Bats as potential biocontrol agents in an agricultural landscape, Levubu Valley: Diet, activity and species composition of bats in macadamia orchards and neighbouring natural habitats

PJ Taylor[1], D Mkhari[1], T Mukwevho[1], A Monadjem[2], MC Schoeman[3], C Schoeman[4] and JN Steyn[1]

[1]Department of Ecology and Resource Management, School of Environmental Sciences, University of Venda
Private Bag X5050, Thohoyandou 0950, South Africa
E-mail: Peter.Taylor@univen.ac.za

[2]Department of Biological Sciences, University of Swaziland
Private Bag 4, Kwaluseni, Swaziland
E-mail: ara@uniswa.sz

[3]School of Biological and Conservation Sciences
University of KwaZulu-Natal
E-mail: schoemanc@ukzn.ac.za

[4]Department of Zoology, School of Natural and Mathematical Sciences, University of Venda
Private Bag X5050, Thohoyandou 0950, South Africa
E-mail: Colin.Schoeman@univen.ac.za

ABSTRACT

Using an ANABAT detector, standardised acoustic transects (20 random points with five minutes recording per point per night) were conducted on five nights during May, October and November 2010 in a macadamia orchard and neighbouring riverine bush on a farm (Welgevonden) in the Levubu Valley, Limpopo Province. Additional continuous recordings were made on two nights in the vicinity of a household in the middle of the macadamia orchard. Based on a total of 165 ANABAT call files, twelve species of bats were recorded of which five common species were recorded across all habitats. Bats were recorded in both macadamia orchards and riverine bush; whilst activity was significantly higher in the riverine bush than in the macadamia orchard, species richness and composition did not vary between habitats. Faecal pellets and culled prey remains were collected from two night roosts of the slit-faced bat (*Nycteris thebaica*) on a neighbouring macadamia farm (Laatsgevonden) during four months (July, August, October and November) in 2010. The dietary niche of this species was broad (as shown by other studies) and varied significantly as indicated by the method used (faecal pellets or culled parts) and month of collection. Although stinkbugs of the Family Pentatomidae (major pests of macadamia) could not be identified specifically from culled parts or faecal pellets using microscopic examination of arthropod fragments, faecal pellets (but not culled parts) revealed an important component (20%) of bugs (Hemiptera; which may have included Pentatomidae), much higher than recorded in previous studies conducted in natural habitats. Future work will focus on a DNA bar-coding approach to probe the diet of this and other species of bats in a more detailed taxonomic context to investigate the extent to which bats feed on particular agricultural pests in a subtropical fruit-growing area.

UITTREKSEL

’n ANABAT-toestel is vir vyf nagte gedurende Mei, Oktober en November 2010 op die plaas Welgevonden in die Levubuvallei, Limpopo Provinsie gebruik om die teenwoordigheid van vlermuise in makadamiaboorde en in die aangrensende rivier-oewerbos te bepaal. Die opnames wat elk vyf minute lank geduur het, is in 20 ewekansig gekose punte in gestandaardiseerde transekte gemaak. Verdere opnames is daarna vir twee nagte in die omgewing van die plaasopstal gemaak wat in die boord geleë is. In totaal is 165 opnames gemaak.

Uit dié totaal is twaalf vlermuisspesies geïdentifiseer waarvan vyf algemene spesies is wat in al die habitatte teenwoordig was. Ten spyte van die feit dat die vlermuis-aktiwiteit betekenisvol hoër was in die rivier-oewerbos as in die boorde, was daar geen verskil tussen die spesieverskeidenheid en samestelling van die verskillende habitatte nie. Mismonsters en prooi-oorblyfsels van die *Nycteris thebiaca* (slit-faced bat) is gedurende die maande Julie, Augustus, Oktober en November 2010 by twee vlermuis-oornagpunte op die aangrensende makadamiaplaas (Laatsgevonden) versamel. Die metodes (misontleding en prooi-oorblyfsels) wat gebruik is om die voedingsnis van dié spesie te bepaal, asook die maand waarin die gegewens versamel is, het aangetoon dat hul voedingsnis wyd strek en betekenisvol wissel. Dié gevolgtrekking word gerugsteun deur ander studieprojekte. Hoewel mikroskopiese ontledings van die prooi-oorblyfsels en mis nie die aanwesigheid van stinkbesies van die Pentatomidae-familie (die belangrikste pes van makadamias) aangetoon het nie, het die mis (maar nie die prooi-oorblyfsels nie) wel 'n belangrike komponent (20%) skildbesies van die orde Hemiptera (waaronder die familie Pentatomidae sorteer) bevat. Hierdie statistiek bevat syfers wat beduidend hoër is as vorige soortgelyke ondersoeke wat in natuurlike habitatte gedoen is.

Toekomstige studies sal op 'n DNS-koderingsbenadering fokus om die dieet van hierdie en ander vlermuisspesies in 'n meer breedvoerige konteks te bestudeer om op dié wyse die omvang van jag deur vlermuise op landboupeste te bepaal.

