Compatible components of the integrated pest management of *Bathycoelia distincta* (Hemiptera: Pentatomidae)

By

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Declaration

I, Shaun Manzini, hereby declare that the work contained in this thesis is my original work and has yet to previously, in its entirety or part, be submitted to any university for a degree. The thesis does not contain other persons' writing unless expressly acknowledged and referenced accordingly

Signature……………………

Date 20 July 2024**……………………**

Abstract

Two-spotted stink bug, *Bathycoelia distincta* Distant (Hemiptera: Pentatomidae), is a major pest of macadamia. It causes damage by directly feeding on macadamia kernel throughout the season and reducing nut quality by inducing nut abscission, premature nut abortion, necrotic lesions, deformed kernels, discoloration, or translucent blemishes on the kernel. Currently, growers rely on chemical insecticides to control stink bug populations. However, with overreliance and extensive use of chemical insecticides, the *B. distincta* has developed some resistance to certain active ingredients Furthermore, there is no doubt that agricultural intensification is the primary driver of the loss of biodiversity globally. Therefore, there is a need to develop Integrated Pest Management (IPM) strategies to effectively minimize the damage caused by *B. distincta* and the loss of biodiversity due to agricultural intensification. The goal of this study was to investigate the role of landscape context, elevation, flying vertebrates, and pesticide application in shaping arboreal arthropod assemblages and macadamia nut quality metrics and evaluate the efficacy of alternative pest control measures to control *B. distincta*, explicitly focusing on entomopathogenic fungi and egg parasitoids *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Platygastridae). Landscape context and elevation were the principal factors that explained species richness and arthropod size. Species richness and insect size decreased with increasing pesticide application rate; however, it was not significant. Insect damage did not vary in response to pesticide application, but the proportion of sound kernels decreased significantly with increasing pesticide application. All entomopathogenic fungal treatments were effective against *B. distincta*, with chitinase enzyme leading to a 95% mortality rate. Under field conditions, EPFs and a chemical knock-down spray showed no significant difference between the treatments. All parasitoid treatments significantly affected *B. distincta* egg mortality, with two pairs reaching a 100% parasitism rate. The processes that increase sound kernel recovery seem to be related to an increase in invertebrate community diversity and arthropod size, and although the mechanisms related to this are not always clear, the results suggest that arthropod diversity could be a good proxy for these processes. On the other hand, the study results showed that EPFs and stink bug egg parasitoids, *Trissolcus basalis*, may be used as biological control agents against *B. distincta.* Overall, the thesis emphasizes the importance of reducing pesticide application and using biological control agents, promoting more sustainable agricultural practices, and supporting biodiversity to improve ecosystem service provided by pest natural enemies.

Keyword: Two-spotted stink bug; Macadamia; Integrate Pest Management; Pesticides; Nut quality metrics

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Dedication

I dedicate this work to honouring the life of an extraordinary Arachnologist, my supervisor Professor Stephan Hendrik Foord. Prof, our thesis received good grades. Rest in peace Prof.

Chapter 1

Introduction and Literature Review

Introduction

Bathycoelia distincta Distant (Hemiptera: Pentatomidae), commonly known as the two-spotted stink bug, is frequently found in southern and east African countries, including South Africa, Zimbabwe, Malawi, Kenya, and Mozambique (Linda 2021). It was first identified in the Levubu subtropical growing region, South Africa, in 1984 as a pest of macadamia by the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) (Schoeman 2018). It represents about 90% of all the heteropterans found in macadamia orchards, but its abundance differs with macadamia tree phenology (Schoeman 2014).

Bathycoelia distincta is a hemimetabolous insect and has five nymphal instars. It is characterized by piercing and sucking mouthparts and wings that are hard, thin, and but thick at the base. The first instars remain on the empty eggshells as a cluster and do not feed on the host plant. Feeding on the host plant and dispersal commence during the second instar phase. The sexually mature females glue their eggs on the stems and leaves of the host plant (De Villiers & Schoeman 2015). *Bathycoelia distincta* has a long proboscis, allowing it to feed on the kernel. The average length of the proboscis of adults is about ±13.6mm, which is twice the length of other species found in macadamia (Bruwer et al. 2021).

The current methods used to monitor and control this pest species include the combination of insects' pathogens and a low rate of chemical insecticides, tree branch shaking (Schoeman. 2011), and chemical insecticides (Schoeman and Mohlala 2012). However, with the challenges arising from the use of chemical pesticides, there is a need to diverge from synthetic insecticides to other environmentally friendly control measures. Thus, this objective has influenced the development of Integrated pest management (IPM) (Tozlu et al. 2019). IPM is an ecologically integrated approach used for crop protection to combat pests by using single or simultaneous methods while reducing the application of chemical pesticides (Dent 2000; Rao & Tanweer 2011; Stenberg 2017). IPM programs include using available or complete information about the pest, such as its life cycle, control measures, and how it interacts with its surrounding environment (Schoeman 2014). IPM's goal is not to eliminate pests but to maintain pest population and damage below the economic threshold (Paredes et al. 2021).

There are currently eight principles (P) of IPM, and they follow a logical sequence as described below: P1) Suppression and Prevention, which focuses on creating a cropping system that is less prone to pests and preventing pests from becoming dominant in the system if available. P2) Monitoring is based on monitoring pest populations, and this approach varies with pests as they vary with economic thresholds. P3) Decision making: this principle is based on making decisions through pest monitoring information and using the established economic threshold levels. P4) focuses on a non-chemical approach; it advocates for using biological control agents and the difficulties underlying their satisfaction with pest control. P5, 6, and 7) deal with selecting pesticides, reducing pesticide usage, and anti-resistance approaches. These principles promote the use of less detrimental pesticides to the environment, partial application, reduction of pesticide dosage, and reduced chance of pests developing resistance and secondary pest outbreaks. P8) Evaluation, the last principle, encourages farmers to evaluate the efficacy of the adopted measure of managing and controlling pests (Barzman et al. 2015). The South African macadamia industry is growing at a rapid rate. Therefore, IPM strategies are needed to maintain pest pressure below the economic threshold.

Literature review

Damage by *B. distincta* on macadamia kernels

Bathycoelia distincta is a major pest of macadamias (Schoeman 2018). It causes damage by directly feeding on macadamia kernel throughout the season (Schoeman 2014). The damage associated with stink bugs can be classified into two categories: early stink bug damage and late stink bug damage. Early stink bug damage occurs during the early development of the kernel and causes abscission, premature abortion (Schumm et al. 2020), necrotic lesions, and deformed kernels (Bruwer et al. 2021). Late stink bug damage occurs during the late season (when the fruit has matured) and is noticeable by discoloration or translucent blemishes on the kernel (Bruwer et al. 2021). Overall, this type of damage results in crop loss and reduces nut quality, thus rendering the kernels unsuitable for the market (Linda 2021). In South Africa, the macadamia industry has a profit loss estimated at ZAR 112 million annually due to *B. distincta* (SAMAC 2023).

Current methods used to control *B. distincta* in macadamia

Stink bugs are highly mobile insects; thus, controlling these kinds of species becomes difficult. However, several strategies are used to control the stink bug population around macadamia orchards. These strategies include using insecticides and some form of IPM strategies (Linda 2021; Schoeman 2014).

Synthetic insecticides

There is a limited number of insecticides that are currently registered to be used for stink bug control in macadamia, and all the insecticides belong to the five Insecticides Resistance Action Committee (IRAC) (Linda 2021). However, with the impact insecticides have on public health (Tozlu et al. 2019), environmental security (Wang et al. 2019), pest resistance (da Silva et al. 2015; Linda 2021), and non-target species (beneficial insects), insecticides are continuously withdrawn. In addition, spray coverage is poor in macadamia due to tree heights, and it has been speculated that *B. distincta* might have developed resistance against some pesticides, such as pyrethroids (Linda 2021; Schoeman 2014; Schoeman 2018). Other challenges associated with synthetic insecticides are unacceptably high residue in produce, mainly noticed when Aldicarb GR 150 g/kg was used to control stink bugs (Schoeman 2014). However, they were promising as kernel damage was reduced, but processors rejected nuts due to high residue levels discovered. Some of the insecticides, such as Cypermethrin and Deltamethrin, were able to control the stink bug population; however, after three times of recurring applications, they led to secondary pest outbreaks (Schoeman 2014)

Integrated Pest Management (IPM)

IPM has several elements that have been applied from the pre-pesticides era and are in practice; these include biological, physical, cultural, and chemical pest approaches (Rao & Tanweer 2011). Current groundbreaking IPM methods used in macadamia include:

Physical approach- Pest monitoring and scouting

This approach includes the removal of pest eggs (Rao & Tanweer 2011) and counting stink bugs obtained during scouting (Linda 2021). The main goal of this approach is to use sampling techniques and detect stink bugs availability in the orchard after arrival (Linda 2021). Schoeman (2011) suggested monitoring and scouting techniques to detect hemipterans in macadamia orchards; these techniques include tree branch shanking in the morning, knock-down using dichlorvos (chemical spray), nut dissection, inspecting the husk and kernel for stink bug damage and egg scouting searching for stink bug egg. However, this approach becomes difficult to implement since stink bugs are highly mobile, and there are no reliable scouting and monitoring techniques (Linda 2021; Schoeman 2014).

Cultural approach -Trap crop.

The cultural approach aims to reduce pest damage below economic injury levels by disrupting pest-adapting behaviors (Linda 2021). A trap crop is one of the cultural approaches; it focuses on habitat manipulations, and it is used to distract pests. As a result, pest damage is reduced. These crops can either be within the field or at the landscape level (Rao & Tanweer 2011). Trap crops are essential for macadamia orchards because they increase functional diversity and arthropod diversity (beneficial insects), which also contribute to reducing pest infestation (Radzilani et al. 2012). Several plant species have been reported to host the two-spotted stink bug in macadamia. These plant species include sun hemp, cowpea, sunflower (Linda 2021), and avocado. Although all plant species indicated the potential to host the two-spotted stink bug, the species occurred in low numbers (Schoeman 2014).

Biological approach

Biological approaches are natural substances. As a result, they are environmentally safe, contribute to sustainable crop production, and provide ecological viability (Rao & Tanweer 2011). Biocontrol uses living organisms such as pathogens, predators, and parasitoids to control pest insects and reduce pest population density (Stenberg 2017), thus making their service in the agricultural sector worldwide worth billions of dollars (Kolkert et al. 2020). In other words, natural pest control using natural enemies is the most economically significant ecosystem service to agriculture (Kolkert et al. 2020). However, it is important to note that for biological control to be effective, it depends on the ecosystem functionality, host specificity, desirous feeding capacity, high adaptability, high resistance to pathogens and parasites, and spatial and temporal dispersal (Rao & Tanweer 2011).

- *Vertebrate predators (bats and birds) in macadamia*

Vertebrate predators protect crops from insect pest damage and are important in agriculture; thus, maintaining natural vegetation for these natural enemies can be beneficial (Kolkert et al. 2020). In South Africa, bats have been reported to play an ecologically and economically significant ecosystem service by consuming and suppressing major macadamia pest, stink bugs (Taylor et al. 2013; Weier et al. 2018; Weier et al. 2019). In addition, it has been estimated that biocontrol by birds and bats contributes about USD-5000 ha/year (Linden et al. 2019). Although the identity of bats providing significant pest suppression in macadamia has been reported (Weier et al. 2019), some natural aspects, such as how they affect arboreal invertebrate diversity and how increasing distance affects predation across the landscape, remain understudied.

- *Invertebrate predators and parasitoids in macadamia*

Five species belonging to the order Hymenoptera have been identified as being hosted by *Bathycoelia* sp. These parasitoids belong to three different families, Scellonidae *(Trissolcus sp. A, Trissolcus* sp. *B,* undetemine sp), Eulophidae (*Pediobius* sp) and Pteromalidae (*Pachyneuron* sp) (Bruwer 1992; Schoeman 2009).The recently tested parasitoid under laboratory conditions is *Trissolcus basalis* (see report by Kilner 2015). *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Platygastridae) is a universally known hemipteran biological control agent (Sahayaraj 2014) for which its uses in IPM programs are well documented (Foti et al. 2017). The female *Trissolcus* sp. deposits eggs in the stink bug eggs, and as a result, the developing wasp starts consuming the developing nymphs inside the egg, and the wasp emerges after 14-16 days as an adult. *Trissolcus basalis* can mate and produce fertile eggs within a few hours of hatching, and they have been used to control stink bugs (Kilner 2015). However, studies have not reported their efficacy in controlling *B. distincta* under field conditions.

Thesis Objectives

Here, the thesis explored the role of landscape context, elevation, flying vertebrates, and pesticide application in shaping arboreal arthropod assemblages and macadamia nut quality metrics. Specifically, the thesis aimed to understand how arboreal arthropod community varies across intensification levels in response to landscape context and how nut quality metrics vary with orchard intensification levels. The study also evaluated potential biological agents for *B. distincta* and identified EPF that affects nymphs and adults and egg parasitoids by quantifying the efficacy of entomopathogenic fungi and egg parasitoids *T. basalis* against *B. distincta.*

Thesis layout

The thesis is comprised of four chapters, in which two key data chapters have been prepared as standalone scientific papers that will be submitted for publication. Therefore, there might be some form of recurrence between the chapters. The purpose of this study was to quantify and document measures of IPM of *B. distincta* that can be applied in the macadamia industry to combat this pest. The first data chapter (Chapter 2) assessed arboreal invertebrate community composition, diversity, and nut quality metrics along an elevational gradient of macadamia farming intensification by specifically quantifying the following:

1. The relationship between arboreal dwelling arthropod diversity and four key drivers: landscape context, flying vertebrates, elevation, and pesticide application rates.
2. The relationship between pesticide application rates and nut quality metrics.

The last data chapter (Chapter 3) aimed at evaluating the efficacy of two potential biocontrol agents, specifically evaluating the following:

1. The efficacy of EPF with and without chitinase enzyme against two-spotted stink bugs (*in vitro*).
2. The efficacy of EPF without chitinase enzyme under field conditions.
3. The efficiency of *Trissolcus basalis* against *Bathycoelia distincta* determines the effective release rate of *T basalis.*

4. The effect of age and generation of *Trissolcus basalis* parasitism rate against *Bathycoelia distincta*.

The last chapter of this thesis (Chapter 4) deals with the study's conclusion, limitations, and recommendations. The measures that are in line with IPM that can be applied in the macadamia industry to mitigate pest pressure and conservation of biodiversity and the ecosystem services that they provide.

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Chapter 2

The role of landscape context, elevation, flying vertebrates, and pesticide application in shaping arboreal arthropod assemblages and macadamia nut quality metrics.

Abstract

Land use change and agricultural intensification have led to a decrease in farmland biodiversity and its ecological functions. Arthropod decline in many terrestrial ecosystems is linked to agricultural intensification. Considering their critical functional role in ecosystem processes and services, they severely threaten human well-being and ecosystem function. Information about the impact of agricultural intensification on many crops and our understanding of the response of different arthropod taxa is still limited, thus hindering the development of mitigation measures. Here, the study assessed invertebrate community composition, diversity, and nut quality metrics along an elevational gradient of macadamia farming intensification. Landscape context and elevation were the principal factors that explained species richness and arthropod size. Species richness and insect size decreased with increasing pesticide application rate; however, it was not significant. Abundance was only explained by elevation. The community composition of higher-elevation orchards approached that of the surrounding remnant vegetation. Insect damage did not vary in response to pesticide application, but the proportion of sound kernels decreased significantly with increasing pesticide application. Processes that increase sound kernel recovery seem to be related to an increase in invertebrate community diversity and arthropod size, and although the mechanisms related to this are not always clear, our results suggest that arthropod diversity could be a good proxy for these processes.

Keywords: Agricultural intensification; Body size; Ecological intensification; Ecosystem services

Introduction

Balancing biodiversity conservation and sustainable food production is a critical global challenge (Johnson et al. 2017). One key focus in applied ecology and conservation is understanding the impact of agricultural intensification on biodiversity, production, and environmental health (Tilman et al. 2001). Agricultural intensification enhances outputs by increasing the input of fertilizers (Erb et al. 2013), pesticides, and expanding field size, leading to a simplified landscape with tiny fragments of remnant vegetation (Bianchi et al. 2006). There is mounting evidence that agricultural intensification and expansion are the most significant drivers of the biodiversity crisis (Maxwell et al. 2016; Wurz et al. 2022) and the reduction of biodiversity-based ecosystem functioning and services that underpin production in agriculture (Ramankutty et al. 2018).

