# Katydid (Orthoptera: Tettigoniidae) bio-ecology in Western Cape vineyards

by

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# Declaration

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# Summary

Many orthopterans are associated with large scale destruction of crops, rangeland and pastures. *Plangia graminea* (Serville) (Orthoptera: Tettigoniidae) is considered a minor sporadic pest in vineyards of the Western Cape Province, South Africa, and was the focus of this study. In the past few seasons (since 2012) *P. graminea* appeared to have caused a substantial amount of damage leading to great concern among the wine farmers of the Western Cape Province. Very little was known about the biology and ecology of this species, and no monitoring method was available for this pest. The overall aim of the present study was, therefore, to investigate the biology and ecology of *P. graminea* in vineyards of the Western Cape to contribute knowledge towards the formulation of a sustainable integrated pest management program, as well as to establish an appropriate monitoring system.

No detailed surveys have as yet been undertaken to assess the assemblage structure of katydids in vineyards and to verify their taxonomic status. By conducting a survey in vineyards located in the greater Stellenbosch region of the Western Cape, the identities of the katydid species present and their pest status was determined. A monitoring method was developed by adapting a generic sampling system for monitoring key arthropod pests in vineyards. Due to the perfect camouflage of adult katydids within the vine canopy, surrogate methods for monitoring this pest were investigated. Besides determining the basic biology and ecology of *P. graminea* within vineyards, aspects of its physiological ecology with implications on its mating behaviour were investigated. Furthermore, natural enemies that could potentially be used as environmentally-friendly biological control agents against this pest were identified.

Three Phaneropterinae species were identified, namely *P. graminea*, *Eurycorypha lesnei* Chopard and a *Phaneroptera* species. Due to the similarity between the *Plangia* and *Eurycorypha* species, an ID-key was compiled for easy identification by growers. *Plangia graminea* was found to be the primary katydid pest in vineyards monitored. There was only one generation per year, with an overwintering egg stage. The monitoring of katydid eggs could potentially be used to monitor *P. graminea*, as eggs were positively and significantly correlated with katydid numbers and could allow early prediction estimates of katydid populations in vineyards. Temperature appeared to be an important environmental factor enhancing population outbreaks, as it influenced katydid development, but could also affect mating success of male katydids. It was found that there was a significant metabolic cost associated with the mating calls of *P. graminea* males. This study identified two natural control agents that could potentially be incorporated into an integrated pest

management program for the control of *P. graminea*, namely hymenopteran egg parasitoids and an entomopathogenic fungus.

The outcomes of this study aim towards the development of a practical, sustainable and environmentally-friendly integrated pest management program. Future research should focus on validating a monitoring method in the field, establishing an economic threshold, testing the efficacy of entomopathogenic fungi in the laboratory and in the field, and investigating the mechanisms involved in habitat preferences of hymenopteran egg parasitoids.

# Opsomming

Baie sprinkaanagtiges is geassosieerd met grootskaalse vernietiging van gewasse en weivelde. *Plangia graminea* (Serville) (Orthoptera: Tettigoniidae), plaaslik bekend as "krompokkels," word beskou as 'n sporadiese plaag in wingerde in die Wes-Kaap Provinsie, Suid-Afrika, en was die onderwerp van die huidige studie. In die afgelope paar seisoene (vanaf 2012) het *P. graminea* ansienlike skade aangerig in wingerde, wat tot groot kommer onder die wynboergemeenskap van die Wes-Kaap gelei het. Baie min inligting is bekend oor die biologie en ekologie van die plaag. Geen moniteringsmetode is vir die plaag beskikbaar nie. Die algehele doel van die studie was daarop gemik om die biologie en ekologie van *P. graminea* in wingerde in die Wes-Kaap te ondersoek, om verworwe kennis beskikbaar te maak vir die formulering van 'n volhoubare geïntegreerde plaagbestuurprogram, asook om 'n geskikte moniteringsisteem te ontwerp.

Tot dusver was daar nog geen gedetailleerde opname van die groeperingsstruktuur van krompokkels in wingerde, en hul taksonomiese status nie. Die studie het beoog om die krompokkel spesies in wingerde te identifiseer, en om hul plaagstatus te bepaal deur 'n opname in wingerde geleë in die groter Stellenbosch streek van die Wes-Kaap uit te voer. 'n Moniteringsmetode, gebasseer op 'n generiese steekproefnemingsisteem vir die monitering van sleutel artropode wingerdplae was ontwerp. Aangesien volwasse krompokkels baie goed gekamoefleerd is tussen wingerdblare, is surrogaat metodes vir die monitering van die plaag ondersoek. Benewens die bepaling van die insek se biologie en ekologie, is ondersoek ook uitgevoer aangaande die fisiologiese-ekologie van die insek in verband met implikasies rakende die insek se paringsgedrag. 'n Verdere doel van die studie was om die natuurlike vyande, teenwoordig in wingerde, te identifiseer wat moontlik gebruik kan word as omgewingsvriendelike biologiese beheermetodes teen die plaag.

Drie Phaneropterinae spesies was geïdentifiseer, naamlik *P. graminea, Eurycorypha lesnei* Chopard, en 'n *Phaneroptera* spesies. Weens die groot ooreenkomste tussen *Plangia* en *Eurycorypha* spesies, is 'n ID-sleutel saamgestel wat deur die wingerdboere gebruik kan word vir maklike identifikasie. *Plangia graminea* was die primêre krompokkel plaag in die wingerde wat gedurende die studie gemoniteer was. Net een generasie was teenwoordig, met 'n oorwinterende eier-stadium. Die monitering van krompokkel eiers kan potensieel gebruik word vir die monitering van *P. graminea*, aangesien daar 'n positiewe en beduidende korrelasie was tussen die getal eiers en die aantal krompokkels. Die eiers kan ook gebruik word vir vroegtydige voorspellingsberamings van krompokkel populasies in wingerde. Dit blyk dat temperatuur 'n belangrike omgewingsfaktor is tot bevolkingsuitbrake, aangesien dit 'n invloed gehad het op die

ontwikkeling van krompokkels, asook die paring sukses van mannetjie krompokkels. Daar is bevind dat daar ‘n beduidende metaboliese koste geassosieer is met die paringsroepe van *P. graminea* mannetjies. Die studie het twee natuurlike agente geïdentifiseer wat moontlik in ‘n geïntegreerde plaagbestuurprogram ingesluit kan word vir die beheer van *P. graminea*, naamlik, eier parasiterende wespies en ‘n entomopatogeniese swam.

Die doelstellings van die huidige studie mik na die ontwikkeling van ‘n praktiese, volhoubare en omgewingsvriendelike geïntegreerde plaagbestuurprogram. Toekomstige navorsing kan fokus op (i) die ontwerp en toepassing van ‘n moniteringsmetode in die veld, (ii) die bepaling van ‘n ekonomiese drempelwaarde, (iii) om die effektiwiteit van die entomopatogeniese swam in die laboratorium en in die veld te toets, en (iv) om die meganismes betrokke by habitatsvoorkeure van eier parasiterende wespies te ondersoek.

This thesis is dedicated to

**Wim J. Tijmens**

# Biographical sketch

Marcé Doubell earned his Bachelor of Science degree in Conservation Ecology and Entomology from Stellenbosch University, South Africa, in 2013. For his Honours project, he studied the importance of different landscape elements for biological diversity within a transformed agricultural landscape using butterflies as indicator species. In 2014, he enrolled for his Master of Science degree in Entomology at Stellenbosch University with the project title "*Katydid (Orthoptera: Tettigoniidae) bio-ecology in Western Cape vineyards*". His keen interest in nature and insects developed early in his childhood when he spent most of his time exploring the African savannas surrounding his small hometown, Hazyview, adjacent to the Kruger National Park, South Africa. However, it was in his final year of his BSc degree, after completing an Entomology course, when he decided to pursue an academic career in Entomology. His current scientific interest is to develop more sustainable and environmentally friendly ways to control agricultural pests in the Cape Winelands of Southern Africa, and he is highly motivated to work in the field of Integrated Pest Management.

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# Preface

This thesis is presented as a compilation of 6 chapters. Each chapter is introduced separately and is written according to the style of the journal *African Entomology* to which Chapters 2, 3 and 5 will be submitted for publication. Chapter 4 was submitted and accepted in *Journal of Experimental Biology*. However, for the purpose of consistency within the thesis it is presented in the same style as the other chapters.

**Chapter 1** **General introduction and project aims**

**Chapter 2** **Research results**
Identification of katydid (Orthoptera: Tettigoniidae: Phaneropterinae) species present in vineyards of the Western Cape - with notes on their morphology, eggs and assemblage structure

**Chapter 3** **Research results**
Monitoring of katydids (Orthoptera: Tettigoniidae: Phaneropterinae) in vineyards in the Western Cape, South Africa - with insights gained on their biology, ecology, and seasonal dynamics

**Chapter 4** **Research results**
Physiological ecology – the metabolic costs of sexual signalling in the chirping katydid *Plangia graminea* (Serville) (Orthoptera: Tettigoniidae) are context dependent: cumulative costs add up fast

**Chapter 5** **Research results**
Natural enemies of *Plangia graminea* Serville (Orthoptera: Tettigoniidae) in vineyards in the Western Cape, South Africa; and their potential for biological control

**Chapter 6** **General discussion and future research recommendations**

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# CHAPTER 1

## General introduction and project aims

The Orthoptera is a major group of insects widely distributed over all the earth's surface except for the coldest parts. To date, more than 20 000 orthopteran species have been described (Eades *et al.* 2015), most species being from the southern hemisphere, especially from the savannah and tropical regions. This order of orthopteroid insects includes those terrestrial insects commonly referred to as locusts, short-horned grasshoppers, crickets, katydids and other related groups not known by vernacular names (Rentz 1991; Gangwere *et al.* 1997). Some of the largest living insects are included in this order, but most are medium-sized to large (Rentz 1991). In many parts of the world they form a major component of the fauna, as they are often abundant as singletons or forming swarms. The Orthoptera represent a range of functional feeding types and biologies, including being phytophagous and carnivorous, diurnal and nocturnal, geophilous (ground living), phytophilous (living on plants), cavernicolous (inhabiting caves), myrmecophilous (inhabiting nests of ant colonies) and fossorial (burrowing) (Rentz 1991). Detailed accounts of the order are given by, amongst others, Beier (1955), Kevan (1982) and Gangwere *et al*. (1997).

Many orthopterans are also associated with large scale devastation of crops, rangeland and pastures. They have plagued mankind since recorded time with locust outbreaks documented in early Chinese literature and biblical writings (Rentz 1991). At present, plagues of grasshoppers and locusts are still responsible for the destruction of many types of crops and cause food shortages in many parts of the world. Similarly, crickets attack many crops including tuberous crops, coffee and tea, and katydids cause damage to orchards, cereals and other cultivated crops (Gangwere *et al*. 1997). *Plangia graminea* (Serville) is considered a minor sporadic pest in the vineyards of the Western Cape Province, South Africa, and is the focus of this study.

## Classification and systematics

The most important aspect of studying organisms for scientific purposes is to firstly determine their taxonomic status and ensure correct identification. As the taxonomic state of the *Plangia* species complex is somewhat confused (Hemp *et al.* 2015), some detailed information is provided here on the classification and systematics of the Tettigoniidae. The order Orthoptera is subdivided into two monophyletic suborders, Ensifera and Caelifera (Song *et al.* 2015). The former represent the long-horned katydids and crickets, and the latter the short-horned grasshoppers and locusts.

A key to the suborders of Orthoptera has been provided by Rentz (1991) and is further divided into a number of superfamilies, families and subfamilies. A key to these families, as adapted from Rentz (1991), is provided in Appendix A.

The superfamily Tettigonioidea with its family Tettigoniidae and subfamily Phaneropterinae (see key trail: 1, 4, 5, 7 in Appendix) and *Plangia graminea* (Serville), a species of the genus *Plangia* Stål within the subfamily Phaneropterinae, will form the main focus and subsequently due to its relevance for the present study be discussed in further detail.

## Superfamily Tettigonioidea

The Tettigonioidea is the largest superfamily in the Ensifera. In addition to the characteristics provided in the key, the taxon can be characterised by a number of features. The most characteristic feature is that of the antennae, usually longer than the body. Auditory tympana are located on the fore tibia. The fore wing is rarely absent, and when present, the left fore wing usually overlaps the right one. In the fore wing the most anterior branch of the cubitus (Cu, or the CuA-branch as such) fuses at least with part of the length of the media (MP-branch); CuP is unbranched and runs straight to the margin in the hind wing. The cubital region of the male fore wing is usually specialised for stridulation with the vein CuP principally modified. This vein is bent towards the posterior margin of the wing to resume its longitudinal course. Its reflexed portion is thickened and toothed ventrally. Abdominal segments 8 (female) and 9 (male) bear a subgenital plate. The male cercus is well sclerotised and inflexible, with styles usually present. An ovipositor is present, although it is sometimes small and laterally compressed with all three pairs of valves well developed. The prothoracic spiracles and the

associated ends of the tracheal system are sometimes modified and enlarged for an auditory function. The legs are rarely modified for digging, even if heavily spined (Rentz, 1991).

**Family Tettigoniidae**

With more than 1 070 genera and more than 6 000 species described worldwide, the family Tettigoniidae is the second largest group of Orthoptera (Gangwere *et al.* 1997; Eades *et al.* 2015). They can be found on all continents, except Antarctica, and occur at all altitudes (Rentz 2010). Their vernacular names vary with regions. In Australia, New Zealand and North America the term katydid is used to identify members of the Tettigoniidae. It was used in the American entomologist C V Riley's Report in 1874, but the term goes at least as far back as 1751, when they were referred to as 'catedidists' by John Bartram in his 'Travels in Pensilvania and Cananda' (Oxford English Dictionary). In the United Kingdom they are known as 'bush-crickets', in France '*sauterelles*', in Portugal (and some parts of Central and South America) '*esperansas*', in Spain '*grillos*', and in Germany '*Laubheuschrecke*' (Nickle & Naskrecki 1997). The reason for the various common names could be because the group is so poorly known (Gwynne 2001), or perhaps a reflection of the general morphological diversity within the family Tettigoniidae (Rentz 2010). The term 'katydid' will be used in the present study .

The external anatomy of katydids is relatively simple with only a few specialised features (Rentz 2010). The morphology of a katydid is illustrated in Figure 1. The pronotum seldom bears a ridge. They have two pairs of wings (Fig. 2). A sword-shaped ovipositor is typically present in the females (Capinera 2004). The spines on the legs (called armature) is of taxonomic importance, usually described in detail and often used in keys to distinguish genera and species (Ragge 1980; Capinera 2004).

The venational patterns as well as the shape, position and other features of the veins of adult wings are often used to identify species. The shape and structure of the fore wing (called the tegmen) is also used for identification (Rentz 2010). A comprehensive account of the venation of tettigoniid wings is given by Ragge (1955).

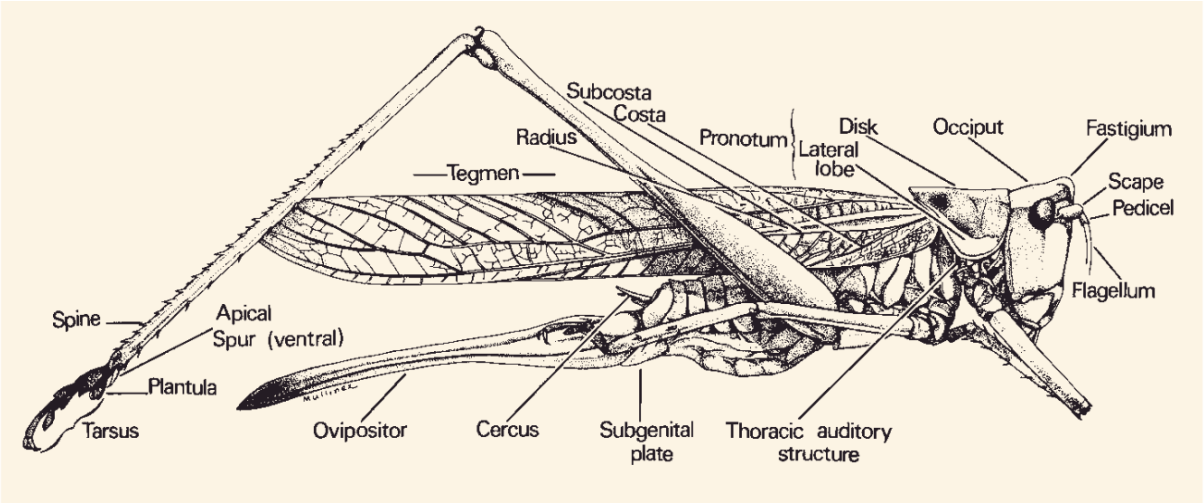



**Fig. 1.** The general katydid body plan. Taken from Rentz (2010).

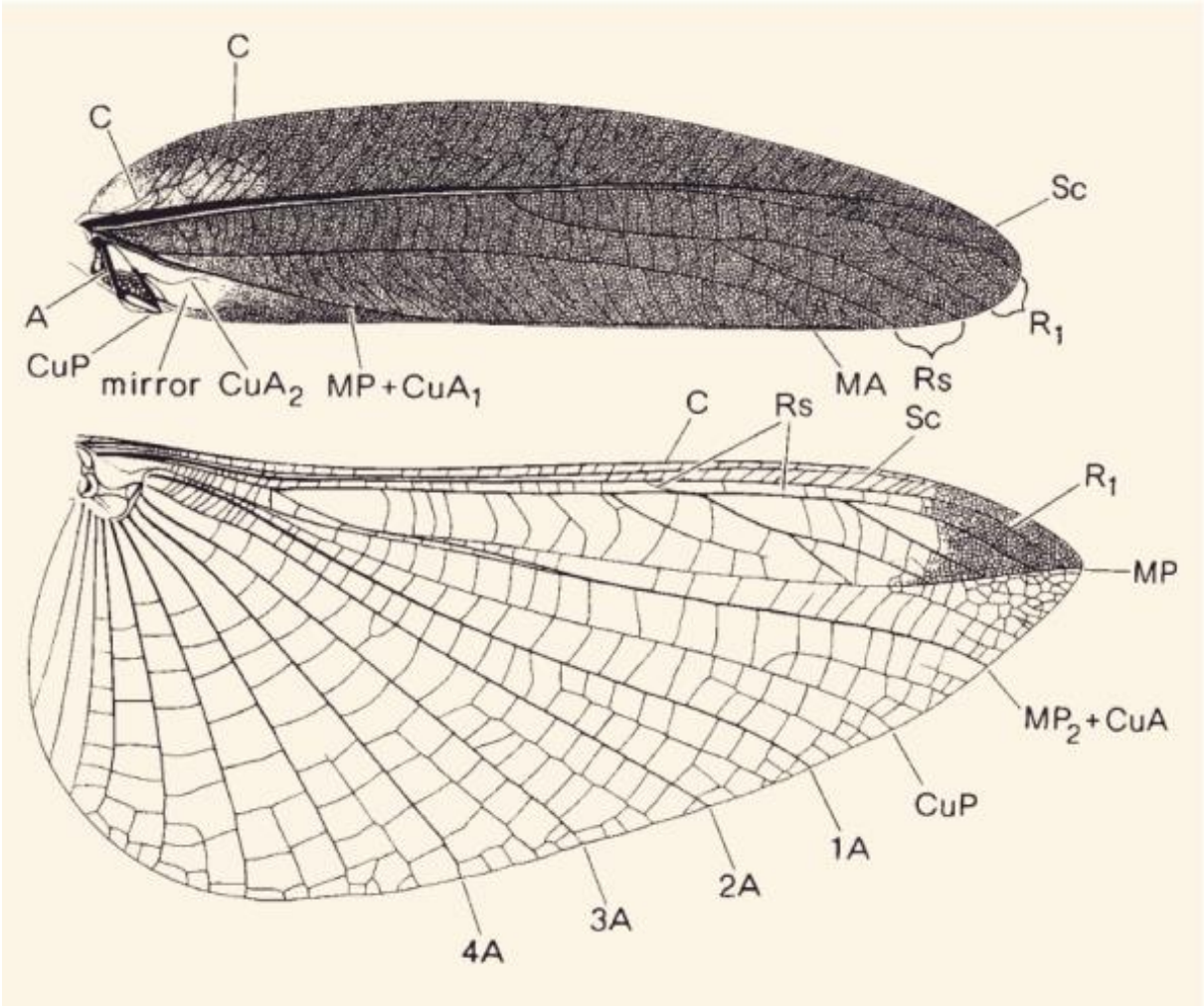



**Fig. 2.** Wing venation of a katydid. Taken from Rentz (2010).

Katydids have an advanced stridulatory specialisation, described by Ragge (1955). The fore wing is folded along the line between CuA and CuP, with the horizontal part being relatively small in fully winged species, and the first fold of the hind wing in the MP area (Rentz 1991). In the male, the cubito-anal regions of the tegmina are modified for stridulation (Imms 1964). An archedictyon is generally present over the whole of the fore wing (except for parts of the male stridulatory apparatus) and sometimes on a small area towards the distal part of the hind wing. In the hind wing, and occasionally in the fore wing, MA is almost always fused to the radial sector (Rs) for a short distance. Most of the anterior branch of CuA (or the vein as a whole) is usually fused with MP over the whole of its distal portion (Rentz 1991).

**Subfamily Phaneropterinae**

Also known as false katydids (or bush katydids), this group is distinguished from others by the absence of spines on the prosternum. Their hind wings are longer than their fore wings, which is also a characteristic feature of this group. They are well known for their acoustic ability and their songs can be heard late in the day and during the evening (Capinera 2004). They occur in most habitats and all known species are herbivorous (Rentz 2010). With more than 2 160 species described in more than 330 genera, Phaneropterinae is the largest and most diverse subfamily of the Tettigoniidae (Nagar *et al*. 2015). With many species and little taxonomic information on South African species, it is also a rather difficult subfamily to deal with. Many of the genera are of uncertain status or in a state of confused taxonomy. This is partly due to genera and species being described without much reference to their relationships (Rentz 2010). Many of the species names date back to the early European scientific expeditions to South Africa, resulting in most of the type material being held in European collections. Moreover, the descriptions of the early describers were often brief and not very informative, often lacking the precise locality of the specimens. With the most critical taxonomic characteristics of a species often not covered in many early works, and with the general lack of illustrations, descriptions often become meaningless (Rentz 2010). The African Phaneropterinae have been reviewed by Ragge (1980) who also provided notes and keys for their identification (see Figs. 3 and 4).

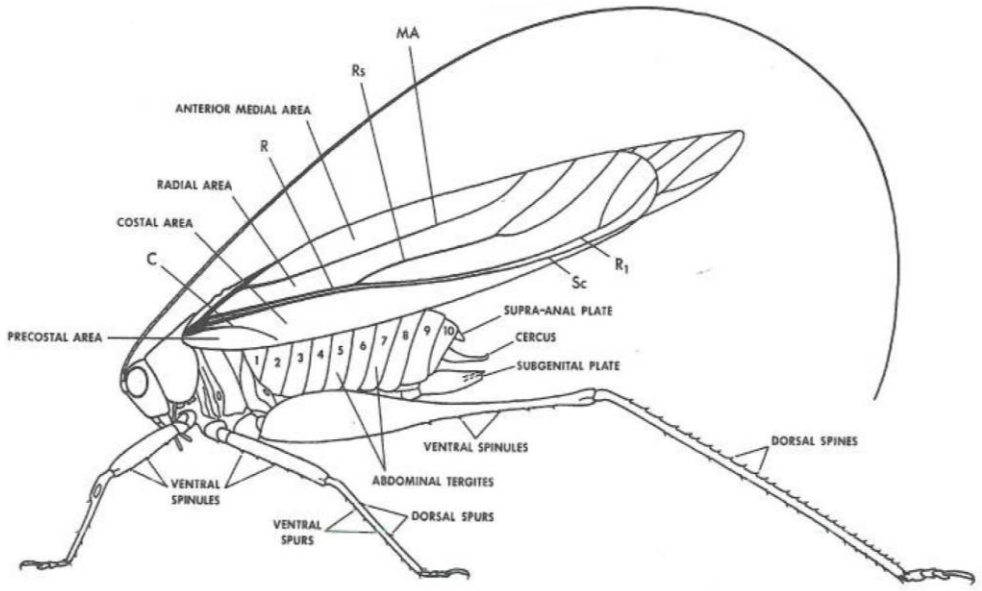



**Fig. 3.** A typical male phaneropterine, showing some of the taxonomically useful characters. Taken from Ragge (1980).

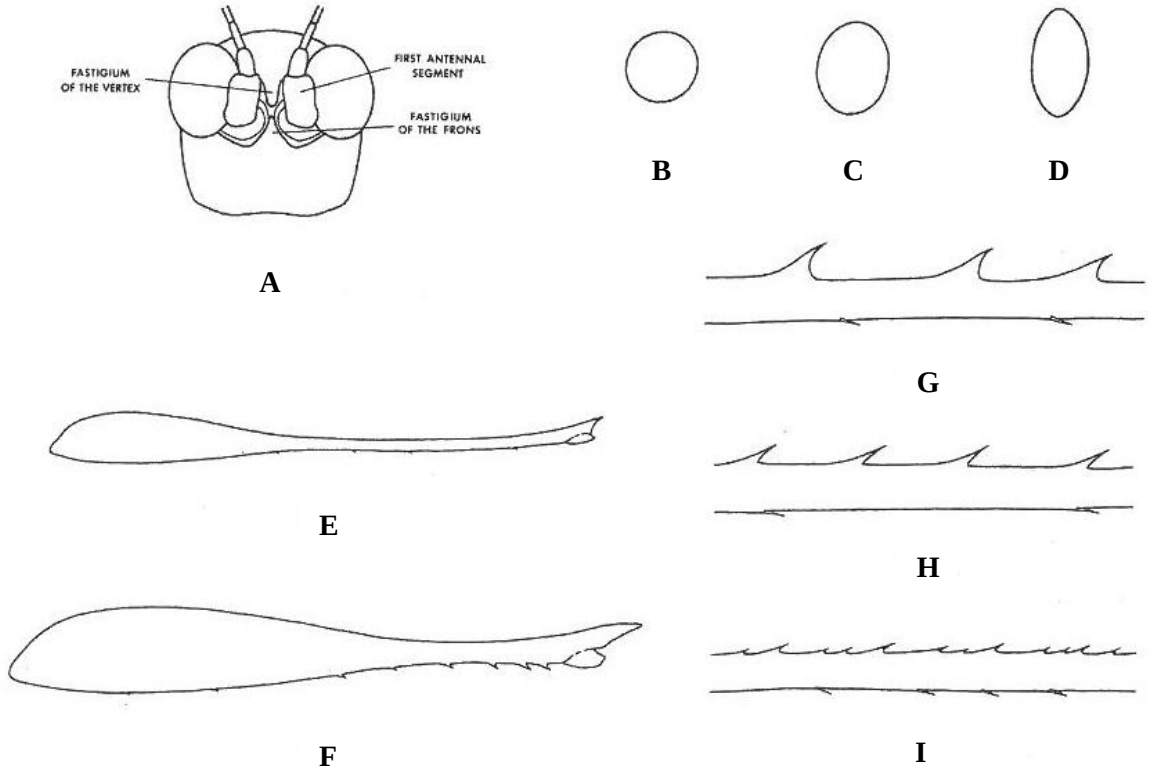



**Fig. 4.** Taxonomic characters of Phaneropterinae. A. Anterodorsal view of part of the head of a typical Phaneropterine, showing the positions of the fastigia in relation to the first antennal segment. B-D. Outlines of Phaneropterine eyes, showing (B) 'circular', (C) 'oval' and (D) 'oval and elongate' shapes. E, F. Lateral view of the left hind femur of (E) *Gabonella cothurnata* and (F) *Oxygonatium huxleyi*. G-I. Lateral view of part of the left hind tibia of (G) *Terpnistria zebrata*; (H) *Atlasacris peculiaris*; (I) *Ducetia fuscopunctata*. Taken from Ragge (1980).

Rentz (1979) also provides some key characteristics with illustrations of the subfamily Phaneropterinae in his illustrated key to the subfamilies of Tettigoniidae:

"Head globose, not usually slanted or frontally flattened. Fore tibia in section approximately square in distal portion, dorsal surface flat or slightly concave, not convex (Fig. 5 A). Proximal tarsomere cylindrical, not laterally sulcate. Ovipositor (Fig. 5 B and C) usually short and upturned flattened laterally, margins usually crenulated. Prosternum unarmed. (Distribution: world-wide)……...…….Phaneropterinae"

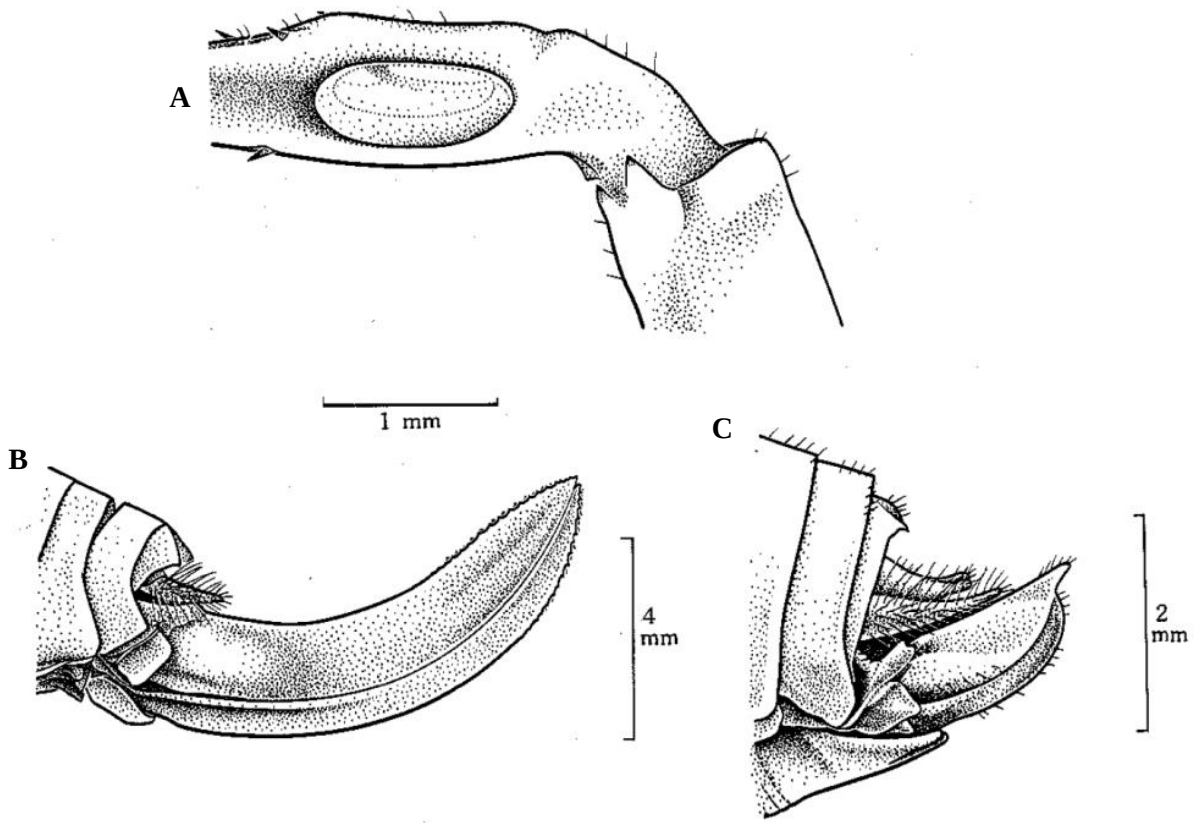



**Fig. 5**. Some distinguishing characteristics of the subfamily Phaneropterinae. A. Fore tibia; B-C. Ovipositor. Taken from Rentz (1979).

**Genus *Plangia* Stål (Tettigoniidae, Phaneropterinae)**

*Plangia* is an African genus occurring south of the Sahara, the Seychelles and Madagascar. It is a rather non-descript genus and mostly classified by negative features. It can be distinguished from its close relatives *Monteiroa*, *Oxygonatium* and *Eurycorypha* by their exceptionally broad fastigia, and from *Plangiodes* by its elongate eyes and frontogenal carinae (Ragge 1980). Currently 12 taxa are listed within the genus *Plangia* (Eades *et al*. 2015). In the key to the

genera of African Phaneropterinae, constructed by Ragge (1980), the *Plangia* genus is identified through the following character steps:

1. Hind femora without a dorsal point at the tip or with a very small point (not as in Fig. 4 E or F);
2. Dorsal spines of the hind tibiae unmodified, of normal shape (Fig. 4 H or I);
3. Fastigium of the vertex at least as broad as the first antennal segment;
4. Eyes circular or oval (Fig. 4 B or C), not elongate. Head almost always without frontogenal carinae;
5. Ninth abdominal tergite unmodified. *Sc* and *R* of the fore wings contiguous except near the apex;
6. Hind wings clearly extending beyond the fore wings;
7. Fastigium of the vertex less than twice as broad as the first antennal segment; and
8. Fastigium of the frons broadly rounded or truncate. Fore and mid tibiae without dorsal spurs except at the apex…….....***Plangia*** Stål

*Plangia* are canopy dwellers and often occur syntopically with other fully winged katydids [e.g. *Eurycorypha* Stål or *Arantia* Stål (Phaneropterinae)]. Very little or nothing is known about the biology, ecology, habitat, distribution and the phylogenetic relationships of these taxa (Hemp *et al*. 2015). Although numerous species have been described from these taxa, the taxonomic status of the species is at present confused and requires further investigation.

***Plangia graminea* (Serville, 1838)**

*Plangia graminea* has a wide distribution range across sub-Saharan Africa. *Plangia graminea* was originally described from the Cape of Good Hope, Cape Province, South Africa by Serville (1839). The type specimen appears to be lost (Eades *et al.* 2015). There are a number of specimens held in various collections that show a degree of variability of this species in terms of colour pattern, wing length and variations in stoutness and length of the male cerci, and shape of the subgenital plate, suggesting that more than one species might be hidden in the *P. graminea* complex (Hemp 2013). Therefore, *P. graminea* is in need of revision (Hemp 2013).

The *P. graminea* complex was reviewed by Hemp *et al.* (2015), and *P. compressa* (Walker) was synonymised with *P. graminea*. However, the authors did not include another morphologically similar *Plangia* sp., namely *P. unimaculata* Chopard, in their revision - which is also present in the Western Cape (Chopard 1955). Hemp *et al.* (2015) suggest that only one

*Plangia* species is present in the Cape and, therefore, the focal species of this study is assumed to be *P. graminea* until further taxonomic investigations can be done to prove otherwise.

Original description of *P. graminea* extracted and translated from Serville (1839):

"(Length about 10 lines). It resembles *Phylloptera laurifolia*, but it is significantly smaller. Body yellowish green. Head of the same colour, without advanced front projection. Prothorax smooth, plain, green, sometimes becoming yellowish. Lateral carinae of the disc fairly well pronounced, bordered constantly by a more or less distinct pale yellow longitudinal line. Elytra green, opaque, somewhat shiny, oval, ending in rounded tip. Their longitudinal vein a little oblique, but not overly branched; stridulating organ rippled, yellowish in the centre of the left elytron. Hind-wings transparent, pointed at the end, at rest the protruding part is opaque green (hind-wing extends past the elytron at rest). Ovipositor short, greenish yellow; subanal plate barely exceeding the male abdomen.

Antennae and legs green; forelegs wider on the inside, covered with a membrane; femur slightly spiny on the lower side; upper carina of hind legs lined with fine spines. Male and female.

The male differs greatly from that of *laurifolia* by the shape of the subanal plate which is much shorter and only forked at the end. In the male of *laurifolia*, the plate is extended beyond the abdomen, and divided into two long branches at the end.

The Cape of Good Hope. My collection"

**General biology and ecology of katydids**

Katydids are diurnal, nocturnal or both (Belwood 1990) leading to a variety of different lifestyles within the group to fill a broad spectrum of ecological niches reflected in their diverse behavioural patterns (Bailey & Rentz 1990) and habits. This makes these insects attractive to most fields of research, which has been hardly explored to date. In South Africa, all but a small minority of katydid species are nocturnal.