INTRODUCTION

Bats are major predators of night-flying insects, including crop pests (Williams-Guillén *et al*., 2008; *Kalko et al*., 2008; Jones *et al*., 2009; www.batcon.org). Insect pests cause untold damage to agricultural production worldwide and macadamia plantations in South Africa are no exception, stinkbugs (Hemiptera: Family Pentatomidae) having been identified as the major pests (Ironside, 1973; Jones & Caprio, 1992; Jones *et al*., 1992; Jones & Caprio, 1994; Vincent *et al*., 2001). Given the large volumes of insects consumed by bats (up to 100% of their body mass, or up to 2000 mosquito-sized insects per night), bat predation of pest insects has been shown to have a significant economic benefit to farmers (Cleveland *et al*., 2006; Boyles *et al*., 2011; Kunz *et al*., 2011). However, few studies have quantified this benefit (but see Boyles *et al*., 2011) and none in Africa. There is an urgent need to demonstrate the value of bats to agriculture in South Africa. Increasing local bat populations (*e.g*. by means of providing artificial bat roosts or bat houses) may provide an effective and less environmentally destructive means of controlling insects pests than simply increasing chemical pesticide use which may ultimately prove counterproductive if populations of beneficial top insect predators like insectivorous spider, bats and birds decline through poisoning.

Whilst much work has been done on the diet of southern African bats (Monadjem *et al*., 2010; Schoeman & Jacobs, 2011), no local studies have related this to the potential role of bats in crop protection (as has been shown in natural and agro-ecosystems in the Americas: Kalko *et al*., 2008; Williams-Guillén *et al*., 2008; Boyles *et al*., 2011). One local exception was a recent study of the diet of two species of free-tailed (Family Molossidae) bats in a sugar cane monoculture, bordering a nature reserve in Swaziland (Bohmann, 2010) which used a DNA sequencing approach to accurately identify the proportions of different insect families in the diet and to positive identify two major lepidopteran sugar cane pests, *Eldana saccharina* and *Mythimna phaea*. A parallel study (Noer, 2010) demonstrated (using bat detectors and radio-tracking) the preference of free-tailed bats to feed over sugar cane rather than an adjacent natural reserve.

The present study aimed firstly to investigate the activity patterns and community composition of bats in a macadamia orchard and adjacent riverine strip of bush over a few months in 2010 at a single farm (Welgevonden) in the subtropical fruit-growing area of the Levubu Valley in Limpopo Province. Secondly, in order to begin to establish baseline data on the diet of different bat species resident in the area, with a view to identifying possible insect pest families, genera and species, we collected faecal pellets from two known roosts of bats in the area, a day-roost of the Angolan free-tailed bat (*Mops condylurus*) and a night roost of the slit-faced bat, *Nycteris thebaica*. Additionally, discarded prey remains were also obtained from underneath the feeding stations of the latter roost. In this report we present data only on the analysis of the diet of *N. thebaica* as samples from the other species are awaiting analysis. Previous studies of the diet of slit-faced bats have analysed either faecal pellets (*e.g*. Bowie *et al*., 1999; Schoeman & Jacobs, 2011) or culled remains (*e.g*. La Val & La Val, 1980; Seamark & Bogdanowicz, 2002); our study analysed both in samples collected at the same time, to test the hypothesis that the two methods should provide different results. Samples of faecal pellets were also collected and stored in 90% ethanol for later analysis (in a separate study) using DNA sequencing methods (as described above; Bohmann, 2010). Given the foraging strategy of the slit-faced bat, which is capable of flying slowly in cluttered space and gleaning stationary insects from the ground or branches of vegetation (Bowie *et al*., 1999), we hypothesised that this species may be expected to prey on stinkbugs in macadamia orchards.

MATERIALS AND METHODS

Study sites

Figure 1 shows the location of the two study farms, Welgevonden and Laatsgevonden, in the Levubu Valley subtropical fruit-growing area in the southern foothills of the Soutpansberg Range, as well as detailed satellite photographs of the two farms showing the location of random sample points (20 in macadamia orchards and 20 in riverine bush) at Welgevonden and the location of two night roosts of the slit-faced bats (*Nycteris thebaica*) at Laatsgevonden. The two farms form part of an area under extensive commercial agriculture, including monocultures of macada-