Arthropod decline is linked to agricultural intensification (Vasconcelos et al. 2022), posing a severe threat to human well-being and ecosystem function (Wagner 2020), considering their critical functional role in ecosystem processes and services (Isaacs et al. 2009). Beneficial arthropods, including predators and parasitoids, aid in pest control, while native bees play a significant role in crop pollination. All these ecosystem services contribute to maintaining agricultural production and reducing the need for pesticide input (Isaacs et al. 2009). Power (2010) and Gardiner et al. (2009) have shown that monocultural fields are associated with high pest damage and tend to have a small population of natural enemies. The lack of food resources and habitat negatively affected the abundance and diversity of natural enemies (Landis et al. 2000). On the other hand, both the abundance and diversity of natural enemies tend to increase towards complex landscapes because natural habitats provide alternative resources and are less affected by pesticide application, acting as a refuge for beneficial arthropods (Bianchi et al. 2006; Landis et al. 2000). Theis et al. 2003, observed a strong effect of non-crop habitat in increasing parasitism and reducing insect herbivory.

Other impacts of pest natural enemies are provided by flying vertebrates and were reported in macadamia orchards at Levubu, Limpopo (Bouarakia et al. 2023b; Weier et al. 2018). Flying vertebrates such as birds and bats also provide pest control services in agricultural fields (Kunz et al. 2011). Experimental work has shown that bats and birds can reduce the damage of major macadamia pests belonging to the order Lepidoptera (family Tortricidae: Macadamia nut borer) (Bouarakia et al. 2023b) and Hemiptera (family Pentantomidae: Two-spotted stink bugs) since they have been reported to be consumed by bats (Weier et al. 2019). Several studies have estimated the economic value of insectivorous bats and birds and have indicated that insectivorous bats and birds play a significant role in pest control (Linden et al. 2019; Taylor et al. 2018). However, these effects can be mediated through trophic cascade in arthropod communities initiated by predation of both birds and bats (Maas et al. 2016)

Besides the effect of agrarian intensification on biodiversity and associated function, it is crucial to understand factors that shape community structures and whether these factors vary geographically (Lessard et al. 2011). Abiotic and biotic gradient components of mountainous ecosystems have a considerable potential to improve our understanding of species richness patterns, species distribution, and conservation (McCain & Grytnes 2010). Monotonic decreases in species richness with increasing elevation have been widely observed (McCain 2005; Rahbek 2005). Elevation is a proxy for a whole range of conditions and resources, such as temperature (McCain 2005), available area, and agricultural intensification, all of which tend to decrease with elevation. Therefore, we should observe lower diversity at higher elevations, while the negative impact of agriculture should be lessened at higher elevations (see Vasconcelos et al. 2022).

High landscape heterogeneity is associated with increased abundance and diversity (Thies & Tscharntke 1999). Understanding the impact of landscape heterogeneity and other environmental elements that drive the diversity of arthropod communities is essential to manage agricultural production sustainably (Ericksen et al. 2009). Although agrarian intensification is associated with arthropod declines, information about the effect of agricultural intensification on many crops is still lacking (Vasconcelos et al. 2022). Therefore, examining the impact of intensification along an elevational gradient and including landscape context may provide offer valuable insights into the impact of agricultural intensification.

Globally, the macadamia industry is growing rapidly, and South Africa remains the largest producer of macadamia worldwide, with about 72 652 hectares under cultivation (van Zyl & Fourie 2023). The major expansion of macadamia orchards occurs in three provinces:

Mpumalanga, KwaZulu-Natal, and Limpopo, and results in biodiversity loss (Botha 2018), affecting not only species harboured by the remnant vegetation but also species that provide essential ecosystem services (Taylor et al. 2018). Macadamia production are threatened by various insect pest from order Hemiptera: Pentatomidae (Two spotted stink bugs) (Schoeman 2013) and Lepidoptera: Tortricidae (Macadamia nut borer and False codling moth) (Schoeman 2009). These pests are a critical threat to the macadamia industry in South Africa. They can damage the husk and kernel. Specifically, stink bug causes direct feeding damage by inserting their proboscis into the nut and thus inducing kernel quality losses (Jones & Caprio 1994), with a benchmark loss of 121 million ZAR caused by stink bugs during the 2022 growing season (van Zyl & Fourie 2023).

Currently, the macadamia industry relies on broad-spectrum insecticides such as pyrethroids and organophosphate, which have been developed to control stink bug populations in macadamia orchards (Pal & Fourie 2023). However, long-term, repeated use of insecticides against stink bugs might prompt the development of resistance, subsequently resulting in increasing pest populations (Sosa-Gómez et al. 2020). For example, intensive use of insecticides has reduced the susceptibility of the two-spotted stink bug (Schoeman 2014). However, although synthetic insecticides have a quick response, they are extremely easy to use, often cost-effective, and efficient. The improper use of insecticides affects non-target species, such as beneficial insects (Jafar et al. 2013). Some studies have reported uncoupled responses of canopy arthropod diversity to environmental drivers (e.g., Vasconcelos et al. 2022), yet it is not entirely clear how landscape context, pesticide application, and intensification in general simultaneously influence arboreal arthropod diversity. Moreover, there is limited information about different arthropod groups or taxa and whether they are vulnerable equally to intensification (Wagner 2020)

This study focused on arboreal arthropods, which represent complex communities with high taxonomic diversity and functional groups that tend to be sensitive to agricultural intensification (Santos et al. 2007). Therefore, the study explored the role of agricultural intensification in affecting invertebrate diversity and nut quality along an elevational gradient by 1) quantifying the relationship between arboreal dwelling arthropod diversity and four key drivers, landscape context, flying vertebrates, elevation, and pesticide application rates and 2) quantifying the relationship between pesticide application rates and nut quality metrics. Specifically, the study aimed to understand how arboreal arthropod community varies across intensification levels in response to landscape context and how nut quality metrics varies with orchard intensification levels.

Materials and methods

Study area

The study was conducted in the Levubu subtropical fruit growing area, 27km west of Thohoyandou, along the south-eastern slopes and foothills of the Soutpansberg mountain range, Limpopo Province, South Africa (Fig. 2.1). The Soutpansberg mountain is a center of biodiversity and endemism, and it is located within the UNESCO Vhembe Biosphere Reserve (Hahn 2010). The Levubu valley receives about 1000 mm of rainfall during the summer months between November and April. Daily maximum temperature in the region often exceeds 35 °C but rarely reaches 40 °C and daily minimum temperature rarely falls below 15 °C in summer, whereas the region experiences cold temperatures in winter with daily maximum temperatures often reaching 25 °C and minimum temperatures of 10 °C. The region is dominated by large-scale monocultures of macadamia, avocados, guavas, commercial afforestation, and bananas (Mucina & Rutherford 2006) and it is the oldest region in terms of macadamia cultivation. This region is one the regions that have the highest unsound kernel recovery rates in macadamia production, mainly caused by early stink bug damage, late stink bug damage, and nut immaturity (SAMAC 2023). Although the region is dominated by monocultural agricultural landscapes, the region still has some remnant vegetation patches that support biodiversity and their ecosystem services (Taylor et al. 2018). The remnant patches in and around the orchards are classified as Soutpansberg Mountain Bushveld or Tzaneen Sour Bushveld (Mucina & Rutherford 2006). These patches still harbour a variety of wildlife, including vervet monkeys, bush pigs, and small antelopes (Linden et al. 2019), but very little extraordinarily little is known about the invertebrates in these remnants.

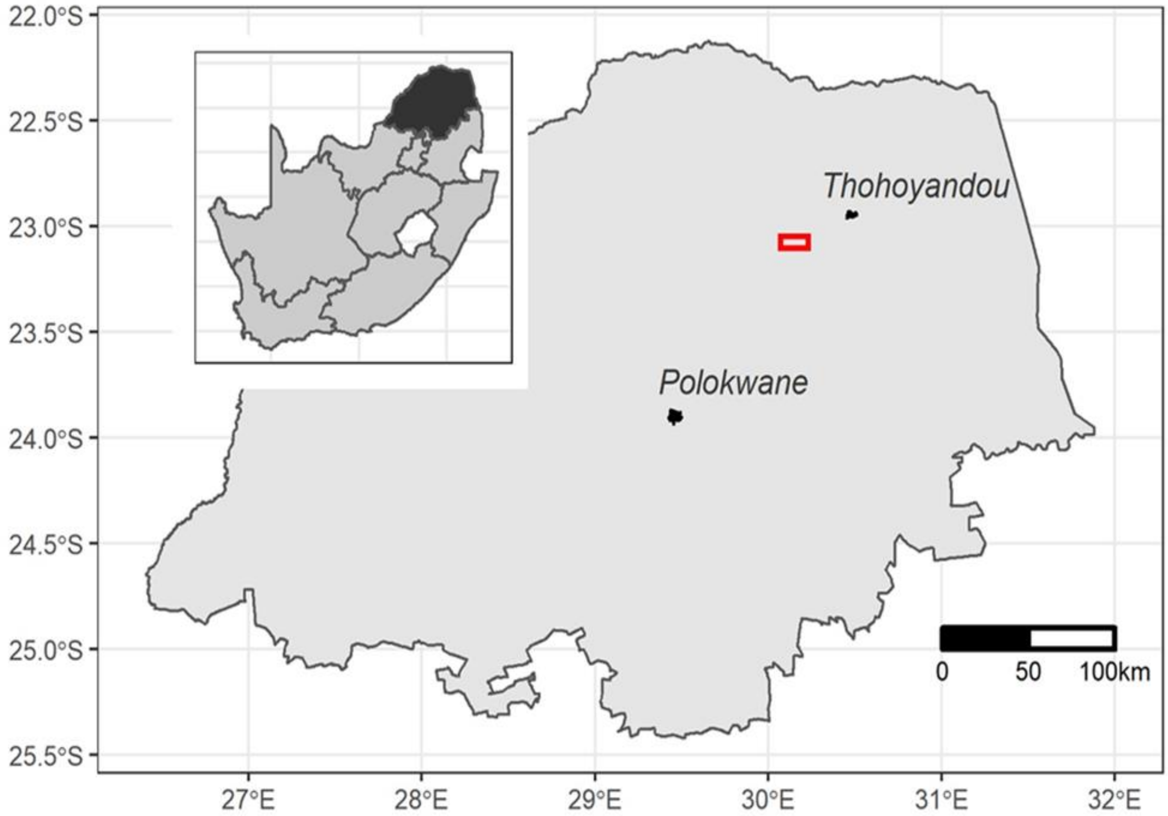

Figure 2.1. Map showing the Levubu region where the study was conducted.

Sampling design

Arboreal invertebrate diversity sampling was conducted from June to July 2021 and replicated across six commercial macadamia orchards in the region. The farms were more than 5 km apart and stratified across an elevational gradient ranging from 700m a.s.l – 1330m a.s.l (Appendix 2. A). On each farm, landscape context was incorporated by stratifying samples across remnant natural vegetation, the edge of the orchards bordering the remnant natural vegetation (edge), and 50m inside the orchard (interior). The orchard edge and interior included a treatment and control sampling unit. A treatment consisted of two macadamia trees enclosed by a cage with dimensions of 5 x 10 x 5m covered with a nylon mesh of size 2 x 2cm (Bouarakia et al. 2023b), excluding large arboreal vertebrate taxa (birds, bats, and vervet monkeys) but allowing invertebrates access. The control consisted of two trees adjacent to exclusion cages (Fig. 2.2A and B). Each farm, therefore, had five sampling units, one sampling unit in the natural vegetation, two sampling units on the edge (control and treatment) of the orchard, and two in the interior (control and treatment) for a total of 5 sampling units x 6 farms = 30 assemblages.

Arboreal invertebrates were sampled using a portable thermal fogger (Electronic Ignition super fogger T.H 130A) with a Dynafog-UVL solution containing Deltamethrin 1.0%, Permethrin 0.5%, and Piperonyl Butoxide 6.0%. Sheets (9 x 6x 9 m) were placed below trees (Fig. 2.2D). Trees were fogged for five minutes (Adis et al. 1997) and searched for 30 minutes after fogging. Branches were shaken 20 minutes into the search period (Schoeman 2020). Sampled invertebrates were stored in 70% ethanol mixed with propylene glycol. Specimens were identified in the laboratory to order and morphospecies level.

Figure 2.2.The A) control plot, which includes two trees, B) treatment where flying vertebrate predators (bats and birds) were excluded, C) the orchard edge bordering natural vegetation, and D) remnant natural vegetation being fogged.

Functional traits

Functional trait-based studies have provided an understanding of how species respond to environmental changes and how community structures are influenced by these responses (Mouillot et al. 2013). Body size in insects explicitly affects their metabolic rate (Harrison et al. 2014), how fast they move (Yang 2000), individual growth (Angilletta Jr et al. 2004), and how often they encounter prey or how many prey they consume (Kalinkat et al. 2015). Ecological and physiological traits can also be assessed using size as a proxy (Jacob et al. 2011), while changes in size distribution within assemblages can indicate environmental stress (McGeogh 2007). The body size of one individual per morphospecies per sample was measured in millimeters using a stereo microscope (Carl Zeiss Discovery V12, Jena, Germany).

Nut quality metrics and pesticide application

Data for this part of the study was collected on ten farms; the four additional farms included had the same treatment as the six farms where insects were sampled. Macadamia nuts were collected in each sampling unit (4 sampling units x 10 farms = 40 samples) during the region's 2020/2021 growing season harvesting period. Macadamia cultivars varied across farms. However, Pahala was the most common cultivar in our sampling units. Nuts were collected weekly or biweekly in each sampling unit, de-husked manually, and stored in a ventilated room for drying and weighing. Dry nuts in the shell were cracked, and kernels were weighed and assessed for any quality defect using a standard procedure to classify and identify categories of unsound kernels. Defects that are less common, such as germination, shell marks, dark marks, and fungus, were classified as 'rest.' These defects included mouldy, germinating kernels and kernels with shell marks or brown centers (Bouarakia et al. 2023a).

Pesticide application schedules for the 2020/2021 growing season were obtained from farm managers (Appendix 2. A and 2.B) and converted to the total active ingredient (kg/ha), which was then used as a covariate in models and is referred to pesticide application rates. Total kernel recovery was calculated as the proportion of whole kernel weight per weight of dry nuts-in-shell. Immaturity, the weight of immature kernels, was calculated as a proportion of the total weight of kernels. Immature kernels have a rubbery texture, are small in size, and have an abnormal shape due to premature nut drop and the lack of oil accumulation (Schoeman, 2009). Oil content is an essential determinant of kernel quality (Mason & McConachie, 1994). Mature kernels were separated from immature kernels by floating kernels in a saline solution, and floating kernels were considered mature (Lee et al., 2011). Insect damage was classified as "early stink bug damage," which is caused by stink bug feeding nuts during the early stage of development, "late stink bug damage," which results from stink bug feeding on mature nuts, and damage caused by Tortricid moth species (Appendix 2. C).

Statistical analysis

All statistical analyses were performed using R, version 4.3.1 (R Development Core Team, 2023). A generalised linear mixed model (Zuur et al. 2009) with a log link function and negative binomial distribution with the package lme4 (Bates et al. 2014) was used to explore to what extent landscape context, elevation, flying vertebrates, and pesticide application rates and their interaction explain variation in species richness and abundance after inspecting for overdispersion in the data set. A linear mixed model with an identity link function and a Gaussian distribution was used to model size. In this instance, size is the community mean of size (CWM_{size}) and is calculated as the size weighted by the relative abundance of each morphospecies in the sample (Lavorel et al. 2007). Farms were included as random factors in our models. The model with the lowest AIC (Akaike Information Criterion) value, including models with $\Delta AIC < 2$ compared to the best model, were included in the results. The pseudo-R^2 of the best model was calculated using conditional (R^2c) and marginal (R^2m) values (Nakagawa & Schielzeth 2013). The conditional values account for the proportion explained by fixed and random factors, and the marginal values account for the proportion explained by fixed factors only. Generalized linear latent variable models (GLLVM) were used to do both constrained and unconstrained ordination using the gllvm package (Niku et al. 2019) and comparing model performance using AIC values. Ants (Formicinae) were not included as ants are known to be eusocial organisms (Parr & Bishop 2022), the presence of ant colonies is not a true reflection of relative abundance but affected by the proximity of nests. The relationship between pesticide application and nut quality parameters was modelled using Pearson product-moment correlations.

Results

Arboreal invertebrate diversity

A total of 6 115 arboreal invertebrates were collected, representing 12 orders. Araneae was the most abundant (36%), followed by Hemiptera (18%), Coleoptera (12%), Hymenoptera (10%), Psocoptera (8%), Neuroptera (5%), Diptera (4%), Blattodea and Orthoptera (3%), Dermaptera and Phasmida were the least abundant (<0.1%).

The best model for species richness included landscape context and its interaction with elevation. The second-best model included pesticides (Table 2.1). Species richness was significantly higher in the remnant vegetation compared to the interior (50m) but did not differ significantly from the edge (Fig. 2.3a), increased with elevation (Fig. 2.3b), and decreased with increasing pesticide application (Fig. 2.3c). The interaction between elevation and natural vegetation was significant, with the effect of elevation decreasing significantly in natural vegetation, suggesting that there is a weak and even negative effect of elevation on species richness in the remnant natural vegetation (Fig. 2.3b).