## Food and feeding

Katydids have a broad spectrum of feeding habits, ranging from species that are omnivorous to highly specialised phytophagous species with their biology finely tuned to the phenology of their host-plant. Some feed on flowers and foliage, others on seeds and fruit whereas some are highly specialised to feed on pollen and nectar. Herbivorous species consume a wide variety of plants ranging from the foliage of trees and shrubs to grasses. Many, however, are opportunistic and will feed on any food source available – including their dead relatives. Some are exclusively predatory. Sometimes katydid nymphs differ in feeding habits from those of their adults. A number of groups, for example the Phaneropterinae, are primarily foliage feeders. Some katydids within these groups prefer the more proteinaceous parts of the plant such as the flowers, but can also develop successfully on the leaves alone (Rentz 2010).

## Reproduction

The reproduction of katydids revolves around communication. Most males produce calling songs detected by females, who when receptive, proceed to the source of the song. Research shows that females can be particularly selective when choosing a mate. Based on the quality of the male song, female katydids detect the fittest males to mate with (Rentz 2010).

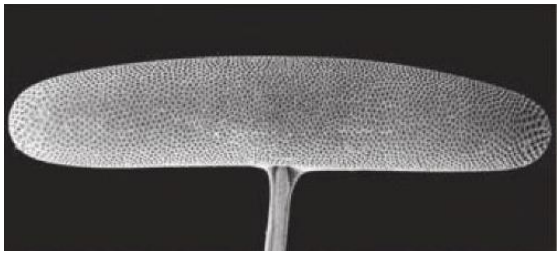
In the act of mating, the male transfers a spermatophore containing the sperm package. Included with the spermatophore is a nuptial gift, the spermatophyllax, which is eaten by the female (Lehmann 2012). This is a source of nutrition for the female and formation of her eggs and as such is the male's contribution to the development of their offspring. As the spermatophyllax is eaten by the female, sperm enters her receptacle, the spermatheca, an internal structure near the tip of her abdomen. The eggs pass the spermatheca as they are being deposited and are fertilised on their route (Rentz 2010). An extensive review of the reproductive behaviour and evolution of katydids (Tettigoniidae) is given by Gwynne (2001).

The abdomina of the male and female katydid have unique characters with those of the male usually more obvious. Insect genitalia can be considered to operate like a 'lock-and-key' mechanism. The male's genitalia are specifically designed to fit into those of a female of the same species. If the coupling is not possible, successful mating will not happen. In this manner,

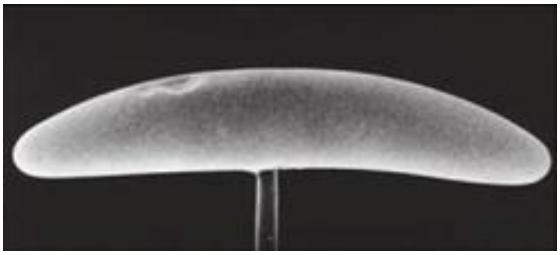
some tettigoniids have evolved some very unique genital structures (Rentz 2010). In most katydid species the cerci appear to be distinctive structures enabling, by comparing the cerci of most males, an accurate identification. The tip of the cercus in the Phaneropterinae is also distinctive, but it usually requires the use of a high-powered microscope to clearly observe this particular structure (Rentz 2010).

## Eggs

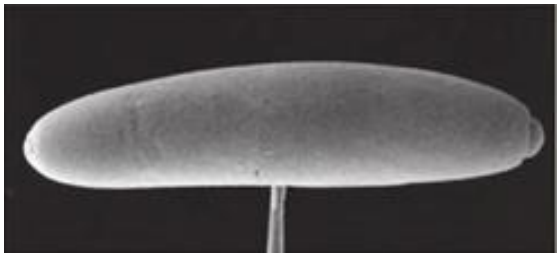
Katydids oviposit their eggs in and on a variety of substrates. Although katydid eggs are not as diverse as those of, for example, stick insects (Phasmatodea), they do have distinctive subtle features which need to be studied under high magnification. As is expected from such a diverse group of insects, there is a wide range of egg-types (Fig. 6). The micropyles are contained on the depressed dorsal area of the egg. The egg is fertilised when the micropylar area passes by the opening of the spermatheca as the egg is laid. For most species, the appearance and position of the micropyle on the egg is characteristic and species-specific (Rentz 2010).



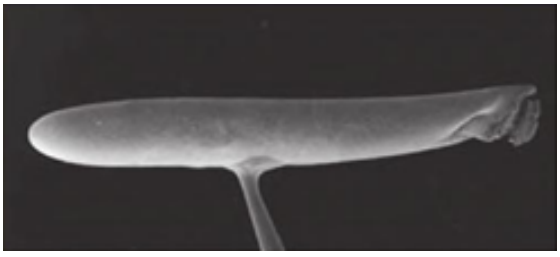
The egg of *Chlorobalius leucoviridis* has a simple lattice-like pattern.



The egg of *Neophisis ecmurra* lacks an obvious lattice pattern.



The eggs of Tympanophorinae species have a cap at one end



The cap on the top of the egg of *Indiamba malkini* is apparently for protection or to transport air to the egg which is laid in wood.

**Fig. 6.** Some katydid egg-types. Taken from Rentz (2010).

The ovipositor of most katydid females is sharply pointed and elongated, and specially designed for laying eggs in the ground. There are four parts to the ovipositor, each operating alternately; with the ovipositor going a little deeper into the ground with each stroke. The shape of the ovipositor is often indicative of the egg-laying site. If the ovipositor is elongate and of uniform dimensions, the eggs are usually laid in hollow grass stems. If the eggs are laid in plant material, either living stems or dead wood, the ovipositor is usually sickle-shaped. Some katydids even lay their eggs in the tissue of plant galls (Rentz 2010). Many species have elaborate saw-like, toothed ovipositors which are specifically designed to saw into specific leaf or bark substrates for oviposition [e.g. Naskrecki & Bazelet 2011: *Austrodontura* (Phaneropterinae)].

The eggs are laid during summer, develop over winter and hatch the following spring. This is the general life history of most katydids and one generation per year appears to be the norm for most species in temperate regions (Gwynne *et al*. 1988; Rentz 2010). However, eggs of some species undergo diapause and several European species require up to three winter seasons before the eggs hatch. On the other hand, species in the tropics hatch without going through a dormant period, only requiring about 50 days to develop (Rentz 2010), resulting in generations to overlap with eggs, nymphs and adults at the same time and in the same place. Hartley (1990) provides a detailed account of the egg biology of the Tettigoniidae. One aspect that the current study focussed on was monitoring and identification of the eggs of *P. graminea*, to establish a potential monitoring system for growers.

**Growth and development**

The eggs usually hatch at dawn. The hatchlings (called nymphs) look like tiny wingless versions of the adult; however, the nymphs of many groups look completely unlike the adult. This is especially true for phaneropterine species. Some nymphs resemble spiders and others assassin bugs or ants, while others resemble floral parts as a strategy to escape predation. This mimicry lasts until after the first or second moult, at which point they are less susceptible to predation. As the nymphs mature they rely on other camouflage strategies, normally resembling plants or parts of plants. Many species utilise a combination of structure, colour and behaviour that make them almost invisible in their surroundings (Rentz 2010).

For katydids to grow, they have to shed or moult their skins periodically. Depending on climatic conditions and the species, the nymphs moult from four to nine times. When a katydid is ready to moult, it usually hangs upside down at the tip of a twig or in vegetation. Moulting usually happens at night when relative humidity is sufficiently high to moisten and shed the old skin (Rentz 2010).

**Dimorphism and polymorphism**

Sexual dimorphism and colour polymorphism has been observed in many katydid species. In all species, males of leaf-mimicking katydids are smaller than their female counterparts (Castner & Nickle 1995). Colour polymorphism is also common in this subfamily with species belonging to the genera *Pterochroza*, *Mimetica*, and *Typophyllum* displaying up to seven different colour morphs (Castner & Nickle 1995). Similarly, Brits & Thornton (1981) noted that males of *Ruspolia differens* were also smaller than females and often green or brown in colour. The North American oblong-winged katydid, *Amblycorypha oblongifolia* (De Geer), displays an array of different colour morphs ranging from green, yellow, orange to pink (Crew 2013) (Fig. 7).


**Fig. 7.** Illustrated examples of the different colour morphs of *Amblycorypha oblongifolia*. Taken from Wikipedia (2015).

**Natural enemies of katydids**

Katydids form a source of food for many invertebrates and vertebrates and are attacked by many parasites (Rentz 2010). They are preyed upon by spiders, lizards, frogs and birds. Bats are known to home in on calling katydids. To evade predators, katydids have evolved various

techniques that help them escape from predation (Belwood 1990). Examples of these include the colour and pattern of camouflage and to confuse bats many katydid species having evolved various stridulation strategies (Rentz 2010; Belwood 1990).

Flies of the family Tachinidae and digger wasps (family Sphecidae) are examples of insect predators. Ormiine tachinids locate male katydids by listening to their calling songs (Rentz 2010). Parasitism of *Orchelimum* katydids by *Ormia lineifrons* (Tachinidae) has been recorded by Shapiro (1995). A number of parasitic wasps from various families parasitize the eggs of katydids (UC IPM 2015). Other insect predators include bugs (Heteroptera) that manage to ambush katydids much larger than they are. Ants are a constant threat and some katydid species are specialised to feed on other species of katydids (Rentz 2010).

Katydids fall victim to Gordian worms (Nematomorpha: Gordioidea). These horse-hair-like worms are internal parasites that occupy the greater part of the host's abdomen. Other parasites include gregarines and entomopathogenic fungi (Rentz 2010). *Beauveria bassiana* and *Metarhizium acridum* fungal strains are being used as biological control options against locusts and grasshoppers (Lomer *et al.* 2001) and have been tested against katydid pests, for example *Uvarovistia zebra*, yielding promising results (Mohammadbeigi & Port 2013).

The twisted-wing parasites, Strepsiptera, also infect katydids (Rentz 2010). *Sexava nubila* Stål, *Segestidea noveaguineae* and *Segestes decorates* Redtenbacher *(*Brancsik) (Orthoptera: Tettigoniidae) are parasitized by female *Stichtrema dallatorreanum* Hofender (Strepsipera) (Kathirithamby *et al.* 2001). These katydid species are all pests on oil palm in Papua New Guinea. The potential of Strepsiptera parasites as novel biocontrol tools for use in an integrated pest management system against the katydid pests of oil palm has been investigated by Kathirithamby *et al.* (1998). They concluded that *Stichotrema dallatorreanum* (Strepsiptera) has an effect on the fecundity and overall fitness of its tettigoniid hosts and therefore may have potential as a biocontrol agent.

Some parasites attack katydids but do not seem to harm or kill them. The auditory trachea of a number of species are often infested by tiny mites. Blood-red mites attached to the tegmina

(wings) or on the intersegmental membranes of a katydid feed off the blood of the host (Rentz 2010). In southern France a few populations of the genus *Platycleis* were found to be infested with larval mites of the trombidiid genus *Eutrombidium* (Samways 1977).

## Katydid sound production and hearing

Katydid sound and hearing are probably the most fascinating and best studied aspects of katydid biology. The unique features of tettigoniids' obvious auditory communication have been used by physiologists and functional anatomists to investigate processes of sound production and reception and many biologists dedicate themselves to 'learn the language' of these insects. For the purpose of this chapter the workings of katydid sound and hearing will only be discussed briefly as follows:

### Sound

Tegminal stridulation, of the 'file and scraper' method, is most commonly used by katydids to produce sound (see Fig. 8). It entails a file of minute teeth (pars stridens) located on the underside of the left tegmen (1) moving over a raised vein, or hardened scraper (plectrum), on the upper surface of the right tegmen (2) when the wings are opened and closed. The mirror (3), surrounded by a sclerotised U-shape frame, resonates and amplifies the sound caused by the tooth strikes across the plectrum (Morris & Pipher 1967; Bailey 1970; Bailey & Broughton 1970; Ewing 1989; Bailey 1991; Morris & Mason 1995; Greenfield 1997; Desutter-Grandcolas 2003; Rentz 2010; Grant 2014). In some species there is also a vestigial file on the right tegmen that does not seem to play any role in stridulation (Rentz 2010). Since the stridulatory apparatus is being used to produce the sound to which females respond, the shape and structure of the tegmen is extremely detailed (Rentz 2010) and the calls are very species-specific. Therefore, the length, shape and number of teeth in the stridulatory file are unique for each species and often used in taxonomy (Rentz 2010, Heller *et al.* 2015) (Fig. 9).

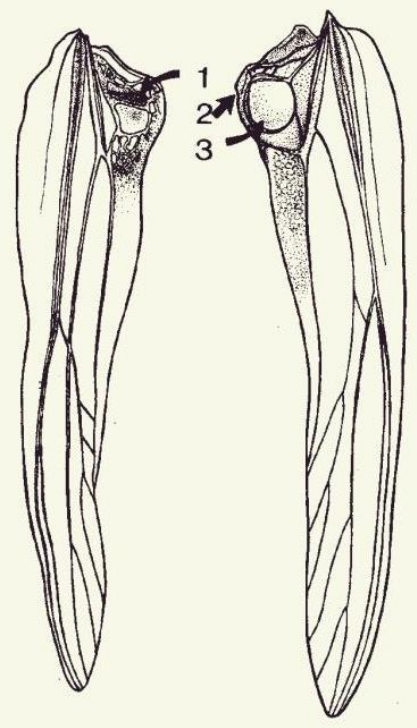
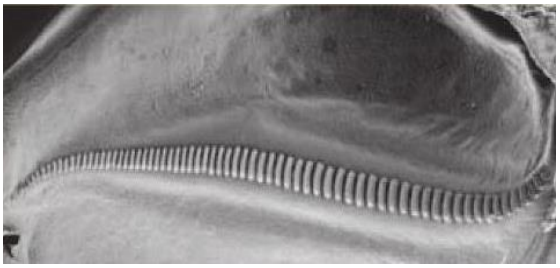
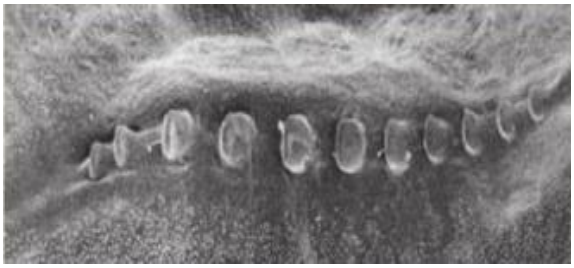



**Fig. 8.** The 'file and scraper' mechanism of a katydid. Taken from Rentz (2010).

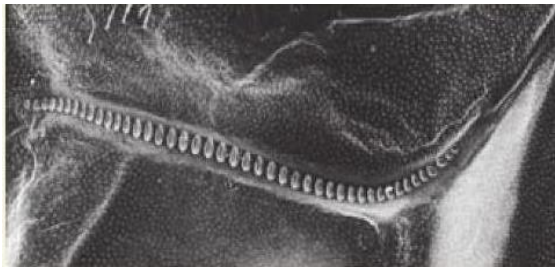
Although acoustic signalling in katydids is predominantly a masculine feature and the females usually only play the role of a silent receiver (Robinson 1990), the females in some families (especially Phaneropterinae) also possess sound producing structures (Nickle & Carlysle 1974; Rentz 2010). However, the signals produced by females consist only of one or a few fleeting ticks in response to the male sound. Nickle & Carlysle (1974) provide more information on the morphology and function of female sound-producing structures.



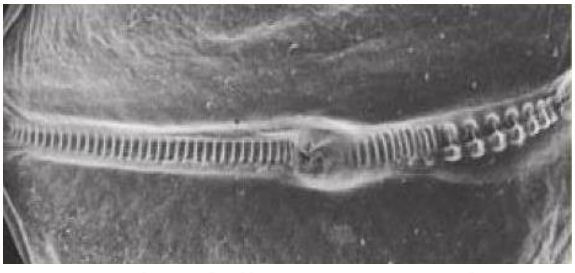
*Requena kerla* (Listroscelidinae)



*Zaprochilus australis* (Zaprochilinae)



*Austrophlugis debaari* (Listroscelidinae)



*Tympanophora kalbarri* (Tympanophorinae)

**Fig. 9.** Scanning electron microscope photographs of different types of stridulatory files of male katydids. Taken from Rentz (2010).

## Hearing

The structure of the file and scraper produces sound of a rather narrow range of frequencies and, therefore, it is often thought that katydids only detect the calls of conspecifics. However, studies in Western Australia revealed that male katydids are able to hear the calls of other species and will move away from nearby callers to avoid rivalry (Rentz 2010). Signal reception is also critical for detection of predators (Rössler *et al*. 2006), resulting in anti-predator behaviour (Grant 2014).

**Fig. 10.** The auditory tympanum on the fore tibia of a Phaneropterine katydid. Taken from Rentz (2010).

Katydids hear sounds by way of tympanal organs (thin membranes) located on the fore tibiae (Grant 2014) (Fig. 10). Hearing by katydids is a very complex subject and has been studied widely (Rentz 2010). The intricacies of katydid hearing are too detailed to discuss here. For comprehensive discussions of hearing by katydids Bailey (1990), Gwynne (2001) and Gerhardt & Huber (2002) should be consulted.

## Economic importance

Some katydids produce sporadic outbreaks and become obvious when their population numbers greatly increase. This occurs generally in response to favourable weather conditions. Other species of katydids become strongly gregarious at times and swarming behaviour, much like locusts, can be observed in some species. This can result in serious agricultural damage (Bailey & McCrae 1978; Rentz 1991). The Mormon Cricket (*Anabrus simplex* Haldeman) is probably one of the most devastating examples of swarming katydids, forming bands that travel more or less in a consistent direction for days or even weeks (Bailey & McCrae 1978).

Katydids can have detrimental effects on horticulture. The controlled conditions of glasshouse orchid culture and shade-houses provide ideal conditions for some katydid species that feed on new shoots and developing flowers (Rentz 2010). Predatory species have also been noted to ravage the stock of insect and butterfly zoos (Rentz 2010). Katydids feed on developing fruits

of citrus, causing the fruits to become disfigured (Fig. 11) and, therefore, reducing the market value resulting in economic loss to producers (Rentz 2010; UC IPM 2015).

Katydids are pests on a variety of different crops in many parts of the world. Table 1, based on findings in available literature, provides a summary of katydid pest species and the affected crops in different parts of the world.

Not all katydids in large numbers, however, have a negative economic impact on their cohabiting communities. *Ruspolia nitidula* (Nsenene grasshoppers), an abundant katydid in Uganda, is considered a delicacy and eaten by a large section of the population; and, in addition, provides a valuable source of income for many people in central Uganda (Agea *et al*. 2008).

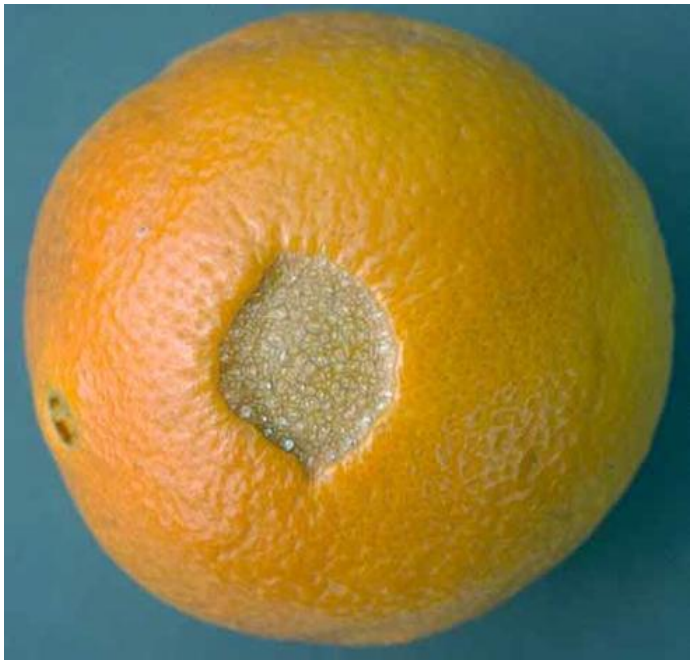


**Fig. 11.** A single circular scar on a mature navel orange from forktailed bush katydid, *Scudderia furcata*, feeding when the fruit was young. Photo by Elizabeth E. Grafton-Cardwell. UC IPM (2015).

**Table 1.** Summary of recorded crops affected by katydids based on findings in available literature sources.

| Common Name | Scientific name | Region/Locality | Crop | Source |
|---|---|---|---|---|
| Forktailed bush katydid | *Scudderia furcata* Brenner | USA, California | Pear, citrus and stone fruit | Bentley *et al*. (2002)<br>Varela (2008)<br>UC IPM (2015) |
| Mediterranean katydid | *Phaneroptera nana* Fieber | USA, California | Pear orchards | Varela (2008) |
| Angularwinged katydid | *Microcentrum retinerve* (Burmeister) | USA, California | Citrus | UC IPM (2015) |
| Mormon Cricket | *Anabrus simplex* Haldeman | Western United States | Sagebrush rangelands | Redak *et al*. (1992) |
| Unknown | *Idiarthron subquadratum* Saussure & Pictet<br>*Idiarthron atrispinum* (Stål) | Central America | Coffee | Reyes de Romero (1986) |
| Anatolian Bright Bush-cricket | *Poecilimon anatolicus* Ramme | Turkey | Cereals and vegetables | Tutkun & Unal (1986) |
| Sexava | *Segestes decorates* Redtenbacher<br>*Segestidia defoliaria defoliaria* Uvarov<br>*Segestidea novaeguineae* (Brancsik)<br>*Segestidea gracilis gracilis* (Willemse)<br>*Sexava nubila* (Stål)<br>*Sexava coriacea* (Linnaeus) | Papua New Guinea | Oil palm, bananas, coconuts | Kathirithamby *et al*. (1998)<br>Young (1998)<br>Kathirithamby *et al*. (2001) |
| Unknown | *Uvarovistia zebra* (Uvarov) | Iran | Field crops and rangeland grass | Mohammadbeigi & Port (2013) |
| Leaf katydids | *Plangia* species | Uganda | Agroforestry (*Alnus* species) | Nyeko *et al*. (2002) |
| Senene | *Ruspolia differens* (Serville) | East Africa | Multiple (including maize and tobacco) | Bailey & McCrae (1978)<br>Blair (1990) |
| Armoured bush crickets (ABC) | *Acanthoplus speiseri* Brancsik<br>**Acanthoplus discoidalis* (Walker) | southern Africa (Botswana and Namibia) | Cereals (sorghum, pearl millet)<br>*Attacks nestling Red-billed Quelea | Wohlleber (1994)<br>Green & Holt (2003)<br>*Cheke *et al*. (2003) |
| Wart-biter | *Decticus verrucivorus* (Linnaeus) | Iran | Cereals, cotton | Gentry (1965) |

### *Plangia graminea*, a South African pest in vineyards

In the past few seasons (since 2012) *P. graminea* appears to have caused a substantial amount of damage leading to great concern among the wine farmers of the Western Cape Province, South Africa (Allsopp 2012). The species is widespread in South Africa and has also been collected as far north as Kampala in Uganda (Allsopp 2012). Very little is known about the biology and ecology of this species as it has not been studied yet. The majority of information available is from observations made by growers. Furthermore, no detailed surveys have taken place to assess the assemblage structure of katydids and verify their taxonomic status.

The immature stages of *P. graminea* appear early in the season (September/October) and feed on young foliage of the vine, especially at night. The feeding can extend to young grape clusters later in the season (Annecke & Moran 1982; Ferreira & Venter 1996). Unlike other pests that typically feed from the edge of the leaf inwards, these katydids can start eating from anywhere on the leaf (Fig. 12 A). The nymphs are dark brown to black with orange legs and resemble toxic Leaf beetles (Chrysomelidae) with the characteristic long antennae also readily noticeable (Fig. 12 B). As they mature they change in colour and the adults are usually green, resembling leaves (Fig 12 C). The wings of the adults have a rounded outside edge that give this katydid a hump-backed appearance, hence the local Afrikaans name “krompokkel” (Allsopp 2012) which literally translates back to “crooked-fatty”.


**Fig. 12.** Examples of (**A**) feeding damage on leaves; (**B**) "krompokkel" nymphs; (**C**) *P. graminea* adult [Photo: Allsopp (2012)], in vineyards of the Western Cape.

These katydids are often associated with invasive hosts in disturbed areas (P. Grant, pers. comm.) and can sometimes also be abundant in pine plantations. However, their natural host in the Western Cape is believed to be *Rhus angustifolia*, an abundant shrub in this area (H. Geertsema, pers. comm.). The eggs are typically oval shaped. Observations in vineyards indicate that the eggs are laid under the loose bark of grapevines (Allsopp 2012).

Reasons for the recent outbreaks are unknown, but favourable climate could be an attributing factor as well as changes in pesticide usage. Broad spectrum pesticides that may have supressed population outbreaks are being used far less as farmers are leaning towards more sustainable, environmentally-friendly practices (Allsopp 2012). There are currently no registered chemical control measures for this pest.

**Integrated Pest Management**

Integrated Pest Management (IPM) can be defined as:

"a pest management system that in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest population at levels below those causing economic injury" - (Smith & Reynolds 1966).

IPM is increasingly being used to control orthopteran pests. Although it involves the use of 'biopesticides' or biological control, the adroit use of conventional chemical pesticides is often inevitable. Knowledge of the ecology of the target pest in context of the phenology of the infested crop is crucial with each orthopteran pest having its own independent dynamic and the crop being a man-made extension of its natural habitat (Jago 1997). When a suitable set of interventions has been determined, the decision to initiate one or more IPM components is based on insight into the short-term future and current ecological conditions, seasonal advancement of the pest population and the vulnerability of the crop to the physical elements such as rainfall and soils of the farm ecosystem (Jago 1997). Monitoring is therefore a crucial component of any IPM program and the first step to developing an IPM plan is to determine an effective monitoring method specifically suited for the pest and its agricultural system (Luckmann & Metcalf 1994).

A generic monitoring system has been developed for the key arthropod pests of table grapes by De Villiers & Pringle (2008), however, it does not include the grape pest *P. graminea*. Furthermore, an economic threshold has not yet been established for this pest.

**The Grapevine**

Grapes have been grown in Mesopotamia from as early as 6 000 BC, making it one of the earliest cultivated fruits according to archeologists (Allsopp *et al*. 2015). Grapes were introduced into Syria about 5 000 BC, and from there, viticulture spread into Palestine, eventually reaching Egypt and Greece (Baker & Waite 2003; Allsopp *et al*. 2015). Further, with the expansion of the Roman Empire throughout Europe, vine culture eventually reached the British Isles and the present world-wide range of Western society – a range that later engulfed the southern tip of Africa some 300 years ago (Bagnall 1961; Baker & Waite 2003; Allsopp *et al*. 2015).

It was Jan van Riebeeck who first introduced the vine to the southern part of Africa after his arrival in 1652 when he came to establish a replenishment facility at the Cape. Today, South Africa is one of the world's major wine producing countries, ranking 7$^{th}$ in overall volume production and producing 4.2% of the world's wine (WOSA 2014). Recent statistics from "South African Wine Industry Information & Systems" (SAWIS) show that 99 680 hectares of vines producing wine grapes are under cultivation in South Africa. According to a Macro-economic Impact study commissioned by SAWIS, the wine industry contributed R36 145 million to the annual GDP of South Africa in 2013, of which approximately 53% remained in the Western Cape. Furthermore, the study found that the wine industry provided (directly and indirectly) employment opportunities to some 300 000 people in South Africa, and was responsible for the employment of 167 494 persons in the Western Cape alone (SAWIS 2015).

In South Africa, the main production areas are found in the provinces of the Western Cape and the Northern Cape, however, commercial producers of grapes are also present in Limpopo and Mpumalanga (James 2013; Allsopp *et al*. 2015). The "Wine of Origin Scheme" (WO) divides the production areas into demarcated regions, districts and wards. James (2013) provides a

comprehensive discussion on the WO system, and detailed maps of regions, districts, and wards are provided by SAWIS (2015).

*Vitis vinifera* is a deciduous sun-loving plant, which does best in regions that experience warm, dry summers, frost-free springs and wet winters cold enough to induce dormancy, but not too harsh as to damage the plant (Baker & Waite 2003; Hurndall 2005). Grapevines have four main phenological stages. The first stage of growth is bud-break (September – October). Bud-break is followed by the period of bloom (November – December) which is followed by the final growth stage, *véraison* (January – March). After *véraison* the grapes are ready to be harvested (Araujo 2014 and references therein). After the harvest period the grapevine will go into winter dormancy and the cycle will continue the next season. These phenological stages are influenced by environmental factors, such as climate; therefore, their durations may vary from year to year (Conradie *et al.* 2002). Moreover, different cultivars grow differently and their growth stages may vary slightly, leading to an overlap of stages within a group of cultivars.

Agricultural systems are under continuous pest pressure and grape production is no exception. In South Africa grapevines are host to 35 insect pests from various families (Allsopp *et al.* 2015). The most important pest species are treated in the following table (Table 2) with regard to type of damage, management practices, and their natural enemies.

**Table 2.** Summary of the main insect pests found on grapevines in South Africa, with emphasis on the type of damage, management practices and natural enemies. Adapted from Allsopp *et al*. (2015).

| Common name | Scientific name | Feeds on | Management | Natural enemies | Further reading |
|---|---|---|---|---|---|
| BUGS | HEMIPTERA | | | | |
| Leafhoppers | Family Cicadellidae | | | | |
| Grapevine leafhopper | *Acia lineatifrons* (Naudé)<br>*Mgenia fuscovaria* (Stål) | L<br>L, V | Seldom requires control; chemical control options available when necessary | Nymphs preyed on by generalist predators (spiders, ladybird beetles), parasitic wasps (no specific ID) | Marais (1989) |
| Phylloxerans | Family Phylloxeridae | | | | |
| Grapevine phylloxera | *Daktulosphaira vitifoliae* (Fitch) | L, R | Effects reduced by good growing conditions. No insecticide registered. Resistant rootstocks | No natural enemies have been identified locally | De Klerk (1981) |
| Armoured scale insects | Family Diaspididae | | | | |
| Red Scale | *Aonidiella aurantii* (Maskell) | Br, Sh | Biological control agents: *Chilocorus nigrita* (Fabricius), *Aphytis spp., C. bifasciata.* Preventative control (mineral oils) and Corrective control (methomyl - emergencies only to prevent resistance) | Parasitic wasps (aphelinids and encyrtids). Predators include ladybird beetles, various lacewing species and a predatory mite, *Cheletogenes ornatus* | Grout *et al*. (1989) Grout & Richards (1991) |
| Pernicious scale | *Diaspidiotus perniciosus* (Comstock) | Br, Sh | Chemical control (dormant season treatments) | Parasitic wasps (families Encyrtidae and Aphelinidae). Predators include various species of ladybird beetles | |
| Ground pearls | Family Margarodidae | | | | |
| Ground pearl<br>Ground pearl<br>Ground pearl | *Margarodes capensis* Giard<br>*Margarodes greeni* Brian<br>*Margarodes prieskaensis* (Jakubski) | R<br>R<br>R | No chemical registered and no resistant rootstocks known | No information on the natural enemies of these ground pearls | De Klerk (1981)<br>De Klerk *et al.* (2011) |

| Common name | Scientific name | Feeds on | Management | Natural enemies | Further reading |
|---|---|---|---|---|---|
| Ground pearl | *Margarodes termini* Giard | R | | | |
| Ground pearl | *Margarodes vredendalensis* De Klerk | R | | | |
| Mealybugs | Family Pseudococcidae | | | | |
| Grapevine mealybug | *Planococcus ficus* (Signoret) | B, Br, Bs, L, Sh, T, V | Regular monitoring. If 2% of grapevines infested, natural enemies can be purchased. Ant control (chemicals) when 20% of the vines are infested with ants. Overwintering mealybug controlled by applying suitable insecticide to dormant grapevines<br><br>Biological and chemical control | Indigenous and introduced natural enemies control grapevine mealybug e.g. various parasitic wasps, the indigenous encyrtid, and various predatory ladybird beetles. Other generalist predators such as lacewing larvae also contribute to the natural control of mealybugs in vineyards | De Klerk (1981)<br>De Villiers & Pringle (2008)<br>Mgochecki & Addison (2009)<br>Nel (1983)<br>Walton & Pringle (2004) |
| Long-tailed mealybug | *Pseudococcus longispinus* (Targioni Tozzetti) | B, Br, Bs, L, Sh, T, V | | Hymenopteran parasitoids, ladybird beetles and lacewing larvae | Wakgari & Giliomee (2004) |
| THRIPS | THYSANOPTERA | | | | |
| Thrips | Family Thripidae | | | | |
| Western flower thrips<br>Guava thrips | *Frankliniella occidentalis* (Pergande)<br>*Heliothrips sylvanus* Faure | B, F, L, Sh<br>L | Monitor with blue sticky traps. Develop resistance to pesticides very rapidly, which limits available control options. Do not disturb/mow weeds and other flowering plants that also attract thrips near vineyards while the grapes are flowering | Predatory insects such as lacewings, ladybird beetles, hover flies, anthocorid bugs and predatory mites | Allsopp (2010)<br>Lewis (1997)<br>Schwartz (1989) |
| BEETLES | COLEOPTERA | | | | |
| Snout beetles, weevils | Family Curculionidae | | | | |

| Common name | Scientific name | Feeds on | Management | Natural enemies | Further reading |
|---|---|---|---|---|---|
| Black snout beetle | *Eremnus atratus* (Sparrman) | L | Integrated management approach using chemical, physical and cultural control | Wide range of natural enemies including entomopathogenic fungi and nematodes, mites, dipteran and hymenopteran parasitoids, protozoans, ants, spiders, predatory beetles and birds | Nel (1983) |
| Speckled weevil | *Eremnus cerealis* Marshall | B, BS, Bu, L, Sh | | | |
| Vine weevil | *Eremnus chevrolati* Oberprieler | B, BS, Bu, L, Sh | | | |
| Grey weevil | *Eremnus setulosus* Boheman | B, BS, Bu, L, Sh | | | |
| White fringed weevil | *Naupactus leucoloma* Boheman | B, BS, Bu, L, Sh | | | |
| Banded fruit weevil | *Phlyctinus callosus* (Schönherr) | B, BS, Bu, L, Sh | | | |
| Sciobius weevil | *Sciobius tottus* (Schönherr) | L | | | |
| Bud nibbler | *Tanyrhynchus carinatus* Boheman | Bu | | | |
| FLIES | DIPTERA | | | | |
| Fruit flies | Family Tephritidae | | | | |
| Mediterranean fruit fly | *Ceratitis capitate* (Wiedemann) | B | Biological control (not always regarded as successful). Monitoring populations with lure-baited traps, control of host plants, sanitation, application of fruit fly bait, the use of bait stations, augmentative releases of parasitoids and the use of the sterile insect technique (SIT). Fruit flies are best managed by an area-wide, integrated approach incorporating as many of the above-mentioned practices as possible | Entomopathogenic fungi, nematodes, and bacteria, microsporidian, ants and spiders. Hymenopterous parasitoids make the largest contribution to biological control | Barnes (2008)<br>De Meyer *et al*. (2002)<br>White & Elson-Harris (1992) |
| Natal fruit fly | *Ceratitis rosa* Karsch | B | | | |
| BUTTERFLIES, MOTHS | LEPIDOPTERA | | | | |
| Carpenter moths | Family Cossidae | | | | |

| Common name | Scientific name | Feeds on | Management | Natural enemies | Further reading |
|---|---|---|---|---|---|
| Apple trunk borer | *Coryphodema tristis* (Drury) | Br, T | No chemical control is available. Remove and burn infested plant material | No information available | De Klerk (1981)<br>Myburgh & Basson (1960) |
| Leaf roller moths | Family Tortricidae | | | | |
| Pear leaf roller | *Epichoristodes acerbella* (Walker) | B, Bs, Bu, F, L | Monitor with pheromone traps. Remove infested vine water-shoots at the beginning of the season. Inspect grape bunches for leaf roller larvae biweekly until harvest. Weed management | Parasitic wasps | Blomefield & Du Plessis (2000)<br>De Villiers & Pringle (2007)<br>De Villiers & Pringle (2008) |
| False codling moth | *Thaumatotibia leucotreta* (Meyrick) | B | Pheromone monitoring system. Orchard sanitation (Citrus) and biological control (*T. cryptophlebia*). Effectively controlled in peach and nectarine orchards using insecticides | The hymenopteran egg parasitoid, *Trichogrammatoidea cryptophlebia* Nagaraja (Trichogrammatidae). Larval parasitoids (mostly wasps, but also a few flies). *Orius* bugs (Anthocoridae) prey on FCM eggs and assassin bugs (Reduviidae) can attack FCM larvae. Ants are also very effective predators of FCM pupae. Two virus species and two species of entomopathogenic fungi have also been recovered from FCM larvae | Blomefield (1989)<br>Daiber (1976)<br>Moore & Kirkman (2008) |
| Hawk moths | Family Sphingidae | | | | |
| Silver-striped hawk moth<br>Arum hawk moth<br>Large striped hawk moth | *Hippotion celerio* (Linnaeus)<br>*Hippotion eson* (Cramer)<br>*Hippotion osiris* (Dalman) | L<br>L<br>L | Insecticidal control not warranted due to their sporadic occurrence, however, stomach and contact | Eggs parasitized by parasitic wasps (family Trichogrammatidae). Larvae parasitized by parasitic flies (family | Pinhey (1962) |

| Common name | Scientific name | Feeds on | Management | Natural enemies | Further reading |
|---|---|---|---|---|---|
| Grapevine hawk moth | *Theretra capensis* (Linnaeus) | L | poisons are effective. Destroy larvae on site | Tachinidae) and by pteromalid, braconid and ichneumonid wasps | |
| Forester moths | Family Agaristidae | | | | |
| Trimen's false tiger | *Agoma trimenii* (Felder) | L | No insecticides registered, however, table grape producers attempt control by targeting the larvae with insecticides registered for the control of other lepidopteran pests of grapevines, or with products containing *Bacillus thuringiensis* | No information available | Pretorius *et al*. (2012) |
| Owlet moths | Family Noctuidae | | | | |
| African bollworm | *Helicoverpa armigera (*Hübner) | B, L, Sh | Chemical control available | Egg and larval parasitoids (tachinid flies and parasitic wasps). Predators include ground and ladybird beetles, ants, earwigs, lacewings, anthocorid and mirid bugs, predatory mites and spiders. | De Villiers & Pringle (2007)<br>De Villiers & Pringle (2008)<br>Van den Berg *et al*. (1988) |
| Tomato moth | *Spodoptera littoralis* (de Boisduval) | L, Bs | No insecticides registered for this pest. Hand collecting in small plots | Parasitic tachinid flies and encyrtid, pteromalid, braconid and ichneumonid wasps | |
| CRICKETS, KATYDIDS, GRASSHOPPERS, LOCUSTS | ORTHOPTERA | | | | |
| Katydids | Family Tettigoniidae | | | | |

| Common name | Scientific name | Feeds on | Management | Natural enemies | Further reading |
|---|---|---|---|---|---|
| Krompokkel | *Plangia graminea* (Serville) | Bu, L, Sh | No insecticides registered for this pest | No information available | |

## Aims and objectives

The overall aim of the present study was to investigate the biology and ecology of *P. graminea* in vineyards of the Western Cape to contribute knowledge towards the formulation of a sustainable integrated pest management program, as well as to establish an appropriate monitoring system.