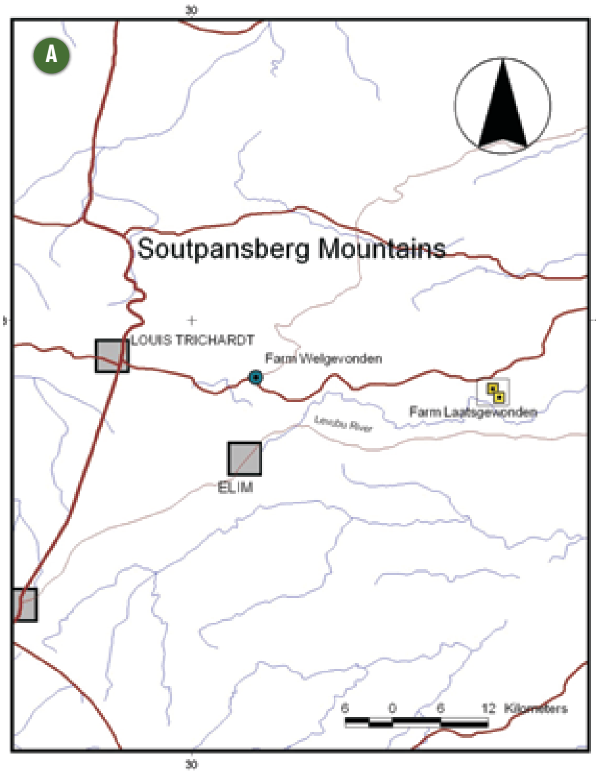

Figure 1. General location of study farms (A) and detailed SPOT 5 satellite photos of Welgevonden (B) and Laatsgevonden (C) farms showing the location of random acoustic recording points (B) and the location of two slit-faced bat (*Nycteris thebaica*) night roosts (C) used to sample culled parts of faecal pellets for dietary analysis. In (B) KM refers to points in macadamia and KR to points in riverine bush.

mia, avocados, bananas, as well as extensive areas used for commercial forestry. The night roost from which most samples were obtained for dietary analysis (lower yellow square in **Figure 1c**) is bordered to the east, west and north by macadamia orchards and to the south by semi-natural savanna vegetation.

Weather data

Hourly rainfall, temperature and humidity data were available for the periods under study through data kindly by Dr Dries Alberts, supplied from his weather station on a neighbouring farm.

Acoustic analyses

An ANABAT detector (www.titley.com.au) was used to make recordings of the ultrasonic vocalisations of bats which were stored automatically on a SD memory card and later analysed by the ANALOOK programme. The ANABAT system is widely used for bat surveys worldwide and has the advantage of being able to record huge volumes of bat call data automatically and efficiently (Gannon *et al*., 2003; O'Farrell *et al*., 1999; Milne *et al*., 2004; Monadjem *et al*., 2007). Since the ANABAT system may lose information relating to amplitude and harmonics of bat calls (Fenton *et al*., 2001), we will use a second Pettersson D240X heterodyne/time expansion bat detector (www.batsound.com) to obtain superior time-expanded recordings of each species sampled so as to verify the identity of calls obtained by ANABAT and to obtain variables (such as peak frequency) which cannot be obtained with ANABAT. However, the ANABAT system continues to be the method of choice for studies aimed at establishing habitat use of bats (Gannon *et al*., 2003; O'Farrell *et al*., 1999; Monadjem *et al*., 2007).

Using a randomised sampling protocol, active acoustic sampling with the ANABAT bat detector was undertaken during the peak feeding period of bats (90 minutes after dusk). Using GIS (ArcView 3.1) we generated 20 random sites in each of two habitats, a defined area of macadamia orchard and a neighbouring area of riverine bush on Farm Welgevonden (Figure 1). Points were placed randomly within the pre-defined area and set to be at least 30 m apart from each other and the habitat border (since 30 m was understood to represent the limit of sensitivity of the detector). GPS points for each site were recorded and used to locate sites in subsequent sessions. Each site was numbered from 1 to 20. Using a random number generator in Excel, we randomly chose the starting point for each sample session and for each habitat. Sample sessions comprised two nights. Recording commenced at dusk in either the macadamia or riverine bush habitat (the order was also randomly decided for each session). On the first day we sampled ten points in each habitat, with exactly five minutes of recording time per sample point. On the second night, we started with a different habitat and proceeded to sample the remaining ten points in each habitat.

After an initial pilot session in July 2010 when only ten sites were recorded in the macadamia orchard, recordings in both habitats were carried out in October and November 2010. Whilst two nights were sampled in November as described above, we sampled three nights in October due to incomplete sampling of the macadamia habitat on the first night (only 15 points sampled). To supplement data on species occurrences from these standardised walked transects, so as to compile a complete species list for the area, we also opportunistically recorded bats at a fixed point (a household situated in the middle of the same macadamia orchard) over several nights between March and May 2010. Although it was not possible to directly compare the activity of bats between the fixed point and transect recordings (since recordings were not made on the same nights), the species identified from calls collected from fixed-point and transect recordings were used to compare in general terms the species composition in the vicinity of the house and away from the house within the same macadamia orchard. For the purpose of this report we present data from fixed point recordings (at the household) made on two nights (24 March and 1 May 2010) when continuous recordings were made for approximately 90-100 minutes after dusk, *i.e*. approximately comparable with the total time of recording made during transects (5 minutes per point x 20 points = 100 minutes).

Identification of bat species from their recordings was based largely on the availability of reference call libraries obtained from mistnetting and recording identified individuals of each species. These reference calls (and associated summaries of means and ranges of call parameters, such as minimum and maximum frequency, frequency at the knee, duration and slope) were obtained from mistnetting and ANABAT acoustic recordings carried out at several localities in the northern Limpopo, including Lajuma Mountain Reserve and Buzzard Mountain in the Soutpansberg (recordings made by PJT) and from Lapalala in the Waterberg Biosphere Reserve (call library provided by Dr Sandie Sowler). We also referred to published ANABAT call parameters of a number of southern African species (Monadjem *et al.,* 2010). In cases where species identity was unclear, calls were labelled according to their family (in most cases calls could be unambiguously assigned to family) and the call parameter, frequency at the knee.