Table 2.1 Results of GLMM for species richness and abundance (log link function and negative binomial distribution) and LMM (identity link function and Gaussian distribution) for size in response to landscape context, pesticide application rate (total active ingredient), treatment and elevation and their interactions as co-variates. The difference in AIC (ΔAIC) between the best and the next best and the null models are also given. The null model included random effects only (farm). Marginal R2 (R2m), measuring variation explained by fixed effects only, and conditional R2 (R2c), measuring variation explained by both fixed and random effects, are given.

Response	Model	AIC (Best model)	ΔAIC (Second best)	ΔAIC (null)	R2m	R2c
Species richness	~landscape context × elevation	233.1	234.2	256.73	0.74	0.85
Best model equation: Y = $e^{3.6\pm0.1+\ 0.55\ \pm0.1 elevation^{***}+0.2\pm0.09 interiorVSedge^{*}+0.4\pm0.1 interiorVSremnant^{***}-0.13\pm0.1 elevation{:}edge-0.75\pm0.13 elevation{:}remanant^{***}}$ Second best model equation: Y = $e^{3.7\pm0.2+\ 0.4\ \pm0.1 elevation^{*}+0.2\pm0.09 interiorVSedge^{*}+0.4\pm0.1 interiorVSremnant^{***}-0.1\pm0.1 pesticide\ application\ rates-0.13\pm0.1 elevation{:}edge-0.75\pm0.13 elevation{:}remanant^{***}}$						
Abundance	~elevation	336.6	337.5	340.6		
Best model equation: Y = $e^{5.3\pm0.1+\ 0.2\ \pm0.1 elevation^{*}}$						
Size	~landscape context × elevation	92.6	94..6	101.5	0.71	0.83
Best model equation: $Y = 5.6 \pm 0.8 + 2 \pm 0.8 elevation + 0.5 \pm 0.35 interiorVSedge + 1 \pm 0.5 interiorVSremnant - 0.6 \pm 0.35\ elevation{:}\ edge - 1 \pm 0.5 elevation{:}\ remnant$ Second best model equation: $Y = 5.8 \pm 0.4 + 2 \pm 0.4 elevation + 0.5 \pm 0.35 interiorVSedge + 1\ 0.5 interiorVSremnant - 0.01 \pm 0.4 pesticide\ application\ rates - 0.6 \pm 0.35\ elevation{:}\ edge - 1 \pm 0.5 elevation{:}\ remnant$						

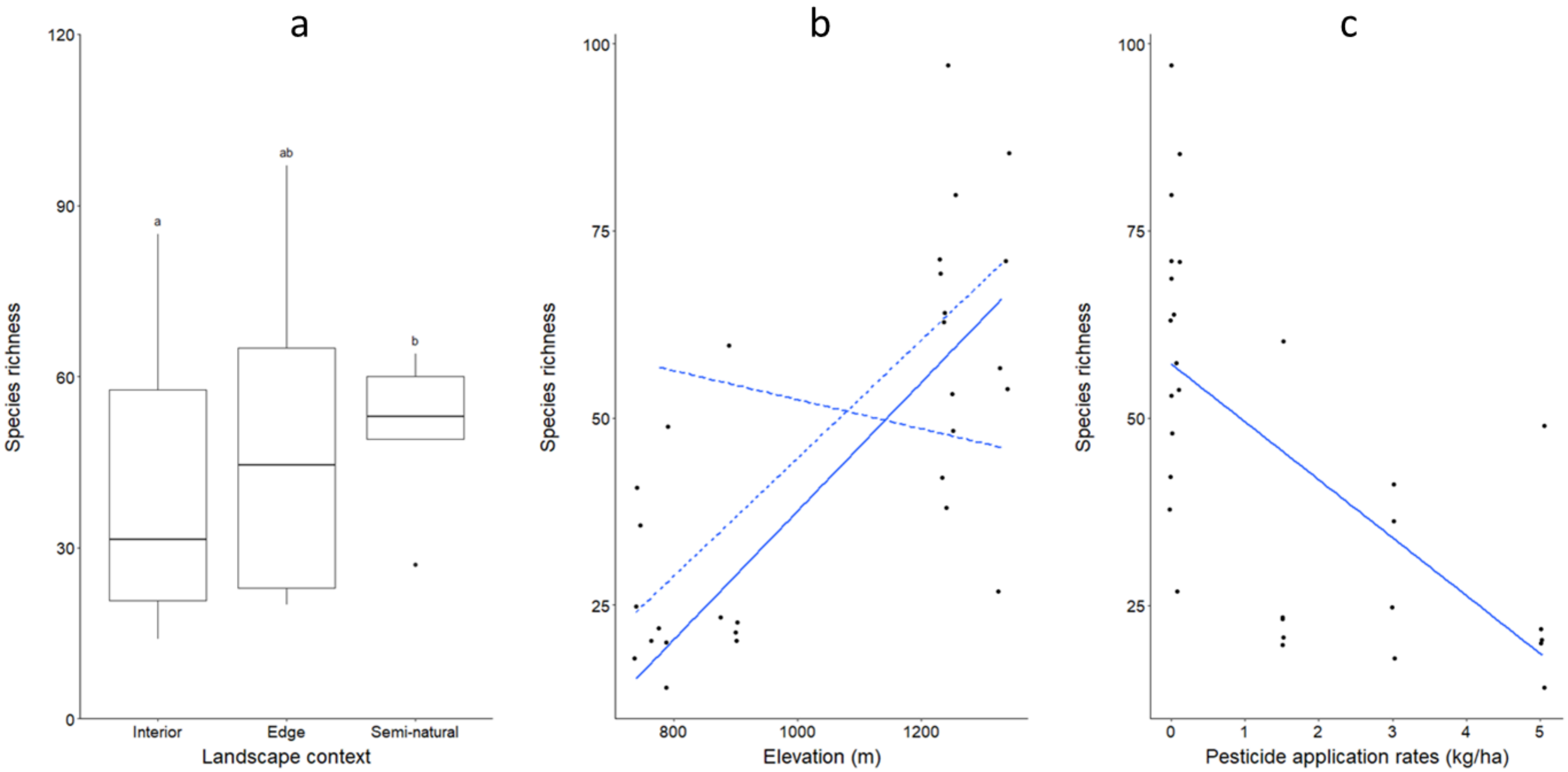

Figure 2.3.The relationship between species richness and a) landscape context, b) elevation and c) total active ingredients applied (kg/ha). Models with significant interactions between elevation and landscape context have the following legend: solid = interior, en-dash = edge and em-dash =remnant natural vegetation.

The best model for abundance only included elevation, with insect abundance increasing at higher elevations (Fig. 2.4). The second-best model included a quadratic term for elevation, suggesting that there is a hump-shaped response of abundance to elevation (Table 1; Fig. 4.).

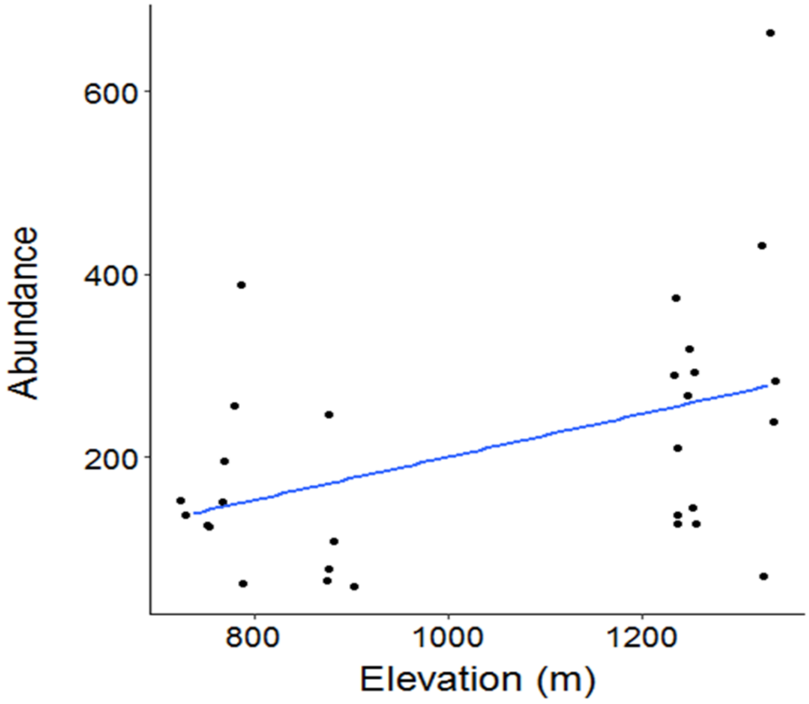

Figure 2.4. The relationship between arthropod abundance and elevation.

The best model for insect size included landscape context and elevation, and the second-best model included elevation, landscape context, and pesticide. Size increased with elevation and interacted with landscape context. The interaction of elevation was most pronounced in the interior of orchards compared to the edge and least pronounced in the remnant vegetation (Fig. 2.5a). Size decreased with increasing pesticide application rates (Fig. 2.5b). Landscape context was also included in the best model, and the pattern suggests an increase in size from the interior to the edge through to the natural vegetation. However, multiple comparisons suggest that they did not differ significantly between the treatments (Fig. 2.5c).

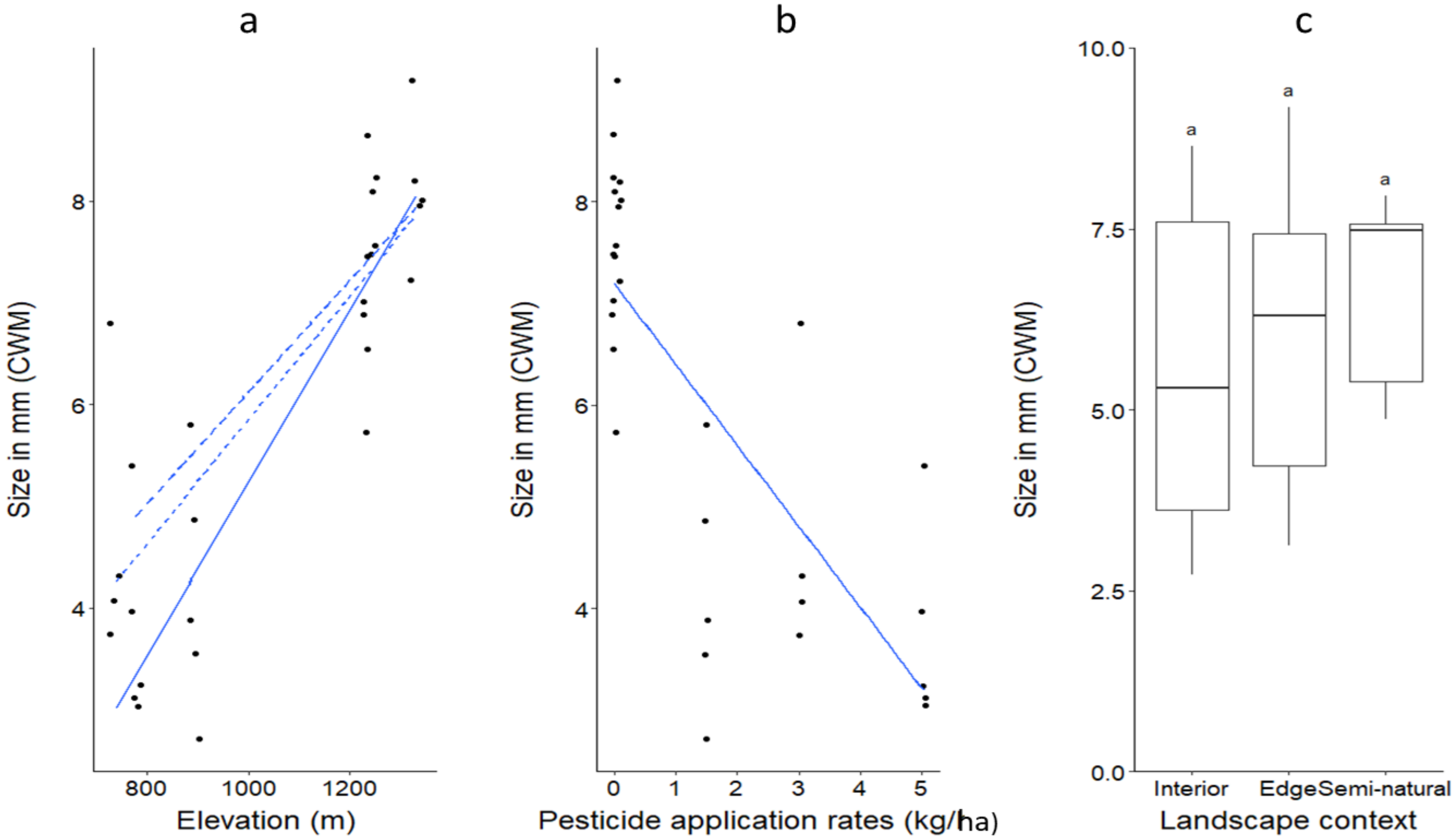

Figure 2.5.The relationship between community weighted mean of size (mm) and a) elevation, b) pesticides, and c) landscape context.

The model for community composition included farm and treatment and had the lowest AIC, 1946.2, compared to 2021.5 of the unconstrained models. Lower-elevation farms varied more in composition (Fig. 2.6) than the higher-elevation farms. Much of the variation in treatment differences is due to samples taken in the remnant vegetation with typical taxa such as Hymenoptera, Neuroptera, Mantodea, and Coleoptera. Orders associated with the lower elevation farms included Psocoptera, Dermaptera, Diptera, and Phasmida.

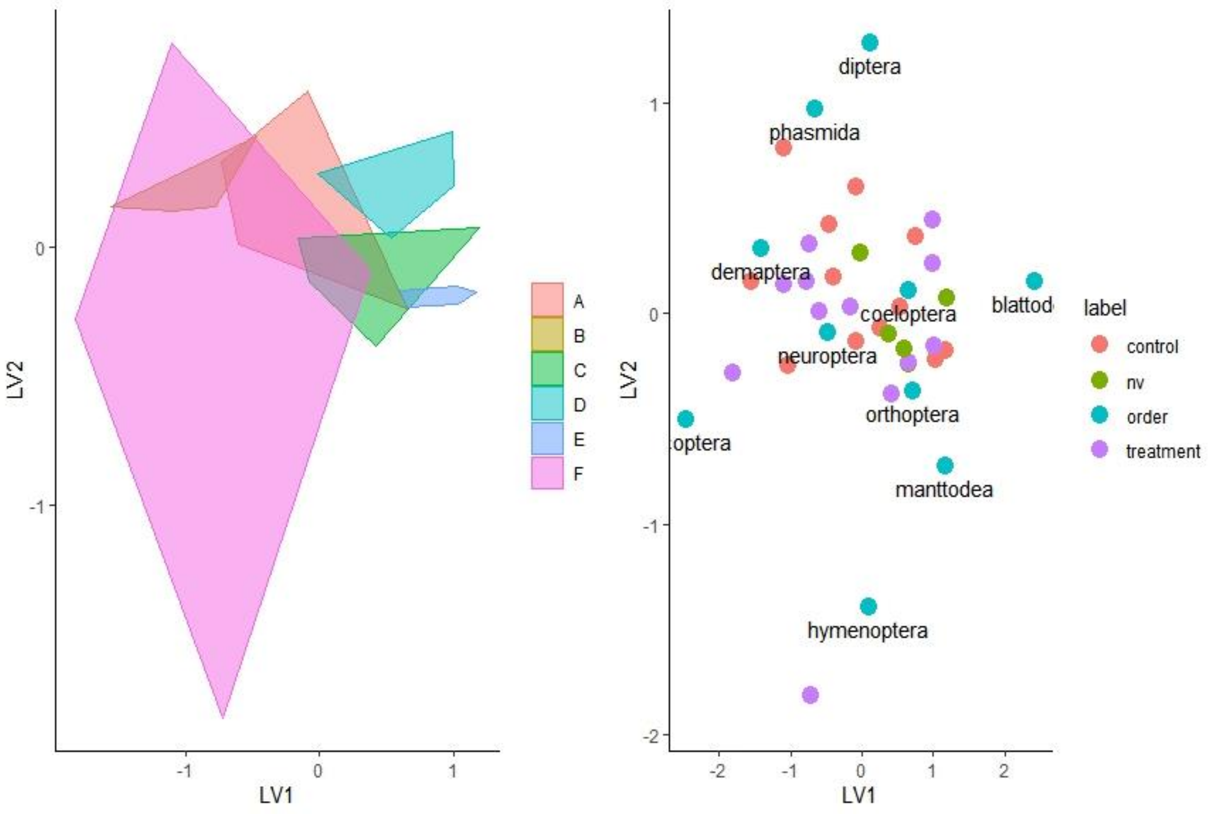

Figure 2.6.Bayesian ordination of order-level community composition across the six farms.

