats with a mammal nest present once, in which case the nest seemed abandoned for some time.

The collection of bat faecal pellets, for a parallel project on bat diet, also suggests that the occupancy of bat houses is much higher than what we observed by recording the presence of individual bats during our monthly visits, as we often collected faecal pellets under unoccupied bat houses. While alternating between different roosts is well known especially for pregnant bats (Kerth et al. 2001; Reckardt & Kerth, 2006) as well as fission and fusions behaviour (Kerth & König 1999), our study focused on the use of bat houses as a day roost and different occupancy numbers might be observed when conducting nightly visits.

As visits were conducted monthly, we could not exclude the possibility that signs of bat presence such as faeces were washed away by rain or disintegrated in the sun beforehand. We did, therefore, not include this in our analyses. However, this variable should be kept in mind for future studies and might be a possible variable to use for studying winter roost occupancy during the dry months in northern South Africa, especially if data is collected over several years.

All of the bat houses in this study are freestanding with no direct cover by trees or houses to either side. While we were unable to analyze the influence of the cardinal direction respectively sun exposure on bat house occupancy, we suggest that future studies should consider this variable, particularly if bat houses are mounted onto the walls of houses and

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receive shadow from at the back. It would also be ideal to, additionally, test bat houses mounted back-to-back to provide additional insulation as proposed by Kiser and Kiser (2004) and control temperature variation between all houses of different designs.

It should also be noted that we found dead bats in or under bat houses on three occasions. Although we can currently not make an informed statement regarding the cause of these deaths, we would like to advice caution when it comes to placing bat houses within orchards, which are frequently sprayed with pesticides. We recommend to rather erect bat houses at the edges of orchards, in some distance to the crops which will be sprayed.

From the experience gained so far in northern South Africa, the bat houses we assume the warmest and best insulated, which were mounted freestanding on poles and in sets worked best to attract bat house occupancy. Further research is necessary and should focus on co-habitation of bat houses and displacement behavior as well as add a greater variation in altitude and distance of bat houses to water. There is also a vast scope to experiment with different colors, designs and position of bat houses and to look into the preferred microclimate of different bat species especially their response to temperature variation within bat houses.

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Chapter 4: Insect pest consumption by bats in macadamia orchards established by molecular diet analyses

Abstract

The diet of insectivorous bat species is difficult to study and the least invasive tool to gain information on these predators' foraging preferences is the study of their faecal pellets. The aim of this study was to determine whether bats consumed insect pest species in macadamia orchards, with the additional goal of incentivising farmers to adopt a more integrated pest management approach (IPM). We used a molecular approach to get insight into insectivorous bat diet, analysing pellets with fluorescent-labelled and species-specific primers for the cytochrome oxidase I (COI) gene. Faecal pellets were collected from captured individuals or from trays installed underneath bat houses and roosts between July 2015 and April 2017 in the Levubu region, Limpopo, South Africa. Four of the main insect pests, two moth (Lepidoptera: Tortricidae) and two stinkbug (Hemiptera: Pentatomidae) species, were collected for species-specific primer development and assay optimisation. We extracted DNA from the faecal pellets and amplified the target regions of pest species present. To verify the results of the fragment analyses we also sequenced all PCR products.

All the species or families of bats from which pellets were collected foraged on at least one of the four major insect pests, with insect pest sequences obtained and confirmed from 54 out of 103 samples (55.6%). Bats consumed insect pests throughout the macadamia growing seasons and are much more generalist and presumably opportunistic feeders than previously assumed. Nearly all species and families of bats analysed foraged on both the Lepidopteran and Hemipteran insect pest species. In conclusion, bats appear to be important for pest control and we suggest that farmers should maintain or restore (semi-) natural vegetation inside and adjacent to their farms. Adding water sources and roosting opportunities, and minimizing pesticide treatments furthermore promotes bat activity.

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1. Introduction

A growing body of literature emphasizes the value of the ecosystem service insectivorous bats provide in agricultural landscapes (Boyles et al., 2011; Cleveland et al., 2006; Kunz et al., 2011; Lopez-Hoffman et al., 2014; Maas et al., 2013; Puig-Montserrat et al., 2015; Russo et al., 2018; Taylor et al., 2018; Wanger et al., 2014). Most of these studies are based on exclusion experiments or avoided-cost models that use estimated levels of pest predation by bats. Moreover, evidence from exclusion experiments remain limited in so far as they fail to incorporate estimates of predation outside exclusion constructions, particularly that of high-flying Molossidae (McCracken et al., 2008; Voigt et al., 2018). South Africa is the world's largest producer of macadamia nuts since 2014 (DAFF 2016), with the annual loss from insect pest damage to macadamia crop being estimated at about 15.23 million USD, with an estimated ecosystem service of 59-139$ (USD) per hectare provided by insectivorous bats, which help suppress stinkbug pest species (Schoeman, 2009; Taylor et al., 2018). Furthermore, Weier et al. (2018) recently showed that bat activity within macadamia orchards in northern South Africa nearly doubles during the macadamia growing season, suggesting that prey availability of insect pests may be higher at this time of the year, thus attracting more insectivorous bat species. To determine whether insect pest species are consumed by bats and to offer incentive to farmers for a more integrated pest management approach (IPM), it is important to provide evidence of insect pest consumption by bats across a wide scale of agricultural systems.

The nocturnal and volant hunting behaviour of insectivorous bat species makes it difficult to study their diet, especially studying predation through direct observation (Brown et al., 2015; Clare et al., 2009). The least invasive tool to gain information on the diet of these predators is the study of bat faecal pellets. A common approach is the morphological analysis of prey items in bat faecal pellets (Lee & McCracken, 2002; Lee & McCracken, 2005; Leelapaibul et al., 2005; Taylor et al., 2017; Whitaker et al., 1996; Whitaker, 1988). Although this approach gives insight into the variability of prey-items consumed to order and, sometimes, family level, it usually lacks species level information (Whitaker, 1988). More recently, various molecular approaches such as next generation sequencing with a universal insect-prey primer pair have been used to study trophic interactions and variability in insectivorous bat diet (Aizpurua et al. 2018; Alberdi et al., 2018; Arrizabalaga-Escudero et al. 2015; Bohmann et al., 2011; Clare et al., 2013, 2011, 2009; Krauel et al., 2018; Mata et al.,

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2016; Taylor et al., 2017; Zeale et al., 2011). Recent studies suggest a high and diverse level of insect pest consumption (44 different insect pest species) by individual insectivorous bat species; Miniopterus schreibersii in Europe (Aizpurua et al., 2018) and Tadarida brasiliensis in the USA (Krauel et al., 2018) using a Next Generation Sequencing (NGS) approach. Insectivorous bats have also been shown to forage on major insect pest species in rice cultivation (Puig-Montserrat et al., 2015), pecan orchards (Brown et al., 2015) and cotton fields (McCracken et al., 2012).

In this study, we consider a molecular approach to insectivorous bat diet analyses from bat faecal pellets using fluorescent-labelled primers, each specifically designed to amplify a single insect pest species, in a multiplex PCR reaction. We targeted the highly polymorphic regions in the mitochondrial cytochrome oxidase I (DNA-barcoding) gene, to design species-specific primers. This method allows makes use of capillary electrophoresis, a technique more often used in genotyping (Beja-Pereira et al., 2009; Blacket et al., 2012). Once primers have been developed, they can be used to study predation of the same insect pest species by bats in different agricultural systems or by other vertebrates. The main objectives of this study were to determine the level of predation on major insect pests by insectivorous bat species in macadamia orchards and to assess whether there is a correlation between the macadamia growing cycle, respectively season, and the prevalence of these insect pest species in the faecal pellets. We also aim to offer incentive and recommendations to farmers such as macadamia growers to include bats in an integrated pest management approach (IPM). We hypothesized that pest predation will be higher during the macadamia growing season when insect pests peak in their abundance (Weier et al., 2018) and that the prevalence of Hemiptera or Lepidoptera insect pests in faecal pellets will depend on the species or foraging group of bats (Monadjem et al., 2010).

2. Methods

2.1 Study area

The study was conducted in the subtropical fruit growing area of Levubu, Limpopo province, South Africa between the towns Thohoyandou (22°59'03.7 S, 30°27'12.8 E) and Makhado/Louis Trichardt (23°03'03.6 S, 29°55'12.7 E). Levubu is located within the valley of the Levuvhu River and accounts for the second highest production of macadamia in South Africa. The study area is subtropical and receives its main rain in the summer season

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between November and April with around 1000 mm of annual rainfall. Other dominant land use types in the study area are pecan, avocado and banana orchards as well as pine and gum plantations (Taylor et al., 2017). There are natural vegetation patches remaining in and around most orchards and plantations, which are classified as ‘Soutpansberg Mountain Bushveld’ and ‘Tzaneen Sour Bushveld’ by Mucina and Rutherford (2006).

2.2 Sample collection

2.2.1 Faecal Sample collection

Between July 2015 and April 2017, we collected bat faecal pellets using two different methods to determine the prevalence of insect pests in faecal pellets. Twenty-one bat houses had been mounted on poles in four orchards in the study area by the company ’EcoSolutions’ (Johannesburg, SA) in 2014. Eighteen of these bat houses (in sets of three) on three different farms and two *Nycteris thebaica* roosts were fitted with trays to collect pellets from those occupied by bats. We noted occupancy of bat houses to species or family level to keep disturbance minimal. Additionally, we collected pellets from individuals captured with mist nets or harp traps (Permit No. 001-CPM403-00010) on the farm Schoonuitzicht. Each captured individual was identified to species level whenever possible by using the identification key of Monadjem et al. (2010). All bats were kept in a cloth bag for at least one hour until their release, in order to collect pellets from the bag. Release calls of captured bats were taken with either the Anabat SD2 (Titley Scientific) or the Batlogger M (Elekon AG). All collected pellets were stored in 70% ethanol in microcentrifuge tubes. A total of 103 samples were used for molecular analysis, each containing between one to ten individual pellets. The number of individual pellets per sample used for analyses was recorded, although by the time of analysis some pellets had disintegrated in the storage medium. Those samples were estimated to three pellets. In total 47 samples were obtained from trays underneath bat houses and 56 samples from bats which were captured. Bat houses were monitored for a parallel study from June 2016 to July 2017, during which we observed a maximum number of five individual bats in one bat house.