We calculated relative abundance (activity levels measured as number of bat passes per 10 minute interval) and species richness for each habitat and sample session and tested for significant differences in mean values obtained between macadamia and riverine habitats. Differences in species composition between the two habitats were tested by means of chi-squared tests.

Dietary analysis

Faecal pellets and discarded prey remains of *Nycteris thebaica* were collected on a monthly basis between July and December 2010 by placing one or two 1 x 2 m boards covered in "Gladwrap" under the nightly feeding stations (night roosts) of bats located in two closely spaced night roosts in sheds located on Farm Laatsgevonden and an adjacent farm. Boards were placed approximately one week prior to collection. Culled (discarded) parts of insect prey were sorted into orders and, where possible, families and the number of individual parts counted. Pellets were kept in ziplock bags in a fridge prior to analysis, but some were placed in 90% ethanol for a related study aimed at using a DNA bar-coding approach to identify prey remains (Bohmann, 2008).

A minimum of five pellets was analysed for each month as described by Whitaker (1988). Each pellet was teased apart under 70% ethanol and the arthropod exoskeleton fragments were identified to order (or family if possible) using the standard key in Whitaker (1988), drawings in Scholtz and Holm (1985) and a reference collection of arthropods (insects and spiders) trapped with malaise traps during two nights

Table 1. Summary of activity (number of ANABAT call files or "bat passes") recorded during acoustic transects conducted during six nights of 2010 at Farm Welgevonden, Levubu Valley, Limpopo Province.

Sample date	Activity (no. of passes)	
	Macadamia	Riverine
3-May	25	NA
1-Oct	4	17
2-Oct	1	12
8-Oct	3	15
20-Nov	6	3
21-Nov	2	8
Summary statistics (excluding May)		
Sum	16	55
Mean	3.2	11
SD	1.92	5.61
N	5	5

Table 2. Summary of number of bat species (species richness) recorded during acoustic transects conducted during six nights of 2010 at Farm Welgevonden, Levubu Valley, Limpopo Province.

Sample date	Species richness	
	Macadamia	Riverine
3-May	9	NA
1-Oct	3	6
2-Oct	1	6
8-Oct	4	6
20-Nov	2	4
21-Nov	1	4
Mean (Excl. May)	2.2	5

Table 3. Activity (number of ANABAT bat call files or "bat passes") of 11 species recorded by acoustic surveys conducted in March, May, October and November 2010 in a macadamia orchard (M), adjacent riverine bush (R) and the vicinity of a homeastead (H) in the middle of the macadamia orchard at Farm Welgevonden, Levubu Valley, Limpopo Province. Two vespertilionid species could not be unequivocally identified and were classified by F_{KNEE} as "vesper55" (possibly *Miniopterus natalensis*) and "vesper65". Another call which could not be assigned to family was characterised by a narrow-band, short duration call structure with F_{KNEE} of 31 kHz.

	Sampling periods													
FAMILY Species	1-Oct		2-Oct		8-Oct		20-Nov		21-Nov		3-May	March	May	TOTAL
	M	R	M	R	M	R	M	R	M	R	M	H	H	
MOLOSSIDAE														
Chaerephon pumilus	1	1		1						1	4	5	7	20
Tadarida aegyptiaca		1	1	1		3						1		7
Mops condylurus	1	1		2		2				1	8			15
VESPERTILIONIDAE														
Pipistrellus hesperidus		5		1		3	3			3	3	3	8	29
Scotophilus dingani	2	8		6	2	6	1	3	2		2	22	6	58
Neoromicia nanus							1				1		1	3
Myotis tricolor											1			1
"Vesper55"						1					2		2	3
"Vesper65"							1							1
EMBALLANURIDAE														
Taphozous mauritianus		1		1	1	1				3	3	5	8	20
RHINOLOPHIDAE														
Rhinolophus simulator												4		4
UNKNOWN														
"Narrow-31"											1			
TOTAL	4	17	1	12	3	16	6	3	2	8	25	40	32	165

in December 2010 at the nearby farm of Welgevonden. The sample from the malaise trap was also sorted into orders and families of arthropods and the number of each category counted to obtain a crude measure of the insect availability in the area during two nights in November 2010 and January 2011. We estimated the percentage by volume of the different prey orders in each pellet. Differences in the proportion of different prey orders between months were tested by chi-squared analysis. Combined proportions of different prey categories for all months were compared between the pellets, the culled parts and the insects collected in the malaise trap.