Nut quality metrics

In nut quality metrics, unsound kernels were due to immature nuts (Fig. 2.7). Insect kernel damage did not vary significantly with the amount of pesticide applied (Fig. 2.8a, b, and c). Nut immaturity was positively correlated and significantly increased with increasing pesticide application (R=0.71, $p < 0.01$) (Fig. 2.8d). There was a strong negative correlation between sound kernel recovery and pesticide application (R=-0.6, $p < 0.01$) (Fig. 2.8e and f).

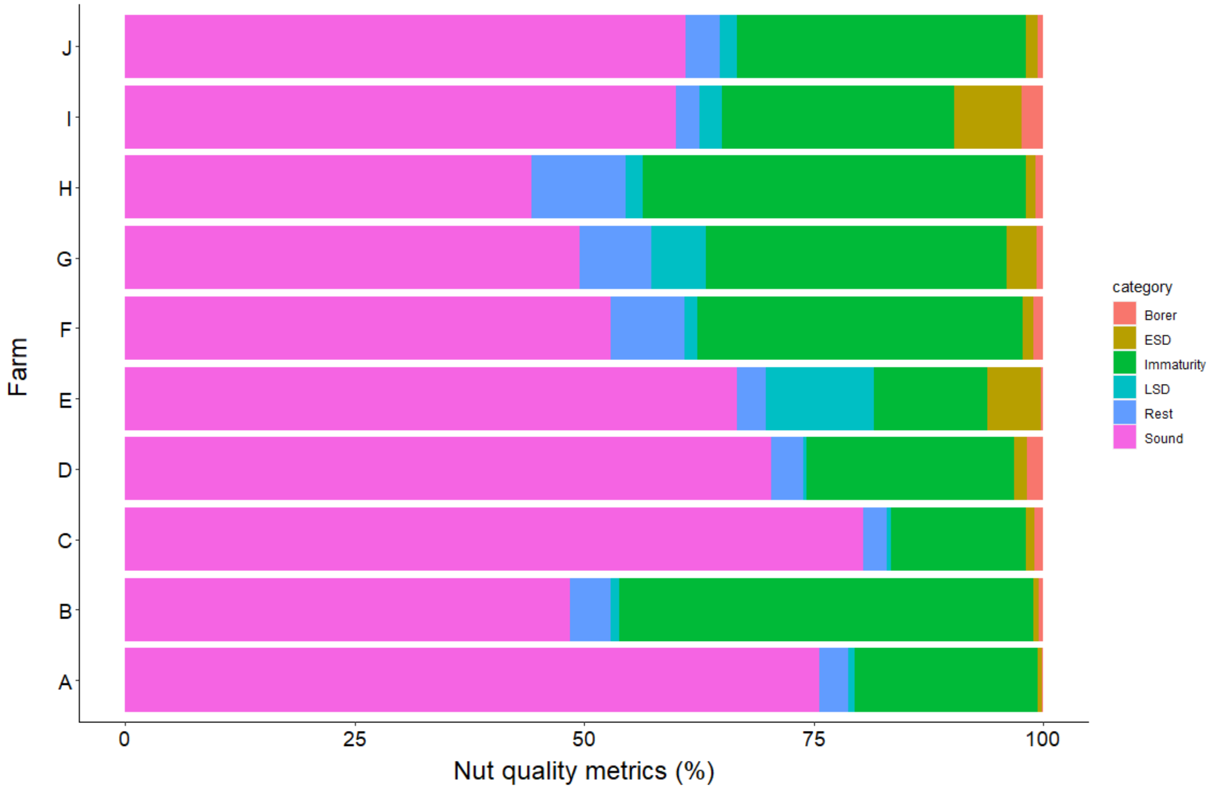

Figure 2.7.Nut quality metrics for the 2020/21 season for ten sampled farms. The metrics included both major economic kernel defects and less economic kernel defects. Borer explains the damage caused by nut borer larvae (*Thaumatotibia batrachopa* and *Cryptophlebia illepida*), stink bug damage is explained by both late stink bug damage (LSD) and early stink bug damage (ESD), immaturity represents immature kernels, rest explained damage caused fungi, germination and brown spots and sound explained all kernels that were in good quality.

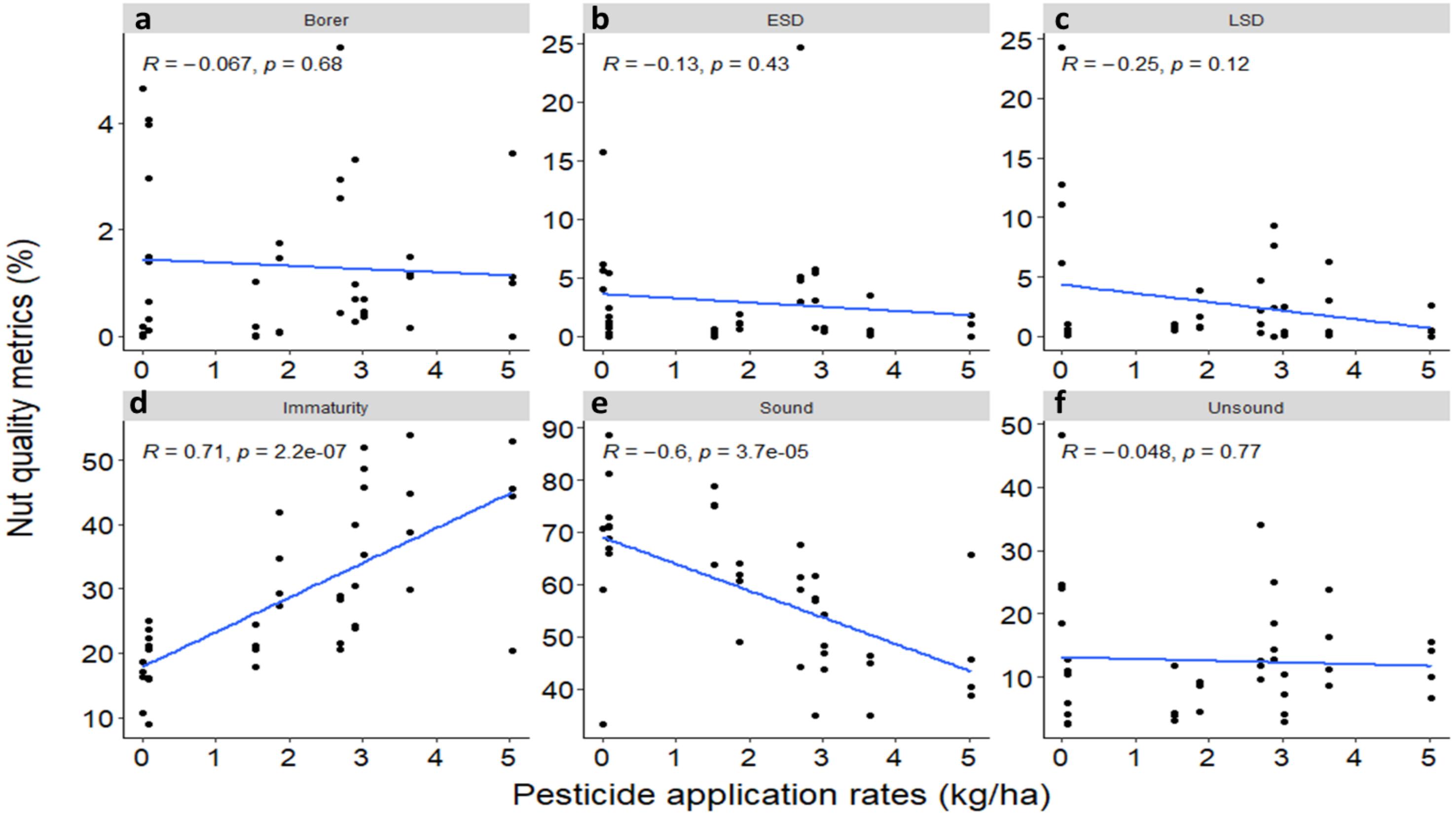

Figure 2.8.Pearson product-moment correlations between active pesticide application and nut quality metrics that include a) borer damage caused by *Thaumatotibia batrachopa* and *Cryptophlebia illepida*), b) late stink bug damage (LSD), c) and early stink bug damage (ESD), d) immaturity represents immature kernels, e) sound explained all kernels that were in good quality and f) unsound kernels.

Discussion

This study observed a strong relationship between macadamia farming intensification and elevation on arboreal arthropod diversity. As expected, the study found a consistent and significant negative impact of pesticide application rates on invertebrate diversity but not insect damage metrics. In overall nut quality metrics deteriorated with increased pesticide application, i.e., pesticide application rate did not explain significant amounts of variation in insect damage and were associated with an overall decrease in sound kernels. Species richness increased with elevation; however, this trend was reversed in remnant vegetation, while flying vertebrate predators did not affect arthropod diversity.

Landscape context significantly shapes species richness, with an increase from simplified landscape to natural vegetation. The observed pattern suggests individual spillover from the remnant vegetation into the orchard. In agricultural landscapes, species distribution and diversity rely on spatial arrangements (typical measures such as edge length and size of the habitat) and the connectivity of the semi-natural habitat (Marja et al. 2022; Schirmel et al. 2016; Tscharntke et al. 2012). The significant differences depicted by species richness between remnant natural vegetation and field edge and with field interior suggest that this pattern might have also resulted from edge effects. Edges are often considered to have higher biodiversity due to individual spillover from adjacent habitats and species associated with edge habitats (Ries et al. 2017). Field edges facilitate spillover and tend to be keystone features in the agricultural landscape as they are neighbouring semi-natural vegetation, which are stable and relatively undisturbed by crop management practices (Gallé et al. 2020).

Farm intensification negatively affects agrobiodiversity and their ecological functions (Duflot et al. 2022) by reducing the availability of microhabitats and creating homogeneous systems (Marasas et al. 2010), thus influencing arthropod movement to undisturbed habitat field margins and seminatural vegetation. González et al. (2017), indicated that edges facilitate easy access to resources provided by both habitats (remnant vegetation and crop fields). Although landscape context significantly affected species richness, abundance was not affected by landscape context. However, we observed a high abundance of individuals of the same species in the interiors of the orchard, e.g., Psocoptera was more abundant in the orchard interior than in the edge and remnant vegetation. Recently Marja et al. (2022) noted that landscape complexity only increased species richness, and agri-environmental schemes increased both species richness and abundance. Arthropod diversity and abundance in crop habitat is affected by management intensity (Epstein et al. 2000; Markó & Kádár, 2005).

Richness decreases with elevation (Joseph et al. 2019; Röder et al. 2010; Stevens, 1992) and this pattern was only observed for the remnant vegetation. This result suggests that temperature and reduced habitat diversity might have contributed to the observed pattern. Temperature plays a significant role in the life history of arthropods (Sinclair et al. 2003), as it affects, among other aspects, generation turnover per year (Hodkinson 2005). High elevations are associated with cooler temperatures (Eneva & Coolbaugh 2009) which influence arthropods by reducing their growth and developmental rate (Hodkinson 2005). Due to cooler temperatures, higher elevations are likely to have less insect abundance compared to lower elevations, which is not what we observed, in this study, insect abundance increased with elevation. In addition, some insects at higher elevations are predicted to have fewer generations due to cooler temperatures. For example, *Bathycoelia distincta* is predicted to complete less or not to complete a single generation at high elevations or at temperatures below 17°C (Muluvhahothe, 2023).

The observed increase with elevation in interior and field edge opposes the area heterogeneity trade-off hypothesis (AHTO) (Allouche et al. 2012; Hortal et al. 2013), which suggests that species richness decreases at an elevated level of habitat diversity due to available area per habitat decreases. Landscape heterogeneity and area are crucial factors that influence species richness. The potential aligned with large areas is that they can harbour large populations than small ones, and many species with different ecological needs are associated with heterogeneous landscapes (Allouche et al. 2012). However, our results suggest that the increase in species richness with elevation was explained by increased habitat diversity at higher elevations due to less agricultural intensification.

We observe an increase in body size from the interior to the edge into remnant vegetation. These results are consistent with the recent studies reported by Gelle et al. (2020) and Rischen et al. (2023), where they observed smaller body sizes for carabids and spiders in the interior of crop fields. They observed that spider species found within crop fields tend to be smaller in size mainly due to their ability to recolonize disturbed habitats by ballooning. Agricultural practices across the elevational transect might explain the observed pattern, as we have noted that in the higher elevation, agroecological interventions, such as having lower pesticide application rates, have been implemented compared to the lower elevation.

It is essential to know whether pest pressure is likely to surpass the established economic threshold injury levels and cause significant damage rather than knowing the pest abundance at a critical time (Paredes et al. 2021). However, it is also essential to understand the effects of management practices on maintaining pest pressure below economic threshold injury levels. Insect damage metrics were not correlated with pesticide application, while immaturity was the most principal factor affecting kernel recovery and positively related to pesticide application. Orchard age, water availability, and soil might have contributed to the observed pattern of immaturity. Immaturity could be the result of water availability and nutrient status of orchards (Klein et al. 2015), while field history, soil content (texture), and soil properties may also play a role (Duflot et al. 2022). In our study, Macadamia cultivation at lower elevations started earlier compared to sites higher up in the mountain, and there is less macadamia coverage at high altitudes (Anders et al. 2023). However, there is convincing evidence in this study to conclude that increasing pesticide usage might be causally related to kernel recovery rates. This could include pollinator diversity, with cross-pollination resulting in lower levels of immaturity (Anders et al. 2023), and pest control by generalist predators, resulting in smaller amounts of fungal infections. Further evidence also suggests that kernel immaturity is linked to *Thaumatotibia batrachopa* larval damage to husks (Bouarakia et al. 2023b)..

Although we only sampled in winter, the comparative nature of the sampling design provides insights into variation in invertebrate assemblages across a relatively large environmental gradient (700m a.s.l – 1330m a.s.l). However, a longer sampling period would have provided an opportunity to explore temporal patterns further and provide valuable insights (see van Schalkwyk et al. 2020). While there is no doubt that agricultural intensification has successfully increased production to meet the global food demand, long-term impacts may not be sustainable. Our results raise questions around awareness of the negative impact of management practices on agrobiodiversity and the concomitant contribution it makes to production. Arthropods provide vital ecosystem services to agriculture (Goehring et al., 2002). However, we have a limited understanding of the crucial role played by arthropods in regulating natural pest populations (Bianchi et al., 2006). Further intensification of macadamia production will lead to declines in arboreal arthropod diversity, including beneficial taxa, leading to increasing pest pressure and reduced nut quality. Reduced use of agrochemicals, increasing landscape heterogeneity, and softening the edge could contribute to the stability of agroecosystems and be more cost-effective (Santos et al., 2007; Vasconcelos et al. 2022). Our results suggest that ecological intensification is a viable alternative to intensive farming practices (Ullah et al., 2020) and advocate for implementing IPM strategies in macadamia management practices.

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Chapter 3

Entomopathogenic fungi and egg parasitoid, *Trissolocus basalis* (Hymenoptera: Scelionidae) as a potential control for *Bathycoelia distincta* (Distint) (Hemiptera: Pentatomidae)

Abstract

Bathycoelia distincta (Hemiptera: Pentatomidae), commonly known as the Two-spotted stink bug, is a major pest on macadamia in South Africa. It causes damage by directly feeding on macadamia kernels throughout the growing season. Currently, farmers rely on applying chemical insecticides to control their populations; however, the effects of extensive insecticide application on human health, environmental health, and the development of pest resistance are alarming. There is a need to evaluate biological control measures that can be used to control this pest. The study assessed the efficacy of entomopathogenic fungi (with and without chitinase enzyme) and egg parasitoid *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Platygastridae) against *B. distincta*. All EPF treatments were effective against *B. distincta*, with chitinase enzyme leading to a 95% mortality rate. Under field conditions, EPFs and a chemical knock-down spray showed no significant difference between treatments. All parasitoid treatments significantly affected *B. distincta* egg mortality, with two pairs reaching a 100% parasitism rate. The parasitism rate decreased with age but increased with the parasitoid second generation. However, both were not significant. Environmental factors influenced the performance of EPFs under field conditions. The results suggest that two pairs of parasitoids are sufficient to produce a 100% parasitism rate and further indicate that female parasitoids can co-exploit a patch without being aggressive to each other for some time. However, parasitism rate results for four pairs suggest an intra-specific competition and the decrease in parasitism rate with age suggests age-specific fecundity. The study results suggest that EPFs and stink bug egg parasitoid *T. basalis* can be used as biological control agents against *B. distincta.*

Keywords: Pathogenicity; density; Intra-specific competition; Age-specific fecundity

Introduction

Pentatomidae, commonly known as stink bug, is the largest group in the family Pentatomoidea and the third largest group in the Heteropteran family (McPherson & McPherson 2000). They are polyphagous and phytophagous except for the subfamily Asopinae, which are predatory species (McPherson & McPherson 2000; Panizzi et al. 2000). Pentatomidae are hemimetabolous with a life cycle consisting of eggs, nymphs, and adults. The sexually mature females glue their eggs on the stems and leaves of the host plant (De Villiers & Schoeman 2015). The first instars are gregarious, generally inactive, and do not feed on the host plant but remain clustered together on the empty eggshells, thus acquiring specific symbionts by ingesting secretions covering eggshells (ABE 1995; Prado & Almeida 2009b). The symbionts play a significant role in improving the fitness of the insects (Bistolas et al. 2014; Prado & Almeida 2009a). Feeding on the host plant and dispersal commence during the second instar phase. They are characterised by piercing and sucking mouthparts and wings that are hard, thin, and thick at the base. Pentatomids, specifically phytophagous pentatomids, are major problems in the agricultural system as they cause crop damage by feeding directly on the crop and transmitting pathogens (McPherson & McPherson 2000).