The objectives of the study were:

i) to identify the katydid species present in vineyards of the Western Cape and determine their pest status and assemblage structure (if more than one species)

ii) to investigate the general biology, ecology and seasonal population dynamics of katydids in vineyards

iii) to develop and establish an appropriate monitoring system for katydids based on the generic pest monitoring system developed by De Villiers & Pringle (2008); and

iv) to identify natural enemies present in vineyards that could potentially be used as biological control agents against this pest

The chapters that follow are presented as separate publishable papers and, for this reason, some repetition in the different chapters is unavoidable.

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# CHAPTER 2

## Identification of katydid (Orthoptera: Tettigoniidae: Phaneropterinae) species present in vineyards of the Western Cape - with notes on their morphology, eggs, and assemblage structure


## ABSTRACT

Many katydid species in the subfamily Phaneropterinae (Tettigoniidae) are considered pests in agricultural systems due to their characteristic feeding behaviour – nearly exclusively herbivorous. *Plangia graminea* (Serville) is a sporadic pest in vineyards in the Western Cape. However, it is uncertain whether *P. graminea* is the only phaneropterine species causing damage to vineyards. Moreover, the taxonomic uncertainty of the *P. graminea* species complex begs the question whether more than one *Plangia* species is present, or if *P. graminea* is the only *Plangia* species present in the Western Cape. A survey of katydid species was performed in vineyards in the greater Stellenbosch region of the Western Cape. Katydids collected during the survey were identified. Three Phaneropterinae species were identified namely *P. graminea*, *Eurycorypha lesnei* Chopard and an unidentified *Phaneroptera* species. *Plangia graminea* appears to be the only *Plangia* species present; however, I suggest that *P. unimaculata* Chopard should be included in the taxonomic review of the *P. graminea* species complex. *Plangia graminea* and *E. lesnei* have the same general appearance upon first inspection and therefore I determined key morphological features that can be used to distinguish between the two species. *Plangia graminea* and *E. lesnei* differ in morphology with regards to their wings, male stridulatory files, their nymphs and their eggs.


## INTRODUCTION

The family Tettigoniidae, commonly referred to as katydids, is the second largest group of Orthoptera (Gangwere *et al.* 1997). Their great diversity and wide range of biological habits have made them attractive study organisms in many fields of research including ecology, behaviour, physiology and functional anatomy (Bailey & Rentz 1990). This is especially true for the Phaneropterinae - the largest and most diverse subfamily of Tettigoniidae (Nagar *et al.* 2015). Many genera of this subfamily are of uncertain status or in a state of confused taxonomy

(Ragge 1980; Rentz 2010), attracting the attention of many taxonomists (e.g. Brunner von Wattenwyl 1878; Bey-Bienko 1954; Ragge 1980; Mugleston *et al.* 2013; Heller *et al.* 2014; Kang *et al*. 2014; Hemp *et al.* 2015).

Morphologically, phaneropterines are easily recognised by the length of their hind wings, which surpass the forewings (Heller *et al.* 2015). The shapes of their eggs are also diagnostic, with all eggs studied so far being flat [Bey-Bienko 1954; however see Massa (2013) for a unique modification], a feature probably adapted to oviposit their eggs into plant tissues (Heller *et al.* 2015). Phaneropterines are also known for their acoustic communication. Like most katydids, sound is produced through tegminal stridulation of the 'file and scraper' method (see Greenfield 1997). Since males produce calling songs to attract conspecific females, the calls are species-specific and the shape, length and number of teeth in the stridulatory file are unique for each species (Rentz 2010). The stridulatory files are now often figured in descriptions of new species or taxonomic revisions and are a useful tool for species determination (Heller *et al.* 2015).

All known phaneropterines are herbivorous (Rentz 2010) and, therefore, many phaneropterine species are considered pests (e.g. Tutkun & Unal 1986; Bentley *et al*. 2002; Nyeko *et al.* 2002; Varela 2008; UC IPM 2015). *Plangia graminea* (Serville) is a sporadic pest in vineyards in the Western Cape, South Africa (Ferreira & Venter 1996; Allsopp 2012). However, the taxonomic status of the species is at present confused and it is possible that many species could constitute a *P. graminea* complex (Hemp 2013). A recent review of this complex has synonymized *Plangia compressa* (Walker) with *P. graminea* (Hemp *et al.* 2015). It is uncertain, however, whether *P. graminea* is the only katydid species present in vineyards in the Western Cape, or if there is perhaps a mélange of phaneropterine species causing damage in this area. By conducting a survey in vineyards located in the greater Stellenbosch region of the Western Cape, I aim to identify the katydid species present, and determine key morphological characteristics that can be used to distinguish between the species. I focused on the morphology of the wings, male stridulatory files, eggs and general habitus of nymphs of the katydids.

## MATERIALS AND METHODS

*Species identification and assemblage structure*

Vineyards on three wine farms were surveyed for katydids from February – June 2013 (Table 1). Katydids were caught and placed in zip-lock bags and transported back to the laboratory at Stellenbosch University. The animals were identified to species level by consulting relevant literature and taxonomic experts. The number of each species was counted before placing them in locally produced perspex vivaria (42 × 33 × 32 cm) to establish laboratory colonies. Katydids were provided with vine leaves and supplemented with lettuce to feed on. Photographs of key characteristics of species were taken and an ID-key was compiled that can be used for easy identification by growers. Eggs that were laid in the laboratory by the different species were reared to determine differences in nymph morphology.

**Table 2.** Coordinates of wine farms surveyed for katydids from February – June 2013 in the greater Stellenbosch region of the Western Cape.

| Farm | Coordinates |
|---|---|
| 1 | **S** 33° 52’ 13.74” |
| | **E** 18° 51’ 43.63” |
| 2 | **S** 33° 51’ 08.02” |
| | **E** 18° 56’ 17.21” |
| 3 | **S** 33° 57’ 14.00” |
| | **E** 18° 54’ 38.00” |

*Wings and male stridulatory files*

Two species that morphologically resembled each other were differentiated based on Ragge (1980) and Hemp *et al.* (2015), and species identification was confirmed by Dr Piotr Naskrecki (Museum of Comparative Zoology, Harvard University). To further assist in the morphological differentiation between these two species, their wings were inspected in terms of venation and male stridulatory files. One pair of wings of each species was bleached to accentuate the wing venation. The wings were soaked in 10% potassium hydroxide (KOH) for approximately 2 hours, and then rinsed with a 10% hydrogen chloride (HCl) solution. After the wings had dried, they were mounted between two glass microscope slides. The wings were photographed with

a LG G4 cellphone camera and the stridulatory files, along the underside of the left forewing, were photographed using a Leica MZ 16A automontage microscope with a Leica DFC 290 fixed digital camera. The venation of the right wing was traced using Adobe Photoshop CC 2015 (Adobe System Incorporated) and an INTUOS pro pen tablet (Model: PTH-651, Wacom Co. Ltd., Japan) to illustrate the veins more clearly. The lengths of the stridulatory files were measured as the linear distance between ends (Hemp *et al.* 2015), and the numbers of teeth were counted.

## *Eggs*

### *Imaging of eggs*

Eggs of the two species were removed from laboratory colonies. Eggs were mounted on aluminium stubs using double-sided adhesive conductive carbon tape. The eggs were then sputter coated with a thin layer of gold to make the surface electrically conductive. Images were taken using a Zeiss MERLIN FEG Scanning Electron Microscope (SEM), with an accelerating voltage of 3 kV, at the Central Analytical Facilities (CAF) laboratories, Stellenbosch University. The SEM images indicate the surface structure of the eggs.

### *Egg size measurements*

Eggs (*N*=25, for each species) were collected from laboratory colonies kept at Stellenbosch University. The eggs of two species were photographed using a Leica MZ 16A automontage microscope with a Leica DFC 290 fixed digital camera and Leica Application Suite (LAS) v.2.7. Software. Egg length (mm) and width (mm) were measured using the measuring tool provided by the LAS software. Length measurements were taken from the egg apices, and width measurements from the widest region of the eggs (Fig. 1).

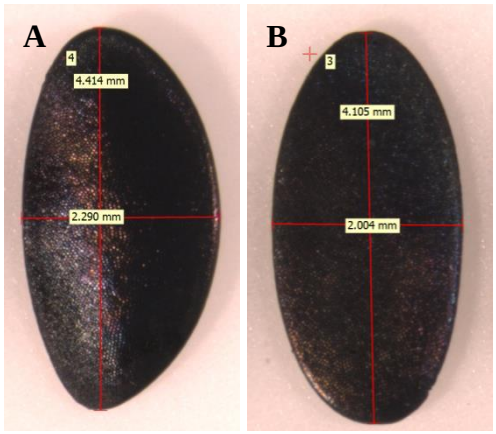


**Fig. 1.** Eggs of **(A)** Species 1 and **(B)** Species 2, indicating position of length and width measurements (mm).

## RESULTS AND DISCUSSION

*Species identification and assemblage structure*

Three species in three different genera of the subfamily Phaneropterinae were found in vineyards in the greater Stellenbosch region of the Western Cape. One species in the genus *Plangia* Stål, *Plangia graminea* (Serville); another species in the genus *Eurycorypha* Stål, *Eurycorypha lesnei* Chopard; and a *Phaneroptera* sp. that could not be identified to species level due to the need for major taxonomic review of this genus. *Plangia* and *Eurycorypha* are closely related genera and have the same general appearance (Ragge 1980). Although many species have been described in these two genera, little or nothing is known about their biology and ecology (Hemp *et al.* 2015). Species within these two genera are fully alate and are therefore highly mobile. They also have the ability to adapt to a wide range of habitats (Hemp *et al.* 2015). *Plangia* and *Eurycorypha* are canopy dwellers and often coexist without interference (Hemp *et al.* 2015).

Although *Plangia* and *Eurycorypha* have the same general appearance, *Eurycorypha* is a morphologically uniform genus and can be recognised from its head alone with a very broad fastigium verticis, frontogenal carinae and elongate eyes (Ragge 1980). *Eurycorypha* is the largest genus of African Phaneropterinae, and is also in need of taxonomic review (Ragge 1980; Hemp *et al.* 2013). The tenth abdominal tergite of *Eurycorypha* males is often characteristic and can be used for species identification (Ragge 1980). The tenth abdominal tergite of *E.*

*lesnei* males can be used for the identification of this species (Fig. 2) (see original description by Chopard 1935). *E. lesnei* has been recorded in the Gauteng and North-West Provinces of South Africa (Bazelet & Naskrecki 2014). This is therefore the first official account of this species in the Western Cape.

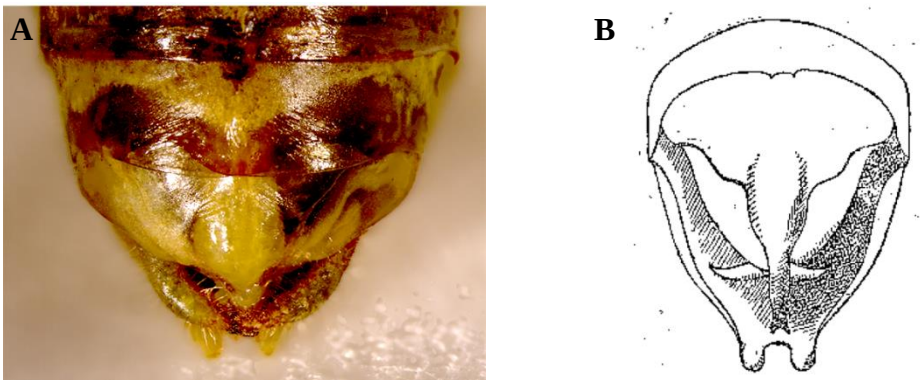


**Fig. 2.** (A) Tenth abdominal tergite of a *Eurycorypha lesnei* male collected in a vineyard located in the greater Stellenbosch region of the Western Cape; (B) illustration of the tenth abdominal tergite of a *E. lesnei* male taken from the original description by Chopard (1935).

For non-taxonomists, however, *P. graminea* and *E. lesnei* may look similar and could easily be confused without close inspection. Therefore, I have compiled an identification key that includes characteristics that can be used by growers to distinguish between the two species (Table 2). The *Phaneroptera* sp. is different in appearance compared to the other two species, with its hind wings extending further beyond the forewings in comparison with other Phaneropterinae (Ragge 1980). The wings are also more slender and elongate compared to *P. graminea* and *E. lesnei* (Fig. 3).

**Fig. 3**. *Phaneroptera* species found in vineyards in the greater Stellenbosch region of the Western Cape. Photo credit: Jaco Smit.

**Table 2.** Identification key for growers: Most conspicuous characteristics used to distinguish between *Plangia graminea* and *Eurycorypha lesnei* adults in vineyards of the greater Stellenbosch region of the Western Cape.

| Characteristic | Photo | | *P. graminea* | *E. lesnei* |
|---|---|---|---|---|
| | *P. graminea* | *E. lesnei* | | |
| Elytra spot (males) |  |  | Dark brown spot on the stridulatory area of the tegmina. | No spot present |
| Female ovipositor |  |  | Short, broad, strongly curved with orange – brown colour towards the apex | Narrower, longer, less strongly curved, greenish yellow |
| Abdomen (male and female) |  |  | Bright blue, purple, reddish colouration on the dorsal side of the abdomen | Abdomen pale yellowish-green |
| Tympanum (ear) on front leg |  |  | Brown – black colouration on the inside of the tympanum | Pale yellowish-green colour |

It should also be noted that adult *P. graminea* display an array of different colour morphs ranging from green, brown, and pink (Fig. 4). Colour polymorphism is common in the subfamily *Phaneropterinae* (Castner & Nickle 1995), and similar colour morphs have been observed in the North American oblong-winged katydid, *Amblycorypha oblongifolia* (De Geer) (Crew 2013). Leaf-mimicking katydids use crypsis as a primary defense mechanism to avoid predation (Belwood 1990). The oval shaped wings of phaneropterines resemble leaves. The colour of the katydid should match the colour of the background substrate to increase the survival value of crypsis (Belwood 1990). Adaptive morphological colour change has been observed in the predatory *Saginae* (Kaltenbach 1990). These colour changes may even occur in adults (Kaltenbach 1990), with the ability to change colour diminishing only in the last weeks of adult life (Kaltenbach 1970). The colouration of the vineyard leaves also change from green to red, dark tan, orange or yellow in autumn, therefore, the different colour morphs observed in *P. graminea* could increase the value of crypsis as a primary defense mechanism in this species.


**Fig. 4.** Colour polymorphism: (**A**) green, (**B**) brown, (**C**) pink; observed in *Plangia graminea* individuals found in vineyards of the greater Stellenbosch area, Western Cape.

*Plangia graminea* and *E. lesnei* nymphs look unlike the adults, and also unlike each other – especially the first two instars (Fig. 5). It is therefore easier to distinguish between the two species during their immature stages. Nymphs of the *Eurycorypha* genus are characterised by their ant-like appearance and behaviour (Ragge 1980; Hemp *et al.* 2013). Nymphs of *E. lesnei* are no exception (Fig. 5 B). Records show that *Eurycorypha* nymphs live together with *Camponotus* and *Myrmicaria* ant species (Ragge 1980; Hemp *et al.* 2013). *Anoplolepis* spp. are widely distributed dominant ant species that forage in vineyards in the Western Cape (Addison & Samways 2000), therefore ant mimicry in *E. lesnei* nymphs could be a valuable defense mechanism to avoid predation from these ants.

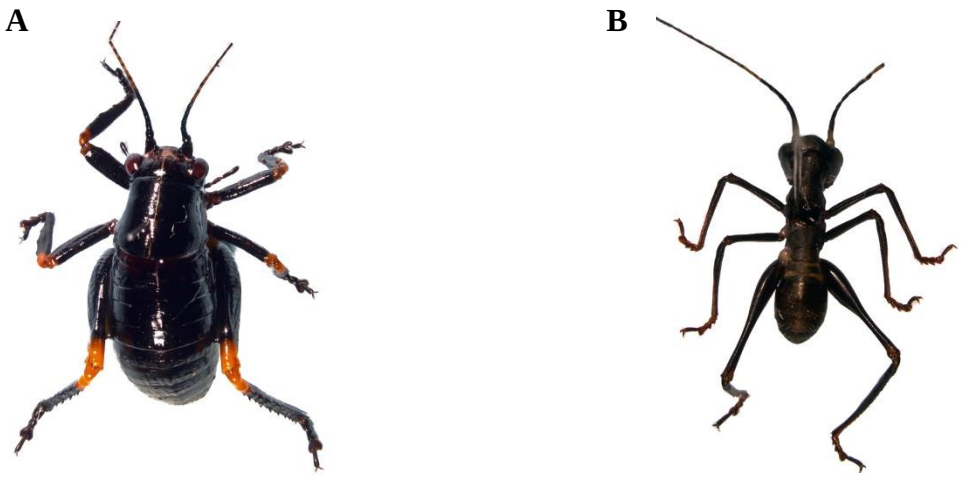


**Fig. 5.** Photos of a **(A)** *Plangia graminea* nymph, and a **(B)** *Eurycorypha lesnei* nymph, found in vineyards in the greater Stellenbosch region of the Western Cape.

At the commencement of population surveys (February 2013) *P. graminea* was the most dominant species (Fig. 6). *E. lesnei* was found at lower abundance levels. Later in the season (April - May 2013) *E. lesnei* increased in abundance, and both *P. graminea* and *E. lesnei* populations decreased when the vineyards reached the period of winter dormancy (June – August 2013). The first *Phaneroptera* sp. was observed in May 2013, and another in June, therefore this species was not included in Fig. 6, due to poor representation.

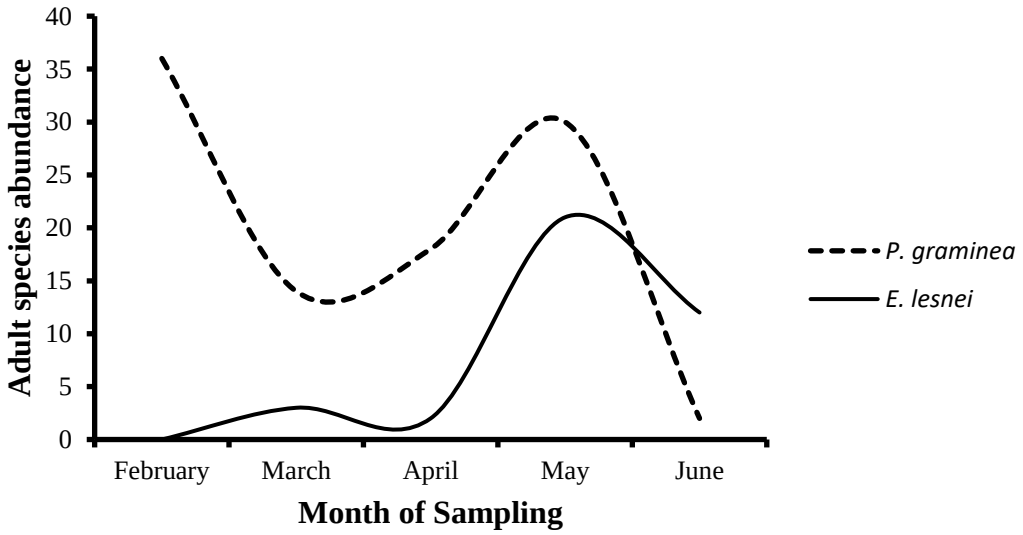


**Fig. 6.** Abundance of two adult katydid species, *Plangia graminea* and *Eurycorypha lesnei*, occurring in vineyards of the greater Stellenbosch area from February 2013 – June 2013.

*Wings and male stridulatory files*

Venational patterns on the tegmen of adult katydids can also be used to identify species (Rentz 2010). The venation on the forewing of both *P. graminea* and *E. lesnei* is typical of that of Tettigoniids (Fig.1, Chapter 1, Ragge 1955). This is, however, the first documentation of the wing venation for *P. graminea* and *E. lesnei* respectively. An archedictyon is present over the whole of the fore wing, except for parts of the stridulatory apparatus. The ambient vein lies along the margin of the wing. The costa (C) is submarginal, poorly developed and barely noticed in both species. The subcosta (Sc) of both species is well developed reaching the anterior margin near the tip of the wing. The radius (R) is situated immediately behind the subcosta. Sc and R are closely approximated in both species, which is often the case in leaf-mimics (Ragge 1955). The radius divides into R1, which is unbranched; and Rs, a pectinately branched radial sector. $R_1$ of *P. graminea* fades away near the tip of the wing, whereas $R_1$ of *E. lesnei* curves down towards the anterior margin and reaches the tip of the wing. The media (M) is situated behind the radius and divides into two branches, MA and MP, near the base of the wing of both species. MA of both species reaches the posterior margin of the wing at approximately two thirds of the length of the wing. On the *E. lesnei* wing, MP fuses with $Cu_{1a}$ and reaches the posterior margin at the proximal third of the wing. For *P. graminea* MP and $Cu_{1a}$ is closely approximated, and MP reaches the posterior margin at the proximal third of the wing (Fig. 7). The wing venation appears to be a characteristic feature for both these species, and can be used for species differentiation in addition to characteristics described in Table 2.

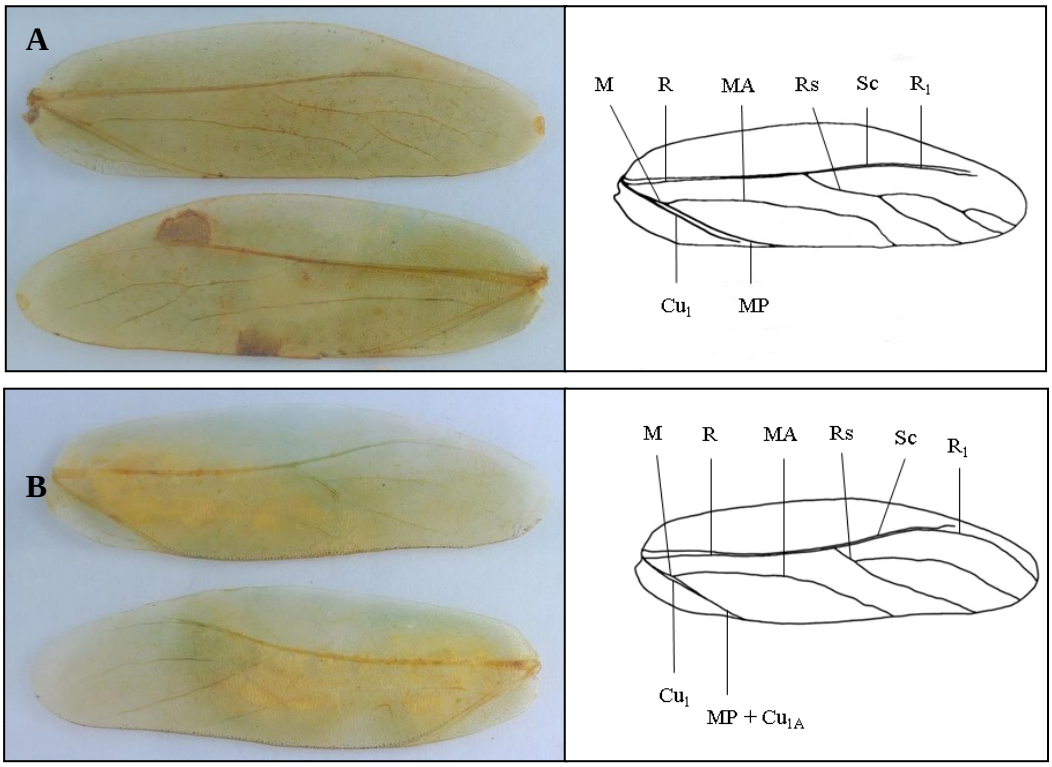


**Fig. 7.** Bleached wings of **(A)** *Plangia graminea* ♀ and **(B)** *Eurycorypha lesnei* ♀; with illustrated venational patterns, collected in vineyards in the greater Stellenbosch region of the Western Cape. Vein nomenclature according to Ragge (1955).

The stridulatory file is situated along the underside of the left tegmen, as in all tettigonioids (Heller *et al.* 2015). The number of teeth in phaneropterine stridulatory files varies (Heller *et al.* 2015). Some species of the genus *Hemielimaea* (Brunner von Wattenwyl) have up to 380 teeth (Ingrisch & Gorochov 2007) while *Elimaea rosea* (Brunner von Wattenwyl) has only 12 (range = 10-14) teeth (Ingrisch 2011). For *P. graminea* I counted 67 teeth (file length = 2.5 mm), which matches the description by Hemp *et al.* (2015). This is the first description of the stridulatory file of *E. lesnei*, for which I counted 118 teeth and measured a file length of 2.6 mm (Fig. 8).

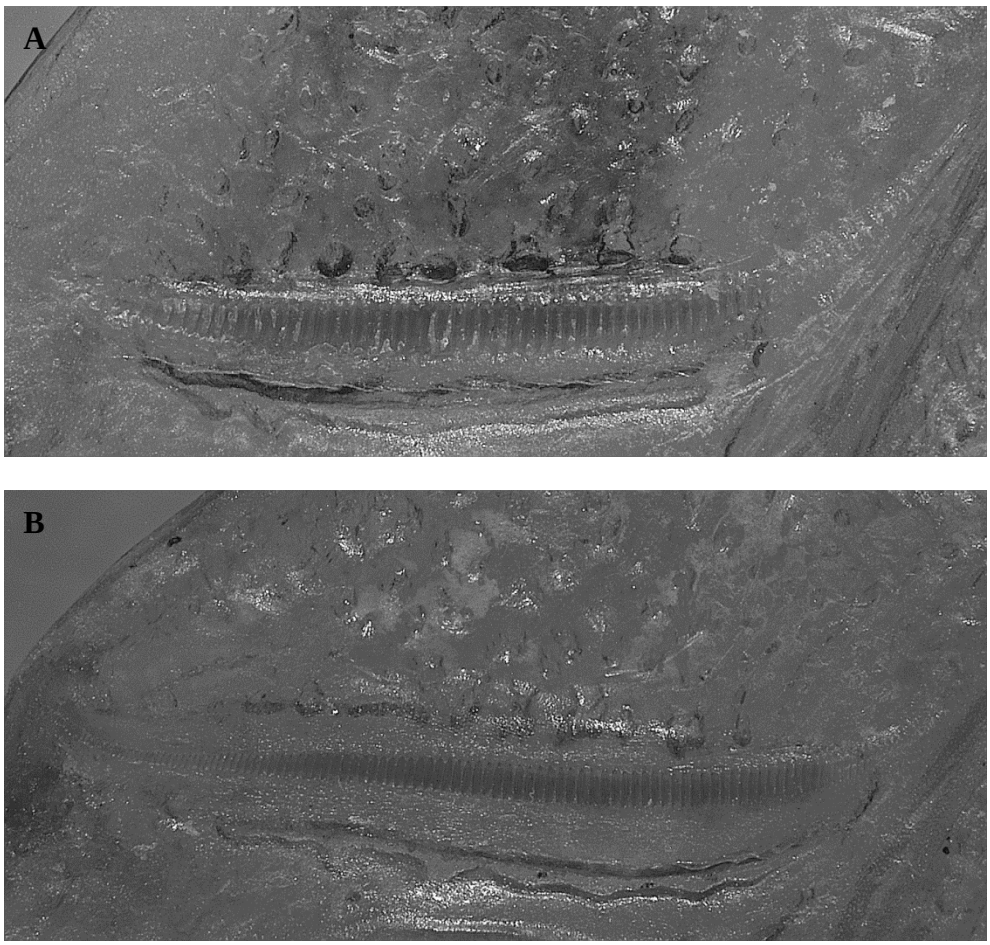


**Fig. 8.** Male stridulatory files of two katydid species; (A) *Plangia graminea* and (B) *Eurycorypha lesnei*; collected in vineyards located in the greater Stellenbosch region of the Western Cape.

*Eggs*

The eggs of *P. graminea* and *E. lesnei* look very similar. The eggs are black, oval shaped and flat. Only when the eggs are studied under high magnification could distinctive subtle features be observed (Fig. 9). The appearance and position of the micropyle on the egg are often characteristic of most species. Micropyles are usually contained on the depressed dorsal area of the egg (Rentz 2010) (Fig. 9 C). The micropyle of *E. lesnei* eggs could not be located on the SEM photographs. The surface structure of *E. lesnei* eggs has an argyle pattern, while the pattern on *P. graminea* eggs appears hexagonal (Fig. 9 B, D). Moreover, there is a significant difference in the size of the eggs, with eggs of *P. graminea* being slightly longer and wider than eggs of *E. lesnei* (Fig. 10).

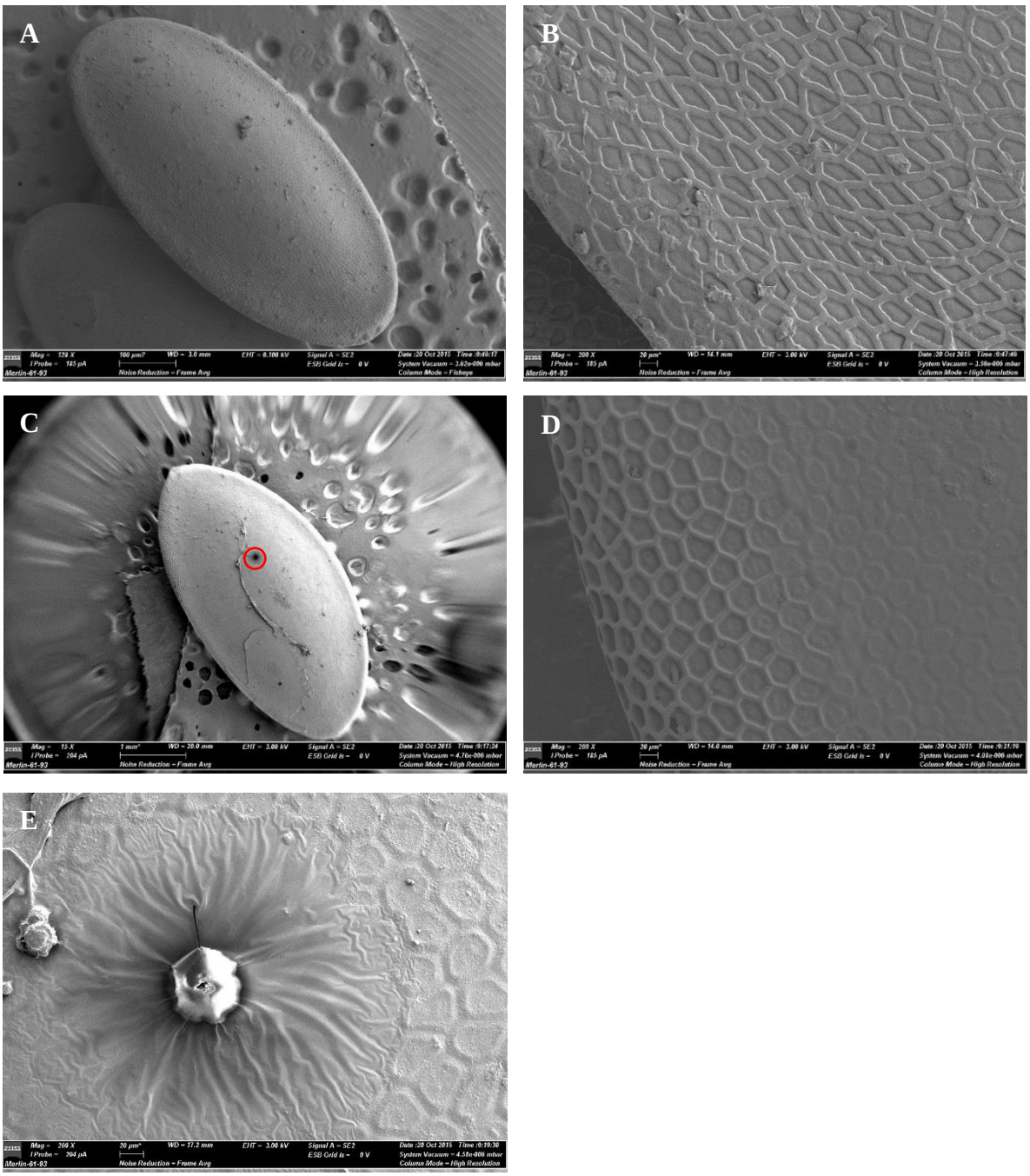


**Fig. 9.** SEM photographs of (**A**) *Eurycorypha lesnei* egg, (**B**) surface structure of *E. lesnei* egg, **(C)** *Plangia graminea* egg with position of micropyle encircled in red, **(D)** surface structure of *P. graminea* egg, **(E)** micropyle of *P. graminea* egg (Mag = 269 X).