2.2.2 Pest insect collection

The South African Subtropical Growers’ Association (SUBTROP) provided samples for genetic analysis of the four insect pest species used for primer development and optimisation; the twin-spot stinkbug (*Bathycoelia distincta,* Distant), the green vegetable bug (*Nezara viridula,*

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Linnaeus*)*, the macadamia nut borer (*Thaumatotibia batrachopa,* Meyrick) and the litchi moth (*Cryptophlebia peltastica,* Meyrick). All sample insects had been collected on macadamia orchards within the study area. The two Lepidopteran species (*T. batrachopa* and *C. peltastica*) were caught with pheromone traps, while the Hemipteran species (*B. distincta* and *N. viridula*) were collected during scouting, a chemical knockdown method using dichlorvos (Schoeman, 2012). All samples were preserved individually in 70% ethanol.

2.3 Molecular methods

2.3.1 Insect DNA extraction and amplification

The insects were rinsed in distilled water (dH_2O) for 20 minutes and air-dried to remove alcohol traces. The whole insect was transferred to a 2ml tube with 5µL of Solid Tissue Buffer. A sterilized glass rod was used to homogenise the insects after which 90µL of Solid Tissue Buffer, 100µL dH_20 and 5µL Proteinkinase K was added. The samples were incubated overnight at 55°C and extracted using the ZR Genomic DNA™-Tissue MiniPrep kit for ‘Whole blood serum and plasma’ (Zymo Research, Irvine, USA). We deviated from the manual by preheating the Elution Buffer to 55°C and eluting in 15µL followed by adding another 15µL, 5 minutes later. We then waited 3-5 minutes before centrifuging at maximum speed for 30 seconds. We visualized 5µL of each sample against 2µL of Kappa Universal ladder (1Kb) on 2.5% agarose gels, stained with Ethidium bromide.

2.3.2 Species-specific primer development

A fragment of the insect cytochrome oxidase I (COI) gene was required for all four species to design species-specific primers. We followed the PCR protocol by Tembe et al. (2014) using universal insect COI primer pair LCO1490 (5′-ggtcaacaaatcataaagatattgg-3′) and HCO2198 (5′-taaacttcagggtgaccaaaaaatca-3′) (Folmer et al., 1994). PCR products were visualized on agarose gels as described above to confirm amplification. PCR products were enzymatically purified using Exonuclease I and Shrimp Alkaline Phosphatase according to manufacturer’s protocol (New England Biolabs, Inc).

All sequencing reactions were performed by Inqaba Biotechnical Industries (Pty) Ltd, using the BrilliantDye™ v3.1 Terminator Cycle Sequencing Kit (NimaGen BV, Nijmenden, The Netherlands). The sequencing products were purified using the ZR-96 DNA Sequencing Clean-up Kit™ protocol (Zymo Research, Irvine, USA), and run on the ABI PRISM™ 3500xl

Genetic Analyser (Applied Biosystems, Thermo Fisher Scientific, Carlsbad, USA). Bioedit version 7.2.6.1 (Hall, 1999) was used to edit and align extracted sequences from five *B. distincta*, eight *C. peltastica,* six *N. viridula* (including Genebank Accessions KR044112.1, KR037758.1 and KJ642019.1*)* and four *T. batrachopa* (including Genebank Accessions KP083436.1 and KP083437.1).

Sequence data and cytochrome oxidase I (COI) Genbank accessions for *Bathycoelia distincta*, *Cryptophlebia peltastica*, *Thaumatotibia batrachopa* and *Nezara viridula* were downloaded into CLC Main Workbench (QIAGEN) from NCBI. Sequence data was assembled, and polymorphic regions between each species identified. Species-specific primers were initially developed using the software Primer-BLAST (https://www.ncbi.nlm.nih.gov/) and tested with the 'Multiple Primer Analyzer' (https://www.thermofisher.com/) for primer-dimer formation, amplified fragment length, melting Temperature and percentage of CG content. Four primer pairs were chosen to be at least 23bp in length, with melting temperatures over 65°C to maintain similar annealing temperatures over 60°C. Primers were designed to amplify fragments of various sizes between ~100 and 300bp to distinguish between each target using fragment analysis. Primers were tested for species specificity by PCR and gel electrophoresis and confirmed by fragment analysis, carried out at Inqaba Biotechnical Industries (Pty) Ltd. The forward primers were labelled with fluorophores (Table 3). Optimization of the multiplex assay was performed using all four primer sets and template DNA from each insect species (Table A7).

2.3.3 Multiplex fragment analysis of DNA extracted from bat pellets

The Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, USA) was used to extract DNA from the faecal pellets. The Thermo Scientific™ NanoDrop™ OneC spectrophotometer was used to perform DNA quantification and quality evaluation before the multiplex PCR amplification. Samples that did not meet quality standards (280/260 between 1.4-1.8 and 230/260 between 1.4-2.0) were cleaned and concentrated using DNA Clean & Concentrator (Zymo Research, Irvine, USA). Target regions were amplified using Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs, MA 01938, USA). PCR reactions were performed in volume of 25µL according to manufacturer's specifications adding 10ng of gDNA, 10mM of each primer and nuclease free water (AMRESCO LLC, OH 44139, USA).

Table 3 Summary of the primers used to detect the four different macadamia insect pest species in bat faecal samples, listed with the markers (dye) used and the expected size of PCR products (bold letters indication the so-called 'GC-clamp' at the 3'-end of each forward and reverse primer).

Species	Forward Primer (5'-3')	Reverse Primer (5'-3')	Primer length (bp)	Dye	Fragment Size (bp)
Thaumatotibia batrachopa	GGAGCTGGGACAGGAT**GAACAG**	CCTCCTCCAGCTGGGT**CAAAA**	22/21	ATTO-532	319
Cryptophlebia peltastica	TGGAGCAGGTACTGGATGA**ACAGT**	AAAGAGCTGTAATACCAACAGCTC**AGACA**	24/29	ATTO-550	222
Nezara viridula	CCCTTTTAATAGTAAGAAGATTAGCAGAATCTG**GAGCA**	CTGCGCCTAGGATTGATGATAC**TCCTG**	38/27	ATTO-565	158
Bathycoelia distincta	GTTTATCCACCTCTATCAAGTAATTTATCACATAGAG**GAGCA**	AGGTAATGATAATAATAGAAGTAGGGCTGTAATTCC**AACGG**	42/41	6-FAM	216

Thermocycler conditions were set to the following program: Initial denaturation at 98°C for 30 seconds. The first 20 cycles were set to 98°C for 10 seconds, 65°C for 10 seconds and 72°C for 10 seconds followed by the second 20 cycles of 98°C for 10 seconds, 68°C for 10 seconds and 72°C for 10 seconds. The final extension was set to 72°C for 30 seconds and holding at 4°C. Due to the suspected low concentration of target gDNA for *B. distincta*, *C. peltastica*, *T. batrachopa* and *N. viridula* in the bat pellets, fragments were prepared by adding 4µL fluorescently labelled PCR amplicons to the LIZ500 sizing standard and Hi-Di^{TM} Formamide (Thermo Fisher Scientific, Carlsbad, USA) mixture and denatured at 95°C for 5 minutes. After denaturation, the fragments were run on the ABI PRISM™ 3500xl Genetic Analyser (Applied Biosystems, Thermo Fisher Scientific, Carlsbad, USA). The data was analysed using GeneScan™ Software and interpreted using GeneMapper® v5.0. (Applied Biosystems, Thermo Fisher Scientific, Carlsbad, USA).

2.4 Data analyses

2.4.1 Primer verification and Sequence analyses

Samples that produced successful profiles from the fragment analysis were amplified in simplex using unlabelled species-specific primers and sequenced as described above by

Inqaba Biotechnical Industries (Pty) Ltd, to verify effectiveness of the four primer sets. To test for false positive or negative fragments, all sequences were aligned in BioEdit version 7.2.6.1 (Hall, 1999) and compared against the NCBI nucleotide sequence database, using the software BLAST (ncbi.nlm.nih.gov). Since there was no *B. distincta* sequences available on GenBank, closest identity to *Bathycoelia indica* (Dallas, 1851) vouchers in BLAST was used for primer verification.

2.4.2 Statistical analyses

We split our nearly two-year data set, from July 2015 to April 2017, into a high season (December to end of May) and a low season (June to end of November) as previously used to distinguish the macadamia growing season from the off-peak season (Weier et al., 2018). All statistical analyses was conducted with R (version 3.4, R Core Team 2017). The R-package ‘lme4’ (Bates et al., 2015) was used to fit a generalized linear model (GzLM) with a binomial distribution. We analysed the relationship of the response variable ‘presence or absence of insect pests’ in faecal samples and the predictor variables ‘number of pellets’ in each sample, month of collection, season (low or high) and method by which pellets were obtained (from roosts or caught bats). We used the R-package ‘ResourceSelection’ (Lele et al., 2019) to test model fit with the Hosmer Lemeshow goodness of fit test, which was not significant (p= 0.69) and confirmed our model is a good fit.

2.4.3 Release call identification

AnalookW (version 0.3.8.13; Corben 2006) or Bat Explorer (Elekon AG, Version 1.10, http://www.elekon.ch) were used to analyse the recorded calls by referring to Monadjem et al. (2010) and Taylor et al. (2013) for call identification. Because of the difficulty to distinguish small Vespertilionidae, we classified them all into one group with the exception of Neoromicia nana, which has a very distinct echolocation call.