RESULTS AND DISCUSSION

Activity and species composition

Based on five nights in October and November 2010 on which paired, standardised acoustic transects were conducted in two habitats (macadamia orchard and riverine bush), we found higher activity (**Table 1**; 11 passes per night in riverine bush compared to 3.2 passes per night in macadamia) and species richness (**Table 2**; mean of 5.0 species per night in riverine bush compared to 2.2 in macadamia) in riverine bush than in macadamia habitats. Using paired t-tests, the difference in activity levels was significant ($P = 0.02$), whereas that of species richness was not ($P > 0.05$). Data from the unpaired 3 May 2010 trial transect of macadamia orchard were not included in statistical analyses; however, this winter data point represented an extreme outlier demonstrating much higher activity (25 passes during 10 five-minute recording points) and species richness (nine species) than any of the five summer samples undertaken in both habitats. Clearly this strange result requires to be tested by repeated sampling of both habitats during winter.

When pooling recordings from acoustic transects as well as two complete evenings (24 March 2010 and 1 May 2010) of recordings at the homestead, a total of 12 different species of bats were recorded on Farm Welgevonden (n = 165 call files) (**Table 3**) representing at least four families, Molossidae (three species), Vespertilionidae (six species), Emballanuridae (one species), Rhinolophidae (one species) and an unidentified call which could not be assigned to family. Although activity levels based on continuous recording at a fixed point (homestead) could not be directly compared in an unbiased way with the results of the transects, it is nevertheless clear that nightly activity levels around the house were relative high, 32-40 calls per 90 minutes (approximately) of recording, compared with 1-17 calls per 50 minutes (10 points x 5 minutes) per night for the habitat transects.

Five species were common and generally found in all habitats (macadamia, riverine and vicinity of house): the yellow house bat, *Scotophilus dinganii* (58 calls), the African pipistelle, *Pipistrellus hesperidus* (29 calls), the Mauritian tomb bat, *Taphozous mauritianus* (20 calls), the little free-tailed bat, *Chaerephon pumilus* (20 calls) and the Angolan free-tailed bat, *Mops condylurus* (15 calls). The results from the recordings at the house are somewhat biased as the roof houses a colony of *S. dinganii* leading to elevated levels of activity due to individuals emerging and returning to the roost. The relatively high number of *T. mauritianus* calls in the vicinity of the house may also be due to individuals roosting on the outside walls of the house or on the bark of nearby tall palm trees which occurred on the property (common roost sites for this species; Monadjem *et al*., 2010). A roost of *Mops condylurus* was known to occur on a neighbouring farm but no individuals were recorded at the farmhouse at Welgevonden, although occasional calls were recorded in the macadamia and riverine transects (possibly commuting individuals). When considering only data from paired acoustic transects (five nights) there was no significant association between habitat (macadamia or riverine) and species or family (chi-squared tests; $P > 0.05$). A total of eight species were recorded in macadamia (excluding the 3 May sample) and seven species in riverine bush, of which six species were common to both habitats (Table 3).

Activity levels did not seem to be associated with temperature or rainfall as would be expected (colder nights with fewer insects should mean lower activity of bats). Night time temperatures in October and November did not vary much, dropping from above 20°C at dusk to between 15°C and 20°C minimum (**Figure 2**). Paradoxically, during the coldest month in May when trial recordings were done in the macadamia orchard (minimum temperature of 14°C), activity was much higher than during the warmer summer months. Sampling in October was done when conditions were very dry before the main rain season had commenced (only 100 mm received between 1 May and 1 October 2010). During the sampling over 20 and 21 November, although no rain was recorded on the sample nights, rainfall (64 mm) had fallen in the previous week. Despite obvious availability of insects (including frequent observed emergences of winged alates of Isoptera) following recent rains, activity of bats in the riverine habitat in November was noticeably lower (3-8 passes per night) than in October (12-17 passes per night). This could be partly explained by the fact that 21 November was a full moon whilst 1-2 October coincided with the new moon. Insect and bat activities are known to vary with moon cycle.

Dietary analysis

A crude estimate of insect availability during summer in the general study area was obtained by two nights of insect collecting at Welgevonden using malaise traps (**Table 4, Figure 3**). As this was part of a pilot study only, insect trapping was not conducted simultaneously with acoustic recordings of bats, and only one insect sampling method (malaise traps) was used (future sampling will use both malaise and light traps associated with bat acoustic recordings). Nevertheless, the sample provided a crude preliminary estimate of insect availability on a macadamia farm where bat sampling was undertaken. Diptera, Lepidoptera, Hymenoptera and Hemiptera were the most abundant orders represented (Table 4, Figure 3).

Based on 121 culled part fragments collected over four months in 2010 (July, August, October and November), counts were obtained by insect order and month (**Table 5**). Nine orders were presented, confirming the relatively broad dietary niche of this species (Bowie *et al*., 1999). A chi-squared test showed significant association between month and insect order, reflecting significant variation in diet between months (chi-square = 113.48; 24 degrees of freedom; P value < 0.05); thus Lepidoptera were absent in winter (July) but common in summer months, and cockroaches (Blattodea) occurred with much higher frequency in some months (July, November) compared with others. Combining months, the diet was dominated by Blattodea with substantial proportions of Orthoptera and Lepidoptera, but other items featured much less importantly (*e.g*. Hemiptera comprised < 2%) (**Figure 4**). Apart from a higher proportion of Blattodea and slightly lower proportions of Lepidoptera and Orthoptera in our study, our results agreed quite closely with those of two KwaZulu-Natal