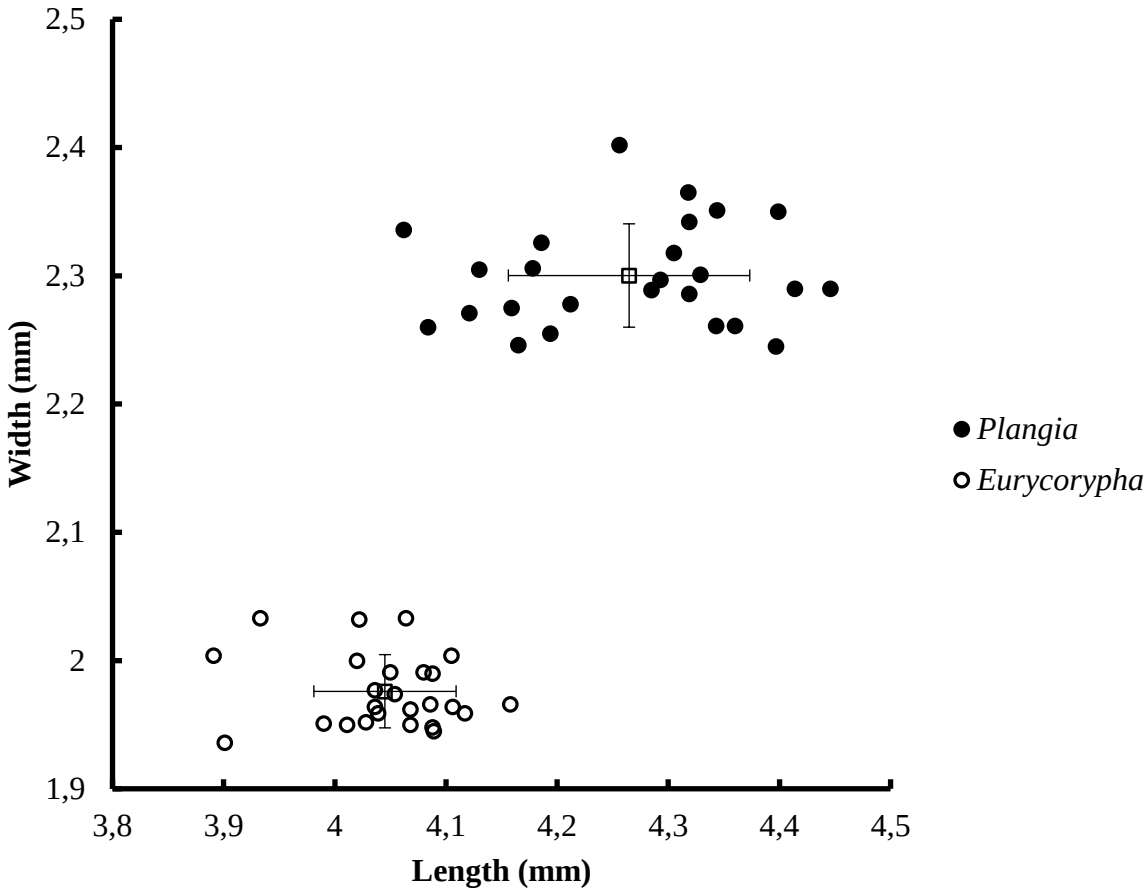


**Fig. 10.** Length and width measurements (mean ± S.E.) of *Plangia graminea* and *Eurycorypha lesnei* eggs collected in vineyards of the greater Stellenbosch region, Western Cape.

## CONCLUSION

*Plangia graminea* was thought to be the only katydid pest in vineyards in the Western Cape; however, the present study identified two more phaneropterine species in vineyards in the greater Stellenbosch region, namely *Eurycorypha lesnei* and a *Phaneroptera* species. Several morphological characteristics have been identified in this study to differentiate between *P. graminea* and *E. lesnei*, due to their close resemblance. Key characteristics include the male elytra spot, the female ovipositor, colouration of the abdomen, and the tympana (ears) on the front leg. The venation on the fore wings of these two species was documented for the first time and can be used for species determination. Moreover, the male stridulatory files differ in size, shape, and number of teeth. The number of teeth in the stridulatory file of *E. lesnei* was recorded here for the first time, with 118 teeth. This study provides the first documentation of the size, shape, and surface structure of *P. graminea* and *E. lesnei* eggs. The eggs of *P. graminea* have a significantly longer length and width compared to *E. lesnei* eggs. It is unclear whether *E.*

*lesnei* and *Phaneroptera* sp. populations may also cause damage to vineyards. Since few *Phaneroptera* sp. were observed during this survey, it seems unlikely that this species occurs in high enough numbers to cause any substantial damage. Further monitoring is, however, required to determine the pest status of all three katydid species. Furthermore, I believe that *P. unimaculata* could also form part of the *P. graminea* complex and further taxonomic revision that includes this species should be conducted to further untangle the *Plangia* complex.

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# CHAPTER 3

## Monitoring of katydids (Orthoptera: Tettigoniidae: Phaneropterinae) in vineyards in the Western Cape, South Africa - with insights gained on their biology, ecology, and seasonal dynamics


## ABSTRACT

In a complex of three phaneropterine species present in Western Cape vineyards, South Africa, *Plangia graminea* (Serville) is considered a sporadic pest. However, it is unknown whether two other species, *Eurycorypha lesnei* Chopard and a *Phaneroptera* species, also contribute towards the damage. Very little information is available on the biology and ecology of katydids in vineyards. Moreover, no monitoring method is currently available for this pest. A monitoring method was adapted from the generic sampling system for monitoring key arthropod pests in vineyards. Vineyards were monitored in the greater Stellenbosch wine production region of the Western Cape from January 2014 to March 2015. Katydid biology in terms of egg laying sites, life-cycle and development rates, and seasonal dynamics within vineyards was investigated during this study. *Plangia graminea* was found to be the primary katydid pest, constituting more than 80% of the katydid population present in vineyards monitored. Their eggs were laid within the bark of the vine. Nymphs hatched from eggs early in the vine growing season (mid-September) and nymph-to-adult development lasted approximately 2 ½ months. Three nymphal instars were observed during this study. Optimum temperature for katydid development was 25°C. A peak in viable egg density was observed in winter (end-July) and katydid density reached a peak early-November. Viable eggs were found in vineyards 10 weeks prior to observation of katydids in vineyards. Viable eggs correlated well with the number of katydids observed after adjusting for this lag time ($r = 0.404$, $P < 0.001$). Therefore, egg monitoring could potentially be a surrogate monitoring method for this pest that would allow early prediction estimates of katydid populations in vineyards.

## INTRODUCTION

In the Western Cape of South Africa, *Plangia graminea* (Serville) is considered a sporadic pest in vineyards (Allsopp 2012). The nymphs feed on the young foliage of the vine, and later in the season, feeding can extend to fruit clusters (Ferreira & Venter 1996). Feeding by *P. graminea* is seldom of economic importance, but sporadic outbreaks may cause economic damage (Ferreira & Venter 1996; Allsopp 2012). In the past few seasons, since 2012, they appear to have caused a substantial amount of damage leading to great concern among the farmers in Western Cape vineyards (Allsopp 2012). For example, a vineyard block which typically yields 7-8 tons of grapes only yielded 3.5 tons after a katydid outbreak (farm manager, pers. comm., 2013). Very little is known about the biology and ecology of this species (Allsopp 2012). It is also unknown whether *P. graminea* is the only katydid pest, or if other phaneropterine species present (*Eurycorypha lesnei* Chopard, and a *Phaneroptera* sp., Chapter 2) also contribute to the damage.

Katydids oviposit their eggs in and on a variety of substrates. The shape of the ovipositor is often indicative of the egg laying site. If the eggs are laid in plant material e.g. leaves or wood, the ovipositor is usually sickle-shaped. The eggs are laid during summer, develop over winter and nymphs hatch the following spring. This is the general life history of most katydids [reviewed in Rentz (2010)]. Based on the phenology of the vines in the Western Cape, with a winter dormancy period, I expect *P. graminea* to have the same life history with an overwintering egg stage. Depending on climatic conditions and the species, the nymphs moult anything from four to nine times (Rentz 2010). Adult katydids are fully alate, and the wings of phaneropterines (leaf-mimicking katydids) resemble oval leaves (Belwood 1990). Phaneropterines use crypsis as a primary defence mechanism to avoid predation, and the colour of their wings usually match the background substrate [Belwood 1990; e.g. *Plangia multimaculata* Hemp (Hemp *et al.* 2015), and *P. graminea* (present study, Chapter 2)]. The perfect camouflage of *P. graminea* within the vine canopy makes monitoring this pest a challenge. A generic monitoring system has been developed for key arthropod pests in vineyards (De Villiers & Pringle 2008); however, this sampling system does not accommodate katydid pests.

This study aims to determine whether *P. graminea* is the primary katydid pest in vineyards located in the greater Stellenbosch region of the Western Cape. Furthermore, I investigate their biology and ecology in terms of egg laying sites, life cycle, optimal temperature for development, and seasonal dynamics within vineyards. Since no monitoring method is currently available for this pest, recommendations on how to monitor this pest in vineyards are also proposed. This information seeks to provide baseline knowledge on the biology and ecology of katydids in this region, and further aims to provide valuable information for the development of an integrated pest management (IPM) strategy.

## MATERIALS AND METHODS

### *Study sites*

The study was conducted on four farms situated in the Stellenbosch and Paarl wards found within the Stellenbosch district and Coastal region of the "Wine of Origin Scheme" in the Western Cape fruit production area of South Africa (Table 1). This region is typified by a Mediterranean climate with winter rainfall, and is a regional biodiversity hotspot (Born *et al.* 2007). In the monitoring sites, cover crops grown between vine rows included *Triticale v. Usgen* 18 (Gramineae) (every second row) and natural weed cover (Fig. 1). Cover crops were planted during the wet winter months and killed off with herbicide during spring to prevent competition with vine roots. Weeds were controlled as needed, depending on growth during the season, also with herbicides or mechanically.

**Table 3**. Location, block size, cultivar, and crop/weed cover of vineyards sampled for katydids in the greater Stellenbosch wine growing region of the Western Cape, South Africa.

| Farm | Coordinates | Elevation (m) | Block size (ha) | *Vitis vinifera* cultivar | Weed cover/cover crops used |
|---|---|---|---|---|---|
| 1 | **S** 33º 52’ 13.74”<br>**E** 18º 51’ 43.63” | 229 | 8.76 | Sauvignon Blanc | Triticale |
| 2 | **S** 33º 53’ 43.69”<br>**E** 18º 53’ 32.95” | 256 | 0.39 | Shiraz | Triticale |
| 3 | **S** 33° 51’ 08.02”<br>**E** 18° 56’ 17.21” | 250 | 5.57 | Cabernet Sauvignon | Triticale/natural weed cover |
| 4 | **S** 33º 52’ 19.68”<br>**E** 18º 53’ 21.32” | 338 | 3.01 | Sauvignon Blanc | Mixture of Triticale, barley and grazing vetch. |


**Fig. 2.** Some examples of vineyards used for monitoring in the greater Stellenbosch area of the Western Cape, with dates at which photos were taken **(1)** May 2013 (austral autumn), **(2)** October 2014 (austral spring), **(3)** November 2013 (early summer), **(4)** December 2014 (summer).

*Seasonal monitoring*

The monitoring system of De Villiers & Pringle (2008) was adapted for katydids as described below, and was carried out from January 2014 to March 2015, following preliminary assessments (March 2013 – December 2013) to determine the most effective method. Five evenly spaced rows were selected in each vineyard block and in each row, four evenly spaced plots, consisting of approximately five vines between trellising posts, giving a total of 20 plots per block (De Villiers & Pringle 2008). Occasionally all sampling had to be postponed due to rain. Monitoring was performed according to the phenology of the vine and also the different life stages of the katydids. Katydids (adults and nymphs together), katydid-eggs and leaf damage were monitored as described in the following sections. Since katydids, especially the adults, are cryptic and nocturnal, I wanted to determine whether the number of eggs or leaf damage assessments could be used as surrogates for the number of katydids in a monitoring system.

*Eggs*

Eggs were sampled by stripping pieces of the bark off the two main cordon arms for 15 cm on either side of the main stem as well as 15 cm down the main stem (only one vine per plot i.e. 1/5 vines per plot) (Fig. 2). Bark stripping is sometimes recommended as a practice to manage vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) populations in vineyards and is not detrimental to vines (Daane *et al*. 2012). The pieces of bark were then taken back to the laboratory and searched for eggs. In winter months (June 2014 – August 2014), during the dormant stages of the vine, sampling was conducted only once per month and only eggs were sampled from the cordons and main stem since neither leaves nor katydids were present during this stage. The eggs were carefully removed from the bark and were separated as being "viable" or "non-viable." Eggs were classified as viable if they were fully intact and contained some substance within the egg which could easily be determined by gently pressing the egg with forceps. Non-viable eggs were damaged or empty shells either because they were not fertilised, parasitised or they were hatched eggs from the previous season. Eggs out of which katydids have hatched could be identified by the presence of a white skin left behind at the apex of the egg.

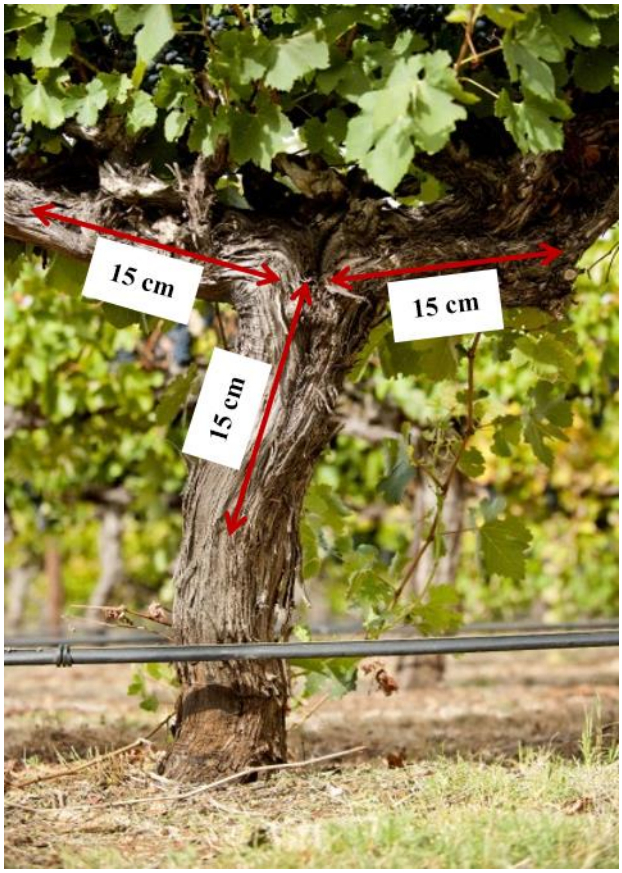


**Fig. 2.** Sampling technique used for monitoring bark for katydid eggs, indicating areas of the vines sampled.

*Laboratory reared eggs*

Viable eggs collected in vineyards during March 2013 – August 2013 were placed in incubators (MRC Ltd., Model LE-509, Holon, Israel) at five different temperatures (15, 20, 25, 30 and 35°C) on 11 September 2013, to determine the optimal temperature for hatching and instar development. A total of 50 eggs were placed in plastic containers (11.5 × 7 cm) on top of absorbent paper together with damp pieces of cotton for humidity at each temperature. Eggs were inspected every 24 hours to see whether nymphs had hatched and the cotton wool was moistened daily. The number of nymphs hatched was noted daily and instars were observed to monitor their development. Katydids were provided with vine leaves and lettuce to feed on.

*Katydid nymphs and adults*

After bud break (September 2014), the shoots and leaves became visible and these were inspected in addition to the cordons and main stem for the presence of katydid nymphs. At this stage the katydid nymphs were darkly coloured and easy to spot on the leaves (Fig. 12 B, Chapter 1). Physical katydid counts were made by visually searching through the leaves within the plot (five vines between the two trellis posts). This was performed by two people standing on either side of the row to prevent katydids being missed because they sheltered on the other side. Immatures were collected and placed in zip-lock bags. They were transported back to the laboratory where they were identified to species level and placed in locally produced perspex vivaria (60 × 40 × 70 cm) to establish a laboratory colony. Katydids were provided with vine leaves and lettuce to feed on. The laboratory colony was observed daily and notes on their biology, ecology and behaviour were made for qualitative purposes.

When inflorescences developed into bunches and katydids reached adulthood, it was no longer practical to do a visual search for adult katydids between the leaves since leaf density was high and adult katydids were much more camouflaged and elusive compared to the younger instars. Adults were also largely nocturnal. A different method for counting adult katydids was therefore required. Adults were counted by shaking the vines three times (three pulses of continuous shaking) and counting the individuals that flew away or dropped to the ground - one person shaking and the other counting. This method proved to be more effective compared to visual inspections and night counts, as determined during preliminary assessments. However, individuals of different species could not be identified using this method. Night counts were done by following male calls, but this was found to be impractical as ideally the whole population needs to be sampled. Adult katydids were caught when possible and transported back to the laboratory to be identified to species level. They were then placed in vivaria to supplement the laboratory colony.

*Leaf damage*

To characterise katydid leaf damage, katydids caught in the field were placed in vivaria (60 × 40 × 70 cm) containing potted vines on which they were allowed to feed. No other insects were present in the vivaria. The leaf damage was carefully observed and compared to the damage

caused by another key insect pest, the Banded Fruit Weevil *Phlyctinus callosus* (Schoenherr) (Allsopp *et al.* 2015). Weevils were placed in a container with no other insects and intact vine leaves. The leaves were inspected for damage after one day and compared to katydid-damaged leaves.

In field sites, within each plot, a leaf damage assessment was conducted by randomly selecting 10 leaves within the plot; 5 leaves in the lower region and 5 leaves in the upper region of the leaf canopy. The leaves were inspected and the presence or absence of possible katydid damage was noted. Rows and plots were chosen at random with each sampling effort to avoid resampling the same vines and plots as much as possible. Leaf assessments were conducted from January 2014 to May 2014. From May onwards the quality of the leaves rapidly deteriorated until leaf senescence and it became difficult to distinguish katydid damage from other damage. Leaf assessments recommenced from October 2014 (when damage was first observed in the new season) to March 2015.

*Statistical analysis*

A Probit-analysis, run in R version 3.3.1 (R Core Team 2013), was performed to determine the $LD_{50}$ and $LD_{90}$ values of katydids reared at different temperatures. The temperatures were regarded as treatments and the number of days was considered to be the dose (i.e. survivorship was the dose response at different temperatures). Probit analyses are often used to assess insect mortality related to temperature (Tang *et al.* 2000). The correlation between the total number of eggs and number of katydids; the number of viable eggs and number of katydids; and percentage leaf damage and number of katydids was determined by constructing 2-D scatterplots using STATISTICA v.13.2. (StatSoft, Tulsa, OK, USA). A Cross-correlation Function was constructed to determine the lag time between the number of viable eggs and the number of katydids observed using STATISTICA v.13.2. After adjusting for the lag time between viable eggs and katydids, the correlation between the number of viable eggs (+ lag time) and the number of katydids observed was reconstructed using a 2-D scatterplot.

## RESULTS AND DISCUSSION

*Plangia graminea* was the most abundant species, and accounted for *ca.* 82% of the katydids collected in vineyards. *Plangia graminea* is therefore the primary katydid pest in the vineyards monitored during this study. *Eurycorypha lesnei* and the *Phaneroptera* sp. were present in low numbers, and accounted for *ca.* 14% and 4%, respectively. Therefore, the latter two species do not appear to be pests at present.

### *Eggs*

The eggs were typically oval shaped and black (Fig. 3, A). The eggs were predominantly laid in rows or clumps within the bark of the vine (Fig. 3, B), but may also be placed between epidermal layers of vine leaves or grass blades (Fig. 3, C & D). Females make a cut into the edge of the leaf with their serrated, sickle-shaped ovipositors and deposit their eggs into the tissue between the upper and lower surface tissue of the leaf. Eggs were also observed in the bark of surrounding pine trees, and at the base of pine needles on branches. In the laboratory, eggs have been laid in plastic, mesh fabric, duct tape and glue residue (own observations).

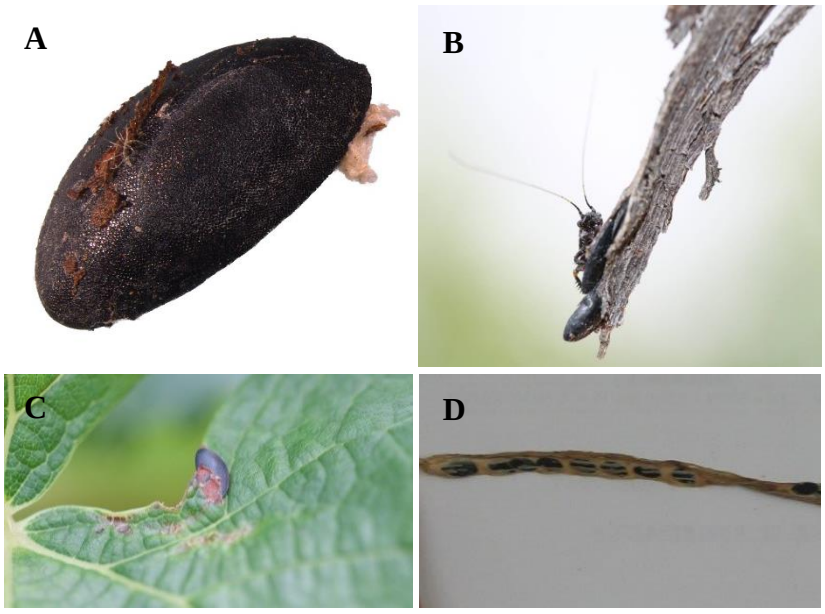


**Fig. 3.** (**A**) Example of a hatched *Plangia graminea* egg, and *P. graminea* eggs laid in (**B**) the bark of the vine, (**C**) the edge of a vine leaf, and (**D**) a grass blade; found in the greater Stellenbosch region, Western Cape.

*Laboratory reared eggs*

Katydids hatched at all temperatures except at 30 and 35ºC (Fig. 4). Nymphs hatched earliest (17 September 2013 – 2 October 2013) at 25ºC, and latest at 15ºC (2 October 2013 – 31 October 2013). Of a total of 50 eggs (per temperature treatment) that were placed in the incubators at the start of the trial, only three nymphs hatched at 15 and 25ºC. Four nymphs hatched at 20ºC; however, one instar (hatching date, 2 October 2013) perished after 9 days due to a handling error and was therefore excluded from Fig. 4. Instar development was suboptimal at 15ºC, with only one individual reaching second instar. Although 15ºC was unsuitable for adult development, one individual survived to 1$^{st}$ instar and another reached 2$^{nd}$ instar before they perished. One of the three hatchlings at 15ºC perished after only 6 days, possibly indicating that this temperature is not favourable for katydid development, although a larger sample size would be necessary in order to determine this. Although only 3 moults were observed (i.e. 3 instars) (Fig. 4), it is possible that there are more instars, since the nymphs consumed their exuviae after moulting. Therefore, a moulting event could have been overlooked due to the exuviae being consumed before inspection. In future, nymphs could be marked with paint on the thorax to assist detecting each moult. For *Ruspolia differens* (Serville) five and six instars were recorded for males and females, respectively (Thornton & Brits 1981), and five nymphal stages for East African *Eurycorypha* species (Hemp *et al.* 2013). At 20ºC, the total duration of the life cycle was 134 days (*N*=1) and the total adult duration was 68 days. At 25ºC the total adult life duration was 61 days, bringing the total duration of the life cycle to 140 days (*N*=1) (Fig. 4). Similar development times were observed for *R. differens* individuals reared under laboratory conditions in South Africa; with the total average duration of the life cycle being 147 days and the adult life duration 72 days (Brits & Thornton 1981).

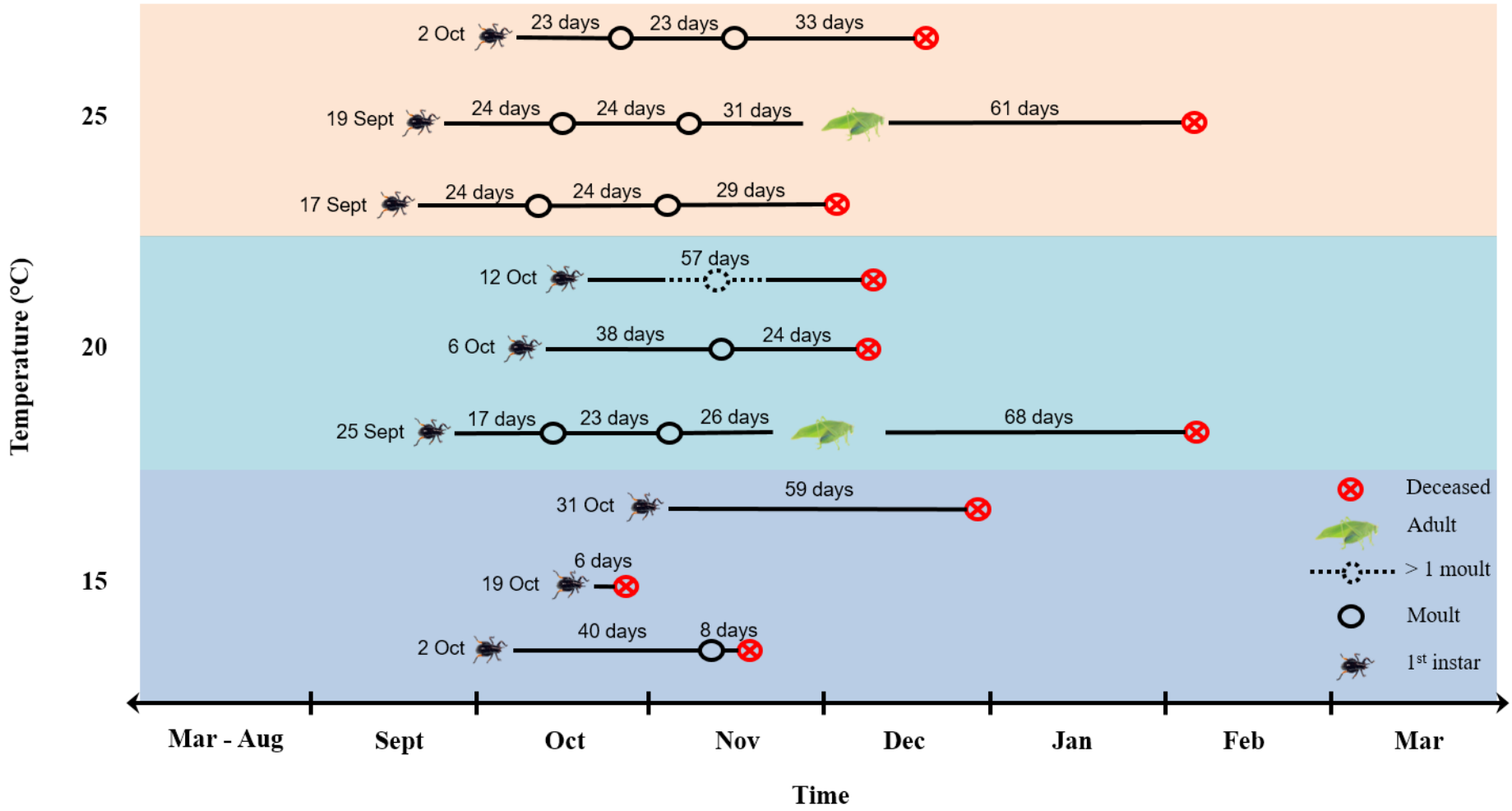


**Fig. 4.** Hatching dates and developmental rates for *Plangia graminea* reared at different temperatures (15, 20, 25ºC) in incubators at Stellenbosch University, Western Cape. Eggs were collected in vineyards located in the greater Stellenbosch region of the Western Cape from March – August 2013.

A probit analysis indicated that 20ºC had the highest $LD_{50}$ (107.915 ± 10.265 S.E), followed by 25ºC (99.975 ± 11.682 S.E.). However, 25ºC had the highest $LD_{90}$ (152.84 ± 28.991 S.E.), followed by 20ºC ($LD_{90}$ = 148.08 ± 21.686 S.E.) (Table 2, Fig. 5). Development was stunted at 15ºC and no katydids emerged at 30 and 35ºC. Therefore, the optimum temperature for katydid emergence and development in the laboratory was between 20-25ºC. However, the extremely low success of hatching and successive moulting indicate that the physiological needs of the katydids were not met under these laboratory conditions, and that these results were inconclusive. As probit analysis is most often used to assess insect mortality in high/low temperature post-harvest storage assessments (e.g. Tang *et al*. 2000), this may not be the most effective method to assess life table parameters. Temperature-dependant development models (Briere *et al*. 1999) are more appropriate for this purpose, but could not be used in the current study due to lack of a viable laboratory colony with sufficient sample sizes. Suitable insect-rearing methods for *P. graminea* are required to acquire such data.

**Table 2.** LD50 and LD90 values for *Plangia graminea* reared at different temperatures in incubators at Stellenbosch University, Western Cape.

| **Temperature (ºC)** | **LD50 ± S.E.** | **LD90 ± S.E.** |
|---|---|---|
| 15 | 62.272 ± 10.883 | 115.03 ± 26.253 |
| 20 | 107.915 ± 10.265 | 148.08 ± 21.686 |
| 25 | 99.975 ± 11.682 | 152.84 ± 28.991 |
| 30 | 0 | 0 |
| 35 | 0 | 0 |

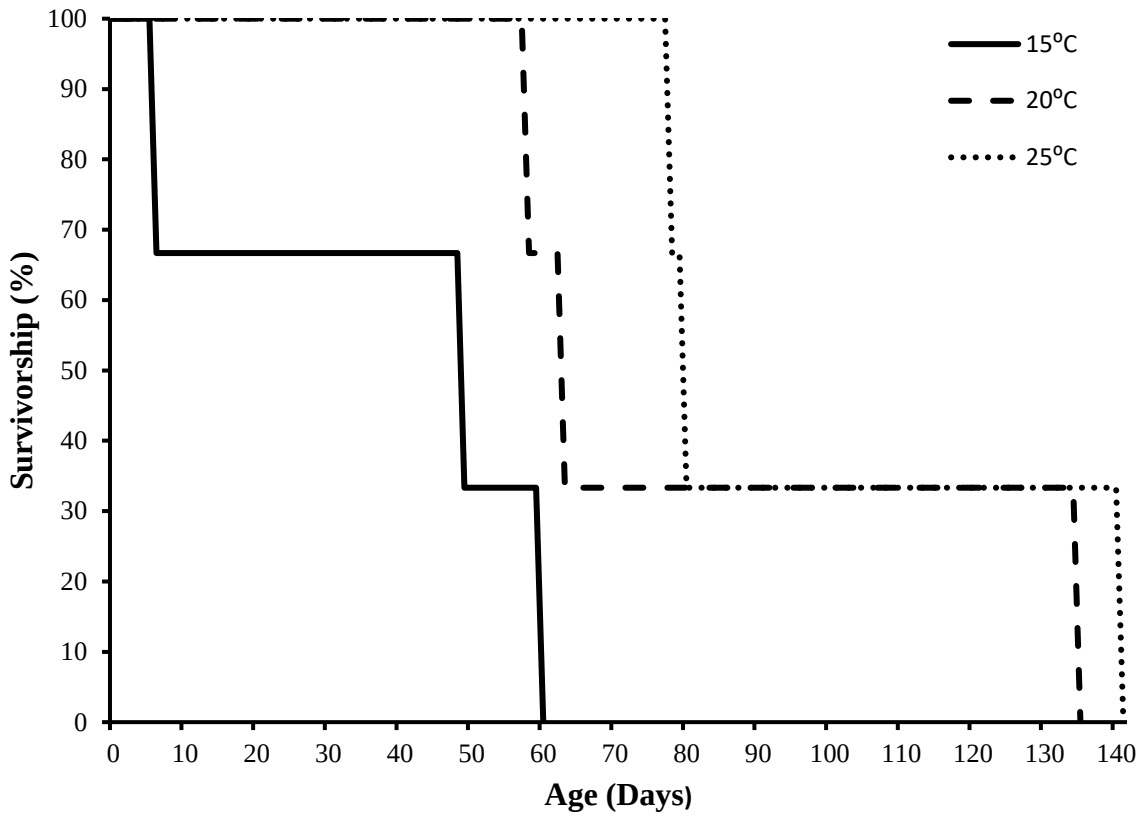


**Fig. 5.** Survivorship curve for *Plangia graminea* individuals reared at different temperatures (15, 20, 25ºC) in incubators at Stellenbosch University.

*Katydid nymphs and adults*

The seasonal cycle of *P. graminea* and *E. lesnei* is graphically represented for eggs, nymphs and adults in Fig. 6. The collective representation of the two species, *P. graminea* and *E. lesnei*, was necessary since it was difficult to distinguish between the adults of the two species using the counting method of shaking the vines. However, *P. graminea* was identified as the primary katydid pest. Moreover, at times when nymphs and adults were present at the same time, both stages were counted without distinction and, therefore, katydid density refers to both life stages. The reason for not making the distinctions between *P. graminea* and *E. lesnei*, and between nymphs and adults, was that I wanted to customize a monitoring method that could easily be adopted by growers.

Mating was first observed in adult katydids in late December (personal observation). Egg laying followed soon after, and continued until late austral-summer before vineyards entered the period of winter dormancy. In late April 2014 the leaves started to drop, and the remainder of the leaves were dry and brittle. Katydids were still present during May 2014, and although most were adults, a couple of late instars were also observed during this time. From the end of May 2014, katydid numbers dropped to zero and from June 2014 to end of August 2014 there were no leaves present. Katydid eggs were largely present throughout the year. The eggs overwintered and represented the new generation of nymphs for the next season. *Plangia graminea* seemed to have only one generation per year, which appears to be the case for most species in temperate regions (Gwynne *et al.* 1988; Rentz 2010).

Katydid nymphs hatched in spring, early in the season (mid-September 2014) at the onset of bud break. The nymphs developed and predominantly fed on the young foliage of the vine. From observations made in the laboratory, only three instars could be distinguished, as was also found during egg incubation experiments at different temperatures (Fig. 4). Populations reached a peak in early-November 2014, followed by a drastic decrease during December 2014. The decline in population numbers may have been due to pesticide treatments in an attempt to supress other pest populations e.g. weevils, or extreme heat conditions experienced during this time (farm managers, pers. comm.). Adult katydids were first observed middle to late-November 2014, approximately 2 ½ months after the first instars were observed in vineyards, which corresponded to the time passed from first instar to adult observed in laboratory observations (Fig. 4). Adult katydids, being cryptic in vineyards, may also have attributed to lower counts during December 2014, as observers' eyes were still untrained and they were more difficult to spot. After mid-December 2014 most katydids observed were adults (> 70% in Dec; > 80% in Jan 2015). The adults remained in the vineyard for the remainder of the season.

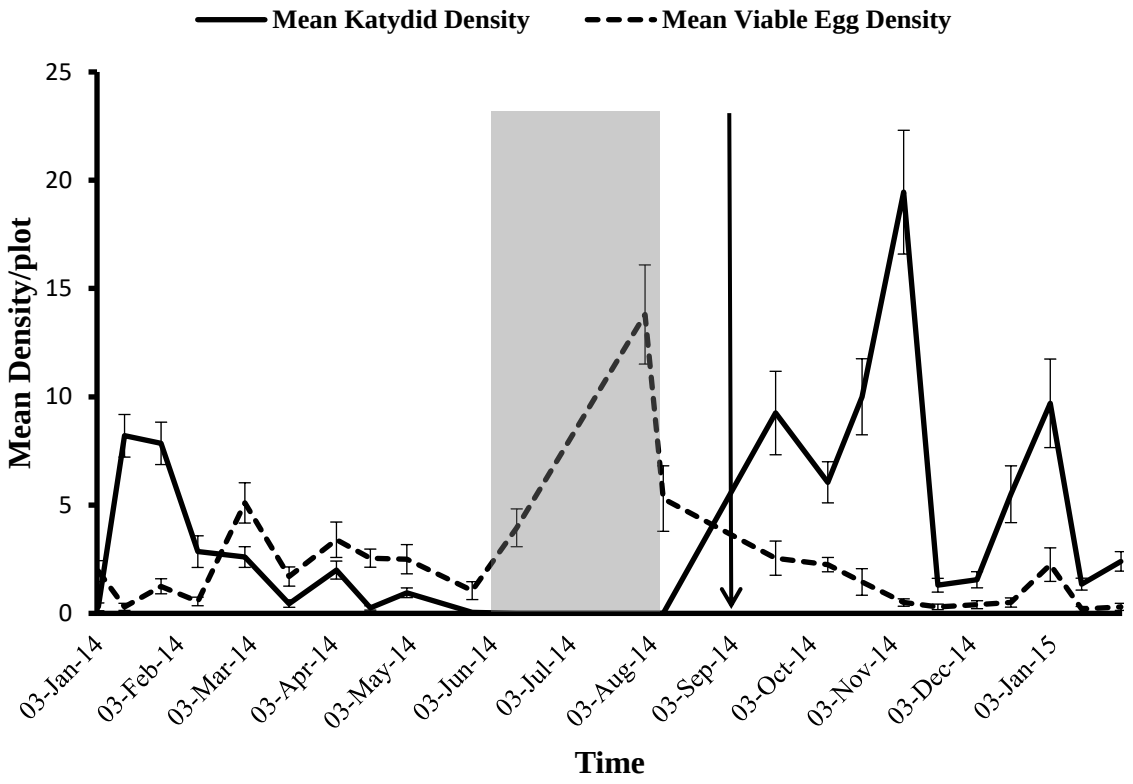


**Fig. 6.** Mean density (± SE) of katydids (*Plangia* and *Eurycorypha* spp. combined) and viable eggs in four vineyards located in the greater Stellenbosch area from January 2014 to March 2015. Grey zone represents the dormant period (no leaves) while the arrow indicates bud break.