3. Results

3.1 Fragment analyses and sequencing

Fragment analyses yielded profiles for 63 of the 103 samples testing positive for one or more pest insect species (61%) with a total of 92 positive fragments. Of the 92 positive fragments sequenced, we obtained sequences from 79 fragment. A total of 13 sequences could not be

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blasted and were excluded from further analyses. We confirmed 57 of the 79 fragments as true positives. Twelve of the remaining 22 fragments turned out to be amplifications of one of the other three pest insect-species.

Primer specificity could be confirmed to 100% for the primer pairs designed for *B. distincta* (all 26 sequences obtained from fragments were confirmed by blasting) and *N. viridula* (all 12 sequences obtained were confirmed by blasting). The primer pairs for *C. peltastica* (18 confirmed out of 30 sequences) and *T. batrachopa* (one confirmed out of 14 fragments) were not sufficiently specific. From the 30 positive sequences obtained for *C. peltastica*, ten were confirmed as amplifications of *T. batrachopa*. From a total of 14 positive sequences obtained for *T. batrachopa*, two were confirmed amplifications of *B. distincta*. Additionally, we detected four false negatives within the samples. One sample showed no positive fragments but contained a positive sequence for *N. viridula*. Three samples tested positive for one pest-species but contained a positive sequence for a second species. Adding four positive sequences and one additional positive sample to the data, we obtained sequences from insect pest species from 54 of the 103 pellet samples (55.6%) with a total of 73 insect pest sequences. Looking at the results for the individual insect pest species, we confirmed 17.5% of the samples positive for *C. peltastica*, 1% positive for *T. batrachopa,* 12.36% positive for *N. viridula* and 26.78% for *B. distincta*.

3.2 Influence of season and other environmental variables on pest detection

The step-wise selection of the GzLM for the ‘presence or absence of insect pests’ in faecal samples only retained the variable low season (macadamia off-growing season) which had no significant effect on the detection of insect pests in bat faecal pellets (β= -0.64, SE= 0.42, p=0.13). Sample size was similar with 46 samples from the low season and 57 from the high season and except for April (N=1) sample size per month ranged from 5-20 samples. For the high season, we yielded a total of 37 positive fragments for the four insect pest species and 24 negative samples and for the low season a total of 36 positive fragments and 15 negative samples (Figure 12). The predictor variables ‘number of pellets’ in each sample, month of collection and the method by which pellets were obtained (from roosts or caught bats) had no significant effect on the detection of insect pests in faecal pellets.

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3.3 Pest consumption of the different species/families

Our sequencing results showed that all bat species/families consumed insect pest species (Table 4). Whereas, all species and families except *Myotis bocagii* (N=2) and *Rhinolophus simulator* (N=1) foraged on both the Lepidopteran and Hemipteran pest species. Most of the Vespertilionidae species (except *M. bocagii*) foraged on all four insect pest species. The Molossidae consumed all but one insect pest (*C. peltastica*) and *Nycteris thebaica* consumed one of the Hemipteran species (*B. distincta)* as well as one of the Lepidopteran species (*C. peltastica).*

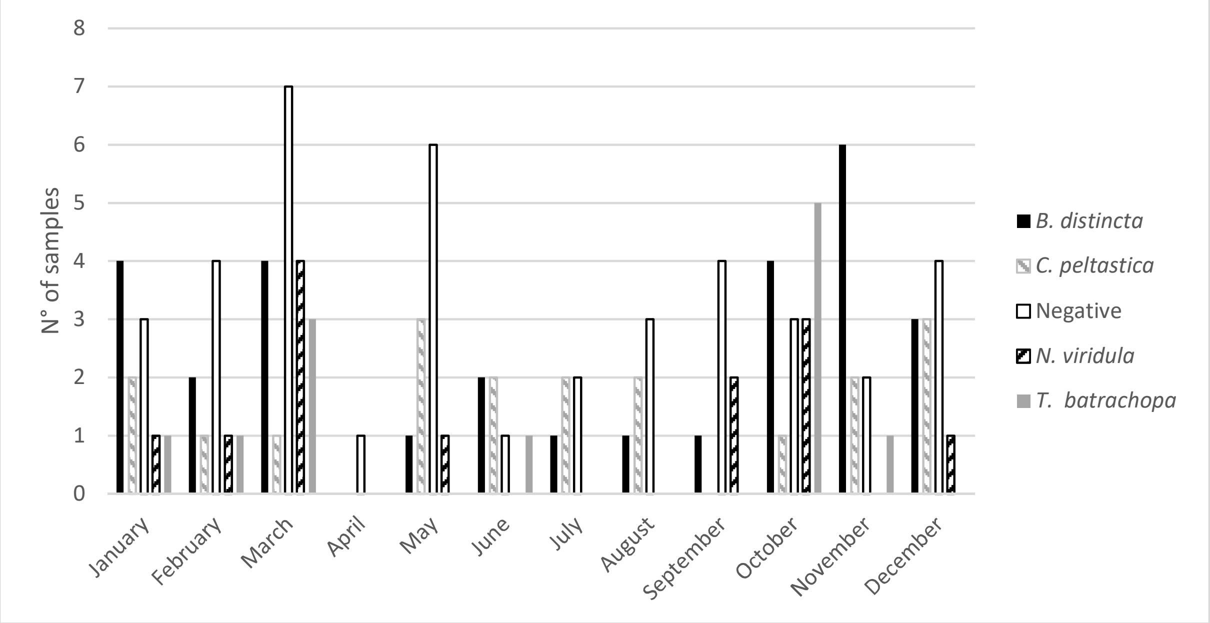

Figure 12 Four macadamia insect pest species detected in bat faecal samples by using a PCR-sequencing approach shown over the different months. Samples in which no pest species were amplified are indicated as “Negative”.

Hemiptera species are listed as one of the major families of prey insects included in the diet of two out of the small Vespertilionidae recorded in the study area and as a possible prey of *S. dinganii* but not at all for *N. nana* (Monadjem et al. 2010; Appendix Table A8). However, we found a high prevalence of Hemiptera pest species in the faecal samples of *N. nana, S. dinganii* and the small Vespertilionidae (Table 4). Likewise, Lepidopteran species are not known to be a major prey item in the diet of *S. dinganii* but were quite prevalent in our analyses of faecal pellets. *N. thebaica* was also confirmed to forage on moth (*C. peltastica*) as well as stinkbug (*B. distincta*) species.

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Table 4 Showing the total number of negative faecal samples as well as the positive sequences detected for each of the insect pest species in the pellets of the different species or families of bats (+ indicating that some samples disintegrated in the storage medium and were estimated to three pellets).

Species/Family	**N° of pellets**	**N° of negative samples**	**N° of positive samples for each insect pest**			
			Cryptophlebia peltastica	*Thaumatotibia batrachopa*	*Bathycoelia distincta*	*Nezara viridula*
small Vespertilionidae	148+	18	**8**	**5**	**13**	**5**
Scotophilus dinganii	177+	14	**9**	**4**	**10**	**4**
Myotis bocagii	6	1	0	0	**1**	0
Neoromicia nana	30+	3	**1**	**1**	**3**	**2**
Rhinolophus simulator	5	0	0	0	0	**1**
Molossidae	33	3	0	**2**	**1**	**1**
Nycteris thebaica	12	0	**1**	0	**1**	0

4. Discussion

All families of bats for which we collected faecal pellets (Molossidae, Nycteridae, Rhinolophidae and Vespertilionidae) have been confirmed to forage on at least one of the four pest insects. Our results provide some interesting new insights into the foraging ecology of the different species (Monadjem et al. 2010; Table A8). The limited number of pest species detected in *M. bocagii* (N=2) and *R. simulator* (N=1) faecal samples is most likely a result of the small sample size collected for those species. The Vespertilionidae species, for which a large sample size was collected, appear to be highly generalist predators with most species consuming both Hemiptera and Lepidoptera insect pests. Generally, our results suggest that all the insectivorous species and families recorded in the study area are much more generalist and presumably opportunistic feeders than previously assumed with more than half of the samples analysed (55.6%) containing sequences of at least one of the four insect pests.

Our results for *C. peltastica* (17.5% of the samples positive) are similar to the results of McCracken et al. (2012) looking at consumption of corn earworm moths (*Helicoverpa zea*) by bats in Texas, United Stated. The study found between 34.4 and 17.3% of samples in

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triplicate qPCR reactions positive for corn earworm (McCracken et al., 2012). A study looking at presence of rice borer moth (*Chilo supressalis*) in bat faecal pellets in Iberia, during two peaks in abundance of this moth, found 20% and 50% of samples were positive for the rice borer moth (Puig-Montserrat et al. 2015). The results of another study (Brown et al., 2015) focusing on the consumption of insect pests by bats in pecan orchards, in Georgia and Texas, found 1.4% of samples positive for pecan nut casebearer moths (*Acrobasis nuxvorella*), 3.8% for hickory shuckworm moths (*Cydia caryana*) and 5.4% for corn earworm moths (*Helicoverpa zea*). The same study also directly sequenced 22 insect pieces from bat faecal pellets, of which seven were confirmed to be *N. viridula (*Brown et al., 2015). Our results confirme a wide range of pest insect consumption by species such as *S. dinganii* and are in line with previous studies looking at insect pest consumption by individual *Miniopterus schreibersii* in Europe and *Tadarida brasiliensis* in the USA (Aizpurua et al., 2018; Krauel et al., 2018). *M. schreibersii* was confirmed to forage on 44 different insect pest species and 94% of faecal samples contained insect pest species (Aizpurua et al., 2018). Likewise, faecal samples of *Tadarida brasiliensis* also contained a range of 44 insect pest species (Krauel et al., 2018).

Our study shows that multiplex fluorescent-fragment analyses can be an efficient tool to screen bat faecal samples for specific insect species but our results also tell a cautionary tale about primer specificity. Even though, optimization assays of the primer sets showed target specificity, the primer sets for the Lepidopteran species (*T. batrachopa* and *C. peltastica*) were not sufficiently specific and sequencing of target products showed cross amplification as well as amplification of non-target species. Therefore, it is imperative to sequence PCR products for the initial validation of primers.