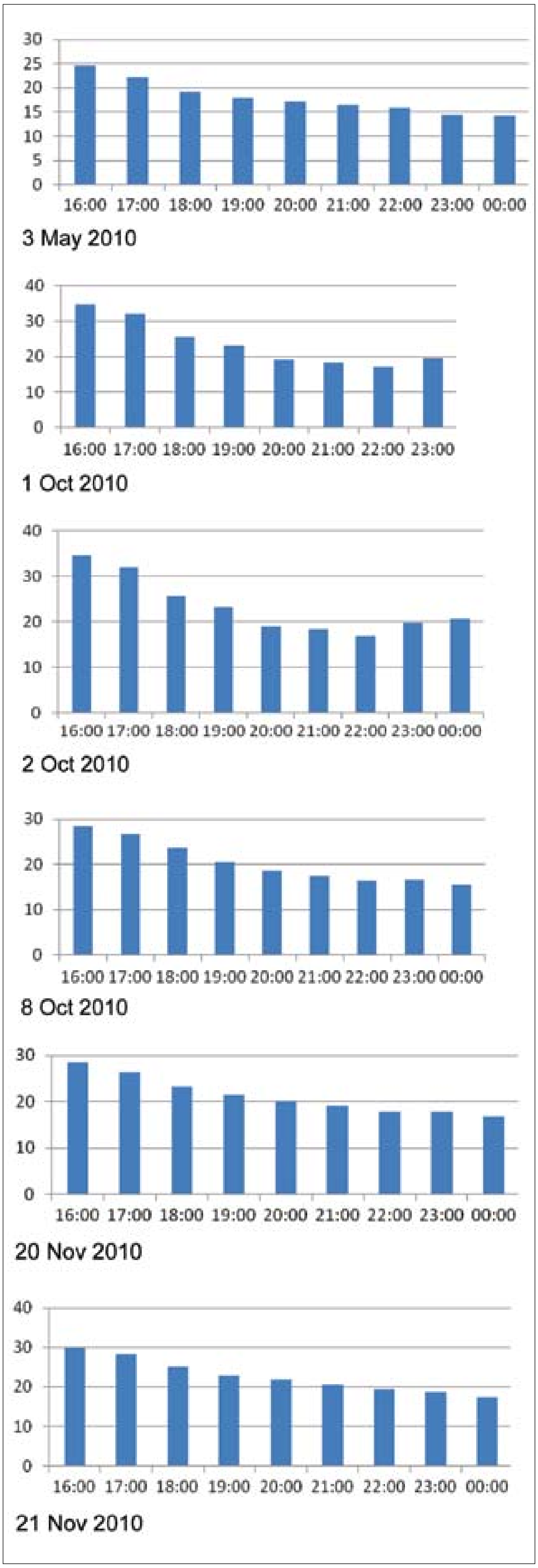

Figure 2. Hourly temperatures for six nights in 2010 on a farm neighbouring the study farm (Data from D. Alberts).

Table 4. Number of insects caught in the Malaise trap at Farm Welgevonden during two nights in November 2010 and January 2011.

Order of insects	Total number of insects per order	Total percentage of insects per order
Hemiptera	14	9
Diptera	38	24
Hymenoptera	19	13
Blattodea	3	2
Coleoptera	11	7
Orthoptera	4	3
Lepidoptera	64	42

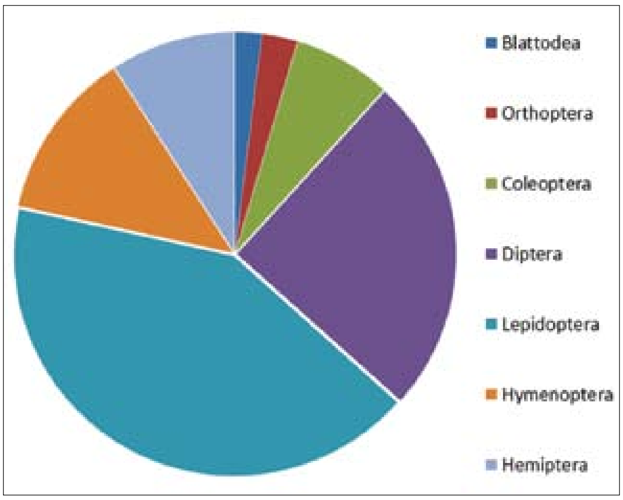

Figure 3. Pie chart showing proportion of different arthropod orders in samples from two nights collecting with Malaise trap on farm Welgevonden.

studies of culled parts in this species (La Val & La Val, 1980; Seamark & Bogdanowicz, 2002).