Since katydids are cryptic and difficult to spot, it could be more feasible to monitor katydid eggs instead of katydid adults. For this to be a practical surrogate monitoring method, however, there should be a significant correlation between the number of katydids observed and the number of katydid eggs observed (specifically viable eggs since only viable eggs will contribute to the next season's katydid population). However, simply comparing the number of viable eggs against the number of katydids gave a poor correlation (r = -0.063, $P = 0.168$). This is to be expected since there is a lag period from the time eggs are laid to when katydids are observed. A cross-correlation to determine the lag period between viable eggs and katydids indicated a lag period of 5 sampling intervals, i.e. 5 two-week intervals (10 weeks), between the time viable eggs were observed and when katydids were observed in the vineyards (Fig. 7). By shifting the number of viable eggs 10 weeks ahead, the peak in viable egg density corresponded with the peak in katydid density observed in vineyards (Fig. 8). This increased the correlation between the number of viable eggs and the number katydids to a significant level (r = 0.404, $P < 0.001$) (Fig. 9). There was also a significant correlation between the total

number of eggs (viable and non-viable combined) and the number of katydids, without having to adjust to the lag period (r = 0.233, $P < 0.001$). Egg monitoring could, therefore, potentially be used to predict katydid populations in vineyards because the association was found to be significant but should be supplemented with further visual monitoring of nymphs and adults as the correlation was found to be poor.

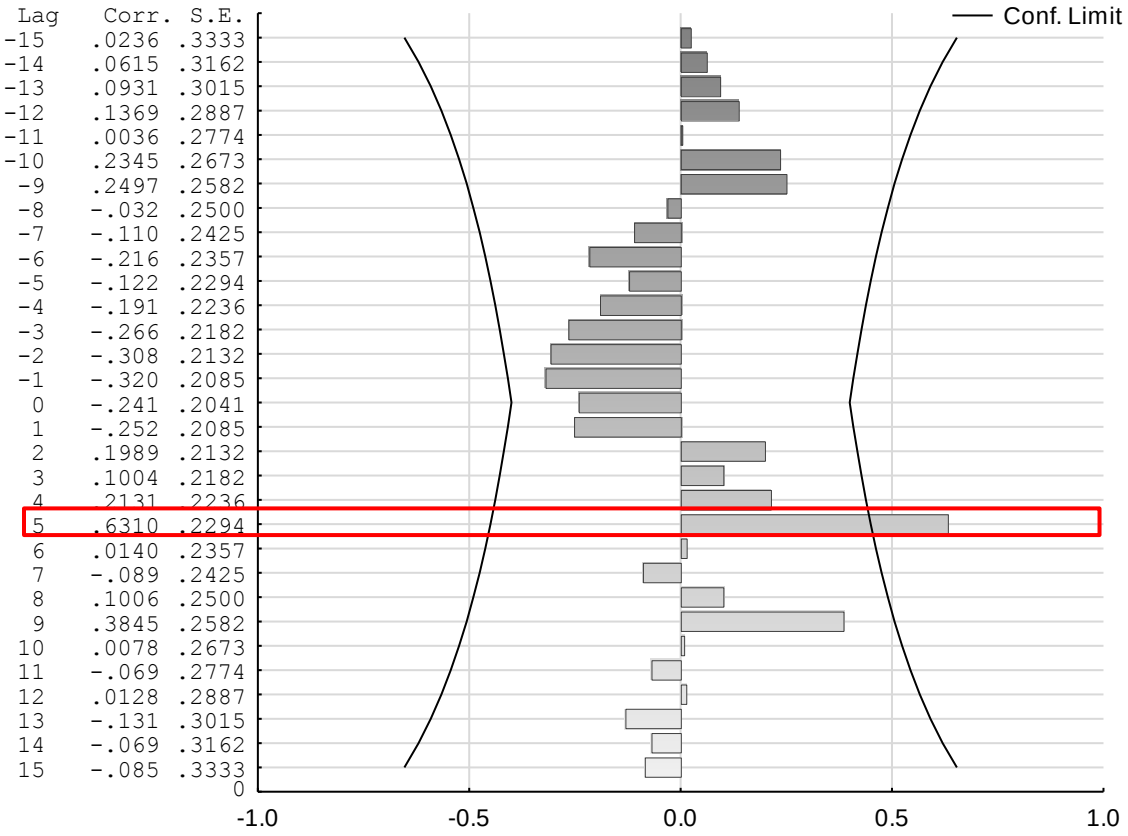


**Fig. 7.** Cross-Correlation Function determining the lag time between viable eggs and the number of katydids observed in vineyards located in the greater Stellenbosch area of the Western Cape. The red box designates the lag time, measured in the number of biweekly sampling intervals.

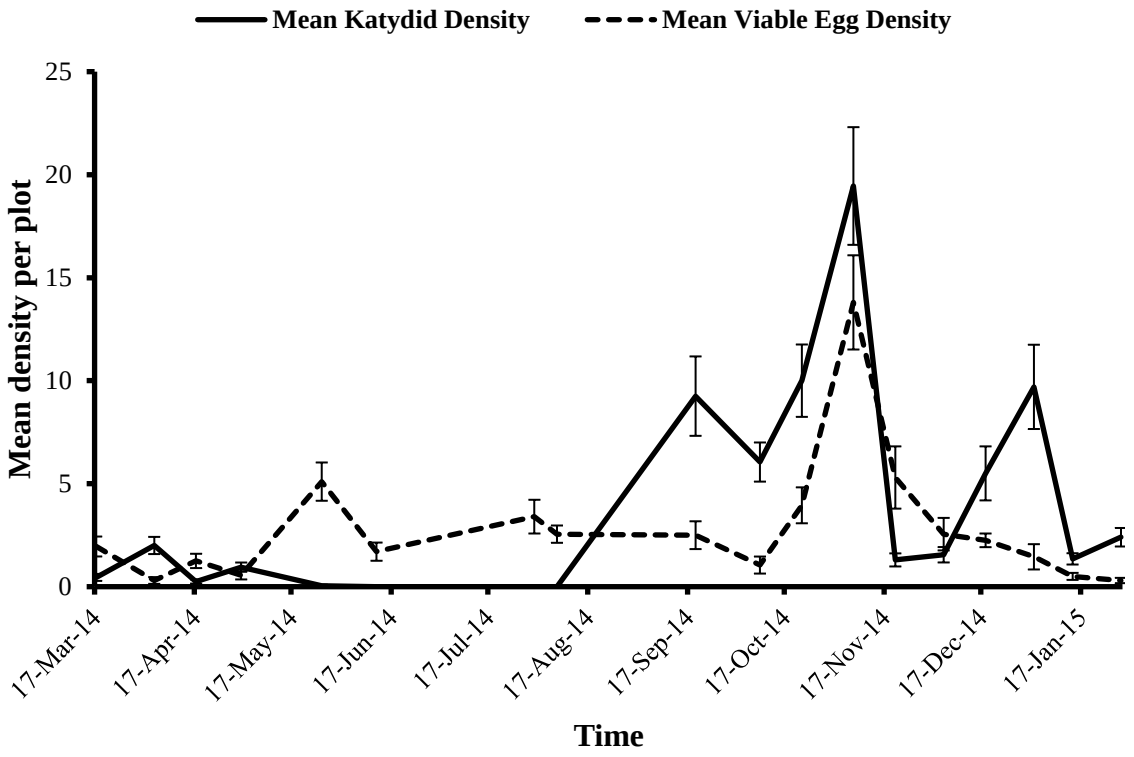


**Fig. 8.** Mean katydid density (± S.E.) (*Plangia* and *Eurycorypha* spp., adults and nymphs) and the adjusted viable egg density (+ 5 sampling intervals, based on the Cross-Correlation Function) in vineyards located in the greater Stellenbosch area from March 2014 to March 2015.

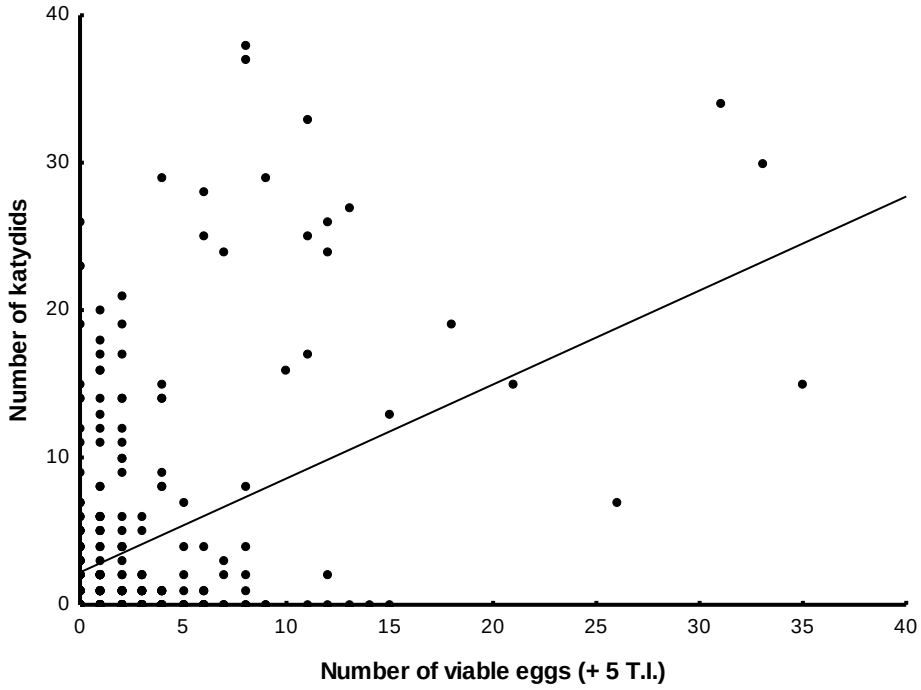


**Fig. 9.** Scatterplot illustrating the relationship between the mean number of katydids (*Plangia* and *Eurycorypha*, adults and nymphs) and the number of viable eggs observed (*Plangia* and *Eurycorypha*), after lag time adjustment (+ 5 sampling intervals), in vineyards located in the greater Stellenbosch region of the Western Cape.

*Leaf damage*

The immature stages of katydids feed on young leaves of the vine and later, after fruit set, the feeding can extend to young fruit clusters (Ferreira & Venter 1996). Adults have also been observed feeding on ripe berries (own observations, Fig. 10, D). The resultant leaf damage looks like that of Banded Fruit Weevil, *P. callosus*, damage (Ferreira & Venter 1996; and present study), especially leaf damage caused by immature katydids. Both katydids and weevils can start feeding on the leaf from anywhere (Fig. 11), and do not necessarily need to feed from the edge of the leaf inwards, contrary to the comment made by Allsopp (2012) that "unlike snoutbeetles, which typically feed from the edge of the leaf inwards, long-horned grasshoppers will start eating the leaf from anywhere." However, the larger holes in the centre of the leaf and the greater extent of feeding from the edge of the leaf could be used to distinguish adult katydid damage from weevil damage (Fig. 11). However, damage caused by katydid nymphs looks very similar to that of weevil damage, and this may therefore not be a practical monitoring measure for the grower.


**Fig. 10.** Feeding damage caused by *Plangia graminea* in vineyards of the greater Stellenbosch area. (**A**) Adult *P. graminea* feeding from the edge of the leaf inwards, (**B**) typical leaf damage in a vineyard, (**C**) adult *P. graminea* damage, (**D**) adult *P. graminea* feeding on ripe berries [Photo: Allsopp (2012)].

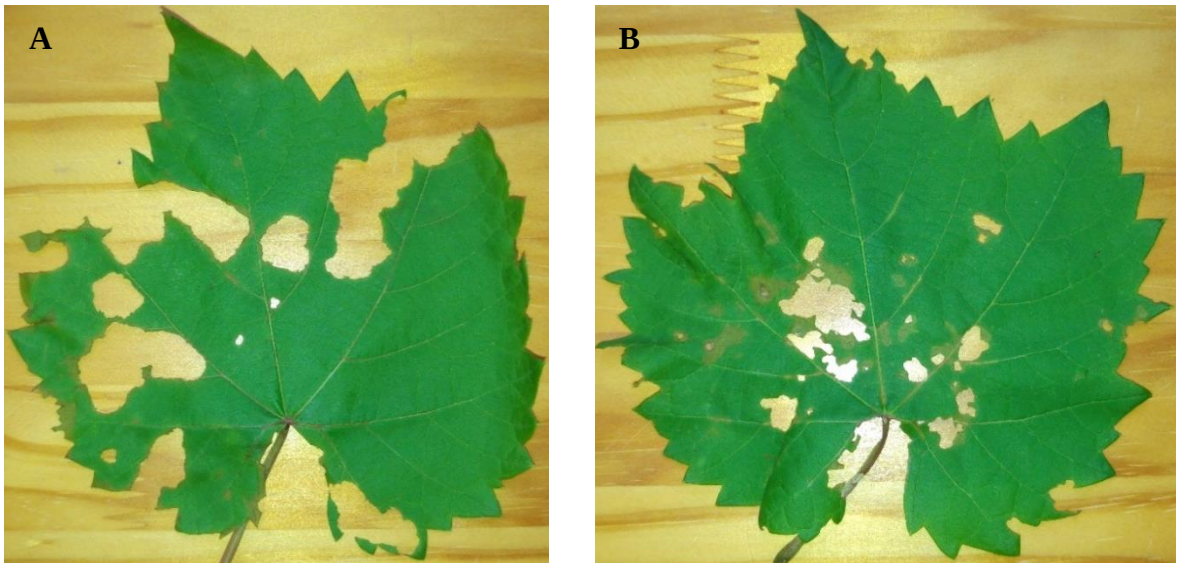


**Fig. 11.** Comparison between (**A**) adult *Plangia graminea* leaf damage and (**B**) *Phlyctinus callosus* damage on grapevine leaves in vineyards of the greater Stellenbosch area, Western Cape, South Africa.

Leaf damage was positively and significantly correlated with the number of katydids observed (r = 0.222, $P < 0.001$; Spearman r = 0.23, $p < 0.01$) (Fig. 12). However, the correlation was found to be poor. The actual number of katydids present could have been much higher than the number of katydids observed, due to the cryptic and elusive nature of katydids. Moreover, leaf damage could potentially have been overestimated in the field due to the similarity between katydid and weevil damage (observed during this study), and snail damage (Ferreira & Venter 1996).

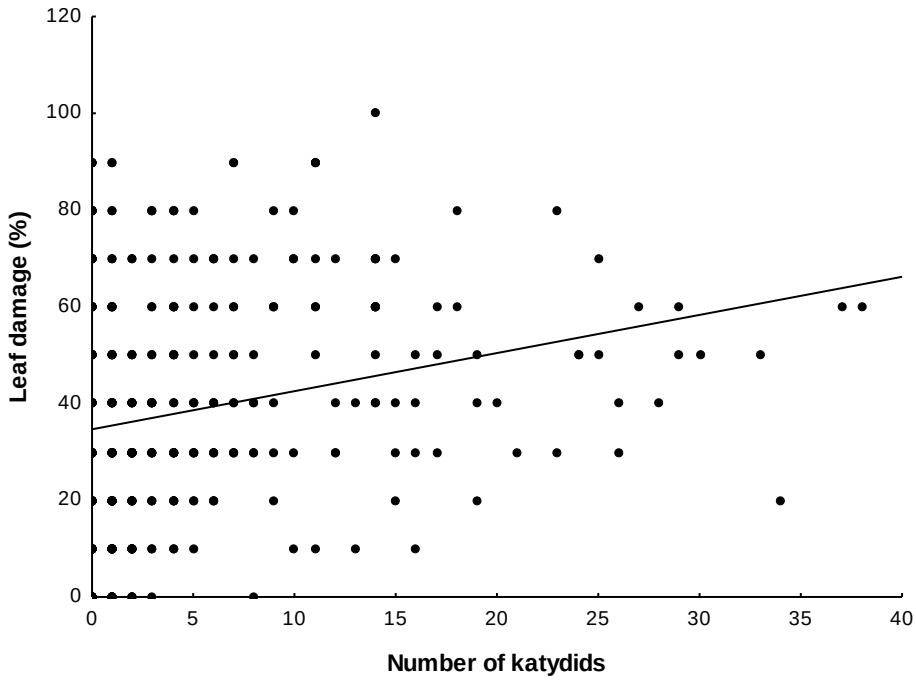


**Fig. 12.** Scatterplot illustrating the relationship between the mean number of katydids (*Plangia* and *Eurycorypha*) and the percentage leaf damage observed on farms located in the greater Stellenbosch region of the Western Cape.

## CONCLUSION

*Plangia graminea* was found to be the dominant katydid species present in Western Cape vineyards and is, therefore, the primary katydid pest. This study provides the first information on the basic biology and ecology of this pest. This is also the first record to determine the overwintering strategy of *P. graminea* in vineyards, determining that katydids overwinter as eggs during the winter months. Temperature appears to be an important environmental factor influencing the development of katydids. The optimum temperature recorded in the laboratory for katydid development was 25°C. Katydid eggs appear to be a good surrogate for katydid monitoring. Viable eggs were significantly and positively correlated with the number of katydids observed after adjusting for a 10-week lag time. The 10-week lag time could potentially allow early prediction estimates of katydid populations within vineyards, allowing growers valuable time to plan their management strategies in advance. In comparison with egg monitoring, leaf damage assessments appear to be a less reliable monitoring tool and is not recommended here due to potential overestimation of damage due to other pests (notable the weevil *P. callosus*). Monitoring of nymphs, separately, could be investigated further as they are easily recognisable and different in appearance from other similar katydids. It is strongly recommended that future research focus on establishing reliable and effective rearing methods for *P. graminea*, as a laboratory colony allows for more detailed temperature dependent development models and life tables to be established. As this katydid is a sporadic pest in South African vineyards, research potential without laboratory colonies is limited.

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# CHAPTER 4

**Physiological ecology – the metabolic costs of sexual signalling in the chirping katydid *Plangia graminea* (Serville) (Orthoptera: Tettigoniidae) are context dependent: cumulative costs add up fast[1]**

## ABSTRACT

Katydids produce acoustic signals via stridulation, which they use to attract conspecific females for mating. However, direct estimates of the metabolic costs of calling to date have produced diverse cost estimates and are limited to only a handful of insect species. Therefore, in this study, I investigated the metabolic cost of calling in an unstudied sub-Saharan katydid, *Plangia graminea*. Using wild-caught animals, I measured katydid metabolic rate using standard flow-through respirometry while simultaneously recording the number of calls produced. Overall, the metabolic rate during calling in *P. graminea* males was 60% higher than the resting metabolic rate (0.443±0.056 versus 0.279±0.028 ml $CO_2$ $h^{-1}$ $g^{-1}$) although this was highly variable among individuals. Although individual call costs were relatively inexpensive (ranging from 0.02 to 5.4% increase in metabolic rate per call), the individuals with cheaper calls called more often and for longer than those with expensive calls, resulting in the former group having significantly greater cumulative costs over a standard amount of time (9.5 h). However, the metabolic costs of calling are context dependent because the amount of time spent calling greatly influenced these costs in my trials. A power law function described this relationship between cumulative cost ($y$) and percentage increase per call ($x$) ($y=130.21x^{-1.068}$, $R^2=0.858$). The choice of metric employed for estimating energy costs (i.e. how costs are expressed) also affects the outcome and any interpretation of costs of sexual signalling. For example, the absolute, relative and cumulative metabolic costs of calling yielded strongly divergent estimates and any fitness implications depend on the organism's energy budget and the potential trade-offs in allocation of resources that are made as a direct consequence of increased calling effort.

[1]This chapter was submitted and accepted in *Journal of Experimental Biology*

## INTRODUCTION

Calling is a conspicuous way of attracting a potential mate and is thus thought to be associated with elevated energy usage and evolutionary fitness costs (Symes *et al.* 2015). These signals have associated metabolic costs that are influenced by sexual selection and constrained by abiotic and biotic factors (Greenfield 1997). Surprisingly little information is available, however, on the energetic costs of calling, and there are few firm theoretical expectations (White *et al.* 2008). The wing muscles used by tettigoniids during stridulation are of the very fast synchronous type and in some species the wing stroke (WS) frequency during stridulation may even exceed that of flight (Stevens & Josephson 1977). Based on the high frequency of wing muscle contraction during stridulation, one would expect calling in these insects to incur a pronounced metabolic cost (Heath & Josephson 1970). This expectation has been confirmed for three trilling katydid species - *Neoconocephalus robustus* (Scudder 1862), *Euconocephalus nasutus* (Thunberg 1815) (Conocephalinae) (Stevens & Josephson 1977), and *Mecopoda* sp. (Erregger *et al.* 2017) - through increased oxygen uptake rates (an indirect measure of metabolic rate, MR) and a rise in thoracic temperature during stridulation (Heath & Josephson 1970; Nespolo *et al.* 2003; Erregger *et al.* 2017). Empirical evidence from other Orthoptera indicate diverse estimates of energy expenditure, typically measured in terms of indirect calorimetry (either as $CO_2$ production or $O_2$ consumption rates, or converted to MR) during acoustic signalling (e.g. Prestwich & Walker 1981; Kavanagh 1987; Prestwich & O'Sullivan 2005; White *et al.* 2008; Erregger *et al.* 2017). However, the cost of work performed depends on the elastic contribution to mechanical efficiency. Because insect muscle efficiency depends on its resilin content and the role of elastic tension in the cuticle (Dickinson & Lighton 1995), any predictions of direct metabolic costs might be influenced by the relative amount of elasticity in a system and this may, in turn, vary in a species-specific manner (e.g. Burrows *et al.* 2008; reviewed in Qin *et al.* 2012). It is therefore unclear how metabolically expensive calling activity is, and particularly what the fitness consequences of elevated MR might be if raised a few percent above baseline resting levels. Regardless, this is particularly significant for understanding the evolution of calling and sexual selection from theoretical and empirical perspectives (e.g. White *et al.* 2008; Erregger *et al.* 2017; reviewed in e.g. Gerhardt & Huber 2002).

Signalling to attract conspecifics is predominantly a male feature in the majority of Orthoptera species, owing to the increased risk of predation and higher reproductive investment for females (Kavanagh 1987; Bailey 1991; Riede 1998; Korsunovskaya 2009). In katydids, sound is produced by tegminal stridulation, by specialised forewings (tegmina) that are rapidly opened and closed. During the closing stroke, a file of minute teeth (pars stridens) along the underside of the left forewing moves over a hardened scraper (plectrum) on the upper surface of the right forewing. Each individual tooth strike across the plectrum causes an associated membrane (mirror) surrounded by a sclerotised U-shaped frame to resonate and amplify sound (Greenfield 1997). Thus, a complete cycle of the wing opening and closing (one WS) can generate a single chirp, comprising of multiple pulses of sound, which are the smallest amplitude modulations within signals (Bailey *et al.* 1993). Wing stroke rates (WSRs) may vary from a few to several hundred per second, and are characteristic in each species for a given temperature (Prestwich & Walker 1981). If a few WSs (i.e., chirps) are followed by a pause and then more WSs, the calling pattern is referred to as chirping. However, if many WSs occur continuously, the calling song is termed a trill (Gerhardt & Huber 2002). Katydids have a very wide range of calling strategies in terms of the temporal structure of the call (Bailey *et al.* 1993). Calls may be nearly continuous, such as in many coneheads (Tettigoniidae: Conocephalinae; Josephson 1973; Counter 1977), or extremely brief sounds, as in the chirps of some phaneropterines (Tettigoniidae: Phaneropterinae; Heller 1990).

For many invertebrate species, the energy expenditure during calling may approach or even surpass the level reached during other activities (Prestwich 1994). Calling can therefore expend a large proportion of the insect's total daily energy budget, especially if maintained over a long period (Prestwich & Walker 1981). Moreover, the cost of calling is additional to other metabolic or fitness costs that can be involved during mating (Calow 1979). For example, the production and exchange of a nuptial gift in the form of a spermatophylax is an important reproductive strategy in katydids (Lehmann 2012). As reproductive investment by the male entails more than just the donation of its sperm, any metabolic cost of calling could energetically constrain its mating behaviour (Arak 1983). Furthermore, because reproductive success of singing insects is closely related to their calling success, they are under selective pressure to optimise their calling efficiency (Bennet-Clark 1998). If females prefer males that invest more energy in their calls, their genetic material will spread through the population and, consequently, result in greater fitness (Bailey *et al.* 1993). If this is indeed the case, it becomes

important to determine a robust estimate of the metabolic costs or energetic consequences of calling and understand the contexts in which trade-offs might be made (Symes *et al.* 2015).

Previous studies examining the metabolic costs of calling in Orthoptera have mainly focused on a handful of species of crickets and mole crickets, most of which call relatively continuously (Prestwich & Walker 1981; Kavanagh 1987; Lee & Loher 1993; Prestwich & O'Sullivan 2005; White *et al.* 2008). To my knowledge, the metabolic cost of calling has only been investigated in four katydid species; of which three produce trilling calls (Stevens & Josephson 1977; Erregger *et al.* 2017). In the fourth species, Bailey *et al.* (1993) focused on the energetic costs of calling in the chirping katydid, *Requena verticalis* (Walker 1869) (Listroscelidinae). The present study aims to add to Bailey *et al.* (1993)'s findings, and the global database across taxa, by investigating the metabolic cost of calling in an unstudied sub-Saharan katydid, *Plangia graminea* (Serville 1838) (Phaneropterinae) (Hemp *et al.* 2015), which also produce chirping calls. I predict that, as in the case of the other orthopterans, there is a cost associated with calling; but, as found with *R. verticalis*, the cost is likely to be relatively low compared with the costs observed in trilling katydids. However, I also aim to assess diverse metrics of calling costs and how these might influence understanding of the costs. I therefore specifically compare the estimates of calling costs in absolute, relative and cumulative terms, including consideration of the power (dB) of sound produced. To better understand the intrinsic variability of my estimates, I aimed to perform a comprehensive repeatability assessment of metabolic rates and calling cost estimates across my trials for controlled and more variable conditions, using temperature in the latter case. My final study objective was to compare the relative amounts of these costs between different activity states by interspecific comparison of literature estimates to date (following e.g. White *et al.* 2008) in both ordinary and phylogenetically informed statistical approaches.

## MATERIALS AND METHODS

### *Animals*

The metabolic cost of sound production was measured in adult male *P. graminea* katydids (*N=11*). All individuals were collected in vineyards surrounding Stellenbosch in the Western Cape of South Africa and were kept in vivaria with *ad libitum* access to lettuce, grapevines and

water. The vivaria were kept at room temperature (25±5ºC) in an air-conditioned laboratory at Stellenbosch University.

*Experimental design*

Experimental trials were conducted during late austral summer. Experiments were typically run within two weeks of collection but at randomised start dates to minimise any laboratory acclimation effects (e.g., Terblanche *et al.* 2004). Combined respirometry and calling trials were started just before dusk and continued throughout the night to cover the period when katydids usually sing in the field (Stevens & Josephson 1977), except for two individuals on which trials were conducted during the day and night. Only one experimental trial consisting of a single male katydid was conducted per night, and each male was only tested once. Individuals were randomly selected from the vivarium and weighed to the nearest 0.1 mg using a digital microbalance (Model MS104S, Mettler Toledo, Greifensee, Switzerland) before and after their metabolic rates were measured. After each trial, the male was placed in a designated vivarium for used males to prevent it from being selected more than once.

Each experimental respirometry and calling trial consisted of three phases: (1) initial baseline period, (2) respirometry and calling period with a katydid and (3) second baseline period. During the baseline periods, respirometry measurements were taken without a katydid for ~ 10-min to measure potential instrument drift and to allow for baseline corrections, which were typically negligible. During the respirometry and calling period, a katydid was placed inside the 50 ml respirometry cuvette coupled to an open flow-through system. Respirometry consisted of simultaneous measurements of $CO_2$ and $H_2O$ production using a standard flow-through, push-system respirometry set-up. Compressed air, generated by an aquarium pump, was passed through sodalime and Drierite (W. A. Hammond Drierite Co., Xenia, OH, USA) scrubber columns to remove $CO_2$ and $H_2O$. Scrubbed air was fed through a flow control valve (Model 840, Side-Trak, Sierra Instruments, Monterey, CA, USA) and regulated at a fixed rate of 200 ml $min^{-1}$ using a mass flow control unit (Sable Systems International, MFC-2, Las Vegas, NV, USA). Thereafter, air flowed through the zero channel of an infra-red gas analyser (Li-7000, Li-Cor, Lincoln, NE, USA) and through the cuvette containing the katydid. Air leaving the cuvette then entered the gas analyser through another channel, resulting in

differential recordings of insect $CO_2$ and $H_2O$ production logged at 1 Hz. The output of the analyser ($V_{CO2}$ and $V_{H2O}$) was recorded *via* Li-7000 software on a standard desktop computer. Data were exported as text files into a respirometry software program (Expedata Data Acquisition & Analysis Program, Sable Systems International, Las Vegas, NV, USA) for further analysis.

Air temperature inside the respirometry cuvette was recorded at 1 Hz using a 36-standard wire gauge Type T thermocouple connected to a PicoLog TC-08 digital recording logger, with data captured by the standard PicoLog software (PicoLog for Windows 5.20.3, Pico Technology, UK). Temperature recordings were temporally synchronised with respirometry and audio recordings. Animal activity was monitored using an infrared activity detector (AD-2 Activity Detector, Sable Systems International, Las Vegas, NV, USA). The cuvette containing the insect was wrapped with aluminium foil to improve activity detector readings, and placed inside an insulated cooler box container with a sound recording device. Temperature was allowed to vary (i.e. not strictly controlled) during trials, as constant temperature might not have encouraged natural calling to be induced.

Calls produced during experimental trials were acoustically monitored and recorded in real-time using a Song Meter wildlife recorder (Model SM2+, Wildlife Acoustics, Inc., Concord, MA, USA) fitted with an omnidirectional weatherproof acoustic microphone for SM2 [sensitivity: -36±4dB (0dB=1V/pa@1KHz), frequency response: 20Hz–20 000Hz; Model SMX-II, Wildlife Acoustics, Inc., Concord, MA, USA] directly onto the left channel of the recorder. Sound recordings were made at a sample rate of 96kHz (16-bit resolution). The recorder was pre-amplified to 48dB gain and digitally configured to an additional 12dB gain. Sound recordings were made continuously in 10-min intervals, with no gaps between consecutive recordings. For all experimental trials, the start and end-times of sound recordings were synchronised with the start and end-times of MR measurements. The total duration of the baseline, respirometry and calling periods were recorded for each experimental trial.

*Respirometry and temperature data processing and analysis*

In Expedata, $CO_2$ and $H_2O$ data were transformed from ppm to ml $CO_2$ $h^{-1}$ and ppt to mg $H_2O$ $h^{-1}$ respectively. Using the marker tool in Expedata, the data were divided into the two baseline periods and the intermediate 10-min time intervals that correspond with the acoustic recording intervals per individual. $H_2O$ data were discarded as the lag times were too large to analyse meaningfully. After correcting for baseline drift, the mean $CO_2$ production values for each 10-min interval were extracted for each individual. $CO_2$ production rate ($V_{CO2}$; ml $CO_2$ $h^{-1}$) was then converted to oxygen consumption rate [$V_{O2;}$ ml $O_2$ $h^{-1}$) and to microwatts (µW) assuming a respiratory quotient (RQ) of 0.84 (Lighton 2008) and an oxyjoule equivalent of 20.3 J $ml^{-1}$ (Lighton *et al.* 1987). Resting metabolic rates (RMRs) were considered to be the lowest MR 10-min interval recorded per individual, which was confirmed as intervals without activity by visually inspecting the activity detector recordings. Katydids were quiescent for the majority of time during the respirometry trials, and when small activity bouts were observed these never coincided with calling periods. Calling metabolic rates (CMRs) were considered to be the MR recorded during the 10-min interval with the highest calling rate for every individual. Temperature data of each individual were exported from the PicoLog software and the mean temperature was calculated for all 10-min intervals that correspond to the audio and respirometry recordings.

*Acoustic data processing and metrics of calling costs*

The Song Meter recorder stored each 10-min audio recording as individual uncompressed ".WAV" files logged to a 32 GB memory card by the Song Meter device. All audio files were analysed in Raven Pro (v. 1.5; Cornell Lab of Ornithology, Bioacoustics Research Program, NY, USA) and extraneous low-frequency sounds were filtered out using the band filtering feature [specifically, Raven uses the Window Method for FIR filter design; see Oppenheim *et al.* (1998) and Charif *et al.* (2010) for a complete description of this method]. The number of chirps (or WSs) were counted in every audio file over each 10-min interval for all individuals using the Band Limited Energy Detector (BLED). Target signal parameters for the BLED were acquired by making multiple selections for each parameter on the spectrogram and then by extracting the most appropriate values from the selection table provided by Raven for each parameter. Selections were made according to the following measures: Minimum and maximum frequencies (kHz) determined the frequency range of the pulses in which the detector

searched; minimum and maximum duration (ms) specified the length of signal that could be considered a single detection (or one chirp); and minimum separation corresponded to the time interval between adjacent chirps (i.e., chirp intervals) [for a complete description of this method, see Mills (2000)]. After the BLED was run, a visual scan through the spectrogram was performed to ensure all detections were accurate and that no calls were missed. The number of chirps counted by the BLED represented the number of calls made during a specific interval and were correlated with the corresponding respirometry interval to obtain an estimate of metabolic rate relative to a specific calling effort. From 18 respirometry trials, 11 individuals called sufficiently throughout the trial to be included in analyses. The other seven individuals either did not call or only chirped briefly once or twice, thus limiting us from comparing calling and resting periods; therefore, they were excluded. Additionally, the peak power (dB) of the calls (detected by the BLED) was extracted from the selection table provided by Raven for a subset of 6 individuals (individuals 6-11). I could only do these analyses in a subset of individuals as the harddrive storing individuals 1-5 calling data was corrupted. The dB values of the calls were averaged over their respective 10-min intervals and represented the call power for corresponding time intervals.

The cost of calling was estimated using a set of different metrics for each individual. The metrics employed were: (1) MR (ml $CO_2$ $h^{-1}$); (2) mass-specific MR ($MR_{ms}$; ml $CO_2$ $h^{-1}$ $g^{-1}$); (3) MR per call (ml $CO_2$ $h^{-1}$ $call^{-1}$); mass-specific $MR_{ms}$ per call (ml $CO_2$ $h^{-1}$ $g^{-1}$ $call^{-1}$); (4) Δcost of calls (calculated as the difference between $CMR_{ms}$ and $RMR_{ms}$ and expressed per call), 5) percentage cost of calls (metabolic cost of calling expressed as the % change over resting rates divided by the number of calls), and finally 6) the cumulative energy cost of calling inferred from the total number of calls and each call's relative cost summed over time. The cumulative cost was calculated over the longest single contiguous period (17:30 PM to 03:00 AM, i.e 9.5 h) for each individual trial. However, individuals 6 and 9 had only 9 h overlap during this period, and individual 4 only had 3.5 h overlap. As a result, the cumulative cost calculated for individuals 4, 6 and 9 over their respective time periods was extrapolated to a 9.5 h period.