However, we assume that the two stinkbug primers developed and verified for specificity in this study, can now be used quickly and cost-effectively to expand faecal diet analyses to other agricultural systems or to study pest predation by other vertebrate species. The green vegetable stinkbug (*N. viridula*) is considered a pest across many agricultural systems worldwide including soybean and cotton (Prado, 2009; Tillman, 2006). Whereas, the two-spotted stinkbug (*B. distincta*) is indigenous to Southeast Africa but also a major (or minor) pest to crops other than macadamia such as avocado (SAMAC, 2016; Schoeman, 2013).

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The sample size (number of pellets in each sample) had no influence on the probability of detecting one or more of the four insect pest species in our study. We frequently detected pest insects in small samples (N<3). Nonetheless, studies focusing on the overall foraging behaviour of bats suggest a sample size of 20 pellets per bat species (for each location and collection event) or five pellets per individual bat, and confidence does evidently increase with sample size (Whitacker et al., 2009; Whitacker et al., 1996).

Further insight could be gained by extending this molecular approach to other agroecosystems and by comparing pest predation in extensive and lower intensity or organic systems (Park, 2015). Furthermore, the majority of research, including this study, is currently focusing on common insectivorous bat species, presumably providing ecosystem services such as pest control for the most part. However, as suggested by Russo et al. (2018), rare species such as the *Rhinolophus sp.*, which are 'gleaners', might also play a key role in suppressing certain pest insect species and will be affected considerably more by ongoing land-use change and, possibly, displacement by common species. The low sample size for *Rhinolophus sp*. (N=1) but also for the *Myotis sp*. (N=2) in our study provides very limited insight into the foraging prevalence of insect pests for those species. However, insect pests have been detected in samples from both bat species and future studies should aim to compare the prevalence of pest insects in the diet of both common and rare bat species with a similar sampling effort.

Furthermore, molecular approaches such as fragment analyses of bat faecal pellets could be used for the detection and monitoring of dispersal of new insect pest species through bat faecal pellets (Russo et al., 2018). Apart from information about the foraging ecology of bats regarding insect pest consumption, our study also provides an indication on the presence and absence of insect pest species in macadamia orchards throughout the year over a 22 months period (July 2015 to April 2017). Our results suggest that the indigenous *B. distincta* was present in macadamia orchards year-round, whereas *N. viridula* was absent from bat faecal pellets from June to September (Figure 1). *T. batrachopa* was absent in May and from July to October, whereas *C. peltastica* was only absent from bat faecal pellets in September (Figure 1). While, monitoring of insect pest species in macadamia orchards is usually limited to the macadamia growing season (De Villiers& Joubert, 2003) our results are in line with the previous suggestion of immigration (with nutset in October) and emigration (with the end of the harvest) of *B. distincta* populations to and from macadamia orchards (Schoeman, 2013).

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However, it also shows that at least some of the *B. distincta* population remains in or around macadamia orchards after macadamia harvest.

In conclusion, the results of this study may incentivize farmers such as macadamia growers to consider bats in their integrated pest management approach (IPM) in order to enhance or maintain natural pest control. Reviewing the findings of other studies (Crisol-Martínez et al., 2016; Fuentes-Montemayor et al., 2011; Park, 2015; Weier et al. 2018) natural and semi-natural vegetation promote bat activity and potentially biological control of major crop pests in agricultural landscapes circumglobally. The study of Weier et al. (2018) highlights that the conservation of bat species and the promotion of their ecosystem services requires macadamia growers to keep natural and semi-natural vegetation patches intact. Apart from conserving natural vegetation it is, therefore, recommended to maintain fallow periods by practicing extensive and rotational agriculture in the vicinity of orchards for example in cattle farming (Starik, 2016). Previous studies have suggested that bat activity is positively influenced by the presence of natural and artificial waterbodies (Park, 2015; Sirami et al., 2013; Stahlschmidt et al., 2012). Adding artificial roosting opportunities to orchards might further improve pest control provided by bats (Puig-Montserrat et al., 2015). It is also essential to monitor the effects of implementing new agroecosystem management approaches and extend management beyond farm boundaries. The implementation of 'Agri-environment schemes (AES)' on 18 farms in Scotland, which each followed at least three schemes of managing 'field margins or beetlebanks'; 'hedgerows'; 'water margins; and 'species-rich grasslands'; did not improve bat activity, however, the presence of periphery woodlands had a positive influence on bat activity (Fuentes-Montemayor et al., 2011).
We, therefore, suggest farmers should maintain and restore natural and semi-natural (fallow) vegetation, provide roosting opportunities in and around macadamia orchards and add water sources such as ponds and dams to promote bat activity. Furthermore, we strongly advise against scheduled sprays and recommend keeping pesticide treatments to a minimum possible threshold. While it remains largely uncertain what direct effects pesticides have on bats, it becomes increasingly clear that pesticides are one of the major factors leading to the current mass insect species extinction and, therefore, a threat to vertebrates such as bats depending on an abundance of insect prey (Mineau & Callaghan, 2008; Sánchez-Bayoa & Wyckhuys, 2019).

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Chapter 5: General discussion and conclusion

The key contributions of this study are the provision of incentive and advice to farmers for a more integrated pest management approach (IPM), to conserve bat species and potentially promote their ecosystem service of pest control. This study was conducted in the subtropical fruit growing area of Levubu, Limpopo province, South Africa between June 2015 and April 2017.

Accelerating land use change is associated with the loss of species and their ecosystem services. An ever-growing human population and agricultural intensification has led to a worldwide threat of extinction to about one quarter of all bat species and their numbers keep decreasing at an alarming rate (Boyles et al., 2011; Jones et al., 2003; Mickleburgh et al., 2002), despite the valuable ecosystem service of pest control provided by bats (Ducummon, 2000; Fenton, 1997; Kalka et al., 2008; Leelapaibul et al., 2005; McCracken et al., 2012; Williams-Guillen et al., 2008). This decline is mainly attributed to the loss and fragmentation of habitats, roost sites and feeding opportunities, with agricultural intensification and related land use changes being by far the most frequent threat listed for bats in some 550 IUCN Red List bat species accounts (see Fig. 1.3 in Voigt and Kingston, 2016). The loss and degradation of natural habitats is predicted to accelerate until 2050 (Foley et al., 2005; Tilman et al., 2001; Tscharntke et al., 2012). With 28,000 ha of land covered by macadamia orchards (SAMAC, 2017), South Africa is the world's leading producer of macadamia nuts since 2014, accounting for an annual production of over 44 thousand tons in 2014 and 46 thousand tons in 2015 (DAFF, 2016; Taylor et al., 2018). The South African macadamia industry continues to grow with a minimum of 1900 ha planted in 2016 alone (DAFF, 2016). The current annual loss from insect pest damage to the macadamia crop has been recently estimated at 200 million ZAR or about 15.23 million USD (Taylor et al., 2018).

The main objective of this study was, therefore, to gain insight into the foraging and roosting behaviour of insectivorous bat species on a temporal and spatial scale in macadamia orchards, South Africa.

The importance of natural vegetation and the influence of bug abundance

Chapter One showed that increasing macadamia cover, often at the expense of natural vegetation, reduced the activity for the clutter edge feeding bat guild in the macadamia

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growing season (high season). In contrast, bush cover promoted activity of the clutter edge guild in the off-peak season (low season). Macadamia cover has a negative effect on the activity of the clutter edge guild in the high season, with low activity in the orchard center (high cover) and activity increasing in a linear way with decreasing orchard cover at the orchard edge (low cover). These results suggest that the clutter edge guild prefers foraging close to the edges of the orchards rather than in the center (Crisol-Martinez et al., 2016; Ewers & Didham, 2006), while the open-air guild prefers semi-natural habitats (fallow). When numbers of pest arthropods drop in the macadamia orchards, the natural land use type, bush, becomes a more important foraging habitat and thereby increased the activity of the clutter edge guild.

The analysis of the foraging activity of bats, based on feeding buzzes, corroborates the conclusion that the natural vegetation positively influences the activity of the clutter edge guild, and hence potentially biocontrol of macadamia pests. It is assumed that the preference for the natural vegetation type (bush) by the clutter edge guild and the semi-natural vegetation type (fallow) by the open-air guild is explained by their wing morphology. While open-air feeders have a ‘high wing-loading’ for flying fast above vegetation, they prefer open areas without obstacles, as the classification ‘open-air’ suggests. The clutter edge guild has a lower wing-loading and intermediate wing size (compared to the open-air and clutter guilds) and a preference to hunt at the ‘edges’ of vegetation such as forests (Monadjem et al., 2010a). In addition, there was a positive relationship between bat activity (number of passes for both groups) in the macadamia orchards and abundances of Hemipteran collected in light traps across the season. These important findings may support the assumption that bats play an important role in pest control by limiting stinkbug species and efficiently tracking the major pests of macadamia (Taylor et al., 2017; Taylor et al., 2013a; McCracken et al., 2012).

In the low season, the clutter edge guild responded with an increase in bat activity with distance from settlements. The settlements in the study area are farmhouses, most of which house bat roosts in their roof spaces, whereas the macadamia trees do not provide any roosting opportunities such as tree hollows (Taylor et al., 2013b). Therefore, the settlements appeared to provide roosting opportunities for maternity colonies. Based on the species list for the study area obtained from recordings, live captures and bat house observations, the majority of bat species give birth and raise their young during the macadamia high season.

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Supported by local observations, at least one clutter edge bat (*Scotophilus dinganii*) is known to roost in farmhouses. As previously shown, home range and foraging distance decreases significantly while bats are lactating (Clark et al., 1993; Henry et al., 2002; O'Donnell, 2001), which coincides with the high season and might explain why the activity of the clutter edge guild is less dependent on the vicinity of settlements during low season. Additionally, the higher pest species abundance provides a higher prey availability during the high season, with all settlements located in the immediate vicinity of the macadamia orchards (Taylor et al., 2017; Taylor et al., 2013a). If these assumptions are correct, the results on settlement distance suggest that the clutter edge guild in the orchards included a high ratio of female bats. This also suggest that bat houses, in particular colony houses, might further improve bat activity on macadamia orchards given the decrease of natural roost sites (such as mature trees) and the desire of excluding bats from houses by most farmers.