Based on microscopic analyses of faecal pellets over the same four months (n = 5 pellets analysed per month), once again a chi-squared test revealed significant differences in diet between months (**Table 6**; Chi-square test: 6197, 79 degrees of freedom, P < 0.5). In winter (July and August), Lepidoptera and Coleoptera occurred at migh higher proportion than in summer months. Orthoptera and Hemiptera varied dramatically from month to month (from 0 to 20% and 0 to 39% respectively). Significantly, when months were combined, Hemiptera comprised a much higher proportion of the total diet (20%) compared to the results of the culled parts (2%) (**Figure 4** and **5**). This was expected due to the foraging habits of this species, whereby larger prey with sharp and unpalatable parts (such as cockroaches, crickets and some moths) would be expected to be well represented amongst culled parts whereas smaller softer-bodied insects (such as Diptera, Hymenoptera and some smaller Hemiptera) would be more likely to be consumed whole and represented in faecal pellets but not culled parts.

From their foraging strategy (a slow-flying gleaner capable of hearing low-intensity prey-generated nois-

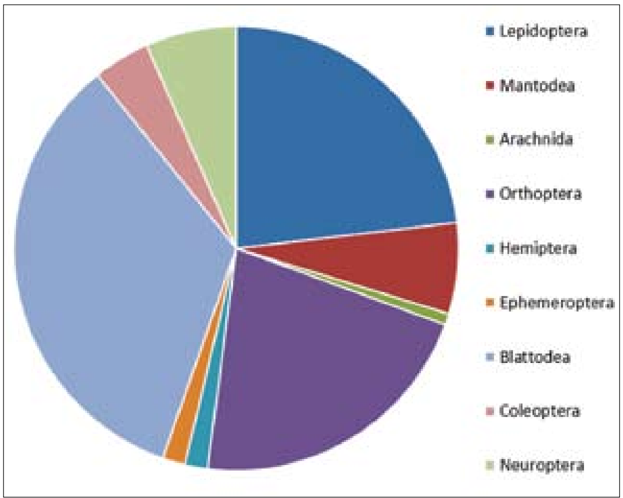

Figure 4. Pie chart showing proportions (by counts) of arthropod orders found in the culled parts.

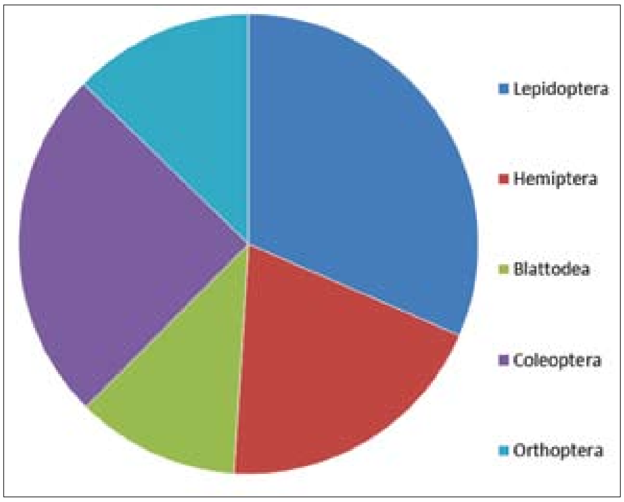

Figure 5. Pie chart showing proportion (by volume) of different arthropod orders in faecel pellets.

Table 5. Numbers of fragments of insect orders identified by microscope analyses of culled parts of *N. thebaica* collected from night roost on Farm Laatsgevonden.

Order of insects	July	August	October	November	Total percentage of insect per order
Blattodea	16	4	3	18	33.88
Neuroptera	0	0	0	8	6.61
Ephemeroptera	0	0	2	0	1.65
Coleoptera	3	0	2	0	4.13
Hemiptera	0	2	0	0	1.65
Orthoptera	9	13	4	0	21.48
Arachnida	0	1	0	0	0.83
Mantodea	0	8	0	0	6.61
Lepidoptera	0	10	14	4	23.14

Table 6. Proportion (percentage) by volume of insect orders identified by microscope analyses of faecal pellets of *N. thebaica* collected from night roost on Farm Laatsgevonden.

Order	July	August	October	November	Mean of insect order
Lepidoptera	40	60	6	20	31.5
Hemipetera	12	0	39	27	19.5
Blattodea	9	7	30	0	11.5
Coleoptera	28	33	5	33	24.75
Orthoptera	11	0	20	20	12.75

es from non-volant prey; Bowie *et al*., 1999), we expected that slit-faced bats should be capable of feeding on stinkbugs of the Family Pentotomidae which would be locally plentiful in the macadamia orchards surrounding the study roost. Indeed we observed large numbers of cockroaches, a common item in the culled parts (**Table 7**), moving at night in piles of leaf litter between rows of macadamia trees. It seems conceivable that the bats may consume some bugs whole, explaining their absence in the culled part samples (however, Seamark & Bogdanowicz, 2002 recorded up to 12.6% of Hemiptera in culled parts of this species). Although Hemiptera comprised 20% of the diet according to faecal pellets, it was not possible to identify fragments to family level. The use of a DNA bar-coding approach holds promise to identify prey remains from faecal pellets to a lower taxonomic category (family, genus or even species; Bohmann, 2010). An ongoing study at the University of Venda is using this approach to isolate arthropod DNA (cytochrome oxidase-I gene) from faecel pellets of bats and to match this with sequences available on Genbank and the Bar-code of Life (BOLD) databases, as well as sequences obtained from localy-obtained samples of stinkbug species (sequences of which are not yet available on pubic gene databases).