*Repeatability assessment*

Repeated measurements of mean MR and number of calls recorded over the 10-min intervals during respirometry trials of the 11 calling males were used to estimate the repeatability of MR and various metrics of calling costs. The intraclass correlation coefficient (ICC) was estimated following methods described by Wolak *et al.* (2012) in the *icc* package (v. 2.3.0) run in R version 3.3.1 (R Core Team 2013). Measurements tested for repeatability were MR (ml $CO_2$ $h^{-1}$); $MR_{ms}$ (ml $CO_2$ $h^{-1}$ $g^{-1}$); number of calls; MR per call (ml $CO_2$ $h^{-1}$ $call^{-1}$); $MR_{ms}$ per call (ml $CO_2$ $h^{-1}$ $g^{-1}$ $call^{-1}$); Δcost of calls and percentage cost of calls. These repeatability estimates were obtained from the full data set ($N$=11, allowing temperature variation) as well as only using a subset of the data, ($N$=10, 10-min sections representing controlled temperature conditions between 22 and 24°C), to test whether repeatability is affected by extrinsic factors – temperature in this case. One individual was excluded from the subset of data because the mean temperature during its trial was >27ºC. To assess whether temporal autocorrelation might be influencing the repeatability results, I examined this per individual in STATISTICA (StatSoft, Tulsa, OK, USA) using time-series forecasting tools on the 10-min extracted data and found little to no significant autocorrelation.

*Interspecific data comparison*

To better understand the range of variation I observed and place it into context of other activities and species, RMR and CMR of *P. graminea* measured in this study were compared with MRs of other resting Orthoptera, calling Orthoptera and flying insects. Values for MRs and body mass were compiled from the literature (Table S1, *Journal of Experimental Biology* online supplementary information). Mass specific MRs were converted back to MR by multiplying with fresh (wet) body mass. Data were available in a variety of units, and these were converted to microwatts (µW) assuming an RQ value of 0.84 and an oxyjoule equivalent of 20.3 J $ml^{-1}$. When necessary, MR data were adjusted to 25°C assuming a $Q_{10}$ of 2.0, with MR roughly doubling with a 10°C increase in temperature (Nespolo *et al.* 2003; Terblanche *et al.* 2007; Irlich *et al.* 2009; reviewed in Dell *et al.* 2011), which was also the case in *P. graminea* here. In the case where several studies had measured the same species' MR, I calculated the mean across these studies. All data were normalised by logarithmic ($log_{10}$) transformation.

RMR of 393 insect species from 16 orders and 87 families were obtained from published data (Chown *et al.* 2007). For this study, however, I only focused on a sub-set of those data and included the 32 species of resting Orthoptera for the purpose of clarity on the graph and also because of the level of variation that exists in RMR amongst insects for various physiological, ecological and evolutionary reasons (see discussions in e.g. Nespolo *et al.* 2003; White 2011; White & Kearney 2014). Additionally, I obtained CMR values of 14 orthopteran species from previously published data (Table S1), and flying MR (FMR) values for 56 insect species from six orders from Niven & Scharlemann (2005). FMR values were included as an upper boundary on metabolic rates that might be expected across the Insecta (following e.g. White *et al.* 2008). The effect of phylogenetic signal on the relationship of metabolic rate to body mass was investigated by means of a phylogenetic generalised least-squares analysis (PGLS) analysis (details in Appendix B).

*Statistical analysis*

Statistical tests were performed using STATISTICA v.13 (StatSoft, Tulsa, OK, USA) or R v. 3.2.4 using the 'lme4' library for the linear mixed-effects model. Data were checked for normality using the Shapiro-Wilk test. RMR and CMR data were compared using appropriate pairwise tests for dependent samples based on the normality of the distribution of the data. When comparing mean RMR with mean CMR, the RMR was expected to be the lowest MR period for each individual when it was not calling, and CMR was considered to be the MR during the period with the highest calling rate for each individual. However, I discovered that the period with the lowest MR for each individual was not necessarily a period without calls for every individual. This was true for three individuals. Therefore, I decided to include both scenarios in the analysis; (1) comparing the RMR at no calls ($RMR_n$) with CMR, and (2) comparing the RMR at the lowest MR period (even if an individual did call during this period, $RMR_c$) with CMR. I also compared $RMR_n$ and $RMR_c$ with each other. Non-parametric sign tests were used to compare $RMR_n$, $RMR_c$ and CMR with each other because the data were not normally distributed.

To test whether temperatures at $RMR_n$, $RMR_c$ and CMR were similar, dependent samples *t*-tests were performed to compare the three pairs. Temperature data at RMR and CMR were

compared using dependent samples *t*-tests, after verifying that the dependent variable was approximately normally distributed and that there were no significant outliers.

For the repeatability assessment, significantly different ICC values between the complete dataset and the subset of data were determined through inspection of the 95% confidence intervals of the respective parameters. If the 95% confidence intervals of the parameters did not overlap between the complete dataset and the subset, the ICC values were considered significantly different from each other ($P<0.05$).

RMR and CMR values for *P. graminea* measured in this study were compared with RMR and CMR measurements of other orthopteran species and FMRs of flying insects based on literature on scaling of energy use. To investigate how RMR and CMR of *P. graminea* compared with RMRs, CMRs and FMRs of other species, *P*-values were determined using prediction levels in relation to the respective regression lines, following Cooper & Withers (2006). In all tests, I assumed $P=0.05$ as the critical value for rejecting a null hypothesis.

**RESULTS**

The insects were typically quiescent during daylight hours in the laboratory, as they are in the field (Stevens & Josephson 1977). At dusk they became active and started calling. However, placing them in a dark chamber during the day was sufficient to disrupt quiescence. Two trials were initiated during the day and in both cases the insects started calling within an hour of placement in the darkened respirometry chamber. The other nine trials were started just before dusk and continued until the next morning. Activity detector readings showed that the animals were mostly inactive for the entire time, and traces showed very little activity even when calling. Calling never coincided with other activities.

Overall, calling activity was significantly positively correlated with $V_{CO2}$ ($R=0.73$, $P<0.0001$), where $V_{CO2}$ increased with an increase in number of calls, although this was variable among individuals (Fig. 1A). However, when comparing a generalised linear model (GLM) versus a

general linear mixed-effects (GLME) model (accounting for individuals as random effects), I found that the GLME model is a significantly better model (ΔAIC: 626.8) and, therefore, accounting for individuals is important. There was no relationship between the power of calls and $V_{CO2}$ ($P$=0.189) (Fig. 1B), and call power was not related to Δcost ($P$=0.76) or percentage cost ($P$=0.521) as estimates of the metabolic cost of calling (Fig. 1C, D).

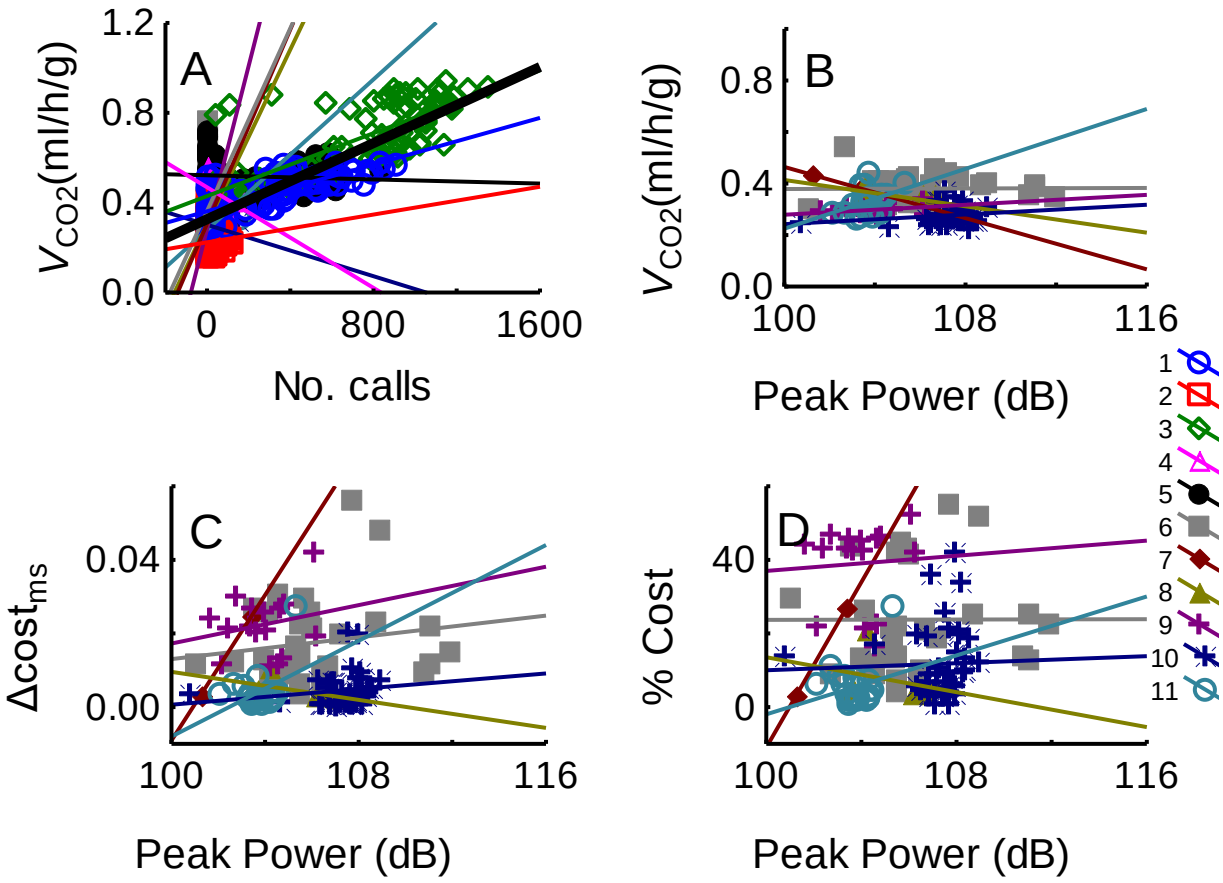


Fig. 1. **Scatterplots showing the relationship between various calling (sound) estimates and metabolic rate of call cost estimates in katydids.** (**A**) $V_{CO2}$ was significantly positively correlated with number of calls (y=0.0004x+0.327; $R$=0.73, $P$<0.0001). (**B**) $V_{CO2}$ versus peak power of calls recorded (overall trend: $P$=0.189). (**C**) Metabolic cost of calling expressed as the difference between the mass-specific metabolic rate during calling minus mass-specific resting metabolic rate when not calling divided by the number of calls plotted against peak calling power recorded (overall trend: $P$=0.76). (**D**) Metabolic cost of calling expressed as the percentage change over resting rates divided by the number of calls versus peak calling power (overall trend: $P$=0.521). Each individual is shown as a unique line colour as well as the overall trendline is shown in black [bold indicates statistical significance (only in the case of A)]. Note that power graphs were only for a subset of six individuals for which these data could be estimated.

The mean total number of calls per trial was 347.6±137.8 (range: 9-1350). The mean percentage increase in MR from RMR to CMR was 60.1±12.7% in calling males (maximum 154%). However, the mean (±s.e.m.) percentage increase per call (or per WS) was *ca.* 1±0.5% (Table 1). Expressing the cost of calling in different metrics yielded different results. An individual with a relatively high calling rate experienced a small percentage increase in MR (Individual 5, call rate=826; absolute increase=13.6%), whereas an individual with a low calling rate (Individual 9, with a maximum calling rate of 9) experienced an absolute increase in MR of *ca.* 50% (Table 1). However, the cost of calling for a 'cheap' caller with a high calling rate accumulated rapidly over time and, therefore, Individual 5 experienced a high cumulative cost (3735.2 $CO_2$ ml $h^{-1}$ $g^{-1}$) compared with a more expensive caller (Individual 9, 19.8 ml $CO_2$ $h^{-1}$ $g^{-1}$) with a low call rate (Table 1). A power law function described this relationship between cumulative cost and percentage increase per call ($y=130.21x^{-1.068}$, $R^2=0.858$), where y is cumulative cost and x is percentage increase per call.

Table 1. **Summary statistics from *Plangia graminea* respirometry and acoustic recordings.** Percentage increase in metabolic rate (MR, $CO_2$ ml $h^{-1}$ $g^{-1}$) from resting metabolic rate (RMR, $CO_2$ ml $h^{-1}$ $g^{-1}$) to calling metabolic rate (CMR, $CO_2$ ml $h^{-1}$ $g^{-1}$), and expressed per call (at maximum calling effort) for 11 *Plangia graminea* individuals respectively. Cumulative costs of calling estimated as the mean cost of calling multiplied by the number of calls per individual over 9.5 h. Note that estimates are for 10 min interval summaries and are conditional on the number of calls being at maximum (full details in Materials and Methods).

| Individual *Plangia* | Body mass (g) | Mean temperature (°C) | RMR (Not Calling) (ml $CO_2$ $h^{-1}$ $g^{-1}$) | CMR (max. call effort) (ml $CO_2$ $h^{-1}$ $g^{-1}$) | Number of calls | Increase in MR (%) | Increase per call (%) | Cumulative cost (ml $CO_2$ $h^{-1}$ $g^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.64 | 24.6 | 0.287 | 0.568 | 905 | 98.0 | 0.1 | 2468.7 |
| 2 | 0.83 | 21.5 | 0.170 | 0.226 | 118 | 33.0 | 0.3 | 191.0 |
| 3 | 0.77 | 27.1 | 0.359 | 0.915 | 1350 | 154.5 | 0.1 | 4614.6 |
| 4 | 0.88 | 26.0 | 0.385 | 0.407 | 159 | 5.7 | 0.04 | 579.2 |
| 5 | 0.70 | 27.4 | 0.476 | 0.540 | 826 | 13.6 | 0.02 | 3735.2 |
| 6 | 0.79 | 22.8 | 0.258 | 0.456 | 49 | 76.8 | 1.6 | 122.0 |
| 7 | 0.58 | 22.0 | 0.233 | 0.433 | 62 | 86.2 | 1.4 | 139.0 |
| 8 | 0.65 | 21.9 | 0.244 | 0.357 | 38 | 46.5 | 1.2 | 89.1 |
| 9 | 0.60 | 22.7 | 0.219 | 0.326 | 9 | 48.6 | 5.4 | 19.8 |
| 10 | 0.75 | 22.0 | 0.223 | 0.319 | 150 | 43.1 | 0.3 | 318.1 |
| 11 | 0.68 | 21.8 | 0.213 | 0.330 | 158 | 54.9 | 0.3 | 320.7 |
| Mean±s.e.m. | 0.71±0.03 | 23.6±0.7 | 0.279±0.028 | 0.443±0.056 | 347.64±137.80 | 60.1±12.7 | 1.0±0.5 | 1145.2±500.3 |

Sign tests indicated that both $RMR_n$ and $RMR_c$ were significantly different from CMR, ($Z$=3.015, $P$=0.003), but they were not significantly different from each other ($Z$=1.155; $P$=0.248) (Fig. 2A). There was no significant difference in air temperature within the respirometry cuvette between $RMR_n$ (23.6±3.1°C) and $RMR_c$ (22.9±2.2°C) ($t$=-1.384, $P$=0.196, $N$=11) as well as $RMR_n$ and CMR (23.5±2.0°C) ($t$=0.229, $P$=0.824, $N$=11), but there was a small yet significant difference between $RMR_c$ and CMR ($t$=-2.329, $P$=0.042, $N$=11) (Fig. 2B). From here onwards, I refer to $RMR_n$ as RMR and exclude $RMR_c$ from further analysis, as there was no significant difference in mean $RMR_n$ and $RMR_c$, and no significant difference in temperature at $RMR_n$ and CMR. Across all individuals for the 'no calling' periods only, the $RMR_n$ was positively related to temperature [y=0.0320±0.002x-0.410±0.040 (mean±s.e.m.); $R^2$=0.39; $F_{1,529}$=329.18, $P$<0.0001] and had a typical $Q_{10}$ effect ($Q_{10}$=2.09).

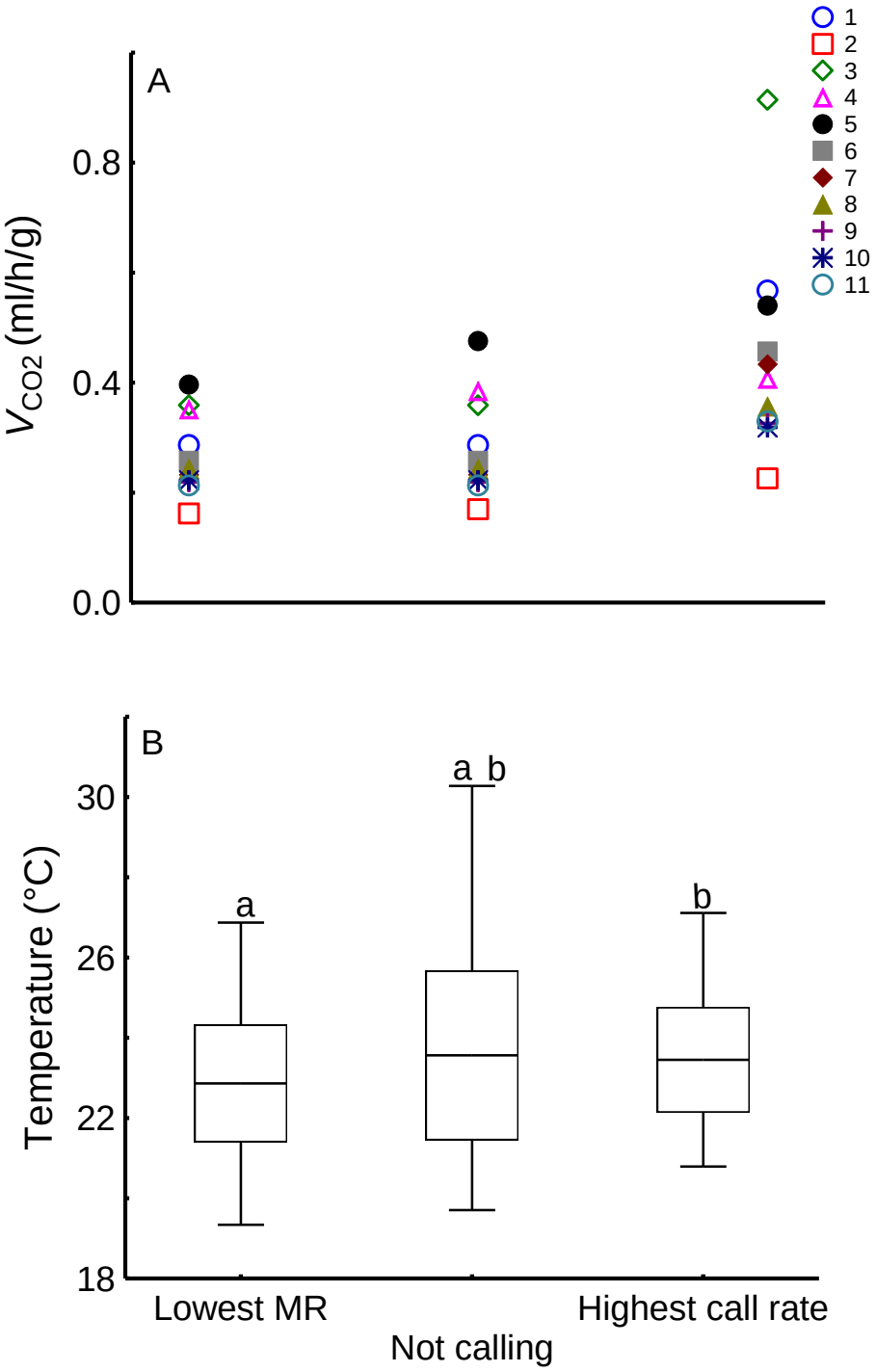


Fig. 2. **Comparison of mean $CO_2$ production rates and temperature in different metabolic and activity states. (A)** Mean $CO_2$ production ($V_{CO2}$; ml $CO_2$ $h^{-1}$ $g^{-1}$) in respirometry trials from 11 individuals. (**B**) Air temperature (°C) measured inside the respirometry cuvette during each of the 11 experimental trials in A. The data are presented for the mean of the lowest MR interval, the period when katydids were not calling and the interval with the highest calling rates recorded. Box plot boundaries show the 95% confidence intervals and the solid horizontal line is the mean. Error bars above and below the box indicate minimum and maximum temperatures. Different letters indicate statistically significant homogeneous groups.

Mean $CO_2$ production rates for calling males were 0.443±0.056 ml $CO_2$ $h^{-1}$ $g^{-1}$. This was an increase of approximately 1.6 times the mean resting rate of 0.279±0.028 ml $CO_2$ $h^{-1}$ $g^{-1}$ (Table 1). The energy required for production of the calling song was the total energy used during calling minus the resting metabolic rate (Kavanagh 1987). For *P. graminea* this was 2280.34-1430.25=850.09 µW.

In the complete dataset (with temperature varying between ca. 20 and 30ºC), repeatability was high for MR, $MR_{ms}$ and the number of calls produced, but low for the various measurements of cost of calling (MR per call, Δcost and %cost). However, when considering only the subset of data representing a controlled temperature range (22-24ºC), repeatability increased for all parameters, and significantly so for MR per call, $MR_{ms}$ per call and Δcost (Table 2).

Table 2. Repeatability estimated from the intraclass correlation coefficient (ICC) with 95% confidence intervals, sample size (*n*), mean number of observations per *Plangia graminea* individual (*k*), for various metabolic or calling cost measurements using the complete dataset (with temperature variation ~20-30 °C) and a subset of the data (measurements only between 22 and 24 ºC).

| | ICC (Lower 95% CI – Upper 95% CI) | | | | | |
|---|---|---|---|---|---|---|
| | Complete dataset | *n* | *k* | Subset (22-24°C) | *n* | *k* |
| MR (ml $CO_2$ $h^{-1}$) | 0.7757 (0.6231 - 0.9149) | 11 | 41.2 | 0.8462 (0.7083 - 0.9496) | 10 | 12.3 |
| $MR_{ms}$ (ml $CO_2$ $h^{-1}$ $g^{-1}$) | 0.7616 (0.6041 - 0.9086) | 11 | 41.2 | 0.8154 (0.6600 - 0.9382) | 10 | 12.3 |
| Number of calls | 0.5741 (0.3890 - 0.8086) | 11 | 41.2 | 0.8631 (0.7362 - 0.9557) | 10 | 12.3 |
| MR/Call (ml $CO_2$ $h^{-1}$ $call^{-1}$) | **0.0892 (0.0334 - 0.2614)** | **11** | **41.2** | **0.5040 (0.2919 - 0.7846)** | **10** | **12.3** |
| $MR_{ms}$/Call (ml $CO_2$ $h^{-1}$ $g^{-1}$ $call^{-1}$) | **0.1005 (0.0396 - 0.2840)** | **11** | **41.2** | **0.5604 (0.3458 - 0.8189)** | **10** | **12.3** |
| ΔCost | 0.0938 (0.0359 - 0.2708) | 11 | 41.2 | 0.4712 (0.2625 - 0.7629) | 10 | 12.3 |
| $\Delta Cost_{ms}$ | **0.1041 (0.0416 - 0.2911)** | **11** | **41.2** | **0.5130 (0.3002 - 0.7903)** | **10** | **12.3** |
| % Cost | 0.2008 (0.0978 - 0.4531) | 11 | 41.2 | 0.5467 (0.3322 - 0.8108) | 10 | 12.3 |

$$\Delta Cost = \frac{MR - RMR^{*}}{Number\ of\ calls}$$

$$\Delta Cost_{ms} = \frac{MR_{ms} - RMR^{*}_{ms}}{Number\ of\ calls}$$

$$\%\ Cost = \left[\frac{MR}{RMR} \times 100\right] \div Number\ of\ calls$$

*RMR = Lowest MR period recorded per individual; $MR_{ms}$ = mass-specific MR

Values in bold text indicate significantly different ICC values between the complete dataset and the subset (22-24ºC) for specific parameters

In the interspecific comparison, resting, calling, and maximum recorded calling MR of *P. graminea* all fall within the 95% prediction level of resting Orthoptera ($P$=0.822, 0.65 and 0.203, respectively) as well as the 95% prediction level of calling Orthoptera ($P$=0.062, 0.132 and 0.29, respectively), but are all significantly lower than MR from flying insects ($P$<0.001) (Fig. 3). There was significant phylogenetic signal for FMR-mass scaling, but not in the case of RMR or calling Orthoptera mass scaling (Table S2, Fig. S4, Appendix B).

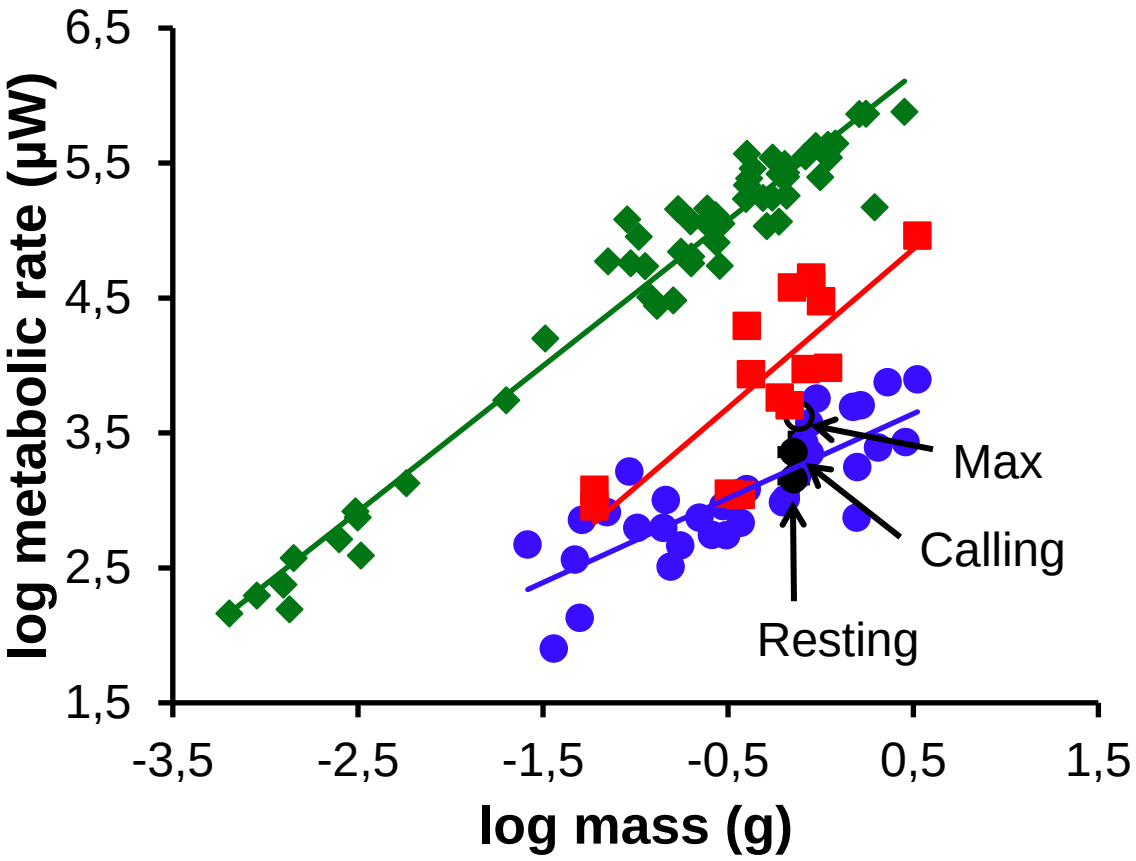


Fig. 3. **Scatterplot showing the linear relationships between metabolic rate (MR, µW) and body mass (*M*, g) for resting, calling and flying insects.** Resting and calling MR of *Plangia graminea* is shown in black filled circles (mean±s.d. [standard deviation]). The maximum metabolic rate recorded in this study for a calling *P. graminea* individual is shown as a black open circle. Metabolic rates of flying insects [green filled diamonds; ordinary least-squares (OLS) regression (y=mx+c): y=1.081x+5.617; phylogenetic generalised least squares (PGLS) regression: y=0.906x+5.245], resting Orthoptera (blue filled circles; OLS: y=0.626x+3.33; PGLS: y=0.561x+3.351), and other calling Orthoptera (red filled squares; OLS: y=1.176x+4.274; PGLS: y=1.27x+4.32). Metabolic rates are normalised to 25°C assuming a $Q_{10}$ of 2.0 (our estimate for *P. graminea* $Q_{10}$ was 2.09). PGLS fits are not shown here since they were virtually indistinguishable from the OLS lines in each case (Fig. S4). Names of species included in this graph with their respective values are provided in Table S1.

## DISCUSSION

While it is widely postulated that the energetic costs of sexual signalling are likely to be high (e.g. Heller & von Helversen 1993), and the efficiency of calling is often argued to be significant in the evolution of sexual signalling (e.g. Gerhardt & Huber 2002) and in trade-offs or the evolution of calling (Symes *et al.* 2015), the direct estimates of costs typically do not support these views. Indeed, the metabolic costs of communication are often small (or insignificant) relative to other activities based on direct estimates (e.g. Bailey *et al.* 1993; White *et al.* 2008). Therefore, I provided a detailed comprehensive assessment of diverse cost metrics expressed in different ways and across more controlled versus more variable conditions. One major novel outcome of the approach I undertook is that it provides strong support for the view that an estimate of metabolic cost of calling or communicative sound production depends heavily on the context. More specifically, the cumulative costs are pronounced for those individuals with low calling metabolic cost per call and are described by a power law function. If cumulative energy costs were extrapolated to multiple nights spent calling and/or calls were sustained for a long period owing to e.g. extrinsic factors (e.g. windy or noisy environments), for example, then such energy-related expenses could well become a significant proportion of an insect's lifetime energy budget and result in significant trade-offs. My results show a small but significant metabolic cost associated with calling effort in *P. graminea* although the approach I used likely maximised these costs, an inadvertent consequence of my efforts to obtain robust metabolic rate estimates during calling bouts. The CMR of *P. graminea* males was significantly higher than their RMR based on these estimates. The RMR for *P. graminea* (0.332 ml $O_2$ $h^{-1}$ $g^{-1}$ at ~22-27°C) is similar to that of most other insects (0.30-0.48 ml $O_2$ $h^{-1}$ $g^{-1}$; Bailey *et al.* 1993) and to that predicted from the scaling relationship in Chown *et al.* (2007) (predicted MR of a 0.71 g individual=3.138 µW vs. my estimate of MR=3.155 µW; $t_{391}$=0.653; $P$=0.743). The MR of calling in *P. graminea* (0.443 ml $CO_2$ $h^{-1}$ $g^{-1}$) is elevated to approximately 1.6 times over resting levels. This translates to an approximate 60% increase in RMR in the 11 calling males analysed in this study, and the energy required for the production of the calling song (±850 µW).

Although I observed a significant increase in MR owing to calling in this study, this increase is perhaps relatively low when compared with other calling Orthoptera (Fig. 3). This can be explained by the nature of the calling song. The calls of chirping species consist of short bursts

of sound followed by a pause (Lee & Loher 1993; Gerhardt & Huber 2002), with WSRs an order of magnitude slower than species that produce trilled calls (Weissman *et al.* 1980), where a number of sound chirps are produced in rapid succession without extended pauses (Prestwich & O'Sullivan 2005). The order of magnitude greater WSRs of trilling species mean that more mechanical work is performed, assuming constant costs, which in turn, translates to the higher rates of metabolism reported in these species (Prestwich & Walker 1981; Kavanagh 1987; Lee & Loher 1993; Hack 1998; Prestwich & O'Sullivan 2005). However, it is important to note that muscle work may not necessarily correlate with calling effort owing to elasticity which can result in muscle contractions being far more efficient than might be expected (e.g. Dickinson & Lighton 1995; Qin *et al.* 2012). Therefore, actual muscle work depends on the elastic contribution of resilin and the cuticle which, in turn, could mask the detection of metabolic costs of calling and influence estimates of mechanical efficiency. In jumping insects [e.g. the froghoppers (Hemiptera, Cercopoidea)], energy needed for jumping is stored by means of a composite structure of chitinous cuticle and resilin (Burrows *et al.* 2008). In the same way, energy needed for wing movement in katydids to produce their calling songs can perhaps be stored, similar to the sound-producing tymbals of cicadas (e.g. *Cyclochila australasiae*, Bennet-Clark 1997; *Tympanistalna gastrica*, Fonseca & Bennet-Clark 1998), and therefore, work performed may be low while sounds appear costly. Like *P. graminea*, there are many other orthopterans that produce chirping songs. The elevation in MRs reported for three chirping crickets *Acheta domesticus* (1.5x resting; Hack 1998), *Teleogryllus comoddus* and *Teleogryllus oceanicus* [both ca. 2x resting; Lee & Loher 1993; however, Kavanagh (1987) reported a fourfold increase] - is similar to the values reported for two chirping katydids *R. verticalis* (Bailey *et al.* 1993) and *P. graminea* (present study), both of which experienced an increase of approximately 1.6x their resting rates. By contrast, elevated MRs reported for trilling species ranged from 5-13 times that of their respective resting MRs (Stevens & Josephson 1977; Prestwich & Walker 1981; Kavanagh 1987; Bailey *et al.* 1993; Lee & Loher 1993; Hack 1998; Prestwich & O'Sullivan 2005; White *et al.* 2008; Erregger *et al.* 2017). The conehead katydids *E. nasutus* and *N. robustus*, produce trilling calls and experience a more than sixfold increase in MR during stridulation (Stevens & Josephson 1977). This is fourfold the increase reported for *P. graminea*, which is a similarly sized katydid. Interestingly, the trilling tree cricket *Oecanthus quadripunctatus* reaches a calling MR comparable to that of *P. graminea* during stridulation, even though it has a significantly smaller body size (Prestwich & Walker 1981). In other words, the relative cost of calling likely varies across taxa and is

partly dependent on the nature of the call (see also Erregger *et al.* 2017) and the number of file teeth struck per WS (Prestwich & Walker 1981).

Resting and calling metabolic rates are significantly lower compared with the MR-mass scaling relationships of flying insects (Fig. 3), a result that is largely in keeping with previous studies (e.g. Prestwich & Walker 1981; White *et al.* 2008). For example, White *et al.* (2008) reported that the CMR of the mole cricket, *Gryllotalpa monanka*, is only 10% that of the MR predicted for a 0.89 g insect based on the scaling relationship that they derived for flying insects using data acquired from the available literature ($MR=59.7\ M^{0.82 \pm 0.09\ [95\%\ CI]}$). Using the same trendline to predict the FMR for an insect with the average mass of *P. graminea* (0.71 g), the CMR for *P. graminea* would be less than 1% that of the predicted value (0.38 ml $O_2$/h). In contrast, a flying female of the same mass consumes a similar amount in only 30 s spent searching for a male, assuming similar muscle mechanical efficiencies between different activities (e.g. flying and calling). It is interesting to note that the same set of muscles used to move the wings during flight, are used by katydids and crickets during stridulation (Stevens & Josephson 1977; Lee & Loher 1993). However, Stevens & Josephson (1977) reported that the katydids *E. nasutus* and *N. robustus* had WSRs an order of magnitude higher during stridulation than a similar-sized desert locust during flight. Even so, the MR of the wing muscles for the two katydids was less than in a flying locust. Why, then, is flight so much more expensive than stridulation? Weis-Fogh (1964) concluded that the aerodynamic work during flight is three to five times greater than the inertial work, i.e., the work required to accelerate the oscillating wings of a flying locust. This suggests that there are other aspects of flight that add to the overall metabolic cost of flight and likely contribute to this variation.