The use of bat houses for insectivorous bat species as day roosts

Another key objective of this study was to identify which factors influencing bat house occupancy in macadamia orchards.

This study may confirm previous assumptions in that the microclimate of bat houses respectively insulation, sun exposure and colour are important factors influencing bat house occupancy (Fukui et al., 2010; Gerell, 1985; Kerth et al., 2001; Lourenço & Palmeirim, 2004; Rueegger, 2016; Shek et al., 2012). However, this study was limited to 31 bathouses and results should be considered with caution, as the small sample size did not provide many replications within designs, set up and locations. Furthermore, only one set of bat houses was used to compare temperature variations between bat houses.

Bats generally preferred the black bat houses, in the set of black and white and the 6-chamber models, the central bat house in the set of three. However, the 6-Chamber models were mounted flanked either side by other bat houses, which provided additional insulation. This suggests that insulation might have affected the preference by bats rather than the bat house design. The Rocket box design did particularly well in the United States and Canada with 62% overall occupancy (Kiser & Kiser, 2004) but was the last design erected during this study in May 2016. Therefore, occupancy of the Rocket boxes might still increase with time (Kiser & Kiser, 2004). However, one possibly important component the Rocket box and the Colony bat house are missing, compared to the other designs erected, is a landing pad.

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Yellow-bellied house bats were by far the most recorded (42 times compared to nine observations of small plain-faced bats). This species is naturally tree cavity roosting but well known to utilize anthropogenic structures particularly rooftops (Monadjem et al., 2010b). The distance to water, the altitude above sea level and the height of bat houses did not significantly influence bat house occupancy in this study. While water availability is known to influence bat activity (Crisol-Martínez et al., 2016; Grindal et al., 1999; Rainho & Palmeirim, 2011), the bat houses in this study were erected within 2 to 680 meters from the closest water source, all within the known home range of even small plain-faced bats (Monadjem et al., 2010a). Therefore, the distance to water might become more significant in regions that are more arid than this subtropical study area. There might also be a difference in this response during dry season, which I suggest should be analysed once a large data set becomes available. The altitude above sea level did range from 607 to 932 meters in this study and did not provide a great climatic variation.

While a number of other animals were observed to occupy bat houses during this study, the present bat house design did not seem to attract birds such as reported by Dodds and Bilston (2013). Interestingly, while there are contrasting observations on whether wasps displace bats from bat houses (Rueegger, 2016), co-habitation with wasps was not observed but instead co-habitation of bats with active beehives. However, tree squirrel and/or dormice might be able to displace bats. Only one bat house was found occupied by bats while a small mammal nest was present, however, in this case the nest seemed abandoned for some time.

The collection of bat faecal pellets also suggests that the occupancy of bat houses is much higher than what was observed by recording the presence of individual bats during monthly day visits, as faecal pellets were often collected underneath unoccupied bat houses. While alternating between different roosts is well known especially for pregnant bats (Kerth et al., 2001) as well as fission and fusion behaviour (Kerth & König, 1999) this study focused on the use of bat houses as a day roost and different occupancy numbers might be observed when conducting nightly visits.

All of the bat houses in this study are freestanding with no direct cover by trees or houses to either side. While it was not possible to analyse the influence of the cardinal direction respectively sun exposure on bat house occupancy, future studies should consider this variable, particularly if bat houses are mounted onto the walls of houses and receive shadow

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from at the back. It would also be ideal to additionally test bat houses mounted back-to-back to provide additional insulation as proposed by Kiser and Kiser (2004). Generally, future studies should aim at a higher sample size with more replication of designs, variation in bat house locations and comparing temperature between several sets of bat houses in different locations.

It should also be noted that dead bats were found in or under bat houses on three occasions. Although I can currently not make an informed statement regarding the cause of these deaths, I would like to advice caution when it comes to placing bat houses within orchards, which are frequently sprayed with pesticides. I recommend to rather erect bat houses at the edges of orchards, in some distance to the crops which will be sprayed.

Consumption of macadamia-pests by insectivorous bat species

All families of bats of which faecal pellets were analysed for this study (Molossidae, Nycteridae, Rhinolophidae and Vespertilionidae) foraged on one or more of the four pest insect species. Looking at the foraging ecology of the different species according to Monadjem et al. (2010a), these results provide some interesting new insights (Table A6). All species and families except *Myotis bocagii* and *Rhinolophus simulator* foraged on both the Lepidopteran and Hemipteran pest species, which might be a result of a small samples size of pellets collected for those two species. Especially, the Vespertilionidae species seem to be very generalist predators. Hemiptera species are only listed as one of the major families of prey insects included in the diet of two out of the small Vespertilionidae recorded in the study area and as a possible prey of *Scotophilus dinganii* but not at all for *Neoromicia nana* (Monadjem et al., 2010a). However, a high prevalence of Hemiptera pest species was found in the faecal samples of *Neoromicia nana, Scotophilus dinganii* and the small Vespertilionidae (Table 4). Likewise, Lepidoptera species are not known to be a major prey item in the diet of *Scotophilus dinganii* but were quite prevalent in the analyses of *S. dinganii* faecal pellets. Generally, these results suggest that all the insectivorous species and families recorded in the study area are much more generalist and presumably opportunistic feeders than previously assumed with more than half of the samples analysed (55.6%) containing sequences of at least one of the four pest insects.

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In conclusion, this study supports the findings of other studies (Crisol-Martinez et al., 2016; Fuentes-Montemayor et al., 2011; Park, 2015) in that natural and semi-natural vegetation promote bat activity and potentially biological control of major crop pests in agricultural landscapes. The study highlights that the conservation of bat species and the promotion of their ecosystem services requires farmers to keep natural and semi-natural vegetation patches intact and that bat activity might be improved by adding artificial roosting opportunities (bat houses) to orchards. Both foraging guilds rely on natural or semi-natural vegetation and various roosting opportunities are important especially in the breeding season. The study also suggests that warm and well insulated bat houses mounted freestanding on poles and in sets work best in northern South Africa. There is a vast scope to experiment with different colors and position of bat houses in order to promote bat activity through the provision of roosting opportunities in macadamia orchards. All of the species or families of bats from which faecal pellets were collected have been confirmed to forage on at least one of the four pest insects and the bat species have shown to be much more generalist and presumably opportunistic feeders than previously assumed. Thus, this study provides incentive and advice to farmers for a more integrated pest management approach (IPM).

Practical recommendations and research outlook

The molecular results of this study should incentivize farmers to include bats in an integrated pest management approach (IPM). Additionally, further insight could be gained by extending molecular approaches to other agroecosystems, by applying a combination of methods such as microscopic and multiplex fragment analyses (providing consumption levels and prevalence of pest species) and by comparing pest predation in extensive and lower intensity or organic systems (Park, 2015). Furthermore, molecular approaches such as fragment analyses of bat faecal pellets could be used for the detection and monitoring of dispersal of new pest insect species (Russo et al., 2018).

This study also showed that bat houses on and around macadamia orchards are colonized by bats relatively quickly and that occupancy is highest in times of high prey availability, including pest insect species. Deriving from this study and the reviewed literature, bat houses seem to work best in sets or adjacent to other bat houses or roosting opportunities (Rueegger, 2016). As found in studies from other regions, warm and well-insulated bat

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houses seem to benefit occupancy rates in northern South Africa. There is still a vast scope for testing bat houses for successful colonisation, notably on the African continent, and for experimentation with different colors, designs and positions of artificial roosts. Other important factors, that need to be investigated in future bat house studies are the distance to water, especially in more arid region, as well as altitudinal variations respectively temperature gradients. The vicinity of natural vegetation might be another important factor influencing bat house occupancy (Kiser & Kiser, 2004), however, this requires a larger variation in distances between bat houses and natural vegetation than provided in our study. Similarly, monitoring should start before the erection of bat houses and include the monitoring of possible changes in local species composition and turnover between rare and common species. Although many Molossidae species are also known to use anthropogenic structures for roosting, the Vespertilionidae *S. dinganii* was by far the most recorded species occupying bat houses in this study (Monadjem et al., 2010b). Monitoring of bat houses with cameras such as in the study of Kerth et al. (2001) might give further insight into displacement behaviour within different bat species or between bats and other animal species. This study recommends against placing bat houses in the close vicinity of crops that are frequently treated with pesticides and it should be an implicit goal of future studies to investigate the physiological effect of agricultural pesticides on bats. Unfortunately, accessibility and safety issues did not allow for radio tracking of bats in this study, which would have provided further insight into habitat use, foraging and roosting behaviour of insectivorous bat species in and around macadamia orchards. However, with ongoing technical advances in producing lighter and more cost effective GPS tracking collars and/or the use of conservation detection dogs, future studies might be able to fill persisting knowledge gaps, particularly for African bat species, and further the conservation of bats in intensive agricultural systems (Monadjem et al., 2009; Park, 2015; Taylor, 2000).

The study also showed that on a temporal and spatial scale natural and semi-natural vegetation are important habitats promoting bat activity and potentially biological control of major crop pests in macadamia orchards. Apart from conserving natural vegetation it is, therefore, also recommended to maintain fallow periods by practicing extensive and rotational agriculture in the vicinity of orchards for example in cattle farming (Starik, 2016). Additional to maintaining and restoring natural and semi-natural (fallow) vegetation and providing roosting opportunities in and around macadamia orchards, adding water sources

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such as ponds and dams might further promote bat activity. Previous studies have suggested that bat activity is positively influenced by the presence of natural and artificial waterbodies but future studies should try to gain more insight into the effect of both types of waterbodies on bat activity in agroecosystems (Park, 2015; Sirami et al., 2013; Stahlschmidt et al., 2012). It is also essential to monitor the effects of implementing new agroecosystem management approaches and extend management beyond farm boundaries. As the study of Fuentes-Montemayor et al. (2011) showed, the implementation of 'Agri-environment schemes (AES)' on farms in Scotland did not improve bat activity; however, the presence of periphery woodlands had a positive influence on bat activity.