Although Hemiptera have not previously been recorded to be predominant in the diet of this gleaning forager, either from culled parts (*e.g*. Laval & Laval, 1980; Seamarck & Bogdanowicz, 2002; although the latter study recorded up Hemiptera to comprise 12.6% of the diet in summer months at Stainbank Nature Reserve in Durban) or from stomach contents (Whitaker & Black, 1976) or faecal pellets (Bowie *et al*., 1999; Schoeman, 2006), our study found a high proportion (20%) of Hemiptera in faecal pellets (but not culled parts), compared to the above-mentioned studies which were all undertaken in natural areas.

CONCLUSION

Our preliminary results of acoustic surveys (165 ANABAT call files) showed 12 species of bats to be present in three recognised habitats (macadamia orchard, riverine bush and the vicinity of the homestead) on a macadamia farm in the Levubu Valley. Activity of bats in the riverine habitat was significantly higher than in adjacent macadamia orchards, although localised high activity of bats was recorded in the vicinity of the homestead located in the middle of the orchard. Overall, species richness and composition did not vary significantly between the three habitats. The most frequently recorded species were *S. dingani* (a beetle-specialist which forages in clutter-edge habitats), *P. hesperidus* (a clutter-edge forager which prefers bugs), *T. mauritianus* (an open-air foraging moth specialist), and two open-air foragers which feed predominantly on moths, flies and bugs (*C. pumilus*, *M. condylurus*) (Monadjem *et al*., 2010). Although stinkbugs (Hemiptera: Pentotomidae) are the primary pest of macadamia and other subtropical fruits, a host of other insect species such as false codling moths are implicated in damage to subtropical fruits. Thus it is important to assess the diet of all the above-mentioned bat species, using both conventional microscopic and DNA bar-coding approaches.

Species of the family Nycteridae (slit-faced bats) were not recorded by the acoustic survey, simply be-

Table 7. Comparison of diet in *Nycteris thebaica* between this study and studies at four other South African sites (Schoeman, 2006). SD = standard deviation.

Locality	Laatsgevonden		Algeria, W. Cape		De Hoop, W. Cape		Goodhouse, N. Cape		Sudwala, Mpumalanga	
No of pellets	(20)		(51)		(30)		(30)		(22)	
Prey category										
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lepidoptera	31.5	±46.257	34.9	±27.3	50	±2.16	66.7	±15.3	45	±7.1
Coleoptera	24.75	±34.449	21.3	±17.6	18.8	±17.5	3.3	±5.8	0	
Hemiptera	19.5	37.166	1.5	±4.5	2.5	±5	0	0	0	
Diptera	0		3.8	±10.6	0		0	0	0	
Hymenoptera	0		1.6	±4.6	0		0	0	0	
Trichoptera	0		0		0		0	0	0	
Othoptera	12.75	±32.260	23.5	±19.6	11	±13	23.3	±25.2	17.5	±10.6
Neuroptera	0		0		0		0	0	0	
Emphemeroptera	0		0		0		0	0	0	
Arachnida	0		13.5	±27.4	5	±10	6.7	±12	12.5	±17.7
Mantodea	0		0		0		0	0	25	±34.4
Other	11.5	±25.449	0		7.5	±113	0	0	0	

cause these bats, often termed "whispering bats", emit very weak echolocation signals which evade detection by bat detectors at distances of more than a metre. The presence of *Nycteris* (species undetermined) at Welgevonden was confirmed by observations of bats with long ears night-roosting in an outhouse close to the main house. Based on the small size and relatively shorter ears of the observed individuals (which we did not succeed in catching), it was suspected that the bats could belong to the species, *N. hispida* (PJT and JNS, personal observation; Monadjem *et al*., 2010). Two characteristic night-roosts suspected of belonging to the common *N. thebaica* (containing culled parts of non-flying prey items) were located some 20 km east of the Farm Welgevonden on the Farm Laatsgevonden and a neighbouring farm. Regular collection of culled parts and faecal pellets from these roosts formed the basis of the dietary component of this study. It remains to be seen from future DNA bar-coding analysis, whether the increased proportion of Hemiptera found in faecal pellets of this species compared with other studies undertaken in natural areas, may be explained by opportunistic foraging of stinkbug pests in macadamia orchards.

Future studies also need to target dietary studies of a wider range of bat species and to conduct insect trapping in parallel with bat acoustic recordings and collections of faecal samples to assess insect availability and hence selectivity of bat foraging. Since agricultural entomologists can forecast times of the year when stinkbug numbers will peak in orchards (S. Schoeman, personal communication), bat studies should be conducted during such peak periods to avoid temporal biases which may lead to false conclusions concerning consumption of pest insects by bats. Radio-tracking studies will also be carried out to determine movement patterns of particular species of bats in an agricultural landscape dominated by subtropical fruits in the Levubu Valley; a likely candidate for this study is the bug-specialist, *P. hesperidus*, which is considered likely to feed on stink bugs. Finally, future studies should compensate for potential biases due to the moon cycle.

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