In South Africa, stink bugs are an economic pest of macadamia with *Bathycoelia distincta*, Distant (Hemiptera: Pentatomidae), commonly known as the two-spotted stink bug (Schoeman 2018). *Bathycoelia distincta* was first detected in the Levubu subtropical growing region, Limpopo, in 1984 as a pest of macadamia by the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) (Schoeman 2018). It causes damage by directly feeding on macadamia kernels throughout the season as it attacks macadamias throughout their life cycles (Schoeman 2014). It has a long proboscis, which allows it to feed on the kernel. The average length of the adult proboscis is about ±13.6mm, which is twice the length of other stink bug species found in macadamia (Bruwer et al. 2021).

The damage associated with stink bugs can be classified into two categories: early stink bug damage and late stink bug damage, with a combined annual profit loss estimated to be about ZAR 112 million (SAMAC 2023). Early stink bug damage occurs during the early development of the kernel and causes abscission, premature abortion (Schumm et al. 2020), necrotic lesions, and deformed kernels (Bruwer et al. 2021). Late stink bug damage occurs during the late season (when the nut has matured), which is noticeable by discoloration or translucent blemishes on the kernel (Bruwer et al. 2021). Overall, this type of damage results in crop loss and reduces nut quality, thus rendering the kernels unsuitable for the market (Linda, 2021).

The production of macadamia nuts in the presence of *B. distincta* becomes difficult without some chemical intervention (De Villiers & Schoeman 2015). Therefore, current methods used to monitor and control this pest species include the combination of insect pathogens, tree branch shaking, and chemical insecticides (Schoeman 2014; Schoeman & Mohlala 2012). However, with the problems associated with synthetic pesticide application on health, environmental health, non-pest species, and pest resistance, there is a need to develop Integrated pest management that can be used to control the pest species. The main aim of IPM is to ensure that there is less application of chemical pesticides so that there is less disturbance to biological control agents, thus leading to biodiversity conservation (Rao & Tanweer 2011). IPM has several elements that have been applied from the pre-pesticides era and are in practice; these elements include biological approach, physical approach, cultural approach, and chemical pest approach (Rao & Tanweer 2011). In this chapter, we will focus only on the biological approach.

The biological control approach is considered the cornerstone of IPM and uses living organisms such as pathogens, predators, and parasitoids to control pest insects (Stenberg 2017). This approach is a non-chemical method, is eco-friendly, contributes to sustainable crop production, and provides ecological viability (Rao & Tanweer 2011; Stenberg 2017). One approach as a form of biocontrol is by using pathogens such as entomopathogenic fungi (EPF). These pathogens generate enzymes such as chitinases, proteases, esterases, and lipases, which break down insect cuticles and allow them to penetrate and colonize the insect body, subsequently causing insect death (Dalla Nora et al. 2021) There are about 1200 species of fungi that have been described as insect pathogens (Abdullah 2019), these include *Beauveria bassiana* (Silva-Santana et al. 2022) and *Metarhizium anisopliae* (Jackson & Jaronski 2009). On the other, there are soil dwelling bacterium such as *Bacillus thuringiensis (Bt)* (Mohan et al. 2014), and enzymes such as Chitin (Chitinase enzyme) (Binod et al. 2007) which are known pathogenic to insects.

Several studies have shown that EPFs are useful for the biological control of stink bugs such as rice stalk stink bug, *Tibraca limbativentris* Stal (da Silva et al. 2015), Green shield bug, *Palomena prasina* L (Erper et al. 2016), Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål, 1855) (Tozlu et al. 2019), brown stink bug, *Euschistus heros* F (Silva-Santana et al. 2022). However, earlier trials on EPFs conducted by Schoeman (2014) reported promising and disappointing results when evaluating the efficacy of *B. bassiana* on stink bug species occurring in macadamia orchards. The results indicated that *B. bassiana* isolates in the laboratory showed significant pathogenicity; however, under field conditions, the results were disappointing but promising when *B. bassiana* was combined with low rates of Beta cyfluthrin 125 g/L SC @ (1 ml/100 L) (Schoeman 2014).

Another biological control approach is using egg parasitoid wasps. Egg parasitoids can be used as biocontrol agents in agricultural systems because they significantly regulate their host population (Birkmire et al. 2021). Among the solitary parasitoids species that regulate stink bugs species, the scelionid egg parasitoid *Trissolcus basalis* (Wollaston, 1858) (Hymenoptera: Platygastridae) is one of the essential biocontrol agents universally known for its ability to regulate the population of cosmopolitan pest, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Waterhouse 1998). *Trissolcus basalis* has been reported to occur in tropical and subtropical regions (Corra-Ferreira & Moscardi 1996). Its ability to locate and accept a host is known to be mediated by chemical cues (Colazza et al. 2004). The female can mate and produce fertile eggs within a few hours of hatching. The female parasitoids deposit eggs singly in the stink bug eggs, and the developing wasp larvae feed on the developing nymphs inside the egg (Kilner 2015). The developing wasp larvae undergo three phases: instar larvae,pre-pupal, and pupal; adults emerge after 12-15 days (El-Husseini et al. 2006).

Unlike other industries, such as citrus, where the IPM program has been archived, in the Southern African Macadamia Association (SAMAC), true IPM is yet to be archived (Schoeman 2014). However, some studies have identified potential biological control agents that can control stink bugs in the macadamia industry. These studies identified egg parasitoids hosted by *B. distincta*, which include two species belonging to the genus Trissolcus (Bruwer 1992) and entomopathogenic fungi for controlling *B. distincta* (Linda 2021), with little evidence of efficacy evaluation. Therefore, using the groundbreaking findings from these studies, the current study aimed to evaluate the efficacy of entomopathogenic fungi and *Trissolcus basalis* against *B. distincta,* and the study objectives were to a) to evaluate the efficacy of 1x109 cfu/g *Bacillus thuringiensis* + 1x108 cfu/g *Beauveria bassiana* + 1x108 cfu/g *Metarhizium anisopliae*

(EPFs) with and without chitinase enzyme against two-spotted stink bugs (*in vitro*), b)to evaluate the efficacy of EPFs without chitinase enzyme under field conditions, c) quantify the efficiency of *T. basalis* against *B. distincta* determine the effective release rate of *T. basalis*, and d) to evaluate the effect of age and generation of *T. basalis* parasitism rate against *B. distincta.*

Materials and methods

Bathycoelia distincta and Trissolcus basalis culture and maintenance

Bathycoelia distincta colony was initiated from hand-collected eggs, nymphs, and adults from commercial macadamia orchards in the Levubu region, Limpopo, South Africa, during the 2020-2021 production season. Collected eggs, adults, and nymphs were taken to the laboratory, placed in a rearing plastic cage (33 × 24 x 16 cm) with the floor lined with a paper towel, and fed *ad libitum* of sweetcorn (*Zea mays*). Paper towels were used as oviposition substrates for field-collected adults, and small cotton balls were used to maintain humidity and as a water source for Instar 1. Eggs were collected twice weekly and placed inside a plastic cage (18 x 12.5 x 5cm) for further use for *B. distincta* rearing and experiments. To prevent fungal development and infection, individuals were transferred to clean, sterilized rearing cages with fresh food and water *ad libitum* with the above-described diet and method twice a week. Inbreeding was avoided by constantly introducing field-collected eggs and individuals into the colony. The eggs, nymph, and adult rearing cage were placed in a walk-in climate chamber at 25°C, 75% relative humidity, and 16:8h (L:D) photoperiod at the University of Venda (Dingha & Jackai 2017; Geng & Jung 2018). *Trissolcus basalis* parasitoids were supplied by Koppert Biological Systems, Inc. to initiate and maintain the parasitoid culture. The parasitoid culture was maintained at the University of Venda laboratory, supplied with a sugar solution as a diet, and kept at 25°C, 75% relative humidity with a 16:8h (L:D) photoperiod.

Entomopathogenic fungi and bioassay

In vitro efficacy of entomopathogenic fungi with and without chitinase enzyme against sucking bugs

We evaluated the efficacy of $1x10^9$ cfu/g *Bacillus thuringiensis* + $1x10^8$ cfu/g *Beauveria bassiana* + $1x10^8$ cfu/g *Metarhizium anisopliae* (EPFs) with and without chitinase enzyme

(Enzyme) against the two-spotted stink bug. A randomized design was used with three life stages (1-3 instar nymph, 4-5 instar nymph, and adults) replicated three times. Three individuals per stage and treatment were placed inside a rearing cage and provided food and water ad libitum. Treatments were applied using a 1L calibrated fine spray bottle. The number of dead nymphs and adults was recorded daily for ten days. All the cadavers found were removed from the rearing cages, placed in a petri dish with filter paper and cotton soaked with distilled water, and placed in an incubator at 27°C in the dark until the development of fungal hyphae.

Treatments are as follows:

Treatment 1: Control (1L water)

Treatment 2: 2ml Enzyme / 1 L water (1:500 dilution)

Treatment 3: 10g EPFs / 1 L water (1:100 dilution)

Treatment 4: 20g EPFs / 1 L water (1:50 dilution)

Treatment 5: 40g EPFs / 1 L water (1:25 dilution)

Treatment 6: 10g EPFs + 2ml Enzyme / 1L water

Treatment 7: 20g EPFs + 2ml Enzyme / 1L water

Treatment 8: 40g EPFs bh+ 2ml Enzyme / 1L water

Efficacy of entomopathogenic fungi against sucking bugs in macadamia orchards (field application).

We evaluated the efficacy of 1x10^9 cfu/g *Bacillus thuringiensis* + 1x10^8 cfu/g *Beauveria bassiana* + 1x10^8 cfu/g *Metarhizium anisopliae* (EPFs). Field applications were done with a calibrated 2000L mist blower (Jacto) at a water volume rate of 2000 L/ha. Applications of EPFs were done at 20 g/ha and tested against chemically treated control trees (trees sprayed with dichlorvos: organophosphate 1000g/L). Ten randomly selected data trees were scouted after applications. All the insects that dropped to the ground onto a black plastic sheet placed out the evening before the application were collected, identified, and counted.

Efficacy of T. basalis against the B. distincta eggs

The efficacy of *T. basalis* was quantified using 14 newly laid *B. distincta* eggs (one egg packet) placed in rearing cages with zero (control), one, two, and four mated female *T. basalis* adults (treatment) replicated five times. Mated female *T. basalis* were assigned to *B. distincta* for 48h. After 48h, the parasitoid wasps were removed from the rearing cages, and the eggs were left for development for ten days under 25°C, 75% relative humidity with a 16:8h (L:D) photoperiod. After ten days, the parasitized eggs were counted and presented as a percentage. The effect of age on egg parasitoids was evaluated using 24h old *T. basalis* wasp pair provided with different densities of two-spotted stink bug newly laid eggs (1-24h) as follows: 2, 4, and 8 egg packets for 48h. After 48h, the eggs were removed, replaced with newly laid eggs, and replicated five times. To measure the efficacy of the first filial generation (F1), the newly emerged (24h) parasitoid wasps were provided with densities similar to those above for 48h and replicated five times. Egg packets were left for seven days to observe whether stink bug nymphs emerged or 14 days for parasitoids to emerge. Stink bug nymph and parasitoids that emerged were scored individually per egg. The number of eggs that have been parasitized and non-parasitized were counted and presented as a percentage of the total number of viable eggs.

Statistical analysis

All statistical analyses were performed using R, version 4.3.1 (R Development Core Team, 2023). Shapiro-Wilk test of normality was used to test normality on stink bug mortality data, where necessary data transformations were performed to ensure homoscedasticity. The effects of treatments, interactions, and other factors were tested using type II analysis of variance (ANOVA) using Satterthwaite's method. The significance of effects was tested using multiple comparisons of least-square means after Tukey adjustment on a confidence level of 95%. Entomopathogenic fungi under field application were analysed by Analysis of Variance (ANOVA) using Satterthwaite's method to test for effects of interactions, treatments, and other factors in a generalised linear model with Poisson data distribution (count data). The dependent variable was the percentage parasitism in a linear model testing the outcomes of the treatment effect. The model residuals were tested for normality using a Shapiro-Wilk test, and data were tested to ensure homoscedasticity. A one-way analysis of variance (ANOVA) was applied to test for the effects of the treatments on the dependent variable. The significance of effects was tested using multiple comparisons of least-square means after Tukey adjustment on a confidence level of 95%. The generation and age data were tested for normality and homogeneity of variance on the model residuals using the Shapiro-Wilk test. The generation and age did not meet the assumptions of the linear model. The effects (interactions) of treatments between age and egg densities, and generations and egg densities were analysed using a Generalised Linear Model (GLM) assuming a Gaussian Distribution and an identity link function with a type II Sum of Squares ANOVA. A comparison between generations, age, and egg densities was performed using Dunn's post hoc, and the latter tests were used to determine the effect of generation and age.

Results

Efficacy of EPFs against B. distincta (in vitro)

All EPF treatments were effective against *B. distincta* (ANOVA: $F_{(7.683)} = 50.19$; $P<0,0001$). The effects of treatments on *B. distincta* life stages varied significantly with observation dates (days after application) (Table 3.1). Mortality after ten days ranged from 50-95% for four treatments (chitinase enzyme leading with 95%, followed by one dose of EPFs and chitinase enzyme at 60%, two doses of EPFs and chitinase enzyme at 55%, and the last half dose of EPFs and chit 50%). The remaining treatments were less than 50% (Fig. 3.1). For instar 1-3, after five days, the chitinase enzyme was applied independently, and a double dose of EPFs significantly increased the mortality rate relative to the control (Appendix 3. A). A single dose of EPFs with chitinase enzyme significantly increases the mortality rate relative to the control after seven days. After nine days, a double dose of EPFs increased the mortality rate significantly relative to the control. For instar 4-5, a significant increase in mortality rate was noted only after 48 hours on chitinase enzyme; other treatments did not show any significant increase in mortality rates (Appendix 3.B & C).

Table 1.1. Summary results of the effect of entomopathogenic fungi on *B. distincta* survival rate, life stage, and observational days.

Parameters	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Treatment	7	27.11	3.87	50.19	**< 0.0001*****
Stage	2	19.35	9.67	125.39	**< 0.0001*****
Days After Application	9	9.37	1.04	13.49	**< 0.0001*****
Stage: Days After Application	18	2.14	0.12	1.5412	0.06992.