The majority of research is currently focusing on common insectivorous bat species, assumingly providing ecosystem services such as pest control for the most part. However, as suggested by Russo et al. (2018) rare species such as 'gleaners' might also have a key role in suppressing certain pest insect species and will be effected considerably more by ongoing land-use change and consequently competition and, possibly, displacement by common species.

Similarly, research is focusing on the consumption of pest species and ecosystem service provisions in order to provide economic incentive for a more IPM approach. However, it is suggested equally important for the conservation of insectivorous bat species to gain insight into foraging habitats that promote non-pest species during off-peaks in pest insects and foraging habitats that are important for prey insects, for example during larval stages (Arrizabalaga-Escudero et al., 2015). Another important knowledge gap, which needs to be closed to sustain bat populations and their long-term ecosystem services concerns migratory bat species and migration routes in light of fatalities mostly through wind farms (Boyles et al., 2011; Dechmann et al., 2017; Popa-Lisseanu & Voigt, 2009; Zeller et al., 2009).

Finally, studies gaining insight into the ecosystem service provision by and effective approaches for the conservation of nectarivorous and frugivorous bat species in agroecosystems should not be neglected (Ghanem & Voigt, 2012; Kunz et al., 2011).

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Appendices

Figure Appendix A1. Picture of the example from Fritz Weber Entomologiebedarf' (http://www.fritz-weber-entomologiebedarf.de/) used to build the light traps to catch arthropods for this study, Levubu, Limpopo, South Africa

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Table Appendix A2. Numbers of Hemipteran and Lepidopteran caught in light traps for this study, Levubu, Limpopo, South Africa.

Date	Month	Farm	Arthropods (total)	Hemiptera	Lepidoptera
03/11/2015	November	F	43	3	27
04/11/2015	November	A	103	4	64
05/11/2015	November	J	71	0	40
06/11/2015	November	D	215	16	96
07/11/2015	November	B	202	25	110
08/12/2015	December	B	214	26	81
11/12/2015	December	D	671	157	40
16/12/2015	December	J	351	104	68
02/01/2016	January	D	234	70	36
07/01/2016	January	B	172	27	108
08/01/2016	January	A	120	43	33
09/01/2016	January	F	224	56	122
31/01/2016	January	D	197	22	61
01/02/2016	February	B	182	31	68
02/02/2016	February	F	136	5	50
05/02/2016	February	A	96	27	36
07/03/2016	March	B	202	5	49
08/03/2016	March	J	102	11	10
13/03/2016	March	F	193	50	24
28/03/2016	March	A	328	41	96
07/04/2016	April	B	78	0	33
09/04/2016	April	D	156	2	65
10/04/2016	April	F	52	0	60
16/04/2016	April	A	101	2	28
04/05/2016	May	J	83	0	35
06/05/2016	May	B	90	1	46

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08/05/2016	May	F	113	1	75
10/05/2016	May	A	65	0	47
01/06/2016	June	F	57	9	22
02/06/2016	June	B	153	2	48
03/06/2016	June	D	45	1	14
05/06/2016	June	J	80	1	9
10/06/2016	June	A	97	6	30
06/07/2016	July	D	45	5	11
07/07/2016	July	B	41	2	16
12/07/2016	July	J	8	0	4
23/08/2016	August	F	171	0	112
24/08/2016	August	D	83	0	44
25/08/2016	August	A	84	2	64
26/08/2016	August	J	45	1	30

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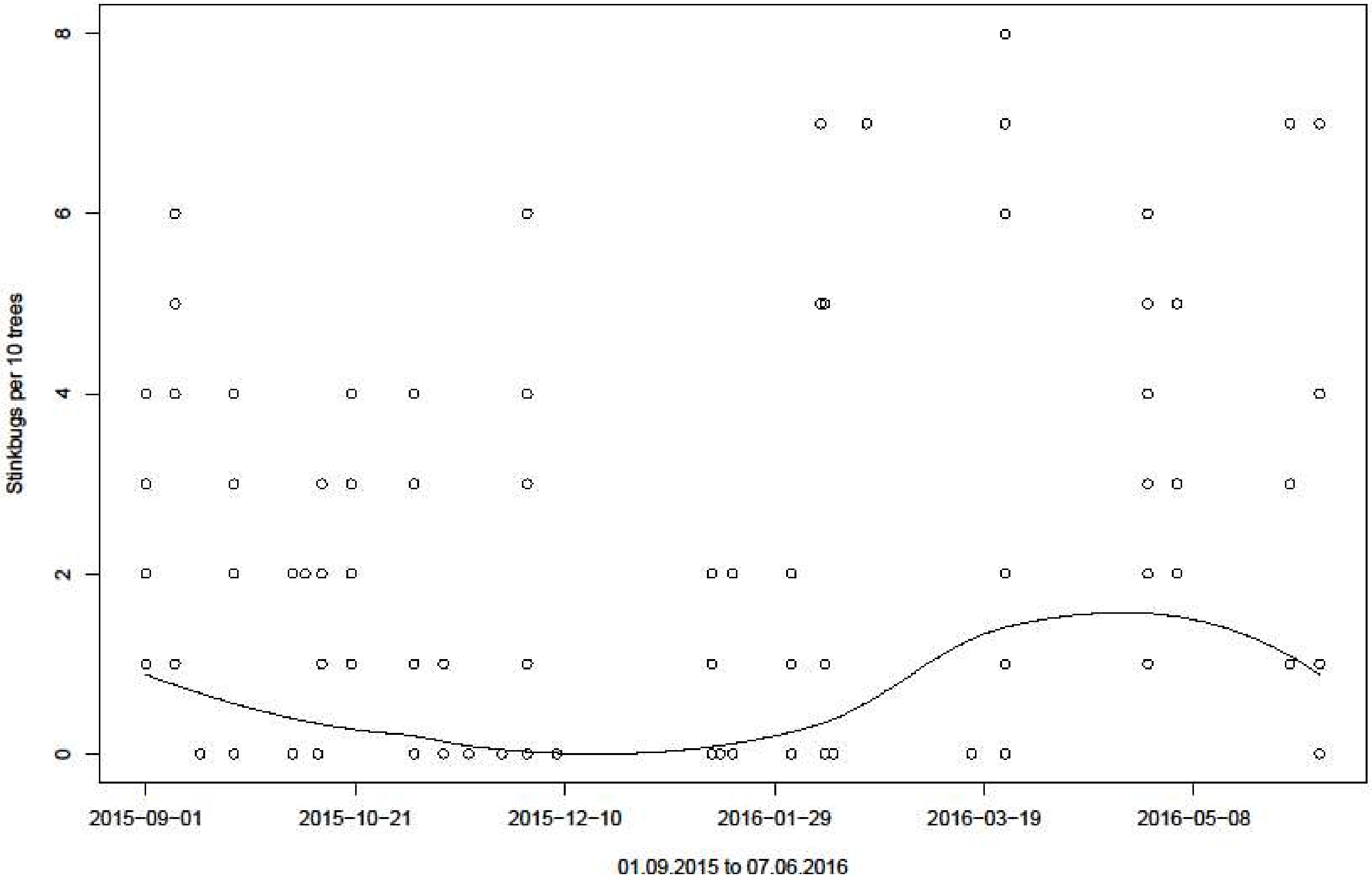
Figures Appendix A3. Showing the stinkbug scouting data per 10 trees for two of the orchards and the moth scouting data for three of the orchards, collected in different blocks, Levubu, Limpopo, South Africa.