It is widely accepted that ambient or body temperature affects many aspects of the functional performance of insects, including biochemical and physiological processes (Nespolo *et al.* 2003; Terblanche *et al.* 2007; Irlich *et al.* 2009; Dell *et al.* 2011; Halsey *et al.* 2015). Therefore, an increased MR may be a result of increased temperature and is not necessarily due to activities such as calling. Moreover, it has been shown that calling in itself may also increase the insect's body temperature (Heath & Josephson 1970; Stevens & Josephson 1977, Erregger *et al.* 2017). This is especially true for species that produce trilling calls, where WSRs are high and sound is produced almost continuously (Bailey *et al.* 1993). The thoracic temperature of

the katydid *N. robustus*, for example, is 5-15°C higher than that of the environment when producing its trilling calls (Heath & Josephson 1970). Stevens & Josephson (1977) also reported an average increase of 16.6°C in body temperature of calling *N. robustus* specimens. These katydids seemingly depend on this heat production in order to achieve greater acoustic power outputs (Heath & Josephson 1970; Stevens & Josephson 1977). Where chirping species are concerned, however, heat production as a result of calling is low and thoracic temperatures typically remain similar to ambient levels (Bailey *et al.* 1993). In this study, although there was a typical $Q_{10}$ effect overall, the variation in temperature was relatively small within individuals over each trial and therefore the mean levels remained quite similar between MR and calling cost estimates within individuals.

Although the overall increase in MR with calling was significant for the 11 individuals analysed in this study, there was considerable variation noted among individuals. The total number of calls produced per individual ranged from nine to 1350, and the percentage increase in RMR ranged from 5 to almost 155% (Table 1). In addition to temperature, body mass is another immediate determinant of MR in insects (e.g. Nespolo *et al.* 2003; Chown *et al.* 2007; Riveros & Enquist 2011) which may have attributed to the variation among individuals in this study. However, the differences in mass in *P. graminea* were not to such a degree that could explain the level of variation found in MR recorded here. The more plausible explanation for the variation in MR experienced by the males in this study is that some males merely called less actively than others.

Male crickets can facultatively adjust their calling strategy to fit local conditions (Hack 1998). According to Hack (1998), the relative prevalence of calling and non-calling strategies among conspecific male crickets appears to be mediated by population density. For example, the daily calling durations of individuals in *Gryllus campestris* field populations vary widely, and males within the same population vary independently of each other (Rost & Honegger 1987). Presumably, changes in the social environment or small-scale interactions among individuals, rather than larger-scale changes in the ecological or physical environment [e.g. temperature (Walker 1983)], which would affect individuals in the same population similarly, give rise to this variation (Hack 1998).

Previous studies on field crickets have indeed shown that increases in local density cause males to abandon calling for a non-calling mate-searching strategy, and to abandon site defence (Alexander 1961; French & Cade 1989; Hissmann 1990; Cade & Cade 1992). Although both strategies can occur at high densities, the majority of males in high density environments pursue a non-calling rather than calling strategy (e.g. Hissmann 1990). Although katydids are distantly related to crickets and no studies have established whether katydid mating behaviour is similarly affected by population density, it is possible that the density of males kept in vivaria in this study may have caused some of the males to switch to a non-calling strategy and presumably caused some of the variation in calling frequency recorded during the respirometry experiments. This behaviour, however, should be more prevalent in trilling species because a trilling male expends energy at roughly the same rate, whether calling or walking, and therefore, would be more prone to switch between the two strategies (Hack 1998). In contrast, the energy cost differential between calling and walking in chirping species is much greater, making it less beneficial for chirpers to abandon a calling strategy (Hack 1998).

The ICC values reported for MR of *P.graminea* in this study are within range of what is expected from measurements of other insects over shorter time-scales (<24 h) (e.g. Marais & Chown 2003; Nespolo & Franco 2007; reviewed in Wolak *et al.* 2012). Under conditions with temperature variation, repeatability of MR and number of calls of *P. graminea* was high but for some estimates of the cost of calling, repeatability was low (Table 2). However, under conditions where costs were estimated only for a controlled temperature range (22-24ºC), repeatability increased significantly. This indicates that repeatability, and costs associated with activities such as calling, are context-dependent. Most importantly, estimates of metabolic costs depend on the context in which they are measured and how such costs are expressed. Partly this is an issue of choice of units of measurement (e.g. percentage increase vs absolute increase will naturally yield divergent estimates of cost) but also because a 'snapshot' view of energy costs may be wholly inadequate. Estimates of the cumulative energy cost of calling inferred from the total number of calls and each call's relative costs in my study yielded an entirely different view. From this analysis individuals with a 'cheap' call spend far more time calling and thus incur a high cumulative cost; individuals with expensive calls spend very little time calling and have low cumulative cost. This is a novel and important demonstration of the value of an energy budget approach to considering the problem of communication and its energetic consequences.

## CONCLUSION

The MR of calling *P. graminea* males was found to be significantly higher compared to periods of RMR, therefore, calling is associated with increased metabolic costs. Since male katydids produce calls to attract conspecific females for mating, calling is closely related to their reproductive success. However, this cost is additional to other metabolic costs involved during mating (such as the production of a spermatophylax), and other metabolically expensive daily activities such as flying and feeding. The increased metabolic cost associated with calling could therefore constrain a male katydid's mating behaviour. Considering the overall $Q_{10}$ effect of temperature on the metabolic rate of an animal, increased temperature experienced in the field may demand trade-offs to be made in terms of the animal's daily energy budget. Elevated temperature experienced in vineyards could, therefore, potentially result in reduced calling activity of male katydids and, in turn, decrease their reproductive success. This would result in lower population levels in vineyards the following season.

## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| BLED | band limited energy detector |
| CMR | calling metabolic rate |
| FMR | flying metabolic rate |
| ICC | intraclass correlation coefficient |
| MR | metabolic rate |
| $MR_{ms}$ | mass-specific metabolic rate |
| RMR | resting metabolic rate |
| $RMR_c$ | resting metabolic rate at the lowest MR period (even if an individual did call during this period) |
| $RMR_n$ | resting metabolic rate at no calls |
| RQ | respiratory quotient |
| $V_{CO2}$ | carbon dioxide production rate |

| | |
|---|---|
| $V_{O2}$ | oxygen consumption rate |
| WS | wing stroke |
| WSR | wing stroke rate |

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# CHAPTER 5

## Natural enemies of *Plangia graminea* Serville (Orthoptera: Tettigoniidae) in vineyards in the Western Cape, South Africa; and their potential for biological control


## ABSTRACT

*Plangia graminea* (Serville) is a sporadic pest in vineyards in the Western Cape which causes damage to the foliage and grape berries of the vine. There are currently no registered control measures for this pest, resulting in the need for the development of an Integrated Pest Management strategy. I investigated the natural prevalence of key biological control agents present in vineyards that can potentially be incorporated into an Integrated Pest Management strategy for the control of this pest. Two species of hymenopteran parasitoids, from the *Anastatus* and *Baryconus* genera, targeted the eggs of *P. graminea*. The level of egg parasitism in vineyards was estimated to be 22%. One entomopathogenic fungus, *Metarhizium anisopliae*, was isolated from a *P. graminea* individual collected in a Stellenbosch vineyard indicating that this pest is susceptible to entomopathogenic fungi. The virulence of the commercially available mycoinsecticide, Green Muscle® (*Metarhizium acridum*), was evaluated against *P. graminea* adults. However, due to high control mortality no conclusions could be made thus far regarding the efficacy of this product against this pest. Other natural predators of *P. graminea* present in vineyards include preying mantids, spiders, birds, and chameleons.


## INTRODUCTION

Of the three species in a complex of katydids present in Western Cape vineyards, South Africa, *Plangia graminea* (Serville) is sporadic and causes damage to the leaves and berries of grapevines. There are currently no control measures registered for this pest (Allsopp 2012), while pesticides used against other pests in vineyards do not appear to have much effect on suppressing katydids. This and the movement towards more environmentally-friendly control methods create the need for the development of a sustainable and environmentally-friendly management strategy that can be incorporated into an Integrated Pest Management (IPM) program.

Microbial control agents (MCAs) are important components of IPM systems, because they are selective and safe for non-target organisms and allow other natural enemies to function (Lacey & Shapiro-Ilan 2008). Entomopathogenic fungi (EPF) are promising MCAs and play an important role in the natural regulation of Orthoptera populations worldwide (Goettel *et al.* 1995). *Beauveria bassiana* (Balsamo) and *Metarhizium acridum* (Driver & Milner) J.F. Bisch., Rehner & Humber (2009) are examples of fungi that are successfully being used as biopesticides for control against orthopteran species; with *Metarhizium* being a key control agent in the development of IPM strategies for several locust and grasshopper species in Africa (Lomer *et al*. 1999; Lomer *et al.* 2001), and Australia (e.g. Green Guard®, Milner 2002). Green Muscle® (active ingredient: *M. acridum* IMI 330180) is an example of a registered mycoinsecticide that is effectively being used against locusts and grasshoppers in Africa. Although most EPF research in Africa has been done on acridid species, laboratory research done by Mohammadbeigi & Port (2013) on the long-horned grasshopper *Uvarovistia zebra* (Uvarov) indicated that suitable *B. bassiana* and *M. anisopliae* isolates can be used to control tettigoniids as well. Entomologists at the University of California are also currently developing *Metarhizium* fungal candidates as biopesticides to control katydid pests of nectarines in California and on oil palm trees in Papua New Guinea (Miller 2011).

Hymenopteran egg parasitoids (parasitic wasps) have also been widely used in the biological control of agricultural pests in many parts of the world (Austin & Dowton 2000; Mills 2010). The fact that they do not only attack the host egg, but also kill the host within the egg (i.e. killing the pest before it can cause any damage) makes them attractive candidates for the use of biological control and IPM (Hassan 1993). They have the advantage of being able to actively search for host eggs whereas pathogens must wait for chance encounters and suitable environmental conditions (Lacey & Shapiro-Ilan 2008). Hymenopteran egg parasitoids, e.g. *Trichogramma* species, have short generation times and can be mass-reared with relative ease on hosts that feed on stored food products, enabling their commercial production (Hassan 1993; Smith 1996; Parra & Zucchi 2004; Mills 2010). A number of parasitic wasps from various families parasitize the eggs of katydids (UC IPM 2015).

Katydids are also a food source for many other animals including bats, birds, spiders, and predatory insects e.g. mantids (Belwood 1990). Since there is no information currently

available on the natural enemies of *P. graminea*, this study aims to determine the natural prevalence of EPF, hymenopteran egg parasitoids and other natural predators that target different life stages of *P. graminea* in vineyards in the Western Cape. Furthermore, I aim to test the virulence of the commercially available EPF, Green Muscle®, against *P. graminea* adults. This study aims to provide valuable information for the development of an IPM strategy.

## MATERIALS AND METHODS

The study was conducted on four farms situated in the Stellenbosch and Paarl wards found within the Stellenbosch district and Coastal region of the "Wine of Origin Scheme" in the Western Cape fruit production area of South Africa (Table 1). This region is typified by a Mediterranean climate with winter rainfall, and is a regional biodiversity hotspot (Born *et al.* 2007). In the monitoring sites, cover crops grown between vine rows included *Triticale v. Usgen* 18 (Gramineae) (every second row) and natural weed cover. Cover crops were planted during the wet winter months and killed off with herbicide during spring to prevent competition with vine roots. Weeds were controlled as needed, depending on growth during the season, also with herbicides or mechanically.

**Table 4.** Farm localities of vineyards surveyed during this study, with cover crops used between vine rows.

| Farm | Coordinates | Cover crops |
|---|---|---|
| 1 | **S** 33º 52' 13.74"<br>**E** 18º 51' 43.63" | Triticale |
| 2 | **S** 33º 53' 43.69"<br>**E** 18º 53' 32.95" | Triticale |
| 3 | **S** 33° 51' 08.02"<br>**E** 18° 56' 17.21" | Triticale/natural weed cover |
| 4 | **S** 33º 52' 19.68"<br>**E** 18º 53' 21.32" | Mixture of Triticale, barley and grazing vetch. |

The monitoring system of De Villiers & Pringle (2008) was adapted for katydids. One vineyard block per farm was monitored. Five evenly spaced rows were selected in each vineyard block and in each row, four evenly spaced plots, consisting of approximately five vines between trellising posts, giving a total of 20 plots per block (De Villiers & Pringle 2008).

*Eggs and egg parasitoids*

One vine per plot (i.e. 1/5 vines per plot) was sampled for katydid eggs. Eggs were sampled by stripping pieces of the bark off the two main cordon arms for 15 cm on either side of the main stem as well as 15 cm down the main stem. The pieces of bark were then taken back to the laboratory and searched for eggs. The eggs were carefully removed from the bark and were separated as being "viable" or "non-viable." Eggs were classified as viable if they were fully intact and contained some substance within the egg which could easily be determined by gently pressing the egg with forceps. Non-viable eggs were damaged or empty shells either because they were not fertilised, parasitized or they were hatched eggs from the previous season. Eggs out of which katydids have hatched could be identified by the presence of a white skin left behind at the apex of the egg. Viable eggs were counted and reared in plastic containers (11.5 × 7 cm), from September 2013 to April 2014. The number of hymenopteran parasitoids that emerged from the katydid eggs was recorded daily. The level of egg parasitism was calculated as the percentage of eggs out of which parasitoids emerged from the total number of viable eggs reared during this study. Parasitoids that emerged from the eggs were placed in glass vials, which were sent for identification by Dr Simon van Noort at the Iziko South Africa museum, Cape Town.

*Entomopathogenic fungi (EPF)*

Katydids were monitored within the vine canopy of each plot. I searched for katydid cadavers, moribund katydids or katydids that showed signs that they may have been infected with an EPF. These signs include poor coordination, loss of orientation, excessive grooming, jerky movements and no feeding. Moreover, orthopterans may climb up high on the plant and bask in the sun as a behavioural fever response, in which they thermoregulate to a higher temperature to restrict fungal growth (Lomer *et al.* 2001). Cadavers and insects that showed signs of

possible infection were transported back to the laboratory where they were incubated until external fungal growth could be observed.

*Fungal bioassay*

The virulence of the commercially available Green Muscle® was evaluated against *P. graminea* adults.

*Insect collection*

In May 2014, *P. graminea* adults were collected in a Stellenbosch vineyard (33º 52' 19.68" S; 18º 53' 21.32" E) in the Western Cape, South Africa. Upon collection, individuals were placed into plastic zip-lock bags with vine leaves and the bags were then placed into a plastic container with ice-packs. The ice-packs kept the container cool which decreased insect activity. In this way the katydids could be transported to the laboratory unharmed.

*Preparation of inoculum*

Green Muscle® was obtained from a local supplier [Becker Underwood BioAg SA (Pty) Ltd.]. A stock formulation was prepared by adding one gram (minimum $5 \times 10^{10}$ spores/gram) of Green Muscle® to 1 litre of sterile distilled water. Due to the hydrophobic nature of the conidia, TWEEN® 80 (0.01%) was added to the solution to lower the surface tension. The stock formulation was prepared the day before application as suggested by the Green Muscle® User Handbook. On the day of application the conidial suspension was prepared by mixing the stock solution with a magnetic stirrer for 5-10 min. Thereafter, 10 ml of the suspended conidia in the stock formulation was added to 90 ml of sterile distilled water. This solution was mixed with a magnetic stirrer for another 5 min. The concentration of this suspension was determined using an improved Neauber haemocytometer. It was then adjusted by means of dilution to give the desired concentration of $5 \times 10^6$ spores/ml. This conidial suspension was used as the primary stock formulation to inoculate the insects with the desired spore concentrations (spores/insect).

*Bioassay*

The bioassay involved treatments with two different spore concentrations ($2.5 \times 10^4$ and $5 \times 10^4$ spores/insect) and two control treatments (dd $H_2O$ and dd $H_2O$-TWEEN® 80 solution),

with 20 insects per treatment. For the two control treatments insects were inoculated with 10µl of sterile distilled water and a distilled water-TWEEN® 80 solution, respectively, on the dorsal side of the abdomen underneath the wings just behind the thorax using a micropipette. To attain a concentration of $2.5 \times 10^4$ spores/insect, a 50% dilution of the $5 \times 10^6$ spores/ml stock suspension was formulated using distilled water to acquire a conidial suspension with $2.5 \times 10^6$ spores/ml. After mixing the $2.5 \times 10^6$ spores/ml solution with a magnetic stirrer for 5 min, insects were inoculated with 10µl of this conidial suspension resulting in $2.5 \times 10^4$ spores/insect. For the last treatment, insects were inoculated with 10µl of the $5 \times 10^6$ spores/ml stock suspension, therefore $5 \times 10^4$ spores/insect, using the same technique as described for the control and $2 \times 10^4$ spores/insect treatments. This concentration was chosen because $5 \times 10^4$ spores/insect caused 95% mortality in adult Desert Locusts at 30°C within 5 days (Green Muscle User Handbook 1999). The control treatments were applied first, followed by the other two treatments, working from low concentration to high. After inoculation, the insects were placed in 5 litre plastic containers (cages) with mesh-fabric lids. Each treatment had 4 cages with 5 insects per cage to minimise contact with each other and to prevent cannibalism. The cages were kept in a temperature controlled room at 25°C and a 12:12: L:D cycle. The insects were provided with fresh, washed lettuce to feed on and cages were cleaned daily. Mortality was recorded daily for 17 days and dead insects were removed from the cages.

## RESULTS AND DISCUSSION

### *Egg parasitoids*

Two species of parasitic wasps (Hymenoptera) emerged from the katydid eggs. The wasps emerged from the eggs by chewing a circular hole out of the egg-shell. Eggs that were parasitized could therefore easily be distinguished from eggs out of which katydids had hatched in the field and laboratory (Fig. 1 A, B). The wasps could only be identified to genus level (Fig 2 A-C), since both genera are in need of taxonomic revision (S. van Noort, pers. comm.). One species belonged to the genus *Anastatus* (*Anastatus*) (Eupelminae; Eupelmidae; Chalcidoidea) (Fig. 2 A, B). There are two subgenera in this genus with about 25 described species for the Afrotropical region and many undescribed species (S. van Noort, pers. comm.). This species belongs to the nominate subgenus *Anastatus*, hence the repeat of the genus name as subgenus in brackets. Sexual dimorphism is extreme in the eupelmids, with females (Fig. 2 A) looking very different from the males (Fig. 2 B). *Anastatus* spp. are primary endoparasitoids of a wide variety of insect eggs, including Orthoptera. The other species belongs to the genus *Baryconus*

(Scelioninae; Platygastridae; Platygastroidea) (Fig. 2 C). *Baryconus* spp. have been recorded as endoparasitoids of Tettigoniidae eggs. This particular species is close to *B. africanus* (Dodd), but may be one of the other described species recorded from the region, or more likely an undescribed species (S. van Noort, pers. comm.).


**Fig. 1. (A)** Katydid eggs from which parasitic wasps emerged; **(B)** Katydid egg from which katydid nymph emerged.

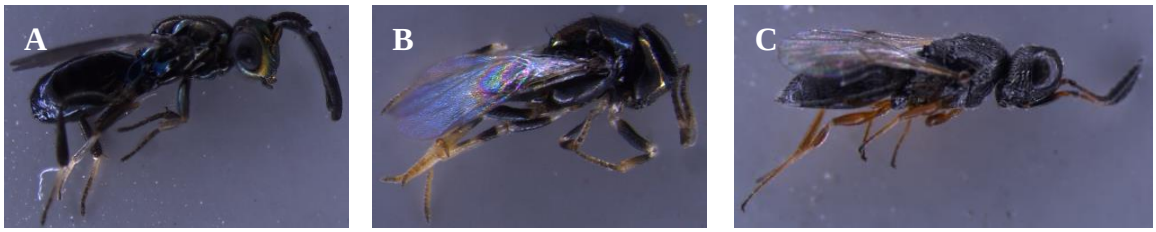


**Fig. 2.** Parasitic wasps that emerged from katydid eggs. **(A)** Female and **(B)** male *Anastatus (Anastatus)* sp. (Eupelminae; Eupelmidae; Chalcidoidea); **(C)** *Baryconus* sp. (Scelioninae; Platygastridae; Platygastriodea).

The parasitoids started hatching early in November and continued to emerge until early-April, reaching a peak in January (Fig. 3). The level of egg parasitism in the vineyards monitored between September 2013 and February 2014 was estimated to be *ca.* 22%. Surveys and monitoring efforts on parasitism rates of hymenopteran parasitoids of grasshopper and locust egg pods recorded parasitism rates ranging from <10% to >30% (Baker *et al.* 1996; Lockwood & Ewen 1997).

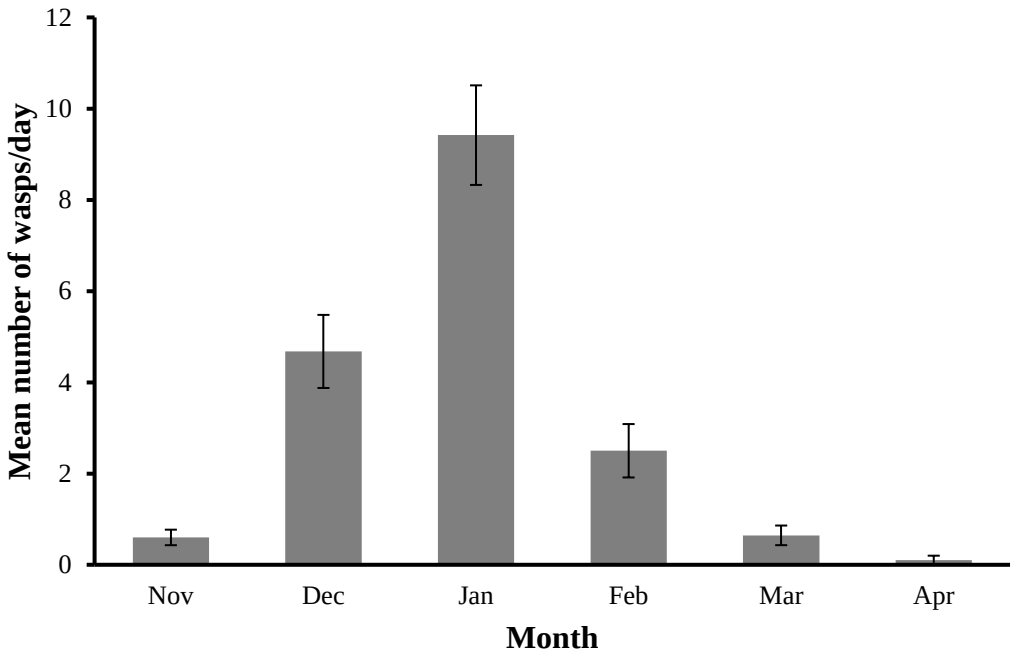


**Fig. 3.** Mean (± S.E.) of wasps emerging from katydid eggs that were collected in vineyards in the greater Stellenbosch region of the Western Cape, November 2013 – April 2014.

This indicates that these wasps are important natural control agents for katydids and their efficacy could be optimized by determining optimal environmental conditions. The prospect of using hymenopteran egg parasitoids for the control of orthopteran pests is appealing due to their long historical use as successful biological control agents (Lomer *et al.* 2001). One example of a hymenopteran egg parasitoid that was successfully introduced as a biological control agent is *Scelio pembertoni* Timberlake, which was imported to Hawaii for the control of the grasshopper *Oxya chinensis* (Thunberg) (Clausen 1978).

*Entomopathogenic fungi (EPF)*

One *P. graminea* individual collected in a Stellenbosch vineyard was found to be infected with an EPF (Fig. 4). It was identified as *Metarhizium anisopliae* and pure cultures of this isolate were sent to the National Collection of Fungi, ARC-PPRI, in Pretoria and accessioned as PPRI 12353. Infected cadavers or diseased insects are rarely observed in the field, as they are rapidly scavenged by ants or taken by other predators (Lomer *et al.* 2001). The natural prevalence of *Metarhizium* was found to be low in West Africa showing prevalence levels of 2%-6%,

although it was the most common grasshopper pathogen in this region (Shah *et al.* 1994; Lomer *et al.* 2001). Considering that *M. acridum*, e.g. Green Muscle®, is already being used as an effective mycoinsecticide against orthopteran pests in Africa and that *P. graminea* is susceptible to *Metarhizium*, the prospect of using this EPF as a biological control agent against *P. graminea* is attractive.

**Fig. 4.** *Plangia graminea* female infected with *Metarhizium anisopliae* (EPF), collected in a vineyard in Stellenbosch, Western Cape.

*Green Muscle® Bioassay*

After four days, percentage mortality was higher for the two fungal treatments, 50% and 65% for the 2.5 x $10^4$ and 5 x $10^4$ concentrations respectively, compared to 10% and 20% for the TWEEN 80® and water control groups respectively (Fig. 5). However, after four days, control mortality escalated rapidly, with TWEEN 80® mortality even exceeding that of the 5 x $10^4$ concentration after day 6. The 2.5 x $10^4$ treatment was the first to reach 100% mortality after 10 days, followed by the TWEEN 80® control (15 days), with the water and 5 x $10^4$ treatment both achieving 100% mortality after 17days. Since the bioassay was conducted relatively late in the season, high control mortality could be attributed to the fact that the katydids used were aged and may have been more susceptible when subjected to either control or fungal treatments.

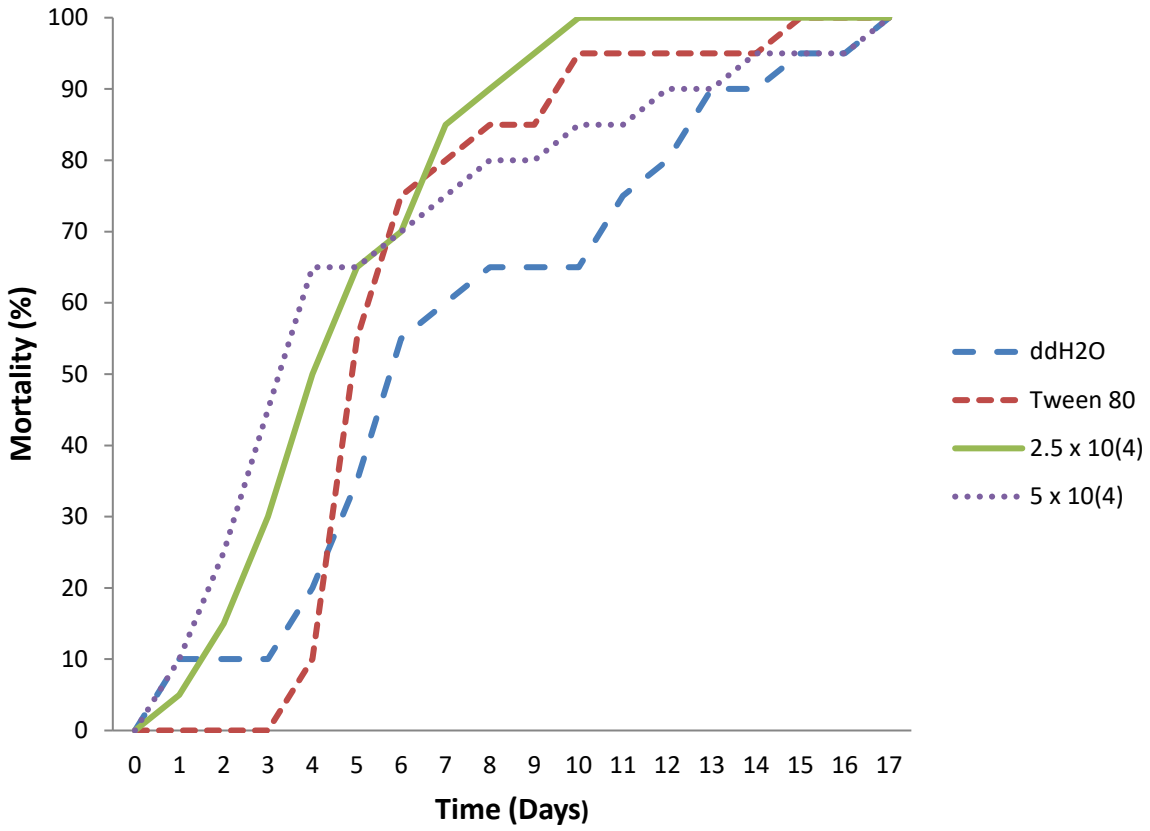


**Fig. 5.** Percentage mortality of *Plangia graminea* adults after being treated with the commercial Green Muscle® mycoinsecticide and control treatments. Treatments: ddH2O = distilled water control; TWEEN® 80 = distilled water + TWEEN® 80 control; 2.5 x 10(4) = 2.5 x $10^4$ spores/insect; 5 x 10(4) = 5 x $10^4$ spores/insect.

*Other predators*

Various other predators were also present in vineyards including praying mantids, spiders and birds (Fig. 6). Immature preying mantids were observed feeding on *P. graminea* nymphs (Fig. 6 B), and the larger adult mantids were observed feeding on *P. graminea* adults (own observations). Chameleons were also occasionally seen (Fig. 7). *Plangia graminea* individuals were also found impaled on vine twigs (Fig. 6 G). This is characteristic hunting behaviour of the Fiscal Shrike *Lanius collaris* Linnaeus (Harris & Arnott 1988). Sand wasps (Sphecidae), especially species of the genus *Sphex* L., are known to hunt katydids which they use to provision their nests (Gess & Gess 2014).


**Fig. 6.** Some predators of katydids found in vineyards in the greater Stellenbosch region of the Western Cape (A-G), and other parts of southern Africa (H). (**A, B)** Praying mantids (Mantodea: Mantidae); (**C**-**F**) spiders; (**G, H**) birds, (**G**) katydid impaled on vine twig possibly by a Fiscal Shrike (*Lanius collaris*) (photo credit: Janina von Diest), (**H**) katydid caught by a Little bee-eater (*Merops pussilus*) – Zimbabwe.

**Fig. 7.** Cape dwarf chameleon *Bradypodion pumilum* (Gmelin), possible predator of *Plangia graminea*, photographed in a vineyard in Stellenbosch, Western Cape. Photo credit: Henré Nortje.

## CONCLUSION

Hymenopteran egg parasitoids and the entomopathogenic fungus *M. anisopliae* are two promising natural enemies of *P. graminea* that could be incorporated into an IPM strategy for the control of this pest in vineyards in the Western Cape. The hymenopteran egg parasitoids are important natural control agents for katydids and further research should investigate how to optimise the vineyard environment so as to increase parasitism to optimise this natural control system. One way of employing these parasitic wasps in the vineyards would be by means of ecological engineering. Ecological engineering in an IPM context simply refers to the manipulation of agricultural habitats to be more attractive to natural enemies and other beneficial insects (Lacey & Shapiro-Ilan 2008, and references therein) and therefore improve the natural efficiency within an agro-ecosystem. The EPF-infected *P. graminea* individual indicates that this pest is susceptible to *M. anisopliae*. Further bioassays need to be performed to evaluate the virulence of this *M. anisopliae* isolate (PPRI 12353) against *P. graminea*

individuals. Due to the high control mortality, no conclusions could be made regarding the virulence of Green Muscle® against *P. graminea* adults. Further bioassays testing the virulence of this commercial product together with other fungal strains against different life stages of *P. graminea* should form part of future research projects. However, these preliminary results indicate good potential for the treatments. The development of a sustainable IPM strategy for this pest will enable other natural predators e.g. preying mantids, spiders, birds and chameleons, to persist in vineyards which will further aid the natural control of *P. graminea* and other pests.

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# CHAPTER 6

## General discussion and future research recommendations

Katydids are sporadic pests in Western Cape vineyards, South Africa, however, since 2012 population outbreaks appear to have caused substantial damage leading to great concern among farmers. The primary katydid pest in vineyards in the Western Cape was thought to be *Plangia graminea* (Serville) (Ferreira & Venter 1996; Allsopp 2012). The nymphs feed on the young foliage of the vine and later in the season, feeding by adults can extend to grape clusters (Allsopp 2012). At the commencement of this study, very little information on the biology and ecology of this pest was available. Moreover, no chemical treatments for katydids in vineyards are registered, and no monitoring system was available for this pest at the start of this study. Factors causing population outbreaks were also unknown. This study was therefore initiated to investigate the basic biology and ecology of katydid pests in vineyards. The information presented in this study aims to aid the development of a practical, environmentally-friendly, and sustainable method of control for this pest to be incorporated into an Integrated Pest Management (IPM) program. The following sections will summarise the outcomes of this study with emphasis being placed on how this information, together with future research recommendations, could aid the development of a sustainable IPM program.

### Chapter 2: Identification of katydid species present in vineyards in the Western Cape - with notes on their morphology, eggs, and assemblage structure

At the commencement of this study, it was unclear whether *P. graminea* was the only katydid species present in vineyards. Given the great diversity and characteristic feeding behaviour - nearly exclusively herbivorous - of the Phaneropterinae subfamily (Orthoptera: Tettigoniidae), more than one species could be present and cause damage to vines. This study performed the first preliminary taxonomic investigation of katydid species present in vineyards, therefore, providing the first information on the katydid species assemblage in Western Cape vineyards. The results of this study recorded three phaneropterine katydid species in four vineyards located in the greater Stellenbosch wine production region in the Western Cape, namely *Plangia graminea* (Serville),

*Eurycorypha lesnei* Chopard, and a *Phaneroptera* species. However, the extent to which these species contributed towards the damage observed was not yet known, therefore, this was further investigated in Chapter 3. *Plangia graminea* was previously recorded as a minor sporadic pest in vineyards in the Western Cape (Ferreira & Venter 1996; Allsopp 2012). *Eurycorypha lesnei* has previously been recorded in the Gauteng and North-West Provinces of South Africa (Bazelet & Naskrecki 2014), therefore, this is the first official account of this species' presence in the Western Cape Province.

The morphological similarity of the *Plangia* and *Eurycorypha* species resulted in the need to provide an easy identification key that can be used by growers to distinguish between the two species. Key characteristics that could aid the differentiation between the two species included the male elytra spot of *P. graminea*, the shape and colouration of the female ovipositor, coloration of the abdomen and the tympana (ears). The wing venation on the forewings and the size and shape of the male stridulatory files (including numbers of teeth) were also documented for the first time for *P. graminea* and *E. lesnei* [however, the male stridulatory file of *P. graminea* has been described by Hemp *et al.* (2015)]. These are two additional characteristics that could be used for the identification of these species.

This study is also the first, to my knowledge, to document the brown and pink colour morphs of *P. graminea*. Colour variations have been recorded for *Plangia multimaculata* Hemp in the Kilimanjaro area in East Africa, by Hemp *et al.* (2015), who suggested that colour is determined by the surrounding vegetation and may also depend on which tree species the nymphs grow on. The colouration and shape of the wings of these leaf-mimicking katydids increase the survival value of crypsis as their primary defence mechanism against predation (Belwood 1990). This also makes it difficult for a human observer to spot them within the vine canopy, which in turn makes monitoring this pest a challenge. Therefore, in addition to using physical katydid counts in a monitoring system, surrogate monitoring methods were investigated in Chapter 3.

This was the first study to examine the eggs of *P. graminea* and *E. lesnei* under high magnification. Although the eggs of these two species look similar upon first inspection, black, oval shaped and flat; distinguishing characteristics were noted under high magnification. It was further found in the present study that the eggs are significantly different in size, with *P. graminea* eggs having a greater width and length (mm) in comparison with *E. lesnei* eggs. Therefore, species determination is already possible at the egg stage. Although species determination would not be possible through inspection of the eggs in the field, as it requires inspection under high magnification, the use of eggs as a surrogate parameter in a monitoring method for this pest is attractive and was further investigated in Chapter 3.

**Chapter 3: Monitoring of katydids in vineyards in the Western Cape - with insights gained on their biology, ecology, and seasonal dynamics**

A suitable katydid control method could not be investigated before target katydid species were identified and their basic bio-ecology established. Furthermore, the first step in developing an Integrated Pest Management program is to establish an effective monitoring method that is specifically suited for the pest and its agricultural system (Luckmann & Metcalf 1994). This study confirmed that *P. graminea* is the primary katydid pest in Western Cape vineyards, constituting more than 80% of the katydid population in vineyards monitored. A monitoring method for this pest was developed by adapting the generic monitoring system for vineyard pests (De Villiers & Pringle 2008).