Signif. codes : 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

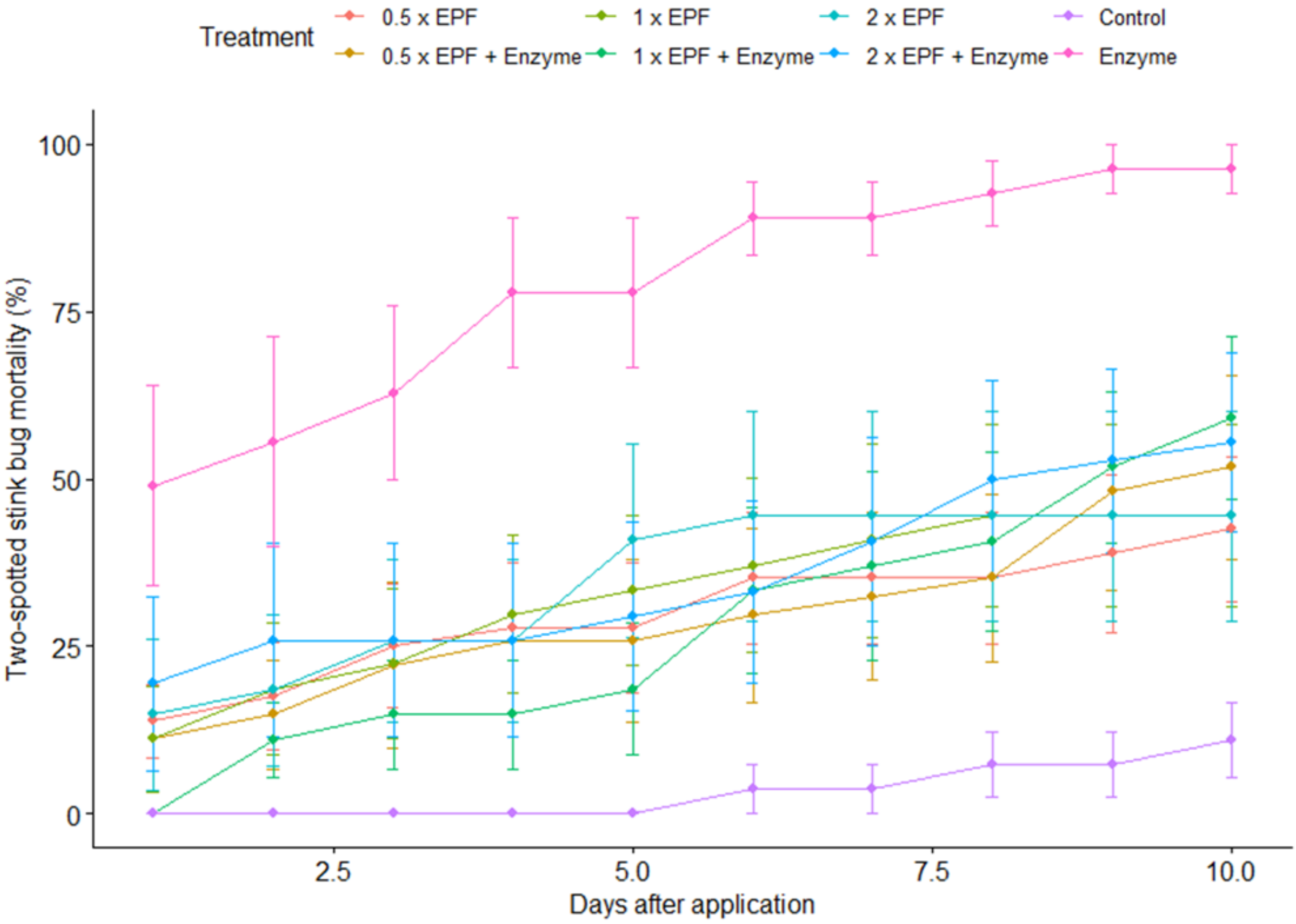

Figure 3.1. Summary of two-spotted stink bug (*B. distincta*) mortality rates after application of treatments.

Efficacy of EPFs against B. distincta (field application)

Overall, the results indicated that EPFs and a chemical knock-down spray showed no significant treatment or target effect (ANOVA: $F_{(3,35)} = 1.68$; $p = 0.19$) (Fig. 3.2.), suggesting that the EPFs performed just as well as a chemical knock-down. The Tukey multiple comparison test confirmed no difference between the treatment outcomes ($p = 0.65$).

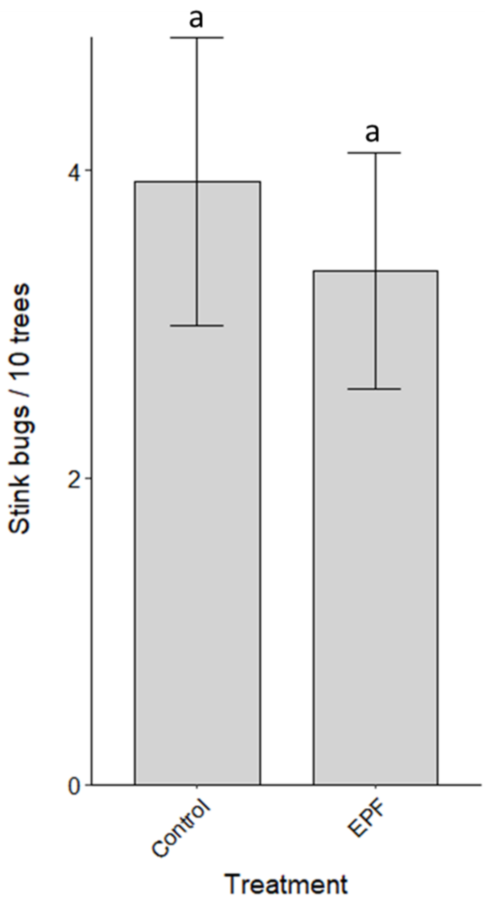

Figure 3.2. The effect of chemical knockdown compared to entomopathogenic fungi on stink bug survival rate.

Although the GLM did not show any significant difference between EPF and chemical knock-down, the interactions between EPF and *B. distincta* showed an increase in mortality, but it was not significant (Appendix 3.D). The trial outcomes to compare the efficacy of EPFs to the chemically treated control are plotted (Fig. 3.3).

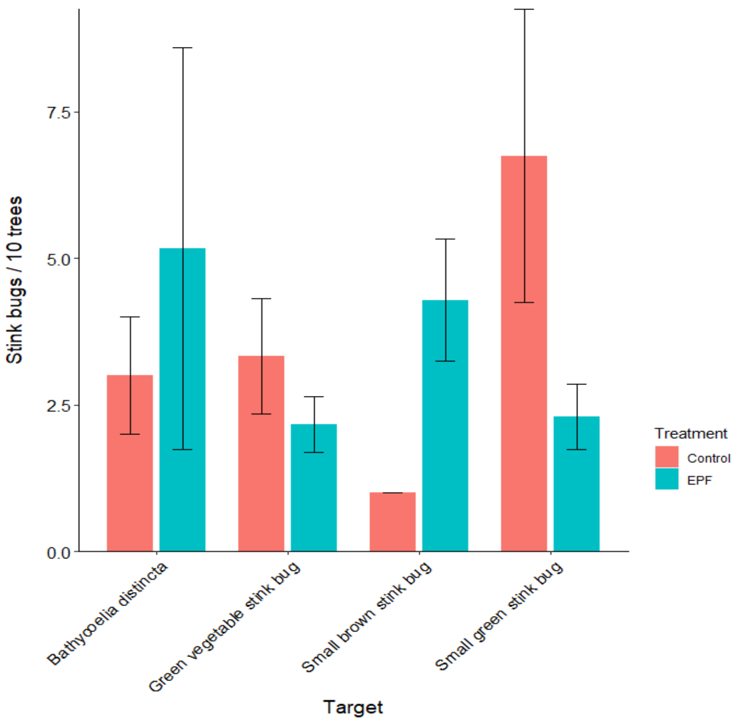

Figure 3.3. Mean ± std. error of results showing the number of stink bugs collected per 10 trees after knock-down treatment with a chemical (control) vs. entomopathogenic fungi.

Efficacy of T. basalis against B. distincta egg packets

All parasitoid treatments significantly affected *B. distincta* egg mortality (ANOVA: F $_{(3.639)}$ =216.04; p = 0.0001). A 100% parasitism rate was obtained in treatment with two pairs of parasitoids only (Table 3.2; Fig. 3.4).

Table 3.2. Summary results of Generalised Linear Model (GLM), showing the outcome effect of *T. basalis* on *B. distincta* egg survival rate.

Treatment	**Estimate Std.**	**Error**	**t value**	**Pr(>|t|)**
Intercept	0.11	0.03	4.01	**<0.0001** ***
four pairs	0.74	0.04	20.00	**<0.0001** ***
one pair	0.64	0.04	17.43	**<0.0001** ***
two pairs	0.89	0.04	23.65	**<0.0001** ***

Signif. codes : 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

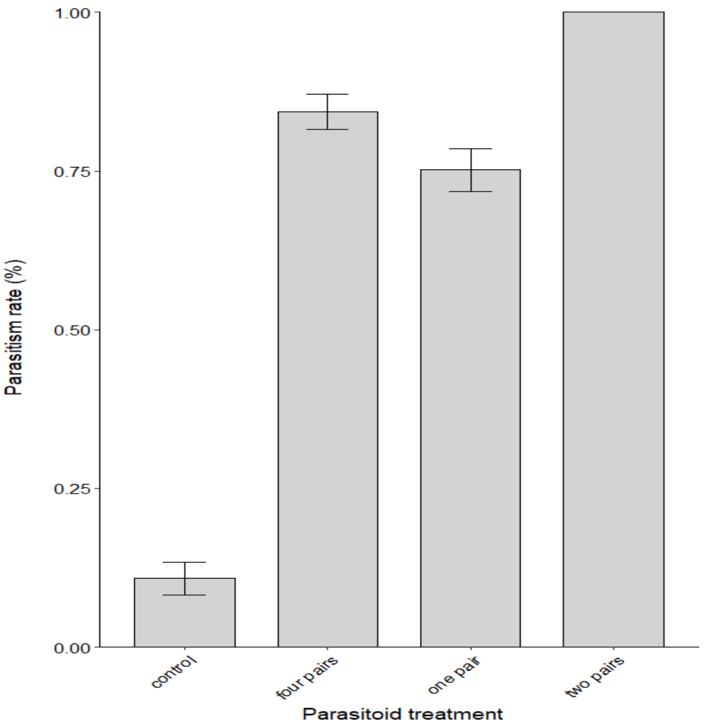

Figure 3.4. Parasitism rate of different densities of *T. basalis* on *B. distincta* eggs packets.

The parasitism rate decreased with wasp age but did not differ significantly (P=0.29). Although the parasitism rate pattern suggests that parasitism decreases with increasing egg densities, the rate did not vary significantly throughout the egg densities provided on day 2. However, day four parasitism rate differed significantly between four and two egg packets but not between two and eight packets (Table 3.3; Fig. 3.5).

Table 3.3. Results of the Generalised Linear Model (GLM) with a Gaussian distribution for the effect of parasitoid age and egg densities evaluated.

Parasitoid age and densities	**Estimate**	**Std. Error**	**t value**	**P value**
Intercept	0.53	0.070	7.57	**<0.0001 *****
Day 4	-0.11	0.10	-1.05	0.2921
Four egg packets	0.28	0.12	2.31	**0.02 ***
Two egg packets	0.33	0.15	2.15	**0.03 ***
Day 4: Four egg packets	-0.19	0.18	-1.04	0.29
Day 4 : Two egg packets	-0.16	0.23	-0.68	0.49

Signif. codes : 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

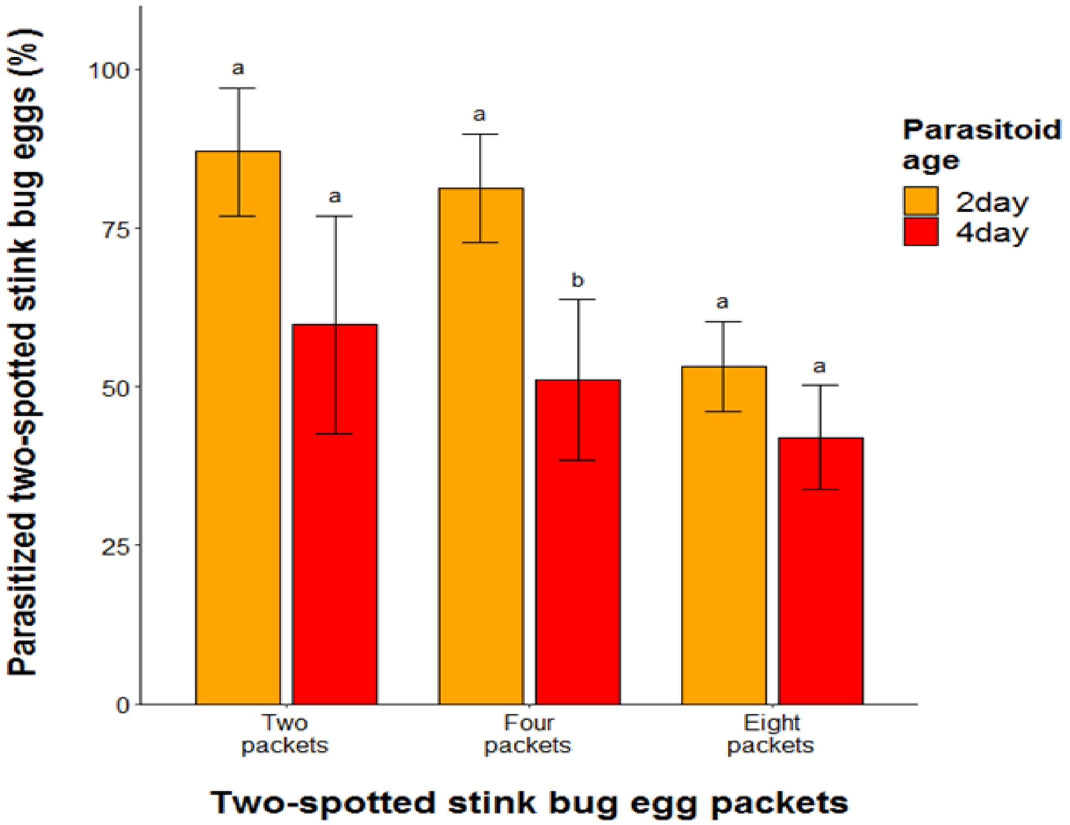

Figure 3.5. Effect of parasitoid age on parasitism rate. Different letters above bars denote significant differences between parasitism rates of parasitoid ages per number of egg packets provided.

There was no significant interaction between parasitoid age and egg density. Parasitoid generation and egg densities significantly influenced parasitism rate (ANOVA: F $_{(1.134)}$ = 32.64; p= 0.0001 & ANOVA: F $_{(2.134)}$ = 5.89; p= 0.001) respectively, with the second generation having a higher parasitism rate compared to the first generation (Table 3.4). The first-generation parasitism rate did not differ significantly throughout egg densities. However, the parasitism rate in the second generation varies significantly between two and four egg packets but not between four and eight packets (Fig. 3.6).

Table 2.4. Results of the Generalised Linear Model (GLM) with a Gaussian distribution for the effect of parasitoid generation and egg densities evaluated.

Parasitoid generation and egg densities	**Estimate**	**Std. Error**	**t value**	**P value**
Intercept	0.53	0.05	10.67	**< 0.001 *****
Second	0.41	0.07	5.79	**<0.001 *****
Four egg packets	0.28	0.09	3.25	**<0.001 ****
Two egg packets	0.34	0.11	3.03	**0.002 ****
Second: Four egg packets	-0.22	0.12	-1.81	0.072.
Second: Two egg packets	-0.28	0.15	-1.80	0.072.

Signif. codes : 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

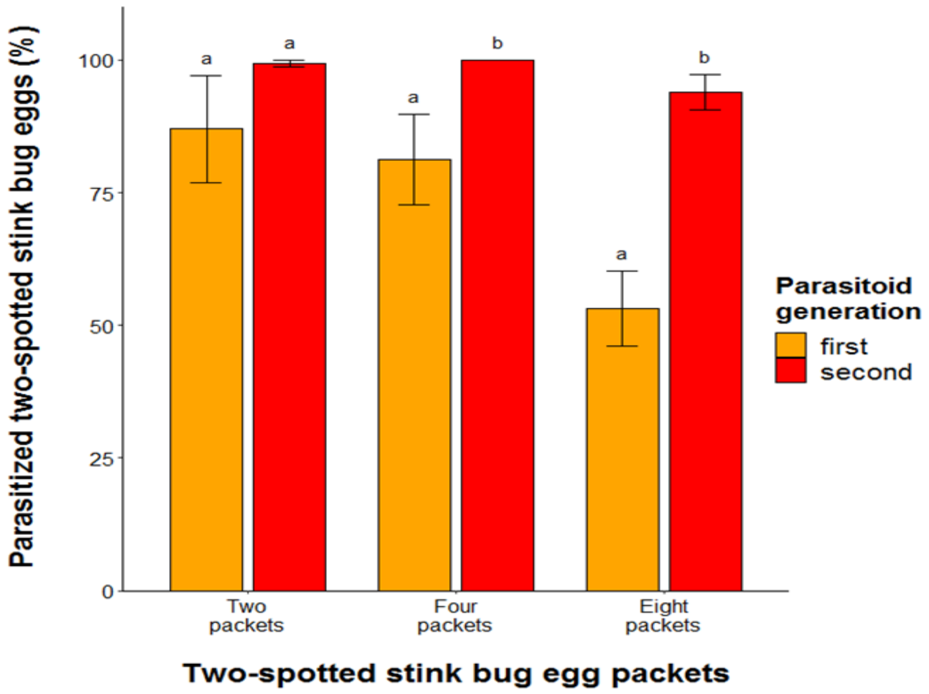

Figure 3.6. Parasitoid generation affects parasitism rate. Different letters above bars denote significant differences between parasitism rates of parasitoid generations per number of egg packets provided.