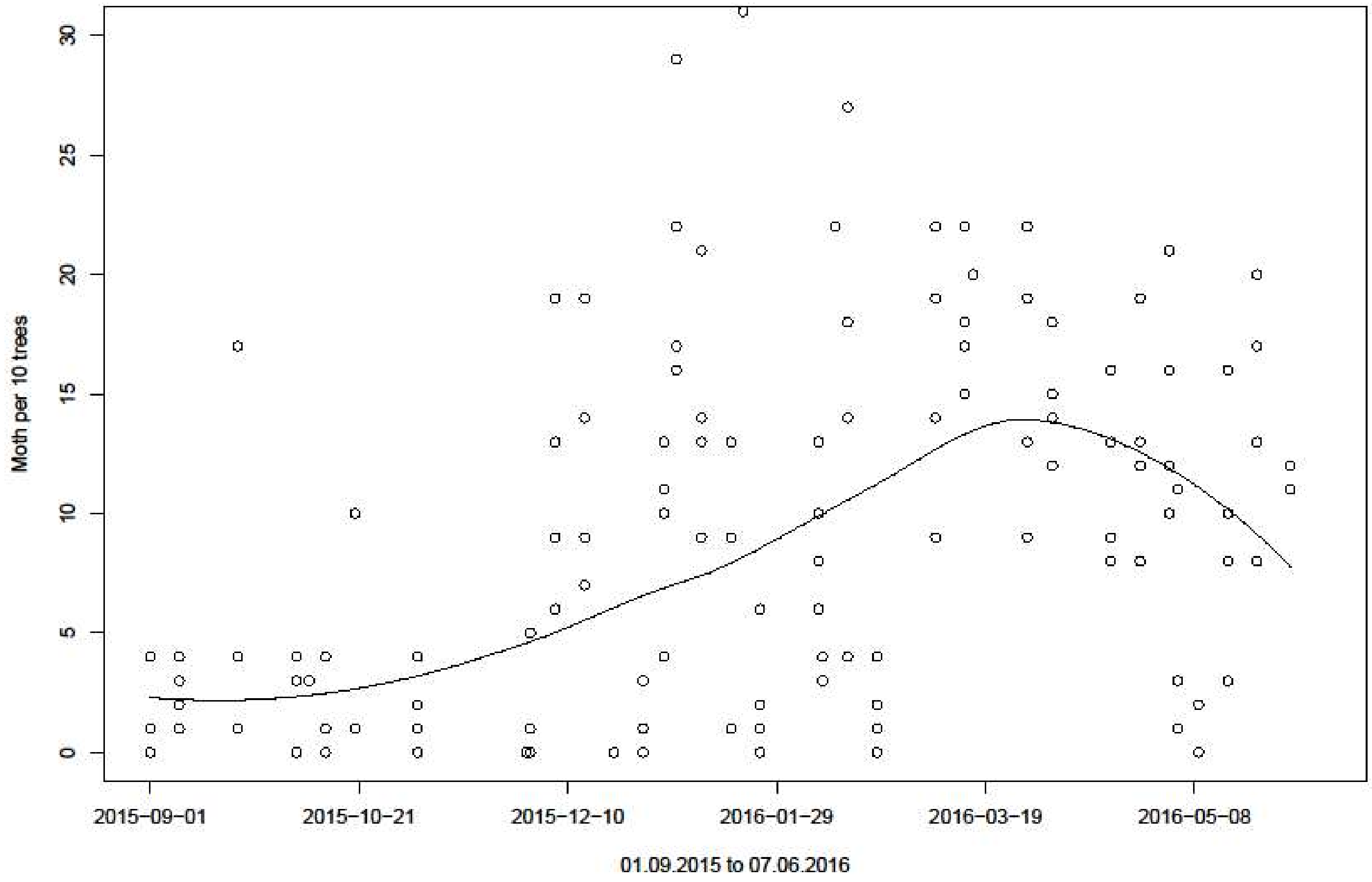

Moth per 10 trees
0
5
10
15
20
25
30
2015-09-01
2015-10-21
2015-12-10
2016-01-29
2016-03-19
2016-05-08
01.09.2015 to 07.06.2016

Table Appendix A4. Species list from live captures (C), bat houses (BH) and analyses of 11 nights of recordings (R) from Levubu, Limpopo, South Africa. Listing all species with their common name, red list status (LC=Least concern; NT=Near threatened), foraging groups, roost and breeding habits according to Monadjem et al. (2010).

Species	**Common name**	**C**	**BH**	**R**	**Red list status**	**Foraging group**	**Roosting in**	**Lactation/Time of giving birth**
Chaerephon ansorgei	Ansorge's Wrinkle-lipped bat		*	Y	LC	Open-air	Rocks/cliffs and **houses/roofs**	Lactating Nov-Jan and April
Chaerephon pumilus	Little Free-tailed bat	Y	*	Y	LC	Open-air	Rocks, trees and **houses/roofs**	Giving birth early Nov, late Jan and April
Mops condylurus	Angolan Mops bat		*	Y	LC	Open-air	Rocks, caves, trees, **houses** and bridges	Giving birth between early Sept and early May
Mops midas	Midas Mops bat		*	Y	LC	Open-air	Rocks/cliffs and **houses/roofs**	Unclear but suggested birth season Dec-March
Myotis bocagii	Bocage's Mouse-eared bat	Y		*	LC	Open-air	Lacking information- only everrecorded in banana leaves	Unknown
Neoromicia capensis	Cape serotine		*	Y	LC	Open-air	Trees and **houses/roofs**	Giving birth late Oct and Nov
Neoromicia nana	Banana bat	Y	*	Y	LC	Open-air	Leaves and **roofs**	Giving birth Nov and early Dec
Neoromicia zuluensis	Zulu Pipistrelle bat	*	*	*	LC	Open-air	Lacking information	Giving birth end of Nov-early Dec
Otomops martiensseni	Large-eared Free-tailed bat			Y	NT	Open-air	Caves and **houses/roofs**	Juveniles have been recorded Oct-May

Pipistrellus hesperidus	African pipistrelle	Y	*	Y	LC	Clutter edge	Lacking information, found in rocks and dead trees	Pregnant female collected in Oct
Pipistrellus rusticus	Rusty Pipistrelle bat	*	*	*	LC	Clutter edge	Trees	Giving birth in Nov
Rhinolophus simulator	Bushveld Horseshoe bat	Y		Y	LC	Clutter	Caves and tunnels	Giving birth around mid-Nov
Scotophilus dinganii	African Yellow House bat	Y	Y	Y	LC	Clutter edge	Trees and **roofs**	Giving birth between Nov and Dec
Tadarida aegyptiaca	Egyptian Free-tailed bat		Y	*	LC	Open-air	Caves, rocks and trees	Young are born Nov or Dec
Taphozous mauritianus	Mauritian Tomb bat		Y	*	LC	Open-air	Rocks, trees and **walls of houses**	Giving birth Oct-Dec and Feb-March

**Possible but could not be confirmed with certainty*

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Table Appendix A5 Measurements (cm) of the bat houses in the study area Levubu, Limpopo, South Africa.

Bat house	4-Chamber	Old George	6-Chamber	Rocket Box*	White&Black	Colony house
	Set of three				Set of two	
Number of sets/houses	7			5	2	1
Height (cm)	60	60	60	60	61	60
Length (cm)	50	50	28	20	43	90
Width (cm)	18	18	30	20	20	30
Inner chamber(s) (cm)	2	3	2	2	4.5	3
Outer chamber (cm)	3	*Slanted*	4	4.5	-	-
Landing pad						
Length (cm)	50	50	28	NA	43	NA
Height (cm)	20	20	15	NA	15	NA
Date erected/maintained	End of 2014/May 2016			May 2016	March 2016	March 2016

Table Appendix A6 Batspecies recorded during the study of bat houses on macadamia orchards, Levubu, Limpopo, South Africa.

Farm	Month	Typ	Bat house	Location	Bats	Species	Cardinal	Altitude	Water	Height
JR	January	Bank	Nursery	Banana	1	Free-tailed bats	NE	793	67	4
DP	January	Set	Black	Pond	1	Mauritian tomb bat	SW	754	2	3.5
AW	January	Set	White	Hill	1	small Plain-faced bat	N	932	680	5
Amana	January	Bank	6-Chamber	Site 2	3	Yellow-bellied house bats	SW	646	85	5
AS	January	Bank	6-Chamber	Dam	4	Yellow-bellied house bats	NE	805	3	2.3
AW	January	Set	Black	Hill	3	Yellow-bellied house bats	N	932	680	5
JR	February	Bank	Nursery	Banana	1	Free-tailed bats	NE	793	67	4
HJ	February	Bank	6-Chamber	Old Shed	5	Yellow-bellied house bats	SW	634	412	4
AS	February	Bank	6-Chamber	Dam	4	Yellow-bellied house bats	NE	805	3	2.3
DP	February	Set	Black	Pond	4	Yellow-bellied house bats	SW	754	2	3.5
AW	March	Set	White	Hill	1	Free-tailed bats	N	932	680	5
Amana	March	Bank	Old George	Site 2	1	Yellow-bellied house bats	SW	646	85	5
HJ	March	Bank	Nursery	Old Shed	2	Yellow-bellied house bats	SW	634	412	4
AS	March	Bank	6-Chamber	Dam	3	Yellow-bellied house bats	NE	805	3	2.3
AS	March	Bank	Nursery	Dam	1	Yellow-bellied house bats	NE	805	3	2.3
DP	March	Set	Black	Pond	4	Yellow-bellied house bats	SW	754	2	3.5
AW	March	Set	Black	Hill	4	Yellow-bellied house bats	N	932	680	5
JR	April	Bank	Nursery	Banana	2	Free-tailed bats	NE	793	67	4
Amana	April	Rocket Box	Rocket box	Dam	2	Yellow-bellied house bats	All	646	155	5
Amana	April	Bank	6-Chamber	Site 2	4	Yellow-bellied house bats	SW	646	85	5
HJ	April	Rocket Box	Rocket box	Site 1	1	Yellow-bellied house bats	All	607	165	4
HJ	April	Bank	Nursery	Old Shed	2	Yellow-bellied house bats	SW	634	412	4
DP	April	Set	White	Pond	1	Yellow-bellied house bats	SW	754	2	3.5
JR	May	Bank	Nursery	Banana	1	Free-tailed bats	NE	793	67	4
HJ	May	Bank	Nursery	Old Shed	1	Free-tailed bats	SW	634	412	4

Farm	Month	Typ	Bat house	Location	Bats	Species	Cardinal	Altitude	Water	Height
AW	May	Set	Black	Hill	1	Free-tailed bats	N	932	680	5
JR	May	Bank	6-Chamber	Dam	1	small Plain-faced bat	W	772	37	4
JR	May	Bank	6-Chamber	Banana	1	small Plain-faced bat	NE	793	67	4
Amana	May	Rocket Box	Rocket box	Dam	3	Yellow-bellied house bats	All	646	155	5
Amana	May	Bank	6-Chamber	Site 2	3	Yellow-bellied house bats	SW	646	85	5
HJ	May	Rocket Box	Rocket box	Site 1	2	Yellow-bellied house bats	All	607	165	4
AS	May	Bank	6-Chamber	Dam	3	Yellow-bellied house bats	NE	805	3	2.3
AW	June	Set	Black	Hill	1	Free-tailed bats	N	932	680	5
JR	June	Bank	6-Chamber	Banana	2	Free-tailed bats	NE	793	67	4
Amana	June	Bank	Nursery	Site 2	1	Free-tailed bats	SW	646	85	5
AW	June	Set	White	Hill	1	Free-tailed bats	N	932	680	5
JR	June	Bank	Nursery	Dam	1	small Plain-faced bat	W	772	37	4
DP	June	Set	Black	Pond	3	small Plain-faced bat	SW	754	2	3.5
Amana	June	Bank	Nursery	Dam	3	Yellow-bellied house bats	NE	646	46	5
Amana	June	Bank	6-Chamber	Site 2	5	Yellow-bellied house bats	SW	646	85	5
AS	June	Bank	6-Chamber	Dam	1	Yellow-bellied house bats	NE	805	3	2.3
Amana	June	Bank	6-Chamber	Site 2	4	Yellow-bellied house bats	SW	646	85	5
AS	June	Bank	6-Chamber	Dam	3	Yellow-bellied house bats	NE	805	3	2.3
JR	July	Bank	6-Chamber	Banana	2	Free-tailed bats	NE	793	67	4
DP	July	Set	Black	Pond	1	Free-tailed bats	SW	754	2	3.5
AW	July	Set	Black	Hill	1	Free-tailed bats	N	932	680	5
Amana	July	Bank	6-Chamber	Site 2	1	small Plain-faced bat	SW	646	85	5
Amana	July	Bank	6-Chamber	Dam	4	Yellow-bellied house bats	NE	646	46	5
Amana	July	Bank	Old George	Site 2	3	Yellow-bellied house bats	SW	646	85	5
AS	July	Bank	6-Chamber	Dam	2	Yellow-bellied house bats	NE	805	3	2.3
Amana	July	Bank	6-Chamber	Site 2	3	Yellow-bellied house bats	SW	646	85	5
AS	July	Bank	6-Chamber	Dam	3	Yellow-bellied house bats	NE	805	3	2.3