Temporally, two peaks in katydid density – each at different life stages – were observed from January 2014 to March 2015. Viable egg density reached a peak in August 2014, and katydid density in November 2014. Utilising a cross-correlation to determine the lag phase between eggs and katydids visible in the canopy, these two peaks aligned well after adjustment for a 10-week lag time, indicating that egg monitoring could be used for early prediction. Furthermore, these two peaks indicate two possible target stages for control measures to be applied. Firstly, hymenopteran egg parasitoids can be utilised to reduce the peak in viable egg density observed in winter (August), therefore, suppressing the pest population before damage can occur at the onset of the new season.

Hatching rates of hymenopteran egg parasitoids, reared from katydid eggs in the laboratory, reached a peak in January 2014 (Chapter 5). This corresponds to the time at which adult katydids were first observed laying eggs during this study. Therefore, increasing the parasitic wasp population at this stage would increase egg parasitism rates in the vineyards, and in turn suppress the katydid population of the following season. Secondly, entomopathogenic fungi in the form of a mycoinsecticide (Chapter 5), could potentially be used to target the second population peak in November. Not only is November a critical time to target this pest due to the high population density recorded at this time, but environmental conditions for EPF appear to be most favourable during this month. Environmental factors such as relative humidity, temperature and UV irradiation have an effect on the efficacy of entomopathogenic fungi in the field (Lomer *et al.* 2001). The optimum temperature recorded for *Metarhizium* IMI 330189 (active ingredient in Green Muscle®) sporulation is 25°C (Thomas & Jenkins 1997). The average maximum temperature recorded during November 2000-2012 was 25°C, and an average maximum relative humidity of 83.6% was recorded from 2008-2012 (weather data acquired from one of the farms monitored during this study). Therefore, environmental conditions experienced during the month of November appears to be favourable for the application of *Metarhizium* as a mycoinsecticide. The peak in katydid density was observed early in November. At this stage nymphs have not matured to adults and are still apterous and, therefore, potentially more susceptible to fungal infection as the wings of adult katydids could act as a barrier for fungal spores to reach the insect-body.

There are many benefits associated with the monitoring of katydid eggs. Firstly, eggs are relatively easy to collect and species determination is possible by investigating the size and surface structure of the eggs (Chapter 2). Secondly, egg monitoring in winter months (June – August) could be used as an early prediction of the expected population density for the following season; given the 10-week lag phase between the time eggs are observed and when katydids are observed in the vineyards. Furthermore, the eggs can be used as an indicator of the parasitism level of katydid egg parasitoids (Hymenoptera) present in the vineyards (Chapter 5). These parasitoids are important natural control agents of katydids in vineyards. Based on this information, growers can make more informed management decisions, therefore, employing more cost-effective control measures.

In comparison with egg monitoring, monitoring leaf damage was found to be a less effective monitoring tool. Although there was a significant correlation between leaf damage and katydid density, this correlation was poor ($r = 0.222$, $P < 0.001$; Spearman $r = 0.23$, $P < 0.01$). The reason for this poor correlation could be that katydid density was poorly reflected by the amount of leaf damage observed, since katydids are well camouflaged and difficult to spot within the vine canopy. Moreover, leaf damage was potentially overestimated due to the similarity between katydid and weevil damage (present study), and damage caused by snails (Ferreira & Venter 1996).

This study also provided the first information on the biology and ecology of *P. graminea*. There is only one generation per year, with an overwintering egg stage. The eggs are predominantly laid within the bark of the vines. The eggs hatch early in the season (mid-September), and nymph-to-adult development takes about 2 ½ months. Only three nymphal stages were observed, however, it is likely that there are more instars since exuviates were consumed before inspection in the laboratory. Determining the exact number of instars could be performed more accurately by marking the thorax of nymphs with paint to aid detection of each moult. Temperature appears to be an important environmental factor influencing population outbreaks, as it influences katydid development, but could also affect mating success of male katydids (Chapter 4). From the laboratory study, it was found that low temperatures (15°C) were inadequate for katydid development, and no eggs hatched at 30°C and 35°C. Complete katydid development was only observed at 20°C and 25°C, and the total duration of the life cycle recorded in the laboratory was 134 and 140 days, respectively, at these two temperatures. A more optimal temperature dependant development model should be establisedh for *P. graminea* to provide more detailed information on temperature tolerances and life table parameters. This is only possible with a large enough laboratory colony.

**Chapter 4: Physiological ecology – the metabolic costs of sexual signalling in the chirping katydid *Plangia graminea***

This chapter was submitted and accepted in *Journal of Experimental Biology*. *Plangia graminea* males produce chirping calls to attract conspecific females for mating. Their reproductive success is, therefore, closely related to their calling success. The results of this study recorded a significant increase in metabolic rate during calling activity of *P. graminea* males. Since calling in *P. graminea* males is additional to other metabolic costs involved during mating, including the production of a spermatophylax, and to other daily activities such as flying and feeding, the increased metabolic cost of calling could energetically constrain its mating behaviour. Furthermore, temperature has an exponential influence on the metabolic rate of an animal, with metabolic rate roughly doubling with a 10°C increase in temperature (Nespolo *et al.* 2003; Terblanche *et al.* 2007; Irlich *et al.* 2009; reviewed in Dell *et al.* 2011). Elevated temperatures experienced in the field could, therefore, necessitate trade-offs to be made by the animal in terms of energy allocation towards different activities based on its daily energy budget. Therefore, increased temperatures could result in reduced calling activity and in turn a decrease in reproduction success leading to lower population levels the following season. This study, therefore, provides baseline knowledge that can be used in the development of a population prediction model based on field temperature and the metabolic rate of *P. graminea*. Initial indications of temperature tolerances investigated in Chapter 3 show that *P. graminea* did not develop optimally at higher temperatures (30°C and 35°C), so potentially this species will not do well based on current climate change predictions of increasing temperatures.

**Chapter 5: Natural enemies of *Plangia graminea* in vineyards in the Western Cape, and their potential for biological control**

Due to emphasis being placed on more environmentally-friendly management practices, research was undertaken to identify natural enemies that could potentially be used as biological control agents against this pest. This study identified two natural control agents that could be incorporated into an IPM program for the control of *P. graminea*, namely hymenopteran egg parasitoids, and an entomopathogenic fungus.

Hymenopteran egg parasitoids appear to be important control agents that are already present within Western Cape vineyards. This study recorded parasitism rates of *ca.* 22% in vineyards monitored in the greater Stellenbosch region of the Western Cape. Two wasp species from the *Anastatus* and *Baryconus* genera were recorded during this study. A peak in wasp emergence was observed in January 2014 from field-collected eggs reared in the laboratory during this study. This corresponds to the time when adult katydids were most abundant and started laying eggs (Chapter 3). The peak of wasp activity in the vineyards from January onwards would, therefore, target the newly laid katydid eggs and are therefore well synchronised with the host. These hymenopteran egg parasitoids could, therefore, potentially be used to suppress the katydid population of the following season. A way of employing these parasitic wasps in vineyards would be by means of ecological engineering, in other words, making the vineyards more attractive to these wasps and, therefore, increasing the natural populations within vineyards. Targeted field monitoring for katydid egg parasitoids in future studies could assess habitat preferences of these wasps. Habitat and plant characters have a strong impact on the parasitism efficacy of parasitic wasps in natural systems as well as in biological control, and a thorough understanding of their habitat and plant preferences is key to the optimization of biological control programs (Romeis *et al.* 2005).

An entomopathogenic fungus (EPF), *Metarhizium anisopliae*, was isolated from a *P. graminea* individual collected in a Stellenbosch vineyard. This isolate, accessioned as PPRI 12353 at the National Collection of Fungi (ARC-PPRI, Pretoria), indicates that *P. graminea* is susceptible to EPF – specifically *Metarhizium*. This makes the use of an EPF as a biological control agent against this pest attractive, also considering that *Metarhizium acridum* is already effectively being used as a mycoinsecticide against orthopteran pests in Africa (Thomas 2000; Lomer *et al*. 2001). Due to high control mortality during the Green Muscle® bioassay trial (100% after 15 days), possibly due to the fact that katydids used were aged, the results of the bioassay performed in this study can be regarded as inconclusive. However, mortality recorded on the fourth day after the bioassay was initiated was higher for the two fungal treatments, 50% and 65% for the $2.5 \times 10^4$ and $5 \times 10^4$ concentrations respectively, compared to 10% and 20% for the TWEEN® 80 and water control groups respectively. These preliminary results indicate further research is warranted for the treatments. Future bioassays testing the virulence of this commercial product together with the

*Metarhizium anisopliae* strain isolated from *P. graminea*, should be performed against different life stages of the katydids. Since EPF invade the host cuticle directly (Niassy *et al.* 2011), immature katydids would potentially be more susceptible to infection, as adult wings could act as a barrier. The ideal time for EPF application appears to be early-November, when katydid population density is at a peak and katydids are still apterous (Chapter 3). Moreover, it appears that this period also coincides with favourable environmental conditions for EPF application. The combined utilisation of hymenopteran egg parasitoids and an EPF could potentially be incorporated into an IPM program for the long-term management of this pest. The wasps can be used to suppress the population before the new season starts (as they would target eggs laid by the previous generation of katydids), while EPF can be used to target population peaks early in the season, which could potentially be predicted through the monitoring of katydid eggs (Chapter 3).

**Future research recommendations**

One major constraint of this study was the difficulty of rearing sufficient numbers of *P. graminea* to allow establishment of a laboratory colony. This would have allowed more detailed experiments to be performed, notably complete life table studies to determine temperature dependent development models; as well as bioassay experiments using EPFs. This should be considered as a longer-term goal in future research projects, as this group appears to be difficult to rear under laboratory conditions. Further complications include having only one generation per year and relatively slower development as well as presence of an overwintering phase; and the sporadic nature of the pest in agricultural systems.

The confused taxonomic state of the *P. graminea* complex creates a need to further untangle this species complex. Considering the pest status of this species, it becomes important that future research focus on a comprehensive taxonomic review of species that may still be hidden within this complex. A recent review of the *P. graminea* complex synonymised *P. compressa* with *P. graminea* (Hemp *et al.* 2015). However, another morphologically similar species, *P. unimaculata*, was omitted in this review. I believe that this species could be integral in the review of this species complex, since its holotype (held at the Museum of Zoology, Lund, Sweden) is fully intact and

well preserved, whereas the holotype of *P. graminea* is lost and most of the body of the holotype of *P. compressa* is missing or damaged (Hemp *et al.* 2015). Specimens should be collected at type localities of the respective *Plangia* species, and data should be gathered following methods described by Hemp *et al*. (2015).

Constructing a life table and degree-day model for *P. graminea* as well as measuring metabolic rate in the laboratory under different temperatures and humidity could further enable predictions regarding their pest status. The measurement of calling frequency and its temperature dependence under controlled laboratory conditions will allow field estimation of body temperature, a critical component of predicting development times of populations in vineyards.

The audible sound produced by male katydids creates a unique opportunity for the development of an acoustic monitoring method for this pest. Microphone arrays (Model SM2+, Song Meter, Wildlife Acoustics, Inc., Concord, MA, USA), could be deployed in vineyards and population estimates could be determined through triangulation of the sound source (Mennill *et al.* 2012). The mating calls are species-specific which would allow identification of species present, while also providing accurate estimates of population densities. This could be a valuable tool in the determination of an economic threshold level for this pest.

Vineyards and adjoining habitats could be surveyed for additional EPF isolates, and the virulence of these isolates can be tested against *P. graminea* together with *M. anisopliae* PPRI 12353 and the commercial Green Muscle® mycoinsecticide. The survey would involve the collection of soil samples and isolation of EPF from these soils using the '*Galleria* bait method' (Zimmermann 1986, Goble *et al.* 2010). The most virulent EPF isolates, determined through laboratory bioassays, could then be tested in small scale field experiments. Field tests are required to establish the efficacy of the EPFs, since a high level of virulence in the laboratory does not necessarily mean that the agent will be effective in the field. Several factors, including suitability to environmental conditions, may play a role in their ability to control the pest in the field (Lomer *et al.* 2001). Small-scale field trails could be adapted from the methods described in Johnson *et al.* (1992).

Finally the mechanisms involved in habitat preferences of parasitic wasps could further be investigated. These mechanisms include plant structure, plant spacing, plant odours, plant colour and food sources (flower morphology and nectar) (Romeis *et al.* 2005). Different coloured sticky traps could be placed out in the field (vineyard and natural habitats) to test their response to colour [methods in Romeis *et al.* (1998)]. This will indicate whether a certain colour of flower is more attractive for the wasps.

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## Appendix A

A key to the ensiferan superfamilies, families and subfamilies (as adapted from Rentz, 1991):

1. Tarsi 3-segmented……..……..…….GRYLLOIDEA.(2)
- Tarsi 4-segmented……...…….……(4)
2. Fore legs fossorial, with broad, flat femur and tibia, and with powerful teeth on both tibia and tarsus……...…**Gryllotalpidae**
- Fore legs normal, not especially modified……..……...(3)
3. Eyes greatly reduced; hind coxae closely approximated ventrally [Small, depressed ant inquilines]……..…….…....**Myrmecophilidae**
- Eyes not reduced; hind coxae well separated ventrally ……..……....**Gryllidae**
4. Fore wings, when present, usually tough, ♂ tegmen usually with stridulatory apparatus; side of abdomen and adjacent inner face of hind femur without stridulatory modifications in either sex.…..……...……TETTIGONIOIDEA-**Tettigoniidae**.(5)
- Fore wings, when present, soft, pliable, without stridulatory apparatus; often side of abdomen and adjacent inner face of hind femur with pegs and modified spines as a stridulatory apparatus in both sexes and nymphs as well as adults…….……...GRYLLACRIDOIDEA.(17)
5. Head prognathous, body form phasmatoid……..…….……(6)
- Head not prognathous, body form not phasmatoid……..…….……..(7)
6. Both sexes apterous, body form extremely slender……...…….PHASMODINAE
- Both sexes winged or ♀ apterous, body form less slender….……...…...ZAPROCHILINAE
7. Head globose, not usually slanted or frontally flattened. Fore tibia in section approximately square in distal portion, dorsal surface not convex. Ovipositor usually short, upturned, laterally compressed. Prosternum unarmed.…….PHANEROPTERINAE
- Without above combination of characters…...……..…….(8)

8. Pronotum massive, posterior margin strongly acute; lateral margins crenulated or dentate. ♂♂ lacking modified stridulatory area of dorsum of tegmen……..……...PHYLLOPHORINAE
- Pronotum not as described above. ♂♂ with stridulatory region on dorsum of tegmen…….……..……...….(9)
9. Antennal sockets strongly rimmed, especially on internal dorsal margins. Thoracic auditory spiracle small, inconspicuous, not hidden by pronotum…….…….……....PSEUDOPHYLLINAE
- Antennal sockets not strongly rimmed. Thoracic auditory spiracle large, elongate, and, in most species, wholly or partially concealed by pronotum……(10)
10. Tibial auditory structure usually open; if closed on one side or both sides, then the slit is directed laterally and the opening is broad and its margins curving. Prosternum armed with a pair of spike-like processes…..…….…..MECOPODINAE
- Tibial auditory structure either open or closed, the slit distinctly directed dorsally in relation to position on tibia; if open, however, the opening is nearly uniform in width. Prosternum armed or unarmed…….……...(11)
11. Prosternum unarmed; small, delicate, highly agile, arboreal and epiphyllic species, greenish or greenish yellow in coloration; tibial auditory structure generally open……MECONEMATINAE
- Prosternum armed or unarmed; combination of other characters not as above……..…(12)
12. Prosternum unarmed; greenish, brachypterous species with the tegmina mostly concealed by pronotum; tibial auditory structure slit-like on both sides, appearing closed; size minute, 5-8 mm….…….…….MICROTETTIGONIINAE
- Lacking above combination of characters……...…….(13)
13. Fore tibia bearing a single apical spur on posterior margin of dorsal surface. Fastigium of vertex as broad as width of 1st antennal segment to half width of same. Hind basitarsus with a plantula which in most species is ½ the length of basitarsus…….……....TETTIGONIINAE
- Lacking at least 2 of the above-listed characters…..……..……..(14)
14. Hind tibia lacking apical spurs on dorsal surface. Posterior portion of lateral lobe of pronotum produced or not produced.……..……..SAGINAE

- Hind tibia with at least an external apical spur on dorsal surface, if not, then posterior portion of lateral lobe of pronotum produced……..……...(15)

15. Frons vertical; mesosternum not spiniform. Sexually dimorphic, ♂♂ winged, ♀♀ apterous…….……TYMPANOPHORINAE

- Without above combination of characters……...……..……...(16)

16. Fore tibia usually bearing 5-7 long, movable, outwardly bowed, opposing spines, the longest of which in many species is as long as or longer than the combined lengths of the first 2 tarsal segments. Fastigium of vertex narrow, strongly laterally compressed, its greatest width less than that of 1st antennal segment in most species and scarcely projected above same and usually sulcate…..……..…….LISTROSCELIDINAE

- Fore tibia of most species with spines not unusually lengthened and not as long as the combined lengths of the first 2 tarsal segments. Fastigium of vertex variable in width and not sulcate…….….CONOCEPHALINAE

17. Tarsi depressed. [1st tarsal segment with plantulae; auditory tympana absent]…….…..…**Gryllacrididae**

- Tarsi compressed…….…….……...(18)

18. Antenna very short, reduced to 10 bead-like segments. Legs and body highly modified. ♂♂ brachypterous, ♀♀ apterous…….....…….....**Cooloolidae**

- Antenna much longer, filamentous. Legs and body not abnormally modified. Sexes not dimorphic for wings, but may be apterous or alate……..…….(19)

19. 1st tarsal segment with plantulae; tibial auditory tympana present in all but one Australian genus……..……....……**Stenopelmatidae**

- 1st tarsal segment without plantulae; tibial auditory tympana absent…….…….…...**Rhaphidophoridae**

## Appendix B

### TESTING FOR PHYLOGENETIC SIGNAL (CHAPTER 4)

Because I expected more closely related species to behave more similarly (i.e. presence of phylogenetic signal in data; Blomberg *et al.* 2003), I conducted three analyses to analyse the presence and extent of phylogenetic signal in the data, and to take this signal into account when analysing the relationship of mass to metabolic rate for three insect behaviours: flying, resting and calling.

Three phylogenetic trees were constructed: for flying insects, for resting Orthoptera, and for calling Orthoptera (Figure S1, S2, S3). For flying insects, a tree was constructed using the R package rotl (Michonneau *et al.* 2016), which searches the Open Tree of Life for taxa and constructs a tree with no branch lengths. Both Orthoptera phylogenies were constructed manually in Newick format according to phylogenetic relationships among subfamilies as published in Song *et al.* (2015) and Chintauan-Marquier *et al.* (2016) and drawn in figtree (http://tree.bio.ed.ac.uk/software/figtree/). In order for the trees to be usable in PGLS analyses, node labels were added and polytomies were resolved randomly to dichotomies using the ape package in R (Paradis *et al.* 2004). Owing to the absence of available information for most species, branch lengths were uniformly set to 1.00 manually for all trees.

In order to test for strength of phylogenetic signal, two metrics were calculated. Blomberg's K was estimated for log metabolic rate of each group of species using the function Kcalc in the picante package in R (Kembel *et al.* 2010). K close to 0 indicates no phylogenetic signal, K approaching 1 indicates a trait signal as would be expected under Brownian motion, and K > 1 indicates a strong phylogenetic signal in the trait (Blomberg *et al.* 2003; Erregger *et al.* 2017). Pagel's λ was estimated in the R package caper (http://www.R-project.org/ package = caper). The λ value ranges from 0 to 1, with the closer the value to 1, the stronger the phylogenetic signal (Pagel, 1999).

PGLS analysis was run in the package caper, using the function pgls. The relationship of log metabolic rate (response variable) to log mass (explanatory) was modelled for each of the three insect behaviours and species groups. The K-value was read in as the calculated value from Kcalc. For each of the three behaviours, an OLS which did not take phylogenetic signal into account was

compared with PGLS using Akaike's Information Criterion (AIC). The lower the AIC value, the better the performance of the tested model (Burnham & Anderson, 2002).

## RESULTS

The relationship of metabolic rate to body mass had a relatively strong phylogenetic signal for flying insects ($\lambda = 0.953$) which approached Brownian motion ($K = 0.635$). For these species, PGLS performed better than OLS, indicating it is important to take phylogenetic signal into account (Table S2, Fig. S4).

For resting and calling Orthoptera, there was no phylogenetic signal in the relationship of metabolic rate to mass ($\lambda = 0.000$; $K_{resting} = 0.301$; $K_{calling} = 0.500$). For both groups of insects, OLS performed better than PGLS, indicating no need to take phylogenetic signal into account (Table S2, Fig. S4).

**Table S2**. Estimates of phylogenetic signal (λ and K) in log metabolic rate for three insect behaviours: flying (all insects), resting (Orthoptera only) and singing (Orthoptera only). Comparison of ordinary least squares (OLS) and phylogenetic least squares (PGLS) models for the relationship of log metabolic rate to log mass for three insect behaviours.

| Dataset | Pagel's λ | Blomberg's K | OLS | | | | PGLS | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $R^2$ | t-value | P | AIC | $R^2$ | t-value | P | AIC |
| Flying | 0.953 | 0.635 | 0.956 | 34.16 | <0.001 | -2.615 | 0.930 | 26.856 | <0.001 | -29.465 |
| Resting | 0.000 | 0.301 | 0.638 | 7.69 | <0.001 | 13.538 | 0.639 | 7.239 | <0.001 | 6.410 |
| Singing | 0.000 | 0.500 | 0.611 | 4.79 | <0.001 | 20.093 | 0.647 | 4.795 | <0.001 | 18.533 |

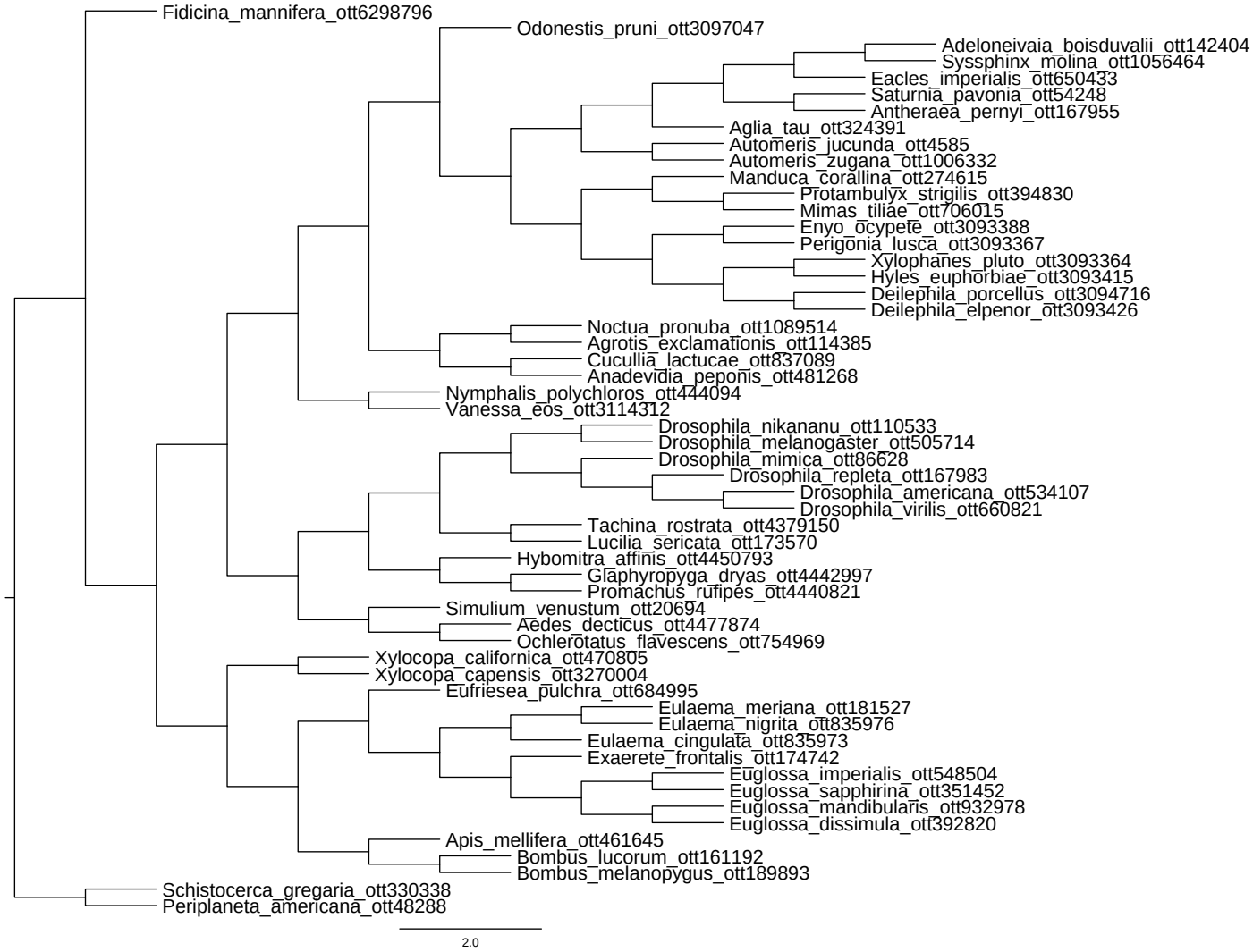


**Fig. S1**. Species tree for flying insects used in phylogenetic signal analyses.

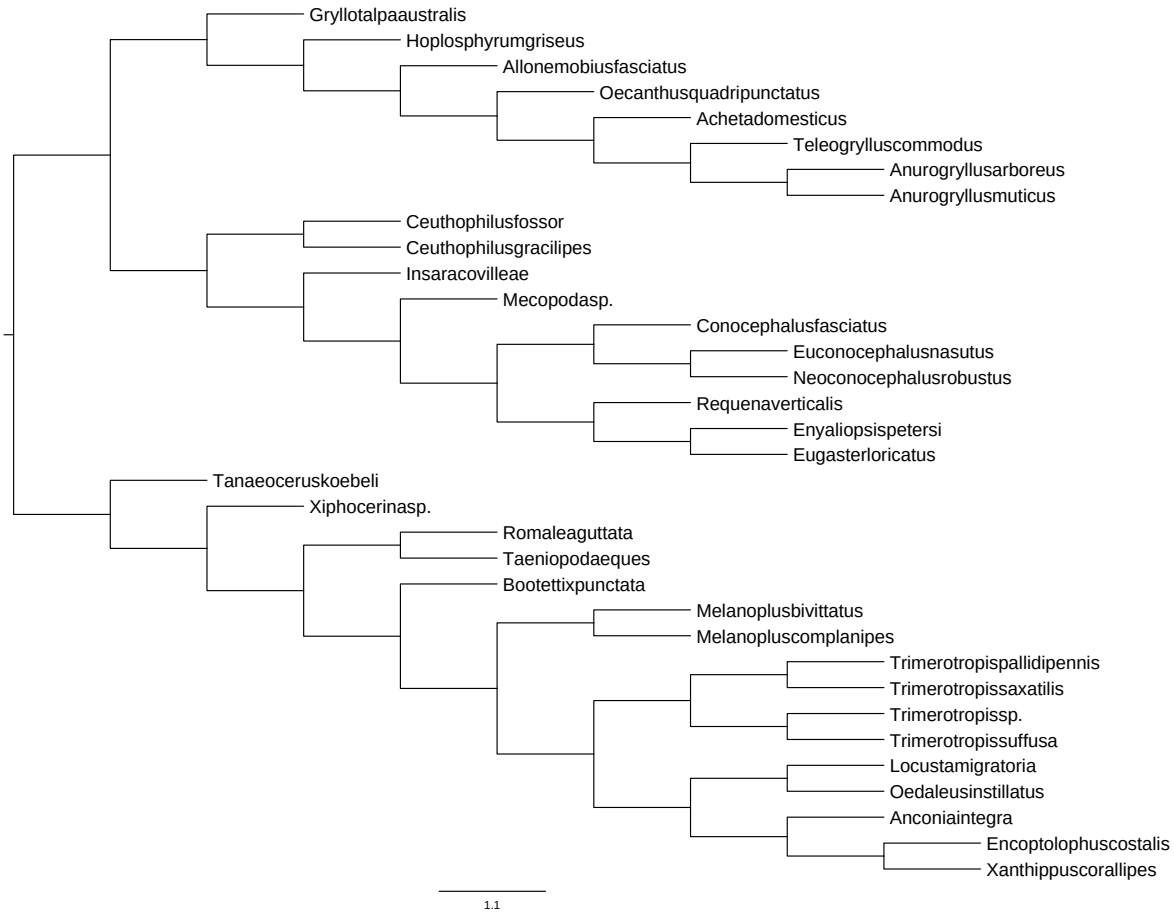


**Fig. S2.** Species tree for resting Orthoptera used in phylogenetic signal analyses.

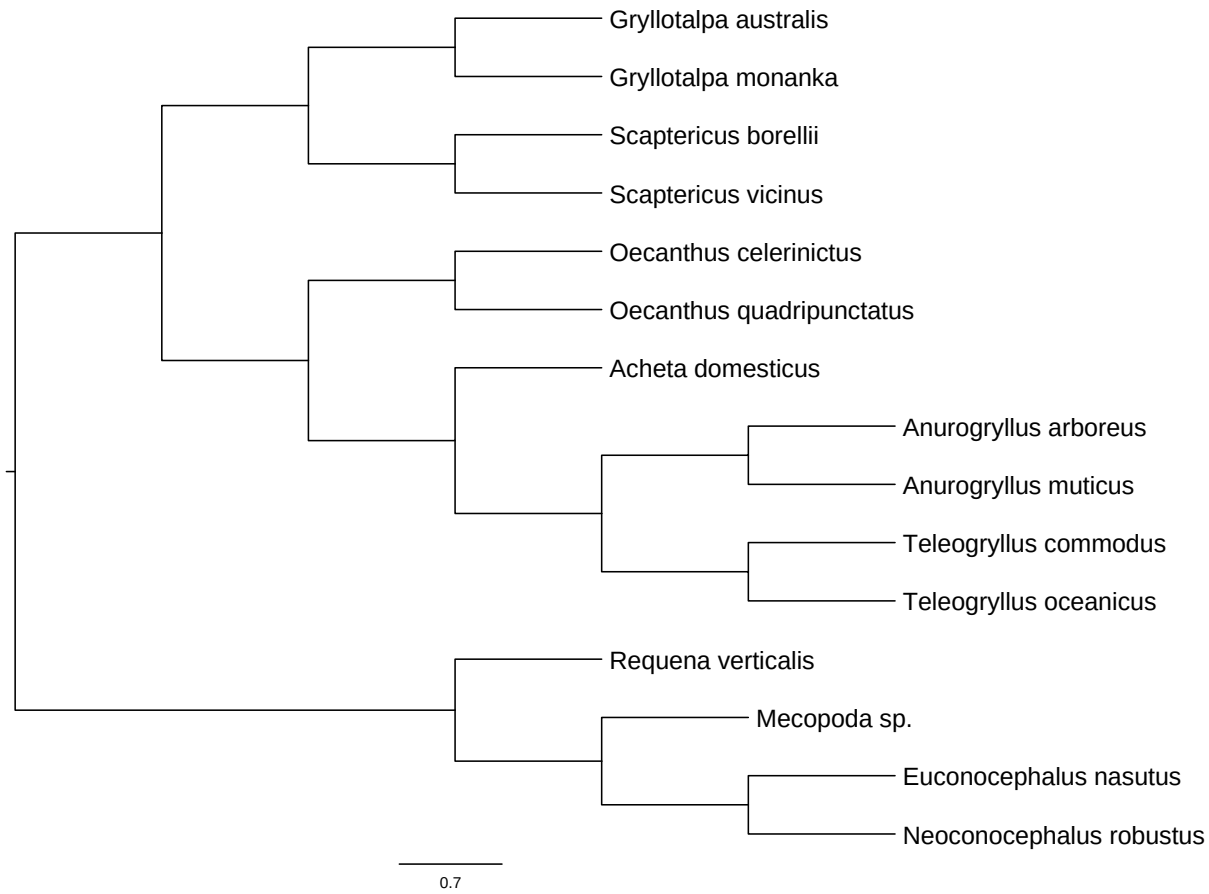


**Fig. S3.** Species tree for singing Orthoptera used in phylogenetic signal analyses.

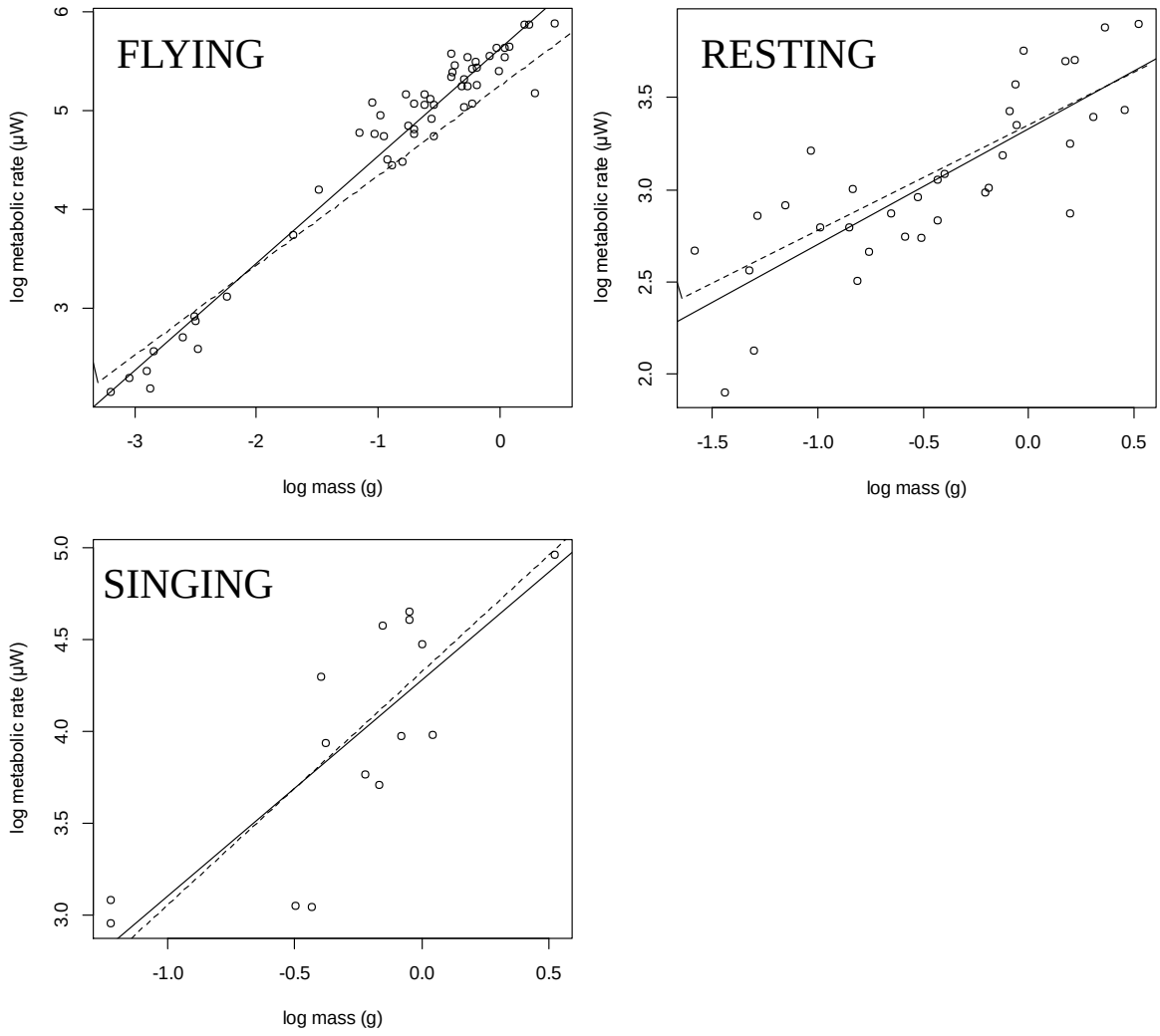


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**Fig. S4.** Relationship of mass to metabolic rate for three insect behaviors: flying insects; resting Orthoptera and singing Orthoptera. Solid line indicates ordinary least square trend and dashed line indicates phylogenetic least squares trend or trend corrected for phylogenetic signal.

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