Discussion

The study investigated the efficacy of egg parasitoids, the influence of generation and age on parasitism rate, and the efficacy of EPF as biopesticides for *B. distincta*. The results demonstrate that EPF with and without chitinase enzyme are pathogenic and can be used to control *B. distincta*, and nymphs are more prone to EPFs than adults. Pathogenicity was also observed under field conditions. However, there was no significant effect between chemical knock-down spray and entomopathogenic fungi, thus suggesting that entomopathogenic fungi performed similarly when compared with chemical knock-down spray. Interestingly, under field conditions, entomopathogenic fungi did not only affect *B. distincta* but included other stink bug species, contributing to damage caused by stink bugs in macadamia orchards. *Trissolcus basalis* was able to parasitise *B. distincta* egg packets, and the effectiveness of *T. basalis* decreased with age and increased with the generation, with the second generation having a higher parasitism rate.

Entomopathogenic fungi (Sankar & Rani 2018) and parasitoids (Péré et al. 2013) are crucial components in agriculture as they regulate insect pest populations. The study results suggest that chitinase enzyme is significantly enhances pathogen entry into host haemocoel compared to combined EPF, with instar 1-3 indicating vulnerability to chitinase enzyme. Similar results were reported and further suggested that the chitinase enzyme results in perforations in the membrane, thus speeding up pathogens' entry into susceptible insects' tissues (Binod et al. 2007). Furthermore, defensive secretions that stink bugs release might have influenced the observed results. These secretions, their ratio, and composition vary with stink bug developmental stages and physiological ages in adults (da Silva et al. 2015). Thus, the vulnerability of 1-3 instars indicates that fungal treatments can easily target them.

The low mortality rate in older nymphs and adults indicates that stink bugs tend to resist fungal infection at these developmental stages. A study conducted under laboratory conditions by da Silva et al. (2015) investigated the susceptibility of rice stalk stink bug, *Tibraca limbativentris*, under different fungal concentrations of *M. anisopliae*, thus noting that with increasing fungal concentrations, older nymphs, and adult mortality increase. However, adults were still resistant to *M. anisopliae* as they survived much longer than older nymphs when exposed to all different fungal concentrations. Thus, increasing fungal concentration in all insect stages enhances their susceptibility. The results suggest that the susceptibility of the *T.*

limbativentris nymphal stages and adults is age-dependent regardless of fungal concentration and hydrocarbon composition in the cuticle and scent gland. The efficiency of fungi is linked with the number of propagules that germinate after the fungi have encountered the host species (Dalla Nora et al. 2021).

Under field conditions, entomopathogenic fungi performed similarly to chemical knock-down spray. Chemical knock-down spray affected mostly other stink bug species than the *B. distincta* population, thus suggesting that *B. distincta* might be developing resistance against chemical pesticides (Schoeman 2014). On the other hand, entomopathogenic fungi affected *B. distincta*, indicating host species specificity. The similarity between the chemical knockdown and the entomopathogenic may be influenced by environmental factors such as temperature, relative humidity, and solar radiation.

Temperature can influence the germination, growth, and quality of the fungi on and in the host and the environment. Low temperatures can influence entomopathogens by reducing their germination and growth rate, thus prolonging successful infection, whereas high temperatures may influence entomopathogens by increasing or decreasing growth rate depending on the temperature required by both host and fungal strain (Zimmermann 2007). The study region's maximum temperatures often exceed 35°C but rarely reach 40 °C, and these temperature values fall between the maximum temperatures reported for entomopathogenic fungi *Beauveria bassiana,* which are 30 – 38°C, with optimum temperatures of 23 -28°C depending on fungal isolates (Muller-Kogler 1965; Roberts & Campbell 1977), thus temperature might have slightly influenced the entomopathogen.

Relative humidity is a crucial environmental factor that can affect the efficacy, survival, and spore germination of entomopathogens. Low or high humidity and high temperatures can affect the fungi's quality and persistence (Zimmermann 2007). Specifically, high temperature and humidity may cause protein denaturation and membrane disorganization, and low humidity with high temperature may damage the DNA, thus resulting in mutation due to loss of nucleotide base causing denaturation (Nicholson et al. 2000). Solar radiation is the most lethal factor that affects the fungal persistence in the field (Zimmermann 2007). A study using simulated sunlight on 65 isolates of *Beauveria bassiana* demonstrated that the fungal survival rate decreases with increasing exposure time. It further indicated that fungal exposure to simulated sunlight for 2 hours was more detrimental to all fungal isolates (Fargues et al. 1996).

Scelionid wasps are important biological control agents for stink bugs and many agricultural pests (Bin & Johnson 1982). In this study, our results demonstrated the efficacy of *T. basalis* against *B. disticta*, and the results suggest that *T. basalis* can be used as a biological control agent for *B. distincta*. Similar efficacy was also recorded for *T. basalis* when controlling *Nezara viridula* (Linnaeus) (Pentatomidae) (Gard et al. 2022). Generally, *T. basalis* is the most crucial biocontrol agent for *Nezara viridula* worldwide (Canton-Ramos & Callejón-Ferre 2010). These results are not surprising as *T. basalis* is also known to parasitise other pentatomids egg masses (Jones 1988; Loch & Walter 1999). A 100% parasitism rate was obtained when using two pairs of parasitoids. These findings suggest that the female parasitoids can co-exploit a patch without being aggressive to each other for some time (Field & Calbert 1998). However, parasitism rate results for four pairs suggest an intra-specific competition. Recently, Gerd et al. (2022) quantified the release rate to control *Neraza viridula* under greenhouse conditions and found that more is less, suggesting that releasing more parasitoids yields less parasitism rate due to intra-specific competition between parasitoids. The parasitism rate was affected by age, with a decreasing parasitism rate on day 4. The results suggest that the decrease in parasitism rate was influenced by age-specific fecundity. These results are not surprising as *T. basalis* age-specific fecundity has been reported to follow a decreasing trend as female age increases (Abdel-Salam et al. 2007). Similar results were also reported for *Trissolcus semistriatus* (Nees, 1834) (Hymenoptera: Scelionidae), with the highest number of eggs on the first day of oviposition and the number of eggs decreasing as the female ages (Kivan & Kilic 2006). Parasitism improves with generations, suggesting that *T. basalis* can successfully parasitise *B. distincta* eggs and establish a viable population.

Natural enemies can provide permanent, immediate, and temporary pest management. In this study, for the first time, we demonstrate that *T. basalis* and EPFs can be used as biological control agents against *B. distincta*. To quantify the efficacy of *T. basalis*, our study was limited to the laboratory environment. Therefore, further studies are still required to quantify the effective release rate under field conditions. The success of an augmentative biocontrol strategy depends on whether natural enemies can establish their population (Pijnakker et al. 2020). Therefore, further studies are needed to evaluate the availability of essential resources such as alternative hosts to support the establishment of parasitoid populations without target pests and primary hosts and food sources such as plant nectars in macadamia orchards.

The availability of these resources will promote the preventative establishment of parasitoids and predators before the arrival of pests (Pijnakker et al. 2020). Pest control rendered by pest natural enemies is vital to the farmers. However, their diversity and abundance are affected by pesticide application and landscape simplification (Maalouly et al. 2013). Therefore, we recommend reducing pesticide application and maintaining natural vegetation strips to enhance predators and parasitoid communities (Maalouly et al. 2013).

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Chapter 4

Conclusion and Recommendations

Conclusion

Although there is increasing evidence for the impact of agricultural intensification and land use change on arthropods and their ecosystem functioning and service (Vasconcelos et al. 2022), the majority of the studies have been conducted in developed countries with highly transformed landscapes and low biodiversity (Katayama et al. 2019). Many of these studies find that high biodiversity is associated with higher ecosystem services. However, a recent review shows that in developing countries and tropical environments in particular, there is very little evidence for this assertion and even fewer studies (Mertz et al. 2007).

Agricultural intensification has helped to increase agricultural production globally to meet the increasing demands, unfortunately, with evident negative impacts on biodiversity and the environment (Bommarco et al. 2013; Potts et al. 2010). Arthropod decline is undoubtedly linked to agricultural intensification (Wagner 2020). This declining trend is also evident in beneficial insects such as pollinators (Tscharntke et al. 2011). It may result in drastic loss and negatively impact ecological and economic processes as pollinators play a significant role in maintaining wild plant communities and crop production (Potts et al. 2010). This highlights that intensified agricultural practice can often disrupt or weaken agroecosystem and long-term food production stability (Tscharntke et al. 2011). Therefore, sustainable management practices are required to mitigate the impacts (Knapp et al. 2022; Vasconcelos et al. 2022).

This study focused on macadamia orchards and their surrounding remnant natural vegetation in a developing country and subtropical region of South Africa. Macadamia are perennial crops (Alam et al. 2016), contributing significantly to the South African economy (Diczbalis et al. 2014). The major pests of the industry include stink bugs and nut borers, which are currently controlled using some form of integrated pest management strategies (Schoeman 2014). This thesis aimed to investigate the relationship of intensification on arthropod assemblages by considering landscape context and transformation and pesticide application rates across ten farms in a production landscape with a gradient of macadamia intensification. The second part focused on the integrated pest management options to control *Bathycoelia distincta*, the economically most significant pest of macadamia, as currently, there is no true integrated pest management program that has been fully archived (Schoeman 2014).

Chapter two investigated the relationship of landscape context (influence of remnant vegetation, orchard edge, and interior), elevation, flying vertebrates, and pesticide application rates on arboreal arthropod diversity (abundance, richness, and size) as well as the relationship of pesticide application rates on nut quality metrics. Diversity was measured on two measures of biodiversity (richness and abundance) and one measure of functional diversity (body size). In this chapter, results show that landscape context, elevation, and agriculture management practices, such as pesticide application rate, are significantly related to species richness and size. Less intensively managed orchards (farms with lower pesticide application rates and have herbaceous cover crops) were associated with and supported more species larger bodied arthropods compared to more intensively managed orchards (farms with higher pesticide application rates and lacking cover crops or surrounded by other crop fields).

A recent study reported that arthropod abundance decreases mainly due to high insecticide and herbicide usage, orchard structure, and lower herbaceous cover and further recommended reducing agrochemical inputs and maintaining herbaceous cover to mitigate the impact of olive farming intensification on canopy-dwelling arthropods (Vasconcelos et al. 2022). Natural habitat patches may often act as a source for pests (Bianchi et al. 2006). However, we highlight the importance of maintaining natural vegetation and minimizing intensive agricultural practices to promote ecological intensification and enhance ecosystem services and functioning, thus promoting sustainable food production. Therefore, remnant natural vegetation in and around conventional farms is a crucial component of the landscape in the farming system and can promote ecological intensification (Bommarco et al. 2013). In this study, remnant vegetation patches supported high arthropod diversity, and a growing literature indicates the importance of natural habitats as they often harbor beneficial insects (predators, parasitoids, and pollinators) (Gaigher et al. 2015; Geldenhuys et al. 2022; Theron et al. 2020). The effect of flying vertebrates was insignificant; however, this does not mean that flying vertebrates are unimportant, but it suggests that their impact may vary across the macadamia growing season. Typically, bat activities tend to double in macadamia orchards during the wet season, signifying prey availability of macadamia insect pests, thus attracting more bat species (Weier et al. 2018).

I observed a decrease in richness at higher elevations in the remnant natural vegetation but not in the orchards. Species richness generally decreases with increasing elevation or displays a hump-shaped response (McCain 2005; Rahbek 2005). Studies by Munyai & Foord (2015) showed that ant richness decreases along an elevational transect across the Soutpabsberg mountain. The increase in species richness, size with elevation, and comparable size throughout the landscape context at a higher elevation is explained by the area heterogeneity trade-off hypothesis (AHTO) and temperature. The increased area heterogeneity supports species richness due to increasing opportunities for niche partitioning (Chocron et al. 2015). On the other hand, temperature is predicted to influence the development and generation turnover of *Bathycoelia distincta* at a higher elevation (Muluvhahothe, 2023).

As expected, arboreal arthropod diversity was also related to insecticide applications. Land cover and agrochemical inputs are key drivers of diversity (Benton et al. 2003; Robinson & Sutherland 2002). In general, pesticides act rapidly and are extremely easy to use, cost-effective, and efficient. However, the improper use of insecticides affects non-target species, such as beneficial insects (Jafar et al. 2013), and long-term usage may lead to the development of resistance (Sosa-Gómez et al. 2020). We expected to find a decrease in insect damage with increasing pesticide application rate. Surprisingly, kernel damages caused by insects were not related to pesticide application rates, and there was a significant positive relationship between the proportion of immature nuts and pesticide application.

Pesticides are used in crops to limit stress induced by insects, microorganisms, nematodes, and competition caused by weeds. However, pesticides can also alter plant health and physiology and subsequently lead to yield loss (Saladin & Clément 2005). Besides the effects of chemicals on crops, we suggest that pollination deficiency might have contributed to increased immaturity in highly intensified orchards due to pesticide usage and degradation of natural habitat, thus resulting in a lack of wild pollinators. A recent study found that managed honeybee colonies increased flower visitation but not nut set (Grass et al. 2018). Maintaining landscape heterogeneity and reducing farming intensity can improve ecological functions for crop production (Duflot et al. 2022). In this study, orchards associated with a high proportion of sound kernels had low pesticide application rates. The positive relationship between arthropod diversity and nut quality and their negative response to pesticides suggests a causal link between the former and support for the concept of ecological intensification.

Chapter three dealt with developing and investigating the efficacy of potential natural enemies of *Bathycoelia distincta*. Firstly, this chapter investigated the efficacy of combined EPFs (*Bacillus thuringiensis* + *Beauveria bassiana* + *Metarhizium anisopliae*) with and without chitinase enzyme against *Bathycoelia distincta* under laboratory conditions and combined EPFS without chitinase enzyme was also evaluated under field conditions. Under both conditions, entomopathogenic fungi were effective against *Bathycoelia distincta*, particularly on nymphs. These suggest that nymphs are more prone to EPFs than adults and further signify that entomopathogenic fungi can be used as a biopesticide to control *Bathycoelia distincta* in macadamia. Although entomopathogenic fungi are crucial components for pest control, their adoption is hindered by the time taken to be effective (Sankar & Rani 2018). Insects infected with entomopathogenic fungi take 3- 5 days to die (BuGtI et al. 2018), and the effectiveness of the strain depends on temperature, incubation period, humidity, host species, and its life stages (Sosa-Gómez & Alves 2000). Despite all the required environmental conditions, using entomopathogenic fungi as biological control is an environmentally friendly alternative to conventional insecticides (Loc et al. 2002; Wu et al. 2014). The results, therefore, recommend using entomopathogenic fungi with other measures within the integrated pest management framework to control the stink bug population.

Lastly, the chapter also investigated the effectiveness of *T. basalis* as a potential biological control of *B. distincta*. Egg parasitoids are essential for controlling stink bugs (Panizzi & Slansky Jr 1985), and species such as *T. basalis* have been used in many countries to control stink bugs (Corrêa-Ferreira 2002). Specifically, *T. basalis* has been used as a biological control agent for its main host, and *T. basalis* has been used as a biological control agent for its main host, *Nezara viridula* (Ferreira 1980). As expected, *T. basalis* was able to parasitise *B. distincta* egg packets and have viable offspring; thus, this observation suggests that *B. distincta* can be considered as a primary host for this solitary egg parasitoid in macadamia orchards. Although our egg parasitoids used in the first-generation trial were reared using *Nezara viridula*, no innate host-acceptance behaviour was observed. Therefore, we suspect that the low parasitism rate in the first generation was due to age-specific fecundity and intra-specific competition between parasitoids at high densities. This observation suggests that although female parasitoids co-exploit a patch (Field & Calbert 1998), releasing more parasitoids might affect the rate of parasitism due to competition (Gard et al. 2022). Therefore, releasing fewer parasitoids might be ideal for archiving a high parasitism rate and maintaining the pest population below the economic threshold. These preliminary results may be useful in evaluating the potential to regulate other Pentatomidae species, which are not economically important but contribute to the damage caused by insects in macadamia orchards.

Study limitations and recommendations

Here the study quantified the impact of landscape context and intensification on arthropod biodiversity associated with macadamia orchards and its implication to nut quality. The study was limited to one growing season; thus, longer-term studies can provide more robust inferences. The taxonomic resolution of the study did not allow for an evaluation of arthropod functional guilds, which would have provided for a more nuanced analysis. Predators play a crucial role in agriculture by suppressing pest species and subsequently reducing the need for hazardous and expensive insecticides. Thus, such work will provide insight into how a range of predator taxa respond to landscape context and intensification. The finding in this work highlights the importance of reducing agricultural intensification, maintaining habitat diversity within crop fields, and conserving remnant natural vegetation, thus promoting ecological intensification.