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Farm	Month	Typ	Bat house	Location	Bats	Species	Cardinal	Altitude	Water	Height
JR	August	Bank	6-Chamber	Banana	1	Free-tailed bats	NE	793	67	4
Amana	August	Bank	6-Chamber	Dam	1	Free-tailed bats	NE	646	46	5
AW	August	Set	Black	Hill	1	Free-tailed bats	N	932	680	5
Amana	August	Bank	Old George	Site 2	2	small Plain-faced bat	SW	646	85	5
HJ	August	Bank	Old George	Site 1	3	Yellow-bellied house bats	S	607	160	4
AS	August	Bank	6-Chamber	Dam	1	Yellow-bellied house bats	NE	805	3	2.3
AW	September	Set	Black	Hill	2	Free-tailed bats/ small Plain-faced bat	N	932	680	5
Amana	September	Bank	6-Chamber	Dam	4	Yellow-bellied house bats	NE	646	46	5
HJ	September	Bank	Nursery	Site 1	4	Yellow-bellied house bats	S	607	160	4
JR	October	Bank	Nursery	Banana	2	small Plain-faced bat	NE	793	67	4
JR	October	Bank	Old George	Dam	1	Yellow-bellied house bats	W	772	37	4
Amana	October	Bank	Old George	Dam	1	Yellow-bellied house bats	NE	646	46	5
Amana	October	Bank	6-Chamber	Site 2	3	Yellow-bellied house bats	SW	646	85	5
HJ	October	Bank	Old George	Site 1	3	Yellow-bellied house bats	S	607	160	4
JR	November	Bank	6-Chamber	Banana	3	Free-tailed bats	NE	793	67	4
Amana	November	Bank	6-Chamber	Site 2	2	Yellow-bellied house bats	SW	646	85	5
HJ	November	Bank	Old George	Site 1	4	Yellow-bellied house bats	S	607	160	4
JR	December	Bank	Nursery	Banana	2	Free-tailed bats	NE	793	67	4
AW	December	Set	Black	Hill	1	Free-tailed bats	N	932	680	5
HJ	December	Bank	Old George	Site 1	4	Yellow-bellied house bats/ Mauritian tomb bat	S	607	160	4
AS	December	Bank	6-Chamber	Dam	5	Yellow-bellied house bats/ Mauritian tomb bat	NE	805	3	2.3

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Figure Appendix A7 Showing the results of Capillary Electrophoresis after the multiplex PCR**.** Each multiplex contained all four primer sets, a negative sample (Neg) and a sample of each of the four insect species *Bathycoelia distincta* (B), *Nezara viridula* (N), *Thaumatotibia batrachopa* (MNB) and *Cryptophlebia peltastica* (FCM/CP).

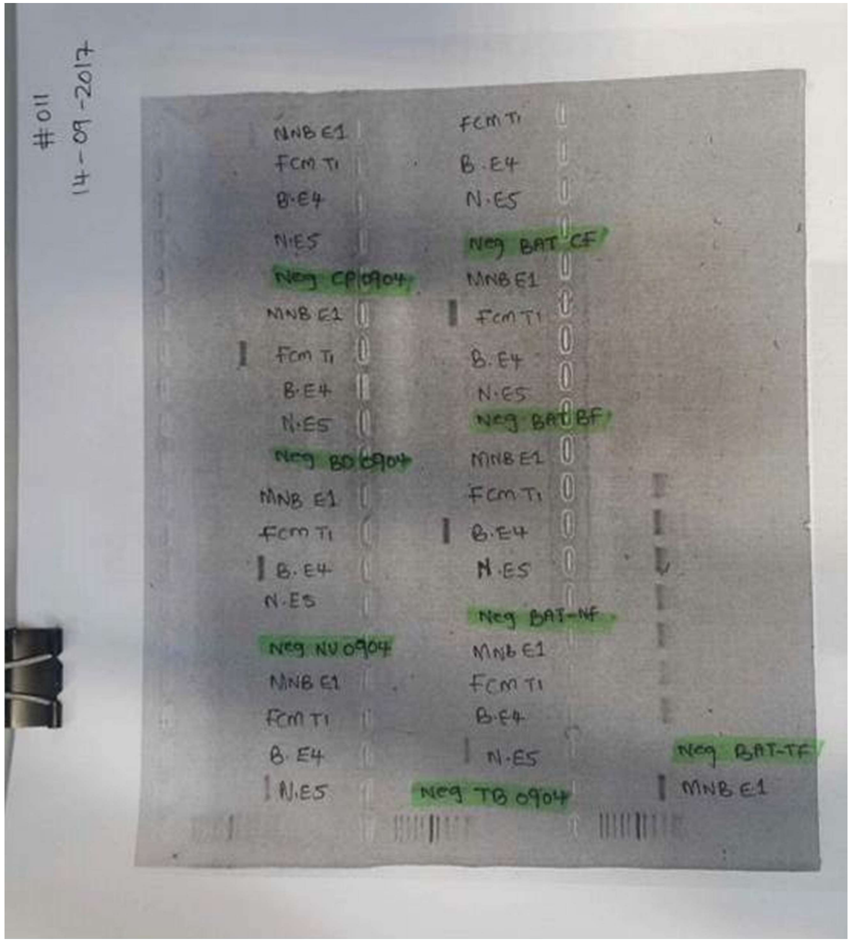

Table Appendix A8 Species which were (Y) or could have been included in species groups (U- Molossidae or small Vespertilionidae) used for the diet analyses collecting faecal pellets from captured bats (C) or trays installed at roosts (R), including species analyses of 11 nights of bat detector recordings (BD) from Levubu, Limpopo, South Africa. Listing all species with their common name, red list status (LC=Least concern; NT=Near threatened), positive detection of one of the two Hemiptera or Lepidoptera pest species through molecular diet analyses (x), foraging groups and main foraging ecology according to Monadjem et al. (2010).

Species	Common name	C	R	BD	Red list status	Hemiptera pest in diet	Lepidoptera pest in diet	Foraging group	Main foraging ecology according to Monadjem et al. 2010
	Molossidae					**x**	**x**		
Chaerephon ansorgei	Ansorge's Wrinkle-lipped bat		U	Y	LC			Open-air	Coleoptera, Trichoptera, **Lepidoptera**
Chaerephon pumilus	Little Free-tailed bat	Y	U	Y	LC			Open-air	Coleoptera, **Hemiptera, Lepidoptera**, Hymenoptera, Diptera
Mops condylurus	Angolan Mops bat		U	Y	LC			Open-air	Coleoptera, **Hemiptera, Lepidoptera**
Mops midas	Midas Mops bat			Y	LC			Open-air	Coleoptera
Otomops martiensseni	Large-eared Free-tailed bat			Y	NT			Open-air	**Lepidoptera**
Tadarida aegyptiaca	Egyptian Free-tailed bat		U	U	LC			Open-air	Coleoptera, **Hemiptera**, Diptera **minorly Lepidoptera**
	Vespertilionidae								
Myotis bocagii	Bocage's Mouse-eared bat	Y		U	LC	**x**		Open-air	Coleoptera, **Hemiptera, Lepidoptera**, Diptera

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Neoromicia nana	Banana bat	Y	U	Y	LC	**x**	**x**	Open-air	Small Coleoptera and **Lepidoptera** may include Diptera
Neoromicia capensis	Cape serotine		U	Y	LC	**x**	**x**	Open-air	Coleoptera, **Hemiptera, Lepidoptera**, Neuroptera, Diptera
Neoromicia zuluensis	Zulu Pipistrelle bat	U	U	U	LC			Open-air	Coleoptera **minorly Lepidoptera**
Pipistrellus hesperidus	African pipistrelle	Y	U	Y	LC			Clutter edge	Coleoptera, **Hemiptera**, **Lepidoptera**, Diptera
Pipistrellus rusticus	Rusty Pipistrelle bat	U	U	U	LC			Clutter edge	Coleoptera, Diptera, **Lepidoptera**
Scotophilus dinganii	African Yellow House bat	Y	Y	Y	LC	**x**	**x**	Clutter edge	Coleoptera **may include Hemiptera**, Hymenoptera, Isoptera, Diptera
	Rhinolophidae								
Rhinolophus simulator	Bushveld Horseshoe bat	Y		Y	LC	**x**		Clutter	**Lepidoptera**, minorly Coleoptera, **Hemiptera**, Isoptera, Orthoptera
	Nycteridae								
Nycteris thebaica	Egyptian slit-faced bat		Y		LC	**x**	**x**	Clutter	Orthoptera, Coleoptera, **Lepidoptera** and many others