Ecological intensification advocates for effective management of supporting ecosystem services and functions to enhance sustainable food production, and this process depends on sustainable management of non-crop habitats (Garratt et al. 2017). Garibaldi et al. (2021) advised that 20% of natural habitat should be retained to maintain regulatory and support services. Furthermore argues for maintaining high-quality levels of natural habitat in landscapes where currently the available natural habitat exceeds the recommended minimum quality levels of natural habitat (Garibaldi et al. 2021). Macadamia establishment is increasing rapidly in South Africa, with about 16 284 ha in Limpopo province covered with macadamia plantation (Shephard 2023), most of the hectares situated in Levubu. Therefore, the study results recommend that 20% of non-crop habitat around macadamia orchards be conserved due to their ability to influence arthropod activities (Blitzer et al. 2012) and to harbour natural enemies that may colonise crop fields and reduce both yield loss and crop damage (Östman et al. 2003). The high pesticide application rate in this study is not associated with the proportion of sound kernels, which suggests that farmers are not using pesticides in a cost-effective way. Therefore, relying on an integrated pest management frame, reducing pesticide application, and promoting biological pest control will be more cost-effective.

Entomopathogenic fungi and egg parasitoids are viable biological agents to control *Bathycoelia disticta* populations in macadamia orchards because they are environmentally friendly, tend to be host-specific, are native to South Africa, and are commercially available. In consideration of macadamia farming practice, recommended future work should evaluate the effective release rate within an integrated pest management framework, as the currently available release rate is effective under greenhouse conditions when controlling *Nezara viridula* (Gard et al. 2022). We further recommend that for effective pest control, egg parasitoids to be released should be reared using the target host species to reduce the chances of innate host-acceptance behaviours. This thesis contributes to the available integrated pest management strategies currently applied in the macadamia industry to maintain pests below economic thresholds.

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Appendix 2. B Elevation, active ingredient applied for a specific target pest, and percentage damage caused by the target pest for the six farms where invertebrates were sampled.

		Target pest: active kg/ha		Percentage damage %	
Farms	Elevation (m)	Stink bug	Nut borers	Stink bug	Nut borers
A	890	1.13	0.36	1.12	0.15
B	739	3.02	0.4	1.4	0.47
C	1241	0	0.08	1.49	0.93
D	1331	0	0.08	1.83	1.78
E	1241	0	0	17.7	0.22
F	777	4.67	0	2.53	1.06

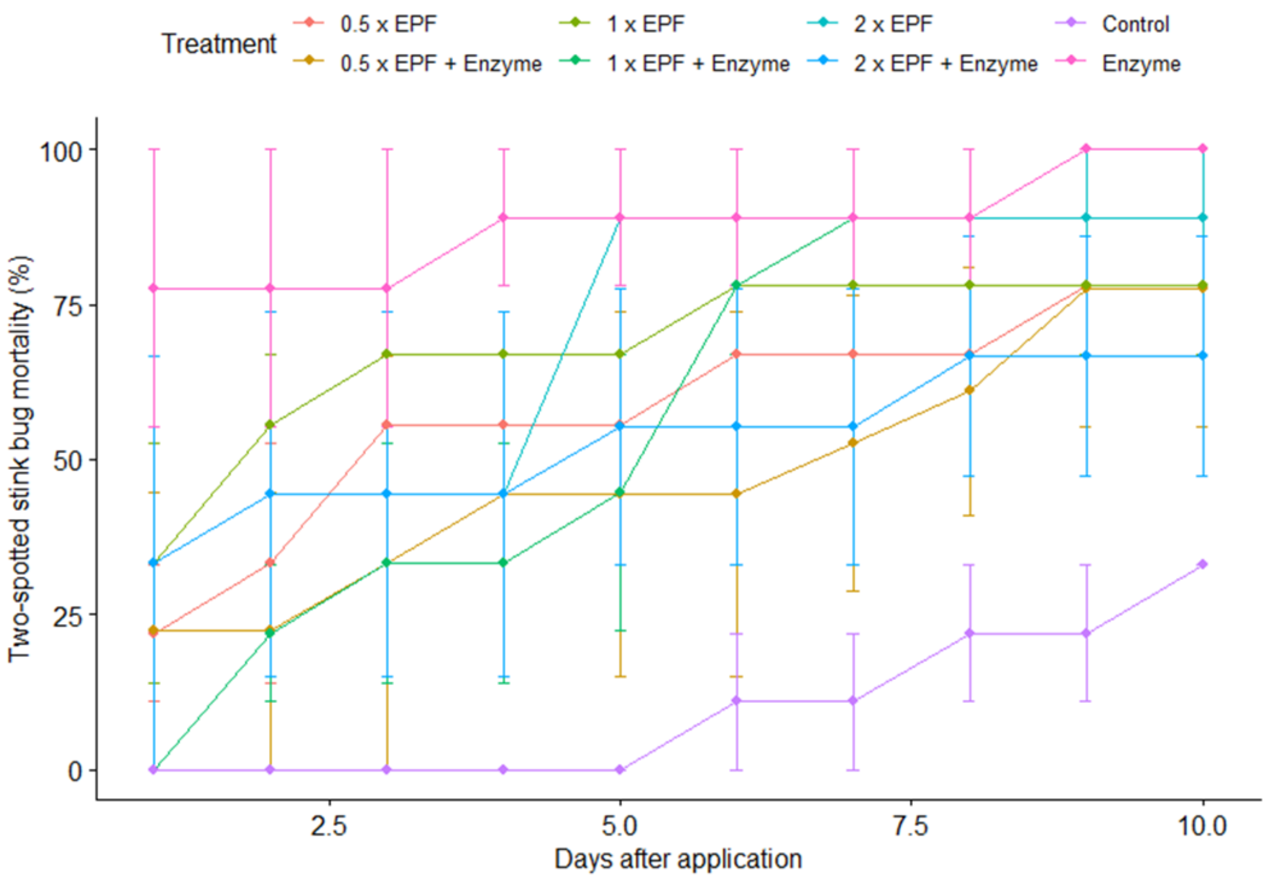
Appendix 2. C Total active ingredients applied per specific target pest, classified by group code and total cost per hectare.

Farm	Target pest	Active kg/ha	Group code of applied insecticides	Cost per hectare (ZAR)
A	stink bug	1.13	3A 13 1B	4000.66
A	borer	0.4	4A	4000.66
B	stink bug	3.02	3A 13 1B	1633.73
B	borer	0	0	1633.73
C	stink bug	0	13	2455.08
C	borer	0.08	11A	2455.08
D	stink bug	0	13	2455.08
D	borer	0.08	11A	2455.08
E	stink bug	0	0	0
E	borer	0	0	0
F	stink bug	4.67	3A 1B 28 3A 9B	8051.89
F	borer	0.36	28 3A 4A 6	8051.89
G	stink bug	2.86	1B 3A	3588.85
G	borer	0.03	18	0
H	stink bug	3.58	1B 3A	2736
H	borer	0.06	4A 3A	2583
I	stink bug	2.42	1B 3A	2685.05
I	borer	0.28	0	0
J	stink bug	1.87	1B 3A	3396.5
J	borer	0.6	18	0

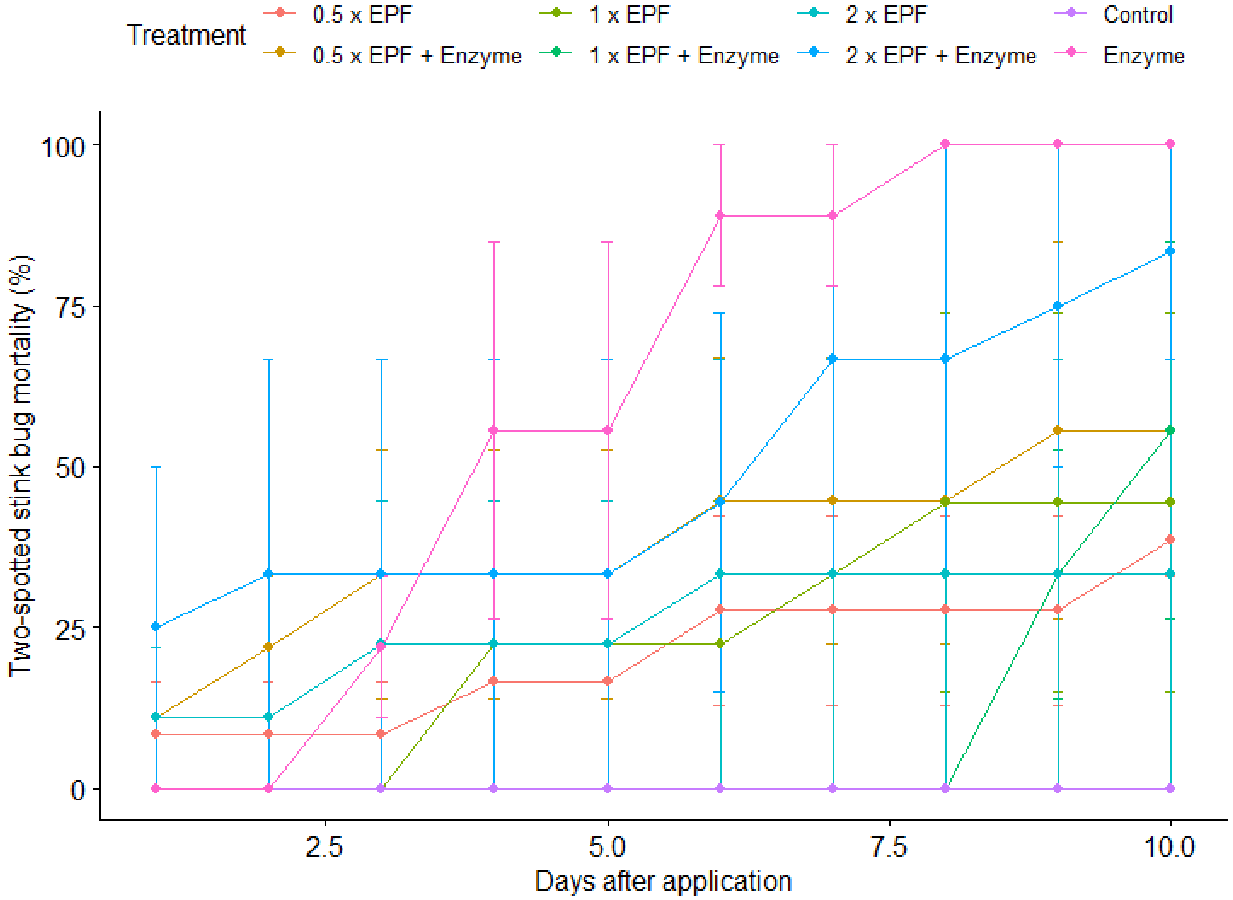
Appendix 2. D Nut quality metrics for nuts harvested during the 2020/21 growth season at all the sampling units across ten farms. The metrics included major and minor economic defects.

Kernel weight (Kg)		Kernel defects %							
Site	Total shelled nuts	Sound kernel	Immature kernel	Early stink bug damage	Late stink bug damage	Nut Borer	Unsound kernel	Total stink bug damage	Total Kernels
A	6992	75.59	20.01	0.39	0.73	0.15	4.40	1.12	96.88
B	11593	48.51	45.21	0.54	0.86	0.47	6.28	1.40	95.59
C	4573	80.40	14.66	0.98	0.51	0.93	4.94	1.49	97.48
D	8601	70.33	22.53	1.41	0.42	1.78	7.14	1.83	96.47
E	3961	66.64	12.35	5.91	11.79	0.22	21.01	17.70	96.90
F	2394	52.89	35.43	1.12	1.41	1.06	11.67	2.53	91.92
G	3695	49.47	32.77	3.23	5.94	0.70	17.76	9.17	92.12
H	5687	44.28	41.76	1.04	1.94	0.79	13.96	2.99	89.82
I	6332	59.99	25.36	7.25	2.42	2.39	14.66	9.67	97.40
J	7034	61.02	31.47	1.25	1.91	0.64	7.51	3.16	96.29

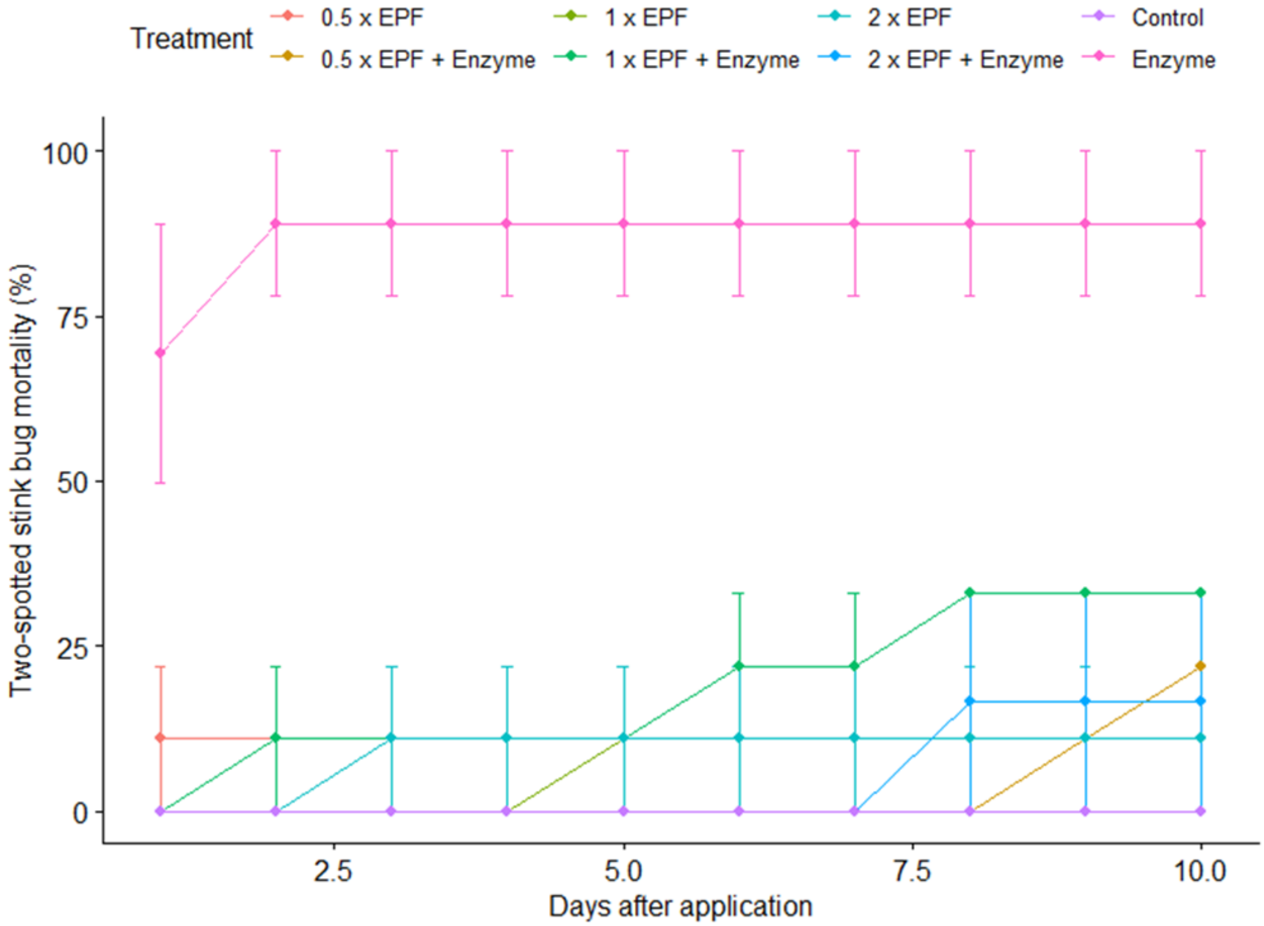
Appendix 3. A Summary on early stages (1^{st} – 3^{rd} instars) of two-spotted stink bug (*B. distincta*) mortality rates after application of treatments.



Appendix 3. B Summary on medium stages (4th –5th instars) of two-spotted stink bug (*B. distincta*) mortality rates after application of treatments.



Appendix 3. C Summary of adults of two-spotted stink bug (*B. distincta*) mortality rates after application of treatments.



Appendix 3. D The effect of entomopathogenic fungi on target pest stink bugs.

Treatment and Target pest	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	1.2040	0.2236	5.384	**0.0001*****
EPF	-0.4308	0.3563	-1.209	0.2266
Small brown stink bug	-1.2040	0.7416	-1.623	0.1045
Small green stink bug	0.7056	0.2950	2.392	0.0168 *
Bathycoelia distincta	-0.1054	0.4655	-0.226	0.8209
EPF x Small brown stink bug	1.8861	0.8126	2.321	0.0203 *
EPF x Small green stink bug	-0.6459	0.4555	-1.418	0.1562
EPF x *Bathycoelia distincta*	0.9744	0.5708	1.707	0.0878.

Signif. codes : 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1